Resource allocation to growth and structure:
The cost of mussel attachment in a dynamic coastal environment

Emily A. Roberts

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2019

Reading Committee:

Emily Carrington, Chair

Jacqueline Padilla-Gamiño

Kenneth Sebens

Program Authorized to Offer Degree:

Department of Biology

University of Washington

Abstract

Resource allocation to growth and structure:
The cost of mussel attachment in a dynamic coastal environment

Emily A. Roberts

Chair of the Supervisory Committee:
Emily Carrington
Department of Biology

Specialized mechanical structures produced by organisms provide crucial fitness advantages, but the production of these materials can result in energy allocation trade-offs, affecting population and species distributions. Producing a higher quality or quantity of structure can result in less energy to invest in other processes such as growth. Energy budget models, including Scope for Growth (SFG), provide a framework with which to investigate energetic trade-offs in organisms. In wave-swept rocky shore habitats, attachment is key to survival. Mytilid bivalves produce byssus, a network of collagen-like threads that tethers individuals to hard substrate. In this dissertation I investigate linkages between energetics, mussel attachment, and growth from three different perspectives. In Chapter 1, I evaluate the linkage between energetics and mussel attachment by perturbing the energetic state of the mussels (*M. trossulus* and *M. galloprovincialis)* by exposing them to a range of temperature and food conditions and evaluating growth and attachment. In Chapter 2, I evaluate an energetic trade-off between byssal thread production and growth by combining a field manipulation with a Scope for Growth model of *M. trossulus*. Specifically, mussels are induced to produce a greater number of threads by severing the byssus, and the cost per thread produced is calculated from the relationship between byssal thread production and growth. Finally, in Chapter 3, I return to the linkage between energetic and mussel attachment, and address this question by calculating SFG, and exposure to other physiological stressors, across a range of natural seawater conditions at two depths over two years, for *M. trossulus* and *M. galloprovincialis*. I then use a stepwise multiple regression analysis to evaluate whether SFG, or other physiological stressors, are the best predictors of growth and attachment. Overall, I find no evidence for a relationship between energetics and mussel attachment for either species in the laboratory manipulation (Chapter 1) or in the field observation (Chapter 3). This work does provide evidence, however, for a trade-off between byssal thread production and growth, and using the SFG model I calculate that the cost of producing a byssal thread is approximately 1 J and that byssal thread production costs range up to 65% of the energy budget (Chapter 2). Together these results suggest that, unlike growth, byssal thread production is not an energetically-constrained trait, and that there is an energetic trade-off between byssal thread production and growth. Field observations (Chapter 3) also suggest that other physiological stressors, co-occurring low dissolved oxygen and pH that are predicted to be exacerbated by global change, affect growth but not byssal thread production. Overall this work suggests that in the field, growth (which includes growth of reproductive tissue) is constrained by both energetic resources and physiological stressors, and that induction of byssal thread production (i.e. cued by waves, etc.) has a cost on growth. Anthropogenic global

change is projected to affect seawater conditions (i.e. lowered pH, dissolved oxygen) and increase ocean wave power. This investigation of the linkages between energetics, growth and investment in a structural material suggests that more severe abiotic stressors may increase energetic constraints, either directly by affecting feeding rates and costs or indirectly through trade-offs. Greater energetic constraints can affect organism size and reproductive output, and may ultimately affect organism fitness and population dynamics.

TABLE OF CONTENTS

List of Figures

List of Tables

Acknowledgements

I wish to thank everyone at the University of Washington who has supported me in my graduate work. I thank my advisor, Emily Carrington, who has been a source of endless support the past seven years. I'd like to thank Emily for inspiring conversations and encouragement in formulating abstract research questions, and for coaching me to help me improve my efficacy and work life balance, and for sharing your wisdom in anticipating and/or solving experimental problems. Thank you for reading countless drafts and for helping me improve my writing.

I thank my committee members, past and present, who have helped shape different aspects of my dissertation, Lauren Buckley, Ken Sebens, Jacqueline Padilla-Gamiño, Dave Beauchamp, Terrie Klinger, and Tim Essington. You have given me many insights in bioenergetics and encouragement in delving into this field, helped me contextualize my work and understand its importance and limitations, and have supported me and given great practical advice.

I thank the staff at Penn Cove Shellfish LLC., including Ian Jeffords, Tim Jones, Jim Nagel, and Zach Loescher, a for your support of the project and for sharing your knowledge of mussel aquaculture and marine ecology with me.

I thank my lab members, Hilary Hayford, Lyda Harris, Matt George, Laura Newcomb, Jaquan Horton, and Rebecca Guenther, and for your guidance and willingness to chat about science and life. I would also like to thank many post-graduate volunteers and undergraduate students including Windy Madden, Michael Pantalos, Brandon Ojogho, Tyler Adamson, Alexis Massey, Nicholas Rose, Pei-Jou Tsai, Silvia Chen, Claire Marvet, and Sarah Champ, for your enthusiasm and endurance in working alongside me in sample processing and/or field experiment setup and maintenance. I thank the Friday Harbor Laboratories community and Biology

Department community, including Michelle Herko, Kristy Kull, Zander Fodor, Will King, Alex Lowe, Sasha Seroy, and Katie Dobkowski and many others for helping me become a better scientist and teacher and for supporting me in my research. Finally, I thank my friends and family for your support over the years.

Chapter 1

Attachment and energetic state: Byssal thread production and growth are not correlated across a temperature and food gradient for two congeneric mussel species

Emily A. Roberts[1,2*] and Emily Carrington[1,2]

1 - Department of Biology, University of Washington, 4000 15th Ave NE, Seattle, WA 98195 USA

2 - Friday Harbor Laboratories, University of Washington, 620 University Road, Friday Harbor, Washington 98250 USA

Keywords: bioenergetics, energy budget, thermal stress, resource allocation, biomechanics, *Mytilus trossulus*, *Mytilus galloprovincialis*

1.1. Abstract

Anthropogenic warming combined with natural climate variability affect global patterns of seawater temperature and marine primary productivity and affect organism survival, growth and physiology. Mussels are habitat engineers that utilize byssal thread structures to attach to hard substrate, a strategy key to survival in dynamic rocky shore habitats. Byssal thread quantity and quality varies according to season and environmental conditions, and temperature and food availability may influence the production of these structures by affecting energy limitation. We hypothesized that temperature has opposing effects on growth rates of the two species and that either (A) byssal thread production is positively correlated with mussel growth rate, or (B) byssal threads are produced constitutively regardless of growth rate. We manipulated seawater temperature and food availability and quantified mussel performance in terms survival, growth, and byssus attachment. We found that over the locally relevant temperature range, *M. galloprovincialis* had high performance as indicated by positive shell and tissue growth and *M. trossulus* had low performance as indicated by minimal shell growth and tissue loss, and a greater loss of tissue with increased temperature. Temperature had opposing effects on each species; temperature increased shell growth of *M. galloprovincialis* but increased tissue loss of *M. trossulus*. Temperature did not, however, significantly affect byssal thread production and there was no significant relationship between byssal thread quality or quantity and shell or tissue growth across the temperature and food gradient for either species. Our results suggest that energy allocation is prioritized towards byssal thread production, and that the production of these structures might remain costly even under conditions of suboptimal temperature and food limitation. We also observed that increasing temperatures strongly decreased *M. trossulus*

survival and increased *M. galloprovincialis* shell growth, suggesting that increasing ocean temperatures may select for *M. galloprovincialis* in Salish Sea mussel populations.

1.2. Introduction

Anthropogenic warming and nutrient input combined with natural climate variability affect global patterns of seawater temperature and marine primary productivity (IPCC 2018). Temperature and food availability are well known to affect the growth, reproduction, and survival of marine organisms and thus represent major drivers in marine ecological systems (Pörtner 2012, Menge 1992, Menge et al., 2003). Key challenges in marine ecology include identifying how thermal stress impact nutrition by affecting consumption, digestion and metabolic rates (Sokolova 2013, Kooijman 2010), how nutrition influences tolerance and acclimatization to physical stressors (Melzner et al., 2011), and how temperature and food availability interact to influence multiple organismal traits (Kellermann et al., 2019). Identifying how various aspects of organism thermal performance (i.e behavior vs. physiology) differ, and contribute to fitness is important in predicting how organisms will respond to natural variability and change due to ocean warming (Monaco et al., 2018, Guderley and Pörtner 2010).

Marine suspension feeders, such as bivalve molluscs, are particularly sensitive to climate variability (Hilbish et al., 2010, Westerbom et al., 2019, Black et al., 2009). Seawater temperature and food availability are both important factors that affect suspension feeder growth, and the interaction between these two factors is context-dependent and may have non-linear synergistic effects on performance (Todgham and Stillman, 2013). For example, in highly dynamic rocky intertidal habitats, temperature is a major factor driving spatial and temporal patterns in mussel growth and physiological stress response (Blanchette 2007, Lesser et al., 2010). In other intertidal and subtidal systems, however, food availability is the main factor driving patterns of mussel growth and stress response (Dowd 2013, Dahlhoff and Menge 1996) and gradients in food limitation may exacerbate or counteract the effect of stressful thermal conditions (Dowd et al., 2013, Blanchette 2007). A further challenge is linking the mechanistic relationships underlying how these factors interact and affect population-level responses (Guderley and Pörtner 2010, Matzelle et al., 2015).

One approach to understanding how environmental temperature and food availability influence organismal growth in field settings is bioenergetics modelling (Nisbet et al., 2012, Matzelle et al., 2014, Filgueira et al., 2011). These models describe the physiological relationship between food availability and temperature in terms of mass balance of temperature-dependent metabolic costs and consumption of food (Kooijman 2010, Kitchell et al., 1977, Widdows and Bayne 1971, Bayne 1976). Temperature generally increases enzyme activity and the overall metabolic cost of tissue maintenance (Q-10 response). As temperature increases further, additional metabolic costs may include temperature-induced oxygen depletion (Pörtner and Farrell 2008) and protein protection and repair (Fields et al., 2012, Lindquist 1986). Food consumption rate is also temperature-dependent, often with species-specific thermal optima (Kitchell et al., 1977). The difference between energetic input and metabolic cost is the surplus energy available for growth, or Scope for Growth (SFG, Widdows and Bayne 1971, Fly and Hilbish 2013). This framework may be used to assess how much energy is available for growth of tissue, shell, and other biomaterials, and how this energy is allocated towards these materials.

While thermal stress and food limitation often are assumed to act additively to influence tissue growth (Kitchell et al., 1977), but these factors may not affect byssal thread production in a proportional way. Energy may be allocated preferentially to certain biological functions, such as stress response and biomaterial production. Greater food availability may act to buffer organisms from abiotic stress by providing additional energetic resources to protect against cellular damage and perform cellular repair (Dowd et al., 2013, Ramajo et al., 2016, but see

Hettinger et al., 2013). In contrast, when resources are depleted due to starvation, organisms may not be able to allocate energy towards alleviating the effects of abiotic stressors. This nutritional buffering capacity is evident in recent ocean acidification research, where greater food availability limits the effect of low pH on bivalve shell maintenance and larval development (Melzner et al., 2011, Thomsen et al., 2013), and coral calcification (Edmunds 2011).

Mussels are dominant habitat-modifying organisms on rocky shores, and the survival and competitive dominance of these organisms depends on their ability to attach securely to hard substrate (Denny 1995). Mussel attachment depends on the quality and quantity of threads in the byssus (Carrington et al., 2002, Carrington et al., 2015). Byssus is a structural biomaterial comprising a network of collagen-like threads that tethers each individual to hard substrate (Bell and Gosline 1996, Waite et al., 1998). Byssus strength varies seasonally in *M. edulis* and *M. trossulus*, with weak attachment during summer when mussels are prone to limited food availability, elevated temperature, as well as reproductive cycles (Carrington et al., 2002, Newcomb 2015, Zardi et al., 2007). When attachment is too weak to withstand hydrodynamic forces, up to 30% of a population may be lost by dislodgement (Carrington et al. 2009).

Production of byssal threads is energetically costly and might be limited by energetic constraints. Of the carbon and nitrogen incorporated into the organic tissues of *M. edulis*, ~8% is used to produce byssal threads for the mussel *Mytilus edulis* (Hawkins and Bayne 1985). Energetically costly activities, such as gamete development, may deplete resources necessary for production of threads regardless of food availability; small mussels induced to spawn produce fewer byssal threads (Babarro et al., 2010). Moreover, starvation decreases byssal thread strength and production in small, but not large mussels (Babarro et al., 2008), suggesting energy reserves are greater and/or thread production may be prioritized for larger body sizes. Altogether, these studies suggest energy limitations may affect byssal thread strength and production, but the energetic allocation trade-offs between growth and byssal thread production, and their dependence on temperature, have not been investigated directly.

Dynamic fluctuations in sea surface temperature and phytoplankton biomass in the Salish Sea may influence mussel growth and attachment in a species-specific way (Khangaonkar et al., 2019, Lowe et al., 2016). *Mytilus trossulus* and *Mytilus galloprovincialis* are found on rocky shores and are commercial aquaculture species in this region (Elliott et al., 2008). These congeneric mussel species differ in their physiological responses to temperature; *M. trossulus* and *M. galloprovincialis* are characterized as a cold-tolerant and warm-tolerant species, respectively (Lockwood and Somero 2011). More specifically, the optimal Scope for Growth temperature is lower for *M. trossulus* than *M. galloprovincialis* (10 °C vs. 25 ºC, Fly and Hilbish 2013). Further, metabolic costs exceed energy available from ingestion (scope for growth, SFG<0) for *M. trossulus* at 15 °C and above. In contrast, ingestion increasingly outweighs metabolic costs for temperatures ranging 10-25 °C for *M. galloprovincialis* (SFG > 0, Fly and Hilbish 2013). Moreover, the two species have different timing in their gametogenic cycles and spawn at different times of the year (*M. trossulus* April – July, *M. galloprovincialis* November - March; *M. trossulus* but formerly called *M. edulis* - Skidmore 1973**,** Strathmann 1987**,** Curiel-Ramirez and Caceres-Martinez 2004), though the reproductive cycle of *M. galloprovincialis* is less well characterized in the Salish Sea. Because of these differences in reproductive cycles and thermal optima, and the potential linkage between energetic resources and byssus production (Babarro et al., 2010, 2008, Clarke., 1999), we hypothesize these two species will differ in their ability to produce attachment byssus in different combinations of temperature and food availability.

To elucidate the role that energetics plays in byssal thread production and attachment strength, we investigated how the effect of food availability combines with the effect of temperature on growth and byssal attachment of two mussel species, *M. trossulus* and *M. galloprovincialis*. In the laboratory, we exposed mussels to factorial combinations of environmentally-relevant temperatures and food availability for 10-weeks, and then measured survival, growth, and byssal thread quantity and quality. We hypothesize that (1) temperature has an effect on growth rate according to known species-specific temperature relationships (Fly and Hilbish 2013), and (2) the effects of food availability and temperature on growth rate are additive for both species. Furthermore, we hypothesize that either (3) energy is allocated evenly to growth and byssal thread production such that growth and byssal thread quantity and quality will have a significant positive relationship across temperature and food treatments, or (4) energy allocation is prioritized towards byssal thread production, such that there is no relationship between growth and byssal thread quantity and quality.

1.3. Methods

1.3.1. Experimental Setup

The effect of temperature and food on the growth and attachment strength of *Mytilus trossulus* and *M. galloprovincialis* was investigated in a fully-factorial experiment over ten weeks (Aug 19 – Oct 31, 2016). There were four temperature levels representing the upper range of local summer seawater temperatures (13, 15, 18 and 21°C) and two food levels (high and low) representing near and below saturating food levels. Each of the three replicates of the eight treatment combinations was established in a ‘mesocosm,’ a 22 L square HDPE food-safe container (S-16971, ULINE), that housed eight individuals of each of the two mussel species (Figure 1.1).

Seawater input to each flow-through mesocosm came from a common supply of 1-micron filtered seawater at a rate of 11 ± 3 L per hour. Incoming seawater was cooled to ~12°C (AquaEuroUSA MC-13HP Max-Chill, Los Angeles CA). To avoid pseudoreplication, each mesocosm was heated independently with a 300W aquarium heater (Jetsu Aquarium Supplies, London UK) controlled by a digital temperature controller (JBJ TRUE TEMP TT-1000, Inglewood CA; ±0.5°C). Within each mesocosm, seawater was circulated using a small pump and aerated with an airstone. Salinity and pH were within the ambient range for local Salish Sea conditions (~28PSU, 7.94 pH NBS scale, Murray et al., 2015).

1.3.2. Mussel Collection and Acclimation

Mussels were collected from commercial aquaculture rafts at Penn Cove Shellfish LLC., Coupeville, WA (48°13'15.1"N 122°42'20.4"W) at ~1m depth in late July 2016 and transported on ice to Friday Harbor Laboratories (FHL). The size and age of the individuals selected were representative of the farmed subtidal mussel populations. *M. trossulus* individuals were ~1 yr old with shell length ranging 55-65 mm, and tissue weight 1.04 ± 0.09 g DW (mean ± SE). *M. galloprovincialis* individuals were slightly older (~1.25 yrs old) and bigger, ranging 75-85 mm in shell length and had a tissue weight of 2.26 ± 0.17g DW. Mussels were scrubbed and immersed briefly in a chlorinated freshwater bath to remove epibionts and the effluent was quarantined as a further precaution to prevent intrastate transfer of epibiont species.

For the week prior to the onset of the experiment mussels were maintained in flowing unfiltered seawater at ambient temperature (13 °C) and fed a supplemental diet of algal paste

(Shellfish Diet 1800, Reed Mariculture, San Jose CA ~0.15% of body weight/day). Mussels were then acclimated to experimental conditions by incrementing temperatures a maximum of 2 °C per day, over 19 days. During this period and throughout the duration of the experiment, food availability was manipulated as described below.

Mussels were fed Shellfish Diet 1800 at a rate of 1.8% dry tissue weight day^{-1} (high food, a maintenance ration, Helm et al., 2004) and 0.18% dry tissue weight day^{-1} (low food level). Mussels were fed hourly rations using programmed peristaltic pumps (Aquamedic EVO 4), which supplied a concentration of approximately 1.0 mg DW L^{-1} and 0.1 mg DW L^{-1} algae each hour in each high and low food mesocosm, respectively. High and low food levels were representative of conditions above and below saturating food levels (Riisgård et al., 2011). Assuming a concentration of 2 billion cells per ml concentration, cell counts were estimated as 25 × 10^{6} cells L^{-1} and 2.5 × 10^{6} cells L^{-1}, and assuming 91.1% AFDW and a conversion factor from 30-50 g C / g Chl we estimate that concentrations were representative of 18-31 ug Chl L^{-1} and 2-3 ug Chl L^{-1} (Jakobsen and Markager 2016). The source of each food treatment was distributed among three separate 4L reservoirs, and to control for the potential effect of the reservoir, reservoirs were randomized among treatments every 4 days. Mesocosms were scrubbed and siphoned weekly to remove detritus.

1.3.3. Survival, Growth, and Attachment

Mussel performance was evaluated in terms of survival, growth, and byssus attachment. At the end of the 10-week experiment, survival of the eight individuals of each species was determined per mesocosm. Shell growth, a non-destructive metric of mussel growth, was calculated as the change in shell length, and was measured with calipers (RMSE ± 0.01mm). Dry tissue weight was determined by removing and drying tissue at 60°C for 48 hours. Condition index (CI) was calculated by dividing dry tissue weight (g) by shell length cubed (mm^3; Crosby and Gale 1990). Tissue growth (g DW), which included gonadal and somatic tissue, was calculated as the difference between final dry tissue weight and estimated initial tissue weight, which was determined from a linear relationship between shell length and tissue mass at the initial timepoint from 24 individuals per species.

Byssal thread production, thread strength, and byssus strength were also measured during the last two weeks of the experiment. Each mussel was tethered to a clear acrylic plate (four rectangular 8” x 4” plates per mesocosm, four mussels of each species per plate) to produce a new byssus. Each mussel was tethered by attaching a nylon thread to the shell with cyanoacrylate glue and tying the thread between regularly spaced holes in the plates such that mussels could move in a limited area, but would not be lost from the plate. The plates were stacked vertically 10 cm apart to ensure adequate water circulation in the mesocosm. After 15 days, the plates and attached mussels were removed from each mesocosm. The adductor muscle of each mussel was severed and the byssal root was dissected from the foot, leaving an intact byssus attached to the plate. In the rare occurrence that a mussel escaped from the nylon tether, the percentage and number of threads that were not attached to the tile substrate (i.e. attached to other mussels, the side of the tank) was determined. Plates with byssus were dried and stored for up to 2 months, then rehydrated in seawater prior to mechanical testing (Brazee 2004).

We adapted the method of Bell and Gosline (1996) to measure the strength of individual byssal threads using a tensometer (Instron 5565, Norwood MA). The number of plaques (and therefore threads) produced by each mussel was counted prior to each test. The mussel root was grasped using a hemostat and connected to a 50 N load cell while the plate on which the thread plaques were attached was clamped to the base of the tensometer. The byssus was extended 5mm/min and force (± 0.0001N) was sampled at 6 kHz. The strength of an individual thread was determined as the rapid change in force when a thread failed, while byssus strength was the maximum load supported (see Figure S1.1). The number of byssal threads that were load-bearing, i.e. not slack or broken, was visually determined over the experiment to ground-truth individual thread strength measurements. The median thread strength of each mussel was determined from the distribution of all thread breaks for each tensile test. For the few occasions where the nylon tether failed to adequately separate the byssi of neighboring mussels, byssus strength was not measured.

1.3.4. Statistical Analysis

All statistical analyses were performed with R software for Mac OSX (version 3.4, R Core Team, 2017). Percent survival was arcsine transformed, thread production was square root-transformed, and all other data were log-transformed. Shapiro-Wilks' tests indicated condition index and thread production were normally distributed, while the other metrics deviated slightly from a normal distribution. A Bonferroni test identified two significant shell growth outliers that were removed from further analyses.

Percent survival was determined as the average percent survival over the three mesocosm replicates, each containing eight mussels (n=3). The effect of temperature and food availability (fixed factors) on percent survival was assessed separately for each species using a two-way ANOVA.

The effects of temperature and food availability on growth and attachment metrics (CI, shell growth, thread production, thread strength, and byssus strength, shell) were assessed using random-intercept, linear mixed-effect models (LME; Zuur et al., 2009) using the *nlme*-package (Pinheiro et al., 2013). Each species was evaluated separately, using an LME model structure with temperature and food availability as fixed factors and mesocosm as a random effect. Wald Chi-square tests (Type II) were run with the *Anova*-function in the *car*-package, and were used to assess the statistical significance of the model parameters (Fox and Weisberg, 2011). Tukey HSD post hoc tests were used to perform pairwise comparisons between treatment combinations.

Shell growth and tissue growth were used as two alternative indices of energetic state, where shell growth was indicative of energetic surplus and tissue growth was an index of energetic scope relative to tissue mass maintenance requirements. For each species, we evaluated the relationship between indices of energetic state and byssus quality and quantity using linear regression analyses where the explanatory variables were shell growth or tissue growth and response variables were thread production or thread strength. Data were averaged by treatment to evaluate these relationships across the range of food and temperature levels.

1.4. Results

Survival of *M. galloprovincialis* over 10 weeks was high across all treatments (96 ± 6% survival; mean ± SD) and did not depend on temperature or food availability (Figure 1.2, Table 1.1, $p = 0.72$ and $p = 0.25$ respectively). Survival of *M. trossulus*, however, was lower overall (ranging 13 – 100% across all mesocosms) and decreased with increasing temperature (Figure 1.2, Table 1.1; $p= 0.046$). After 10 weeks, mean survival for *M. trossulus* at 21°C was only 35% (Figure 1.2) however food availability had no effect on survival of *M. trossulus* (Figure 1.2, Table 1.1; $p = 0.69$).

The tissue mass of *M. trossulus* decreased by 45 ± 29% while that of *M. galloprovincialis* increased by 5.1 ± 0.2% (g DW; means ± SE). Initial *M. trossulus* condition index was 5.1 ± 0.5 (g/cm$_3$), and for *M. galloprovincialis* was 4.5 ± 0.3 (g/cm$_3$; means ± SE). Final condition index was approximately two times greater for *M. galloprovincialis* compared to *M. trossulus* (Figure 1.3; Table 1.2). For *M. trossulus*, final condition index depended on temperature and food (p = 0.02 and p = 0.03 respectively; Figure 1.3; Table 1.2). For *M. trossulus,* mussels at 21°C showed a 24% decrease in condition index compared to mussels at 13°C, and low food availability decreased condition index by 11%. For *M. galloprovincialis,* low food significantly decreased condition index by 11%, but there was no effect of temperature (p = 0.01 and p = 0.52, respectively; Figure 1.3, Table 1.2). There was no significant interaction between food availability and temperature on condition index in either species (p = 0.99 and p = 0.20; Figure 1.3, Table 1.2).

M. trossulus had negligible shell growth overall, and there was no significant effect of temperature or food on shell growth (p = 0.12 and p = 0.14 respectively; Figure 1.3, Table 1.2). For *M. galloprovincialis*, shell growth increased with greater temperature and food availability (p = 0.01 and p < 0.001, respectively). There was no significant interaction between temperature and food availability for either species (p = 0.23 and p = 0.30; Figure 1.3, Table 1.2).

There was no significant effect of temperature or food availability on byssal thread production, thread strength, or byssus strength for either mussel species tested (Figure 1.4; Table 1.3). However, samples sizes for *M. trossulus* thread strength and attachment strength were reduced due to high mortality (60%) and low attachment to the plate substrate at high temperature and statistical power was low (Table S1.1). There was a marginal effect of food on *M. galloprovincialis* thread production (p = 0.06; Figure 1.3; Table 1.2).

Thread production and thread strength was not significantly correlated with shell or tissue for either species (Figure 1.5, Figure S1.2, Table 1.4, Table S1.2). *M. trossulus* and *M. galloprovincialis* thread production was only marginally correlated with shell growth (p = 0.22 and p = 0.15, respectively; Figure 1.5, Table 1.4).

1.5. Discussion

Our 10-week mesocosm experiment with two congeneric mussel species indicated that the effects of temperature and food influenced survival and growth but did not significantly affect byssal thread strength or production. Across this temperature range, *M. galloprovincialis* had overall high performance at higher temperatures, with high survival, positive tissue growth, and increased shell growth. In contrast, *M. trossulus* had overall low performance, with negative tissue growth, minimal shell growth, and with condition index and survival lower at higher temperatures.

1.5.1. Thermal and Nutritional Physiology

M. trossulus survival decreased markedly with increasing temperature while *M. galloprovincialis* survival remained high across all treatments. Average *M. trossulus* mortality after 10 weeks was 60% at 21°C degrees, which is comparable to previous reports at similar temperatures (e.g., 50% mortality after 3 weeks, Braby and Somero 2006; Schneider et al., 2008). *M. trossulus* is known to have a lower tolerance to warm water than *M. galloprovincialis*; warming conditions cause a greater decrease in malate dehydrogenase function, and a greater increase in heart rate and the production of heat shock proteins in *M. trossulus* compared to *M. galloprovincialis* (Fields et al., 2006, Fields et al., 2012, Braby and Somero 2006, Hofmann and

Somero 1996). Long-term extremes in metabolic rate can cause mortality (Priede 1997**)**. In mussels, greater temperatures may cause inefficient circulation and the build-up of anaerobic products (Guderly and Pörtner 2010), as well as decrease immune response and increase susceptibility to disease (Monari et al., 2007, Mackenzie et al., 2014).

Food availability did not influence survival of *M. trossulus*. Within the experimental range of temperatures, this species can have metabolic costs that outpace food input (Fly and Hilbish 2013). Reduced feeding at higher temperatures might cause starvation at a temperature lower than the upper lethal limit (Kitchell et al., 1977). This temperature effect in continually submersed mussels contrasts with the aerial temperature effects observed by Schneider et al., (2010) in intertidal mussels, where greater food availability can decrease mortality caused by heat exposure during low tide as long as seawater temperatures remain near Topt (13 °C).

Across the environmentally-relevant range of seawater temperatures used in this study (13-21°C), the effects of temperature and food had species-specific effects on growth and biomass metrics that are similar to those commonly used in bioenergetic growth models (e.g. DEB; Kooijman 2010). Temperature and higher food availability increased *M. galloprovincialis* shell growth, but condition index only increased under high food availability. These patterns suggest shell growth may be a good index of SFG for this species. SFG typically increases with temperature due to higher clearance rates that are counteracted by only a small increase in metabolic cost (Fly and Hilbish 2013). In contrast, high temperature and high food availability decreased condition index of *M. trossulus* across this same temperature range, and the effects on shell growth were marginal. Overall, this species lost 45% of its tissue mass over the experiment, reflecting an energetic deficit and negative SFG, likely because warming above 12°C decreases clearance rates and increases metabolic costs at higher temperatures (Fly and Hilbish 2013). *M.*

trossulus condition index decreased with greater temperature and lower food availability, as would be expected from known SFG of this species in this range (Fly and Hilbish 2013), and thus condition index (and not shell growth) might be a good indicator of energetic deficit for this species under the experimental range of food availability and temperature conditions. The effects of food availability and temperature did not interact for metrics of biomass or growth (*M. galloprovincialis* - shell growth, *M. trossulus* - condition index), supporting the assumption of bioenergetics theory that the effects of temperature and food availability are additive (Kooijman 2010, Kitchell et al., 1977).

Neither temperature nor food availability significantly affected byssal thread production, thread strength, or attachment for either species. While temperature does not affect thread mechanical properties after they are produced and removed from mussels (George et al., 2018), *M. trossulus* thread production and thread strength is known to be influenced by the seawater temperature in which they are produced (Newcomb 2015). Specifically, in short term experiments (3 day), *M. trossulus* exposed to temperatures above 21°C produced fewer, weaker byssal threads. Our longer term exposures (10 weeks) to a slightly narrower range of experimental temperatures, however, caused such high mortality that few individuals remained to produce threads.

Thread strength is known to vary seasonally in *M. edulis*, weakening in summer (Moeser and Carrington 2006). Our study was conducted in the late summer - early autumn, and it is possible that the threads were too weak, due to the time of year (Newcomb 2015), to observe any effect of an experimental treatment. This idea is supported by our observation that the thread strengths of *M. galloprovincialis* and *M. trossulus* were lower than previously reported (Babarro et al., 2010, O'Donnell et al., 2013, Newcomb 2015). This difference in thread strength could also be due to methodological differences. After byssal threads are produced, they become stronger over a two-week period (George et al., 2018), and we sampled all attached threads, which likely represents a broad distribution of thread ages and strengths for each individual.

1.5.2. Energetic State and Allocation to Byssal Threads

We hypothesized that mussels in conditions that produced a greater energetic surplus would either (1) produce more threads and have stronger attachment strength, or alternatively, (2) produce the same number of threads and have the same attachment strength. Neither thread quantity (thread production) nor thread quality (thread strength) were significantly correlated with shell or tissue growth, supporting our alternative hypothesis that within this range of seawater temperature and food conditions, thread production and attachment may be prioritized regardless of energetic state.

Energetic prioritization of byssal threads over maintenance needs has been proposed previously for other mussel species (Clarke 1999). Under conditions of food availability below tissue maintenance requirement levels, zebra mussels allocated assimilated carbon toward byssus production, and stored reserves may have been used (3 week acclimation; Clarke 1999). Mussels use glycogen as an energy reserve, and under short-term starvation, glycogen stores are not reduced for large individuals but may be depleted for small individuals (*M. galloprovincialis*, Babarro et al., 2008). Short-term starvation does not influence *M. galloprovincialis* byssal thread production, regardless of whether spawning is induced or not (4 day acclimation; Babarro et al., 2010), and starvation reduces small *M. galloprovincialis* thread production, but not the thread production of larger individuals (1 week acclimation; Babarro et al., 2008).

While producing threads may come at a cost to growth and other non-essential processes, other organismal-level processes including reproduction may be prioritized over thread production. Seasonal reproductive cycles may present energy allocation trade-offs for byssal thread attachment (Zardi et al., 2007, Carrington et al., 2002, Moeser and Carrington 2006). While gametogenesis and growth of reproductive tissue might be more energetically costly than loss of gametes during spawning, induction of spawning in the laboratory results in lower thread production for small *M. galloprovincialis* (Babarro et al., 2010) and lower thread strength for *M. edulis* (Hennebicq et al., 2013). On rocky shores, mussel attachment (Carrington 2002, Moeser and Carrington 2006) and byssal thread production (Zardi et al., 2007) are temporally correlated with reproductive cycles. Our experimental manipulation (Aug-Oct) corresponded with the end of the reproductive cycle for *M. trossulus* (April – August), which may explain the lower physical condition, mortality, and weak byssus for this species (Skidmore 1973).

The two species were relatively similar in terms of shell length and initial condition index (*M. galloprovincialis* was 20% greater by length); final *M. galloprovincialis* condition index, however*,* was approximately three times that of *M. trossulus*. Any size difference might have consequences for suspension feeding, metabolic costs, and metabolic reserves (Kooijman 2010). Larger mussels also will have a greater metabolic cost of tissue maintenance (Kooijman 2010, Sebens et al., 2002), but may be able to tolerate stressful situations due to greater glycogen concentration in their tissues or energetic reserves (Babarro et al., 2010), and have a greater gill area which allows for greater clearance rates (related to surface area; Kooijman 2010).

Prioritization of thread production may impact other fitness parameters, and the cost of producing additional threads can decrease growth (Sebens et al., 2017, Carrington et al., 2015). At higher temperatures mussels more readily escaped their tether, and had decreased attachment to the acrylic plate relative to other substrates (e.g., the shells of other mussels; E. Roberts personal observation). Increased thread production under stressful conditions allows mussels to change their position within their local microenvironment but this likely comes at an energetic cost (Sebens et al., 2018, Roberts Chapter 2), but can increase risk of dislodgement (Schneider et al., 2005). Future work should explore the effect of temperature on this behavioral response of mussels, as attachment to surrounding mussels instead of rocky substrate may influence mussel bed attachment.

Within the range of seawater conditions tested, which are representative of the upper range of local summer conditions, *M. trossulus* had a loss of tissue mass and at the greatest temperature had low survival. This species does survive in the Salish Sea region, and if seawater temperatures were lower and food levels were greater, we might have observed growth and high survival. Within this range of seawater conditions, byssal thread production did not correlate with metrics of growth for either species. It is possible, however, that this relationship would be observed across a broader range of temperature and food conditions, ranging from starvation to positive growth for each species. Future work exploring starvation of *M. galloprovincialis* and enhanced food conditions with *M. trossulus* could demonstrate a bioenergetic relationship between thread production and growth, and would disentangle differences due to species and energetic state.

Natural variability in seawater stratification and phytoplankton biomass in the Salish Sea leads to highly variable temperature and food availability (Sutherland et al., 2011, Lowe et al., 2016), with decreased phytoplankton biomass in the summer and early autumn (Mackas et al., 2013, Sutherland et al., 2011). Farmed populations of *M. trossulus* in the Salish Sea can experience mortality events in early autumn (Personal communication, Ian Jeffords), and our experiment demonstrated a relationship, for this species, between temperature and mortality during this time of year. In the Salish Sea, estuarine-oceanographic models predict an overall increase in temperature of ~1.51 °C, and an increase in primary production of up to ~23% by 2095 (Khangaonkar et al., 2019). Our results suggest increased seawater temperature may, over the long-term, enhance *M. galloprovincialis* shell growth and decrease *M. trossulus* survival and have a minimal effect on byssal thread production of either species. The combination of natural variability with long term change will thus very likely have species-specific effects on both natural and farmed mussel populations (Newcomb 2015, Carrington et al., 2016).

1.6. Literature Cited

Azpeitia, K., Ortiz-Zarragoitia, M., Revilla, M. and Mendiola, D., 2017. Variability of the reproductive cycle in estuarine and coastal populations of the mussel *Mytilus galloprovincialis* Lmk. from the SE Bay of Biscay (Basque Country). *International Aquatic Research*, 9:329-350.

Bayne, B. L., R. J. Thompson, and J. Widdows. Physology 1. In Bayne, B.L. ed., 1976. *Marine mussels: their ecology and physiology* (Vol. 10). Cambridge University Press.

Bell, E.C., and J.M. Gosline, 1996. Mechanical design of mussel byssus: material yield enhances attachment strength. *Journal of Experimental Biology.* 199:1005-1017.

Babarro J. M. F., Fernández Reiriz M. J., Labarta U. 2008. Secretion of byssal threads and attachment strength of Mytilus galloprovincialis: the influence of size and food availability. *Journal of the Marine Biological Association of the United Kingdom* 88:783-791

Babarro J. M., Reiriz M. J. 2010. Secretion of byssal threads in Mytilus galloprovincialis: quantitative and qualitative values after spawning stress. *Journal of Comparative Physiology B* 180:95-104

Barber, B.J., 2004. Neoplastic diseases of commercially important marine bivalves. *Aquatic Living Resources.* 17:449-466.

Barber, B.J. and Blake, N.J., 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *Journal of Experimental Marine Biology and Ecology*. 52:121-134.

Bayne, B.L. and Bayne, B.L. eds., 1976. Marine mussels: their ecology and physiology (Vol. 10). Cambridge University Press.

Bouhlel, Z., Genard, B., Ibrahim, N., Carrington, E., Babarro, J.M., Lok, A., Flores, A.A., Pellerin, C., Tremblay, R. and Marcotte, I., 2017. Interspecies comparison of the mechanical properties and biochemical composition of byssal threads. *Journal of Experimental Biology*, 220:984-994.

Blanchette, C.A., Helmuth, B. and Gaines, S.D., 2007. Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at Point Conception, California, USA. *Journal of Experimental Marine Biology and Ecology*, 340:126-148.

Black, B.A., 2009. Climate-driven synchrony across tree, bivalve, and rockfish growth-increment chronologies of the northeast Pacific. *Marine Ecology Progress Series*, 378:37-46.

Braby, C.E. and Somero, G.N., 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology*, 209:2554-2566.

Brazee, S.L., 2004. An interspecific comparison of biomechanical properties and morphometrics of mussel byssal threads (Doctoral dissertation, University of Rhode Island).

Brokordt, K.B. and Guderley, H.E., 2004. Energetic requirements during gonad maturation and spawning in scallops: sex differences in *Chlamys islandica* (Muller 1776). *Journal of Shellfish Research*, 23:25-32.

Carrington E. 2002. Seasonal variation in the attachment strength of blue mussels: causes and consequences. *Limnol. Oceanogr*. 47:1723–33

Carrington, E., George, M., Newcomb, L., Friedman, C., Jefferds, I. and O'Donnell, M., 2016. Losing Their Lifeline? The Effects of Ocean Warming and Acidification on Mussel Attachment. *SEB Brighton*, pp.4-7.

Carrington, E., Moeser, G.M., Dimond, J., Mello, J.J. and Boller, M.L., 2009. Seasonal disturbance to mussel beds: field test of a mechanistic model predicting wave dislodgment. *Limnology and Oceanography*, 54:978-986.

Carrington, E., Waite, J.H., Sarà, G. and Sebens, K.P., 2015. Mussels as a model system for integrative ecomechanics. *Annual Review of Marine Science*, 7:443-469.

Clarke, M., 1999. The effect of food availability on byssogenesis by the zebra mussel (*Dreissena polymorpha* Pallas). *Journal of molluscan studies*, 65:327-333.

Crosby, M., and L. Gale. 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research*, 9:233-237.

Curiel-Ramirez, S. and Caceres-Martinez, J., 2004. Reproductive cycle of coexisting mussels, *Mytilus californianus* and *Mytilus galloprovincialis*, in Baja California, New Mexico. *Journal of Shellfish Research*, 23:515-521.

Dahlhoff, E.P. and Menge, B.A., 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. *Marine Ecology Progress Series*, 144:97-107.

Denny, M., 1995. Predicting physical disturbance: Mechanistic approaches to the study of survivorship on wave-swept shores. *Ecological Monographs*, 65:371-418.

Dowd, W.W., Felton, C.A., Heymann, H.M., Kost, L.E. and Somero, G.N., 2013. Food availability, more than body temperature, drives correlated shifts in ATP-generating and antioxidant enzyme capacities in a population of intertidal mussels (*Mytilus californianus*). *Journal of experimental marine biology and ecology*, 449:171-185.

Edmunds, P.J., 2011. Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. *Limnology and Oceanography*, 56:2402-2410.

Elliott, J., Holmes, K., Chambers, R., Leon, K. and Wimberger, P., 2008. Differences in morphology and habitat use among the native mussel *Mytilus trossulus*, the non-native *M. galloprovincialis*, and their hybrids in Puget Sound, Washington. *Marine Biology*, 156:39-53.

Fields, P.A., Rudomin, E.L. and Somero, G.N., 2006. Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *Journal of Experimental Biology*, 209:656-667.

Fields PA, Zuzow MJ, Tomanek L (2012) Proteomic responses of blue mussel (*Mytilus*) congeners to temperature acclimation. *Journal of Experimental Biology* 215:1106–1116.

Fly, E.K. and Hilbish, T.J., 2013. Physiological energetics and biogeographic range limits of three congeneric mussel species. *Oecologia*, 172:35-46.

Fox J, Weisberg S. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage; 2011. Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

George, M.N., Pedigo, B. and Carrington, E., 2018. Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. *Journal of The Royal Society Interface*, 15:20180489.

Guderley H, Pörtner HO. 2010. Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. *Canadian Journal of Zoology* 88:753–63.

Hawkins, A.J.S. and Bayne, B.L., 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. *Marine ecology progress series. Oldendorf*, 25:181-188.

Helm, M.M., Bourne, N. and Lovatelli, A., 2004. Hatchery operation: Broodstock conditioning, spawning and fertilization. *Hatchery culture of bivalves. A practical manual. FAO Fish. Tech. Paper, Rome*, pp.59-83.

Hennebicq, R., Fabra, G., Pellerin, C., Marcotte, I., Myrand, B. and Tremblay, R., 2013. The effect of spawning of cultured mussels (*Mytilus edulis*) on mechanical properties, chemical and biochemical composition of byssal threads. *Aquaculture*, 410:11-17.

Hettinger, A., Sanford, E., Hill, T.M., Hosfelt, J.D., Russell, A.D. and Gaylord, B., 2013. The influence of food supply on the response of Olympia oyster larvae to ocean acidification. *Biogeosciences*, 10:6629-6638.

Hilbish, T.J., Brannock, P.M., Jones, K.R., Smith, A.B., Bullock, B.N. and Wethey, D.S., 2010. Historical changes in the distributions of invasive and endemic marine invertebrates are contrary to global warming predictions: the effects of decadal climate oscillations. *Journal of Biogeography*, 37:423-431.

Hofmann, G.E. and Somero, G.N., 1996. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitination. *Marine Biology*, 126:65-75.

IPCC, 2018: Summary for Policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P. R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.)]. World Meteorological Organization, Geneva, Switzerland, 32 pp.

Jakobsen HH, Markager S., 2016. Carbon-to-chlorophyll ratio for phytoplankton in temperate coastal waters: Seasonal patterns and relationship to nutrients. *Limnology and Oceanography* 61:1853-1868.

Kellermann, V., Chown, S.L., Schou, M.F., Aitkenhead, I., Janion-Scheepers, C., Clemson, A., Scott, M.T. and Sgrò, C.M., 2019. Comparing thermal performance curves across traits: how consistent are they?. *Journal of Experimental Biology*, 222:193433.

Khangaonkar T., Nugraha A., Xu W., and Balaguru K., 2019. Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. *Journal of Geophysical Research*, 124:3876-3904.

Kitchell, J.F., Stewart, D.J. and Weininger, D., 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). *Journal of the Fisheries Board of Canada*, 34:1922-1935.

Kooijman, S.A.L.M. (2010). *Dynamic energy budget theory for metabolic organisation*. Cambridge University Press.

Lesser, M.P., Bailey, M.A., Merselis, D.G. and Morrison, J.R., 2010. Physiological response of the blue mussel *Mytilus edulis* to differences in food and temperature in the Gulf of Maine. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 156:541-551.

Lockwood, B.L. and Somero, G.N., 2011. Invasive and native blue mussels (genus *Mytilus*) on the California coast: the role of physiology in a biological invasion. *Journal of Experimental Marine Biology and Ecology*, 400:167-174.

Lowe, A.T., Roberts, E.A. and Galloway, A.W., 2016. Improved marine-derived POM availability and increased pH related to freshwater influence in an inland sea. *Limnology and Oceanography*, 61:2122-2138.

Lika, K. and Kooijman, S.A., 2011. The comparative topology of energy allocation in budget models. *Journal of Sea Research*, 66:381-391.

Lindquist, S., 1986. The heat-shock response. *Annual Review of Biochemistry*, 55:1151-1191.

Mackas, D., Galbraith, M., Faust, D., Masson, D., Young, K., Shaw, W., Romaine, S., Trudel, M., Dower, J., Campbell, R. and Sastri, A., 2013. Zooplankton time series from the Strait of Georgia: Results from year-round sampling at deep water locations, 1990–2010. *Progress in Oceanography*, 115:129-159.

Mackenzie, C.L., Lynch, S.A., Culloty, S.C. and Malham, S.K., 2014. Future oceanic warming and acidification alter immune response and disease status in a commercial shellfish species, *Mytilus edulis* L. *PLoS One*, 9:e99712.

Matzelle, A.J., Sarà, G., Montalto, V., Zippay, M., Trussell, G.C. and Helmuth, B., 2015. A bioenergetics framework for integrating the effects of multiple stressors: Opening a ‘black box’ in climate change research. *American Malacological Bulletin*, 33:150-161.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S.N. and Gutowska, M.A., 2011. Food supply and seawater pCO2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PloS One*:e24223.

Menge, B.A., 1992. Community regulation: under what conditions are bottom-up factors important on rocky shores?. *Ecology*, 73:755-765.

Menge, B.A., Lubchenco, J., Bracken, M.E.S., Chan, F., Foley, M.M., Freidenburg, T.L., Gaines, S.D., Hudson, G., Krenz, C., Leslie, H. and Menge, D.N.L., 2003. *Coastal oceanography* sets the pace of rocky intertidal community dynamics. *Proceedings of the National Academy of Sciences*, 100:12229-12234.

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

Monari, M., Matozzo, V., Foschi, J., Cattani, O., Serrazanetti, G.P. and Marin, M.G., 2007. Effects of high temperatures on functional responses of haemocytes in the clam *Chamelea gallina*. *Fish & Shellfish Immunology*, 22:98-114.

Murray, J.W., Roberts, E., Howard, E., O'Donnell, M., Bantam, C., Carrington, E., Foy, M., Paul, B. and Fay, A., 2015. An inland sea high nitrate-low chlorophyll (HNLC) region with naturally high pCO2. *Limnology and Oceanography*, 60:957-966.

Newcomb, L.A., 2015. Elevated temperature and ocean acidification alter mechanics of mussel attachment (Doctoral dissertation).

Nisbet, R.M., Jusup, M., Klanjscek, T. and Pecquerie, L., 2012. Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. *Journal of Experimental Biology*, 215:892-902.

O'Donnell, M.J., George, M.N. and Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. Nature Climate Change, 3, 587-590.

Paul, C., Matthiessen, B. and Sommer, U., 2015. Warming, but not enhanced CO_2 concentration, quantitatively and qualitatively affects phytoplankton biomass. *Marine Ecology Progress Series*, 528:39-51.

Pinheiro J, Bates D, DebRoy S, Sarkar D, Team TRC. _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1–118. October, 2013. Available at: http://CRAN.R-project.org/package=nlme.

Pörtner HO. 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series,* 470:273–90.

Pörtner, H.O. and Farrell, A.P., 2008. Physiology and climate change. *Science*, 322:690-692.

Priede, I.G., 1977. Natural selection for energetic efficiency and the relationship between activity level and mortality. *Nature*, 267:610.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Ramajo, L., Pérez-León, E., Hendriks, I.E., Marbà, N., Krause-Jensen, D., Sejr, M.K., Blicher, M.E., Lagos, N.A., Olsen, Y.S. and Duarte, C.M., 2016. Food supply confers calcifiers resistance to ocean acidification. *Scientific Reports*, 6:19374.

Riisgård, H. U., P. P. Egede, and Barreiro Saavedra, I.. 2011. Feeding behaviour of the mussel, *Mytilus edulis*: new observations, with a minireview of current knowledge. *Journal of Marine Biology*, 2011:1-13.

Schneider, K.R., 2008. Heat stress in the intertidal: comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. *The Biological Bulletin*, 215:253-264.

Schneider, K.R., Van Thiel, L.E. and Helmuth, B., 2010. Interactive effects of food availability and aerial body temperature on the survival of two intertidal *Mytilus* species. *Journal of Thermal Biology*, 35:161-166.

Schneider, K.R., Wethey, D.S., Helmuth, B.S.T. and Hilbish, T.J., 2005. Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. *Marine Biology*, 146:333-343.

Sebens, K.P., Sarà, G. and Carrington, E., 2018. Estimation of fitness from energetics and life-history data: An example using mussels. *Ecology and Evolution*, 8:5279-5290.

Skidmore, D., 1983. Settlement, growth, and survival of *Mytilus edulis* L. in Puget Sound and Assessment of *Mytilus Californianus* for Aquaculture. Thesis, M.S., University of Washington.

Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integrative and comparative biology*, 53:597-608.

Strathmann, M. F. 1987. Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae. University of Washington Press.

Thompson, R.J. and Bayne, B.L., 1974. Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*. *Marine Biology*, 27:317-326.

Thompson, R.J. and MacDonald, B.A., 2006. Physiological integrations and energy partitioning. In *Developments in aquaculture and fisheries science* (Vol. 35: 493-520). Elsevier.

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A. and Melzner, F., 2013. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology*, 19:1017-1027.

Todgham, A.E. and Stillman, J.H., 2013. Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integrative and Comparative Biology*, 53:539-544.

van der Veer, H.W., Cardoso, J.F. and van der Meer, J., 2006. The estimation of DEB parameters for various Northeast Atlantic bivalve species. *Journal of Sea Research*, 56:107-124.

Waite JH, Qin XX, Coyne KJ. 1998. The peculiar collagens of mussel byssus. *Matrix Biology*, 17:93–106.

Westerbom, M., Mustonen, O., Jaatinen, K., Kilpi, M. and Norkko, A., 2019. Population Dynamics at the Range Margin: Implications of Climate Change on Sublittoral Blue Mussels (Mytilus trossulus). *Frontiers in Marine Science*, 6:292.

Widdows, J. and Bayne, B.L., 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *Journal of the Marine Biological Association of the United Kingdom*, 51:827-843.

Zardi, G.I., McQuaid, C.D. and Nicastro, K.R., 2007. Balancing survival and reproduction: seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Marine Ecology Progress Series*, 334:155-163.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer Science & Business Media.

1.7. Tables and Figures

Table 1.1. Summary of two-way ANOVA of the effects of temperature and food on percent survival of two mussel species. Bold font indicates a significant effect (p< 0.05).

Species	Factor	Sum Sq	Df	F	p
M. trossulus	Temp	0.685	3	3.336	**0.046**
	Food	0.011	1	0.167	0.69
	Temp:Food	0.016	3	0.080	0.97
	Residuals	1.095	16		

Tukey HSD	Temp	Group
	13	a
	15	ab
	18	ab
	21	b

	Factor	Sum Sq	Df	F	p
M. galloprovincialis	Temp	0.054	3	0.444	0.72
	Food	0.058	1	1.442	0.25
	Temp:Food	0.060	3	0.493	0.69
	Residuals	0.646	16		

Table 1.2. Summary of linear mixed effects models evaluating the fixed effects of temperature and food availability on condition index and shell growth for two mussel species. The random effect was mesocosm. Bold font indicates a significant effect ($p < 0.05$).

		Condition Index (g / mm^3)			Shell growth (mm)		
Species	Treatment	X_2	Df	p	X_2	Df	p
M. trossulus	Temp	10.08	3	**0.02**	5.90	3	0.12
	Food	4.54	1	**0.03**	2.11	1	0.14
	Temp:Food	0.12	3	0.99	4.26	3	0.23

	Temp	Food	Group	Group
Tukey HSD	13		a	-
	15		ab	-
	18		ab	-
	21		b	-
		Low	a	-
		High	a	-

		Variance	SD	Variance	SD
Random effect	Mesocosm	0.0000	0.005	0.0003	0.02
	Residual	0.0185	0.136	0.0013	0.04

	Treatment	X_2	Df	p	X_2	Df	p
M. galloprovincialis	Temp	2.28	3	0.52	12.40	3	**0.01**
	Food	6.06	1	**0.01**	21.26	1	**<0.001**
	Temp:Food	4.40	3	0.22	3.63	3	0.30

	Temp	Food	Group	Group
Tukey HSD	13		-	a
	15		-	ab
	18		-	ab
	21		-	b
		Low	a	a
		High	b	b

		Variance	SD	Variance	SD
Random effect	Mesocosm	0.0412	0.203	0.0003	0.02
	Residual	0.9374	0.968	0.0086	0.09

Table 1.3. Summary of linear mixed effects models evaluating the fixed effects of temperature and food availability on byssal thread production, thread strength, and byssus strength for two mussel species. The random effect was mesocosm. None of the analyses showed a significant effect ($p<0.05$).

		Thread production (#/mussel)			Thread strength (N)			Byssus strength (N)		
Species	Factor	X_2	Df	p	X_2	Df	p	X_2	Df	p
M. trossulus	Temp	4.71	3	0.19	0.59	3	0.90	5.36	3	0.15
	Food	0.07	1	0.79	0.01	1	0.92	0.15	1	0.70
	Temp:Food	4.65	3	0.20	2.40	3	0.49	0.39	3	0.94
		Variance		SD	Variance		SD	Variance		SD
Random effect	Mesocosm	0.000		0.000	0.000		0.000	0.021		0.15
	Residual	0.045		0.215	0.015		0.123	0.041		0.20
		X_2	Df	p	X_2	Df	p	X_2	Df	p
M. galloprovincialis	Temp	1.39	3	0.71	1.16	3	0.76	2.02	3	0.57
	Food	3.49	1	0.06	2.48	1	0.12	0.22	1	0.64
	Temp:Food	1.25	3	0.74	4.82	3	0.19	2.38	3	0.50
		Variance		SD	Variance		SD	Variance		SD
Random effect	Mesocosm	0.000		0.000	0.0022		0.047	0.0324		0.18
	Residual	0.043		0.207	0.0192		0.139	0.0837		0.29

Table 1.4. Summary of linear regression analyses of the relationship between thread production and tissue and shell growth of two mussel species across all temperature and food treatments. None of the analyses showed a significant effect ($p<0.05$).

	Factor	Sum Sq	Df	F-value	p	R^2
M. trossulus	Tissue growth	10.20	1	0.582	0.47	0.08
	Residuals	105.27	6			
	Shell growth	27.12	1	1.842	0.22	0.23
	Residuals	88.36	6			
M. galloprovincialis	Tissue growth	26.43	1	2.226	0.19	0.27
	Residuals	71.24	6			
	Shell growth	30.72	1	2.753	0.15	0.31
	Residuals	66.95	6			

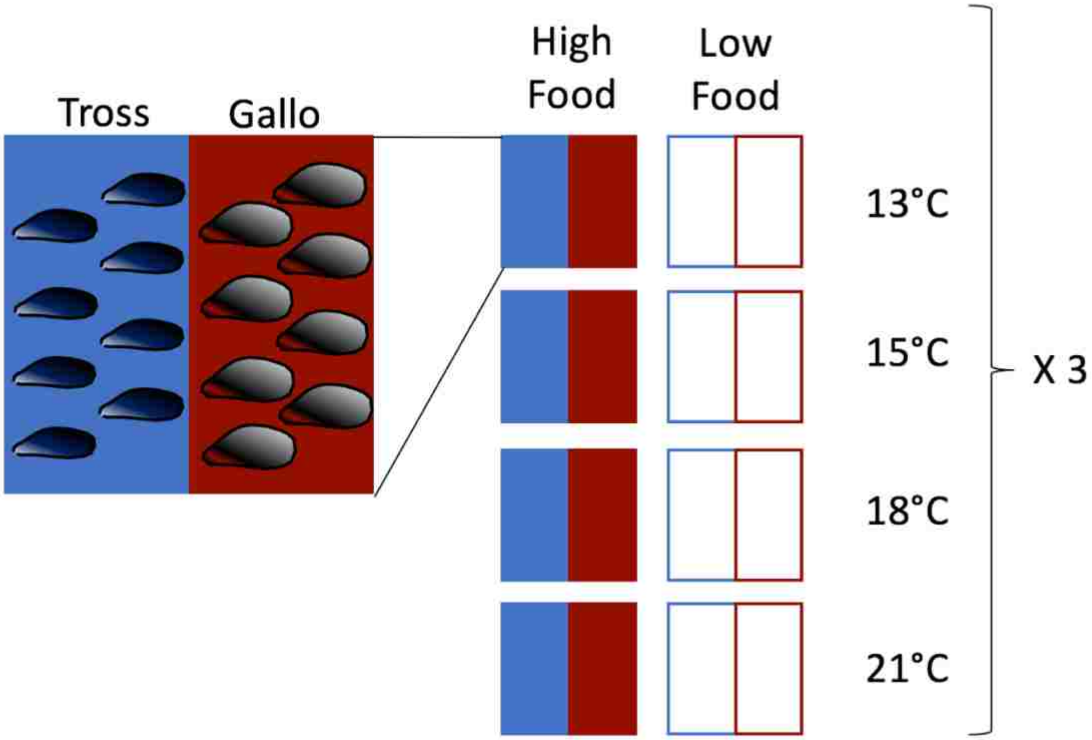

Figure 1.1. Schematic of the experimental design. The experimental design was fully factorial and tested for the effects of species, temperature, and food on mussel survival, growth, and biomaterial production. Each 22L mesocosm contained 8 individuals of each species, M. trossulus (blue) and M. galloprovincialis (red). Each mesocosm was supplied with one of two levels of food availability (high or low) and maintained at one of four levels of seawater temperature. Each treatment combination (=mesocosm) was replicated three times.

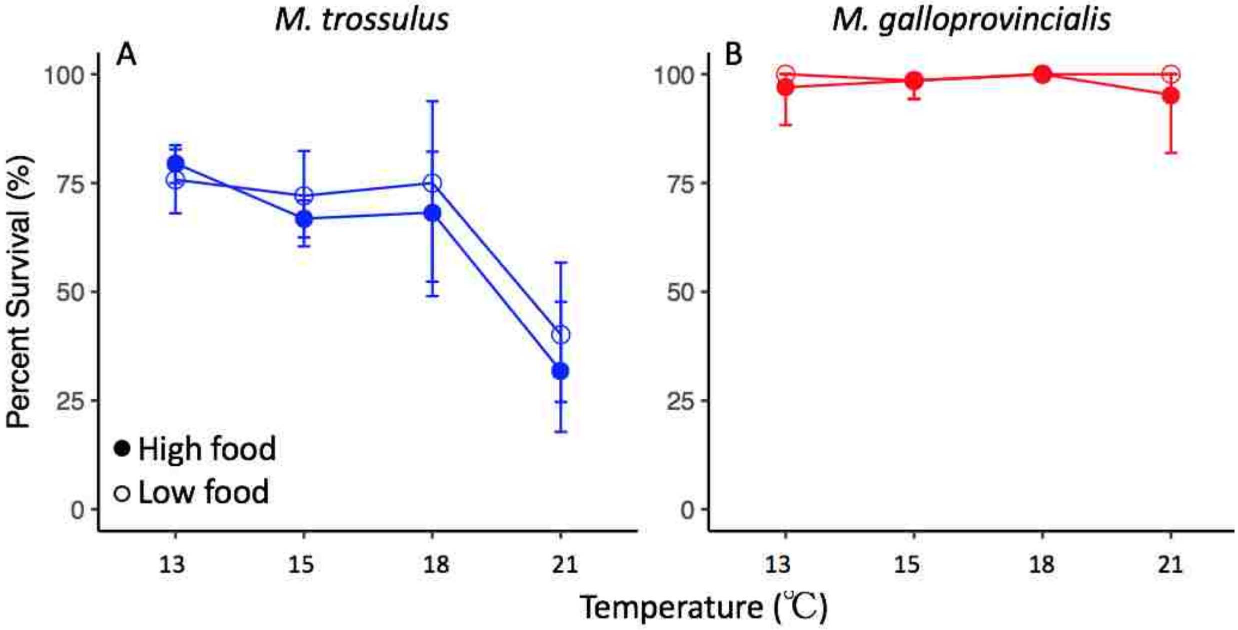

Figure 1.2. Percent survival as a function of seawater temperature at high or low food availability.Percent survival of *M. trossulus* (blue) and *M. galloprovincialis* (red) across a range of seawater temperature under high or low food conditions (closed and open circles, respectively). Symbols and bars represent back-transformed mean ± SE; N = 3 replicate mesocosms per treatment.

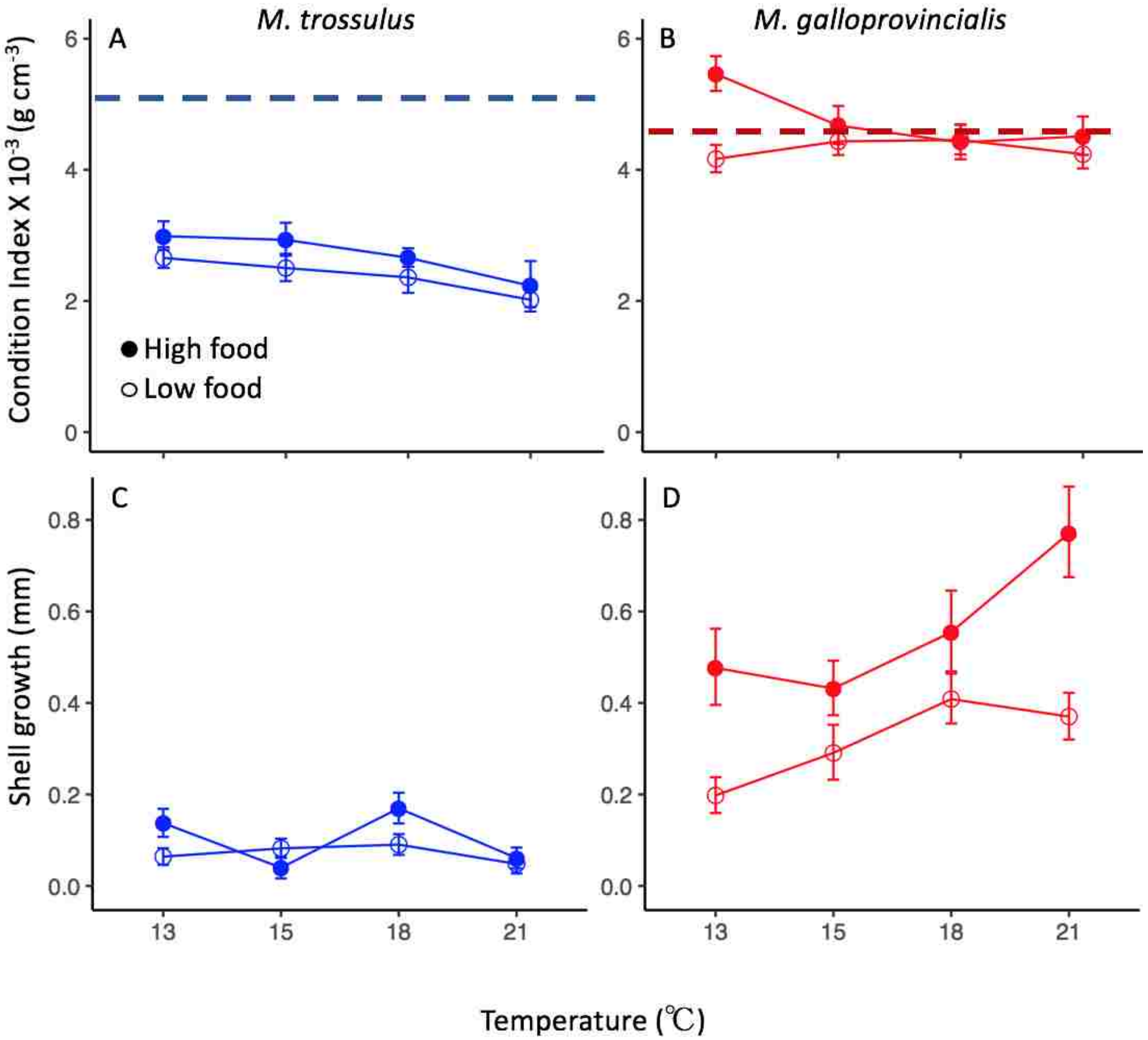

Figure 1.3. Mussel condition and shell growth as a function of seawater temperature at high or low food availability. Condition index (A, B) and shell growth (C, D) of *M. trossulus* (blue) and *M. galloprovincialis* (red) as a function of seawater temperature under high or low food conditions (closed and open circles, respectively). (A, B) Initial condition index of each species is represented with a dashed line. Symbols and bars represent means ± SE of the three mesocosms estimated as fixed effects standard error from LME analyses.

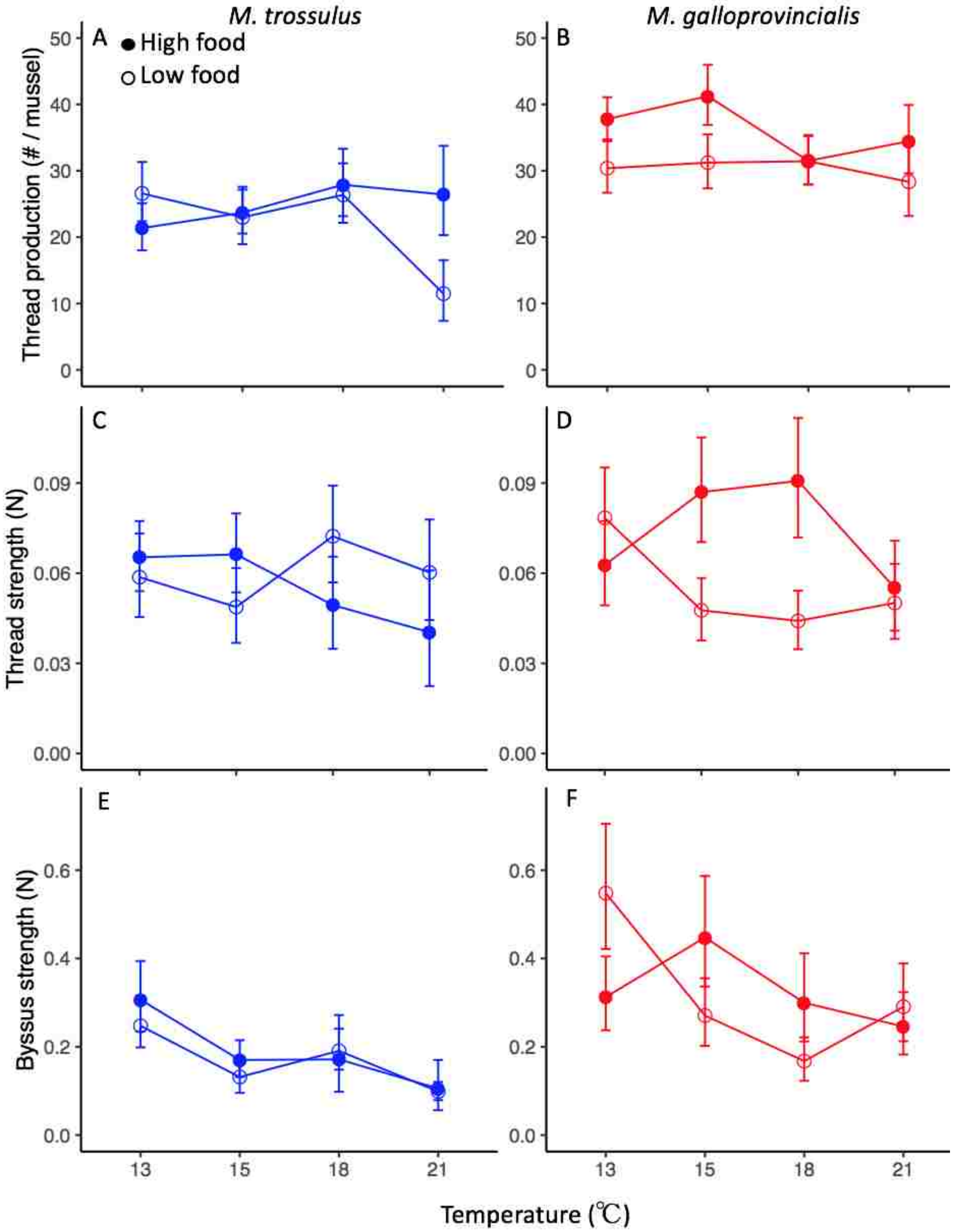

Figure 1.4. Byssus quantity and quality as a function of seawater temperature and at high or low food availability.*M. trossulus* (blue) and *M. galloprovincialis* (red) byssal thread quantity and quality as a function of seawater temperatures under high or low food conditions (closed and

open circles, respectively). (A, B) Byssal thread production per mussel over 15 days; (C, D) byssal thread strength and (E, F) byssus strength. Symbols and bars represent means ± SE of the three mesocosms, estimated as fixed effects error from the LME analyses.

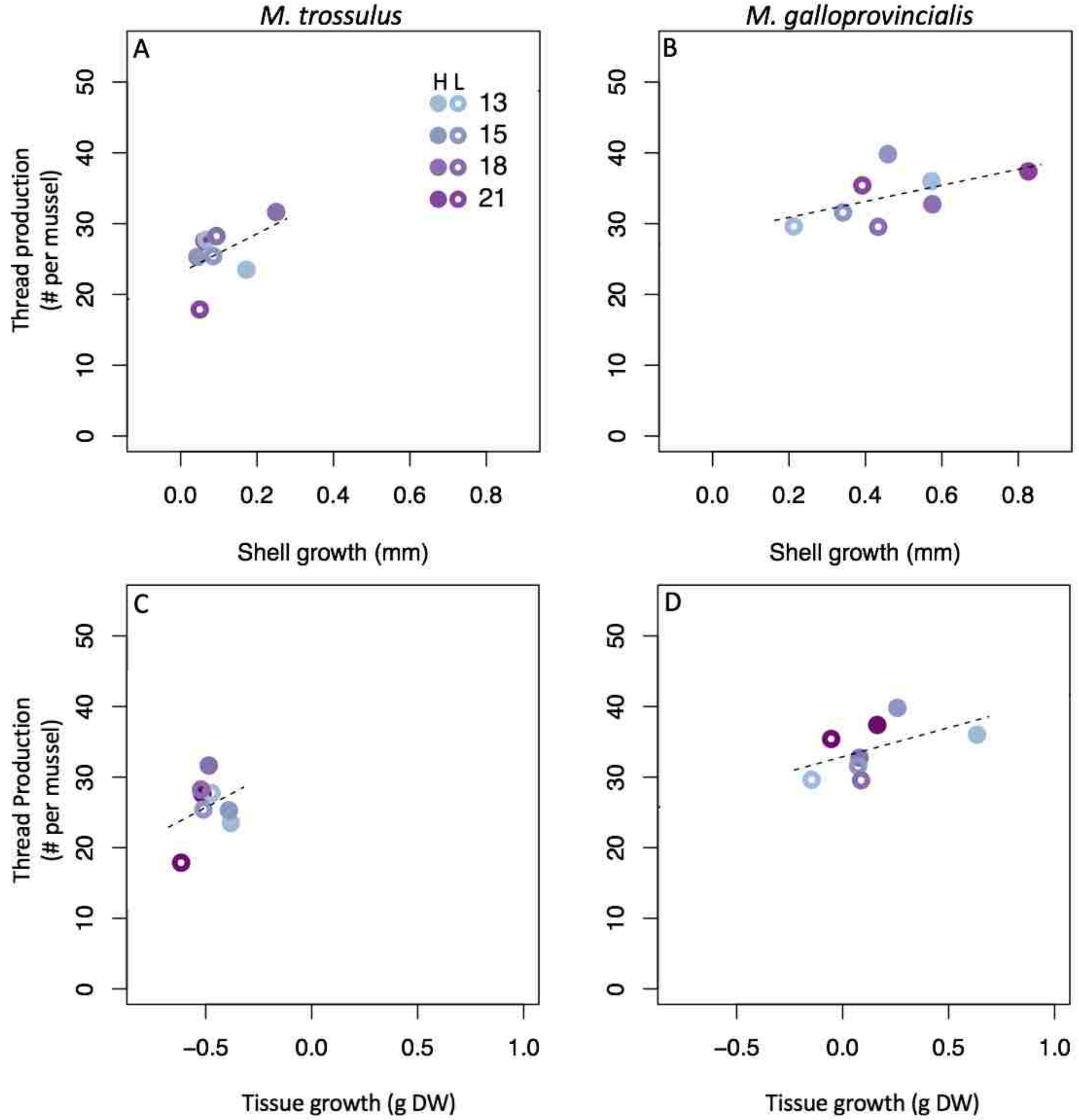

Figure 1.5. Thread production as a function of shell growth or tissue growth. Thread production of *M. trossulus* and *M. galloprovincialis* as a function of shell growth (A, B) or tissue growth (C, D) across the experimental temperature and food levels. Measurements are binned by treatment. See inset for treatment combination.

1.8. Acknowledgements

We thank Jennifer Ruesink, Lauren Buckley, Kenneth Sebens, Jaqueline Padilla-Gamiño, and Rubén D. Manzanedo for insightful discussions that improved the quality of this manuscript. Windy Madden provided valuable assistance in sample processing, and Michael Pantalos provided valuable assistance in developing R code optimization functions. E.A.R. was supported by a National Science Foundation IGERT Program on Ocean Change Fellowship (1068839), and several grants from UW Friday Harbor Laboratories and UW Biology (Edmondson Award, Richard and Megumi Strathmann Fellowship, Kohn Fellowship, and Carrington Student Travel Fellowship). Additional support was provided by the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under project number NA14OAR4170078 (to EC and CS Friedman) through the Washington Sea Grant Program. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U.S. government is authorized to reproduce and distribute this paper for governmental purposes.

1.9. Supplementary Tables and Figures

Table S1.1. Summary of sample sizes for each treatment combination (species:temperature:food) used in the byssal thread strength and byssus strength analyses.

		M. galloprovincialis		*M. trossulus*	
	Temp (℃)	High Food	Low Food	High Food	Low Food
Thread strength	12	14	15	12	13
	15	14	15	10	14
	18	14	13	7	9
	21	15	16	4	3
Byssus strength	12	16	10	13	15
	15	15	14	11	17
	18	11	11	6	12
	21	14	16	3	3

Table S1.2. Summary of linear regression analyses of the relationship between thread strength and tissue and shell growth of two mussel species across all temperature and food treatments. None of the analyses showed a significant effect ($p<0.05$).

	Factor	Sum Sq	Df	F-value	p	R^2
M. trossulus	Tissue growth	3.6×10^{-8}	1	0.002	0.97	0.0003
	Residuals	0.0011	6			
	Shell growth	5.2×10^{-5}	1	0.298	0.61	0.0470
	Residuals	0.0011	6			
M. galloprovincialis	Tissue growth	2.1×10^{-6}	1	0.004	0.95	0.0007
	Residuals	0.0029	6			
	Shell growth	1.4×10^{-6}	1	0.003	0.96	0.0005
	Residuals	0.0029	6			

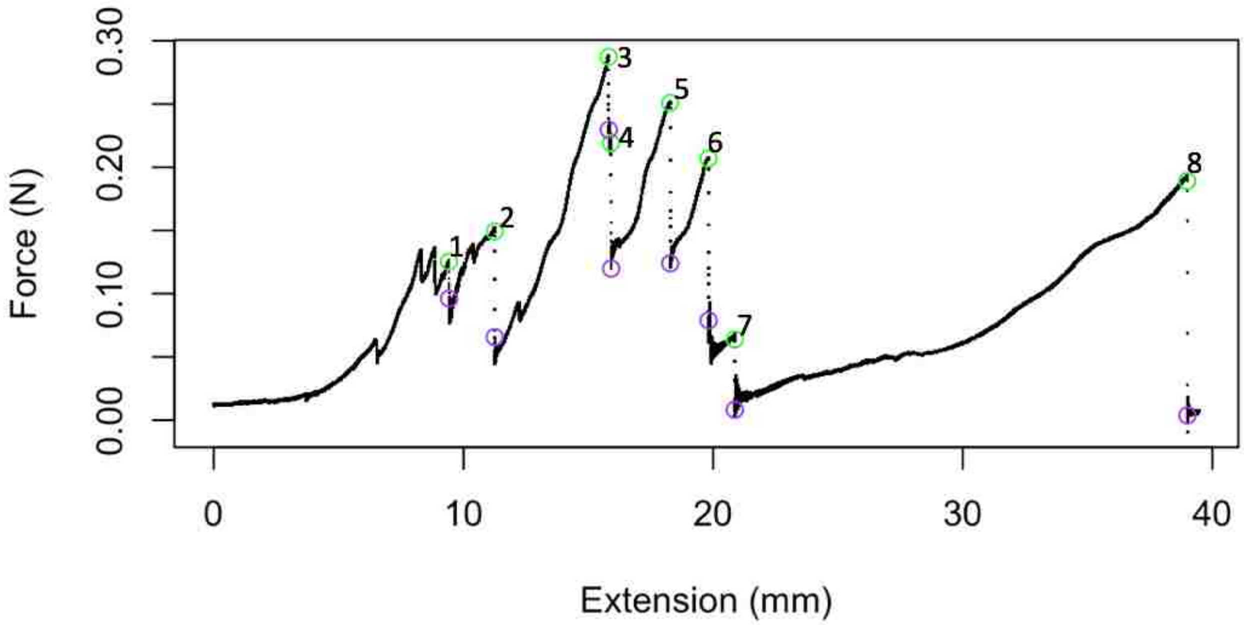

Figure S1.1. Representative force versus extension trajectory of a byssal tensile test.The strength of an individual thread in the network was quantified as the change in force associated with a rapid (catastrophic) drop in load when the thread failed. Eight thread failure events are numbered and the maximum and minimum load associated with each break are indicated by green and purple circles, respectively).

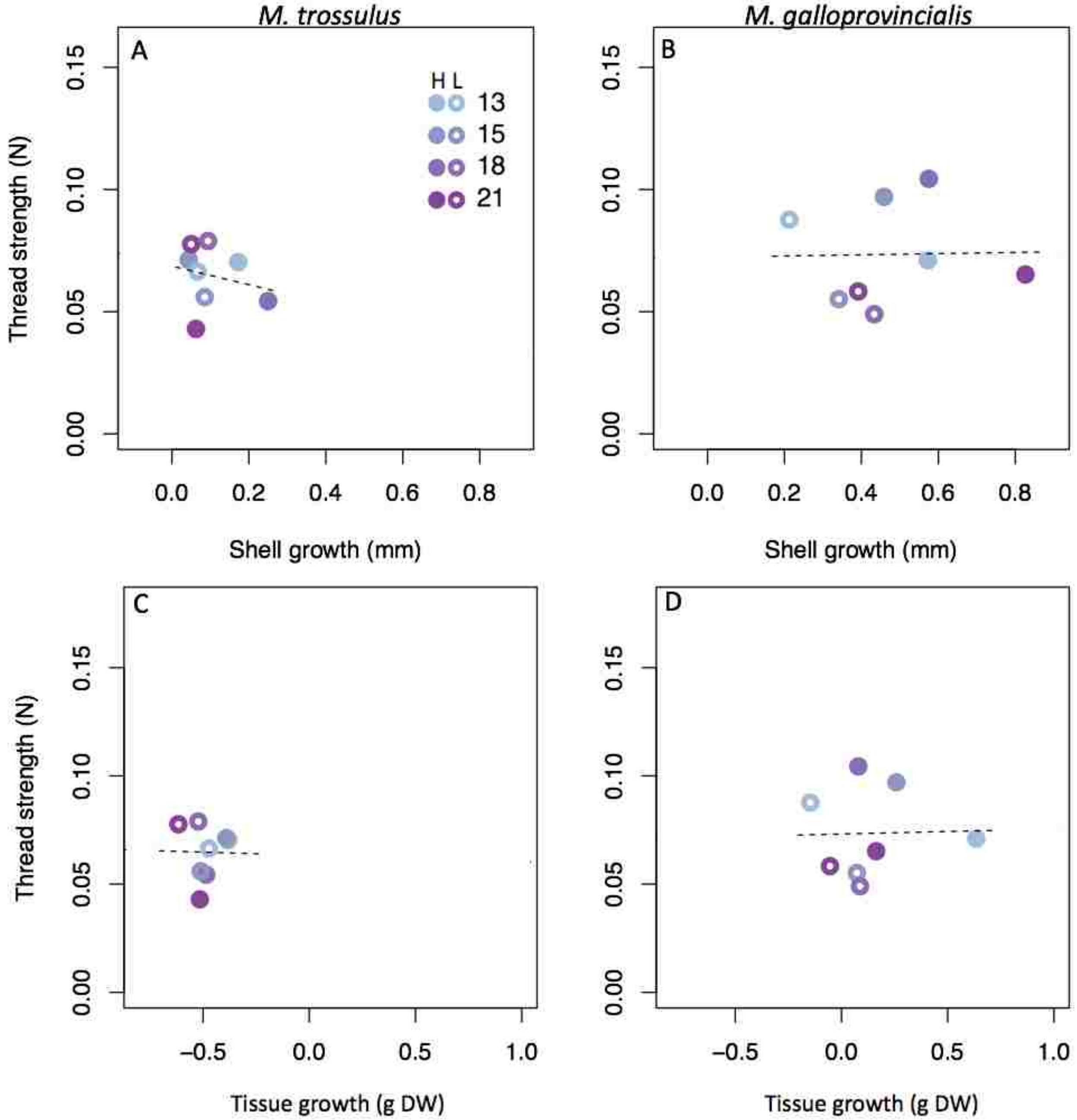

Figure S1.2. Thread strength as a function of shell or tissue growth. Thread strength as a function of shell growth (A, B) or tissue growth (C, D) of *M. trossulus* and *M. galloprovincialis* across the experimental of temperature and food levels. Measurements are binned by treatment. See inset for treatment combination.

Chapter 2

The energetic cost of producing a structural biomaterial: induced production of byssal threads decreases growth of a marine mussel, *Mytilus trossulus*

Emily A. Roberts[1,2*], Laura Newcomb[1,2], Michelle McCartha[2], Katie Harrington[2], Sam LaFramboise[2], Emily Carrington[1,2], Ken Sebens[1,2,3]

1 - Department of Biology, University of Washington, Seattle, WA 98195 USA

2 - Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington 98250 USA

3 - School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195

Keywords: bioenergetics, energy budget, biomaterial, energetic cost, resource allocation, byssus, *Mytilus trossulus*

2.1. Abstract

Specialized mechanical structures produced by organisms provide crucial fitness advantages, but the production of these materials can result in energy allocation trade-offs, affecting population and species distributions. The energy allocation of structural materials can be investigated in organisms where the production of these materials can be readily manipulated. 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. 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. We found that byssal thread removal stimulated increased byssal thread production and decreased growth. The energetic cost of producing byssal threads was 2-8% percent of 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. This work demonstrates that byssus production costs and seasonal differences in mass-specific metabolic costs have a substantial effect on mussel energetic state. Further, we quantify an energy allocation trade-off towards structural material production by combining an experimental manipulation of structural material production with a Scope for Growth model. We propose that energetic constraints from decreased food availability, or greater metabolic costs, can potentially strengthen the trade-off between biomaterial production and growth, with implications for understanding the role of trade-offs in adaptive evolution, and improved natural resource management and conservation practices.

2.2. Introduction

The specialized mechanical structures organisms produce, such as cactus spines, spider silk, 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 affect population dynamics (Sebens et al., 2018) and spatial distributions of organisms (biomechanical ecotype, Reed 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 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 1976), fish bioenergetics (Kitchell et al. 1977) and Dynamic Energy Budgets (Kooijman 2010). Such energy budget models have been developed primarily to evaluate relationships between environmental factors (e.g. energy inputs and temperature-related costs) and organismal processes (soft tissue growth and reproductive output; e.g. Kooijman 2010, Nisbet et al., 2012, Filgueira et al. 2011). 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 1964, Sanders et al., 2018). Experimental manipulations of structure quantity provide 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 2007) and form mussel beds which provide a refuge for associated species by limiting flow (O'Donnell 2008). Byssus also provides an essential function for mussels that are farmed; mussels attach to collector ropes as larvae (Brenner and Buck 2010), and as adults, remain attach to aquaculture lines without a surrounding net that would otherwise limit flow and increase fouling (Korringa 1976).

Byssal thread production accounts for 8 to 10% of the energy budget of mussels. An elemental balance method 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 (i.e. 0-20 per day) or the potential energetic trade-off with other processes such as growth.

The energetic trade-off between thread production and tissue growth can be characterized with a Scope for Growth model (Figure 2.1). Specifically, environmental conditions, such as increased wave disturbance (Van Winkle 1970, 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 et al. 2002, Newcomb 2015) are known to influence mussel byssal thread production and attachment. We hypothesize that experimental manipulation (severing) of byssal threads will increase energy allocation towards byssal thread production and thus take energy away from other organismal processes (Figure 2.1). SFG models also consider how food availability affects tissue growth (Widdows and Bayne 1971). We hypothesized that energy allocation to byssal thread production is prioritized over tissue growth because byssal threads are known to be produced even under starvation conditions (Clarke 1999, Roberts Chapter 1), and starvation does not reduce the production of byssal threads of larger mussels with a large glycogen energetic reserve (Babarro et al. 2008, 2010). We quantified the theoretical variable, SFG, using an index of tissue growth (including gonadal and somatic tissue growth; Sebens et al., 2002) and an energetic conversion factor relating mass to the energy required to produce new tissue (Bayne 1976, Figure 1.1B, C).

In this study, we tested whether mussels allocate energy towards byssal thread production, and away from tissue growth and reproduction, when a higher rate of production is necessary to maintain attachment. In field enclosures, we induced the production of byssal threads above baseline levels by severing threads at different frequencies to quantify the effect of different levels of thread production on mussel growth, and thus cost of thread production. 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.

2.3. Methods

2.3.1. 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. 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.

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 produced 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.

2.3.2. 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 at the beginning and end of the experiment. At the end of the experiment, 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 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).

2.3.3. 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 (1976) and Kitchell et al., (1977), 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 2.2.

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):

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

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 2.2).

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):

$$\mathrm{cost_{non\text{-}byssus}} = \mathrm{b} \times \mathrm{TM_{initial}}^{\mathrm{e}}, \tag{3}$$

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 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 2.2). 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 2.2, 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 2.1; Sebens 2002):

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

where W_{opt} is the energetic optimal size (mg DW), or the size at which the difference between 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} (Supplementary Information 2.1; Table 2.2). 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 2.2).

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):

$$\mathrm{cost_{byssus}} = h \times N_{Th}. \tag{5}$$

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:

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

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

2.3.4. 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:

$$\mathrm{TM}_{\mathrm{initial\_fit}} = \delta \times \mathrm{length}_{\mathrm{initial}}{}^{3}\ , \tag{7}$$

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 2.2). We assumed that the exponent relating length and tissue mass is 3 (Kooijman, 2010) and confirmed this assumption with a separate sampling (see Supplemental Information 2.1). 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:

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

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

$$\mathrm{TM}_{\mathrm{initial}} = \mathrm{TM}_{\mathrm{initial\_fit}} + \mathrm{TM}_{\mathrm{residual}}, \tag{9}$$

2.3.5. 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 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):

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

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 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:

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

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.

2.3.6. Statistical Analysis

All statistical analyses and model calculations were performed with R software for Mac OSX (version 3.4, R Core Team, 2017). Thread production was square root transformed, gonad index was arcsine square root transformed, and shell growth, buoyant weight change, tissue growth, and condition index were log-transformed for statistical analyses. All data met assumptions of equal variances, with the exception of tissue growth, where variance differed among seasons but not within each season. 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. If any significant effects were present, a post-hoc Tukey test was performed to evaluate differences between groups. Preliminary analyses using linear mixed models (LME; Zuur et al. 2009) and confirmed the random effect of the enclosure was not significant (data not shown).

We ran all model parameter estimations as linear models in R where each parameter was estimated as the coefficient for each vector of individual mussel (Eq. 10). 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.

2.4. Results

2.4.1. Field Manipulation of Byssal Thread Production

Byssus severing increased thread production by a factor of five in both seasons (Figure 2.2, Table 2.2). 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 2.2, Table 2.2). 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 2.2, Table 2.2). 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 2.2, Table 2.2). 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 2.2, Table 2.2), probably because gonad tissue was a small proportion. Condition index did not differ significantly among treatments ($p = 0.2$ to 0.7; Figure 2.2, Table 2.2). 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 2.3), but no significant relationship between initial mass and tissue growth for either season ($p = 0.43 - 0.67$; Table 2.3), and no interaction between these two effects on tissue growth ($p= 0.21 - 0.88$; Table 2.3). 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 ($p = 0.007$; Table S2.4, Figure S2.3), but within each treatment an overall positive relationship between byssal thread production and growth ($p = 0.04$; Table S2.4, Table S2.3). In the spring, there was a similar trend but both effects were only marginally significant.

2.4.2. 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 the autumn and spring, and approximately 2/3 of the predicted growth rates had a percent error relative to observed growth of less than 40% (Figure S2.3E, F). The cost of byssal thread production was similar between the two seasons, ranging 1.0 – 1.2 J per thread (Table 2.3). Relative food availability (f, unitless) was 50% higher in the autumn than in the spring (Table 2.3).

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 2.4. 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 2.2), and the proportion of the energy budget allocated towards byssal thread production was 2-4 times greater in the autumn (Figure 2.4, Table 2.5). 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 2.5). 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 2.5).

2.4.3. 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, 29% in spring; Figure S2.2).

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 severed (Figure S2.2). The error introduced by changing *b* by one SE was similar to the magnitude of the population standard error. W_{opt} had no effect on these three model outputs in either season.

2.5. Discussion

The two month-long experiments demonstrated that clipping byssal threads increased byssus production and decreased growth. This trade-off is consistent with reports of constitutive byssal thread production regardless of growth rate or energetic input (Clarke 1999, Roberts

Chapter 1, 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 produced fewer threads, and allocated 2% to 8% of the energy budget towards threads, which is consistent with previous estimates of up to 8% of the carbon and nitrogen budget (Hawkins and Bayne 1985). 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.

The experimental mussels were within a protected enclosure under a dock, without predators or wave forces, but with flushing by currents, and baseline byssal thread production rates may thus have been lower than in rocky shore habitats. In natural wave-swept environments greater hydrodynamic forces induce mussels to produce more byssus (Van Winkle 1970, 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 cue can induce thread production in small mussels and might affect predator escape within mussel aggregations (Brown et al., 2011). Unsuccessful predation could also cause thread breakage, and increase production of new threads. Conditions that cause or require greater thread production can increase the cost of byssus to values high enough to use up the entire energy surplus (i.e. >50%) and limit growth or reproduction entirely.

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 the spring (Skidmore 1983) and it is known that periods of spawning can decrease thread production (Babarro et al., 2010), and byssus attachment strength decreases following seasonal reproductive periods (Zardi et al., 2007, Carrington et al. 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 reduced growth, but within each treatment combination there was no evidence of a trade-off between growth and byssal thread production. In contrast, after accounting for the effect of experimental group, there was a significant positive relationship between thread production and growth in the autumn, and a marginal positive relationship in the spring (Figure 2.1, Table S2.3). Within the same experimental population, variance 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). These results support the hypothesis that thread production and growth could be positively correlated across a broad range of energetic surplus, since individuals with increased growth also have greater resources with which to produce byssal threads (Roberts, Chapter 1), but when a high 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. Although all mussels in these experiments were exposed to the same food concentration (within a season), it is still possible that mussel behavior (closure, pumping) could differ enough to make intake variable even within one treatment group, producing a range of growth rates.

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 coefficient (J/mg DW; Table S2.1), 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 S2.1). DEB theory makes the assumption that this parameter is the caloric density of tissue only when a mussel has been starved, and this energetic conversion factor is lower and would decrease the calculated energy budget and individual thread costs. The energy required to produce tissue mass includes both the energy consumed in anabolism and catabolism as well as the cost of the building blocks of mass in the organism. DEB theory makes 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 (Kooijman 2010), but ultimately both energy and mass are required for growth. Further, mussel shell has been found to use 30-60% of the energy budget for Baltic *M. trossulus*. Accounting for this potentially large component of energy expenditure separately from tissue production might be important to properly account for the amount of energy it takes for a mussel to grow and produce threads.

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. Respiration is variable even within individuals of the same population at the same temperature (Fly and Hilbish et al. 2013, Sanders et al. 2018), and the contribution of its error to bioenergetics model results should be considered.

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 (Gabbot 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 the costs terms or other parameters (i.e. the shape coefficient) are not temporally or spatially explicit (i.e. measured for each season and/or population), *f* used as the catch-all variable to fit the model to the data could have limited explanatory power, supporting the argument that stationary models should be treated with caution (Monaco et al. 2018). The value obtained for a' also differed by season, reflecting different assumptions about lifetime metabolic costs (Sebens 2002). Optimal size theory can be used to obtain this value, but respiration is context-specific and measurements in one season might not reflect lifetime values.

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). Thus 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, Chapter 1). Energetic limitations can thus inform hypotheses about 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).

M. trossulus ranges from CA to Alaska (Elliott 2008), and ocean warming and acidification in this region (Khangaonkar et al. 2019) is likely to decrease attachment strength (Newcomb et al. 2019, O'Donnell et al. 2013, George et al. 2018). Additionally, warming can limit energetic resources necessary for attachment (Roberts, Chapter 1) by lowering intake rates and increasing metabolic costs (Fly and Hilbish 2013). On the US west coast, buoy observations indicate that wave heights are increasing 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 could directly lead to dislodgement and/or increase byssal thread attachment costs. *Mytilus* spp. occur in coastal ecosystems and aquaculture farms globally (Gosling 1992) and thus a promising direction for future work would be to evaluate physiological trade-offs of byssal thread production costs in the context of ocean 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.

2.6. Literature Cited

Allan, J.C. and Komar, P.D. (2006). Climate controls on US West Coast erosion processes. Journal of Coastal Research, 22, 511-529.

Bayne, B. L. and R. C. Newell. (1983). Physiological energetics of marine molluscs. In: Wilbur K.M., Salenddin A.S.M. (eds.) *The 74Mollusca,* Vol 4. Physiology, Part 1. pp. 407-515. Academic Press.

Bayne, B. L., R. J. Thompson, and J. Widdows. (1976). Physology 1. In B.L. Bayne (Ed.) Marine mussels: their ecology and physiology (Vol. 10). Cambridge, MA: Cambridge University Press.

Babarro, J. M. F., M. J. Fernández Reiriz, and U. Labarta. (2008). Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: the influence of size and food availability. Journal of Marine Biological Association of the United Kingdom 88: 783-791.

Babarro, J. M., and M. J. Reiriz. 2010. Secretion of byssal threads in Mytilus galloprovincialis: quantitative and qualitative values after spawning stress. J. Comp. Physiol. B, 180: 95-104.

Bell, E.C. and Gosline, J.M. (1996). Mechanical design of mussel byssus: material yield enhances attachment strength. Journal of Experimental Biology 199, 1005–1017.

Bell, E.C. and Gosline, J.M. (1997). Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. Marine Ecology Progress Series, 159, 197-208.

Bennett, A.F. & Lenski, R.E. (2007) An experimental test of evolutionary trade-offs during temperature adaptation. Proceedings of the National Academy of Sciences, 104, 8649-8654.

Boersch-Supan, P.H. and Johnson, L.R. (2019). Two case studies detailing Bayesian parameter inference for dynamic energy budget models. Journal of Sea Research, 143, 57-69.

Borthagaray, A. I., & Carranza, A. (2007). Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. Acta Oecologica, 31(3), 243-250.

Bonte, D., Verduyn, L., & Braeckman, B. P. (2016). Life history trade-offs imposed by dragline use in two money spiders. Journal of Experimental Biology, 219, 26-30.

Brenner, M. and Buck, B.H. (2010). Attachment properties of blue mussel (*Mytilus edulis* L.) byssus threads on culture-based artificial collector substrates. Aquacultural Engineering, 42, 128-139.

Brody S. (1945). Energetic efficiencies of growth and work processes. Chapter in: Bioenergetics and growth with special reference to the efficiency complex in domestic animals (pp. 37-58). New York, NY: Hafner Publishing Company, Inc.

Brown, K.M., Aronhime, B. and Wang, X. (2011). Predatory blue crabs induce byssal thread production in hooked mussels. Invertebrate biology, 130, 43-48.

Carrington, E. (2002). Seasonal variation in the attachment strength of blue mussels: causes and consequences. Limnology and Oceanography, 47, 1723-1733.

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

Carrington, E., Waite, J.H., Sará, G. and Sebens, K.P. (2015). Mussels as a model system for integrative ecomechanics. Annual Review of Marine Science, 7, 443-469.

Clarke, M. (1999). The effect of food availability on byssogenesis by the zebra mussel (*Dreissena polymorpha* Pallas). Journal of Molluscan Studies, 65, 327-333.

Chehab, E. W., Eich, E., & Braam, J. (2008). Thigmomorphogenesis: a complex plant response to mechano-stimulation. Journal of experimental botany, 60, 43-56.

Cheng, T.K., Hill, D.F., Beamer, J. and García-Medina, G. (2015). Climate change impacts on wave and surge processes in a Pacific Northwest (USA) estuary. Journal of Geophysical Research: Oceans, 120, 182-200.

Crofts, S.B. and Anderson, P.S., (2018). The influence of cactus spine surface structure on puncture performance and anchoring ability is tuned for ecology. Proceedings of the Royal Society B, 285, 20182280.

Denny, M. (1995). Predicting physical disturbance: Mechanistic approaches to the study of survivorship on wave-swept shores. Ecological Monographs, 65, 371-418.

Denny, M. W., & Gaylord, B. (2010). Marine ecomechanics. Annual Review of Marine Science, 2, 89-114.

Dolmer, P. and Svane, I. (1994). Attachment and orientation of *Mytilus edulis* L. in flowing water. Ophelia, 40, 63-74.

Dmitriew, C.M. (2011) The evolution of growth trajectories: what limits growth rate? Biological Reviews, 86, 97-116.

Elliott, J., Holmes, K., Chambers, R., Leon, K. & Wimberger, P. (2008) Differences in morphology and habitat use among the native mussel *Mytilus trossulus*, the non-native

M. galloprovincialis, and their hybrids in Puget Sound, Washington. Marine Biology, 156, 39-53.

Filgueira, R., Rosland, R. & Grant, J. (2011) A comparison of scope for growth (SFG) and dynamic energy budget (DEB) models applied to the blue mussel (*Mytilus edulis*). Journal of Sea Research, 66, 403-410.

Fly, E.K., and Hilbish, T.J. (2013). Physiological energetics and biogeographic range limits of three congeneric mussel species. Oecologia, 172, 35-46.

Gabbott, P.A. (1976). Energy metabolism. In B.L. Bayne (Ed.), Marine Mussels: Their ecology and physiology (pp. 293-355). Cambridge, MA: Cambridge University Press.

George, M.N., Pedigo, B. and Carrington, E. (2018). Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. Journal of The Royal Society Interface, 15, 20180489.

Gosline, J.M., 2018. Mechanical design of structural materials in animals. Princeton University Press.

Gosling, E. (1992). The mussel *Mytilus*: ecology, physiology, genetics and culture. Amsterdam: Elsevier.

Hawkins A.J.S., and Bayne, B.L. (1985). Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies, and maintenance requirements. Marine Ecology Progress Series, 7, 181-188.

Newell R.I.E., Hilbish T.J., Koehn R.K., Newell C.J. (1982). Temporal variation in the reproductive cycle of *Mytilus edulis* (*Bivalvia, Mytilidae*) from localities on the East Coast of the United States. Biological Bulletin, 162, 299-310

Jones, H.D., Richards, O.G. and Southern, T.A. (1992). Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology, 155, 213-237.

Khangaonkar T., Nugraha A., Xu W., and Balaguru K. (2019). Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. Journal of Geophysical Research, 124:3876-3904.

Kitchell, J.F., Stewart, D.J. and Weininger, D. (1977). Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of the Fisheries Board of Canada, 34, 1922-1935.

Koehl, M.A.R. (1996) When does morphology matter? Annual Review of Ecology and Systematics, 27, 501-542.

Kooijman, S.A.L.M. (2010). Dynamic energy budget theory for metabolic organization. Cambridge, MA: Cambridge University Press.

Korringa, P. (1976). Farming marine organisms low in the food chain: a multidisciplinary approach to edible seaweed, mussel and clam production. Amsterdam, Oxford: Elsavier.

Lee, C. Y., S. S. L. Lim, and Owen. M.D. (1990). The rate and strength of byssal reattachment by blue mussels (*Mytilus edulis* L.). Canadian Journal of Zoology, 68, 2005-2009.

Leonard, G.H., Bertness, M.D. and Yund, P.O. (1999). Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology, 80, 1-14.

Lowe, A.T., Roberts, E.A. and Galloway, A.W. (2016). Improved marine-derived POM availability and increased pH related to freshwater influence in an inland sea. Limnology and Oceanography, 61, 2122-2138.

Lurman, G.J., Hilton, Z. and Ragg, N.L. (2013). Energetics of byssus attachment and feeding in the green-lipped mussel *Perna canaliculus*. The Biological Bulletin, 224, 79-88.

Martin, B.T., Zimmer, E.I., Grimm, V. and Jager, T. (2012). Dynamic Energy Budget theory meets individual-based modelling: a generic and accessible implementation. Methods in Ecology and Evolution, 3, 445-449.

Matzelle, A.J., Sarà, G., Montalto, V., Zippay, M., Trussell, G.C. and Helmuth, B. (2015). A bioenergetics framework for integrating the effects of multiple stressors: Opening a 'black box' in climate change research. American Malacological Bulletin, 33, 150-161.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S.N. and Gutowska, M.A. (2011). Food supply and seawater pCO2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. PloS One:e24223.

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

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

Monaco, C. J., Wethey, D. S., & Helmuth, B. (2014). A dynamic energy budget (DEB) model for the keystone predator *Pisaster ochraceus*. *PLoS One*, 9, e104658.

Monaco, C.J. & McQuaid, C.D. (2018). Applicability of Dynamic Energy Budget (DEB) models across steep environmental gradients. *Science Reports*, 8, 16384.

Newcomb, L.A. (2015). Elevated temperature and ocean acidification alter mechanics of mussel attachment (Doctoral dissertation).

Newcomb L.A., George, M.N, O'Donnell, M.J., and Carrington, E. (2019). Only as strong as the weakest link: combined effects of temperature and pCO2 on mussel attachment. Conservation Physiology, 7:coz068.

Nisbet, R.M., Jusup, M., Klanjscek, T. & Pecquerie, L. (2012) Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. Journal of Experimental Biology, 215, 892-902.

O'Donnell, M.J. (2008). Reduction of wave forces within bare patches in mussel beds. Marine Ecology Progress Series, 362, 157-167.

O'Donnell, M.J., George, M.N. and Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. Nature Climate Change, 3, 587-590.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Riisgård, H.U. and Randløv, A. (1981). Energy budget, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Marine Biology, 61, 227-234.

Rumohr H, Brey T, Ankar S. (1987). A compilation of biometric conversion factors for benthic invertebrates of the Baltic Sea. Baltic Marine Biologists Publication, 9. Kiel: Institut für Meereskunde.

Sanders, T., Schmittmann, L., Nascimento-Schulze, J.C. and Melzner, F. (2018). High calcification costs limit mussel growth at low salinity. Frontiers in Marine Science, 5, Art-Nr.

Sarà, G., Palmeri, V., Montalto, V., Rinaldi, A. and Widdows, J., 2013. Parameterisation of bivalve functional traits for mechanistic eco-physiological dynamic energy budget (DEB) models. Marine Ecology Progress Series, 480, pp.99-117.

Sebens, K.P. (1982). The limits to indeterminate growth: an optimal size model applied to passive suspension feeders. Ecology, 63, 209-222.

Sebens, K.P. (1987). The ecology of indeterminate growth in animals. Annual Review of Ecology and Systematics, 18, 371-407.

Sebens, K.P. (2002). Energetic constraints, size gradients, and size limits in benthic marine invertebrates. Integrative and Comparative Biology, 42, 853-861.

Sebens, K.P., Sarà, G. and Carrington, E. (2018). Estimation of fitness from energetics and life-history data: An example using mussels. Ecology and Evolution, 8, 5279-5290.

Semedo A., Weisse R., Behrens A., Sterl A., Bengtsson L., Günther H. (2013). Projection of global wave climate change toward the end of the Twenty-First Century. Journal of Climate, 26, 8269-8288.

Skidmore, D. (1983). Settlement, growth, and survival of *Mytilus edulis* L. in Puget Sound and Assessment of *Mytilus Californianus* for Aquaculture. Thesis, M.S., University of Washington.

Telewski, F.W. and Pruyn, M.L. (1998). Thigmomorphogenesis: a dose response to flexing in *Ulmus americana* seedlings. Tree Physiology, 18, 65-68.

Thompson, R.J. and Bayne, B.L. (1974). Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*. Marine Biology, 27, 317-326.

van der Veer, H.W., Cardoso, J.F. and van der Meer, J. (2006). The estimation of DEB parameters for various Northeast Atlantic bivalve species. Journal of Sea Research, 56, 107-124.

Van Winkle, W. (1970). Effect of environmental factors on byssal thread formation. Marine Biology, 7, 143-148.

Vogel, S., 2013. Comparative biomechanics: life's physical world. Princeton University Press.

Waldbusser, G.G., Brunner, E.L., Haley, B.A., Hales, B., Langdon, C.J. and Prahl, F.G. (2013). A developmental and energetic basis linking larval oyster shell formation to acidification sensitivity. Geophysical Research Letters, 40, 2171-2176.

Waite JH, Qin XX, Coyne KJ. (1998). The peculiar collagens of mussel byssus. Matrix Biology, 17, 93–106.

Walker, J. (2007) A General Model of Functional Constraints on Phenotypic Evolution. The American Naturalist, 170, 681-689.

Widdows J. (1978). Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom, 58, 109-124.

Widdows, J. and Bayne, B.L. (1971). Temperature acclimation of *Mytilus edulis* with reference to its energy budget. Journal of the Marine Biological Association of the United Kingdom, 51, 827-843.

Young G.A. (1985). Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. Marine Ecology Progress Series, 24, 261–271.

Zardi, G.I., McQuaid, C.D. and Nicastro, K.R. (2007). Balancing survival and reproduction: seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. Marine Ecology Progress Series, 334, 155-163.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. (2009). Mixed effects models and extensions in ecology with R. Springer Science & Business Media.

2.7. Tables and Figures

Table 2.1. 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 HSD	Never	a				a				a			
	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 HSD	Never	a				-				-			
	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 HSD	Never	a				a				-			
	Weekly	b				ab				-			
	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 HSD	Never	a				-				-			
	Weekly	ab				-				-			
	Daily	b				-				-			

Table 2.2. 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	$a'=(b*e)/(W_{\mathrm{opt}}{}^{(d-e)}*d)$	Equation from Sebens 1982
			Spr	1.76	0.55		
b	Cost coefficient	J / (day × mg DW)	Aut	0.081	0.019	b=R×(4.75cal/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
C.F.	Energetic conversion factor	J / mg DW	All	21.6	1.6		Table S2.1
Measured values used to calculate input parameters							
W_{opt}	Energetic optimum size	g DW	All	0.72	0.06	-	Unpublished data E.A. 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 × (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 2.3. Summary of multiple linear regression analyses of tissue growth as a function of byssal thread production and estimated initial mass, across all byssus severing frequency treatments.
Bold font indicates a significant effect ($p<0.05$).

		Tissue growth (g)						
Season	Effect	Estimate	SE	Sum Sq	Df	F val.	p	R^2
Autumn	Intercept	0.0	0.1	0.0	1	0.00	0.97	0.20
	Thread production	-0.4	0.2	7.1	1	5.48	**0.02**	
	Initial mass	-0.1	0.1	1.0	1	0.62	0.43	
	Thread production: Initial mass	0.2	0.2	1.41	1	1.60	0.21	
	Residuals			33.90	39			
Spring	Intercept	0.0	0.2	0.0	1	0.00	0.96	0.20
	Thread production	-0.4	0.2	4.6	1	5.23	**0.03**	
	Initial mass	-0.1	0.2	0.16	1	0.18	0.67	
	Thread production: Initial mass	0.0	0.2	0.02	1	0.02	0.88	
	Residuals			29.5	34			

Table 2.4. Summary of parameter estimations of the food scalar (*f*) and the cost per thread (*h*) 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**	1.58 ± 0.10	**<0.001**
Spring	1.16 ± 0.39	**0.005**	1.06 ± 0.04	**<0.001**

Table 2.5. 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 cost (excluding reproductive 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

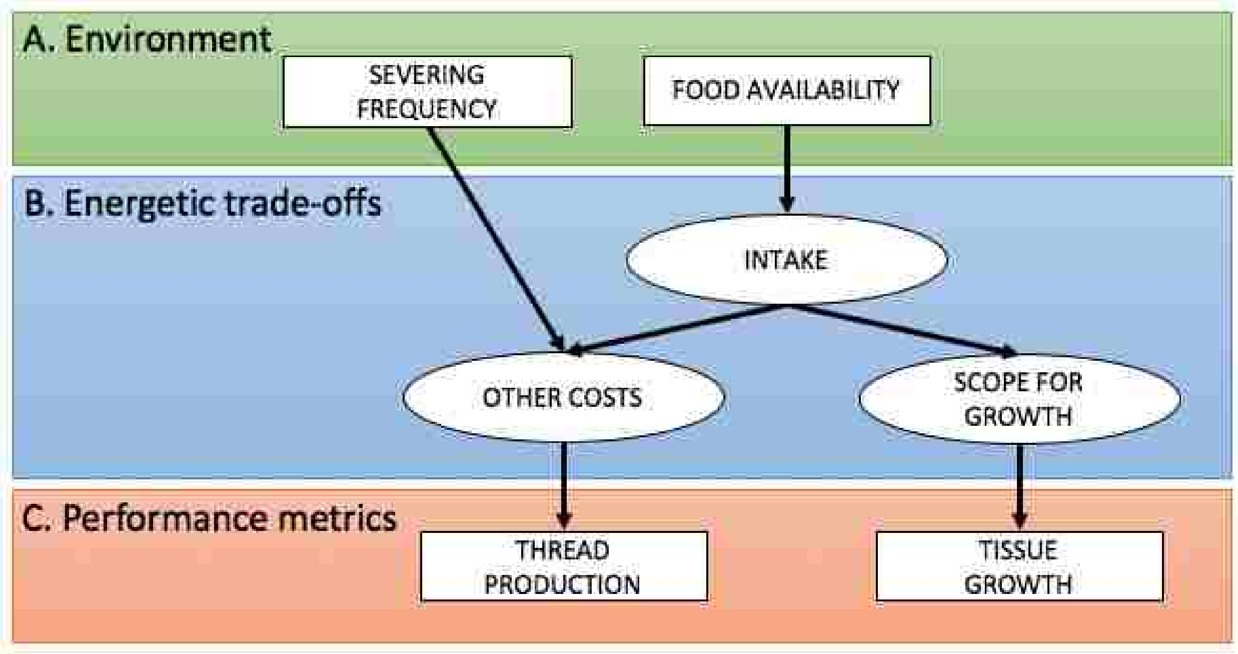

Figure 2.1. Schematic representation of possible energetic trade-offs between mussel byssal thread production and growth using a Scope for Growth framework. Environmental conditions (A), such as increased wave disturbance, predation pressure or, in the case of this study, experimental byssal thread removal by severing can increase byssal thread production and affect other performance metrics (C). We hypothesized that energy allocation to byssal thread production is prioritized over tissue growth, which includes both somatic and reproductive tissue, and 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. See text for details.

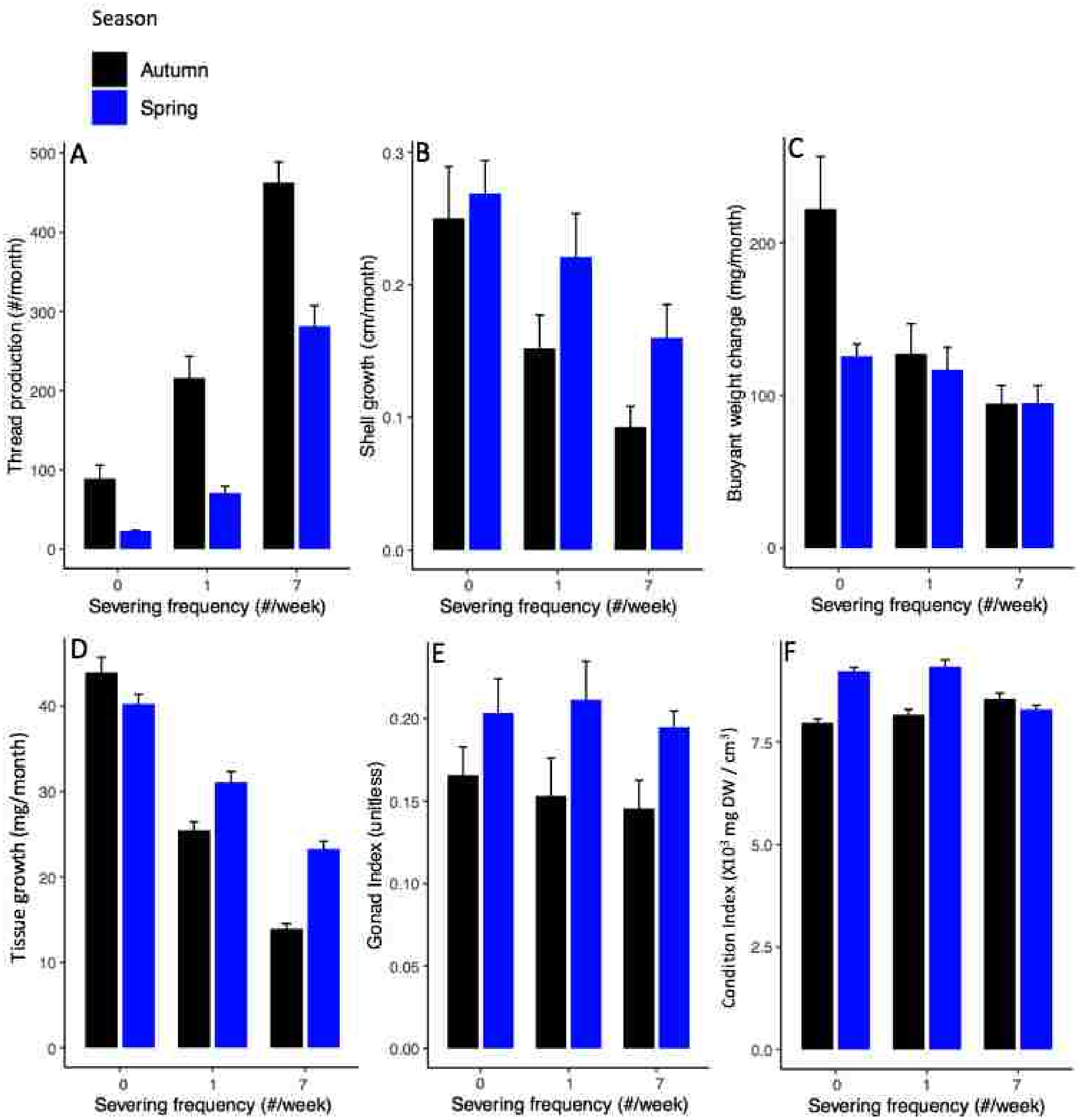

Figure 2.2. Summary of byssal thread production, growth, and mussel condition across a range of byssus severing frequencies in autumn and spring. (A) Thread production, (B) shell growth, (C) buoyant weight change, (D) tissue dry weight growth, (E) final gonad index, and (F) final condition index as a 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). Severing

frequencies of 0, 1 and 7 times per week correspond to the “Never” (control), “Weekly” and “Daily” manipulations described in the text.

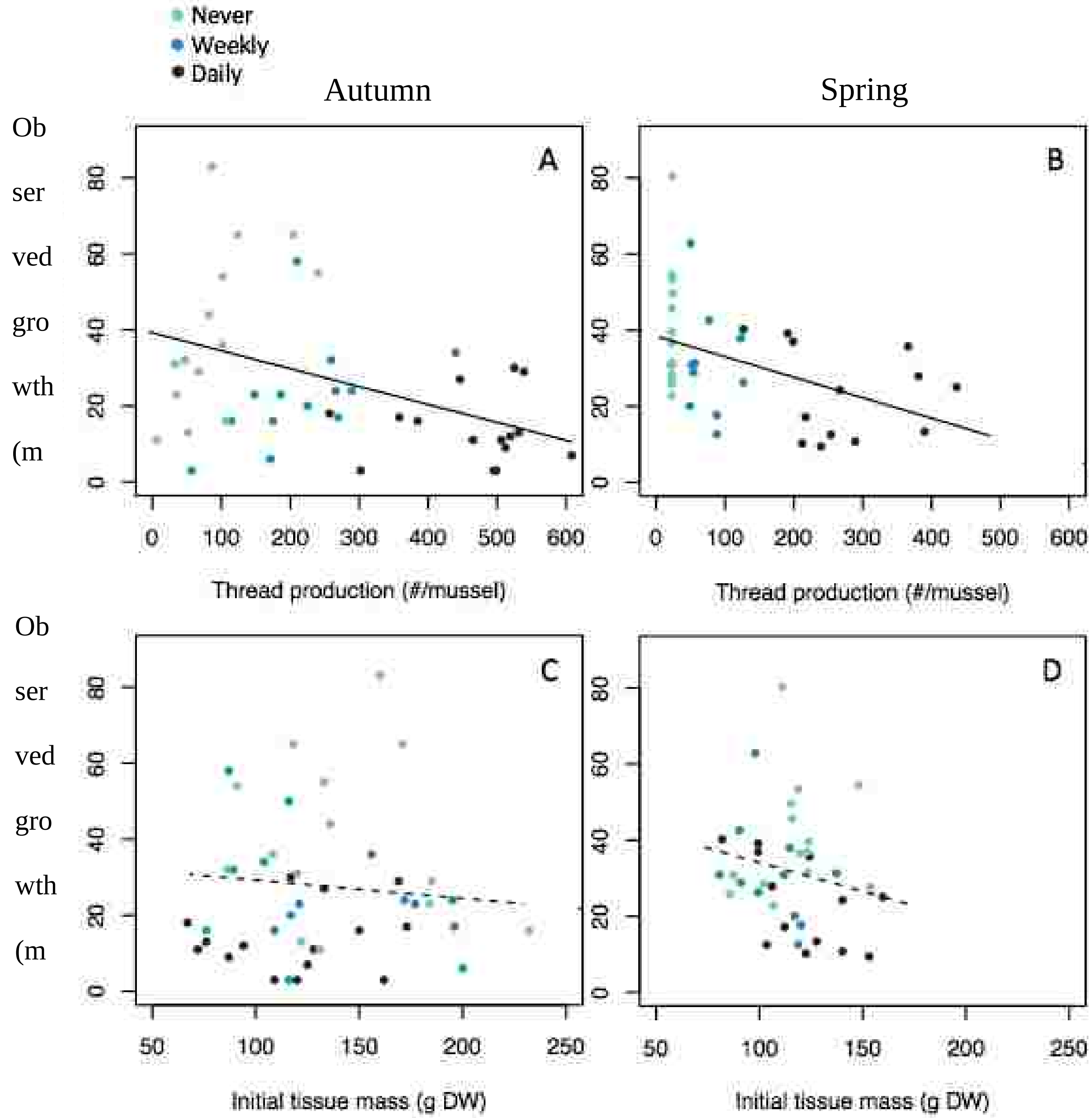

Figure 2.3. Mussel tissue growth as a function of thread production and initial tissue mass. Tissue growth as a function of (A, B) thread production and (C, D) estimated initial tissue mass in the autumn and spring. Symbols represent individual mussels in different severing frequency treatments (see 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 2.3). Observed growth (mg DW) divided by the energetic conversion factor (C.F.) is G_{TM}.

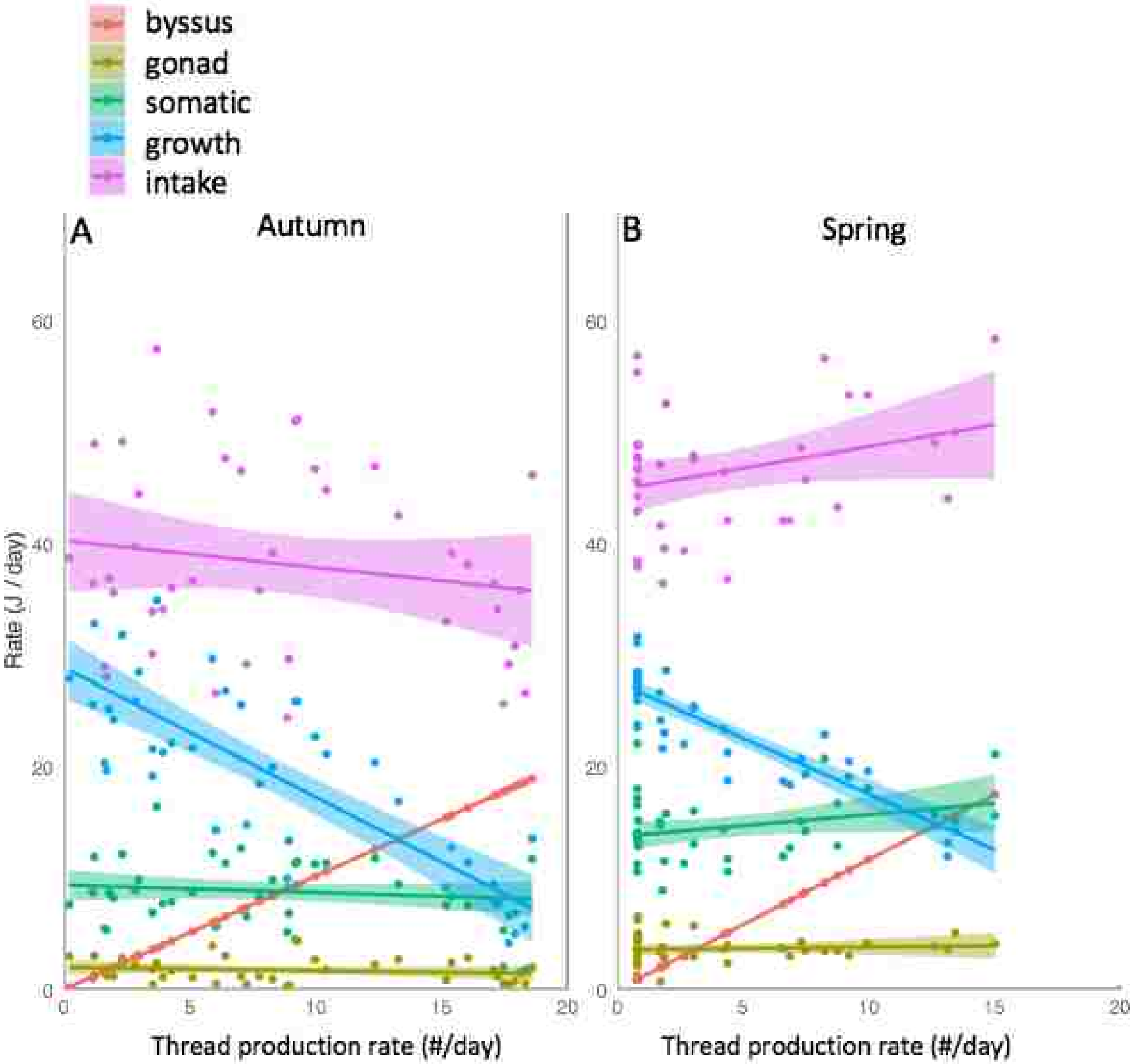

Figure 2.4. Model results for all components of a mussel's energy budget (J per day) as a function of byssal thread production rate (threads per day). Model results are presented for autumn (A) and spring (B) as determined by the two-step optimization.Circles represent calculated values of budget components (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 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.

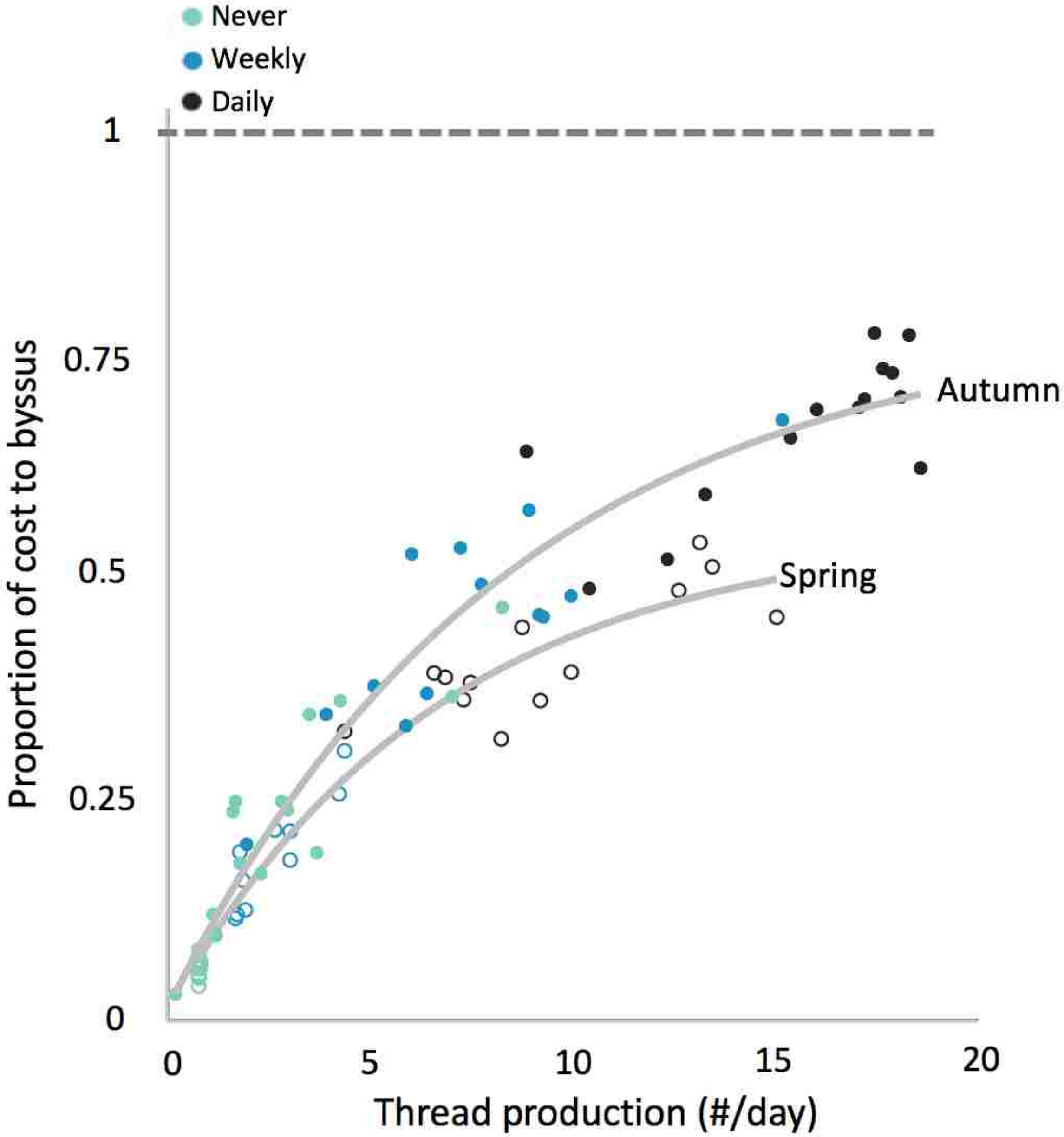

Figure 2.5. Energy allocation towards byssal threads as a proportion of metabolic costs of tissue maintenance. The proportion of total metabolic cost allocated towards producing byssal threads (excluding reproductive costs) as a function of thread production in the autumn (closed circles) and spring (open circles).Symbol colors represent the frequency of severing in the treatment. Each curve is an exponential fit.

2.8. Acknowledgements

We thank Timothy Essington, Lauren Buckley, and Jaqueline Padilla-Gamiño for insightful discussions that improved the quality of this manuscript. We also thank Alex Lowe and Megan Dethier for their involvement in the spring experiment. E.A.R. was supported by a National Science Foundation IGERT Program on Ocean Change Fellowship (1068839), and several grants from UW Friday Harbor Laboratories and UW Biology (Edmondson Award, Richard and Megumi Strathmann Fellowship, Kohn Fellowship, and Carrington Student Travel Fellowship). Additional support was provided by the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under project number NA14OAR4170078 (to EC and CS Friedman) through the Washington Sea Grant Program. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U.S. government is authorized to reproduce and distribute this paper for governmental purposes.

2.9. Supplementary Tables and Figures

Table S2.1. Summary of energy conversion factor measurements for tissue growth of *Mytilus spp.* Comparison of literature-derived values of energetic conversion values for the cost of tissue growth (J / mg dry weight) with Dynamic Energy Budget (DEB) volume-specific cost of growth. Note that energy required per gram of growth (J / mg DW) is up to three times lower in DEB models. Abbreviations: Dutch Wadden Sea (DWS), Phillipson bomb calorimeter (PBC), Gallenkamp calorimeter (GC), Lipid, protein, and fat composition (Lip. Prot. Fat.), Ash free dry weight (AFDW).

				Value	Reported value	
Species	Location	Parameter name	Method	Mean (± SD)	Mean (± range)	Source
Scope for Growth energy density measurements						
M. spp.	Baltic	Conversion factor	PBS		20.8 J/mg DW	Rumohr et al. 1987
M. spp.	Baltic	Conversion factor	GC		20.5 ± 0.5 J/mg DW	Rumohr et al. 1987
M. spp.	Baltic	Conversion factor	PBC		21.7 J/mg DW	Rumohr et al. 1987
M. spp.	Baltic	Conversion factor	GC		21.5 ± 1.9 J/mg DW	Rumohr et al. 1987
M. trossulus	Baltic	Conversion factor	Lip. Prot. Fat		23.6 J/mg DW	Sanders et al. 2018
Mean ± SD				21.6 ± 1.2 J/mg DW		
Dynamic Energy Budget (DEB) energy density measurements						
M. edulis	DWS	Volume-specific costs for growth	AFDW of starved mussels collected in January	7.0 J/mg DW	1750 J/cm^-3	Van der Veer 2006
M. edulis	DWS	Maximum storage density + Volume-specific costs for growth	AFDW maximum size	15.7 J/mg DW	3940 J/cm^-3	Van der Veer 2006

Table S2.2. Summary of linear regression analysis of log tissue weight as a function of log shell length. Bold font indicates a significant relationship ($p < 0.05$).

	Log tissue weight (g)			
	Sum Sq	DF	F value	p
Log length (cm)	400.89	1	6401.3	**<0.001**
Residuals	6.33	101		

	Estimate	SE
Intercept	1.70	0.04
Slope	3.19	0.04

Table S2.3. Summary of one-way ANCOVA of the effects of thread production (covariate) and byssus severing treatment (fixed effect) on mussel tissue growth in two seasons. All variables are transformed using standard z-scores. Bold font indicates a significant effect (p< 0.05).

		Tissue growth (g)				
Season	Effect	Type III Sum Sq	Df	F val.	p	Adj. R-sq
Autumn	Intercept	1.3	1	1.2	0.17	0.36
	Thread production	2.9	1	4.48	**0.04**	
	Frequency	7.5	2	5.73	**0.007**	
	Thread production:Frequency	1.9	2	1.49	0.23	
	Residuals	24.1	37			

	Frequency	Group
Tukey HSD	Never	a
	Weekly	b
	Daily	b

		Type III Sum Sq	Df	F val.	p	Adj. R-sq
Spring	Intercept	2.7	1	3.4	0.07	0.21
	Thread production	2.6	1	3.40	0.07	
	Frequency	2.7	2	1.70	0.19	
	Thread production:Frequency	2.8	2	1.80	0.18	
	Residuals	25.4	32			

Table S2.4. Summary of exponential curve fit for the modeled output of the proportion of cost allocated towards byssus (excluding reproductive costs) as a function of byssal thread production rate for autumn and spring.

	Autumn		Spring	
	Estimate	SE	Estimate	SE
Number of threads at 1/e max cost (tau)	8.4	0.9	6.6	0.7
Maximum proportion of cost to threads (Vmax)	0.77	0.04	0.54	0.03

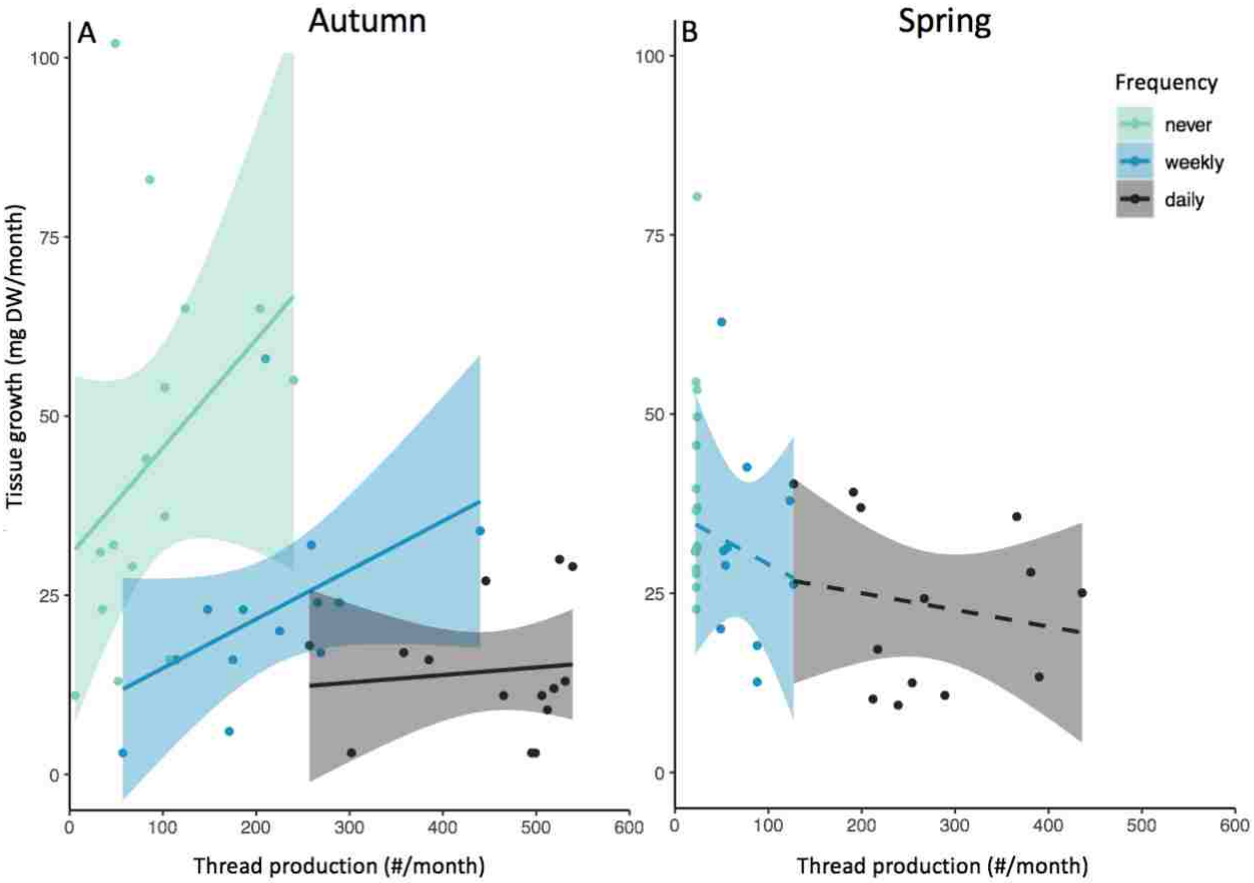

Figure S2.1. Tissue growth as a function of thread production at three severing frequency treatment levels. Manipulation results are shown for the autumn (A) and spring (B) experiments over the range of byssus severing frequencies (see inset for color scheme). Symbols represent individual mussels in different severing frequency treatments and lines are regressions with 95% confidence intervals. After the treatment effect is accounted for, there is a significant positive relationship between thread production and tissue growth in the autumn but only a marginal trend in the spring (Table S2.3).

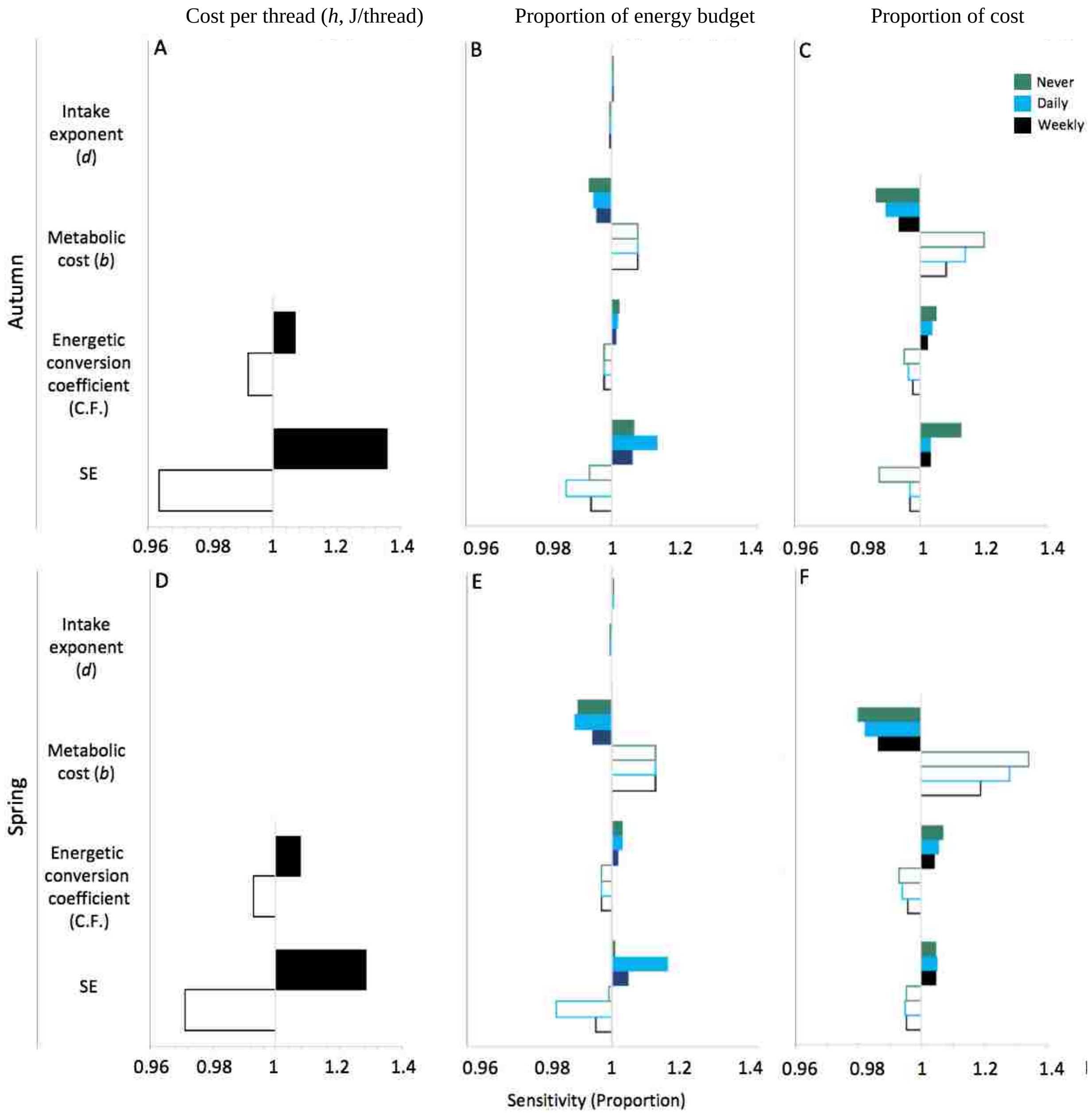

Figure S2.2. Sensitivity analysis using individual parameter perturbation (IPP) of energy allocation calculations in autumn and spring: (A, D) the cost per threads (*h*); (C, F) the proportion of costs allocated to byssus (excluding reproductive costs); and (B, E) the proportion of the energy budget allocated towards byssus production. Model parameters were the intake exponent (*d*), metabolic cost (*b*), the energetic conversion coefficient (C.F.). Also included for comparison is the population error (SE), which is due to variance in the experimental data and is the error reported throughout the paper. The optimum size, W_{opt} had no influence on these model results and is not shown here.

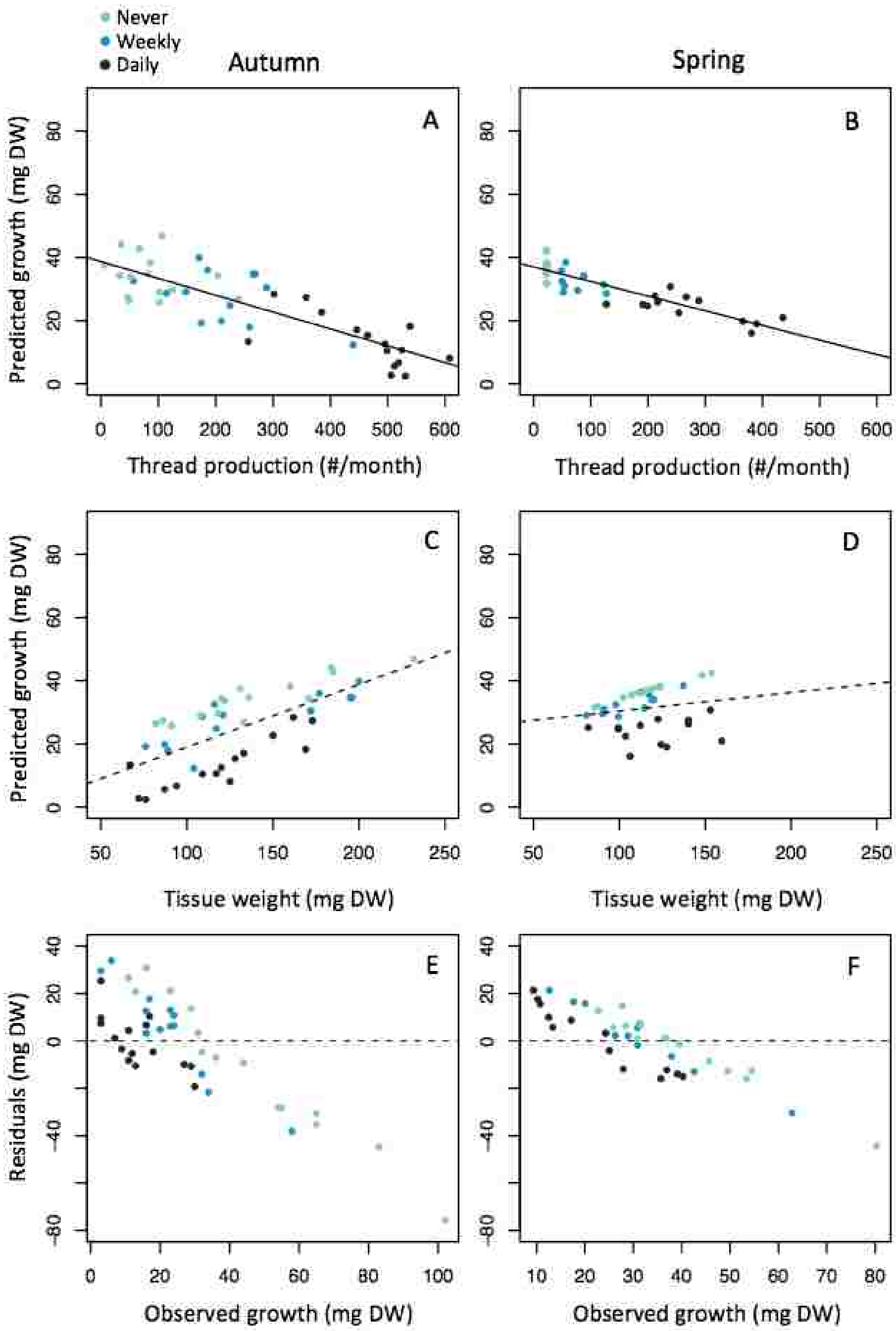

Figure S2.3. Model output across mussel thread production and initial tissue weight. Model predictions of tissue growth for autumn and spring, as a function of thread production (A, B) or initial tissue weight (C, D). (E, F) Residual tissue growth, or the difference between predicted tissue growth (SFG) and observed growth as a function of observed growth.

2.10. Supplementary Information

Supplementary Information 2.1.

The assumption that the exponent relating length and tissue mass was 3 (Kooijman, 2010), and evaluated this assumption with a separate sampling of *M. trossulus*, with a greater size range (0.7 – 5.7 cm length, n = 100). Mussels were collected from Coupeville, WA (48°13'15.1"N 122°42'20.4"W; July 6, 2016) and shell length was determined using calipers, and tissue was removed from the shell, blotted and weighed, and then dried at 60°C to a constant dry weight, as described above. The exponent relating tissue dry weight and shell length was close to three (3.19 ± 0.04, unitless), supporting this assumption (Table S2.2). This sampling of mussels was also used to determine the ratio between wet and dry tissue weight (Table 2.2).

W_{opt} was estimated empirically from the tissue dry weight of the upper 5% quantile of 238 mussels collected during 2014 from nearby experimental enclosures for another study (0.72 ± 0.06 g DW, Roberts, unpub. data; Table 2.2).

Chapter 3

Energetic limitation affects growth but not byssal thread production of two congeneric mussel species

Emily A. Roberts[1,2*] and Emily Carrington[1,2]

1 - Department of Biology, University of Washington, 4000 15th Ave NE, Seattle, WA 98195 USA

2 - Friday Harbor Laboratories, University of Washington, 620 University Road, Friday Harbor, Washington 98250 USA

Keywords: bioenergetics, energy budget, resource allocation, biomaterial, byssus, pH, dissolved oxygen, *Mytilus trossulus*, *Mytilus galloprovincialis*

3.1. Abstract

Anthropogenic warming of the ocean and atmosphere, concurrent with ocean acidification and deoxygenation, have made it even more pressing to quantify the link between these environmental stressors and marine organism population dynamics. One physiological stressor that can affect marine organisms is energetic limitation, which can be caused by low food availability, decreased feeding rates, or increased metabolic costs at suboptimal temperatures. Energetic limitation can affect certain organismal traits, such as growth, but organisms might be able to continue to invest in other traits that present evolutionary trade-offs, regardless of energetic surplus or deficit. Mussels are ecosystem engineers in rocky shore habitats, and they produce byssal threads to attach to hard substrate and aquaculture line. Previous modelling and laboratory studies of mytilid mussel bioenergetics suggest tissue and shell growth are energetically-constrained, while production of byssal threads presents a fitness trade-off and could potentially be a fixed or 'constitutive' response regardless of energetic state. In this study, we conduct a field test with two congener mussel species, *Mytilus trossulus* and *Mytilus galloprovincialis* to determine whether an index of energetic availability, scope for growth (SFG), correlates with growth and byssal thread production, and the extent to which other potential stressors (hypoxia, low pH, low salinity and high temperature) modulate this response. We find a positive correlation between SFG and growth (both tissue and shell) but not byssal thread production. We also find low pH or low DO, two co-varying physiological stressors, negatively affect tissue growth of both species, but only marginally affect byssal thread production. We also observed mortality in the late summer/early autumn that coincides with the periods of greater hypoxia and low pH. Overall, this work suggests that byssal thread production

is independent of energetic constraints for adult mussels, and may thus be considered a 'constitutive' response that represents a fitness trade-off.

3.2. Introduction

Anthropogenic ocean warming, acidification, and deoxygenation (Bopp et al., 2013, IPCC 2019, Kroeker et al., 2011, Keeling et al., 2010) in recent decades have made it even more pressing to quantify the link between environmental conditions and the population dynamics of marine organisms (Kraus et al., 2015, Carrington et al., 2015). One way to quantify this link is to evaluate the relative influence of multiple physiological stressors, such as seawater temperature, pH, and dissolved oxygen, on organism physiology and survival (Sokolova 2013, Gunderson et al., 2016, Folt et al., 1999). While the effects of multiple environmental variables can be evaluated empirically using purely statistical models (e.g. Soberon et al., 2009), the influence of biologically meaningful mechanisms is difficult to evaluate directly (i.e. plant evapotranspiration - Stephenson 1998, Robertson et al., 2003). Ecophysiological models allow for testing the influence of biologically meaningful physiological stressors, which do not necessarily scale linearly with environmental conditions. For instance, ingestion of food saturates at high food levels and thermal responses typically decline precipitously when thermal optima are exceeded (e.g. Schulte et al., 2011). Mechanistic models can be used to quantify the combined effects of food limitation and temperature on organism feeding and metabolic costs (Bioenergetics, e.g. Bayne and Newell 1983). One physiological stressor that can be determined from such bioenergetic models is energetic limitation, which can occur when food availability is low, feeding is limited by temperature, and/or metabolic costs are high (Kitchell et al., 1977). While bioenergetic methods for predicting growth from environmental food and temperature measurements are well developed (e.g. Filgueira et al., 2011, Kooijman 2010, Kitchell et al., 1977), the relative effect of energetic limitation compared to other physiological stressors on organism physiology is less well understood (Jørgenson et al., 2016, Kraus et al., 2015).

Understanding the effect of physiological stressors is especially important in seasonally eutrophic systems, where physical and biological processes shape the ecological niche of resident sessile organisms (Holt 2009, Sarà 1986). Physiological stress, including energetic limitation, can decrease organism growth and can affect reproductive output. However, organisms can adaptively invest in other traits that increase the chance of survival and fitness despite the short-term energetic cost, presenting evolutionary trade-offs (Jørgensen et al., 2016, Sebens et al. 2018). Investment in these traits may be prioritized, independent of energetic constraints, due to behavioral control (e.g., 'flexible traits' such as fish locomotion or swim bladder regulation; Jørgensen et al., 2016, Tang and Boisclair 1995, Strand et al., 2005), investment in rebuilding damaged organs (rebuilding of digestive tract; Dekinga et al., 2001, Secor 2008) or producing structural materials (mussel attachment; Roberts Chapter 2). Bioenergetic models can serve as a predictive tool to evaluate whether physiological responses are energetically-constrained, and serve as a baseline upon which to evaluate the effects of other physiological stressors (Kraus et al., 2015).

Mussels are dominant species on rocky shores globally, are major aquaculture species, and serve as model systems to study the effect of energetic limitation on growth (Van der Veer 2006, Kooijman 2010, Bayne 1976). As sessile organisms, attachment to hard substrate is essential to mussel survival, and there is evidence of an adaptive advantage to allocating energetic resources to attachment (Sebens et al., 2018). Mussels tether themselves to rock and aquaculture line by producing a network of collagen-like threads known as "byssus." Byssal thread attachment strength is affected by both the quality and quantity of byssal threads (Bell and Gosline 1997). Byssal thread production can be induced experimentally by severing the byssus; mussels make more threads when the byssus is repeatedly cut (Roberts Chapter 2). The growth of the soft tissue and shell length of mussels are often uncoupled (Hilbish 1986) and can be limited by energetic constraints (Bayne 2004, Hilbish and Koehn 1985, Filgueira et al., 2011, Beiras et al., 1994, Roberts Chapter 1). In contrast, there are several lines of evidence that production of byssal threads is 'constitutive' and not subject to energetic constraints. First, byssal thread production is key to survival and a model of this trade-off suggests an evolutionary advantage to investing in byssus regardless of energy availability (Sebens et al., 2018). Second, induced byssal thread production (by severing the existing threads) can decrease growth by up to 66% (Roberts Chapter 2). Third, byssus is produced under conditions of energetic limitation (Clarke 1999), and low survival (Roberts Chapter 1), regardless of long-term nutritional or temperature stress (Roberts Chapter 1). While these studies suggest that byssal thread production is not constrained by energetic limitation, this relationship has not been explored in the field, where there is naturally a larger range of food availability. Further, mussel attachment strength varies with depth and/or season (Newcomb 2015, Moeser and Carrington 2006, Carrington et al., 2009, Zardi et al., 2007). This variability suggests that food limitation (Babarro et al., 2008) or other physiological stressors, such as low pH, and DO (George et al., 2018, O'Donnell et al., 2013) and reproductive state (Babarro et al., 2010), could potentially affect byssal thread production in the field.

Mussel energetics may be estimated using a Scope for Growth (SFG) framework, which is based on food availability and the effect of temperature on feeding, rate minus metabolic costs such as cellular maintenance (Figure 3.1A, Bayne 1976, Widdows and Hawkins 1989). We hypothesize that tissue (including gonad) and shell growth are energetically-constrained physiological responses, and increase across a gradient of energetic scope (SFG, Figure 3.1B), and that byssal thread production, as an adaptive investment, is produced constitutively across this same gradient of energetic scope (Figure 3.1C). Other physiological stressors, including acute temperature stress, low salinity, low pH and low dissolved oxygen, might affect measured traits directly, or indirectly by influencing SFG (Figure 3.1A). For example, low pH could decrease carbonate ion abundance and directly affect shell growth (Sanders et al., 2014) or could indirectly limit shell growth by affecting feeding rate or metabolic costs (Seibel et al., 2012). We hypothesize that physiological stress might also affect constitutive responses, such as byssal thread production, independent of energetic scope (Figure 3.1B).

We tested these hypotheses using two congeneric mussel species with differing ecological niches, *Mytilus trossulus* (less tolerant of high thermal stress) and *Mytilus galloprovincialis* (less tolerant of low salinity; Braby and Somero 2006). The effect of temperature on energetic scope is well-described for both species; increasing temperature decreases energetic scope for *M. trossulus*, but increases energetic scope of *M. galloprovincialis* (Fly and Hilbish 2013). In contrast, the physiological stressors of hypoxia and low pH affect both species similarly (Sanders et al., 2014, de Zwaan et al., 1981), We therefore predict both species will display a positive relationship between growth and energetic scope and no relationship between energetic scope and byssal thread production (Figure 3.1B and 3.1C). We also predict that exposure to physiological stressors, including hypoxia and low pH for both species, and low salinity and high temperature for *M. trossulus* and *M. galloprovincialis,* respectively, will cause decreased growth and attachment relative to these baseline energetic predictions (Figure 3.1B and 1C).

Shallow Salish Sea bays, such as Penn Cove on Whidbey Island, offer an excellent natural dynamic coastal system in which to study organismal responses to gradients of physiological stress. Seawater is stratified in this embayment, experiences a long residence time, and local anthropogenic nutrients exacerbate the effects of eutrophication (Ahmed et al., 2019). Penn Cove Shellfish is a mussel aquaculture farm located in a shallow bay (>10m depth) with riverine input from the Skagit Valley and the greater Whidbey Basin watershed. Seawater conditions change hourly and seasonally, depending on tides and other physical mixing processes (Sutherland et al., 2011), presenting a range of physiological stressors that can co-vary. Mussel aquaculture lines extend from the surface to seven meters depth, where intermittent hypoxia and low pH are common (Newcomb 2015, George et al., *in press*).

In this study, we tested whether SFG, an index of energetic stress, correlates with growth and byssal thread production, and if hypoxia, low pH, low salinity (*M. galloprovincialis*) and high temperature (*M. trossulus*) exposure alter growth and thread production relative to baseline SFG values. We measured environmental conditions (temperature, salinity, pH, dissolved oxygen, chlorophyll) as well as the growth, attachment, and survival of two species at two depths. We then quantified the relationship between SFG and somatic growth, shell growth, and byssus production, and used multiple regression analysis to evaluate the effect of SFG in combination with the physiological stressors.

3.3. Methods

Byssal thread production, growth and survival of *Mytilus trossulus* and *Mytilus galloprovincialis* were measured in experimental cages at Penn Cove Shellfish LLC on Whidbey

Island (Coupeville, WA ; 48°13’15.1”N 122°42’20.4”W) over two years (June 2016 – July 2018).

3.3.1. Seawater Monitoring

Seawater conditions were monitored adjacent to experimental cages, continuing the time series initiated by Newcomb (2015), for a total of five years (April 2014 – March 2019). Water quality sondes (YSI EXO2 #599502-00; Yellow Springs, OH, USA) were suspended from ropes on the raft and deployed at 1m and 7m below the surface. Each sonde was equipped with four sensors: temperature and conductivity (accuracy ± 0.5%; YSI #599870), pH (accuracy ± 0.1 pH units; YSI #599701), dissolved oxygen (accuracy ± 1%; YSI #599100-01), and total algae PE (precision 0-100 µg L^{-1}; YSI #599103-01). Water temperature (^{o}C), salinity (psu), pH, dissolved oxygen concentration (DO, mg L^{-1}), and chlorophyll concentration (Chl, µg L^{-1}) were recorded as hourly averages of 10-minute samples and radio-transmitted to a database. Sensors were calibrated monthly against NBS pH standards (YSI #3822), air-saturated DI water (DO), and a 0.625 mg L^{-1} Rhodamine FWT red dye solution (Chlorophyll, Kingscote Chemicals, Miamisburg, OH, USA; #106023). The conductivity sensor was calibrated every three months against a 50,000 µS cm^{-1} conductivity standard (YSI #3169). Missing salinity data at 7m, due to a faulty sensor March-May 2016 and January – June 2017, was estimated from the average difference in salinity between the two depths. Specifically, weekly average salinity was calculated as 1m salinity plus an offset of +5.2 psu up to a maximum of 30 psu.

3.3.2. Mussel Collection and Transplantation

Mussels were collected from aquaculture lines at 1m and 7m depth in spring 2016 and 2017 and transplanted to 12 mesh bags (30 cm long x 10 cm diameter, 3 cm$_2$ mesh size) hung between the lines at their respective depths. At each depth, a subset of the "source" mussels in the mesh bag was periodically transplanted to either 'interval cages' for destructive sampling of mussel thread production and tissue mass, or 'continuous cages' for non-destructive monitoring of shell growth and mortality (Figure 3.2). The cages were constructed from HDPE vexar plastic mesh (15 cm x 15 cm x 20 cm, 1 cm$_2$ mesh size). Each of the six replicate cages at each depth housed four individuals of each mussel species. Both the 2016 and 2017 cohorts of *M. trossulus* were approximately 1 year old at the start of the experiment, and initial shell length ranged 55-65 mm. *M. galloprovincialis* cohorts were approximately 1.25 years old and initial shell length ranged 60-70 mm in 2016 and 50-60 mm in 2017. Due to mortality, the supply of source mussels was replenished in the autumn, at least 1 month prior to their use in a cage.

3.3.3. Interval Cages for Mussel Byssus Production and Condition

Every 1-2 months, from June 2016 – February 2016 and July 2017 – February 2017, 24 mussels of each species were transplanted from the mesh bags at each depth into the six replicate 'interval cages' to quantify byssus production and shell growth over two weeks. Each mussel was tethered to an acrylic plate to prevent aggregation (two rectangular 15 cm x 15 cm plates per cage, four mussels of a single species per plate). The plates were stacked vertically 10 cm apart to ensure adequate water circulation throughout the cage, and the placement of each species on the top or bottom was randomized. Each mussel was tethered by attaching a 20 cm nylon thread to the shell with cyanoacrylate glue and tying the two ends of the thread between regularly spaced holes in the plates such that mussels could move in a limited area but would not be lost from the plate (Moeser et al., 2006). After 15 days, the plates and attached mussels were removed from each cage. The adductor muscle of each mussel was severed and the byssal root was dissected from the foot, leaving an intact byssus attached to the plate (Roberts, Chapter 1). Plates with byssal threads were dried and the number of threads produced by each mussel was counted. The remaining gonadal and somatic tissue was dissected from the shell and dried to a constant weight at 60°C. Total tissue mass (TM, g) was calculated as the sum of gonadal (GTM, g) and somatic tissue mass (STM, g) and condition index (CI, g cm$_{-3}$; Crosby and Gale 1990) was calculated as TM divided by shell length cubed. Somatic index (SI, g cm$_{-3}$) was calculated as STM divided by the shell length cubed and gonad index (GI, unitless) was calculated as GTM divided by TM.

3.3.4. Continuous Cages for Mussel Shell Growth and Survival

In spring 2016 and 2017, 24 mussels were transplanted from the mesh bags at each depth into the six ‘continuous cages’ to monitor shell growth and survival. These enclosures were identical to the ‘interval cages’ except that mussels were not tethered to the acrylic plate and were free to move around one of the two sections in the cage. The same mussels were followed continuously; measurements were made quasi-monthly at the end of each two-week deployment of the ‘interval cages’. Shell growth was calculated as the change in shell length, measured with calipers to the nearest 0.1mm. Survival was determined for each species per cage. The interval between these quasi-monthly shell growth and survival measurements ranged 1-2 months and intervals were therefore normalized to a 30 day period. At the end of the experiment for each cohort (June 2017 or July 2018), mussels were collected and dissected to determine final TM, STM, GTM, CI, SI, GI, and shell length, as described above for ‘interval cage’ mussels. When mussels were lost due to mortality, as occurred in autumn of each year, they were replenished and transplanted from the mesh bags at their respective depths.

Somatic tissue growth (SG, g DW) was estimated for each sampling date, normalized to a 30-day period, using interval measurements of mussel STM and CI and continuous measurements of shell length. Specifically, SG was calculated as the difference between final and initial STM, where initial STM (g DW) was estimated as SI (g cm^{-3}) multiplied by the initial shell length cubed (cm^{3}).

3.3.5. Physiological Thresholds of Stress and Food Limitation

The proportion of time at which seawater conditions exceeded known physiological tolerances for mussels was used as an index of stress from temperature, salinity, DO, pH and food limitation over the course of the 5 years of seawater monitoring. Thermal and salinity tolerances are known to differ between *M. trossulus* and *M. galloprovincialis*; a threshold of >18 °C for *M. trossulus* and >25 °C *M. galloprovincialis* was used, based on byssus production measurements of Newcomb (2015) and supported by heart rate measurements with a lower temperature resolution (14°C vs. 21°C, Braby and Somero 2006). A low salinity stress threshold of <14 was used for *M. galloprovincialis* (Freitas et al., 2017) since 14 psu causes cellular damage and stress response in this species, and <7 psu was used for *M. trossulus* (Riisgård et al., 2014).

For the remaining environmental parameters, threshold values for physiological stress were assumed to be similar for the two species. In comparison to many mobile taxa, bivalves are tolerant to long periods of low oxygen; a conservative threshold for hypoxia (<2 mg L^{-1}; Anestis et al., 2010, Jakubowska et al., 2015) was used, even though the effects of hypoxia may be relevant up to 3.5 mg L^{-1} (Steckbauer et al., 2011). A pH of <7.2 (NBS) was used as a conservative low pH stress threshold, based on previous studies of byssus production and strength (George et al., *in press*, O'Donnell et al., 2013), and were close to pH values shown to additively affect mussel clearance rate and respiration in combination with hypoxia (pH 7.3 NBS, DO 2 mg L^{-1}, Gu et al., 2019). Maximal filtration rate per unit food ranges 0.5 – 6.3 ug Chl a L^{-1} for *Mytilus edulis* (Riisgård 1991, Riisgård et al., 2011), but this relationship is less well characterized for *M. trossulus* and *M. galloprovincialis* (but see Maire et al., 2007 for *M. galloprovincialis*). A value of 6 ug Chl a L^{-1} was used to identify periods of food limitation for both species.

Weekly exposure to physiological stressors was calculated for the five-year timeseries period as the proportion of the hours per week at or beyond the specified threshold. To evaluate the potential effect of exposure to physiological stressors on mussel growth and attachment, exposure to each physiological stressor was calculated as the proportion of the hours per interval beyond the specified threshold.

3.3.6. Energetics and Energy Allocation to Byssus

Seasonal and depth estimates of Scope for Growth (SFG) were determined following the general method of Bayne (1976) and Kitchell et al., (1977), with modifications suggested by Sebens et al., (2018) and Roberts (Chapter 2). The average temperature (°C) and chlorophyll (ug L^{-1}) over each growth period was determined over the two-year study period, and these values were used to calculate SFG (see Supplementary Information 3.1 for details). Briefly, clearance rate, relative food availability, and respiration were first estimated from measured temperature and chlorophyll data for each period using relationships from the literature. Second, SFG was determined from clearance rate and respiration and are reported as a monthly index (g month^{-1}) for each species. Model parameters are reported in Table 3.1. Stepwise multiple linear regression (MLR) was used to evaluate the effects of SFG and other physiological stressors (DO, pH, salinity – *M. galloprovincialis* only, and temperature – *M. trossulus* only) on somatic tissue growth, shell growth, and byssal thread production.

3.3.7. Statistical Analysis

All statistical analyses and model calculations were performed with R software for Mac OSX (version 3.4, R Core Team, 2017). Relationships among seawater conditions at each depth were evaluated using Pearson rank correlation coefficients (alpha = 0.001). SFG calculations were run as a function of average temperature and chlorophyll for each growth period (Eq. 5). All variables were log transformed, except for proportion of time of exposure to physiological stressors, which was arcsine sqrt transformed. Model selection for the stepwise MLR used AIC as a metric of comparison.

3.4. Results

Environmental conditions, including temperature, salinity, DO, pH, and chlorophyll, varied seasonally and with depth (Figure 3.3, Figure 3.4, Table 3.2), similar to the previous observations at this site by Newcomb (2015). In general, there was a greater range in temperature near the surface at 1m depth compared to at 7m depth. Seawater was warmer in the summer and colder in the winter at 1m depth than at 7m (Figure 3.3, Figure 3.4, Table 3.2). Salinity varied with season, especially at 1m depth, with the lowest salinities measured in the autumn and winter

(Figure 3.3, Figure 3.4, Table 3.2). pH also varied seasonally and was on average lower at 7m depth; pH was generally lower in the summer and autumn at 7m depth (Figure 3.3, Figure 3.4, Table 3.2). DO was relatively stable and elevated (10 mg L$_{-1}$) at 1m, but more variable at 7m, where the lowest DO levels were observed July – November (Figure 3.3, Figure 3.4, Table 3.2). Chlorophyll was near or above saturating chlorophyll levels (~6 ug L$_{-1}$) all year except for winter, when chlorophyll was often below threshold levels for maximal consumption rates (<0.5 ug L$_{-1}$; Figure 3.3, Table 3.2).

Temperatures frequently exceeded the thermal physiological stress threshold for *Mytilus trossulus* (>18°C) at 1m depth in the late summer and autumn each year (Figure 3.4, Figure 3.5), but never exceeded the *Mytilus galloprovincialis* threshold. Salinity often dropped below the physiological threshold for *M. galloprovincialis* (14 psu) at 1m depth in the autumn, winter, and spring but did not exceed the *M. trossulus* threshold (Figure 3.4, Figure 3.5). Physiological stress thresholds of low DO and pH were exceeded primarily at 7m depth (Figure 3.4, Figure 3.5). Hypoxia (<2 mg L$_{-1}$) and low pH (<7.2) at 7m depth were most prevalent during the summer and autumn each year, and were temporally correlated (Figure 3.4, Figure 3.5). Hypoxic conditions lasted up to 20 hours per day. Chlorophyll was below saturating levels (< 6 ug L$_{-1}$) in the winter (Figure 3.4, Figure 3.5).

For *M. trossulus*, there was a significant interaction between the effects of season and depth on byssal thread production (Figure 3.6; p = 0.03, Table S3.1, Table S3.2), and in the summer, thread production was 50% greater at the surface than at depth. For *M. galloprovincialis*, there was only an effect of season on thread production, and thread production was 70% greater in the summer than in the winter (Figure 3.6; p = 0.02, Table S3.1, Table S3.2).

Shell growth for both species was variable at 1m, but shell growth at 7m depth remained below <0.25 cm month^{-1} for both species, regardless of season (Figure 3.7). Two major *M. trossulus* mortality events were observed at 7m in October 2016 and October 2017, in which 60% and 30% of experimental mussels died in the 'continuous' cages. A third mortality event occurred at 1m depth in June 2016, with a monthly mortality of 35*%. M. galloprovincialis* had <20% monthly mortality across periods sampled (Figure 3.7).

At the end of each experimental period, GI was 0.2 – 1 times greater at 1m compared to 7m and was typically lowest in summer for both cohorts and both species (Figure 3.8, Table S3.3 and S3.4). CI of both species generally decreased throughout the experimental period (Figure 3.8) and. At the end of the experimental period, there was an interaction between the effects of cohort year and depth on final CI (Table S3.3), and CI was greater at 1m compared to 7m for the first cohort and not the second cohort for both species (Table S3.3). TM remained relatively constant for both species in 2017-2018, although TM was variable in 2016-2017 for *M. galloprovincialis* (Figure 3.8). Shell length increased over the duration of the experiment for both species and cohorts (~30 – 50%, Figure 3.8).

A multiple stepwise regression was run to predict either somatic tissue growth, shell growth, or byssal thread production from SFG and exposure to hypoxia, low pH, and either acute temperature – *M. trossulus* or low salinity – *M. galloprovincialis*. For *M. trossulus* somatic tissue growth, a significant regression was found (F(2, 21) = 11.95, p = 0.003 , Adj-R^2 = 0.49), and both low pH and SFG were significant predictors of somatic tissue growth (p < 0.001, p = 0.001, respectively, Table 3.3). For shell growth of this species, the best regression model (F(3, 19) = 4.03, p = 0.02 , Adj-R^2 = 0.29) included acute temperature stress exposure as a non-significant predictor (p = 0.21, Table 3.3) and SFG and low DO as significant predictors of shell growth (p

= 0.21, p = 0.013, p = 0.031, Table 3.3). For byssal thread production of this species, no significant regression was found (F(1, 22) = 2.66, p = 0.12 , Adj-R_2 = 0.07), and the best model included a marginal effect of low pH exposure (p = 0.12).

For *M. galloprovincialis* somatic tissue growth, a significant regression was found (F(2, 21) = 8.30 , p = 0.002 , Adj-R_2 = 0.39), and both low pH and SFG were significant predictors of somatic tissue growth. For shell growth of this species, the best model was only a marginal regression (F(2, 21) = 3.36, p = 0.05, Adj-R_2 = 0.17) and the effects of SFG and low DO were significant (p = 0.02, p = 0.05, Table 3.3). For byssal thread production of this species, no significant regression was found (F(1, 22) = 4.06, p = 0.06 , Adj-R_2 = 0.12), and the best model included a marginal effect of low salinity exposure on byssal thread production (p = 0.05).

There was a significant interaction between the effects of SFG and depth on somatic tissue growth in both species (*M. trossulus* p = 0.04, *M. galloprovincialis* p = 0.046, Table S3.3) and thread production did not significantly correlate with SFG or depth for either species (range p = 0.14 to p = 0.80, Table S3.3).

3.5. Discussion

Our two-year field experiment with two congeneric mussel species indicated that energetic scope affected somatic tissue and shell growth but not byssal thread production. This field observation is consistent with previous reports of limited growth, but not byssal thread production, in conditions of energetic limitation (Roberts Chapter 1, 2, Clarke 1999), and supports the concept that constitutive production of byssal threads is a fitness strategy that minimizes the risk of dislodgement, and thus maximizes fitness, at a cost to growth and reproduction (Sebens et al., 2018, Roberts Chapter 2).

Estimates of energetic scope provided a valuable baseline from which to evaluate other physiological stressors, besides energetic limitation, that might affect growth and byssal thread production, either directly or indirectly by affecting energetic scope (Figure 3.1). Either low pH or low DO, two physiological stressors that were highly temporally correlated at 7m depth, decreased somatic tissue growth and shell growth in both species of mussels (although the effect on *M. galloprovincialis* was marginal). This negative effect of either low pH or low DO on tissue growth and shell growth is consistent with other studies showing effects of low pH and hypoxia on mussel clearance rate and growth of *M. edulis* (Sanders et al., 2014 and Gu et al., 2019). Other SFG experiments that included additional physiological stressors have shown that low pH and DO levels decrease SFG by over 90% for *M. edulis* (Gu et al., 2019, Sanders et al., 2014).

In contrast, there was no significant effect of SFG and only a marginal effect of physiological stressors on byssal thread production (*M. trossulus* – low pH, *M. galloprovincialis* – low salinity). These results support the idea that physiological stressors, including low pH and low DO, may have the largest effects on responses that are energetically-constrained (e.g. growth) rather than constitutive (e.g. byssal thread production). Physiological stressors can affect energetic intake and increase costs (e.g. reduced feeding, changes in metabolic rate or nitrogen excretion rate; Sanders et al., 2014, Gu et al., 2019), and byssal thread production and other constitutive (or ‘flexible’ traits, sensu Jørgensen et al., 2016) might be affected by physiological stressors that affect key energetic functions, at the extremes. Further work should evaluate the effects of physiological stressors on other such energetically ‘flexible’ traits in comparison to traits that are energetically-constrained, since physiological stressors may have less of an effect on ‘flexible’ traits, than on those that are energetically constrained. Whereas decreased tissue mass might provide an advantage in warmer, hypoxic or high CO_2 conditions by lowering tissue maintenance costs (Sebens 2002), there may be an adaptive advantage to producing byssal threads that are needed for survival, despite the presence of other physiological stressors (Sebens et al., 2018).

Hypoxia (< 2 mg L_{-1}) and low pH (<7.2 NBS) conditions were prevalent at 7m depth, occurring ~25% of the week in late summer-early autumn each year. Observations of low dissolved oxygen are consistent with reports of hypoxia ranging 170-270 days out of the year in Penn Cove, and other shallow bays throughout Puget Sound (Ahmed et al., 2019, Newcomb 2015, George et al, *in press*). DO and pH were tightly correlated (Figure 3.4), and often co-occurred with high energetic scope (Figure 3.3), suggesting that the effects of these physiological stressors and energetic limitation were not observed concurrently in this location.

One common mussel aquaculture practice is mussel thinning, where mussels that grow into dense aggregations are transferred to new aquaculture line in lower densities to improve access to available food. This practice of transferring mussels involves severing the byssus, which induces mussels to produce new threads to reattach to the new line (e.g. Roberts Chapter 2) Our findings suggest that byssus will be produced regardless of whether there is an energetic surplus or deficit. For example, SFG for both species ranged from positive (energetic surplus) to negative (energetic deficit), yet byssal thread production after transplantation averaged 42-44 threads per mussel. We estimated energy allocation to thread production ranged from 12-3% of the energetic surplus to 8-2% of the energetic deficit, for *M. trossulus* and *M. galloprovincialis* respectively, which is consistent with energy allocation to thread production even in conditions of energetic deficit (Hawkins and Bayne 1985). These results are consistent with high byssal thread production even under food and temperature conditions where *M. trossulus* survival is low (Roberts Chapter 1). What is less clear from this research, and merits further study, is whether in conditions of energetic deficit, the additional energetic cost from the induction of byssal thread production causes increased tissue loss and mortality.

We observed >30% mortality of *M. trossulus* (but not *M. galloprovincialis*) between September and October at 7m depth in both years, associated with increases in the physiological stressors of low pH and hypoxia (>25% of the week) and was observed more broadly throughout the farm (Ian Jeffords, Personal Communication). There is a dearth of information comparing lethal effects of hypoxia and low pH on *M. trossulus* and *M. galloprovincialis*. *M. galloprovincialis* and the congeneric species, *M. edulis*, however, can survive over two weeks in hypoxic conditions (*M. galloprovincialis* – de Zwaan et al. 1981, Jørgensen 1980, Theede et al., 1969, Von Oertzen and Schlungbaum 1972). A better understanding of lethal effects of hypoxia, and concurrent low pH, on *M. trossulus* may contribute to understanding of the sensitivity to hypoxia and low pH of this species (Sokovlova 2013).

There was no effect of energetic limitation, and only a marginal effect of physiological stressors on byssal thread production of either species. Despite this lack of evidence for environmental drivers, there was a significant effect of both season and depth on byssus production for *M. trossulus*, and an effect of season on byssus production of *M. galloprovincialis* (Table S3.2). This unexplained variability suggests that other factors, beyond the scope of the study, might influence production of threads of this species. In natural environments wave action can induce production of new byssal threads (Van Winkle 1970, Dolmer and Svane 1994, Lee et al., 1990, Moeser et al., 2006, Carrington et al., 2008), and greater mechanical movement of mussels from seawater currents or storms could increase byssal thread production. Although mussels are “sessile” animals, individuals can move within mussel beds using byssal thread production to do this, and effectively change their microenvironment (e.g. Schneider et al., 2005), but little is known about cues that cause mussels to move and how these cues might vary seasonally.

Previous work has identified a trade-off between reproduction and the strength of mussel attachment (Zardi et al., 2007, Carrington 2009, Moeser et al., 2006). Mussel spawning, evidenced by an abrupt decrease in GI, was observed May – June for *M. trossulus*, and between February and the following summer for *M. galloprovincialis*. This pattern is consistent with reported spawning phenology of these two species (*M. trossulus* – spring through fall, *M. galloprovincialis* – winter through early spring, *M. trossulus* but formerly called *M. edulis* - Skidmore 1983, Strathmann 1987**,** Curiel-Ramirez and Caceres-Martinez 2004). While the aim of this study was not to evaluate trade-offs between reproduction and byssal thread production, we did note a significant relationship between byssal thread production and GI for *M. trossulus.* This relationship was not observed for *M. galloprovincialis*, however, perhaps because our sampling schedule for byssal thread production did not adequately include the months just after spawning of this species (data not shown).

While energetic limitation from external seawater conditions may not significantly affect byssal thread production, a model of fitness trade-offs suggests that short term investment in reproduction at the cost of producing fewer byssal threads does occur and may be evolutionarily adaptive (Carrington et al., 2015). For example, if few mussels ever survive to spawn in the next season, it may be adaptive not to invest in byssus and to put all available energy into reproduction. We made the assumption that SFG was allocated to both somatic and gonad tissue growth each month (Figure 3.1A, Supplementary Information 3.1). To avoid misinterpreting a loss of gametes during spawning as decreased monthly growth, however, we evaluated the correlation between SFG index and growth of only somatic tissue, rather than growth of total tissue (Figure 3.9). Although shell length increased over the course of each year-long experiment, total tissue mass did not substantially increase, suggesting mussels were near their optimum (asymptotic) mass for that location, and additional surplus would go to reproduction rather than to somatic tissue growth (Sebens 2002). Differences in gonad tissue at the end of the experiment could indicate differences in cumulative scope for growth. At the end of each year-long experimental period, we observed greater GI at 1m compared to 7m for both species. This result might be explained by cumulative effects of physiological stressors at depth, since physiological stress of hypoxia and low pH can decrease mussel SFG (*M. edulis* – Sanders et al., 2014, Gau et al., 2019). DEB theory makes the assumption that energy is allocated evenly to somatic and reproductive tissue growth, unless proven otherwise (Kooijman 2010). This assumption merits further review, however, for adult mussels, since mussels that are not gaining somatic tissue mass and are at their asymptotic size (energetically optimal size, EOS, Sebens 2002), these outputs are likely de-coupled. This de-coupling may be masked by assuming that shell and tissue growth are coupled (Kooijman 2010), which previous work has demonstrated is not the case (Hilbish 1986).

The model used in this paper is adapted from the Scope for Growth model by Roberts (Chapter 2). A sensitivity analysis demonstrated that much of the error in the SFG calculation was associated with uncertainty in respiration rate measurements (Roberts Chapter 2). Additional uncertainty may be introduced by the temperature responses of feeding and respiration, additional assumptions for ingestion, and the effects of seasonality. Considering these limitations, the SFG index may be considered a useful index of the relative energetic scope as a function of temperature and food, rather than a precise absolute value with well-characterized uncertainty. A positive correlation between this index and tissue growth not only supports the idea that tissue growth is energetically constrained, but also demonstrates that a proportion of variance in tissue growth is explained by model assumptions. Our model compares well to previous scope for growth models. We made the empirical assumption that feeding saturated at 6 ug L$_{-1}$ (Riisgård et al., 2011). Other models have estimated a range of half saturation coefficients from growth data that suggest a similar saturating value depending on the site (half-saturation, 1-4 ug L$_{-1}$, depending on the site; Rosland et al., 2009, Filgueira et al., 2011). More complex energetics models can include the effect of suspended sediment on intake (Grant and Bacher 1998). Detritus can be an additional source of carbon for mussels (up to 330mg L$_{-1}$, Widdows et al., 1979, Foster-Smith 1975) and while large loads of inorganic matter can increase sorting costs and decrease ingestion, suspended sediment can increase mussel feeding rate (Kiørboe et al., 1981). We made the assumption that the effect of suspended sediment on carbon ingestion was negligible, but future work could incorporate this component.

In summary, we found that both energetic scope and the proportion of time beyond a threshold of physiological stress ($pH < 7.2$ NBS and $DO < 2$ mg L$_{-1}$) were correlated with lower tissue growth of both species, and that mortality of *M. trossulus* was also associated with a period of physiological stress. These results suggest that energetic constraints, and covarying hypoxia and pH at depth, may limit mussel growth but not byssal thread production. Mussel attachment strength depends on both the quality and quantity of byssal threads produced (Bell and Gosline 1997). While there was no effect of environmental conditions on byssal thread quantity, these conditions could affect quality of threads produced either by directly affecting the material after it is produced (pH, DO, George et al., 2018), or potentially by affecting organism energetics (temperature - Newcomb 2015, George et al., 2018) though this remains an open question. Dynamics in byssal thread quality can affect attachment in the field (Carrington 2009, Newcomb 2015, Zardi et al., 2007) and thus survival probability of mussels.

Adjustment of aquaculture practices to manage the effects of energetic limitation and other physiological stressors is a pressing concern. Hypoxic events have increased over the past 20 years in the Salish Sea, and biogeochemical models by Kangaonkar et al., (2018) have shown that approximately half of the duration of hypoxia exposure at Penn Cove is due to land-based nitrogen input (20 out of 50 days). Real time sensing data may provide an 'early warning system' for mussel farmers, but pH and DO data are variable. We present a method of assessing physiological stress that could be used as an early warning indicator; physiological stress was interpreted using thresholds and physiological stress exposure ranged from 0-25% of a week beyond these thresholds. EPA water quality standards where exposure beyond a threshold (<2 mg L_{-1} DO) on any particular day deems that day "low water quality," could be used as well (Ahmed et al., 2019). Alternatively, maintaining sensitive species of mussels, in this case *M. trossulus*, on shorter lines through months that experience sustained hypoxia (e.g. September) might be an effective management practice to limit exposure. Respiration of mussels can have a large effect on seawater DO and pH (Jørgensen 1980) and within the microenvironment of aquaculture lines (George et al., *in press*). Mussel mortality could further exacerbate physiologically stressful conditions (Jørgensen 1980). Management practices that minimize mortality may limit the effect that mussels may have on local oxygen and pH levels.

Although there is a large range in physiological stress in surface water (e.g. top ~10m) in coastal systems, only more modern models of Salish Sea oceanography allow for predictions of seawater in coastal areas and with greater depth resolution near the surface (Khangaonkar et al., 2019, Ahmed et al., 2019). Identification of timing and extent of hypoxia and low pH from these models could directly benefit aquaculture practitioners and help them make sound management decisions, especially in predicting or observing hypoxic and low pH conditions. Identification of relationship between climate indices and mussel health may allow for longer-term (e.g. 3 month) advance management planning. In the short term, a greater capacity to adjust farming practices to natural variability in seawater conditions may minimize losses in mussel farm yields. In the long term, this capacity to adjust management practices to variable seawater conditions may also contribute to a greater capacity to adjust to expected more severe changes in future seawater conditions caused by continuing anthropogenic ocean warming, acidification, and deoxygenation (Ostrom 2009, Bopp et al., 2013).

3.6. Literature Cited

Ahmed, A., C. Figueroa-Kaminsky, J. Gala, T. Mohamedali, G. Pelletier, and S. McCarthy. 2019. Puget Sound nutrient source reduction project volume 1: Model updates and bounding scenarios. Washington State Department of Ecology, January 2019, Publication No. 19-03-001. https://fortress.wa.gov/ecy/publications/documents/1903001.pdf

Anestis, A., H. O. Pörtner, and B. Michaelidis. 2010. Anaerobic metabolic patterns related to stress responses in hypoxia exposed mussels *Mytilus galloprovincialis*. J. Exp. Mar. Biol. Ecol. 394: 123-133.

Babarro, J. M. F., M. J. Fernández Reiriz, and U. Labarta. 2008. Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: the influence of size and food availability. J. Mar. Biol. Assoc. U. K. 88: 783-791.

Babarro, J. M., and M. J. Reiriz. 2010. Secretion of byssal threads in *Mytilus galloprovincialis*: quantitative and qualitative values after spawning stress. J. Comp. Physiol. B, 180: 95-104.

Bayne, B. L. 2004. Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve molluscs. Integr. Comp. Biol. 44: 425-432.

Bayne, B.L. and Newell, R.C., 1983. Physiological energetics of marine molluscs. In: Wilbur K.M., Salenddin A.S.M. (eds.) *The 135Mollusca,* Vol 4. Physiology, Part 1. pp. 407-515. Academic Press.

Bayne, B. L., R. J. Thompson, and J. Widdows. 1976. Physology 1. In B.L. Bayne (Ed.) Marine mussels: their ecology and physiology (Vol. 10). Cambridge, MA: Cambridge University Press.

Beiras, R., A. P. Camacho, and M. Albentosa. 1994. Comparison of the scope for growth with the growth performance of *Ostrea edulis* seed reared at different food concentrations in an open-flow system. Mar. Biol. 119: 227-233.

Bell, E. C., and J. M. Gosline. 1997. Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. Mar. Ecol. Prog. Ser. 159: 197-208

Bopp, L. and others 2013. Multiple stressors of ocean ecosystems in the 21$_{st}$ century: projections with CMIP5 models. Biogeosciences. 10: 6225-6245.

Braby, C. E., and G. N. Somero. 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J. Exp. Biol. 209: 2554-2566.

Carrington, E., G. M. Moeser, S. B. Thompson, L. C. Coutts, and C. A. Craig. 2008. Mussel attachment on rocky shores: the effect of flow on byssus production. Integr. Comp. Biol. 48: 801-807.

Carrington, E., G.M. Moeser, J. Dimond, J.J. Mello, and M.L. Boller, 2009. Seasonal disturbance to mussel beds: field test of a mechanistic model predicting wave dislodgment. Limnol. Oceanogr. 54:978-986.

Carrington, E., J. H. Waite, G. Sarà, and K. P. Sebens. 2015. Mussels as a model system for integrative ecomechanics. Ann. Rev. Mar. Sci. 7: 443-469.

Clarke, M., 1999. The effect of food availability on byssogenesis by the zebra mussel (*Dreissena polymorpha* Pallas). J. Molluscan Stud. 65:327-333.

Curiel-Ramirez, S. and Caceres-Martinez, J., 2004. Reproductive cycle of coexisting mussels, *Mytilus californianus* and *Mytilus galloprovincialis*, in Baja California, New Mexico. J. Shellfish. Res. 23:515-521.

Dekinga, A., M. W. Dietz, A. Koolhaas, and T. Piersma. 2001. Time course and reversibility of changes in the gizzards of red knots alternately eating hard and soft food. J. Exp. Biol. 204: 2167-2173.

de Zwaan, A., D. Holwerda, and P. Veenhof. 1981. Anaerobic malate metabolism in mitochondria of the sea mussel *Mytilus edulis* L. Mar. Biol. Lett. 2:131-140.

Dolmer, P. and Svane, I., 1994. Attachment and orientation of *Mytilus edulis* L. in flowing water. Ophelia. 40: 63-74.

Freitas, R., L. De Marchi, M. Bastos, A. Moreira, C. Velez, S. Chiesa, F.J. Wrona, E. Figueira, and A.M. Soares. 2017. Effects of seawater acidification and salinity alterations on metabolic, osmoregulation and oxidative stress markers in *Mytilus galloprovincialis*. Ecol. Indic. 79: 54-62.

Filgueira, R., R. Rosland, and J. Grant. 2011. A comparison of scope for growth (SFG) and dynamic energy budget (DEB) models applied to the blue mussel (*Mytilus edulis*). J. Sea Res. 66: 403-410.

Fly, E. K., and T. J. Hilbish. 2013. Physiological energetics and biogeographic range limits of three congeneric mussel species. Oecologia. 172: 35-46.

Folt, C. L., C. Y. Chen, M. V. Moore, and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44: 864-877.

Foster-Smith, R. 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J. Exp. Mar. Biol. Ecol. 17: 1-22.

George, M. N., B. Pedigo, and E. Carrington. 2018. Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. J. R. Soc. Interface. 15.

George, M.N., J. Andino, J. Huie, and E. Carrington. 2019. Microscale pH and dissolved oxygen fluctuations within mussel aggregations and their implications for mussel attachment and raft aquaculture. J. Shellfish. Res. *In press*.

Grant, J., and C. Bacher. 1998. Comparative models of mussel bioenergetics and their validation at field culture sites. J. Exp. Mar. Biol. Ecol. 219: 21-44.

Gu, H., Y. Shang, J. Clements, S. Dupont, T. Wang, S. Wei, X. Wang, J. Chen, W. Huang, M. Hu, and Y. Wang. 2019. Hypoxia aggravates the effects of ocean acidification on the physiological energetics of the blue mussel *Mytilus edulis*. Mar. Pollut. Bull. 149: 110538.

Gunderson, A. R., E. J. Armstrong, and J. H. Stillman. 2016. Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. Annu. Rev. Mar. Sci. 8: 357-378.

Hawkins, A. J. S., and B. L. Bayne. 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. Mar. Ecol. Prog. Ser. 25: 181-188.

Hilbish, T. J. 1986. Growth trajectories of shell and soft tissue in bivalves: Seasonal variation in *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 96: 103-113.

Hilbish, T. J., and R. K. Koehn. 1985. The physiological basis of natural selection at the LAP locus. Evolution. 39: 1302-1317.

Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. Proc. Natl. Acad. Sci. U.S.A. 106: 19659-19665.

IPCC, 2019: Summary for Policymakers. In: IPCC Special Report on the Ocean and Cryosphere in a Changing Climate [H.- O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, M. Nicolai, A. Okem, J. Petzold, B. Rama, N. Weyer (eds.)]. *In press*.

Jakubowska, M. and Normant, M., 2015. Metabolic rate and activity of blue mussel *Mytilus edulis I* under short-term exposure to carbon dioxide-induced water acidification and oxygen deficiency. Mar. Freshwater Behav. Physiol. 48: 25-39.

Jones, H. D., O. G. Richards, and T. A. Southern. 1992. Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 155: 213-237.

Jørgensen, C., K. Enberg, and M. Mangel. 2016. Modelling and interpreting fish bioenergetics: a role for behaviour, life-history traits and survival trade-offs. J. Fish. Biol. 88: 389-402.

Jørgensen, B. B. 1980. Seasonal oxygen depletion in the bottom waters of a Danish fjord and its effect on the benthic community. Oikos. 34: 68.

Keeling, R. E., A. Kortzinger, and N. Gruber. 2010. Ocean deoxygenation in a warming world. Ann. Rev. Mar. Sci. 2: 199-229.

Khangaonkar T., Nugraha A., Xu W., and K. Balaguru. 2019. Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. J. Geophys. Res. 124: 3876-3904.

Kiørboe T, Møhlenberg F, and O. Nøhr. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis*. Mar. Biol. 61: 283-8.

Kitchell, J. F., D. J. Stewart, and D. Weininger. 1977. Applications of a Bioenergetics Model to Yellow Perch (*Perca flavescens*) and Walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board. Can. 34: 1922-1935.

Kooijman, S.A.L.M. (2010). *Dynamic energy budget theory for metabolic organization*. Cambridge University Press.

Kraus, R. T., D. H. Secor, and R. L. Wingate. 2015. Testing the thermal-niche oxygen-squeeze hypothesis for estuarine striped bass. Environ. Biol. Fishes. 98: 2083-2092.

Kroeker, K. J., F. Micheli, M. C. Gambi, and T. R. Martz. 2011. Divergent ecosystem responses within a benthic marine community to ocean acidification. Proc. Natl. Acad. Sci. U. S. A. 108: 14515-14520.

Lee, C. Y., S. S. L. Lim, and M. D. Owen. 1990. The rate and strength of byssal reattachment by blue mussels (*Mytilus edulis* L.). Can. J. Zool. 68: 2005-2009.

Maire, O., J.-M. Amouroux, J.-C. Duchêne, and A. Grémare. 2007. Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. Mar. Biol. 152: 1293-1307.

Moeser, G.M. and E. Carrington. 2006. Seasonal variation in mussel byssal thread mechanics. J. Exp. Biol. 209: 1996-2003.

Moeser, G. M., H. Leba, and E. Carrington. 2006. Seasonal influence of wave action on thread production in *Mytilus edulis.* J. Exp. Biol. 209: 881-890.

Newcomb, L.A., 2015. Elevated temperature and ocean acidification alter mechanics of mussel attachment. PhD Thesis. University of Washington.

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

Ostrom, E. 2009. A General Framework for Analyzing Sustainability of Social-Ecological Systems. Science. 325: 419-422.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Riisgård, H.U., 1991. Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus, 1758: dependence on algal concentration. J. Shellfish. Res. 10: 29-36.

Riisgård, H. U., P. P. Egede, and I. Barreiro Saavedra. 2011. Feeding behaviour of the mussel, *Mytilus edulis*: new observations, with a minireview of current knowledge. J. Mar. Biol. 2011: 1-13.

Riisgård, H. U., P. S. Larsen, R. Turja, and K. Lundgreen. 2014. Dwarfism of blue mussels in the low saline Baltic Sea—growth to the lower salinity limit. Mar. Ecol. Prog. Ser. 517: 181-192.

Riisgård, H.U. and Randløv, A., 1981. Energy budget, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Mar. Biol. 61: 227-234.

Robertson, M. P., C. I. Peter, M. H. Villet, and B. S. Ripley. 2003. Comparing models for predicting species' potential distributions: a case study using correlative and mechanistic predictive modelling techniques. Ecol. Modell. 164: 153-167.

Rosland, R., Ø. Strand, M. Alunno-Bruscia, C. Bacher, and T. Strohmeier. 2009. Applying Dynamic Energy Budget (DEB) theory to simulate growth and bio-energetics of blue mussels under low seston conditions. J. Sea Res. 62: 49-61.

Sanders, T., S. Widdicombe, R. Calder-Potts, and J. I. Spicer. 2014. Environmental hypoxia but not minor shell damage affects scope for growth and body condition in the blue mussel *Mytilus edulis* (L.). Mar. Environ. Res. 95: 74-80.

Sarà, M. 1986. Sessile macrofauna and marine ecosystem. Ital. J. Zool. 53: 329-337.

Sarà, G., Palmeri, V., Montalto, V., Rinaldi, A. and Widdows, J., 2013. Parameterisation of bivalve functional traits for mechanistic eco-physiological dynamic energy budget (DEB) models. Mar. Ecol. Prog. Ser. 480: 99-117.

Schneider, K.R., Wethey, D.S., Helmuth, B.S.T. and Hilbish, T.J., 2005. Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. Mar. Biol. 146: 333-343.

Schulte, P.M., Healy, T.M. and N.A. Fangue. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integ. Comp. Biol. 51: 691-702.

Sebens, K. P. 2002. Energetic constraints, size gradients, and size limits in benthic marine invertebrates. Integr. Comp. Biol. 42: 853-861.

Sebens, K. P., G. Sarà, and E. Carrington. 2018. Estimation of fitness from energetics and life-history data: An example using mussels. Ecol. Evol. 8: 5279-5290.

Secor, S. M. 2008. Digestive physiology of the Burmese python: broad regulation of integrated performance. J. Exp. Biol. 211: 3767-3774.

Seibel, B. A., A. E. Maas, and H. M. Dierssen. 2012. Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. PLoS One, 7: e30464.

Skidmore, D., 1983. Settlement, growth, and survival of *Mytilus edulis* L. in Puget Sound and Assessment of *Mytilus Californianus* for Aquaculture. Thesis, M.S., University of Washington.

Soberon, J., and M. Nakamura. 2009. Niches and distributional areas: Concepts, methods, and assumptions. Proc. Natl. Acad. Sci. U.S.A. 106: 19644-19650.

Sokolova, I. M. 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. 53: 597-608.

Steckbauer, A., C. M. Duarte, J. Carstensen, R. Vaquer-Sunyer, and D. J. Conley. 2011. Ecosystem impacts of hypoxia: thresholds of hypoxia and pathways to recovery. Environ. Res. Lett. 6: 025003.

Stephenson, N. 1998. Actual evapotranspiration and deficit: biologically meaningful correlates of vegetation distribution across spatial scales. J. Biogeogr. 25: 855-870.

Strand, E., C. Jørgensen, and G. Huse. 2005. Modelling buoyancy regulation in fishes with swimbladders: bioenergetics and behaviour. Ecol. Modell. 185: 309-327.

Strathmann, M. F. (1987). Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae. University of Washington Press.

Sutherland, D. A., P. MacCready, N. S. Banas, and L. F. Smedstad. 2011. A Model Study of the Salish Sea Estuarine Circulation. Journal of Physical Oceanography, 41: 1125-1143.

Tang, M., and D. Boisclair. 1995. Relationship between respiration rate of juvenile brook trout (Salvelinus fontinalis), water temperature, and swimming characteristics. Can. J. Fish. Aquat.Sci. 52: 2138-2145.

Theede, H., A. Ponat, K. Hiroki, and C. Schlieper, 1969. Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. Mar. Biol. 2: 325-337.

van der Veer, H. W., J. F. M. F. Cardoso, and J. van der Meer. 2006. The estimation of DEB parameters for various Northeast Atlantic bivalve species. J. Sea Res. 56: 107-124.

Von Oertzen, J., and G. Schlungbaum. 1972. Experimentell-ökologische Untersuchungen über O2-Mangel und H2S-Resistenz an marinen Evertebraten der westlichen Ostsee. Beiträge zur Meereskunde 29: 79-91.

Van Winkle, W. 1970. Effect of environmental factors on byssal thread formation. Mar. Biol. 7: 143-148.

Widdows, J., P. Fieth, and C. Worrall. 1979. Relationships between seston, available food and feeding activity in the common mussel Mytilus edulis. Mar. Biol. 50: 195-207.

Widdows, J., Hawkins, A. J. S. 1989. Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. Physiol. Zool. 62: 764-785.

Zardi, G.I., McQuaid, C.D. and Nicastro, K.R., 2007. Balancing survival and reproduction: seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. Mar. Ecol. Prog. Ser. 334:155-163.

3.7. Tables and Figures

Table 3.1. Summary of parameter calculations for the Scope for Growth model, adapted from Roberts Chapter 2. The model had five input parameters, each estimated separately for *M. trossulus* and *M. galloprovincialis* using constants obtained from this and previously published studies. SFG parameter values were derived from the relationship of both respiration and clearance rate with temperature.

Parameter		**Unit**	**Spp.**	**Crit.**	**Slope**	**Int.**	**Equation**	**Source**
Input Parameter								
a'(T)	Intake coefficient	J (d f g^{d})$^{-1}$	Tross	<10°C	8.81	-23.3		
				>10°C	-2.69	83.1		
			Gallo		5.45	7.25		
b(T)	Cost coefficient	J d^{-1} g^{-d}	Tross		7.97	-4.2		
			Gallo		8.99	-16.6		
d	Intake exponent	unitless	Both		0.69	0.01		Jones et al., 1992 *M. edulis*
e	Cost exponent	unitless	Both		1			Van der Veer 2006
C.F.	Energetic conversion factor	J mgDW^{-1}	Both		21.6	1.6		Roberts, Ch. 2
Measured values used to calculate input parameters								
R	Respiration	mlO2 hr^{-1}	Tross		0.0078	-0.004	R = slope × temp + int	Fly and Hilbish 2013 0.469 g DW
			Gallo		0.0088	-0.016		
CR	Clearance rate	ml min^{-1}	Tross	<10°C	0.6801	-1.796	CR = slope × temp + int	
				>10°C	-0.2074	6.413		
			Gallo		0.426	0.5667		

Table 3.2. Summary of seawater temperature (°C), salinity (psu), pH (NBS), dissolved oxygen (mg L$_{-1}$), and chlorophyll concentration (ug L$_{-1}$) at Penn Cove from 2014 – 2019. Reported values are seasonal mean ± SD of hourly measurements at each depth.

Season	**Depth**	**T (^{o}C)**	**n**	**Sal**	**n**	**pH (NBS)**	**n**	**O$_2$ (mg L$_{-1}$)**	**n**	**Chl (µg L$_{-1}$)**	**n**
Spring	1m	12.3 ± 1.9	8519	21.8 ± 3.4	8209	8.10 ± 0.26	8519	11.4 ± 1.8	8519	6 ± 19	7403
	7m	10.4 ± 1.1	6829	26.4 ± 2.0	5299	7.75 ± 0.33	8509	8.7 ± 3.1	8509	3 ± 12	7013
Summer	1m	15.1 ± 1.6	9287	26.1 ± 2.5	8755	8.04 ± 0.24	8068	10.3 ± 2.0	8408	11 ± 3	7363
	7m	12.3 ± 1.3	8912	28.5 ± 1.1	8422	7.64 ± 0.31	8912	6.7 ± 3.1	8912	11 ± 20	7704
Autumn	1m	10.8 ± 2.8	9539	21.1 ± 5.3	9538	7.79 ± 0.24	9538	10.0 ± 1.5	9539	2 ± 6	6497
	7m	11.5 ± 1.5	9506	26.1 ± 3.3	9166	7.60 ± 0.22	9506	6.7 ± 2.6	9506	5 ± 12	8201
Winter	1m	7.3 ± 1.6	9777	19.3 ± 4.4	9775	7.80 ± 0.23	8251	11.2 ± 1.6	9777	1 ± 3	7235
	7m	8.8 ± 1.2	9677	25.0 ± 3.0	8782	7.66 ± 0.16	8249	8.3 ± 2.3	9775	2 ± 8	8490

Table 3.3. Summary of multiple regression analysis of the effects of SFG and other physiological stressors on tissue growth (g DW month^{-1}), shell growth (cm month^{-1}), and thread production over two weeks (# mussel^{-1}) for both cohorts of *M. trossulus* and *M. galloprovincialis* at 1m and 7m depth. Thresholds for pH and DO were the same for both species, but thresholds for temperature and salinity were species-specific (Temp >18 °C for *M. trossulus*; Sal <14 psu for *M. galloprovincialis*). Significant effects are indicated in bold.

Species	Response	Effect	Estimate	SE	Sum Sq	Df	F val.	p	Adj -R^2
M. trossulus									
	Somatic tissue growth (g/month)	Intercept	0.022	0.010	0.012	1	4.9	0.038	0.49
		pH	-0.056	0.013	0.049	1	19.8	**<0.001**	
		SFG	0.053	0.013	0.044	1	17.8	**0.001**	
		Residuals			0.052	21			
	Shell growth (cm/month)	Intercept	0.114	0.014	0.298	1	63.1	<0.001	0.29
		Temp	0.019	0.015	0.008	1	1.7	0.211	
		SFG	0.050	0.018	0.036	1	7.6	**0.013**	
		DO	-0.043	0.018	0.026	1	5.4	**0.031**	
		Residuals			0.090	19			
	Byssal thread production (#/mussel)	Intercept	45	2	48050	1	373.6	<0.001	0.07
		pH	-4	2	342	1	2.7	0.12	
		Residuals			2830	22			
M. galloprovincialis									
	Somatic tissue growth (g/month)	Intercept	0.057	0.041	0.079	1	2.0	0.17	0.39
		SFG	0.207	0.052	0.630	1	15.9	**0.001**	
		DO	-0.159	0.052	0.373	1	9.4	**0.01**	
		Residuals			0.832	21			
	Shell growth (cm/month)	Intercept	0.182	0.018	0.793	1	98.5	<0.001	0.17
		SFG	0.061	0.024	0.052	1	6.4	**0.020**	
		pH	-0.049	0.024	0.033	1	4.1	0.055	
		Residuals			0.169	21			
	Byssal thread production (#/mussel)	Intercept	42	2	43111	1	411.5	<0.001	0.12
		Sal	-4	2	425	1	4.1	0.056	
		Residuals			2305	22			

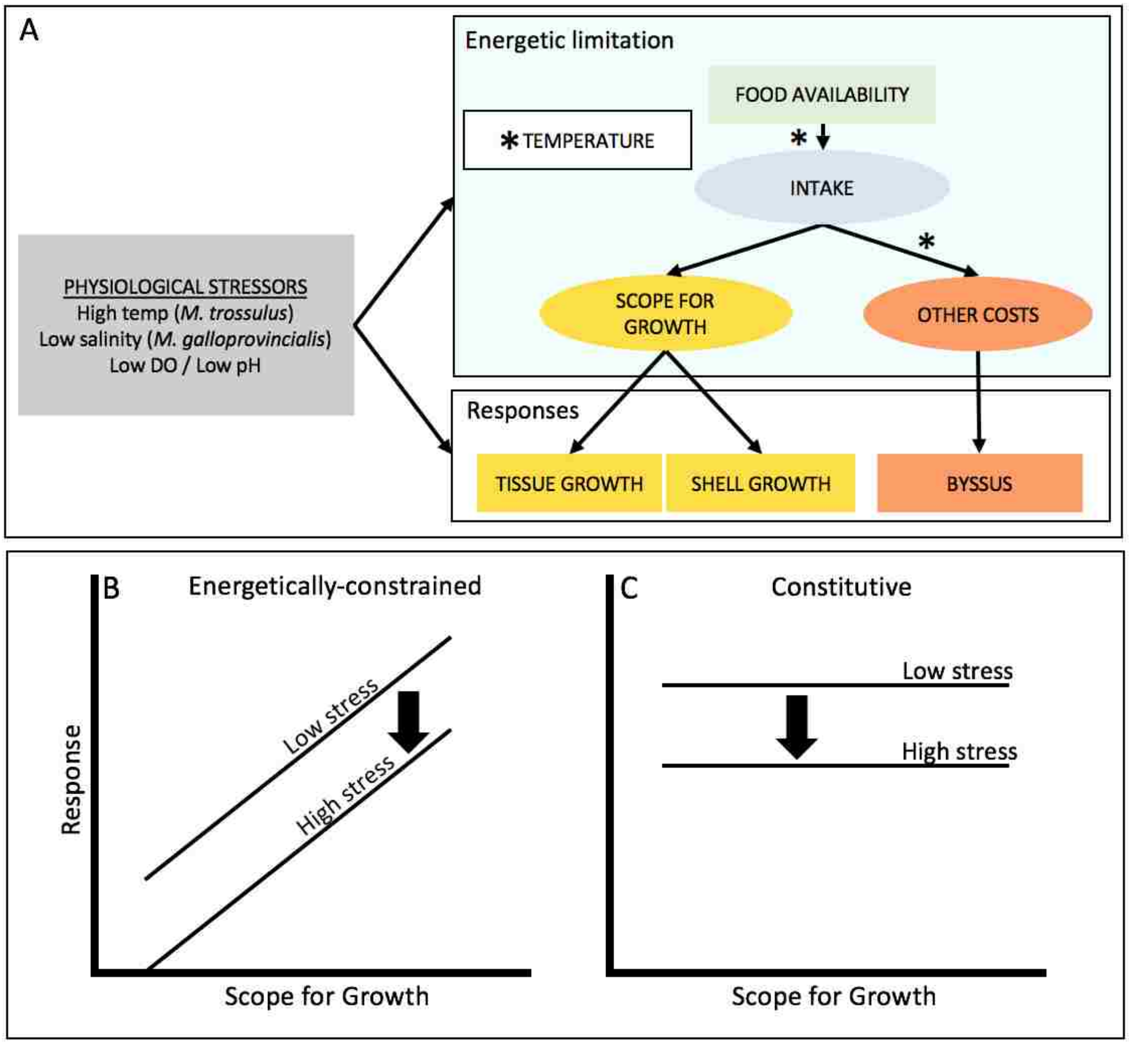

Figure 3.1. Schematic representation of effects of food limitation and other physiological stressors on mussel growth, reproduction, and attachment, using a Scope for Growth (SFG) framework. (A) We hypothesized that food availability and temperature affect the theoretical variable, SFG, which in turn determines energetic allocation to tissue (both reproductive and somatic) and shell growth (B, low stress). Byssus, on the hand, is produced constitutively and is not subject to food limitation (C, low stress). Both energetically-constrained (e.g. food-limited) and constitutive responses are potentially reduced by other physiological stressors, such as high temperature, low salinity, hypoxia or low pH (B and C, high stress). See text for details.

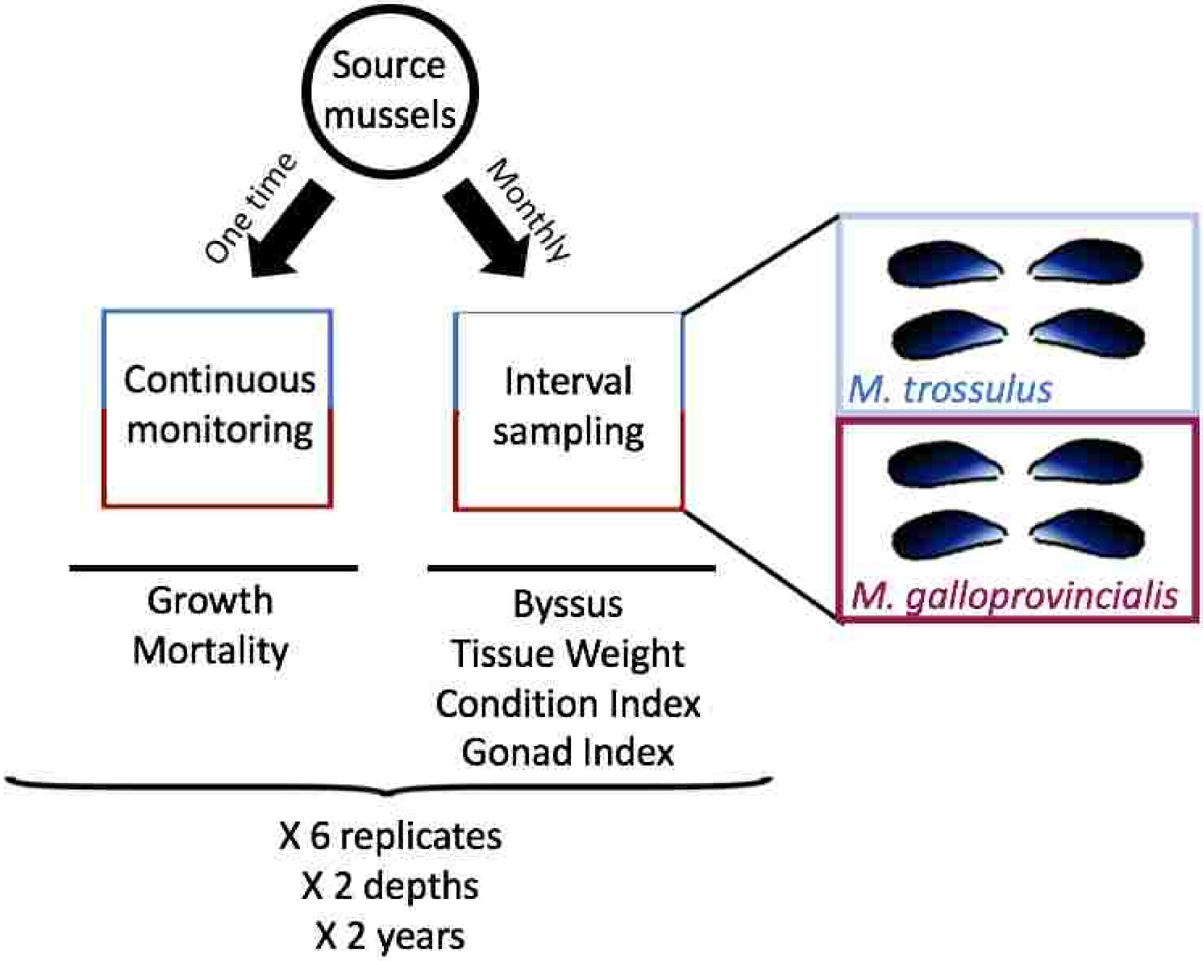

Figure 3.2. Schematic of the experimental transplant design to test for the effects of species and seawater conditions on mussel growth, byssus production, and survival at two depths. Mussels were collected in the spring each year (2016 and 2017) and transplanted into mesh bags at 1m and 7m depth to acclimate. These "source" mussels were transferred into the experimental cages at the same depth for either continuous monitoring or interval sampling. For continuous monitoring, cages were established to follow a single population over time; non-destructive measurements of shell growth and mortality were made quasi-monthly. Byssal thread production, tissue mass, condition index and gonad index, which require destructive sampling, were also measured quasi-monthly by transferring additional source mussels to cages for a two-

week interval. Each cage contained four individuals of each species, *M. trossulus* (blue) and *M. galloprovincialis* (red), and there were six replicate cages at each depth.

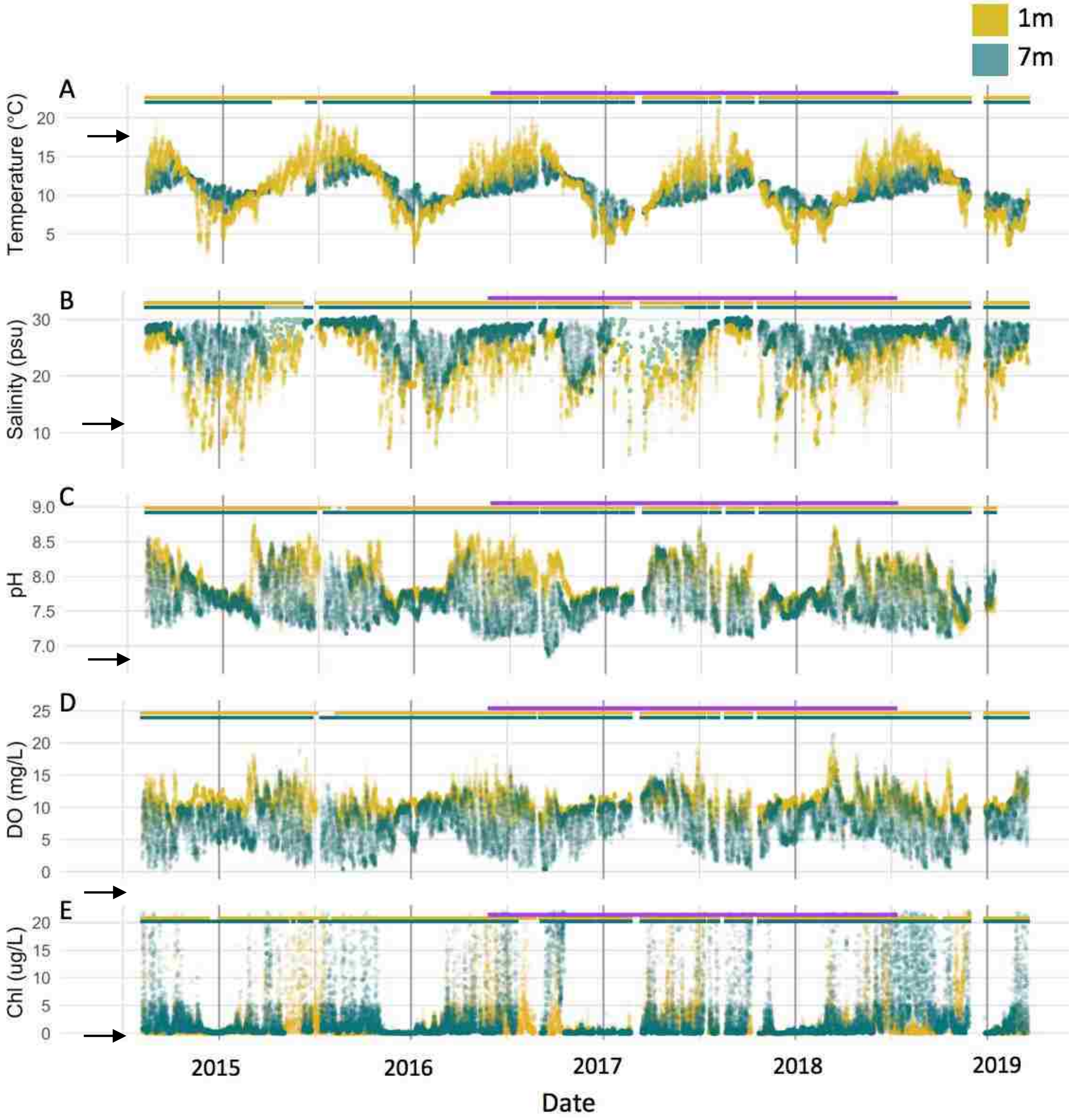

Figure 3.3. Environmental conditions at Penn Cove from 2014-2019. (A) Seawater temperature (°C), (B) salinity (psu), (C) pH (NBS scale), (D) dissolved oxygen (DO, mg L^{-1}), and (E) chlorophyll (Chl, ug L^{-1}). Symbols represent hourly measurements at both shallow (1m, yellow) and deep (7m, dark green) depths. Arrows indicate the threshold for food limitation or other physiological stress identified for each environmental parameter. Horizontal lines represent the

duration of mussel growth data for this study (purple) and as well as periods of data availability at both shallow (yellow) and deep depths (green) over the five years of monitoring; gaps in the lines indicate where data are absent. The light green symbols for salinity at 7m represent daily estimates of missing data, see text for details.

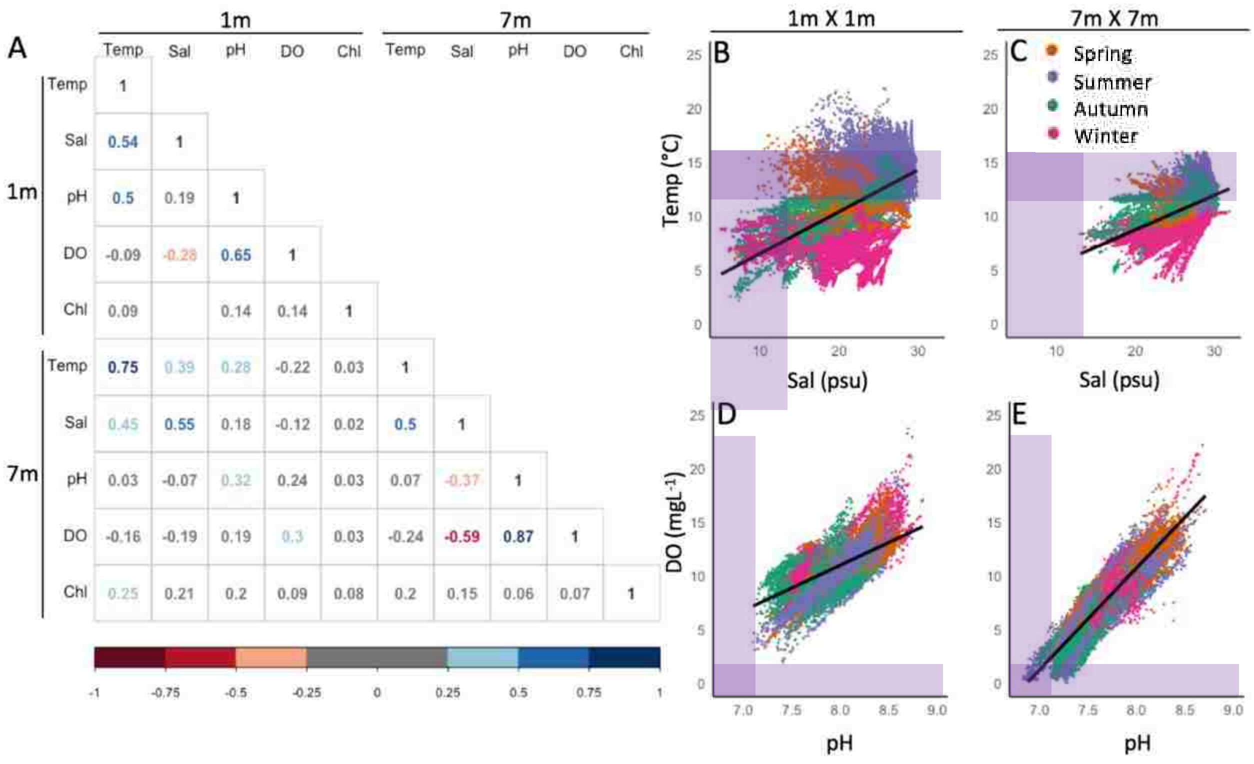

Figure 3.4. Summary of seawater conditions at two depths (1m and 7m) at Penn Cove from 2014-2019. 2014-2015 data are from Newcomb (2015). (A) Correlation coefficients determined from a pairwise Pearson correlation test for all parameters compared across the two depths (alpha = 0.001). (B and C) Temperature (°C) as a function of salinity (psu) at deep (7m) and shallow (1m) depths. Temperature was overall positively correlated with salinity at both depths, but this relationship differed by season and depth (R ranges from -0.22 at 1m depth in the summer to +0.43 at 7m depth in the winter, alpha = 0.01). (D and E) Dissolved oxygen (mg L_{-1}) was positively correlated with pH (NBS scale) at both 1m and 7m depths. Symbols represent hourly data and are color-coded by season (spring = orange, summer = blue, autumn = green, winter = pink). Shaded regions (purple) indicate conditions that exceed the physiological stress thresholds (purple) for temperature, salinity, pH, and DO. Descriptive statistics for each parameter are summarized in Table 3.1.

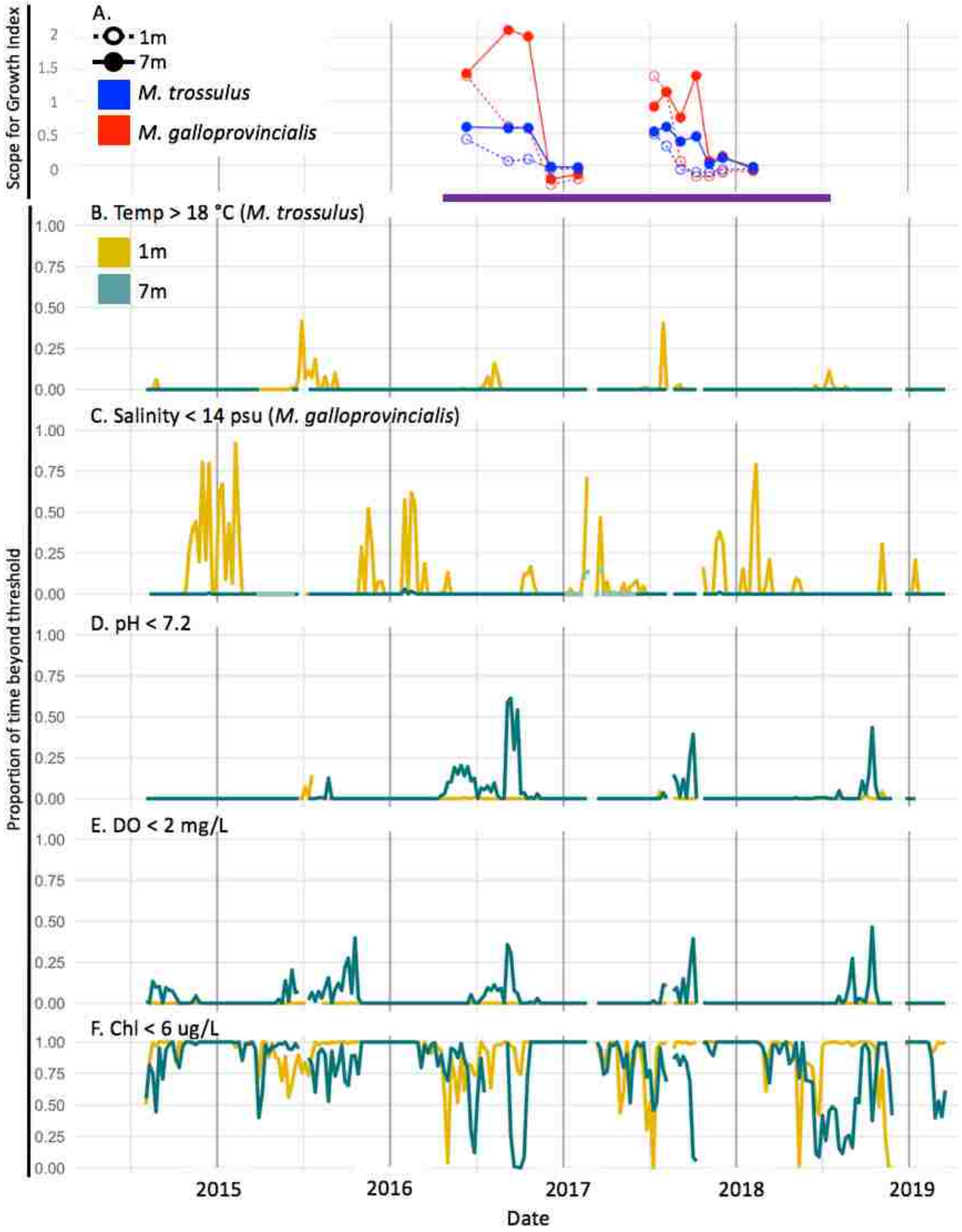

Figure 3.5. Time series of Scope for Growth and physiological stressors. Scope for Growth Index (SFG, A) and the frequency of food limitation and other stressful physiological conditions limitation (B-F) for mussels at Penn Cove from 2014-2019. Symbols represent quasi-monthly

SFG estimates at 1m depth (open circles) and 7m depth (closed circles) for *M. trossulus* and *M. galloprovincialis* (A). Lines represent the proportion of time per week a threshold tolerance was exceeded at 1m depth (yellow) and 7m depth (blue, B-F). (A) High temperature stress (> 18 °C) for *M. trossulus*. (B) Low salinity stress (<14 psu) for *M. galloprovincialis*. The same physiological stress thresholds were used for both species for pH (C, <7.2 NBS scale), DO (D, 2 mg L^{-1}) and food limitation (E, chlorophyll concentrations < 6 ug L^{-1}). The horizontal line (purple) represents the timing of mussel growth and byssus production measurements.

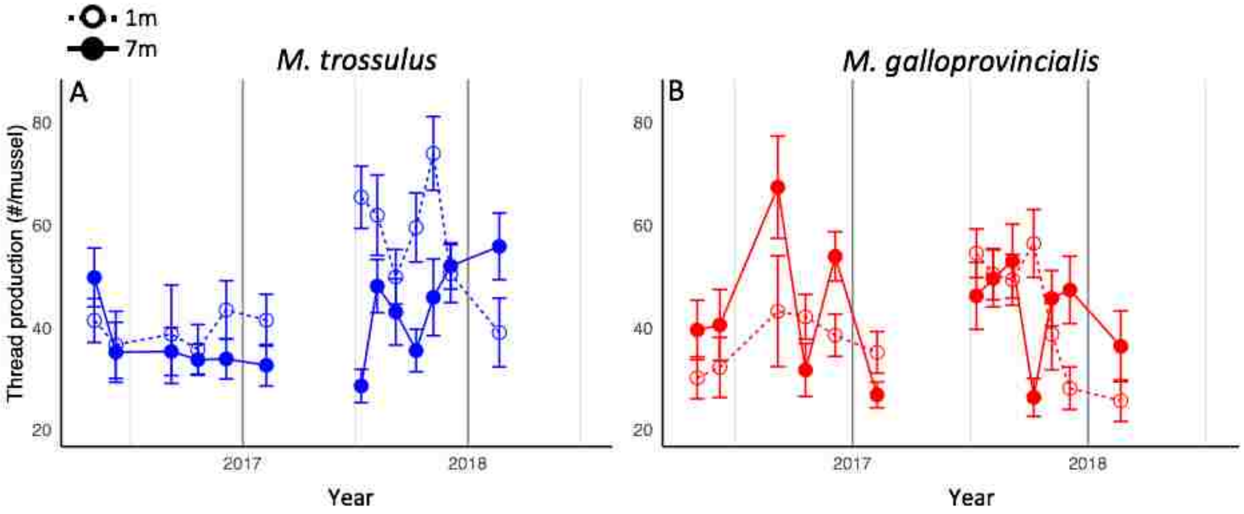

Figure 3.6. Time series of thread production. Thread production (# mussel-1) over two weeks for *M. trossulus* (A) and *M. galloprovincialis* (B) at 1m depth (shallow, open circles) and 7m depth (deep, filled circles). Measurements are for two cohorts of mussels (May 2016 – June 2017 and June 2017 – July 2018) deployed in interval cages. Symbols and bars represent means and SE.

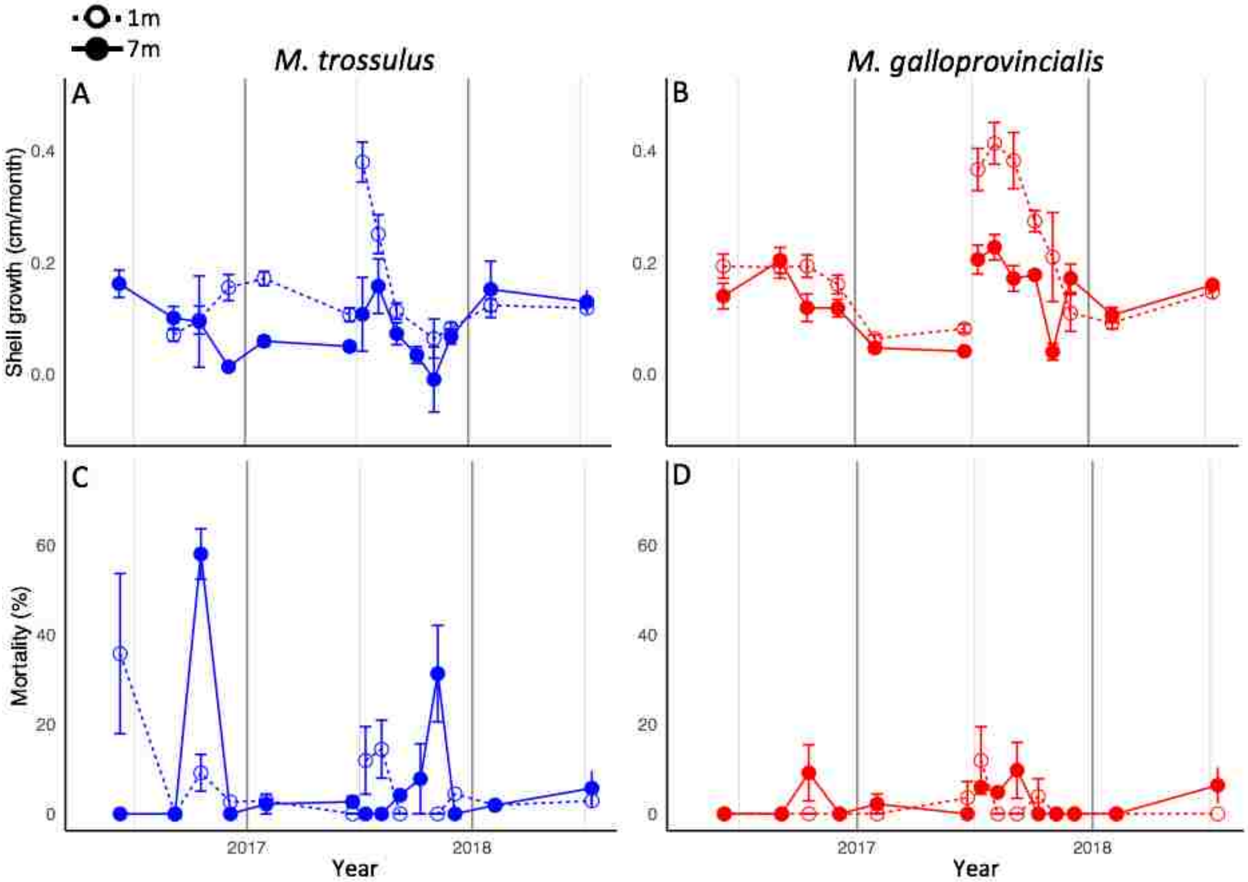

Figure 3.7. Time series of shell growth and mortality. Shell growth (cm month-1) and mortality (%) of *M. trossulus* (A, C) and *M. galloprovincialis* (B, D) at 1m depth (shallow, open circles) and 7m depth (deep, filled circles). Measurements are for two cohorts of mussels (May 2016 – June 2017 and June 2017 – July 2018). deployed in continuous cages. Symbols are means and bars are SE.

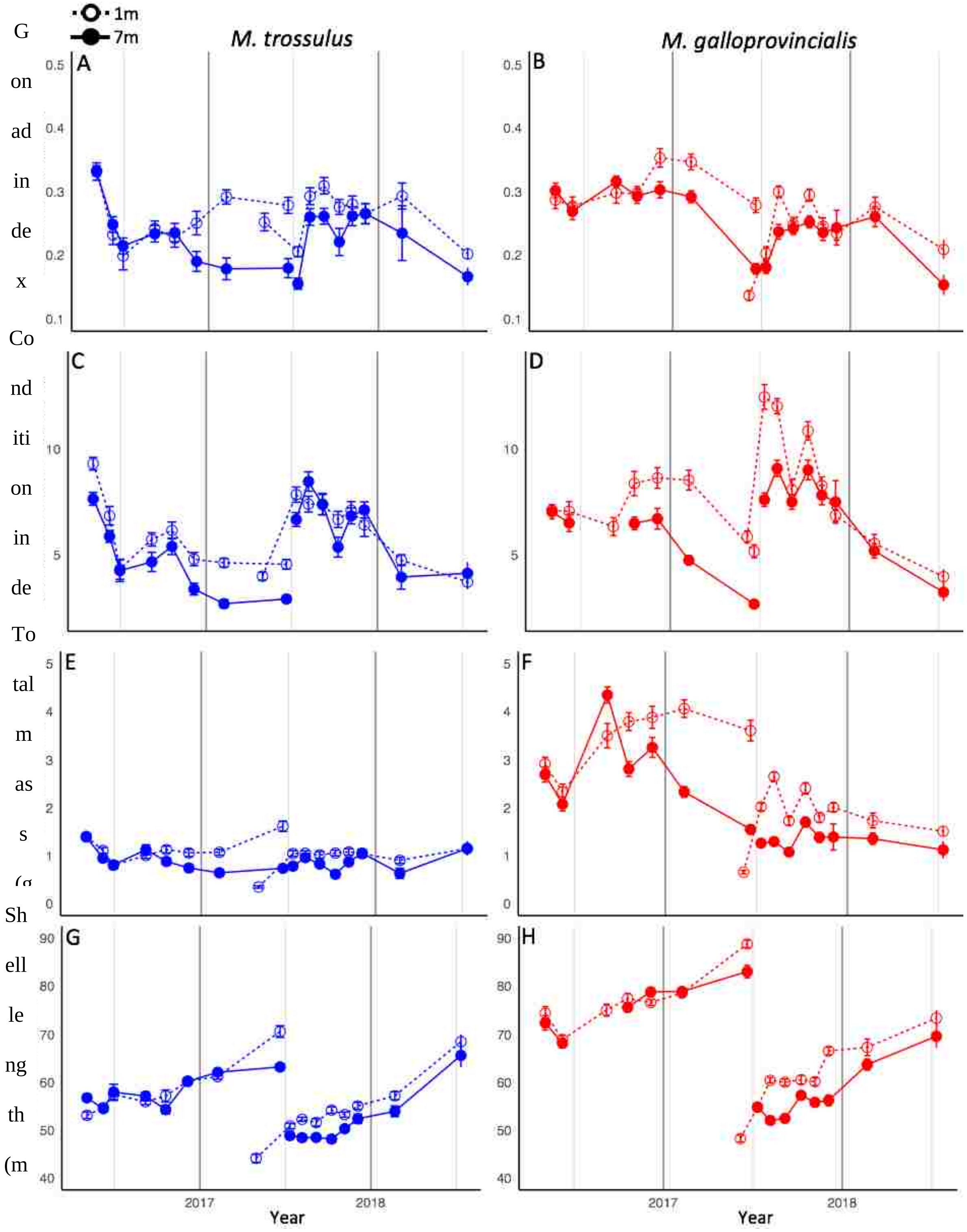

Figure 3.8. Time series of mussel condition and shell length. Gonad index (proportion), condition index (g DW cm$_{-3}$), total tissue mass (TM, g DW), and shell length (mm) of *M.*

trossulus and *M. galloprovincialis* at 1m depth (shallow, open circles) and 7m depth (deep, filled circles), for two cohorts of mussels (May 2016 – June 2017 and June 2017 – July 2018). All measurements are from mussels deployed in ‘interval cages’ except the final measurements for each cohort, which were obtained from mussels in ‘continuous’ cages.

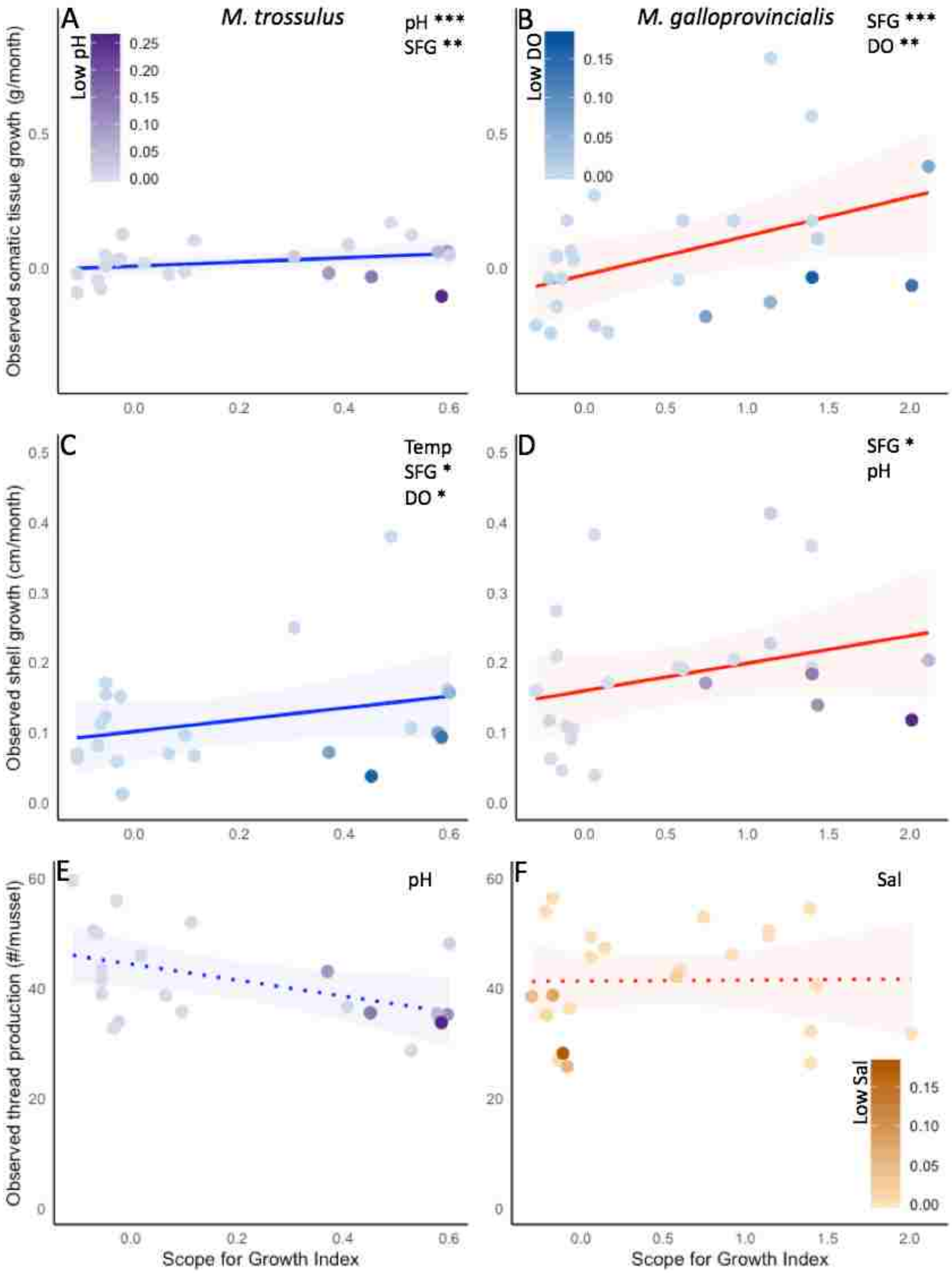

Figure 3.9. Summary of multiple regression analysis of the effects of SFG and proportion of time exposed to other physiological stressors (pH, DO, temp, and salinity)on tissue growth (g DW month^{-1}), shell growth (cm month^{-1}), and thread production over two weeks (# mussel^{-1}) for *M.*

trossulus and *M. galloprovincialis*. Thresholds for pH and DO were the same for both species, but those for temperature and salinity were species-specific (Temp - *M. trossulus*; Sal - *M. galloprovincialis*). Symbols represent monthly averages for a given depth (1m or 7m) and cohort (2016 or 2017), and the legend indicates significant explanatory parameters for each dependent variable. Symbol shading indicates proportion of time exposed to a given stressor (pH = purple, DO= blue, salinity = orange). Statistics are summarized in Table 3.3.

3.8. Acknowledgements

We thank the staff at Penn Cove Shellfish for their assistance with this project, especially Tim Jones, Sam Smith, and Zack Loescher for support on the water. We also thank Laura Newcomb and Jim Nagel for seawater sensor setup and support. We also thank Windy Madden, Hilary Hayford, Lyda Harris, Michael Pantalos, Tyler Adamson, and several undergraduate students, including Brandon Ojogho, Alexis Massey, Nicholas Rose, Pei-Jou Tsai, Silvia Chen, Claire Marvet, and Sarah Champ who provided valuable assistance in field experiment maintenance and/or sample processing. We also thank Timothy Essington, Lauren Buckley, and Jaqueline Padilla-Gamiño for insightful discussions that improved the quality of this manuscript. E.A.R. was supported by a National Science Foundation IGERT Program on Ocean Change Fellowship (1068839), and several grants from UW Friday Harbor Laboratories and UW Biology (Edmondson Award, Richard and Megumi Strathmann Fellowship, Kohn Fellowship, and Carrington Student Travel Fellowship). Additional support was provided by the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under project number NA14OAR4170078 (to EC and CS Friedman) through the Washington Sea Grant Program. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U.S. government is authorized to reproduce and distribute this paper for governmental purposes.

3.9. Supplementary Tables

Table S3.1. Summary of byssus production of *M. trossulus* and *M. galloprovincialis* at 1m and 7m depth from 2016 – 2018. Reported values are seasonal means ± SE of individual mussel measurements.

		M. trossulus		*M. galloprovincialis*	
Season	Depth	Byssal thread production (#/mussel)	n	Byssal thread production (#/mussel)	n
Spring	1m	39 ± 4	25	31 ± 3	22
	7m	45 ± 4	34	40 ± 5	27
Summer	1m	57 ± 4	68	50 ± 3	63
	7m	38 ± 3	78	52 ± 4	73
Autumn	1m	56 ± 3	85	41 ± 3	89
	7m	41 ± 2	88	41 ± 3	81
Winter	1m	40 ± 4	30	29 ± 3	29
	7m	40 ± 4	22	31 ± 4	31

Table S3.2. Summary of ANOVA of the effects of season and depth on byssal thread production of *M. trossulus* and *M. galloprovincialis*.

Species	Season			Sum Sq	Df	F val.	p
M. trossulus	Intercept			211.0	1	3071.5	<0.001
	Season			0.10	3	0.48	0.69
	Depth			0.73	1	10.62	**0.001**
	Season:Depth			0.63	3	3.06	**0.03**
	Residuals			28.99	422		
		Season	Depth				
	Tukey HSD	Spring	1m	ab			
		Summer	1m	b			
		Autumn	1m	b			
		Winter	1m	a			
		Spring	7m	ab			
		Summer	7m	b			
		Autumn	7m	ab			
		Winter	7m	a			
M. galloprovincialis	Intercept			194.3	1	2075.5	<0.001
	Season			0.91	3	3.22	**0.02**
	Depth			0.00	1	0.02	0.89
	Season:Depth			0.13	3	0.46	0.71
	Residuals			38.10	422		
		Season	Depth				
	Tukey HSD	Spring	Both	ab			
		Summer	Both	c			
		Autumn	Both	bc			
		Winter	Both	a			

Table S3.3. Summary of ANOVA of the effects of depth and experimental year on gonad index (GI, %) and condition index (CI, g / cm$_3$) of *M. trossulus* and *M. galloprovincialis*.

		Final GI (%)					
Species	Effect	Estimate	SE	Sum Sq	Df	F val.	p
M. trossulus	Intercept	0.104	0.007	0.082	1	214.54	**<0.001**
	Depth	-0.040	0.010	0.006	1	16.11	**<0.001**
	Year	-0.016	0.005	0.004	1	11.27	**0.001**
	Depth:Year	0.016	0.007	0.002	1	5.55	**0.021**
	Residuals			0.027	71		
M. galloprovincialis	Intercept	0.106	0.007	0.076	1	219.99	**<0.001**
	Depth	-0.035	0.010	0.004	1	12.38	**0.001**
	Year	-0.017	0.005	0.004	1	12.90	**0.001**
	Depth:Year	0.011	0.007	0.001	1	2.47	0.121
	Residuals			0.022	62		

		Final CI (g/cm^3)					
Species	Effect	Estimate	SE	Sum Sq	Df	F val.	p
M. trossulus	Intercept	0.0015	0.0002	0.0000180	1	54.88	**<0.001**
	Depth	-0.0011	0.0003	0.0000048	1	14.70	**<0.001**
	Year	0.0001	0.0001	0.0000001	1	0.35	0.555
	Depth:Year	0.0006	0.0002	0.0000035	1	10.58	**0.002**
	Residuals			0.0000233	71		
M. galloprovincialis	Intercept	0.0018	0.0002	0.0000231	1	78.71	**<0.001**
	Depth	-0.0013	0.0003	0.0000057	1	19.53	**<0.001**
	Year	-0.0001	0.0001	0.0000003	1	0.99	0.324
	Depth:Year	0.0005	0.0002	0.0000022	1	7.50	**0.008**
	Residuals			0.0000182	62		

Table S3.4. Summary of gonad index of *M. trossulus* and *M. galloprovincialis* at 1m and 7m depth from 2016 – 2018. Reported values are seasonal means ± SE of individual mussel measurements.

		M. trossulus		*M. galloprovincialis*	
Season	Depth	GI	n	GI	n
Spring	1m	0.273 ± 0.009	67	0.232 ± 0.011	71
	7m	0.288 ± 0.011	46	0.285 ± 0.009	48
Summer	1m	0.250 ± 0.006	143	0.256 ± 0.006	132
	7m	0.212 ± 0.006	127	0.226 ± 0.006	120
Autumn	1m	0.260 ± 0.007	119	0.285 ± 0.007	119
	7m	0.236 ± 0.008	103	0.269 ± 0.006	97
Winter	1m	0.292 ± 0.011	43	0.314 ± 0.011	42
	7m	0.191 ± 0.017	31	0.276 ± 0.009	44

3.10. Supplementary Information

Supplementary Information 3.1.

The Scope for Growth model is adapted from Roberts Chapter 2 with modifications to include chlorophyll and temperature as inputs. All energy budget calculations are expressed as daily fluxes (in J). Scope for Growth (SFG, J), the energy available for growth (somatic and gonad), was calculated as follows:

$$\mathrm{SFG} = \mathrm{E(Chl, T)} - \mathrm{R(T)} \tag{1}$$

where *E* is the energy intake (J), and *R* is the cost of tissue maintenance (J). Gonadal and somatic tissue maintenance costs are included in the term, *R* (Eq. 1). We made the assumption that a constant fraction of energy is allocated evenly to growth and reproduction (e.g. gamete development; Kooijman 2010), 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):

$$\mathrm{E(Chl, T)} = \mathrm{f(Chl)} \times \mathrm{a(T)} \times \mathrm{TM_{initial}}^{\mathrm{d}}, \tag{2}$$

where *f* is the relative food availability coefficient (unitless), *a(T)* is the energy intake coefficient, and a function of temperature (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 chlorophyll data for each month given critical saturation thresholds (Table 3.1). Food availability was considered equal for all mussels within each month 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 3.2).

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):

$$\mathrm{R(T) = b(T) \times TM_{initial}}^{\mathrm{e}}, \tag{3}$$

where *b(T)* is the mass-specific metabolic cost coefficient and is a function of temperature (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 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^{-1}. *b* was determined as a function of temperature from the autumn measurements of mass-specific oxygen consumption of Fly and Hilbish (2013) for *M. trossulus* and *M. galloprovincialis* from WA. Respiration was estimated as 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 (Table 3.1, Figure 3.1). The autumn values were then converted to daily values to yield the metabolic cost coefficient (*b*; J mg^{-1}; Table 3.1, Riisgård and Randløv 1981).

The energy intake coefficient (*a*, J f^{-1} g DW $^{-d}$) was calculated as a function of temperature (Table 3.1):

$$\mathrm{a(T) = CR(T) \times I_M \times AE}\ , \tag{4}$$

where *CR* is the clearance rate (L hr^{-1}), I_m is the maximum ingestion rate (J), and AE is the assimilation efficiency (unitless) which is the proportion of food assimilated by the mussels. As with respiration, CR was estimated from a linear regression of CR measurements from 5 °C to 20 °C, and the standard error was estimated as the average standard error from each temperature (Fly and Hilbish 2013, Table 3.1, Figure 3.1). The average AE was determined across all temperatures since there was no linear correlation with temperature (Fly and Hilbish 2013, Table 3.1).

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

$$\mathrm{SFG} = \mathrm{f(Chl)} \times \mathrm{a(T)} \times \mathrm{TM_{initial}}^{\mathrm{d}} - \mathrm{b(T)} \times \mathrm{TM_{initial}}^{\mathrm{e}}. \qquad (5)$$

The relationship of estimated SFG and somatic growth, shell growth, and byssal thread production was then evaluated (see text for details).

VITA

Emily Roberts has an individualized undergraduate degree in Biophysics (B.A.) from Oberlin College and a Professional Master's Degree in Marine Biology (M.S.) from Northeastern University, where she focused on the ecological role of UV-protectant sunscreen compounds produced by seaweeds. Emily has also performed research on a variety of topics ranging from marine physiological ecology, including ocean acidification and phytoplankton photobiology, to protein biochemistry and tendon bioengineering. Emily's interest in ecology was first sparked by learning and teaching natural history and landscape ecology in the San Gabriel Mountains near Los Angeles, and in the future hopes to facilitate place-based student research and collaborate within multisector aquaculture networks to increase capacity to adjust to environmental fluctuations and change.