Vol. 704: 35–54, 2023
https://doi.org/10.3354/meps14234

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published January 26

Byssal thread attachment and growth are not correlated across gradients of temperature and food availability for two congeneric mussel species

Emily A. Roberts[1,2,3,*], 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
[3]*Present address:* Keck Science Department, Claremont McKenna College, 925 N Mills Ave, Claremont, CA 91711, USA

ABSTRACT: Anthropogenic warming and natural climate variability affect global patterns of seawater temperature and marine primary productivity and affect organism survival, growth, and physiology. Mussels are ecosystem engineers that utilize byssal thread structures to attach to hard substrate, a strategy key to survival in wave-swept rocky shore environments. Byssal thread production varies according to season and environmental conditions, and temperature and food availability may influence the production of these structures by affecting energy limitation. *Mytilus trossulus* and *M. galloprovincialis* are congeneric mussel species in the Northeast Pacific with cold- and warm-adapted thermal tolerances, respectively. First, we hypothesized that temperature has opposing effects on growth rates of the 2 species. Second, we hypothesized that either (1) byssal thread production is positively correlated with growth rate (the 'production' hypothesis), or (2) there is a trade-off between growth and byssal thread production, and resources are allocated first to byssal thread production rather than growth. Under this 'trade-off' hypothesis, we predicted no relationship between growth rate and byssal thread production. We manipulated seawater temperature and food availability and quantified mussel performance in terms of survival, growth, and byssus attachment. Across all treatment combinations, we found that *M. galloprovincialis* had positive shell and tissue growth and *M. trossulus* had minimal shell growth and a loss in tissue mass. Temperature had opposing effects on each species; temperature increased shell growth of *M. galloprovincialis* but increased tissue loss of *M. trossulus*. Temperature did not 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 over growth.

KEY WORDS: Bioenergetics · Energy budget · Thermal tolerance · Resource allocation · Biomechanics · *Mytilus trossulus* · *Mytilus galloprovincialis*

1. 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 (Menge 1992, Menge et al. 2003, Pörtner 2012). Key challenges in marine ecology include identifying how thermal stress impacts nutrition through its effect on food consumption, digestion, and metabolic rates (Kooijman 2010, Sokolova 2013), 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

*Corresponding author: molly.a.roberts@gmail.com

various aspects of organism thermal performance (i.e. behavior vs. physiology) differ and how they contribute to fitness is key to predicting how organisms will respond to natural variability and change due to ocean warming (Guderley & Pörtner 2010, Monaco & McQuaid 2018). Further, understanding which traits are prioritized over others and in what contexts is crucial to identifying beneficial and maladaptive ways that organisms acclimate and adapt to changing environmental conditions. For example, energy may be allocated preferentially to stress response (Sokolova 2013) and biomaterial production (Roberts et al. 2021), and energy allocation rules such as these may ensure the survival of individuals and persistence of populations in their natural environments.

Population distributions and organismal performance of bivalve mollusks are sensitive to large-scale (Black 2009, Hilbish et al. 2010) and small-scale environmental variability (Westerbom et al. 2019). Seawater temperature and food availability both affect the growth of suspension feeders, and the interaction between these 2 factors is context-dependent and may have nonlinear synergistic effects on performance (Blanchette et al. 2007, Lesser et al. 2010, Zippay & Helmuth 2012, Todgham & Stillman 2013, Tagliarolo et al. 2016). In intertidal systems, where organismal temperature at low tide is dynamic, greater food availability buffers against the effect of high emersion temperatures on mortality (Schneider et al. 2010, Fitzgerald-Dehoog et al. 2012). At extreme temperatures, greater resource availability and/or greater resource allocation towards survival may be adaptive for a population (Fitzgerald-Dehoog et al. 2012). In subtidal systems, the effects of seawater temperature and food availability on performance are often context-dependent. For example, on a set of offshore moorings, the effects of food availability and aquatic temperature on mussel growth counteracted each other (Blanchette et al. 2007), while another set of experimental observations showed food availability was the dominant factor limiting mussel growth (Page & Hubbard 1987). While temperature extremes are common in intertidal environments and cause acute physiological stress, seawater temperature affects both feeding rate and metabolic costs. Energy budget frameworks are used to determine the energy allocation to body tissue growth vs. metabolic costs, and estimate how food intake affects endogenous resource availability for these processes (e.g. Bayne & Newell 1983). A mechanistic understanding of resource availability, as a function of both food and seawater temperature, and how it is allocated to competing organismal processes is foundational for understanding how food availability and temperature act together to affect organisms in dynamic rocky shore ecological communities (Guderley & Pörtner 2010, Matzelle et al. 2015).

One approach towards understanding how environmental temperature and food availability influence organismal growth in field settings are bioenergetics frameworks (Filgueira et al. 2011, Kearney et al. 2012, Nisbet et al. 2012). These conceptual models describe the physiological relationship between food availability, temperature, and organismal growth in terms of mass balance of temperature-dependent metabolic costs and consumption of food (Widdows & Bayne 1971, Kitchell et al. 1977, Bayne & Newell 1983, Kooijman 2010) (Fig. 1A,C). Bioenergetic frameworks do not just represent the 'environment' per se, but the state (body temperature, food chemistry intake) of an organism as a function of its multi-dimensional environment (Kearney et al. 2010). The energetic state can thus be represented as a function of the individual mass/energy of an organism at any time '*t*' and the biophysical environment and nutritional space (Fig. 1B,D) (Kearney et al. 2010). For example, at low temperatures, increased temperature increases enzyme activity and the overall metabolic cost of tissue maintenance (Fig. 1A,C). As temperature increases further, additional metabolic cost often corresponds with decreased respiration (Fig. 1A,C) and is associated with temperature-induced low oxygen availability at the organismal level and systemic decrease in oxygen levels (Pörtner & Farrell 2008) and protein protection and repair (Lindquist 1986, Feder & Hofmann 1999, Fields et al. 2012). Food consumption rate is also temperature-dependent, often with species-specific thermal optima (Fig. 1A,C; e.g. fish: Kitchell et al. 1977; mussels: Widdows & Bayne 1971, Fly & Hilbish 2013). The difference between energetic input and the metabolic cost minus the energy in waste products is the surplus energy available for growth, or scope for growth (SFG; Widdows & Bayne 1971, Bayne & Newell 1983, Fly & Hilbish 2013). SFG may be represented as the difference between 2 thermal performance curves that represent the temperature-dependence of feeding processes and metabolic costs (Fig. 2A,C). A perturbation of temperature will affect feeding rate and metabolic costs, resulting in an effect on energetic scope, as will a perturbation in the amount of food available. As a result, gradients in food and temperatures will produce a range of energetic scope that may be used towards biosynthesis of body tissue and gametogenesis (Fig. 2B,D). This state–space framework (sensu Kearney et al. 2010) may be used to assess the availability of endogenous energetic resources for the growth of

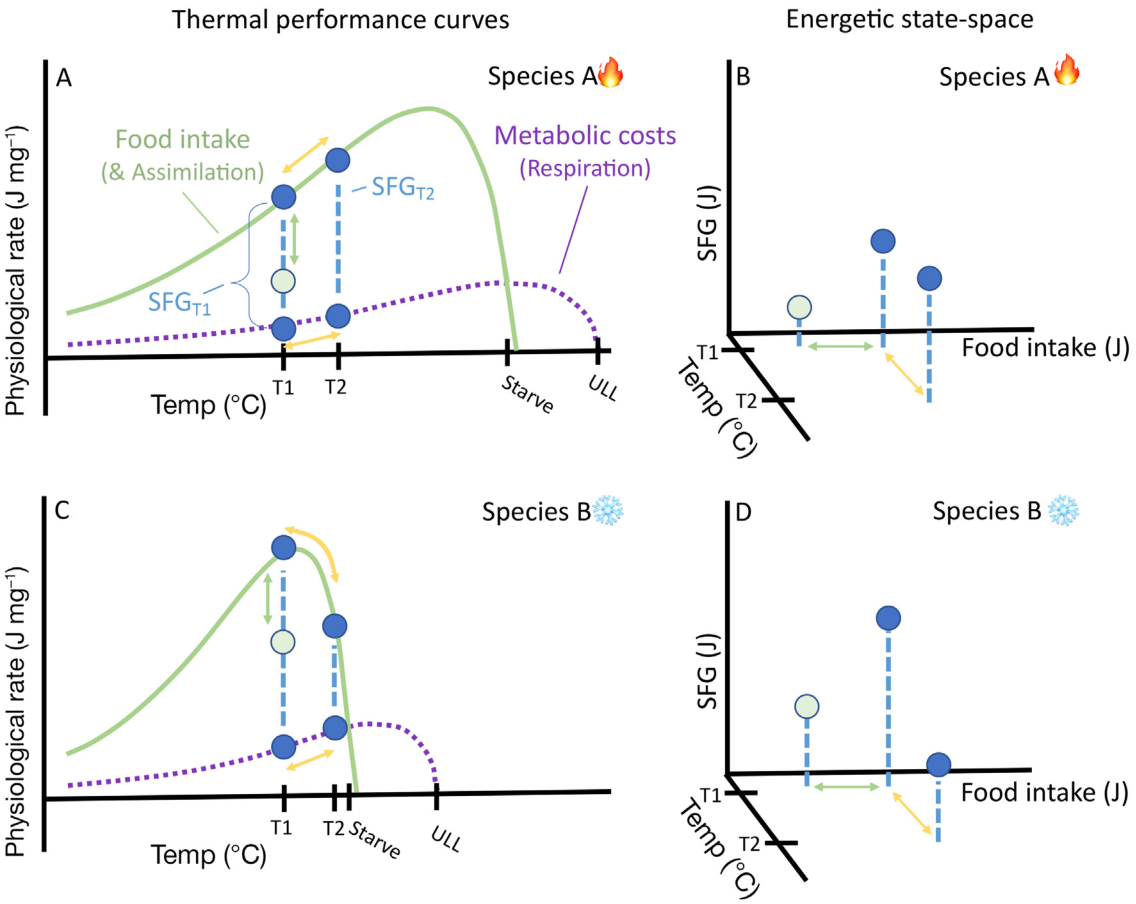

Fig. 1. Conceptual diagram showing the relationships between temperature, food intake, and energetic scope adapted from Kitchell et al. (1977). Species A and Species B have differing energetic state–space landscapes that depend on the influence of temperature and food availability on the species-specific functions of feeding and metabolic costs. (A,C) Scope for growth (SFG, dashed blue lines) is the difference between thermal performance curves for feeding (ingestion and assimilation of energy from food, green) and metabolic costs (respiration, purple) minus excretion (not shown). Food availability affects food assimilation rates (green arrows). Temperature affects assimilation and metabolic rates (yellow arrows). (B,D) Values of SFG from panels A and C (dashed blue lines) are mapped onto a 3-dimensional state–space of temperature and food intake. Species A and Species B differ in their thermal performance characteristics. Species A (panels A,B) can survive in greater thermal regimes than Species B (panels C,D). The upper lethal limit, or the temperature at which respiration ceases, occurs at a higher temperature for Species A. Similarly, the temperature at which costs exceed energy assimilation (starvation temperature) is greater for Species A than for Species B

tissue, shell, and other biomaterials as well as resource allocation towards these materials across a range of environmental conditions.

Thermal stress and food limitation often are assumed to act independently and have a simple additive effect on energetic scope and tissue growth (e.g. Kitchell et al. 1977, Kooijman 2010). It is possible for 2 factors, however, to have smaller or greater effects when applied in combination than when applied individually (interactions known as antagonism and synergism, respectively; reviewed by Sokolova 2021). Greater food availability may act to buffer organisms from the negative consequences of abiotic stressors 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, a 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). Organisms that lack external resources and/or have a depleted energy reserve may not be able to allocate energy towards alleviating the effects of these abiotic stressors and building and maintaining biomaterials. On the other hand, other organisms that feed more may generate additional biomaterial; marine snails

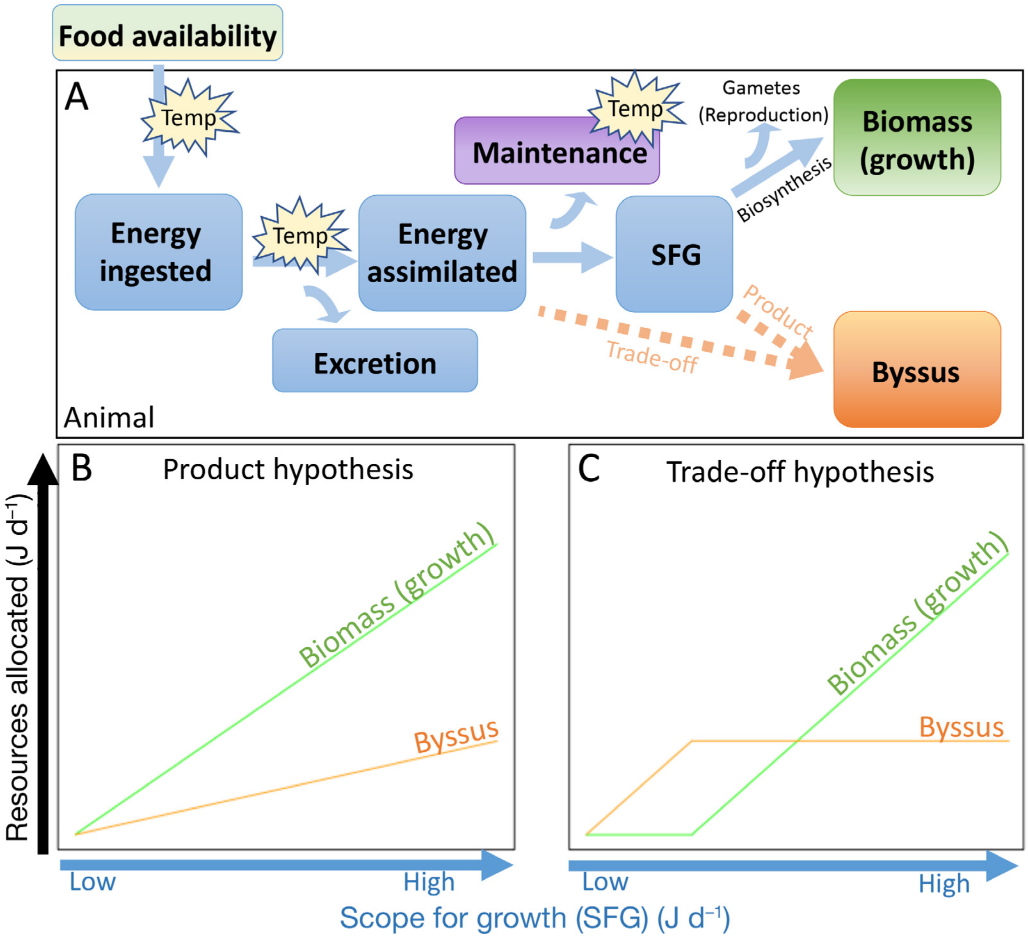

Fig. 2. Schematic of an energetic framework that incorporates byssal threads. (A) The environmental factors food availability and temperature affect organism energetics and biosynthesis of body mass (growth). A fraction of the energy content of food is ingested and a fraction of the energy ingested is assimilated by the organism and used for processes including maintenance and biosynthesis. Scope for growth (SFG) is energy that is assimilated minus metabolic costs and is energy available for biosynthesis of body tissue and gamete production (reproduction). It is unknown if byssal thread production is linked to organism energetics upstream of SFG ('trade-off' hypothesis) or downstream of SFG ('product' hypothesis). (B) Under the product hypothesis, increased SFG causes an increase in byssal thread quality and quantity and an increase in body tissue growth. Our quantitative hypothesis assumes that energy allocated to byssus is proportional to energy allocated to biosynthesis and sums to the value of SFG. (C) Under the trade-off hypothesis, energy is allocated towards byssal thread production regardless of the SFG. As SFG increases, body tissue biosynthesis increases, but there is no change in byssal thread production. Our quantitative hypothesis assumes that, at energy levels with a positive SFG, all energy available is prioritized towards byssus, and then after a threshold value, there is no change in energy allocated to byssal thread production. Again, energy allocation to byssus and biosynthesis sum to the value of SFG

that produced more calcium carbonate shell consumed more food (Palmer 1992).

Mussels are dominant ecosystem-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 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 & Gosline 1996, Waite et al. 1998). Byssus strength varies seasonally in the mussels *Mytilus edulis* and *M. trossulus*, and these species both have weak attachment during summer when mussels experience limited food availability, elevated temperature, and have a high investment in reproduction (Carrington 2002, Moeser & Carrington 2006, Zardi et al. 2007, Newcomb 2015). When attachment is too weak to withstand hydrodynamic forces, up to 30% of a population can be lost by dislodgement (Carrington et al. 2009).

Production of byssal threads is energetically costly. Of the material incorporated into the organic tissues of *M. edulis*, ~8 % of both carbon and nitrogen is used to produce byssal threads (Hawkins & Bayne 1985). The full cost of byssus production can depend on the number of byssal threads produced and is estimated to range over 8–50 % of the energy budget of mussels (*M. trossulus*, Roberts et al. 2021). What is less well understood is resource allocation to byssal threads under different levels of endogenous resource availability. Do mussels in more favorable conditions (i.e. greater food availability and temperatures at which feeding rates are high and metabolic costs are low) also produce a greater quality and quantity of byssal threads? This question can be described in terms of conceptual frameworks that link energetics components (e.g. ingestion, assimilation, metabolic costs) with byssal thread production (Fig. 2A; Roberts 2019; ecomechanics: Carrington et al. 2015). In this conceptual model there is either an 'upstream' cost that competes with energy available for growth of body tissue ('trade-off' hypothesis; Fig. 2A,C), or a 'downstream' fraction of energetic surplus goes to biosynthesis of both body tissue and byssal threads ('product' hypothesis; Fig. 2A,B). In the dynamic energy budget (DEB) model framework, byssal threads might be considered a 'product' (a biomaterial without a maintenance cost associated with it), given that older larger mussels that have a greater energetic surplus for biosynthesis produce a higher quality of byssal threads (Kooijman 2010).

Recent work demonstrates, however, that mussels that are induced to produce more threads by frequently severing the byssal threads (e.g. daily) grow less, supporting the concept that byssal thread production is a cost 'paid' prior to growth (Roberts et al. 2021). The energy allocation rule that byssal thread production is prioritized by adult mussels and is not controlled by the availability of endogenous surplus is further supported by mussel response to starvation. There was a lack of an effect of starvation (Babarro et al. 2008) on byssal thread production of adult (but not smaller juvenile) mussels. However, mussels that were spawned and remained unfed produced lower quality threads, raising questions about the effect of availability of endogenous resources on byssal thread production (Babarro & Reiriz 2010). While evidence supports a trade-off between byssal thread production and growth, it is unknown whether byssal thread quality and quantity itself may vary with energetic scope (or deficit). Experiments demonstrating a short-term effect of temperature on byssal thread quality and quantity (Newcomb 2015) suggest that acute differences in temperature play a role. However, it is unknown whether long-term exposure to a range of temperature and food levels that would be expected to produce a range of growth might not only perturb energetic state and growth, but also have a similar effect on byssus quality and quantity.

Fluctuations in sea surface temperature and phytoplankton biomass in the Salish Sea, Washington, USA (Lowe et al. 2016, Khangaonkar et al. 2019), may influence mussel growth and attachment in a species-specific way. *M. trossulus* and *M. 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 & Somero 2011). The temperature at which SFG is maximized (T_{opt}) can differ between species. Maximal values of SFG for *M. trossulus* occur at lower temperatures than for *M. galloprovincialis* (T_{opt} = 10°C vs. 25°C; Fly & Hilbish 2013). Further, metabolic costs can exceed energy assimilated, producing a negative SFG (<0) for *M. trossulus* at 15°C and above (Fly & Hilbish 2013). In contrast, energy available from the assimilation of food outweighs metabolic costs for temperatures ranging over 10–25°C for *M. galloprovincialis*, producing a net positive SFG (>0) that increases with temperature (Fly & Hilbish 2013). Moreover, the 2 species have different timings in their gametogenic cycles and spawn at different times of the year (*M. trossulus* [formerly *M. edulis*]: April–July; *M. galloprovincialis*: November–March; Skidmore 1973, Strathmann 1987, Curiel-Ramirez & Caceres-Martinez 2004), though the reproductive cycle of *M. galloprovincialis* is less well characterized in the Salish Sea (Northeast Pacific). Because of these differences in reproductive cycles and thermal optima, and the potential linkage between energetic resources and byssus production (Clarke 1999, Babarro et al. 2008, Babarro & Reiriz 2010), we hypothesize that these 2 species will differ in their ability to produce byssus under different combinations of temperature and food availability.

To elucidate the role that energetics play in byssal thread production and attachment strength, we investigated how the effect of food availability combines with the effect of seawater temperature on growth and byssal attachment of 2 mussel species, *M. trossulus* and *M. galloprovincialis*. In the laboratory, we exposed mussels to factorial combinations of environmentally relevant seawater temperatures and food availability for 10 wk and then measured survival, growth, and byssal thread quantity and quality. Growth rate was evaluated both in terms of the change in the size of the organism (shell growth) and the change in the body tissue mass of the organism. Given demonstrated species-specific differences in the relationship between temperature and energetic scope (SFG; Fly & Hilbish 2013), we evaluated the species-specific effect of the range of seawater temperatures commonly experienced by organisms in the Salish Sea on growth and byssus. Firstly, we hypothesize that within the experimental range of seawater temperatures, there is a negative relationship between temperature and growth rate of *M. trossulus* and a positive relationship between temperature and growth rate of *M. galloprovincialis*, given the relationships between temperature and SFG quantified in the literature (Fly & Hilbish 2013). Secondly, we hypothesize that food availability increases growth rate, and more specifically, that the effects of food availability and temperature on growth rate are additive, rather than synergistic or antagonistic, for both species. Thirdly, increased byssal thread production has been shown to decrease growth (Roberts et al. 2021). It is unclear, however, if mussels that are held in conditions that produce greater growth (e.g. greater food availability and optimal temperatures) also produce a greater quality and quantity of threads (Fig. 2A), or if byssal thread production is prioritized over other biosynthesis processes such as the production of body tissue biomass and shell (Fig. 2B). We hypothesized that either (1) byssal thread quantity and quality are positively correlated with growth rate across a range of temperature and food treatments (the 'production' hypothesis), or alternatively, that there is a trade-off between growth and byssal thread production and (2) energy allocation is prioritized towards byssal thread production regardless of the energetic state. Under this 'trade-off' hypothesis, we predicted no relationship between growth rate and byssal thread quantity and quality.

2. MATERIALS AND METHODS

2.1. 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 10 wk (Aug 19–Oct 31, 2016). There were 4 temperature levels representing a range of local summer seawater temperatures (13, 15, 18, and 21°C) and 2 food levels (high and low) representing near and below saturated food levels. Each of the 3 replicates of the 8 treatment combinations was established in a mesocosm, a 22 l square HDPE food-safe container (S-16971, ULINE), that housed 8 individuals of each of the 2 mussel species (Fig. 3).

Seawater input to each flow-through mesocosm came from a common supply of 1 µm filtered seawater and was supplied at a rate of 11 ± 3 l h^{-1} by using drip irrigation emitters to control flow into the mesocosms. Incoming seawater was cooled to ~12°C by immersing the tubing that carried the incoming seawater into a cold bath (AquaEuroUSA MC-13 HP Max-Chill Titanium Chiller). Each mesocosm was heated independently with a 300 W aquarium heater (Jetsu Aquarium Supplies) controlled by a digital temperature controller (JBJ TRUE TEMP TT-1000; ±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 (~28 psu, 7.94 pH; Murray et al. 2015).

2.2. Mussel collection and acclimation

Mussels were collected from commercial aquaculture rafts at Penn Cove Shellfish LLC, Coupeville, Washington (48° 13′ 15″ N 122° 42′ 20″ W), at ~1 m depth in late July 2016 and transported on ice to

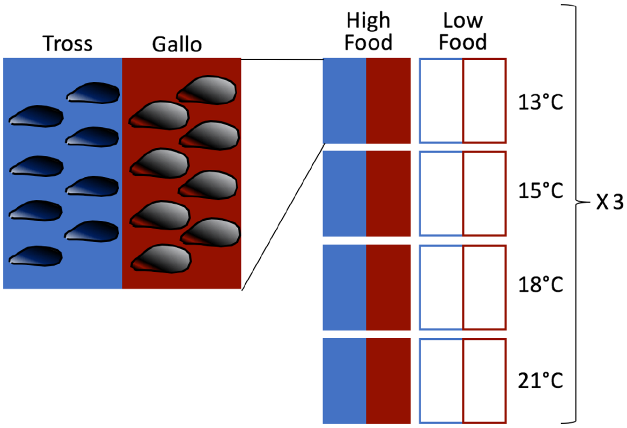

Fig. 3. Schematic of the experimental design. The experimental design was fully factorial and tested for the effects of species, temperature, and food on mussel survival, growth, and biomaterial production. Each 22 l mesocosm contained 8 individuals of each species, *M. trossulus* (Tross, blue) and *M. galloprovincialis* (Gallo, red). Each mesocosm was supplied with 1 of 2 levels of food availability (high or low) and maintained at one of 4 levels of seawater temperature. Each treatment combination (=mesocosm) was replicated 3 times

Friday Harbor Laboratories (FHL). The size and age of the individuals selected were representative of the farmed subtidal mussel populations. *M. trossulus* at this site is grown from a natural set, and populations of both species are established within the region (Elliott et al. 2008). *M. trossulus* individuals were ~1 yr old with shell length 55–65 mm and tissue weight 1.04 ± 0.09 g dry weight (DW, mean ± SE). *M. galloprovincialis* individuals were slightly older (~1.25 yr old) and bigger, ranging 75–85 mm in shell length and a tissue weight of 2.26 ± 0.17 g 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, ~0.15% of tissue DW d^{-1}). Mussels were then acclimated to experimental conditions over a period of 19 d by incrementing temperatures a maximum of 2°C per day until target temperatures were reached and then holding the temperature at the experimental temperature. 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% tissue DW d^{-1} (Reed Mariculture, high food, a maintenance ration, Helm & Bourne 2004) and 0.18% tissue DW d^{-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 per 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). We estimated that food concentrations were 18–31 µg chlorophyll *a* (chl*a*) l^{-1} and 2–3 µg chl*a* l^{-1} for the high food and low food treatment respectively, or 25×10^6 cells l^{-1} and 2.5×10^6 cells l^{-1}, given a food source of 2×10^6 cells ml^{-1}concentration at 91.1% AFDW (Reed Mariculture) and a conversion factor from 30–50 g C g chla^{-1} (Jakobsen & Markager 2016). The source of each food treatment was distributed among 3 separate 4 l reservoirs, and to control for the potential effect of the reservoir, reservoirs were randomized among treatments every 4 d. Mesocosms were scrubbed and siphoned weekly to remove detritus.

2.3. Survival, growth, and attachment

Mussel performance was evaluated in terms of survival, growth, and byssus attachment. At the end of the 10 wk experiment, survival of the 8 individuals of each species was determined per mesocosm. Shell growth, a nondestructive metric of the change in the size of the mussel, was calculated as the change in shell length, and was measured with calipers (RMSE ± 0.01 mm). Tissue DW was determined by removing and drying tissue at 60°C for 48 h. Condition index (CI) was calculated by dividing tissue DW (g) by shell length cubed (mm^3; Crosby & Gale 1990). Tissue growth (g DW), which included gonadal and somatic tissue, was calculated as the difference between final tissue DW and estimated initial tissue DW. Initial tissue DW of the experimental individuals was determined from their initial shell length using a linear relationship between shell length and tissue DW from 24 individuals per species dissected at the initial timepoint.

Byssal thread production, thread strength, and byssus strength were also measured during the last 2 wk of the experiment. Each mussel was tethered to a clear acrylic plate (4 rectangular ~20 × 10 cm plates per mesocosm, 4 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 d, 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 or the side of the tank) was determined. Plates with byssus were dried and stored for up to 2 mo, then rehydrated in seawater prior to mechanical testing (Brazee 2004, Brazee & Carrington 2006, George & Carrington 2018).

We adapted the method of Bell & Gosline (1996) to measure the strength of individual byssal threads using a tensometer (Instron 5565). 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 to which the thread plaques were attached, was clamped to the base of the tensometer. The byssus was extended 5 mm min^{-1} and force (±0.0001 N) 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 Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m704p035_supp.pdf). 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.

2.4. Statistical analysis

All statistical analyses were performed with R software for Mac OSX (v.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 CI and thread production were normally distributed, while the other metrics deviated slightly from a normal distribution. A Bonferroni test identified 2 significant shell growth outliers that were removed from further analyses.

Percent survival was determined as the average percent survival over the 3 mesocosm replicates, each containing 8 mussels (n = 3). The effect of temperature and food availability (fixed factors) on percent survival was assessed separately for each species using a 2-way ANOVA.

The effects of temperature and food availability on growth and attachment metrics (CI, shell growth, thread production, thread strength, and byssus strength) were assessed using random-intercept, linear mixed-effects models (LMEs; 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 & Weisberg 2019). Tukey HSD post hoc tests were used to perform pairwise comparisons between treatment combinations. The statistical significance of the interaction term in the 2-way ANOVA was used to assess whether the effect of the 2 factors was independent and additive or not independent and either synergistic or antagonistic (Slinker 1998). A significance value of $\alpha = 0.05$ was used for all statistical analyses.

Shell growth and tissue growth were used as 2 alternative indices of energetic scope across a state–space defined by gradients of temperature and food availability. Change in the length of the shell was used as an index of the change in the size of the organism and corresponds with volumetric growth (DEB; Kooijman 2010). Tissue growth was used as an index of the change in the mass of the organism and is correlated to energetic scope in net production models (SFG: Widdows & Bayne 1971, Bayne & Widdows 1983, Widdows & Johnson 1988; fish bioenergetics: Kitchell et al. 1977). Shell growth is arguably as vital in a growth process as tissue growth, as shell growth precedes tissue growth (Hilbish 1986). Calcification can also have a lower per unit cost associated with it than soft tissue, suggesting energy allocation to shell growth should not be secondary to organic tissue growth (Palmer 1992). 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.

3. RESULTS

Survival of *Mytilus galloprovincialis* over 10 wk was high across all treatments (96 ± 6% survival; mean ± SD) and did not depend on temperature or food availability (Fig. 4, Table 1; $F_{3,16} = 0.444$, p = 0.72 and $F_{1,16} = 1.442$, p = 0.25, respectively). Survival of *M. trossulus*, however, was lower overall (ranging 13–100% across all mesocosms) and decreased with increasing temperature (Fig. 4, Table 1; $F_{3,16} = 3.336$, p = 0.046). After 10 wk, mean survival of *M. trossulus* at 21°C was only 35% (Fig. 4); however, food availability had no effect on the survival of *M. trossulus* (Fig. 4, Table 1; $F_{1,16} = 0.167$, p = 0.69).

Tissue mass (g DW) of *M. trossulus* decreased by 45 ± 29% (mean ± SE), while that of *M. galloprovincialis* increased by 5.1 ± 0.2% over the course of the experiment. Initial CI was 5.1 ± 0.5 g cm^{-3} (mean ± SE) for *M. trossulus* and 4.5 ± 0.3 g cm^{-3} for *M. galloprovincialis*. Final CI of *M. galloprovincialis* was

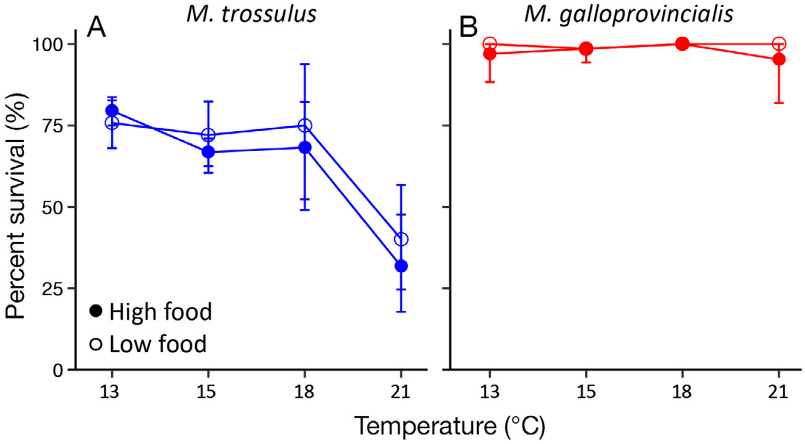

Fig. 4. Percent survival of *Mytilus trossulus* and *M. galloprovincialis* across a range of seawater temperatures under high or low food availability. Symbols and bars represent back-transformed mean ± SE; n = 3 replicate mesocosms per treatment

Table 1. Summary of 2-way ANOVA of the effects of temperature and food on percent survival of *Mytilus trossulus* and *M. galloprovincialis*. **Bold** indicates a significant effect (p < 0.05). For the case where the effect of temperature is statistically significant, Tukey HSD groupings indicate differences among the 4 experimental temperatures

Species	Factor	SS	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	SS	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		

approximately 2-fold that of *M. trossulus* (Fig. 5, Table 2). For *M. trossulus*, the final CI depended on temperature and food (p = 0.02 and p = 0.03, respectively; Fig. 5, Table 2); mussels at 21°C showed a 24% decrease in CI compared to mussels at 13°C, and low food availability decreased CI by 11%. For *M. galloprovincialis*, low food significantly decreased CI by 11%, but there was no effect of temperature (p = 0.01 and p = 0.52, respectively; Fig. 5, Table 2). There was no significant interaction between food availability and temperature on CI in either species (*M. trossulus*, p = 0.99, and *M. galloprovincialis*, p = 0.20; Fig. 5, Table 2).

M. trossulus had overall negligible shell growth, and there was no significant effect of temperature or food on shell growth (p = 0.12 and p = 0.14, respectively; Fig. 5, Table 2). For *M. galloprovincialis*, shell growth increased with higher temperature and greater food availability (p = 0.01 and p < 0.001, respectively). There was no significant interaction between temperature and food availability for either species (p = 0.23–0.30 for both species; Fig. 5, Table 2).

There was no significant effect of temperature or food availability on byssal thread production, thread strength, or byssus strength for either mussel species (Fig. 6; Table 3). However, the sample 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). There was a marginal effect of food on *M. galloprovincialis* thread production (p = 0.06; Fig. 5, Table 2).

Thread strength was not significantly correlated with shell or tissue growth for either species (Figs. 7 & S2, Tables 4 & S2). *M. trossulus* and *M. galloprovincialis* thread production was also not correlated with shell growth ($F_{1,6}$ = 1.842, p = 0.22 and $F_{1,6}$ = 2.753, p = 0.15, respectively) or tissue growth ($F_{1,6}$ = 0.582, p = 0.47 and $F_{1,6}$ = 2.226, p = 0.19, respectively; Fig. 7, Table 4).

4. DISCUSSION

Our 10 wk mesocosm experiment with 2 congeneric mussel species indicated that the effects of temperature and food influenced growth but did not significantly affect byssal thread strength or production. Across the experimental temperature range, *Mytilus 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 at higher temperatures, with negative tissue growth, minimal shell growth, and decreased CI and survival.

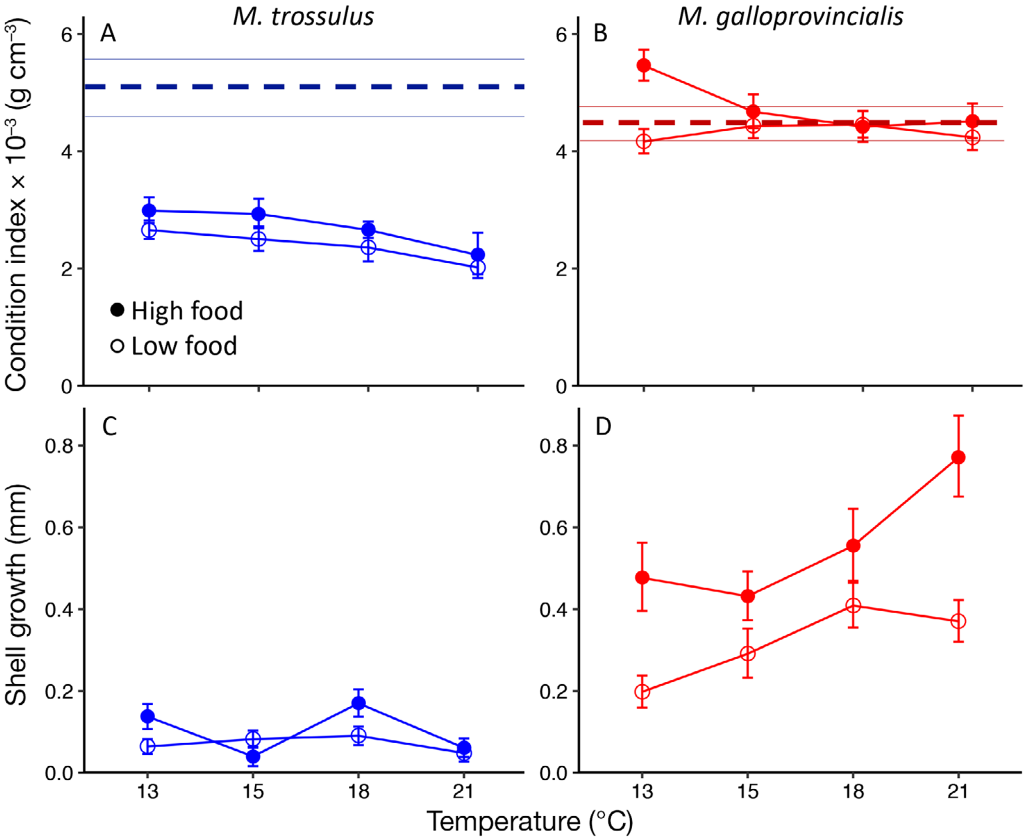

Fig. 5. Condition index (A,B) and shell growth (C,D) of *Mytilus trossulus* and *M. galloprovincialis* as a function of seawater temperature under high or low food conditions. (A,B) Initial condition index of *M. trossulus* (dark blue) and *M. galloprovincialis* (dark red) is represented with a dashed line (mean) and solid lines (±SE). Symbols and bars represent the final condition index as means ± SE, where SE is estimated as fixed-effects standard error from linear mixed-effects model analysis

4.1. 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 wk was 60% in the highest temperature treatment (21°C), which is comparable to previous reports at similar temperatures (e.g. 50% mortality after 3 wk; Braby & Somero 2006, Schneider 2008). *M. trossulus* has a lower tolerance to warm seawater temperatures than *M. galloprovincialis* (Braby & Somero 2006). Compared to *M. galloprovincialis*, warming conditions cause a greater decrease in the function of the *M. trossulus* ortholog of cytosolic malate dehydrogenase, an enzyme involved in mitochondrial energy production and other metabolic pathways (e.g. malate-aspartate/NADH shuttle, Fields et al. 2006). Warming conditions also cause a greater increase in heart rate for *M. trossulus* compared to *M. galloprovincialis* (Braby & Somero 2006). The temperature at which heat shock protein production occurs also differs between species (23°C and 25°C for *M. trossulus* and *M. galloprovincialis*, respectively), but experimental treatment temperatures remained below these values (21°C; Hofmann & Somero 1996, Feder & Hofmann 1999).

While temperature had a significant effect on the survival of *M. trossulus*, food availability did not affect the survival of either species. Greater temperatures can decrease immune response and increase susceptibility to disease (Monari et al. 2007, Mackenzie et al. 2014). Aside from disease, the lack of an effect of food availability could be explained by either (1) at these higher temperatures *M. trossulus* might not have had a feeding rate high enough to take advantage of the greater food supply and buffer against the cost of physiological stress at this temperature (Fig. 1A,C), or (2) mortality was primarily mediated by physiological stress mechanisms besides calorie limitation. In terms of calorie limitation, at the warmer end of the experimental range of temperatures, *M. trossulus* has reduced clearance rates, and

Table 2. Summary of linear mixed-effects models evaluating the fixed effects of temperature and food availability on condition index and shell growth for *Mytilus trossulus* and *M. galloprovincialis*. The random effect was mesocosm. **Bold** indicates a significant effect ($p < 0.05$). Where Temp and/or Food factors are significant, Tukey HSD groupings indicate differences among individual treatment levels

Species			Treatment	Condition index (g mm^{-3})			Shell (mm)		
				χ^2	df	p	χ^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	χ^2	df	p	χ^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

metabolic costs outpace assimilation of food at and above 15°C or 20°C, depending on the season (Fly & Hilbish 2013; Fig. 1C). Over the long term, reduced feeding at higher seawater temperatures could cause calorie limitation regardless of the amount of food available, at a temperature lower than the upper lethal limit (Kitchell et al. 1977). While we might assume calorie limitation causes animals to starve, aerobic scope and aerobic power budgeting theories are more directly linked with survival than calorie limitation in bivalves (Guderley & Pörtner 2010). In this conceptual model, superoptimal (pejus) temperature can increase demand for oxygen and limit the supply of oxygen (Guderley & Pörtner 2010); however, quantifying oxygen supply and demand was beyond the scope of this study. A more general explanation that may explain these results is that in the pejus temperature range, energy allocation to basal metabolic rate increases, resulting in trade-offs over the long term that can impact fitness parameters (reviewed by Sokolova 2021). Animals undergoing food limitation and/or physiological stress often conserve energy by undergoing metabolic depression. While this enables survival over a period of time (e.g. days, months, even years), animals have delayed energy costs, such as dealing with anaerobic end-products. As such, metabolic depression is incompatible with survival over the long term (reviewed by

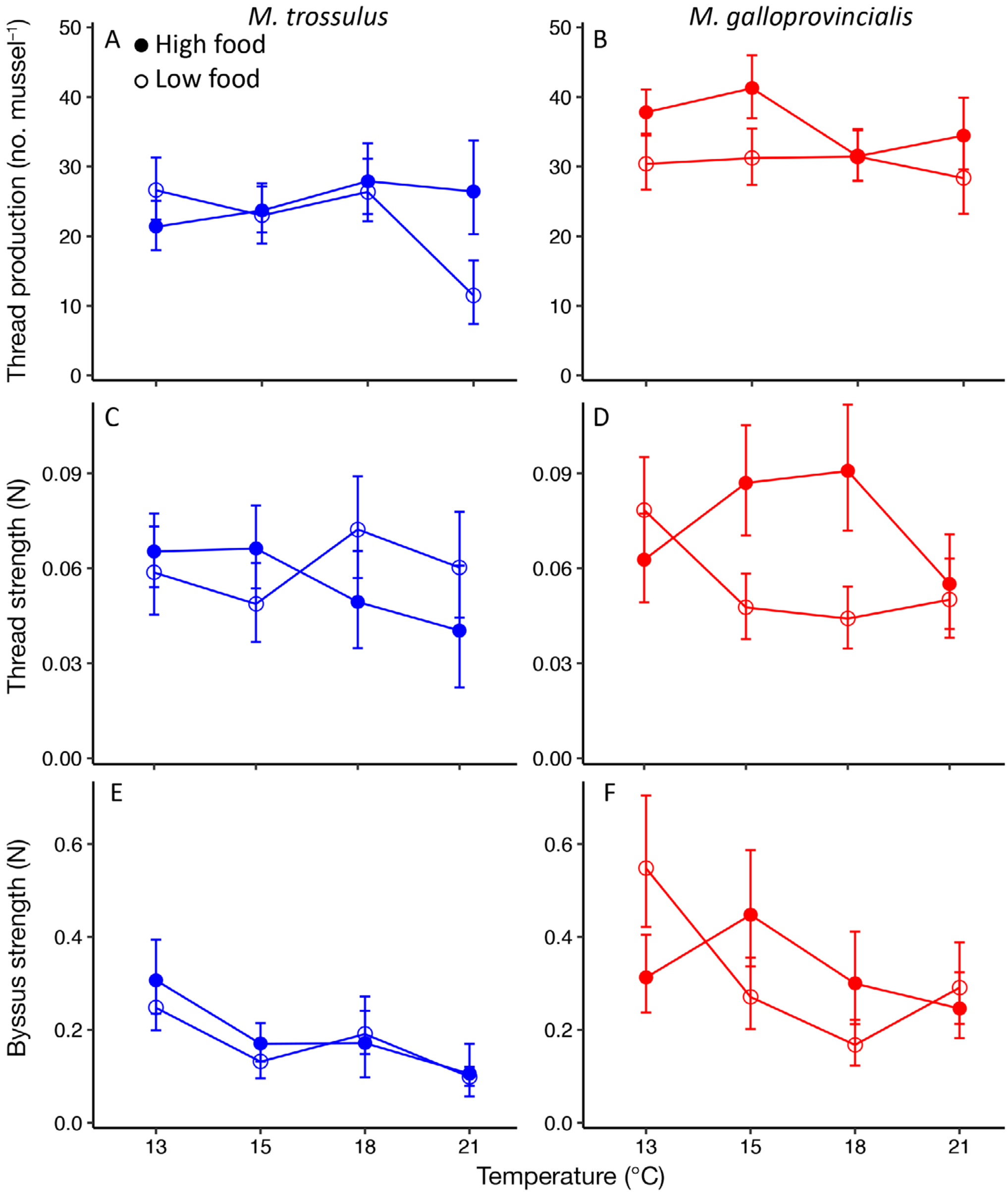

Fig. 6. *Mytilus trossulus* and *M. galloprovincialis* byssal thread quantity and quality as a function of seawater temperatures under high or low food conditions. (A,B) Byssal thread production per mussel over 15 d; (C,D) byssal thread strength and (E,F) byssus strength. Symbols and bars represent means ± SE, where SE is estimated as fixed-effects standard error from the linear mixed-effects model analysis

Sokolova 2021) and could have caused mortality for *M. trossulus* at the greatest experimental temperatures. From a neurophysiological perspective, bivalves undergoing thermal stress modulate oxidative stress and mortality through the catecholamine system and other neuroendocrine systems (reviewed by Liu et al. 2018). Greater temperatures affect the neuroendocrine control of the circulation of seawater in the mantle cavity in bivalves (*Ostrea virginica*: Galtsoff 1928; *M. eduilis*: Catapane et al. 1978, 1979; *Crassostrea virgininca*: Carroll & Catapane 2007), which has implications for feeding, gas exchange, and elimination of waste products. Interestingly, intertidal mussels that are exposed to acute thermal stress at low tide, but experience temperatures near their thermal optimum for feeding when submerged at high tide, have greater survival when supplied with a greater amount of food (Schneider et al. 2010). In summary, the ability to ingest and assimilate food resources is critical for organisms to tolerate and recover from physiological stress, but the link between energetics and survival is complex given

Table 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 *Mytilus trossulus* and *M. galloprovincialis*. The random effect was mesocosm. None of the analyses showed a significant effect ($p < 0.05$)

Species	Factor	Thread production (no. mussel^{-1})			Thread strength (N)			Byssus strength (N)		
		χ^2	df	p	χ^2	df	p	χ^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
		χ^2	df	p	χ^2	df	p	χ^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

the ability of animals to persist in conditions that are ultimately incompatible with long-term survival by delaying costs associated with basal metabolism (metabolic depression; Sokolova 2021). While the link between survival and thermal and nutritional conditions can be complex, changes in organism size and mass are often more directly linked to thermal and nutritional conditions (e.g. Kitchell et al. 1977, Kooijman 2010, Sokolova 2021).

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. Temperature and higher food availability increased *M. galloprovincialis* shell growth, but the CI only increased under high food availability, consistent with the finding that shell growth can precede the growth of soft tissue (*Mytilus trossulus*: Hilbish 1986). These patterns of growth in terms of size and tissue mass are consistent with published relationships between temperature, food level, and SFG for this species. For *M. galloprovincialis,* SFG typically increases with temperature due to higher clearance rates, and this increase is counteracted by only a small increase in metabolic cost (Fly & Hilbish 2013).

In contrast, high temperature and high food availability decreased the CI 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. A loss in tissue mass may reflect an energetic deficit, or negative SFG. Warming above 12°C decreases clearance rates and increases metabolic costs for *M. trossulus* (Fly & Hilbish 2013). Temperature and lower food availability caused a decrease in *M. trossulus* CI, which is consistent with SFG values of this species in this range (Fly & Hilbish 2013). This data suggests that CI (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. Further, the effects of food availability and temperature did not interact for metrics of biomass or growth (*M. galloprovincialis*: shell growth; *M. trossulus*: CI), supporting the simplifying assumption made in bioenergetics models that the effects of temperature and food availability are additive, rather than synergistic or antagonistic (Kitchell et al. 1977, Kooijman 2010).

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 are influenced by the seawater temperature in which they are produced (Newcomb

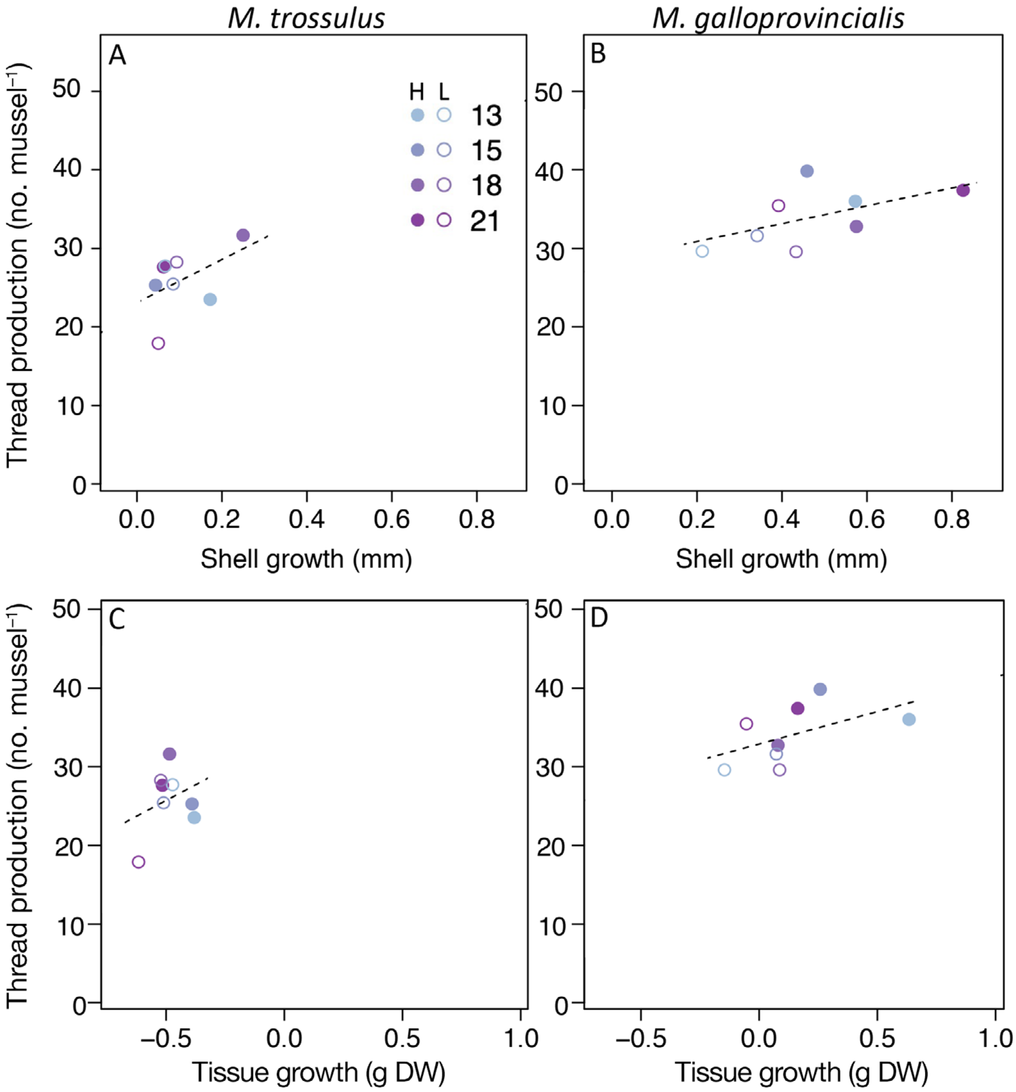

Fig. 7. Thread production of *Mytilus trossulus* and *M. galloprovincialis* as a function of (A,B) shell growth and (C,D) tissue growth across the experimental temperature and food levels. Measurements are binned by treatment. See inset for treatment combination. H (L): high (low) food availability; numbers are experimental temperatures (°C)

2015). Specifically, in short-term experiments (3 d), *M. trossulus* exposed to temperatures above 21°C produced fewer, weaker byssal threads. Our longer-term exposures (10 wk) 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 & Carrington 2006). Our study was conducted in late summer–early autumn, and it is possible that the threads were too weak, due to

Table 4. Summary of linear regression analyses of the relationship between thread production (no. mussel^{-1}) and tissue growth (mg) and shell growth (mm) of *Mytilus trossulus* and *M. galloprovincialis* across all temperature and food treatments. None of the analyses showed a significant effect ($p < 0.05$)

	Factor	SS	df	*F*	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			

the time of the 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 (up to ~0.3 N for both species, Newcomb 2015; also see Babarro & Reiriz 2010, O'Donnell et al. 2013, Newcomb et al. 2019). This difference in thread strength could also be due to methodological differences. After byssal threads are produced, it takes ~8–12 d for the byssal thread plaque, a structure at the distal region of the byssal thread, to achieve full strength (George & Carrington 2018). We sampled all attached threads produced over 2 wk, which likely represents a broad distribution of thread ages and strengths for each individual.

4.2. Energetic state and allocation to byssal threads

Bioenergetic studies range from quantitative models that predict the biological effects of environmental variability to work that deepens our understanding of the complexity of physiological processes involved in energy flux. Our goal was to examine energy allocation assumptions that underly the relationship between the biosynthesis of body mass and that of a key material structure (byssus). Using growth of body tissue (biomass) and shell length growth as proxies for energetic state, and given that *M. trossulus* had a loss in CI, negative tissue, and absence of shell growth, this species appeared to exhibit negative SFG across all experimental treatments. In contrast, *M. galloprovincialis* had positive shell growth and increase in body tissue, suggesting this species likely exhibited positive SFG across all treatments. We hypothesized that mussels in treatments in which there was greater growth 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. The lack of a significant correlation between either growth metric and byssus, and the lack of an effect of treatment on byssus, provides more evidence for the prioritization of byssal thread production during energy limitation ('trade-off' hypothesis) than for the 'product' hypothesis. This idea is further supported by the fact that *M. trossulus* produced byssal threads under conditions that produced a loss in tissue mass and no shell growth.

Prioritization of energy allocation towards byssal threads has been proposed previously. A study by Clarke (1999) found that mussels prioritize energy allocation to byssal threads over many other organismal needs, such as the cost of maintaining tissue. Under conditions of food availability below levels required to maintain their tissue mass, zebra mussels *Dreissena polymorpha* allocated assimilated carbon toward byssus production and most likely used stored reserves to produce byssus (3 wk acclimation; Clarke 1999). Further, byssal thread production can occur despite the loss of glycogen stores in *M. galloprovincialis* (Babarro et al. 2008, Babarro & Reiriz 2010), suggesting that other factors besides energetics may play a role. Mussels use glycogen as an energy reserve, and under short-term starvation, glycogen stores are not reduced in large individuals but may be depleted in 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 d acclimation; Babarro et al. 2010), and starvation reduces small *M. galloprovincialis* thread production, but not the thread production of larger individuals (1 wk acclimation; Babarro et al. 2008).

In the high temperature treatment, *M. trossulus* lost tissue mass, and energy budget calculations suggest that the cost of byssus production is sufficient to account for this loss in mass. A SFG model based on the relationship between thread production induced by mechanical perturbation of byssus and its effect on growth estimated thread production costs at ~1 J thread^{-1} for juvenile *M. trossulus* (Roberts et al. 2021). In the low food, high temperature treatment, *M. trossulus* produced on average 18 threads per mussel. Given an energetic cost of producing a thread of 1 J thread^{-1} (Roberts et al. 2021), we can calculate that producing 18 threads cost an equivalent of ~0.4 g of a loss in growth in terms of tissue mass. In this treatment, *M. trossulus* tissue mass decreased ~0.6 g over the 6 wk experiment. This calculation suggests that the cost of byssus could account for up to 60 % of the overall net loss in tissue. In the other treatments, mussels produced an average of ~25 threads. Assuming a similar cost per byssal thread produced, thread production may have cost ~0.5 g of tissue mass. In comparison, in these treatments, *M. trossulus* tissue mass decreased 0.3–0.5 g over the 6 wk experiment. While the cost of byssus estimate was generated with juvenile mussels and may not be transferrable to adult mussels, these calculations do emphasize that the cost of the production of byssus can be quite large, and that this cost may remain substantial even in conditions producing a low energetic state. Future work quantifying energetic trade-offs between induced byssus production and mussel condition in adult mussels with limited calorie intake may be key to quantifying the cost of byssus for these organisms.

A limitation of this study is that we used growth of body tissue and shell growth as proxies for differences in energetic scope across environmental gradients. Future work that estimates assimilation, metabolic costs, and energetic scope across gradients in temperature and food will provide a clearer understanding of the complex system that is organism energetics. This future work may shine a clearer light on energy allocation rules relating growth and the production of structural biomaterials, such as byssus.

Our experiment did not detect differences in byssus quality and quantity across the range of food and temperature conditions, and did not detect a significant relationship between byssus quality or quantity, with either metric of growth. It is possible, however, that the production of a greater number of byssal threads in energetically favorable conditions might decrease growth substantially, and that this cost could mask a potential positive relationship between energetic state and byssus quality and quantity in controlled conditions. Further experiments that explore the relationship between byssus and energetic state across a broader range of conditions, manipulate byssal thread production behavior, model the cost of byssus as a fixed cost, and evaluate experimental energetic state, might have the sensitivity to uncover a potential relationship between byssus and energetic state. In the present study, the observed growth from September to November correlated with summer SFG estimates using data from the literature, but not fall SFG (Fly & Hilbish 2013, data not shown), highlighting the need for concurrent energetic measurements in future growth experiments that account for seasonality and acclimation to fixed or dynamic temperatures.

While thread production may limit growth, other costly processes including reproduction may be prioritized over growth and/or thread production. Seasonal reproductive cycles may present energy allocation trade-offs for byssal thread attachment (Carrington 2002, Moeser & Carrington 2006, Zardi et al. 2007). Gametogenesis is energetically costly (Seed & Suchanek 1992), and spawning is a highly stressful event that can weaken individuals and cause mortalities (Myrand et al. 2000) and weaken byssus (Lachance et al. 2008). The induction of spawning in the laboratory results in weaker attachment and smaller thread diameter for spawned and unfed *M. galloprovincialis* (Babarro & Reiriz 2010), and lower thread strength for *M. edulis* (Hennebicq et al. 2013). On rocky shores, mussel attachment (Carrington 2002, Moeser & 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; Skidmore & Chew 1985), which may explain the lower physical condition, greater mortality, and weak byssus for this species. The 2 species were relatively similar in terms of shell length and initial condition index (*M. galloprovincialis* was 20 % greater by length); final *M. galloprovincialis* CI, however, was approximately 3 times that of *M. trossulus*. Any size difference might have consequences for suspension feeding, metabolic costs, and metabolic reserves (Kooijman 2010). Larger mussels also have a greater overall metabolic cost of tissue maintenance (Kooijman 2010, Sebens 2002), but may be able to tolerate stressful situations due to greater energetic reserves in the form of stored glycogen concentration in their tissues (Babarro & Reiriz 2010). Like many animals (Gillooly et al. 2001), larger mussels also have a lower mass-specific normalized cost, which could result in lower per-gram maintenance needs that would otherwise compete with other processes. Larger mussels also have a greater gill area and greater clearance rates (related to surface area; Kooijman 2010), which may contribute to stress tolerance as well (Babarro et al. 2008).

Prioritized energy allocation to byssal thread production may impact other fitness parameters, and the cost of producing additional threads can decrease growth (Carrington et al. 2015, Sebens et al. 2018). 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. A. Roberts pers. obs.). 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 et al. 2021) and 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. While the effects of food and temperature on both metrics of growth did not interact, there was a marginal interaction between these 2 factors on thread production, and there was a trend towards mussels subject to high temperature and low food conditions producing few threads. This pattern suggests that chronic high temperature may modulate byssal thread production differently than expected from energetic limitation alone.

Within the range of seawater conditions tested, which are representative of the upper range of local summer conditions, *M. trossulus* showed a loss in tissue mass and at the greatest temperature had low survival. However, this species does survive in the Salish Sea. Mortality of *M. trossulus* in the 21°C temperature treatment did not typically occur within the first few hours or days of exposure, but reached >50% over the course of the 2 mo experiment. Summer temperatures typically range over 12–16°C, with only brief excursions >18°C (Roberts 2019), suggesting that we might expect greater survival in the field wherever higher temperatures are not prolonged. This region also experiences high phytoplankton biomass in warm surface water in the summer months (Lowe et al. 2016). We expect a higher quantity and quality of food in the field compared to the lab, potentially leading to greater growth. SFG models for mytilid mussels have been used to quantify predictions of survival at a range of sites (Fly et al. 2015). Quantified predictions of growth in Salish Sea field conditions are reported in a separate paper (Roberts 2019). In the future, understanding how mussels perform under temperature exposures of different lengths of time may provide a better picture of survival, growth, and attachment in a world where temperature extremes are more common. Models that are based on findings from shorter-term experiments (e.g. a few days or weeks) may predict that *M. trossulus* attachment is more sensitive to temperature (e.g. low attachment above 18°C) and that mortality is less sensitive to temperature (Newcomb et al. 2022).

At this time of the year (August 2016), *M. trossulus* CI in the field was ~2 times greater than that for *M. trossulus* in the lab, shell growth was similar or greater in the field (~0.6 to ~1.2 mm), and mortality in the field in August ranged ~5–60%, depending on depth, which encompasses the range of mortality observed in experimental treatments (Roberts 2019). Within the experimental range of seawater conditions, and during this season, 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 (Sutherland et al. 2011, Mackas et al. 2013). Farmed populations of *M. trossulus* in the Salish Sea can experience mortality events in early autumn (I. Jeffords pers. comm.), 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 that 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. This work provides insight on the perturbation of energetic state as a result of a 2 mo long exposure to gradients in food and temperature conditions. Shorter-term acute thermal stress, as might be experienced with increased frequency and magnitude of marine heat waves, may affect byssus strength and number (Newcomb 2015), and acclimation to thermal stress may influence the trade-off between growth and survival, as has been previously observed in intertidal systems (Schneider et al. 2010, Fitzgerald-Dehoog et al. 2012). 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, Newcomb et al. 2019, 2022).

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, US Department of Commerce, under project number NA14OAR4170078 (to E.C. and C. S. 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 US government is authorized to reproduce and distribute this paper for governmental purposes.

LITERATURE CITED

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

Babarro JMF, Fernández Reiriz MJ, Labarta U (2008) Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: the influence of size and food availability. J Mar Biol Assoc UK 88:783–791

Bayne BL, Newell RC (1983) Physiological energetics of marine molluscs. In: Saleuddin ASM, Wilbur KM (eds) The Mollusca. Academic Press, Cambridge, MA, p 407–515

Bayne BL, Thompson RJ, Widdows J (1976) Physiology 1. In: Bayne, BL (ed) Marine mussels: their ecology and physiology, Vol. 10. Cambridge University Press, Cambridge

Bell EC, Gosline JM (1996) Mechanical design of mussel byssus: material yield enhances attachment strength. J Exp Biol 199:1005–1017

Black BA (2009) Climate-driven synchrony across tree, bivalve, and rockfish growth-increment chronologies of the northeast Pacific. Mar Ecol Prog Ser 378:37–46

Blanchette CA, Helmuth B, Gaines SD (2007) Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at Point Conception, California, USA. J Exp Mar Biol Ecol 340:126–148

Braby CE, Somero GN (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

Brazee SL (2004) An interspecific comparison of biomechanical properties and morphometrics of mussel byssal threads. PhD dissertation, University of Rhode Island, Kingston, RI

Brazee SL, Carrington E (2006) Interspecific comparison of the mechanical properties of mussel byssus. Biol Bull 211:263–274

Carrington E (2002) The ecomechanics of mussel attachment: from molecules to ecosystems. Integr Comp Biol 42:846–852

Carrington E, Moeser GM, Dimond J, Mello JJ, Boller ML (2009) Seasonal disturbance to mussel beds: field test of a mechanistic model predicting wave dislodgment. Limnol Oceanogr 54:978–986

Carrington E, Waite JH, Sarà G, Sebens KP (2015) Mussels as a model system for integrative ecomechanics. Annu Rev Mar Sci 7:443–469

Carroll MA, Catapane EJ (2007) The nervous system control of lateral ciliary activity of the gill of the bivalve mollusc, Crassostrea virginica. Comp Biochem Physiol A Mol Integr Physiol 148:445–450

Catapane EJ, Stefano GB, Aiello E (1978) Pharmacological study of the reciprocal dual innervation of the lateral ciliated gill epithelium by the CNS of *Mytilus edulis* (Bivalvia). J Exp Biol 74:101–113

Catapane EJ, Stefano GB, Aiello E (1979) Neurophysiological correlates of the dopaminergic cilio-inhibitory mechanism of *Mytilus edulis.* J Exp Biol 83:315–323

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

Crosby M, Gale L (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. J Shellfish Res 9:233–237

Curiel-Ramirez S, 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

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

Dowd WW, Felton CA, Heymann HM, Kost LE, Somero GN (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*). J Exp Mar Biol Ecol 449:171–185

Edmunds PJ (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol Oceanogr 56:2402–2410

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. Mar Biol 156:39–53

Feder ME, Hofmann GE (1999) Heat-Shock Proteins, molecular chaperones, and the stress response: Evolutionary and Ecological Physiology. Annu Rev Physiol 61:243–282

Fields PA, Rudomin EL, Somero GN (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. J Exp Biol 209:656–667

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

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*). J Sea Res 66:403–410

Fitzgerald-Dehoog L, Browning J, Allen BJ (2012) Food and Heat Stress in the California Mussel: Evidence for an Energetic Trade-off Between Survival and Growth. Biol Bull 223:205–216

Fly EK, Hilbish TJ (2013) Physiological energetics and biogeographic range limits of three congeneric mussel species. Oecologia 172:35–46

Fly EK, Hilbish TJ, Wethey DS, Rognstad RL (2015) Physiology and biogeography: the response of European mussels (*Mytilus* spp.) to climate change. Am Malacol Bull 33:136–149

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks, CA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Galtsoff PS (1928) The effect of temperature on the mechanical activity of the gills of the oyster (*Ostrea virginica* Gm.). J Gen Physiol 11:415–431

George MN, Carrington E (2018) Environmental post-processing increases the adhesion strength of mussel byssus adhesive. Biofouling 34:388–397

George MN, Pedigo B, Carrington E (2018) Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. J R Soc Interface 15:20180489

Gillooly, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251

Guderley H, Pörtner HO (2010) Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. Can J Zool 88:753–763

Hawkins AJS, Bayne BL (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

Helm MM, Bourne N (2004) Hatchery operation: broodstock conditioning, spawning and fertilization. In: Lovatelli A (ed) Hatchery culture of bivalves. A practical manual. FAO Fish Tech Paper 471. FAO Fisheries Department, Rome, p 59–83

Hennebicq R, Fabra G, Pellerin C, Marcotte I, Myrand B, Tremblay R (2013) The effect of spawning of cultured mussels (*Mytilus edulis*) on mechanical properties, chemical and biochemical composition of byssal threads. Aquaculture 410-411:11–17

Hettinger A, Sanford E, Hill TM, Hosfelt JD, Russell AD, Gaylord B (2013) The influence of food supply on the response of Olympia oyster larvae to ocean acidification. Biogeosciences 10:6629–6638

Hilbish TJ (1986) Growth trajectories of shell and soft tissue in bivalves: seasonal variation in *Mytilus edulis* L. J Exp Mar Bio Ecol 96:103–113

Hilbish TJ, Brannock PM, Jones KR, Smith AB, Bullock BN, Wethey 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. J Biogeogr 37:423–431

Hofmann GE, Somero GN (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. Mar Biol 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. World Meteorological Organization, Geneva

Jakobsen HH, Markager S (2016) Carbon-to-chlorophyll ratio for phytoplankton in temperate coastal waters: seasonal patterns and relationship to nutrients. Limnol Oceanogr 61:1853–1868

Kearney M, Simpson SJ, Raubenheimer D, Helmuth B (2010) Modelling the ecological niche from functional traits. Philos Trans R Soc B 365:3469–3483

Kearney MR, Matzelle A, Helmuth B (2012) Biomechanics meets the ecological niche: the importance of temporal data resolution. J Exp Biol 215:922–933

Kellermann V, Chown SL, Schou MF, Aitkenhea I and others (2019) Comparing thermal performance curves across traits: How consistent are they? J Exp Biol 222:193433

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

Kitchell JF, Stewart DJ, Weininger D (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 SALM (2010) Dynamic energy budget theory for metabolic organisation. Cambridge University Press, Cambridge, MA

Lachance AA, Myrand B, Tremblay R, Koutitonsky V, Carrington E (2008) Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. Aquat Biol 2:119–129

Lesser MP, Bailey MA, Merselis DG, Morrison JR (2010) Physiological response of the blue mussel *Mytilus edulis* to differences in food and temperature in the Gulf of Maine. Comp Biochem Physiol A 156:541–551

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191

Liu Z, Li M, Yi Q, Wang L, Song L (2018) The neuroendocrine-immune regulation in response to environmental stress in marine bivalves. Front Physiol 9:1456

Lockwood BL, Somero GN (2011) Invasive and native blue mussels (genus *Mytilus*) on the California coast: the role of physiology in a biological invasion. J Exp Mar Biol Ecol 400:167–174

Lowe AT, Roberts EA, Galloway AW (2016) Improved marine-derived POM availability and increased pH related to freshwater influence in an inland sea. Limnol Oceanogr 61:2122–2138

Mackas D, Galbraith M, Faust D, Masson D and others (2013) Zooplankton time series from the Strait of Georgia: Results from year-round sampling at deep water locations, 1990–2010. Prog Oceanogr 115:129–159

Mackenzie CL, Lynch SA, Culloty SC, Malham SK (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 AJ, Sarà G, Montalto V, Zippay M, Trussell GC, Helmuth B (2015) A bioenergetics framework for integrating the effects of multiple stressors: Opening a 'black box' in climate change research. Am Malacol Bull 33: 150–161

Melzner F, Stange P, Trübenbach K, Thomsen J and others (2011) Food supply and seawater pCO2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis.* PLOS ONE 6:e24223

Menge BA (1992) Community regulation: under what conditions are bottom-up factors important on rocky shores? Ecology 73:755–765

Menge BA, Lubchenco J, Bracken MES, Chan F and others (2003) Coastal oceanography sets the pace of rocky intertidal community dynamics. Proc Natl Acad Sci USA 100: 12229–12234

Moeser GM, Carrington E (2006) Seasonal variation in mussel byssal thread mechanics. J Exp Biol 209:1996–2003

Monaco CJ, McQuaid CD (2018) Applicability of Dynamic Energy Budget (DEB) models across steep environmental gradients. Sci Rep 8:16384

Monari M, Matozzo V, Foschi J, Cattani O, Serrazanetti GP, Marin MG (2007) Effects of high temperatures on functional responses of haemocytes in the clam *Chamelea gallina.* Fish Shellfish Immunol 22:98–114

Murray JW, Roberts E, Howard E, O'Donnell M and others (2015) An inland sea high nitrate-low chlorophyll (HNLC) region with naturally high pCO_2. Limnol Oceanogr 60: 957–966

Myrand B, Guderley H, Himmelman JH (2000) Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. Mar Ecol Prog Ser 197:193–207

Newcomb LA (2015) Elevated temperature and ocean acidification alter mechanics of mussel attachment. PhD dissertation, University of Washington, Seattle, WA

Newcomb LA, George MN, O'Donnell MJ, Carrington E (2019) Only as strong as the weakest link: structural analysis of the combined effects of elevated temperature and pCO_2 on mussel attachment. Conserv Physiol 7:coz068

Newcomb LA, Cannistra AF, Carrington E (2022) Divergent effects of ocean warming on byssal attachment in two congener mussel species. Integr Comp Biol 62:700–710

Nisbet RM, Jusup M, Klanjscek T, Pecquerie L (2012) Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. J Exp Biol 215:892–902

O'Donnell MJ, George MN, Carrington E (2013) Mussel byssus attachment weakened by ocean acidification. Nat Clim Chang 3:587–590

Page HM, Hubbard DM (1987) Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. J Exp Mar Biol Ecol 111:159–179

Palmer AR (1992) Calcification in marine molluscs: how costly is it? Proc Natl Acad Sci USA 89:1379–1382

Pinheiro J, Bates D, DebRoy S, Sarkar D, Team TRC (2013) nlme: linear and nonlinear mixed effects models. R package version 3.1–118. http://CRAN.R-project.org/package=nlme (accessed October 2019)

Pörtner HO (2012) Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar Ecol Prog Ser 470:273–290

Pörtner HO, Farrell AP (2008) Physiology and climate change. Science 322:690–692

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ramajo L, Pérez-León E, Hendriks IE, Marbà N and others (2016) Food supply confers calcifiers resistance to ocean acidification. Sci Rep 6:19374

Riisgård HU, Egede PP, Barreiro Saavedra I (2011) Feeding behaviour of the mussel, *Mytilus edulis*: new observations, with a minireview of current knowledge. J Mar Biol 2011:1–13

Roberts EA (2019) Resource allocation to growth and structure: the cost of mussel attachment in a dynamic coastal environment. PhD dissertation, University of Washington, Seattle, WA

Roberts EA, Newcomb LA, McCartha MM, Harrington KJ, LaFramboise SA, Carrington E, Sebens KP (2021) Resource allocation to a structural biomaterial: Induced production of byssal threads decreases growth of a marine mussel. Funct Ecol 35:1222–1239

Schneider KR (2008) Heat stress in the intertidal: comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. Biol Bull 215: 253–264

Schneider KR, Wethey DS, Helmuth BST, Hilbish TJ (2005) Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. Mar Biol 146:333–343

Schneider KR, Van Thiel LE, Helmuth B (2010) Interactive effects of food availability and aerial body temperature on the survival of two intertidal *Mytilus* species. J Therm Biol 35:161–166

Sebens KP (2002) Energetic constraints, size gradients, and size limits in benthic marine invertebrates. Integr Comp Biol 42:853–861

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

Seed R, Suchanek TH (1992) Population and community ecology of *Mytilus*. In: Gosling EG (ed) The mussel *Mytilus*: ecology, physiology, genetics, and culture. Elsevier, Amsterdam, p 87–169

Skidmore D, Chew KK (1985) Mussel aquaculture in Puget Sound. Tech Rep WSG 85-4. Washington Sea Grant Program, University of Washington, Seattle, WA

Slinker BK (1998) The statistics of synergism. J Mol Cell Cardiol 30:723–731

Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr Comp Biol 53:597–608

Sokolova I (2021) Bioenergetics in environmental adaptation and stress tolerance of aquatic ectotherms: linking physiology and ecology in a multi-stressor landscape. J Exp Biol 224:jeb236802

Strathmann MF (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, Seattle, WA

Sutherland DA, MacCready P, Banas NS, Smedstad LF (2011) A model study of the Salish Sea estuarine circulation. J Phys Oceanogr 41:1125–1143

Tagliarolo M, Montalto V, Sarà G, Lathlean JA, McQuaid CD (2016) Low temperature trumps high food availability to determine the distribution of intertidal mussels *Perna perna* in South Africa. Mar Ecol Prog Ser 558:51–63

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

Todgham AE, Stillman JH (2013) Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integr Comp Biol 53:539–544

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

Westerbom M, Mustonen O, Jaatinen K, Kilpi M, Norkko A (2019) Population dynamics at the range margin: implications of climate change on sublittoral blue mussels (*Mytilus trossulus*). Front Mar Sci 6:292

Widdows J, Bayne BL (1971) Temperature acclimation of *Mytilus edulis* with reference to its energy budget. J Mar Biol Assoc UK 51:827–843

Widdows J, Johnson D (1988) Physiological energetics of *Mytilus edulis*: scope for growth. Mar Ecol Prog Ser 46: 113–121

Zardi GI, McQuaid CD, Nicastro KR (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

Zippay ML, Helmuth B (2012) Effects of temperature change on mussel, *Mytilus*. Integr Zool 7:312–327

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media, New York, NY

Editorial responsibility: Jana Davis,
Annapolis, Maryland, USA
Reviewed by: J. M. Babarro, T. Sanders, C. J. Monaco

Submitted: October 5, 2022
Accepted: December 16, 2022
Proofs received from author(s): January 23, 2023