

Energetic scope limits growth but not byssal thread production of two *Mytilid* mussels

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ARTICLE INFO

Keywords:

Bioenergetics
Energy budget
Resource allocation
Biomaterial
Byssus
pH
Dissolved oxygen
Mytilus trossulus
Mytilus galloprovincialis

ABSTRACT

Anthropogenic warming of the ocean and atmosphere, concurrent with ocean acidification and deoxygenation, has made it even more pressing to quantify the link between environmental stressors and marine organism population dynamics. In marine environments, low food availability, low feeding rates, and/or increased metabolic costs can cause energetic limitation. Energetic limitation affects some functional traits, such as growth rates of body tissue and reproductive output. Other functional traits that are linked with short-term survival are often prioritized in conditions of energy limitation, despite their energetic cost. Mussels are ecosystem engineers in rocky shore ecosystems, and they produce byssal threads to attach to hard substrate and aquaculture line. Previous 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 the number of byssal threads produced. 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 period of greater hypoxia and low pH. Overall, this work suggests that some functional traits, such as shell and tissue growth, are energetically-constrained while other functional traits, such as mussel byssal thread production, may be best described as a fitness trade-off.

1. Introduction

Anthropogenic ocean warming, acidification, and deoxygenation (Bopp et al., 2013; Keeling et al., 2010; Kroeker et al., 2012; Masson-Delmotte et al., 2021) have emphasized the need to quantify the relationships between environmental conditions and the sizes, abundances, and population dynamics of marine organisms (Carrington et al., 2015; Kraus et al., 2015). One approach to quantifying this link is to evaluate the impact of multiple physiological stressors, such as seawater temperature, pH, and dissolved oxygen, on organism physiology and survival (Folt et al., 1999; Gunderson et al., 2016; Sokolova, 2013). The integration of multiple ecophysiological modeling frameworks and methodologies provides an opportunity to better characterize the effect of multiple environmental factors on organism growth, and survival.

There are two major approaches to using ecological and physiological data to predict future changes in populations; correlative and mechanistic models. Correlative models evaluate the relationship between biological and environmental variables, often working with species distribution and abundance data across spatial and temporal gradients (ecological niche modeling - Soberon and Nakamura, 2009). While correlative models can offer greater precision, due to their simplicity (e. g. evapotransmission of coastal dune plants - Robertson et al., 2003), models that include mechanistic linkages between the functional traits of organisms and their environments (Kearney et al., 2010) may offer insights into biological patterns that may be more generalizable to novel future scenarios (Buckley et al., 2010; Kearney and Porter, 2009). Integrating these methodologies may better inform climate predictions, the uncertainty surrounding these predictions, and future key research

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<https://doi.org/10.1016/j.jembe.2023.151927>

Received 17 November 2022; Received in revised form 25 June 2023; Accepted 28 June 2023

Available online 13 July 2023

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areas necessary for gaining a better understanding what to expect in future novel scenarios.

Environmental factors often have non-linear effects on metabolic rates, feeding rates, and growth (Brown et al., 2004; Bruno et al., 2015; Kitchell et al., 1977). Ingestion of food saturates at high food levels (Kooijman, 2010) and the shape of this curve depends on factors including predator-prey interactions and the time it takes to digest and process food (Holling, 1959). Metabolic rates generally increase exponentially with temperature (metabolic scaling - Brown et al., 2004; Bruno et al., 2015) and decline precipitously when thermal optima are exceeded (Iles, 2014; Schulte et al., 2011). The duration of exposure to hypoxic and anoxic conditions can affect physiology and survival (Steckbauer et al., 2011), while average DO may not fully represent the impact of the duration of exposure to a stressor. Many models of organism growth quantify the effect of temperature on the amount of energy acquired from feeding and the cost of metabolism across this same temperature range (e.g. Bayne and Newell, 1983; Kitchell et al., 1977). For many species of fish and invertebrates, including *Mytilid* mussels, feeding optima and the subsequent decline in feeding at higher temperatures occurs at a lower temperature than that of metabolic rate, leading to a starvation temperature that is below the physiological upper lethal limit of the organism (Bayne and Newell, 1983; Kitchell et al., 1977). While it is crucial to represent non-linear effects of environmental factors on physiology, information theory suggests that limiting complexity of mathematical representations of these relationships is also important when building mechanistic models (Levin, 1992).

The combined effects of food limitation and temperature on organism feeding, metabolic costs, and growth have been quantified with mechanistic models (Bioenergetics Bayne and Newell, 1983). Energetic limitation can occur when food availability is low, feeding is limited by temperature, and/or metabolic costs are high (Kitchell et al., 1977), though the negative effects of energetic limitation on growth and survival may be alleviated by metabolic depression and acclimation (Sokolova, 2021). While bioenergetic methods for predicting growth from temperature and food-driven energetic limitation are well developed (e.g. Filgueira et al., 2011; Kitchell et al., 1977; Kooijman, 2010; Sokolova, 2021, 2013), there is less work that integrates energetic limitation with non-linear effects of other seawater characteristics (dissolved oxygen, pH, salinity) on organism energetics and growth in field environments (Jørgensen et al., 2016; Kraus et al., 2015; but see - Pousse et al., 2020).

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 (Bayne and Newell, 1983; Kooijman, 2010; van der Veer et al., 2006). 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 et al., 2021).

While the growth of the soft tissue and shell of mussels are often uncoupled (Hilbish, 1986) and often limited by energetic constraints (Bayne, 2004; Beiras et al., 1994; Filgueira et al., 2011; Hilbish and Koehn, 1985), there are several lines of evidence that suggest that production of byssus is not subject to the same energetic constraints. First, byssal thread production is key to survival and a quantitative model of this trade-off suggests that there is an evolutionary advantage to investing in byssus even in conditions of low energy availability (Sebens et al., 2018). Second, byssal thread production induced by severing existing threads daily can decrease growth by up to 66% (Roberts et al., 2021). Third, byssus is produced under conditions of energetic limitation (Clarke, 1999), and low survival (Roberts, 2019), regardless of long-

term nutritional or temperature stress (Roberts, 2019). While these studies suggest that byssal thread production is “constitutive,” or 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 (Carrington et al., 2009; Moeser and Carrington, 2006; Moeser et al., 2006; Newcomb, 2015; 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., 2019, 2018; O'Donnell et al., 2013), and reproductive state (Babarro and Reiriz, 2010), could potentially affect byssal thread production in the field.

Mussel energetics can 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 and minus excretion (Fig. 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, Fig. 1B), and that byssal thread production, as an adaptive investment, is produced constitutively across this same gradient of energetic scope (Fig. 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 (Fig. 1A). For example, low salinity could decrease carbonate ion abundance and directly affect the cost of shell growth (Sanders et al., 2018), while hypoxia (Sanders et al., 2014) or low pH (Seibel et al., 2012) could indirectly limit shell growth by affecting feeding rate or metabolic costs. We hypothesize that physiological stress might also affect a constitutive response, byssal thread production, independent of energetic scope (Fig. 1B).

We tested these hypotheses using two congeneric mussel species with differing ecological niches, *Mytilus trossulus*, a species with a lower tolerance of high thermal stress, and *Mytilus galloprovincialis*, a species with a lower tolerance of low salinity (Braby and Somero, 2006; Evans and Somero, 2010). The effect of temperature on energetic scope is well-described for both species; increasing temperature decreases energetic scope for *M. trossulus*, but often increases energetic scope of *M. galloprovincialis* (Fly and Hilbish, 2013). In contrast, there may be less of a difference between the two species of the effect of hypoxia (De Zwaan et al., 1991; Sanders et al., 2014) and low pH. 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 (Fig. 1B and C). 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 (Fig. 1B and C).

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 (~9-10 m 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 (George et al., 2019; Newcomb, 2015).

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 *a*) as well as the growth, attachment,

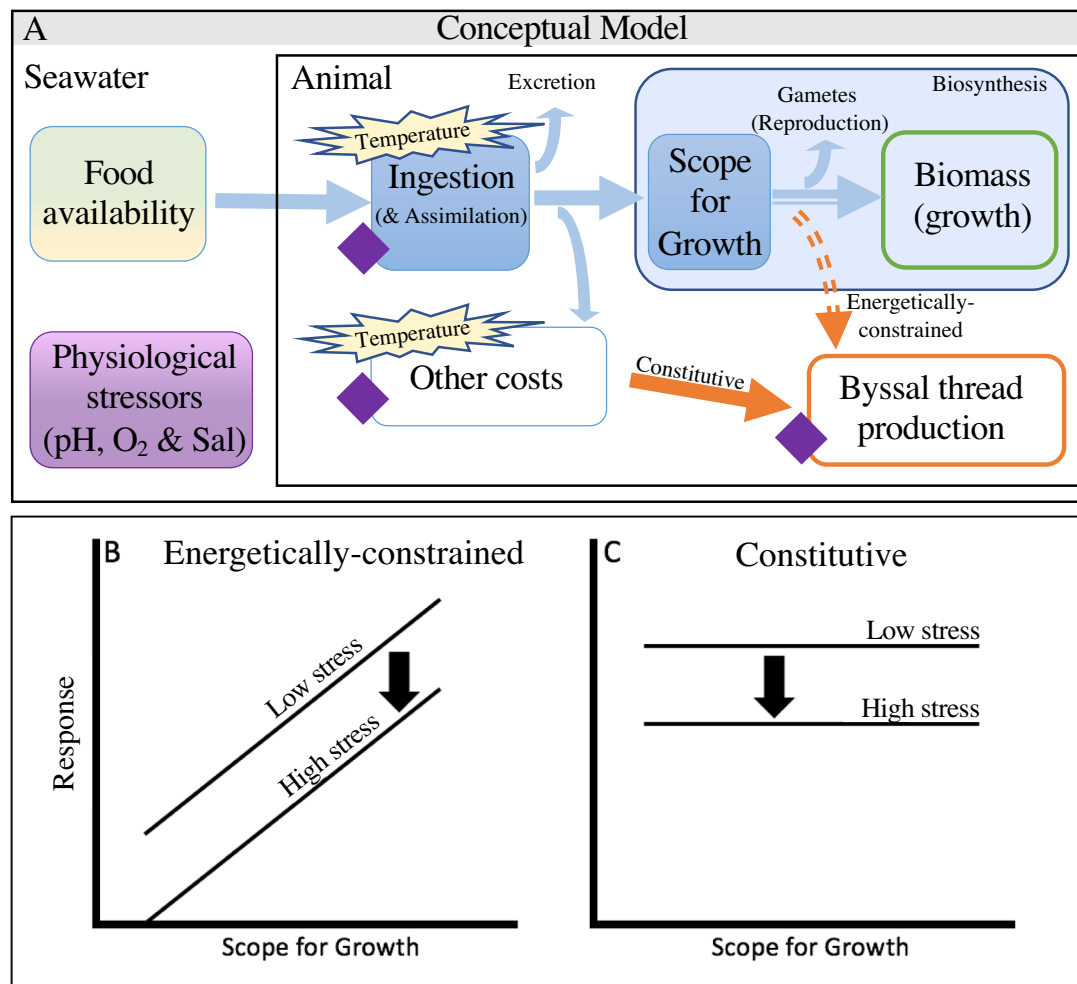


Fig. 1. Schematic representation of effects of food availability and other physiological stressors on mussel growth, reproduction, and attachment, using a Scope for Growth (SFG) framework.

(A) We hypothesized that food availability and temperature affect the theoretical variable, SFG, which in turn determines biosynthesis of tissue (both reproductive and somatic) and shell growth (B, low stress). We hypothesize that byssus, on the other hand, is produced constitutively and is not subject to the same energetic constraints as growth (C, low stress). Both energetically-constrained (e.g. food-limited) and constitutive responses are potentially reduced by other physiological stressors (purple diamond), such as high temperature, low salinity, hypoxia or low pH (B and C, high stress). See text for details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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.

2. 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).

2.1. 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 1 m and 7 m below the surface. Each sonde was equipped with four sensors: temperature and conductivity (accuracy $\pm 0.5\%$; YSI #599870), pH (accuracy ± 0.1 pH units; YSI #599701),

dissolved oxygen (accuracy $\pm 1\%$; YSI #599100-01), and chlorophyll *a* concentration $\mu\text{g L}^{-1}$ (precision 0–100 $\mu\text{g L}^{-1}$; YSI #599103-01). Water temperature ($^{\circ}\text{C}$), salinity (psu), pH, dissolved oxygen concentration (DO, mg L^{-1}), and chlorophyll *a* concentration (Chl *a*, $\mu\text{g L}^{-1}$) were recorded as hourly averages of 10-min 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 *a*, Kingscote Chemicals, Miamisburg, OH, USA; #106023). The conductivity sensor was calibrated every three months against a 50,000 $\mu\text{S cm}^{-1}$ conductivity standard (YSI #3169). Missing salinity data at 7 m, 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 1 m salinity plus an offset of +5.2 psu up to a maximum of 30 psu.

2.2. Mussel collection and transplantation

M. trossulus and *M. galloprovincialis* were collected from aquaculture lines at 1 m and 7 m 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 (Fig. 2). The cages were constructed from HDPE vexas plastic mesh (15 cm × 15 cm × 20 cm, 1 cm² 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 adults and 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.

2.3. 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 growth over two weeks. Each mussel was tethered to an acrylic plate to prevent aggregation (two rectangular 15 cm × 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. Mussels were given a 15 day period to attach because byssal thread production levels off by ~2 weeks after byssus is severed for *M. galloprovincialis* (Babarro et al., 2008). A 2 week period was also chosen for comparison with other studies (Roberts and Carrington, 2023) that rely on evidence that *Mytilus trossulus* byssal thread adhesive strength is maximal after ~2 weeks (George et al., 2018). 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. Plates with byssal threads were dried and the number of threads produced by each mussel was counted. The mantle tissue (Carrington, 2002) and in very ripe mussels, additional gonadal

tissue from the posterior dorsal side of the mussel (Eggermont et al., 2020), was dissected from the shell as gonadal tissue. The remaining somatic tissue was dissected from the shell and gonadal and somatic tissue were 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). Condition index (CI, g cm⁻³) (Lucas and Beninger, 1985) 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.

2.4. 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 0.1 mm. 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, the cages were replenished by transplanting mussels to the cages 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 (cm³).

2.5. 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

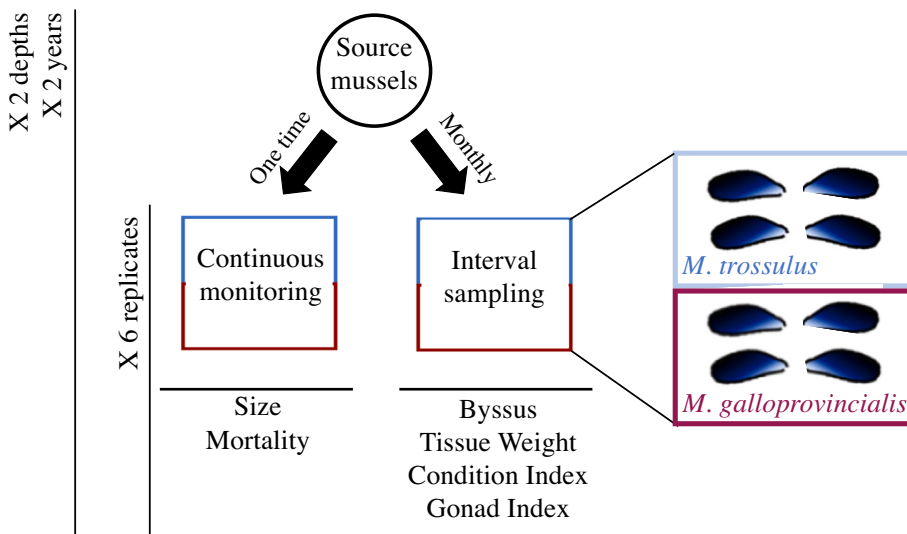


Fig. 2. Schematic of the experimental transplant design to test for the effects of species and seawater conditions on mussel growth, byssus production, and survival at two depths.

Mussels were collected in the spring each year (2016 and 2017) and transplanted into mesh bags at 1 m and 7 m depth to acclimate. These “source” mussels were transferred from the mesh bags into nearby experimental cages suspended from the same mussel farm raft at the same depth either once for continuous monitoring or monthly for interval sampling. For continuous monitoring, cages were established to follow changes in shell growth and mortality of the population over time. These non-destructive measurements 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stress from temperature, salinity, DO, pH and food limitation over the course of the 5 years of seawater monitoring. Upper thermal tolerance thresholds for byssal thread production are known to differ between *M. trossulus* and *M. galloprovincialis*; a threshold of $>18^{\circ}\text{C}$ for *M. trossulus* and $>25^{\circ}\text{C}$ *M. galloprovincialis* was used, based on byssus production measurements of Newcomb et al. (2019, 2022). Heart rate measurements followed a similar pattern to byssal thread production with respect to temperature (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 ($<2\text{ mg L}^{-1}$) (Anestis et al., 2010; Jakubowska and Normant, 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 and Carrington, 2018; 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 $0.5\text{--}6.3\text{ }\mu\text{g Chl a L}^{-1}$ 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 $6\text{ }\mu\text{g Chl a L}^{-1}$ 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.

2.6. 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 (2019). The average temperature ($^{\circ}\text{C}$) and chlorophyll ($\mu\text{g L}^{-1}$) over each growth period was determined over the two-year study period, and these values were used to calculate SFG. In summary, 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 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.

The numerical Scope for Growth model is adapted from Roberts et al. (2021) with modifications to include chlorophyll *a* and temperature as inputs and predict growth dynamically over time. 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:

$$\text{SFG} = E(\text{Chl}, T) - R(T), \quad (1)$$

where *E* is the energy intake (J), and *R* is the cost of tissue maintenance (J) and both are a function of temperature. 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):

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

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 eq. 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 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* (Bayne and Newell, 1983; Jones et al., 1992, Table 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):

$$R(T) = b(T) \times TM_{\text{initial}}^c, \quad (3)$$

Table 1

Summary of parameter calculations for the Scope for Growth model, adapted from Roberts et al. (2021). The model had five input parameters, each estimated separately for *M. trossulus* (abbrev. as *M. t.*) and *M. galloprovincialis* (*M. g.*) 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							
<i>a</i> (<i>T</i>)	Intake coefficient		$<10^{\circ}\text{C}$ $>10^{\circ}\text{C}$	8.81	−23.3		
				−2.69	83.1		
<i>b</i> (<i>T</i>)	Cost coefficient		$<10^{\circ}\text{C}$ $>10^{\circ}\text{C}$	5.45	7.25		
				7.97	−4.2		
<i>d</i>	Intake exponent		$<10^{\circ}\text{C}$ $>10^{\circ}\text{C}$	8.99	−16.6		
				0.69	0.01		
<i>e</i>	Cost exponent			1			
C.F.	Energetic conversion factor			21.6	1.6		
Measured values used to calculate input parameters							
<i>R</i>	Respiration		$<10^{\circ}\text{C}$ $>10^{\circ}\text{C}$	0.0078	−0.004	$R = \text{slope} \times \text{temp} + \text{int}$	Fly and Hilbish, 2013 0.469 g DW
				0.0088	−0.016		
<i>CR</i>	Clearance rate		$<10^{\circ}\text{C}$ $>10^{\circ}\text{C}$	0.6801	−1.796	$CR = \text{slope} \times \text{temp} + \text{int}$	
				−0.2074	6.413		
				0.426	0.5667		

Table 2Summary of seawater temperature (°C), salinity (psu), pH (NBS), dissolved oxygen (mg L⁻¹), and chlorophyll *a* concentration (µg L⁻¹) at Penn Cove from 2014 to 2019.

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

Reported values are seasonal mean ± SD of hourly measurements at each depth.

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 ($e = 1$; Kooijman, 2010; Sará et al., 2013), thus b has units of J mg⁻¹. The equation for 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 within the range from 5 °C to 20 °C, and the standard error was estimated as the average standard error from each temperature ($b(T) = mT + int$, Table 1). Akaike Information Criteria (AIC) comparisons were used to evaluate the appropriateness of using a linear function to approximate the relationship between temperature and respiration over the small temperature range (2–25 °C). Specifically, for *M. galloprovincialis*, the better fit over 5–25°C was a linear function (AICc = 2, BIC = 2), and for *M. trossulus* the better fit was the exponential equation (AICc = 5, BIC = 5). Neither model was strongly supported over the other (AIC comparison were < 10), and the linear model was used to approximate the relationship for both species over this small temperature range. The autumn values were then converted to daily values to yield the metabolic cost coefficient (b ; J mg⁻¹; Table 1) (Riisgård and Randløv, 1981).

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

$$a(T) = CR(T) \times I_m \times AE, \quad (4)$$

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 linear regressions of autumn CR measurements by Fly and Hilbish (2013) of the ‘upslope’ and ‘downslope’ regions of the CR curve, representing the regions below and above the thermal optima across the temperature range of 5 °C to 20 °C. Linear regressions were chosen to approximate these temperature relationships given the few temperatures available to parameterize these functions. The standard error was estimated as the average standard error from each temperature (Fly and Hilbish, 2013, Table 1). The average AE was determined across all temperatures since there was no linear correlation with temperature (Fly and Hilbish, 2013, Table 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:

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

The relationship of estimated SFG and somatic growth, shell growth, and byssal thread production was then evaluated.

2.7. 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. Results

Environmental conditions, including temperature, salinity, DO, pH, and chlorophyll, varied seasonally and with depth (Fig. 3, Fig. 4, Table 2). In general, there was a greater range in temperature near the surface at 1 m depth compared to at 7 m depth. Seawater was warmer in the summer and colder in the winter at 1 m depth than at 7 m (Fig. 3, Fig. 4, Table 2). Salinity varied with season, especially at 1 m depth, with the lowest salinities measured in the autumn and winter (Fig. 3, Fig. 4, Table 2). pH also varied seasonally and was on average lower at 7 m depth; pH was generally lower in the summer and autumn at 7 m depth (Fig. 3, Fig. 4, Table 2). DO was relatively stable and elevated (10 mg L⁻¹) at 1 m, but more variable at 7 m, where the lowest DO levels were observed July – November (Fig. 3, Fig. 4, Table 2). Chlorophyll was near or above saturating levels (~6 µg L⁻¹) all year except for winter, when chlorophyll was often below threshold levels for maximal consumption rates (<0.5 µg L⁻¹; Fig. 3, Table 2).

Temperatures frequently exceeded the thermal physiological stress threshold for *Mytilus trossulus* (>18 °C) at 1 m depth in the late summer and autumn each year (Fig. 4, Fig. 5), but never exceeded the *Mytilus galloprovincialis* threshold. Salinity often dropped below the physiological threshold for *M. galloprovincialis* (14 psu) at 1 m depth in the autumn, winter, and spring but did not exceed the *M. trossulus* threshold (Fig. 4, Fig. 5). Physiological stress thresholds of low DO and pH were exceeded primarily at 7 m depth (Fig. 4, Fig. 5). Hypoxia (<2 mg L⁻¹) and low pH (<7.2) at 7 m depth were most prevalent during the summer

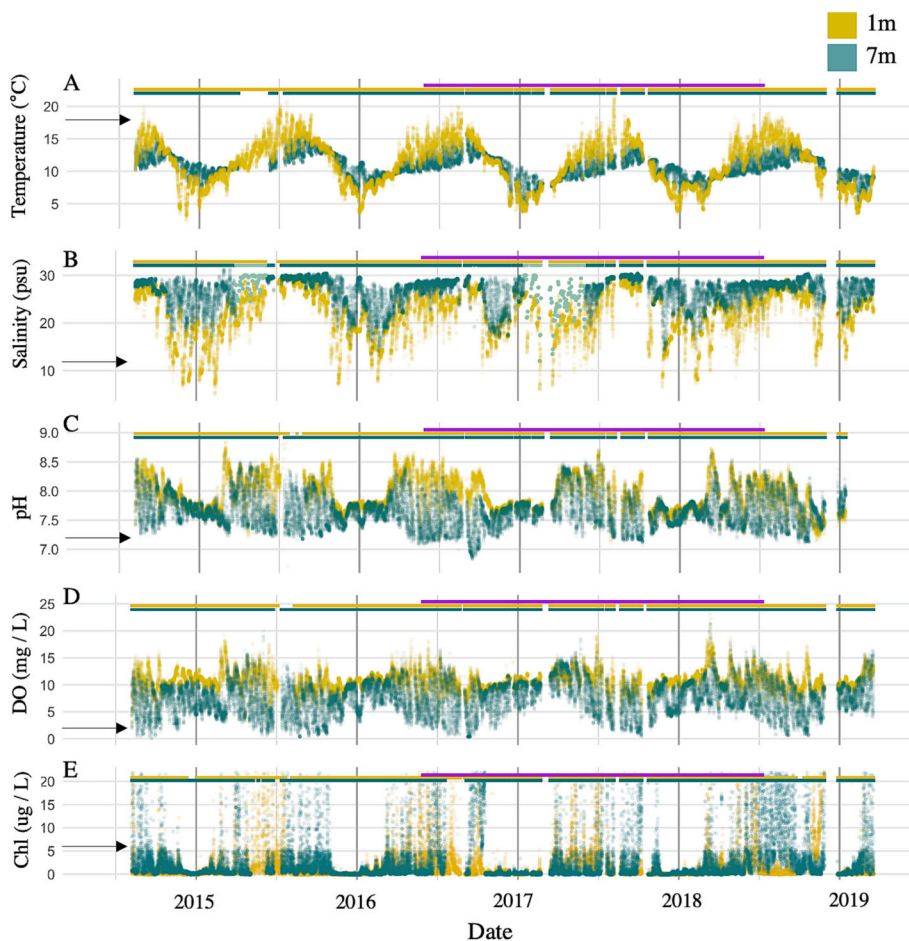


Fig. 3. Environmental conditions at Penn Cove from 2014 to 2019.

(A) Seawater temperature ($^{\circ}\text{C}$), (B) salinity (psu), (C) pH (NBS scale), (D) dissolved oxygen (DO, mg L^{-1}), and (E) chlorophyll (Chl, $\mu\text{g L}^{-1}$). Symbols represent hourly measurements at both shallow (1 m, yellow) and deep (7 m, 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 7 m represent daily estimates of missing data, see text for details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and autumn each year, and were temporally correlated (Fig. 4, Fig. 5). Hypoxic conditions lasted up to 20 h per day. Hypoxia ($<2 \text{ mg L}^{-1}$) occurred up to 30–40% of the week at 7 m depth in late summer-early autumn 2015–2019 (Fig. 5). Low pH (<7.2) occurred up to 50–60% of the week in late summer-early autumn 2015 and 2016 (Fig. 5). Near-anoxic conditions ($0\text{--}0.5 \text{ mg L}^{-1}$) also occurred at 7 m 10–20% of the time during 1–2 weeks in late-August 2015 and mid-September 2016. Lower pH (<7.0) was also observed over these same weeks, and pH remained below 7.0 (NBS scale) for 10–20% of the week during these periods. Chlorophyll was below saturating levels ($<6 \mu\text{g L}^{-1}$) in the winter (Fig. 4, Fig. 5).

For *M. trossulus*, there was a significant interaction between the effects of season and depth on byssal thread production (Fig. 6; $p = 0.03$, Table S1, Table S2), 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 (Fig. 6; $p = 0.02$, Table S1, Table S2).

Shell growth for both species was variable at 1 m, but shell growth at 7 m depth remained below $<0.25 \text{ cm month}^{-1}$ for both species, regardless of season (Fig. 7). Two major *M. trossulus* mortality events were observed at 7 m 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 1 m depth in June 2016, with a monthly mortality of 35%. *M. galloprovincialis* had $<20\%$ monthly mortality across periods sampled (Fig. 7).

At the end of each experimental period, GI was 1.2–2 times greater at 1 m compared to 7 m and was typically lowest in summer for both cohorts and both species (Fig. 8, Table S3 and S4). CI of both species generally decreased throughout the experimental period (Fig. 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), and CI was greater at 1 m compared to 7 m for the first cohort and not the second cohort for both species (Table S3). TM remained relatively constant for both species in 2017–2018, although TM was variable in 2016–2017 for *M. galloprovincialis* (Fig. 8). Shell length increased over the duration of the experiment for both species and cohorts ($\sim 30\text{--}50\%$, Fig. 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 ($F(2,21) = 11.95$, $p = 0.003$, $\text{Adj-R}^2 = 0.49$), and both low pH and SFG were significant predictors of somatic tissue growth ($p < 0.001$, $p = 0.001$, respectively, Fig. 9, Table 3). For shell growth of this species, the best regression model ($F(3, 19) = 4.03$, $p = 0.02$, $\text{Adj-R}^2 = 0.29$) included acute temperature stress exposure as a non-significant predictor ($p = 0.21$, Table 3, Fig. 9) and SFG and low DO as significant predictors of shell growth ($p = 0.21$, $p = 0.013$, $p = 0.031$, Table 3, Fig. 9). For byssal thread production of this species, no significant regression was found ($F(1,22) = 2.66$, $p = 0.12$, $\text{Adj-R}^2 = 0.07$), 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 ($F(2, 21) = 8.30$, $p = 0.002$, $\text{Adj-R}^2 = 0.39$), and both low pH and SFG were significant predictors of somatic tissue growth (Table 3, Fig. 9). For shell growth of this species, the best model was only a marginal regression ($F(2, 21) = 3.36$, $p = 0.05$, $\text{Adj-R}^2 = 0.17$) and the effects of SFG and low DO were significant ($p = 0.02$, $p = 0.05$, Table 3, Fig. 9). For byssal thread production of this species, no significant regression was found ($F(1, 22) = 4.06$, $p = 0.06$, $\text{Adj-R}^2 = 0.12$), and the

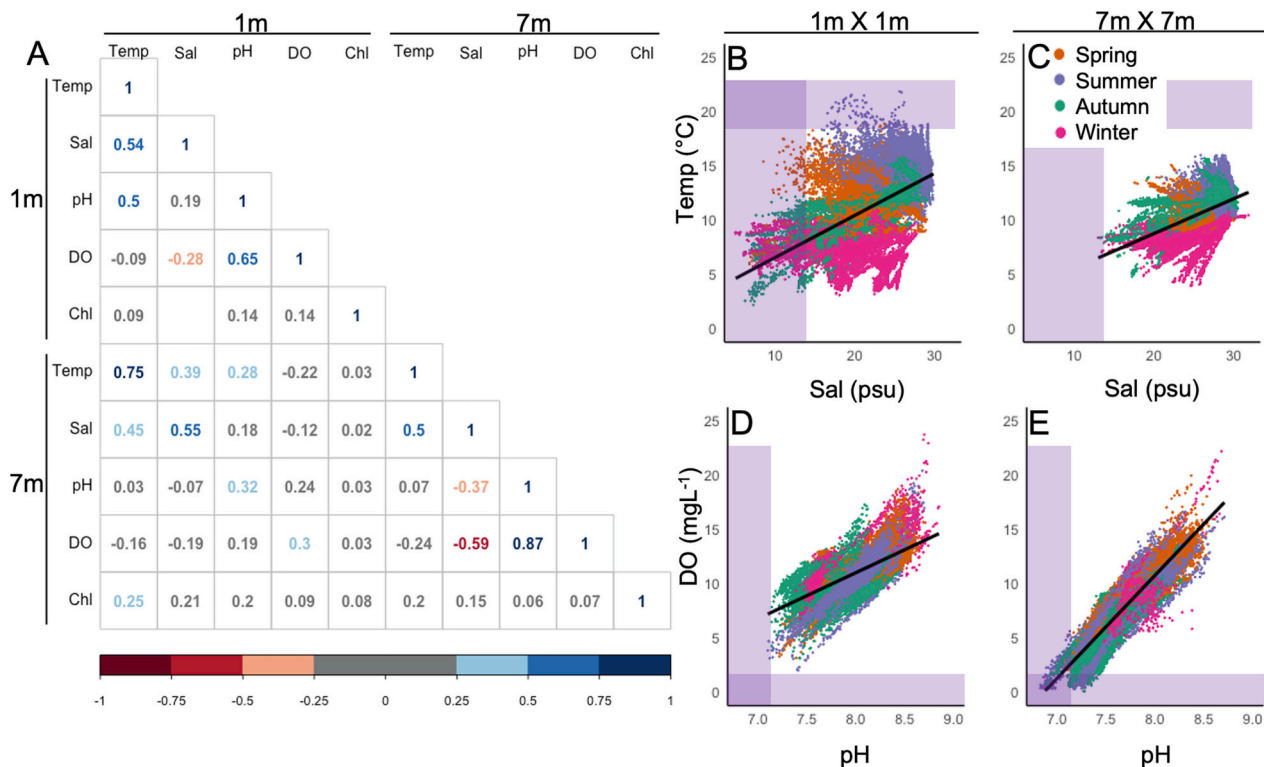


Fig. 4. Summary of seawater conditions at two depths (1 m and 7 m) at Penn Cove from 2014 to 2019. 2014–2015 data are from Newcomb (2015). (A) Correlation coefficients determined from a pairwise Pearson correlation test for all parameters compared across the two depths ($\alpha = 0.001$). (B and C) Temperature ($^{\circ}\text{C}$) as a function of salinity (psu) at deep (7 m) and shallow (1 m) 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 1 m depth in the summer to $+0.43$ at 7 m depth in the winter, $\alpha = 0.01$). (D and E) Dissolved oxygen (mg L^{-1}) was positively correlated with pH (NBS scale) at both 1 m and 7 m depths. Symbols represent hourly data and are colour-coded by season (spring = orange, summer = blue, autumn = green, winter = pink). Shaded regions (purple) indicate conditions that exceeded the physiological stress thresholds (purple) for temperature, salinity, pH, and DO. Descriptive statistics for each parameter are summarized in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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) and thread production did not significantly correlate with SFG or depth for either species (range $p = 0.14$ to $p = 0.80$, Table S3).

4. 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. Byssal thread production was relatively consistent over time except for a trend of lower byssal production during periods of low pH (*M. trossulus*) or low salinity (*M. galloprovincialis*). These field observations are consistent with laboratory reports of limited growth, but not byssal thread production, in conditions of energetic limitation (Clarke, 1999; Roberts and Carrington, 2023), 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 (Roberts et al., 2021; Sebens et al., 2018).

Consistent with laboratory measurements by Fly and Hilbish (2013), SFG estimates were maximal for *M. trossulus* at 10°C , and temperatures at 7 m depth were often closer to this thermal optimum than temperatures at 1 m (Figs. 3, 5). Fly and Hilbish (2013) reported a negative relationship between SFG and temperature for *M. galloprovincialis* in the autumn (Fly and Hilbish, 2013). Consistent with these data, SFG estimations were maximal for *M. galloprovincialis* at deeper depths where

temperatures were cooler (Figs. 3, 5). Estimated SFG was also lower in the fall and winter for both species, reflecting the negative impact of large size and low food availability in the winter season.

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 (Fig. 1). Hypoxia ($< 2 \text{ mg L}^{-1}$) and low pH (< 7.2 NBS) conditions were prevalent at 7 m depth, occurring $\sim 25\%$ 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; George et al., 2019; Newcomb, 2015). DO and pH were tightly correlated (Fig. 4), and periods of longer exposures to low DO and low pH often co-occurred with high energetic scope (late summer and autumn - Fig. 3), suggesting that energetic limitation and was out of phase with energetic limitation (Gunderson et al., 2016).

Either low pH or low DO, which were highly temporally correlated at 7 m 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* (Gu et al., 2019; Sanders et al., 2014). 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). Alternatively, high SFG might buffer against the effect of low DO and low pH might otherwise have on the production of biomaterials such as byssal threads. In Baltic *Mytilus trossulus*, high food availability is

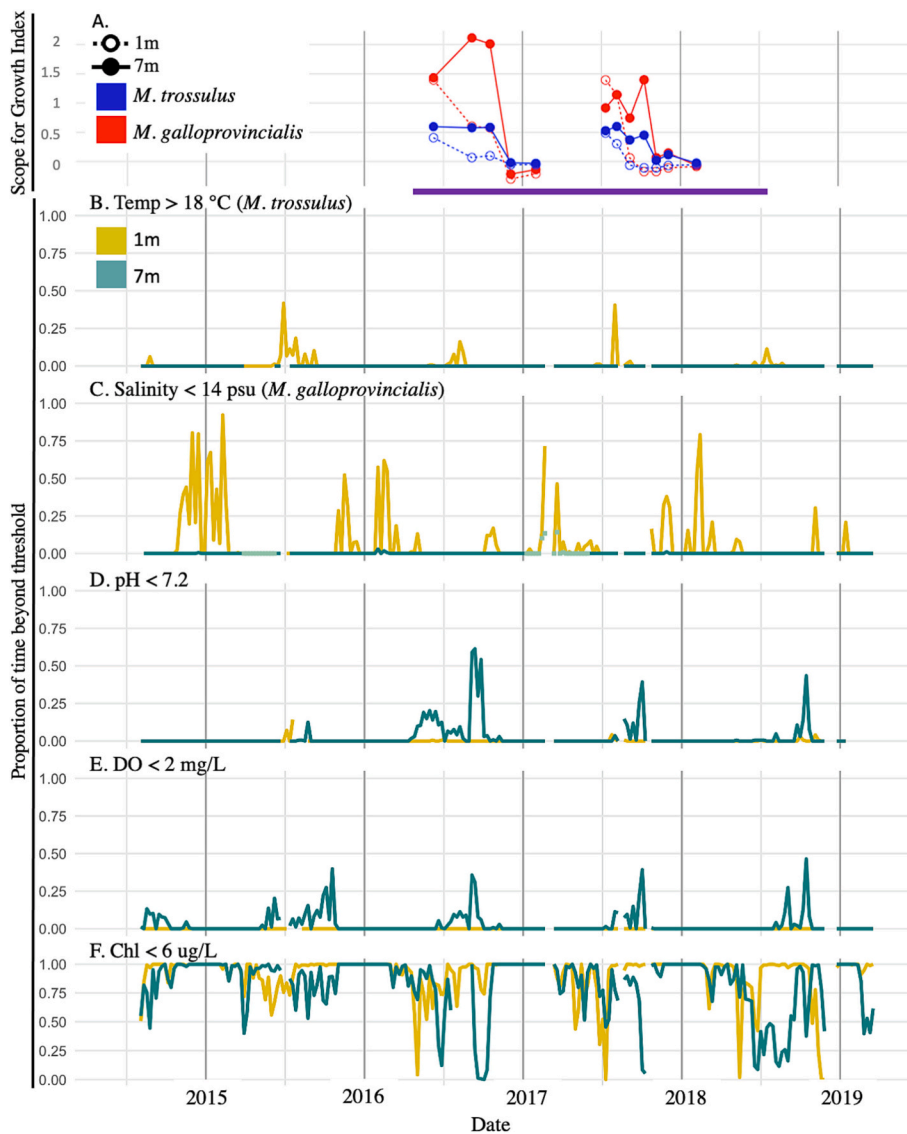


Fig. 5. Time series of Scope for Growth and physiological stressors.

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 to 2019. Symbols represent quasi-monthly SFG estimates at 1 m depth (open circles) and 7 m depth (closed circles) for *M. trossulus* and *M. galloprovincialis* (A). Lines represent the proportion of time per week a threshold tolerance was exceeded at 1 m depth (yellow) and 7 m depth (blue, B–F). (A) High temperature stress ($> 18^{\circ}\text{C}$) 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, $< 2\text{ mg L}^{-1}$) and food limitation (E, chlorophyll concentrations $< 6\text{ }\mu\text{g L}^{-1}$). The horizontal line (purple) represents the timing of mussel growth and byssus production measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

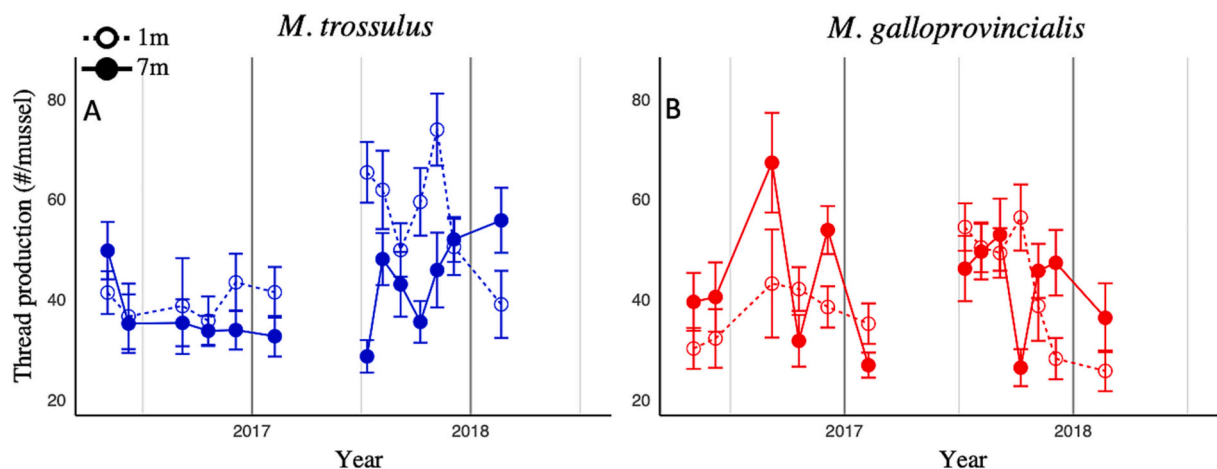


Fig. 6. Time series of thread production.

Thread production ($\# \text{ mussel}^{-1}$) over two weeks for *M. trossulus* (A) and *M. galloprovincialis* (B) at 1 m depth (shallow, open circles) and 7 m 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.

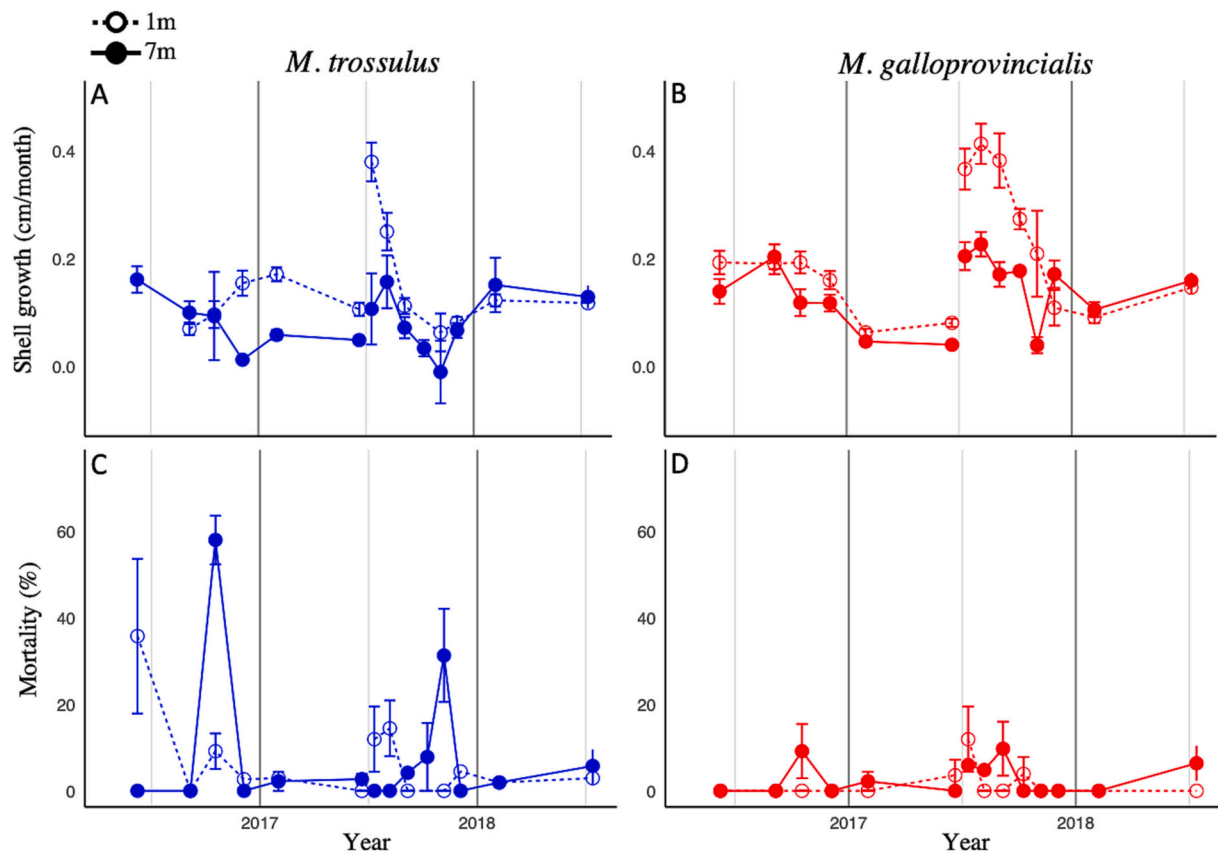


Fig. 7. Time series of shell growth and mortality.

Shell growth (cm month^{-1}) and mortality (%) of *M. trossulus* (A, C) and *M. galloprovincialis* (B, D) at 1 m depth (shallow, open circles) and 7 m 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.

known to limit shell dissolution caused by low pH from enhanced pCO_2 (Melzner et al., 2011). If high SFG does limit the effect of physiological stress on byssal thread production, the relationship between low oxygen and low pH and byssal thread production could be muted.

In contrast to patterns in mussel growth, 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; Gu et al., 2019; Sanders et al., 2014), and byssal thread production and other constitutive, or ‘flexible’ traits (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).

A 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

(Roberts et al., 2021). 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. Based on an estimate of ~ 1 J / thread (Roberts et al., 2021), we estimated energy allocation to thread production ranged from 12 to 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, 2019). 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 7 m depth in both years, associated with increases in the physiological stressors of low pH and hypoxia (>25% of the week). At this time, mortality of *M. trossulus* was also observed more broadly throughout the farm (Ian Jeffords, Personal Communication). Mortality also corresponded with the period where dissolved oxygen levels were between 0 and 0.5 mg L^{-1} for 10–20% of the week over 1–2 weeks in late Summer / early August. 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 (De Zwaan et al., 1991; Jørgensen, 1980; Theede et al., 1969; Von Oertzen and Schlungbaum, 1972). A better understanding of lethal effects of hypoxia, and

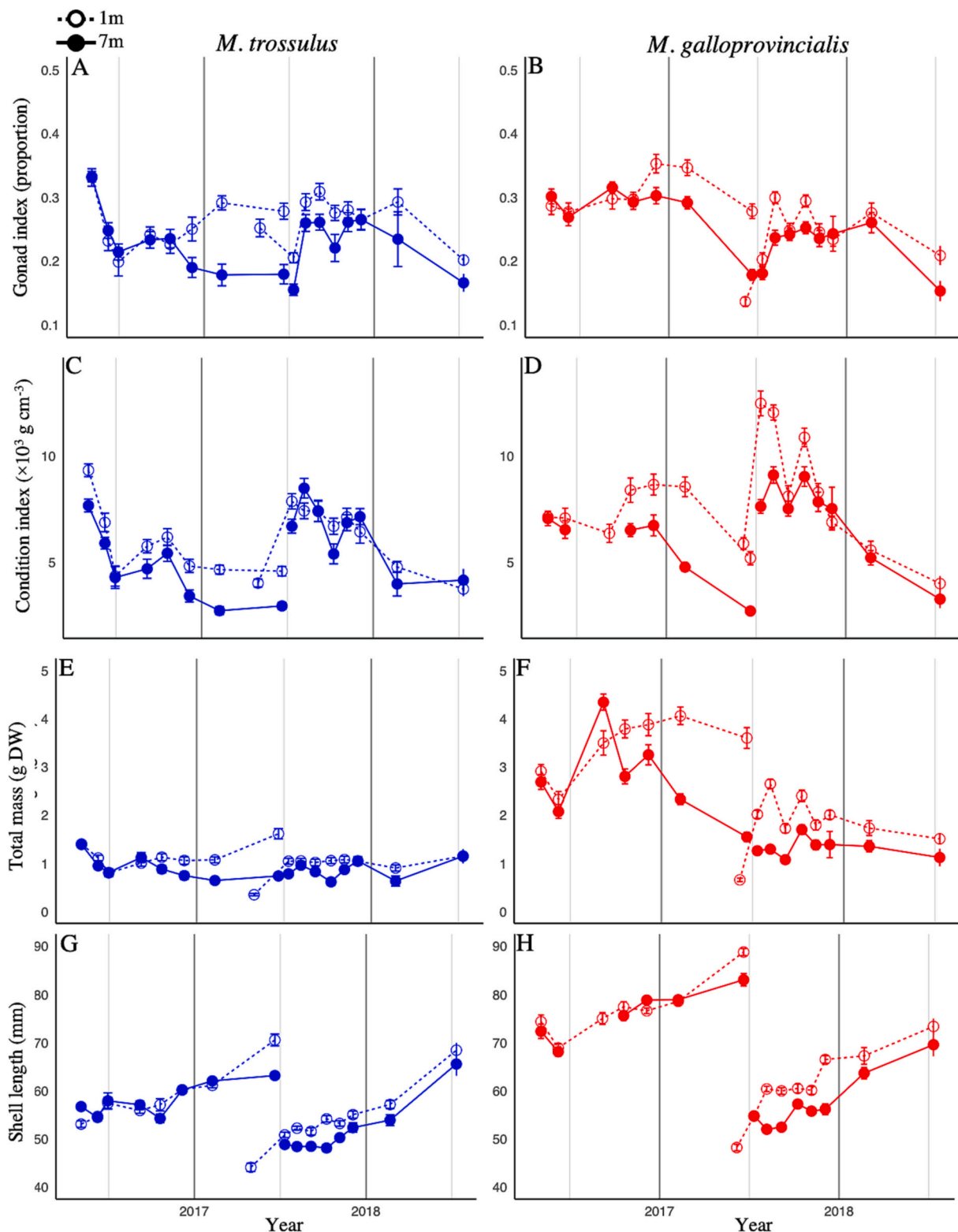


Fig. 8. Time series of mussel condition and shell length.

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 1 m depth (shallow, open circles) and 7 m 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.

concurrent low pH, on *M. trossulus* may contribute to understanding of the sensitivity to hypoxia and low pH of this species (Sokolova, 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 S2). This unexplained variability suggests that

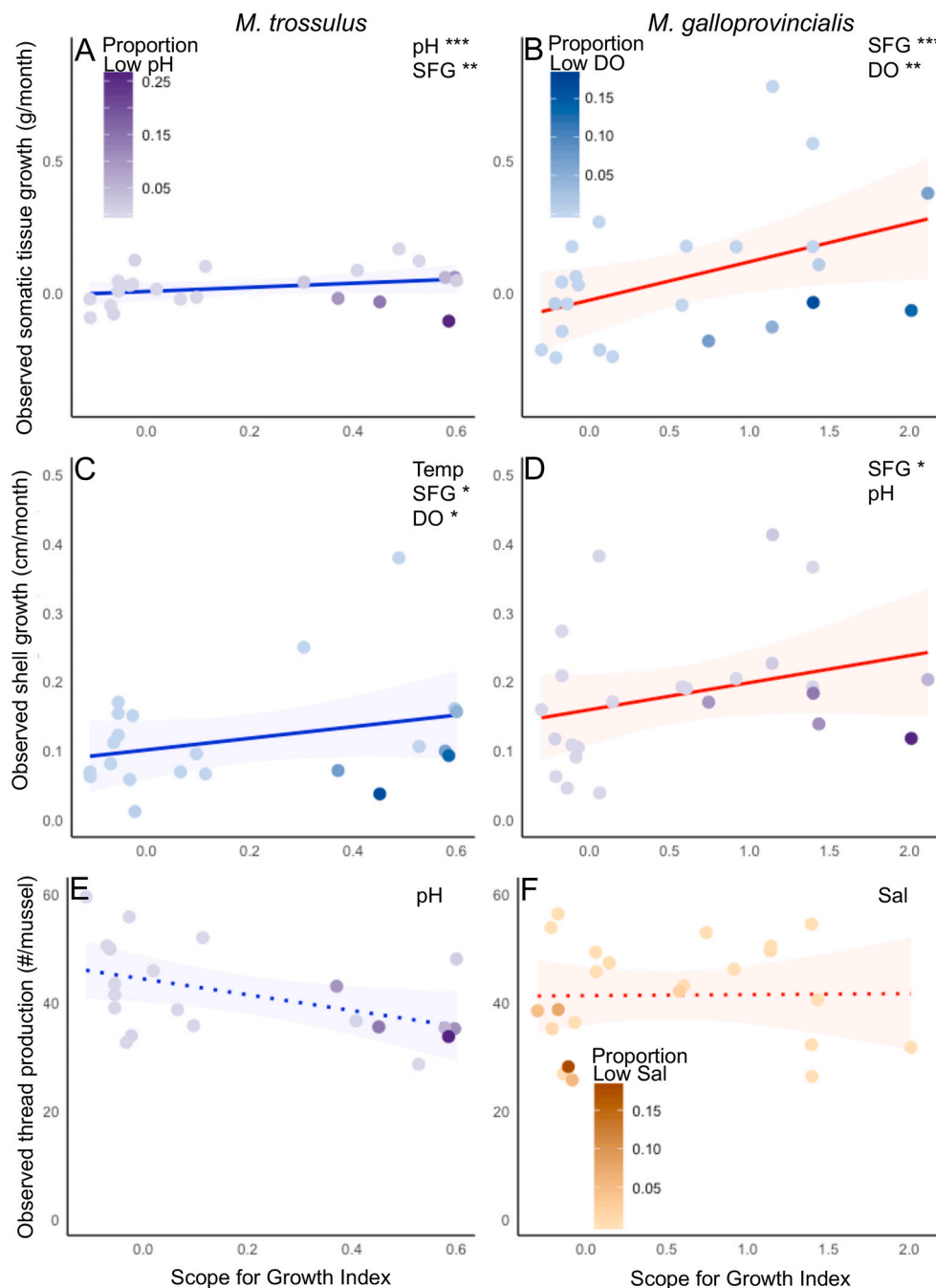


Fig. 9. Summary of multiple regression analysis of the effects of SFG and proportion of time exposed to other physiological stressors (pH, DO, temp, and salinity) on (A, B) tissue growth (g DW month^{-1}), (C, D) shell growth (cm month^{-1}), and (E, F) thread production over two weeks ($\text{\# mussel}^{-1}$) for *M. trossulus* and *M. galloprovincialis*. (A, B) Both SFG and time below a threshold of either pH or DO were significant predictors of tissue growth. (C, D) SFG and time below a threshold of either pH or DO were significant predictors of shell growth. (E, F) SFG was not a significant predictor of byssal thread production. Thresholds for pH and DO used for the analysis did not differ between species, but thresholds for temperature and salinity were species-specific (high temp threshold - *M. trossulus*; low temp threshold sal - *M. galloprovincialis*). Symbols represent monthly averages for a given depth (1 m or 7 m) 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 (Carrington et al., 2008; Dolmer and Svane, 1994; Lee et al., 1990; Moeser et al., 2006; Van Winkle, 1970), and greater mechanical movement of mussels from seawater currents or storms could increase byssal thread production. The site was a shallow bay protected from ocean swell. Wind-generated waves were typically small (less than ~ 2 ft) and much of the water motion was from tidally-driven currents. Although mussels are “sessile” animals, individuals can move within mussel beds using byssal thread production to do this, and effectively change their microenvironment (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 (Carrington et al., 2009; Moeser et al.,

2006; Moeser and Carrington, 2006; Zardi et al., 2007). For *M. edulis*, weak attachment is associated with high gonadal investment in the summer and early fall, and primarily associated with decreased byssal strength, not changes in the number of byssal threads produced (Moeser and Carrington, 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 in the Eastern Pacific ocean (*M. trossulus* – spring through fall, *M. galloprovincialis* – winter through early spring, *M. trossulus* but formerly called *M. edulis*) (Curiel-Ramirez and Caceres-Martinez, 2004; Skidmore, 1983; Strathmann, 1987). 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

Table 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 1 m and 7 m 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
<i>M. trossulus</i>	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	48,050	1	373.6	<0.001	0.07
		pH	-4	2	342	1	2.7	0.12	
		Residuals			2830	22			
<i>M. galloprovincialis</i>	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	43,111	1	411.5	<0.001	0.12
		Sal	-4	2	425	1	4.1	0.056	
		Residuals			2305	22			

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, 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 (Fig. 1A). 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 (Fig. 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 1 m compared to 7 m 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* - Gu et al., 2019; Sanders et al., 2014). 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, or 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 et al. (2021). A sensitivity analysis demonstrated that much of the error in the SFG calculation was associated with uncertainty in respiration rate measurements (Roberts et al., 2021). 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 µg L⁻¹ of chlorophyll *a* (Riisgård et al., 2011). Other models have estimated a range of half saturation coefficients from growth data that suggest a similar saturating value (half-saturation, 1–4 µg L⁻¹, depending on the site) (Filgueira et al., 2011; Rosland et al., 2009). 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 330 mg L⁻¹) (Foster-Smith, 1975; Widdows et al., 1979) and while large loads of inorganic matter can increase sorting costs and decrease ingestion, suspended sediment can increase mussel feeding rate (Kjørboe 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.

Another limitation of this study is that we used autumn SFG data to make predictions for energetic limitation from summer to early spring both years. In laboratory conditions, *M. trossulus* SFG declined at temperatures above 10–15 °C in all seasons that measurements were made (Fly and Hilbish, 2013). For *M. galloprovincialis*, in contrast, SFG temperature relationships vary substantially with season. In the autumn, SFG decreases with temperature, but in the spring and summer, SFG increases with temperature up to 25 °C. This seasonal variation complicates year round predictions of energetic limitation of this species, and future work that seeks to incorporate seasonal variation in thermal performance curves into predictions is needed. This work relied on SFG measurements from the literature obtained from farmed animals collected from the same location in one season. Future work that incorporates feeding experiments in-situ (Galimany et al., 2018), and

modeling methods that account for seasonal variation in the influence of temperature on intake and respiration may improve model accuracy and more realistic future predictions. Despite these model limitations, estimated SFG was still a significant predictor of growth, and a better predictor of growth than species-specific thresholds of temperature (*M. trossulus*) and salinity (*M. galloprovincialis*). One additional advantage to using data from the literature of SFG as a function of temperature is that it can be calculated for average temperatures over the growth period, rather than being measured at the start or end of the growth period.

Altogether, this study demonstrated that both energetic scope and the proportion of time beyond a threshold of physiological stress ($\text{pH} < 7.2$ NBS and $\text{DO} < 2 \text{ mg L}^{-1}$) 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 and DO - George et al., 2018; George and Carrington, 2018), or potentially by affecting organism energetics (thermal response - Newcomb et al., 2019, 2022) though this remains an open question. Dynamics in byssal thread quality can affect attachment in the field (Carrington et al., 2009; Newcomb, 2015; Zardi et al., 2007) and the survival 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 Khangaonkar et al. (2019) 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 to 25% of a week beyond these thresholds. EPA water quality standards where exposure beyond a threshold ($< 2 \text{ mg L}^{-1}$ 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., 2019). 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.

Given the wide-ranging environmental conditions in surface water (e.g. top ~10 m) in coastal systems, newer models of Salish Sea oceanography allow for predictions of seawater in coastal areas and with greater depth resolution near the surface (Ahmed et al., 2019; Khangaonkar 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. By 2095, the Salish Sea is expected to warm 1.5°C , seawater dissolved oxygen is expected to decline by 0.8 mg L^{-1} , and pH is expected to decline by 0.18 pH units (Khangaonkar et al., 2019). The region within the Salish Sea in which this farm is located, Whidbey Basin, is predicted to freshen at a faster rate than the other regions in the Salish Sea (Walker et al., 2022), which has the potential to affect nutrient input, stratification and low oxygen stress locally (Ahmed et al., 2019; Keeling et al., 2010). Climate oscillations, including El Niño Southern Oscillation, also affect wind

speeds regionally (Enloe et al., 2004) and river discharge locally (Bretl, 2019), which may also affect nutrient input, stratification, and low oxygen stress at depth. Our work suggests that these changes in environmental conditions, including low oxygen and low pH at depth may affect growth of *M. trossulus* and *M. galloprovincialis* negatively. This work is also consistent with findings that suggest that *M. trossulus* growth may be more impacted than *M. galloprovincialis* by warmer seawater (Roberts and Carrington, 2023).

Recent advances in Puget Sound biogeochemical models have improved spatial and depth resolution, and evaluate anthropogenic climate forcing of both current seawater conditions and long term trends (Ahmed et al., 2019; e.g. Khangaonkar et al., 2019; Walker et al., 2022). Identifying relationships between climate indices and mussel health may allow for improved farm management planning (e.g. 3–9 months). Considering the mussel farm as a social-ecological system (Ostrom, 2009), a greater capacity to adjust farming practices to natural variability in seawater conditions may minimize losses in mussel farm yields. Additionally, a greater capacity to adjust to short-term fluctuations may also confer a greater capacity to adjust to expected more frequent and more severe fluctuations in future seawater conditions caused by continuing anthropogenic ocean warming, acidification, and deoxygenation (Bopp et al., 2013).

CRedit authorship contribution statement

Emily A. Roberts: Conceptualization, Methodology, Formal analysis, Writing – review & editing, Resources, Funding acquisition, Software, Validation, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Emily Carrington:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Resources, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Emily Roberts reports financial support was provided by National Science Foundation. Emily Carrington reports financial support was provided by Washington Sea Grant. We used established mussel farm rafts owned by Penn Cove Shellfish LLC. to hold both our seawater sensors and animals. This company also provided technical assistance in support of maintaining seawater sensor data live feed, and transported us in their boats from the shore to the rafts.

Data availability

Data will be made available on request.

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-Gamino 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2023.151927>.

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