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**THE IMPACT OF OCEAN ACIDIFICATION ON THE PHYSIOLOGY OF THE BLUE MUSSEL MYSILUS EDULIS**

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THE IMPACT OF OCEAN ACIDIFICATION ON THE PHYSIOLOGY OF THE BLUE MUSSEL MYTILUS EDULIS

BY

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Bachelor of Science, Biology

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THESIS

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Abstract

Bivalve aquaculture garners global ecological and economic benefits, which makes the continued health of bivalve populations paramount. Ocean acidification presents a novel pressure on bivalves. Decreased pH due to acidification limits the carbonate available for bivalves to take-up, thus inhibiting the formation and growth of their calcium carbonate-based shells. The aragonite saturation (Ω) of seawater serves a useful biologic contextualization for pH in studies of acidification and bivalves, as an Ω of 1 indicates that calcium and carbonate ions can bond to form calcium carbonate. Shell health is a central aspect of bivalve fitness as it is the main defense of these organisms against predation and exposure to disease or other unfavorable environmental conditions. Resistance of the shell against crushing force is highly relevant as aquaculture bivalves are reportedly becoming increasingly predated upon by crustaceans in the Gulf of Maine as more invasive crab species become established in the region. Juvenile bivalves seem exceptionally susceptible based on reports from the Gulf of Maine shellfish industry that has recently been losing large portions of their juvenile stock to crab predation.

The blue mussel *Mytilus edulis* was selected for this study as it is a model species in bivalve research and has commercial relevance in the Gulf of Maine. Three cohorts of *M. edulis* were housed for this study. One cohort was housed in aquaria to simulate ambient pH Gulf of Maine seawater, whereas two were exposed to moderately and highly acidified conditions (0.25 pH and 0.5 pH below ambient) in aquaria. Exposure was conducted for three months and enabled examination of the significance of pH, Ω, and the length of exposure time to the physical conditions of resistance to crushing force, lengthwise growth, and mortality.

Mussels with greater shell length had more resistance to crushing force than smaller mussels, and the force required to crush mussels of all length classes increased at a consistent rate of 1.25 lbf per millimeter of shell length within each length class. The average force required
to crush mussels between 40 and 45 mm long was significantly greater than for mussels between 35 and 39.99 mm long. The average force required to crush mussels greater than 45 mm long was also significantly greater than for mussels between 40 and 45 mm long. Exposure time did not have a significant effect on the force required to crush the shells of *M. edulis* housed for the acidification treatment, but resistance to crushing force increased linearly with increased $\Omega$.

Crushing force resistance, standardized against length, was expected to increase by 0.609 lbf/mm per one unit increase of $\Omega$.

Lengthwise growth rates were unaffected by the amount of time that mussels were exposed to treatment, but lower $\Omega$ decreased lengthwise growth rates. For every one unit increase of $\Omega$, lengthwise growth was expected to increase by 176%. Mussels housed at ambient pH levels had the highest average lengthwise growth rate, whereas the average lengthwise growth was lowest in the -0.5 pH treatment. One additional mortality out of one-thousand mussels per day was estimated by the end of the study period, but $\Omega$ did not significantly affect mussel mortality. The number of mussel mortalities in the aquaria increased over the course of the study period, but percent mortality never exceeded 5% of the total cohort in any of the treatment aquaria, and biweekly percent mortality ranged between 0.18% to 4.92%. Acidification may lower blue mussel shell resistance to crushing force and may increase the timeframe when mussels are more actively susceptible to predation by crabs and other damage through the paired effects of acidification on force resistance and lengthwise growth.
Chapter 1: Background and Literature Review

1. Introduction

1.1 Shellfish Aquaculture

1.1.1 Global Shellfish Industry

Mollusks are organisms of critical importance to the aquaculture industry and the environment. In 2019, mollusks accounted for 20% of global aquaculture exports, and mussel exports accounted for 15% of the global mollusk export as well as 1.55% of the global total aquaculture export (FAO 2019). Aquaculture as an industry has increasingly supplanted wild mollusk fisheries and has supplied more than 50% of total global seafood since 2020, and aquaculture oyster production increased to nearly sixty-times the weight obtained through wild harvest internationally from 1950 to 2016 (Botta et al. 2020). This increase may be attributed to the low cost of shellfish rearing in comparison to animals like finfish compared to their high protein and market value per weight, as well as the ecosystem services afforded from bivalve production such as water purification and storm surge protection (Wijsman et al. 2019, Botta et al. 2020).

1.1.2 New England Shellfish Industry

Aquaculture has been a focal point in the United States seafood industry since the late 1900s. The United States government passed the National Aquaculture Act of 1980 to reduce the impact by fisheries on natural systems, increase the independence of the United States from international sources of seafood, and create opportunities for the development of novel products like pharmaceuticals from aquaculture products (1980). Despite federal interest and investment, experts at the turn of the century suggested that aquaculture would not become an established industry in New England (Conkling 2000). By 2015, shellfish aquaculture in New England had an estimated worth of $50 million independent from finfish and other forms of aquaculture (Barnes 2015, Bricknell et al. 2021). Federal programs like the National Oceanic and Atmospheric Administration are responsible in part for nationwide growth of aquaculture, but initiatives based in New England, such as the push for offshore aquaculture of blue mussels, have been key in localized growth (Greene and Grizzle 2006, Lapointe 2013, Fairbanks 2016).

Within the history of the aquaculture industry in New England, the proper care of indigenous species separate from farmed species has been at the forefront of management. New
England has been historically reliant on single-species fisheries, like the famous New England American lobster (*Homarus americanus*) fishery and the now-collapsed Atlantic cod (*Gadus morhua*) fishery, that have driven seasonal work strategies for local fisheries (Pershing et al. 2015, Rickard et al. 2016). Aquaculture initially became popularized in New England as an “off-season” supplement to fishery workers, but aquaculture has increasingly supplanted fisheries with Maine and Massachusetts hosting 40% of Eastern oyster (*Crassostrea virginica*) fisheries in the United States by 2013 (USDA 2013, Rickard et al. 2016). Increased local interest and industry reliance on aquaculture-produced mussels and oysters in New England highlights the relevance of this area as a representative of the aquaculture industry at the national and international scale.

### 1.2 Ocean Acidification

#### 1.2.1 Natural and Anthropogenic Ocean Acidification

Ocean acidification is an anthropogenic phenomenon in which the pH of seawater decreases due to a change in the equilibrium of carbon dioxide (CO$_2$) in the marine system. Acidification events occur naturally, especially in coastal and estuarine environments, due to processes such as saltwater-freshwater mixing and biological processes like respiration. Within coastal and estuarine environments, highly variable pH conditions are naturally driven by high levels of saltwater-freshwater mixing in the late summer and early autumn, as well as by the end of the period of high primary production which typically occurs in the spring and summer (Saba et al. 2019, Hunt et al. 2022). However, atmospheric CO$_2$ concentrations have increased sharply in the past 250 years from about 280 ppmv (parts per million volume) to near 384 ppmv (Doney et al. 2009). This high rate of CO$_2$ loading has been caused by human activity like deforestation and industrialization (Doney et al. 2009, Waldbusser et al. 2016). It is estimated that oceans retain roughly one-third of the carbon released into the atmosphere by human activity, which makes oceans the largest individual carbon sink on Earth (Sabine et al. 2007, Doney et al. 2009). Natural processes, such as saltwater-freshwater mixing, compound with anthropogenically-induced ocean acidification and cause greater variability in the pH of coastal environments than in the open ocean (Doney et al. 2009, Saba et al. 2019, Hunt et al. 2022).
1.2.2 Availability of Calcium Carbonate (CaCO$_3$)

Calcium carbonate (CaCO$_3$) in seawater decreases because of ocean acidification due to the concentration of carbonate ions ([CO$_3^{2-}$]) decreasing in acidified conditions (Salisbury et al. 2008, Saba et al. 2019, Atasaral et al. 2020). CaCO$_3$, especially the metastable form aragonite, is a key compound in bivalve shell formation, and aragonite formation for shell production occurs when the internal aragonite saturation (Ω) of mussels is equal to or greater than 1 (Salisbury et al. 2008, Hiebenthal et al. 2013). Ω is defined by the following equilibrium equation for calcium carbonate and its composite ions in seawater (Eq. 1.1).

$$\Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{\text{CaCO}_3}$$  \hspace{1cm} (Equation 1.1)

Ω cannot be measured independently in seawater and is estimated from water temperature, pH, salinity, and total alkalinity, as well as the equilibrium constants of hydrogen fluoride (K$_f$) and potassium sulfate (K$_s$), the stability constant for boric acid (K$_b$), and the primary (K$_1$) and secondary (K$_2$) dissociation constants for carbonic acid. In recent years, programs in multiple coding languages have been created as accessible Ω estimation tools which have become used in industry and environmental management as ambient Ω estimation may provide insight into conditions which favor or impede bivalve shell formation and recruitment (Salisbury et al. 2008, Jiang et al. 2022).

1.3 Biological Implications of Ocean Acidification for Shellfish

1.3.1 CaCO$_3$ Availability Impact on Shellfish

Carbonate (CO$_3^{2-}$) availability is central to the survival of shellfish, as their shells are mostly composed of CaCO$_3$ (Salisbury et al. 2008, Saba et al. 2019). Ω is frequently the standard for describing the impacts of ocean acidification on bivalves because it provides a useful proxy for their internal conditions which directly relates to their physical health (Salisbury et al. 2008, Atasaral et al. 2020, Carboni et al. 2021, Jiang et al. 2022). Some bivalve species, such as the blue mussel *Mytilus edulis*, are capable of expending energy to maintain internal conditions of high [CO$_3^{2-}$] in acidified environments to facilitate aragonite production for shell formation (Thomsen et al. 2010, Hiebenthal et al. 2013, Waldbusser et al. 2016). Highly aragonite-saturated conditions (Ω > 1.6) appear to best facilitate healthy shell growth in bivalves (Salisbury et al. 2008, Hiebenthal et al. 2013, Waldbusser et al. 2016). Lower [CO$_3^{2-}$] caused by
acidification, limits $\Omega$, which inhibits the formation of aragonite and thereby increases the energy expenditure by bivalves to maintain internal conditions optimal for shell formation (Salisbury et al. 2008, Hiebenthal et al. 2013). Bivalves are present in every ocean on Earth, which puts them on the front lines of the heightened levels of ocean acidification identified in the post-industrial era (Saba et al. 2019, Atasaral et al. 2020, Bricknell et al. 2021).

Resilience against acidification is variable between different bivalve species, but observed and predicted population dynamics suggest that acidified conditions are generally detrimental to bivalve populations (Thomsen et al. 2010, Hiebenthal et al. 2013, Saba et al. 2019, Bricknell et al. 2021). Some species of bivalve, such as *Mytilus edulis*, are observed as tolerant to acidification and exhibit standard levels of metabolism and survival in ambient and acidified environments (Thomsen et al. 2010, Carboni et al. 2021). However, acidification does not occur at a globally standard scale, and the naturally variable pH in coastal environments driven by natural processes has become more extreme because of increased anthropogenic CO$_2$ input (Doney et al. 2009). Population dynamics of bivalves are expected to change drastically in coastal environments due in part to this variability because the effects of ocean acidification on nutrient availability affects the physical conditions of bivalves such as shell formation (Kroeker et al. 2014).

### 1.3.2 Predator-Prey Interactions in Acidified Conditions

Ocean acidification may increase the energy costs of defense maintenance and growth in bivalves, which would be expected to influence the susceptibility of bivalves to predation (Kroeker et al. 2014, Atasaral et al. 2020, Bricknell et al. 2021). Bivalves have evolved calcium carbonate shells as their main defense against predators, which has caused quantifiable coevolution between shell thickness and the claw strength of predatory crustaceans (Zuchsin et al. 2001, Kroeker et al. 2014, Carboni et al. 2021). As a result, the decline of available CO$_3^{2-}$ and increased energy expenditure is expected to inhibit bivalve fitness (Salisbury et al. 2008, Kroeker et al. 2014, Saba et al. 2019, Atasaral et al. 2020). The continuous global trend of ocean acidification would suggest that bivalves, including fisheries-important species, are already experiencing increased stress which will increase in the future (Salisbury et al. 2008, Thomsen et al. 2010, Jewett and Romanou 2017, Bricknell et al. 2021). Although the interaction between
ocean acidification and bivalve predation by crustaceans has been hypothesized, changes in the mechanics of that predation has not been formally studied.

Climate change and resulting changes to the physical conditions of the Gulf of Maine such as acidification, have also enabled colonization by a wide variety of invasive species (Harris and Tyrrel 2001). Among these species are a host of predatory crustaceans, such as Asian shore crabs (*Hemigrapsus sanguineus*) and green crabs (*Carcinus maenas*), which have become increasingly prevalent in New England coastal systems and have largely outcompeted indigenous crustaceans as the main bivalve predator in the Gulf of Maine (Bricknell et al. 2021, Carboni et al. 2021). As recently as 2021, Atlantic blue crabs (*Callinectes sapidus*), another highly efficient predator of mussels, have begun establishing in the Gulf of Maine (Clavero et al. 2022). The continued establishment of major mussel predators such as *C. sapidus* in the Gulf of Maine due to climate change increases the vulnerability of native and aquaculture mussel populations in addition to climate change itself.

1.3.3 Ocean Acidification Impacts on Shellfish Aquaculture

Some species, like the commercially important blue mussel *Mytilus edulis*, have shown resistance to shell degradation in acidified coastal environments, but this resistance may not account for the highly variable pH observed in coastal environments (Thomsen et al. 2010, Hiebenthal et al. 2013). In particular, the Gulf of Maine has been experiencing more severe pH fluctuations over the past sixteen years and the presence of wild blue mussels in that region has also been declining (NOAA-PMEL, [https://www.pmel.noaa.gov/co2/story/GOM](https://www.pmel.noaa.gov/co2/story/GOM), Sorte et al. 2016, Saba et al. 2019, Petraitis et al. 2020). Ocean acidification is a global phenomenon and a key aspect of climate change, and the unique conditions of the Gulf of Maine have made it a model area for research into the expected trends of other coastal and marine systems (Brickman et al. 2021, Bricknell et al. 2021, Hunt et al. 2022). Since the Gulf of Maine is distinct for both its unique physical conditions and its importance to the growing American aquaculture industry, the interaction between its physical conditions and commercially relevant bivalves raised there is central to the continued management of bivalve aquaculture in the region.

Blue mussels have a direct response to increased predator presence like what is being observed in the Gulf of Maine. The presence of nearby predatory crustaceans facilitates thicker shell growth at the expense of soft tissue development (Bricknell et al. 2021). For aquaculturists,
this trade-off presents a substantial issue as a decrease in soft tissue decreases the market value of their product. Blue mussels may become increasingly pressurized to overcompensate for predator defense at the expense of soft tissue development given the increasingly high presence of predatory crustaceans in the Gulf of Maine, which would be detrimental to the productivity of local aquaculture.

1.4 Study Site and Research Questions

1.4.1 Newcastle, NH

Within the Gulf of Maine, Great Bay is an estuary spanning the border between southwest Maine and southeast New Hampshire, USA. The University of New Hampshire Coastal Marine Laboratory (CML) is located at the mouth of the Piscataqua River, in addition to some integrated aquaculture systems maintained by the University and commercial growers (Figure 1). CML hosts a flow-through system which continuously pumps intake water from lower depths of the Piscataqua River. This system enables simulation of tidal conditions in the Piscataqua River within aquaria supplied with the seawater pumped through CML, as work conducted at CML identified low-tide water pumped into CML as being comprised of water outgoing from Great Bay whereas water pumped at high-tide consisted of near-shore water from the western Gulf of Maine (Brown 2006, Hunt et al. 2022).

1.4.2 Research Questions and Hypotheses

How ocean acidification impacts the physical condition of the blue mussel *M. edulis* is the cornerstone of this study. Resistance to crushing force ($F_C$), and growth are expected to decrease in acidified conditions as $\Omega$ decreases in acidified conditions (Salisbury et al. 2008, Atasaral et al. 2020). More direct exposure to environmental conditions such as heating events or microbial pathogens due to the compromised shell conditions caused by lower $\Omega$ is expected to increase the overall mortality of mussels in acidified conditions (Botta et al. 2020). Trends of decreased $F_C$ and growth, as well as increased mortality, are also expected to increase over the course of continuous exposure to acidified conditions for mussels kept in high-density environments. The high-density conditions on aquaculture farms reportedly cause greater rates of mortality in farmed than wild populations due to intraspecific competition for food and greater rates of disease transmission (Capelle et al. 2016, Botta et al. 2020). Whether ocean acidification
and aquaculture farm conditions compound negatively on the physical conditions of blue mussels is extremely relevant to the maintenance of this species in the Gulf of Maine by the aquaculture industry and in the wild.

The shell formation of the blue mussel is hypothesized to be impeded by acidified conditions. Resistance to crushing force is expected to decrease as pH decreases due to lower $\Omega$ in acidified conditions. Lengthwise shell growth rate and mortality, on the other hand, are hypothesized to increase as pH decreases due to lower $\Omega$. 
2. References


Chapter 2: The Impact of Continuous Exposure to Ocean Acidification on the Physiology of the Blue Mussel *Mytilus edulis*

1. Introduction

Mollusks are organisms of critical importance to the aquaculture industry and the environment. Aquaculture has increasingly supplanted wild mollusk fisheries and now supplies more than 50% of total global seafood (Botta et al. 2020). In 2019, Mollusks accounted for 20% of global aquaculture exports, and mussel export accounted for 15% of the global mollusk export as well as 1.55% of the global total aquaculture export (FAO 2019). Shellfish aquaculture is an industry worth at least $50 million in New England and is a staple industry of the region (Barnes 2015, Bricknell et al. 2021). This increase may be attributed to the low cost of shellfish rearing in comparison to animals like finfish compared to their high protein and market value per weight, as well as the ecosystem services begotten by bivalve production such as water purification and storm surge protection (Botta et al. 2020, Wijsman et al. 2019). Heightened reliance on aquaculture reinforces the need for comprehensive assessments of current and future risks to product stock, which includes a wide range of anthropogenic impacts on coastal ecosystems.

Humanity has left significant impacts on every aspect of the natural world. In the oceans, human activity has been linked to phenomena such as sea level rise, the melting of glaciers and the polar ice caps, and increased concentration of dissolved carbon dioxide (CO$_2$) (Jewett and Romanou 2017). Increased CO$_2$ decreases the pH of seawater, creating a more acidic environment.

Mollusks, specifically bivalves, and other organisms like coral are especially susceptible to ocean acidification (Atasaral et al. 2020). The shells of these organisms are composed of calcium carbonate (CaCO$_3$) obtained from the environment (Salisbury et al. 2008). Aragonite saturation ($\Omega$) in seawater decreases in acidified environments because the reaction which produces CaCO$_3$ loses equilibrium because the concentration of carbonate ions ([CO$_3^{2-}$]) trends lower (Salisbury et al. 2008, Atasaral et al. 2020). $\Omega$ is the sum of the following equilibrium equation for calcium carbonate and its composite ions (Eq. 2.1).

$$\Omega = ([Ca^{2+}][CO_3^{2-}])/[CaCO_3]$$  \hspace{1cm} \text{(Equation 2.1)}

Lower CO$_3^{2-}$ concentrations inhibit the formation of shells by bivalves and other organisms, which has direct impacts on the fitness of those organisms (Salisbury et al. 2008, Hiebenthal et
al. 2013, Kroeker et al. 2014, Atasaral et al. 2020). It has been observed that ocean acidification increases the energy costs of defense maintenance, growth, and reproduction in bivalves, which would directly impact predator-prey relationships which may be expected to increase further if current trends of ocean acidification and influx of new predator species continue in the Gulf of Maine (Kroeker et al. 2014, Bricknell et al. 2021).

Predator-prey interactions are a key aspect of all aquatic ecosystems, from microbes to reefs. Bivalves evolved calcium carbonate shells as their main defense against predators, which has caused quantifiable coevolution between shell thickness and the claw strength of predatory crustaceans (Kroeker et al. 2014). Kroeker et al. (2014) considers decreased net calcification a potential driver of greater predation pressure on bivalve species. The continuous global trend of ocean acidification coupled with the impact of acidified conditions on the saturation of biologically reactive forms of CaCO$_3$ would suggest that bivalves, including species that are important to the shellfish industry, are already experiencing increased stress which will increase in the future (Salisbury et al. 2008, Thomsen et al. 2010, Jewett and Romanou 2017, Bricknell et al. 2021). Some species, like the commercially important blue mussel *Mytilus edulis*, have shown resistance to shell degradation in coastal environments which have become acidified, but this resistance may only hold at present conditions and not account for the continued acidification of coastal environments (Thomsen et al. 2010). In particular, the Gulf of Maine has been experiencing major pH fluctuations over the past several decades and the presence of blue mussels in this region has also been declining (NOAA-PMEL, \url{https://www.pmel.noaa.gov/co2/story/GOM}, Sorte et al. 2016, Petraitis et al. 2020).

Blue mussels are a key species in coastal systems and bivalve aquaculture around the globe and is considered a key aquaculture species by every state in New England (Lapointe 2013). Given its ubiquity in coastal environments, interactions between blue mussels and environmental factors such as predation and environmental conditions are well-documented. Although blue mussels are considered resistant against acidification-induced shell degradation at past recorded levels (Thomsen et al. 2010), the extent of this resistance is not well understood, especially when recent, highly variable pH conditions of the Gulf of Maine are considered (NOAA-PMEL, \url{https://www.pmel.noaa.gov/co2/story/GOM}). The implication of this unknown degree of resistance presents a great concern to the growing New England aquaculture industry.
Although bivalve aquaculture is a global industry, it has only recently become established in the Gulf of Maine (Lapointe 2013, FAO 2019, Botta et al. 2020). The threat of predation pressure to blue mussel aquaculture in the Gulf of Maine is not well known, especially in the context of climate change-driven factors like species migration and ocean acidification. Juvenile aquaculture oysters in the Gulf of Maine have become increasingly at risk to predation according to aquaculturists, as invasive crab species continue establishing in the Gulf of Maine (Capelle et al. 2016, Clavero et al. 2022). Asian shore crabs (Hemigrapsus sanguineus) and green crabs (Carcinus maenas) have become increasingly prevalent in New England coastal systems and have largely outcompeted other crustacean species as the main bivalve predator in the Gulf of Maine (Bricknell et al. 2021). Atlantic blue crabs (Callinectes sapidus) are also becoming established in the Gulf of Maine, which would further stress blue mussel populations as C. sapidus are yet another highly efficient predator of mussels in coastal environments (Clavero et al. 2022). Blue mussels have a direct response to increased predator presence like what is being observed in the Gulf of Maine. The presence of nearby predatory crustaceans facilitates thicker shell growth at the expense of soft tissue development (Bricknell et al. 2021).

This evolutionary strategy of favoring shell over soft tissue growth presents a potentially significant issue for aquaculturists as a decrease in soft tissue decreases the market value of their product. Although crabs are absent in offshore aquaculture environments, ocean acidification and lower Ω of seawater would increase the energy required for shell formation, thereby decreasing soft tissue growth and leading to mortality in extreme acidification conditions (Kroeker et al. 2014, Bricknell et al. 2021). The main goal of this thesis project was to quantify the impact of acidified conditions observed in the Gulf of Maine on the strength and growth of blue mussel shells.
2. Materials and Methods:

2.1 Sample Collection and Acclimation

2.1.1 Specimen Sampling Site Description

Blue mussels were sampled from the University of New Hampshire (UNH) Pier in New Castle, New Hampshire (Fig. 1). Individuals between 20 and 50 millimeters in length were selected for this study for the inclusion of multiple length classes in the force analysis. Collected mussels were stored in oyster bags inside a lobster tote at the UNH Pier until the complete cohort was assembled, at which point they were moved to aquaria in the UNH Coastal Marine Laboratory (CML). The number of mussels included in each aquarium was designed to maintain conditions suitable for growth and survival, while also ensuring enough individuals for sampling.
2.1.2 Captive Specimen Care

Nine aquaria were set-up in CML which contained baskets arranged to keep the mussels above the bottom of the aquarium. Mussels were acclimated in the aquaria for one month before the pH treatments were introduced. Aquaria were supplied with filtered (100 μm mesh size) water from the Piscataqua River via a flow-through system in each aquarium that enabled fluctuations in ambient water conditions like temperature and salinity due to seasonal and tidal cycles. Water temperature was continuously tracked in one aquarium and spot-checked during regular maintenance in all others via an electronic thermometer. Since the aquaria were in close proximity, daily checking was sufficient for the identification of abnormal temperatures in any specific aquarium.

*Nannochloropsis* spp. 3600 (68 billion cells per mL) from Reed Mariculture was used to feed the mussels. After consultation with experts at Reed Mariculture, Mook Sea Farm, and the NOAA lab in Milford, Connecticut, a feeding rate of 2-6% mass of feed per gram of wet weight of mussels present in the aquaria would be a suitable representation of real-world conditions. Daily feeding needs were determined, and mussels were fed a solution of feed diluted at a 1:5 ratio with the inflow water to CML. Diluted feed was stored in a refrigeration unit above the
aquaria and predetermined feed volumes were dispensed through plastic tubing on a four-hour cycle (six times per day) via a system of peristaltic pumps (Fig. 2).

Since mussels were removed from the aquaria for sampling and some mortality occurred, the amount of feed was adjusted after each sampling event by the calculated wet weight biomass in each aquarium (Supplementary Material 1). Wet weight biomass was calculated based on the number of mussels per aquaria and an average biomass for individual mussels in the following length classes: <30 mm in length; 30 to 34.99 mm; 35 to 39.99 mm; 40 to 44.99 mm; and >45 mm. The average biomass per length class was based on the wet tissue weights of sixty additional mussels sampled from the UNH Pier (Supplementary Material 1).

2.2 Acidification Treatment
2.2.1 Treatment with pCO₂

Experiments involving multiple levels of pH which simulated present and projected acidified conditions in the Gulf of Maine were conducted with triplication of each treatment level over the course of three months in the summer, like other studies on the impact of acidification on blue mussels (Salazar et al. 1995). Treatment levels reflected variations in Gulf of Maine surface water pH identified from NOAA Pacific Marine Environmental Laboratory (NOAA-PMEL) data, which is between 7.8 to 8.4, with an annual average pH of 8.2 (NOAA-PMEL, https://www.pmel.noaa.gov/co2/story/GOM). An ambient pH treatment served as the control for this experiment; water supplied to the control aquaria was not dosed with pCO₂. Two levels of acidification treatments were conducted. One treatment exposed mussels to slightly acidified conditions of 0.25 pH below ambient levels, whereas the second treatment exposed mussels to a level of acidification 0.50 pH below ambient levels (Table 1). pCO₂ was dosed to aquaria from a storage tank via air stone bubblers.
Table 1: Description of the three pH treatments run for this study and the nomenclature of each sample. Treatments were run in triplicate between three separate tanks.

<table>
<thead>
<tr>
<th></th>
<th>Aquarium 1</th>
<th>Aquarium 2</th>
<th>Aquarium 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C, ambient pH)</td>
<td>C-1</td>
<td>C-2</td>
<td>C-3</td>
</tr>
<tr>
<td>Treatment 1 (T1, -0.25 pH)</td>
<td>T1-1</td>
<td>T1-2</td>
<td>T1-3</td>
</tr>
<tr>
<td>Treatment 2 (T2, -0.50 pH)</td>
<td>T2-1</td>
<td>T2-2</td>
<td>T2-3</td>
</tr>
</tbody>
</table>

2.2.2 Monitoring Conditions in Aquaria

Measurements of pH in the treatment aquaria were taken at 10 hertz by Atlas Scientific probes to the nearest hundredth of a pH unit and recorded to an Excel file every ten minutes. These measurements informed dosing of pCO₂ to the treatment aquaria and corrected the level of dosage for changes to the pH of the flow-through water caused by conditions outside of CML. Each pH probe was calibrated with three levels of commercial NBS buffer (4.01, 7.00, and 10.01). A probe in each treatment aquarium enabled the identification and adjustment of any improper pCO₂ dosage to maintain consistent acidification treatment conditions. pH was tracked in only one of the three control aquaria, as pH was assumed to be identical in all control aquaria because no dosing with pCO₂ was conducted. Faulty probes were removed from the aquarium immediately and replaced. Salinity, oxygen saturation, temperature, and total alkalinity of the flow-through water were measured by a preexisting system at CML to enable calculation of Ω (Hunt et al. 2022).

Figure 3: Specimen of *Mytilus edulis* with the area where force was applied highlighted in red.
2.3 Physical Condition Testing

2.3.1 Crushing Force Testing

The physical resistance of mussel shells to crushing is a key feature of mussels, as it is the main defense of mussels against hunting behaviors exercised by predators like crab species present in the Gulf of Maine (Cornelius et al. 2021). At the start of the sample period, additional blue mussels were sampled from the UNH Pier and tested for vertical force resistance (\(F_C\)) prior to the initiation of the treatments to provide a baseline of data on force resistance in different length classes of Gulf of Maine mussels. Sampling of mussels from the aquaria for \(F_C\) testing began six weeks after the acidification regime had been implemented. Within these samples, most mussels were < 30 mm, and at least one individual represented each of the following length classes: 30 - 34.99 mm; 35 - 39.99 mm; 40 - 44.99 mm; and greater than 45 mm. A greater number of smaller mussels were sampled because smaller mussels and oysters are more vulnerable to invasive crab predation than larger individuals as observed by New England aquaculturists and ecologists (Capelle et al. 2016). The second sampling from the aquaria was conducted two weeks after the first sampling, and the third aquaria sampling was two weeks after the second (four weeks after the first).

Pressure was exerted vertically onto each mussel via a 250-lbf hydraulic press, which simulated the pressure exerted onto mussel shells by predatory crustaceans (Bricknell et al. 2021, Cornelius et al. 2021). Vertical force was applied to the hinge-side of the mussel (Fig. 3) and measured with a load cell and LabView program provided. Mussels were held in place by a loose-fitting zip tie at the opposite end of the mussel, so specimens remained consistently oriented and experienced force from the same angle. The zip-tie was kept loose so there was no pressure on the shell besides what was exerted by the press. Exerted force was measured and recorded in pounds of force (lbf) every ten seconds, and output was reported in Excel files generated by the program during each sampling.

2.3.2 Lengthwise Growth and Mortality Tracking

Mussels were measured lengthwise with electronic calipers on a biweekly basis, starting at the initiation of the acidification treatment. All the mussels within an aquarium were removed
for the duration of the measuring period and returned to their original aquarium once the entire cohort had been measured (~15-minute removal from aquaria). During measurements, the lengths of mussels which had died during the two-week growth period were recorded and marked in the spreadsheet as mortalities. A count of deceased mussels was recorded for each aquarium at the end of the measurement period. Individuals that died were excluded from calculations of the average length for that aquarium on the day of sampling and the calculation of growth which occurred during the two-week period. The number of mussels per length class was also recorded after measurement events to inform how many mussels should be sampled to represent each length class for each Fc sampling event.

Biomass in each aquarium was calculated after sampling events with the following equation:

\[ B = E(bx*nx) \]  
(Equation 2.2)

where B is the sum of wet weight biomass (g) present in each aquarium determined from the average wet weight biomass (b, Fig. S1.1) of a given length class (x) and the number of mussels (n) counted in that length class at the time of sampling. B informed the calculation of daily feed dispensed via the following equation:

Feed g DW/Aquarium =  
(# Mussels/Aquarium)*(0.1 g Mussel DW/ g Mussel WW)*(2 g Feed DW/100 g Mussel DW)  
(Equation 2.3)

where Feed g DW/Aquarium is the dry weight of feed dispensed to a given aquarium and is a function of the number of mussels in an aquarium multiplied by the ratio of 0.1 grams of dry weight per 1 gram of wet weight per mussel, with 2 grams of dry weight feed dispensed per 100 grams of mussel dry weight.

2.4 Data Analysis
2.4.1 Estimation of Aragonite Saturation
The aragonite saturation (Ω) for each aquarium over the course of the study period was estimated from measurements of water temperature, pH (on the NBS scale), salinity, and total alkalinity via the “seacarb” package in R Studio. Ω was calculated as a biologic contextualization for the impacts of marine acidification on blue mussels given the importance of aragonite in bivalve shell formation (Salisbury et al. 2008, Atasaral et al. 2020). The Ω of the inflow water to CML aquaria was assumed to be identical to the Ω of control aquaria because the pH in those aquaria was unaltered by pCO$_2$ dosing.

Constants for other physical conditions relevant to Ω estimation included the equilibrium constants for hydrogen fluoride (K$_f$) and potassium sulfate (K$_s$), the stability constant for boric acid (K$_b$), and the primary (K$_1$) and secondary (K$_2$) dissociation constants for carbonic acid. These values were sourced by “seacarb” based on environmental observations during the study period by (Table 2). Phosphate and silicate concentrations were assumed to be insignificant in the estimation of Ω in this study. The average Ω in each aquarium was determined at the end of the study period. Tukey’s range tests were conducted to determine the level of difference between the averages of the measured pH and estimated Ω between treatments to confirm the intended experimental acidification levels.
Table 2: Constants used for the estimation of $\Omega$. $K_1$ and $K_2$ are the primary and secondary dissociation constants for carbonic acid ($H_2CO_3$), $K_B$ is the equilibrium constant for boric acid ($H_3BO_3$), $K_f$ is the equilibrium constant of hydrogen fluoride (HF), and $K_s$ is the stability constant of hydrogen sulfate ($HSO_4^-$).

<table>
<thead>
<tr>
<th>Constant</th>
<th>Formula</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>$= [H^+]\frac{[HCO_3^-][CO_2^-]}{[CO_2]}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 3633.86/T - 61.2172 + 9.67770*\ln T - 0.011555S + 0.0001152S^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T$ = seawater temperature (°C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S$ = salinity</td>
<td></td>
</tr>
<tr>
<td>$K_2$</td>
<td>$= [H^+]\frac{[CO_2^2^-][HCO_3^-]}{[HCO_3^-]}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 471.78/T + 25.9290 - 3.16967*\ln T - 0.01781S + 0.0001122S^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T$ = seawater temperature (°C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S$ = salinity</td>
<td></td>
</tr>
<tr>
<td>$K_B$</td>
<td>$= [H^+]\frac{[B(OH)_4^-][B(OH)_3]}{[B(OH)_3]}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 0.053105S^{1/2}(T/K)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T/K$ = absolute seawater temperature (K)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S$ = salinity</td>
<td></td>
</tr>
<tr>
<td>$K_f$</td>
<td>$= [H^+]\frac{[F^-][HF]}{[HF]}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= -874/(T/K) - 9.68 + 0.111S^{0.6}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T/K$ = absolute seawater temperature (K)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S$ = salinity</td>
<td></td>
</tr>
<tr>
<td>$K_s$</td>
<td>$= [H^+]\frac{[SO_4^{2-}][HSO_4^-]}{[HSO_4^-]}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$= 141.411 - 4340.704/T$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T$ = seawater temperature (°C)</td>
<td></td>
</tr>
</tbody>
</table>

2.4.2 Models of Crushing Force

$F_C$ was calculated with the following equation:

$$F_C = (F_{\text{max}} - F_{\text{min}})$$  \hspace{1cm} (Equation 2.4)

where $F_{\text{max}}$ (lbf) is the highest amount of pounds of force recorded by the load cell and $F_{\text{min}}$ (lbf) is the lowest. The subtraction of $F_{\text{min}}$ from $F_{\text{max}}$ corrected for any offsetting from zero that may have occurred between the start of the load cell run and the beginning of force exertion on the mussel. Measured $F_C$ was used in the parameterization of Model 2.1:
where \( F_C \) is a function of \(~\) L, which represents shell length (mm). FC was modeled as a Gamma distribution in this model. This generalized linear model was used to determine a significant relationship between \( F_C \) and shell length by length class. A Tukey’s range test was also conducted to identify the difference in the average \( F_C \) of sequential length classes. \( F_C \) was standardized against shell length (\( F_S \), standardized crushing force in lbf/mm) once a significant relationship between shell length and the \( F_C \) had been identified so the potential impacts of \( \Omega \) and exposure time would not be confounded by the potential relationship between \( F_C \) and shell length:

\[
F_S = F_C / L \tag{Equation 2.5}
\]

Values of \( F_S \) calculated from measured \( F_C \) and shell length were then used to parameterize Model 2.2:

\[
F_S \sim \Omega \times \text{Exposure Time} + (1|\text{Aquarium ID}) \tag{Model 2.2}
\]

where \( F_S \) is a function of \( \Omega \) and the duration of exposure time to treatment by the sample date. This generalized linear model was used to determine whether \( \Omega \) and exposure time, as well as the interaction of those two factors, had a significant impact on \( F_S \). Aquarium ID served as a random effect in this model. \( F_S \) data was modeled as a Gamma distribution.

### 2.4.3 General Linear Mixed Effects Model of Growth

Growth was calculated as the change in shell length for each mussel in an aquarium between measurement events. An average overall growth for all mussels in an aquarium was determined from growth measured for all live mussels (Eq. 2.6). To correct for mortalities and removals for sampling, the average length was calculated from the initial date after mortalities and removals for sampling on that date as well as mortalities from the later date were removed from the raw set of lengths data. Removing the lengths of mortalities from the later date accounted for the mortalities which occurred during the growth period, as those mussels would not have contributed to the overall growth of the cohort. Only mortalities recorded on the date of sampling were removed from the later date for calculation of the average length in the aquarium
at that time. This procedure also ensured that the total number of mussels was the same for each date when the average length was calculated with Equation 2.6:

\[ G = \frac{(L_{t+1} - L_t)}{t} \]  
(Equation 2.6)

where \( G \) denotes growth rate (lengthwise, millimeters per day), and is a function of the average length (mm) of mussels at a later measurement date (\( L_{t+1} \)), the average length of mussels in the same aquaria at the previous measurement date (\( L_t \)), and the number of days between those dates (t). Mortalities and mussels removed for crushing force testing during the growth period were not included in the calculations of average length and growth rates.

Calculated lengthwise growth rates (\( G \), mm/day) for each growth period parameterized Model 2.3:

\[ G \sim \Omega \times \text{Exposure Time} + (1|\text{Aquarium ID}) \]  
(Model 2.3)

where \( G \) is a function of \( \Omega \) and the length of exposure time to treatment by the sample date. This general linear mixed effects model was used to determine whether \( \Omega \) and exposure time, as well as the interaction of those two factors, had a significant impact on the growth rates of the mussels. Aquarium ID served as a randomizing effect in this model. Lengthwise growth rate was modeled as a gaussian distribution, and log transformation of the calculated growth rates was conducted to meet the assumptions of a linear model (Newman 1993, Thorson 2019).

### 2.4.4 Generalized Linear Mixed Effects Model of Mortality

Dead mussels, identified as individuals which could no longer close their shell or shells emptied of soft tissue, were counted for each aquarium, and measured lengthwise during the biweekly measurement events. Percent mortality (\( M_P \)) was calculated from the recorded number of mortalities (\( M \)) and the total number of live and dead mussels in the aquaria (\( n \)):

\[ M_P = \frac{M}{n} \times 100\% \]  
(Equation 2.7)

A generalized linear mixed effects model parameterized by the recorded number of mortalities (\( M \)) was used to parameterize Model 2.4:
where the number of mortalities is a function of $\Omega$ and the length of exposure time to treatment. Aquarium ID served as a randomizing effect in this model. Mortality count data was modeled as a Poisson distribution. Estimated percent mortality ($M_p$) was calculated from the number of mortalities estimated by Model 2.4 ($M_{\Omega t}$) and $n$:

$$M_p = \frac{M_{\Omega t}}{n} \times 100\%$$  \hspace{1cm} (Equation 2.8)

2.4.5 Linear Mixed Effects Model Evaluation

A Kolmogorov-Smirnov test, used to test the correctness of the distributions selected for each dataset against a normal distribution and the level of dispersion and presence of outliers in the measured data, was conducted via the “DHARMa” package of R Studio. “DHARMa” was also used to compare the empirical and theoretical 0.25, 0.50, and 0.75 quantiles for the residuals of each model output (Hartig 2022; Supplementary Material 3).
3. Results

3.1 Measured Environmental Conditions and Estimated Total Alkalinity

Dissolved oxygen (μmol kg⁻¹), salinity, water temperature (°C), and pCO₂ of the inflow water to the aquaria were measured over the study period (Fig. 4). Reduced freshwater-saltwater mixing in the study region resulted from a drought that persisted for the duration of the study period (McCarthy et al. 2023). An alternative model that is not reliant upon salinity was used for the estimation of total alkalinity because the expected linear relationship between salinity and total alkalinity was very weak due to low variability in salinity caused by reduced mixing (Supplementary Material 2).

The pH of the treated aquaria was controlled by pCO₂ input and designed to be 0.25 (T1) and 0.50 (T2) pH less than the ambient (C) pH in the control tanks (Fig. 4). The average ambient pH was 7.69 ± 0.01, the average pH of the -0.25 pH treatment was 7.44 ± 0.01, and the average pH of the -0.50 pH treatment was 7.22 ± 0.01. The differences in pH levels between the different treatments, which varied as designed with the ambient pH, were also compared. The Tukey test for the measured pH of each treatment yielded significant differences between each of the treatment groups at the 0.05 alpha level; the -0.25 pH treatment aquaria were 0.25 pH lower than ambient pH levels, and the -0.50 pH treatment was -0.47 pH lower than ambient pH.
3.2 The Effect of pH on Estimated Aragonite Saturation

Aragonite saturation (Ω) was estimated by using the measured values of water temperature, and salinity measured in the flow-through water at CML, the estimated total alkalinity in the flow-through water at CML, and the pH measured in each aquarium over the course of the study period. Ω was highest in aquaria kept at ambient pH and lowest in aquaria at 0.50 pH below ambient levels; aquaria in the -0.25 pH treatment group had Ω between the ambient and -0.5 pH treatment groups (Fig. 5). The average Ω at ambient pH levels was estimated to be $1.13 \pm 0.0027$ over the course of the study period and Ω was assumed to be
identical in the control aquaria because they were not dosed with pCO₂ and received the same water from the flow-through system. In the -0.25 pH treatment aquaria, the average Ω was 0.664 ± 0.0017, and the average Ω in the -0.50 pH treatment aquaria was 0.426 ± 0.0019. The Tukey test identified significant differences between the average Ω of each treatment. The average Ω in aquaria kept at ambient pH was 0.464 greater than the average Ω of the -0.25 pH treatment aquaria, and 0.702 greater than the average Ω of the -0.50 pH treatment aquaria (Fig. 5).

Figure 5: Ω was estimated via the “seacarb” package in RStudio. Three acidification levels were implemented; one control treatment where the pH of the aquaria was unaltered (C); and two control treatments where the pH was slightly (-0.25 pH below ambient, T1) or greatly (-0.50 pH below ambient, T2) altered. pH values were based on historic pH data taken in the Gulf of Maine (NOAA-PMEL, https://www.pmel.noaa.gov/co2/story/GOM).
3.3 The Effect of Shell Length on Crushing Force

Shell crushing force ($F_C$) increased with increasing shell length, especially for mussels greater than 40 mm (Fig. 6). The Tukey test of $F_C$ and length class determined that $F_C$ did not differ significantly between mussels of consecutive length classes, until shell length was greater than 40 mm. Mussels in the 40 - 44.99 mm length class required $63.22 \pm 3.13$ lbf to crush, which was significantly greater by 13.81 lbf than mussels in the 35 - 39.99 mm length class, which required $49.4 \pm 2.56$ lbf to crush ($p = 0.0044$). Mussels greater than 45 millimeters required $83.4 \pm 4.58$ lbf to crush and required significantly more (20.17 lbf) force than mussels in the 40 - 44.99 mm length class ($p = 1.99 \times 10^{-5}$) (Table 3). An increase of 1.25 lbf per millimeter of shell length was maintained between length classes despite the sharp increases in average $F_C$ measured in length classes of mussels greater than 40 mm long ($p = 0.0041$).

<table>
<thead>
<tr>
<th>Length Class</th>
<th>Average $F_C$</th>
<th>$F_C$ Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 mm</td>
<td>34.6 ± 0.978 lbf</td>
<td>A</td>
</tr>
<tr>
<td>30 - 34.99 mm</td>
<td>41.6 ± 2.31 lbf</td>
<td>AB</td>
</tr>
<tr>
<td>35 - 39.99 mm</td>
<td>49.4 ± 2.56 lbf</td>
<td>B</td>
</tr>
<tr>
<td>40 - 44.99 mm</td>
<td>63.2 ± 3.13 lbf</td>
<td>C</td>
</tr>
<tr>
<td>&gt;45 mm</td>
<td>83.4 ± 4.58 lbf</td>
<td>D</td>
</tr>
</tbody>
</table>

Table 3: Averaged $F_C$ (± SE) for blue mussels of different length classes. Length classes were separated into distinct $F_C$ Groupings, signified by a different letter, when the average $F_C$ of one length class was significantly higher than the next lowest length class. Length classes which did not have a significantly different average $F_C$ than the next lowest length class share the same letter with that length class.
3.4 The Effect of Acidification on Crushing Force

The effect of lowering pH decreased the force required to crush the shells of blue mussels, probably because of the impact of reduced pH on $\Omega$, as $F_C$ did not change significantly within each treatment group as the study period continued ($p = 0.235$). $F_C$, standardized against length as $F_S$, averaged $1.50 \pm 0.046$ lbf/mm in the ambient pH treatment over the course of the study, $1.26 \pm 0.041$ lbf/mm in the -0.25 pH treatment, and $1.06 \pm 0.032$ lbf/mm in the 0.50 pH treatment (Table 4). No significant interaction between $\Omega$ and exposure time on $F_S$ was determined ($p = 0.800$). A non-interactive model of $F_S$, $\Omega$, and exposure time was then used to
remove potential masking of effects caused by the inclusion of the insignificant interaction between Ω and exposure time (Model 2.5):

\[ F_S \sim \Omega + \text{Exposure Time} + (1|\text{Aquarium ID}) \]  

(Model 2.5)

This generalized linear mixed effects model of \( F_S \) determined a significant increase in \( F_S \) by 0.609 lbf/mm per one unit increase of \( \Omega \) (\( p = 6.35 \times 10^{-13} \)). Force testing was initiated six weeks after pH treatment began to expose each group to its respective treatment (Fig. 7). A decrease of 0.1 lbf/mm of \( F_S \) per day of exposure time was estimated, although this relationship between exposure time and \( F_S \) was insignificant (\( p = 0.395 \)).

<table>
<thead>
<tr>
<th>Table 4: Averaged crushing force standardized against length (( F_S )) (± SE) of blue mussels from each treatment group over the course of the study period.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample #1 (Aug. 10)</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Control (C, ambient pH)</td>
</tr>
<tr>
<td>Treatment 1 (T1, -0.25 pH)</td>
</tr>
<tr>
<td>Treatment 2 (T2, -0.5 pH)</td>
</tr>
</tbody>
</table>
3.5 The Effect of Acidification on Lengthwise Growth

The growth of the experimental mussels was determined by measuring changes in mussel shell length. Mussels grew in all treatments throughout the 3-month study period, and although lengthwise growth rates differed between treatments, the lengthwise growth rate between all the aquaria did not change significantly over the course of the study period (p = 0.0715). Mussels housed at ambient pH had the highest average lengthwise growth rate of $0.026 \pm 0.0035$ mm/day, followed by mussels in the -0.25 pH treatment group with an average lengthwise growth rate of
0.022 ± 0.0041 mm/day, and the average lengthwise growth rate of mussels in the -0.50 pH treatment was the lowest measured at 0.018 ± 0.0043 mm/day (Table 5). Lengthwise growth rates did not exceed 0.06 mm/day at any point in any of the individual treatment aquaria.

Table 5: Averaged lengthwise growth rates (± SE) of blue mussels from each treatment group over the course of the study period. The averaged lengthwise growth rates

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Average Lengthwise Growth Rate (mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C, ambient pH)</td>
<td>0.026 ± 0.0035 mm/day</td>
</tr>
<tr>
<td>Treatment 1 (T1, -0.25 pH)</td>
<td>0.022 ± 0.0041 mm/day</td>
</tr>
<tr>
<td>Treatment 2 (T2, -0.5 pH)</td>
<td>0.018 ± 0.0043 mm/day</td>
</tr>
</tbody>
</table>

The potential interactive effect of Ω and exposure time on lengthwise growth rate was analyzed using Model 2.3. Ω and exposure time did not have a significant interaction (p = 0.371). As a result, a non-interactive model of lengthwise growth was implemented to prevent potential masking of effects caused by the inclusion of the insignificant interaction between Ω and exposure time (Model 2.6):

\[ G \sim \Omega + \text{Exposure Time} + (1|\text{Aquarium ID}) \]  (Model 2.6)

The generalized linear mixed effects model of growth determined that lengthwise growth rate was higher by 176% per unit increase of Ω (p = 0.004; Fig. 8). Although lengthwise growth rates were estimated to decrease by 1.4% per day of exposure to treatment over the course of the study period across treatment groups, this negative relationship between exposure time and lengthwise growth rate was not significant (p = 0.0544).
Feeding rates were adjusted according to increasing mussel biomass estimated from lengths measured in each aquarium over the course of the study (Fig. 9). Shell length served as a proxy for wet tissue biomass based on measurements taken at the onset of this study for each mussel length class, which led to adjustments in the amount of feed distributed, even when mussels were not removed for sampling, due to growth (Supplementary Material 1).

Figure 8: Lengthwise growth (mm/day) calculated by treatment group at the end of each growth period over the course of the study period (+ SE), with trendlines representing lengthwise growth rates estimated as a function of $\Omega$ and exposure time.
3.6 The Effect of Acidification on Mussel Mortality

The number of mussel mortalities increased significantly over the course of the study period across all treatment groups \((p = 0.003)\) (Fig. 10). Percent mortality did not exceed 5% of the total cohort at any point in any of the treatment aquaria, and the percent mortality ranged from 0.18% to 4.92% over the course of the study period (Fig. 10). \(\Omega\) and exposure time had no significant interactive effect on mortality during the treatment period \((p = 0.474)\), so a non-
interactive model of mortality as a function of $\Omega$ and exposure time was implemented (Model 2.7):

$$\text{Mortality} \sim \Omega + \text{Exposure Time} + (1|\text{Aquarium ID})$$  \quad \text{(Model 2.7)}

The generalized linear mixed effects model for the number of mortalities during this study determined an increase in one additional mortality per one thousand mussel mortalities per unit increase of $\Omega$, but this relationship was not significant ($p = 0.998$). A significant ($p = 0.003$) increase of one additional mortality out of one hundred mussels per additional day of exposure to the acidification treatments was determined across treatment groups.

Figure 10: Percent mortality measured in each treatment group within growth periods over the course of the study (+ SE) plotted with trendlines of the percent mortality estimated as a function of $\Omega$ and exposure time.
4. Discussion

Acidification was hypothesized to decrease crushing force and shell growth because lower seawater pH decreases Ω, which is a metric of carbonate availability in seawater (Salisbury et al. 2008, McGarry et al. 2021). Although salinity generally serves as a proxy of total alkalinity, the area where this study was conducted underwent a drought during the study period which would have limited coastal salinity cycling from freshwater-saltwater mixing (Saba et al. 2019, Hunt et al. 2022, McCarthy et al. 2023). This led to an alternate model being developed and used for the estimation of total alkalinity which did not rely on salinity as a proxy for total alkalinity due to decreased freshwater discharge from inland sources altering chemical cycling in the coastal environment where this study was conducted (Supplementary Material 2). Varied levels of marine acidification were successfully simulated in the treatment aquaria maintained for this study, which caused a gradient of lower Ω from the ambient pH treatment to the lowest pH treatment. Calcium carbonate availability is central to the growth and physical fitness of bivalves like blue mussels, as their shells are built from calcium carbonate obtained from their environment (Salisbury et al. 2008, Atasaral et al. 2020).

The amount of force required to mussel shells was determined to be positively correlated to shell length. As shell length increased, $F_C$ increased in a linear relationship until shell length exceeded 40 mm, when the amount of $F_C$ required increased by 13.81 lbf from the 35 - 39.99 mm length class to the 40 - 44.99 mm length class, and 20.17 lbf from the 40 - 44.99 mm length class to the >45 mm length class. Lower $F_C$ represents less energy exertion required from a predatory crab for successful predation of an individual, so this increase in $F_C$ in larger mussels may indicate a size at which mussels are safe from being crushed by crabs. Length is also an
indicator of age, so it is possible that older, larger mussels are less susceptible to crab predation than younger, smaller mussels (Hastie et al. 2000).

This trend appears to be occurring in the Gulf of Maine already, as higher juvenile mortalities due to predation by invasive crab species in the Gulf of Maine are being reported on aquaculture farms (Capelle et al. 2016). With length being a standard for age estimation in bivalves, lower Ω in the environment may be a driving factor for recently increased mortalities in juveniles in addition to decreased exertion required for a crab to predate upon a small versus a large mussel (Hastie et al. 2000, Capelle et al. 2016). This is likely a factor in the preference of molluscivorous crab species for smaller prey despite the lower edible soft tissue available in smaller individuals (Cornelius et al. 2021). The sharp increase in $F_C$ when mussels were greater than 45 mm in length may indicate a size threshold for predatory crabs, where a different method of predation may become more energy efficient. Sex-specific predatory behaviors have been identified the Asian shore crab *Hemigrapsus takanoi*, which is a close relative of the species *Hemigrapsus sanguineus* invasive to the Gulf of Maine, as males of that species seem to prefer crushing a shell and females pry the shell open (Cornelius et al. 2021). Crab size also directly limits the size of mussels that an individual --or species-- can target, which may indicate that the 45 mm threshold identified in this study represents a size refuge for mussels from this crushing as a predation type (Saxena 2020, Cornelius et al. 2021).

Acidified conditions, which led to decreased Ω, seemed to decrease the force required to crush blue mussel shells irrespective of length ($F_S$), but the length of exposure time did not seem to affect $F_S$. In the context of the increased diversity of invasive crab species in the Gulf of Maine, decreased Ω due to climate change would heighten the susceptibility of bivalves like the blue mussel to predation (Hiebenthal et al. 2013, Kroeker et al. 2014, Atasaral et al. 2020,
Bricknell et al. 2021). Since $\Omega$ seems to affect $F$ and lengthwise growth, the highly variable pH conditions of the Gulf of Maine mean that wild and farmed bivalve populations may be experiencing inhibited shell formation based on the results of this study (NOAA-PMEL, https://www.pmel.noaa.gov/co2/story/GOM, Hunt et al. 2022).

The quality of newly formed shells may also impact interactions with predators, so impacts of shell formation caused by acidified conditions may increase the susceptibility of mussels to predation (Hiebenthal et al. 2013). As mussel shells grow lengthwise, the posterior area of the shell --the area furthest from the shell hinge-- is generally the part held by predators as they exert force on the thicker anterior side of the mussel, where most of the edible soft tissue is located (Cornelius et al. 2021). Further observation of changes in shell density or shell height at the anterior side of the mussel, as well as handling by predatory crustaceans, may provide insight into how acidified conditions impact the interaction between blue mussels and their predators in the Gulf of Maine.

Decreased lengthwise growth would increase the timeframe in which a mussel is most susceptible to predation as its resistance to crushing by predators would be lower for a greater period, as smaller, juvenile mussels are reportedly more susceptible to crab predation (Hastie et al. 2000, Capelle et al. 2016). The presence of a significant relationship between decreased $\Omega$ and decreased lengthwise growth may indicate that acidification may be a stressor which causes decreased growth, whereas the absence of a significant relationship between the duration of exposure to treatment and lengthwise growth may indicate that exposure time to acidified conditions is not a substantial stressor affecting shell formation. Although the rate of lengthwise growth decreased across treatments over the course of the study period, continuous lengthwise growth occurred in aquaria. This sustained growth led to adjustments in the feeding regime even
when the number of mussels did not change over the course of a growth period due to mortalities or removals for sampling.

High-density conditions, which are standard in aquaculture, are known to disfigure mussel shells (i.e., length-width ratio and shell straightness) and reduce soft tissue biomass in comparison to individuals grown in natural conditions (Dionne et al. 2006, Lauzon-Guay et al. 2005). Since mussels in this study were kept in controlled conditions with consistent access to food, which would reduce the competition observed between dense populations of mussels housed in natural systems, the mussels in this study may not have been exposed to the same levels of stress as mussels housed on aquaculture farms where their food is not supplemented and instead is a function of natural water conditions (Capelle et al. 2016).

Acidification is also known to cause shell disfiguration like what has been observed in high-density populations (Hiebenthal et al. 2013). Therefore, mussels kept in high-density aquaculture farms where acidification is prevalent, like the Gulf of Maine, would be expected to grow slower and into a detrimental form with less soft tissue development than mussels in less dense conditions where acidification is less severe. Gulf of Maine aquaculturists may consider decreasing the densities at which they grow mussels and oysters in protective bags and cages, as disfiguration of the shell and decreased growth in high-density conditions may compound with the detrimental effect of acidified conditions on growth and reduce the market value of their product (Lauzon-Guay et al. 2005, Dionne et al. 2006, Ponce et al. 2019).

High-density conditions experienced in captivity on aquaculture farms seem to increase the percent mortality of mussels housed in natural systems, and this stress seems to arise from competition for food when mussels are raised in a natural setting (Capelle et al. 2016). Mussels
in this study were consistently fed based on the biomass present in each aquarium, and mussels were gradually removed over the course of this study for sampling or due to mortality, which would have reduced density-related stress as the study continued. Variable $\Omega$ did not impact percent mortality, and this lack of interaction did not change after continuous exposure to the acidification treatments. Seasonal changes in other water conditions, like water temperature, may have influenced the observed increase in mortality by the end of the study period (Matoo et al. 2021).

Further study into acidification and the physical conditions of blue mussels, or other bivalves, could be conducted in a natural setting for a better representation of the conditions experienced on an aquaculture site. The use of cages and protective bags is recommended for mussels being studied in a natural system as those are the conditions experienced by aquaculture bivalves (Dionne et al. 2006, Capelle et al. 2016). However, this increases the potential for confounding $F_c$ and growth measurements due to the presence of predators in a natural system causing stress responses which could not be simulated in this study (Capelle et al. 2016, Bricknell et al. 2021). A second laboratory-based study which introduces predatory crustaceans like the Asian shore and green crab to mussels housed in aquaria could enable quantification of the impact by predator presence on $F_s$, growth, and mortality, as well as to enable the observation of preferred handling behaviors and times of mussels grown in artificially acidified versus natural conditions by predatory crabs. Consideration of shell length and the most frequently observed predation styles could provide greater information about the length at which juvenile mussels can be more safely transferred from controlled nursery environments to bags and cages in a natural system where there may be predators. Water temperature is a parameter used for the estimation of $\Omega$ so it was not included as a factor of force or growth in this study,
however it could be considered separately in future studies into the impact by climate change on
the physical conditions of bivalves given the relevance of heat events in mollusk health (Sawabe

Although bivalves may have strong natural defenses against climate change and
predation, these adaptations are only resilient to an extent for a given species (Thomsen et al.
2010). In the Gulf of Maine and coastal environments where wild blue mussel populations have
been declining, the combined factors of climate change and predator migration are likely causes
for the historic decline of mussels and other invertebrates (Sorte et al. 2016, Petraitis et al. 2020).
Recent growth in the aquaculture industry alleviates stress on wild populations from fisheries
harvesting, but the byproducts of fossil fuel consumption and coastal development still place
direct pressure on bivalves (Lapointe 2013, FAO 2019). Protective management of coastal and
offshore environments is critical for the maintenance and rehabilitation of damaged and
threatened bivalve habitats.
5. References

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Supplementary Material 1: Biomass per Length Class

Figure S1.1: Biomass (g) of soft tissue shucked from mussels collected from the original sampling site at the UNH Pier in Newcastle, NH at the start of the study period. Weights were averaged by size classes based on individual shell lengths: >30 mm; 30 - 34.99 mm; 35 - 39.99 mm; 40 - 44.99 mm; and >45 mm.
Supplementary Material 2: Estimation of Total Alkalinity

Total alkalinity was measured hourly by the flow-through system in CML as water was pumped from the Piscataqua River for the aquaria in this study, but salinity, which is typically used as a proxy for total alkalinity due to the linear relationship between these variables, variability was lower than expected due to a drought during the study period (Hunt et al. 2022, McCarthy et al. 2023). This lack of variability led to the design of an alternative linear model for total alkalinity which was not reliant upon salinity.

CML hosts automated sensor systems for measurement of the temperature and salinity of intake water (Aanderaa 4319), dissolved oxygen (Aanderaa 4835), and total alkalinity (Contros HydroFIA TA). Seawater temperature data from the Aanderaa system is also used for the calculation of pCO$_2$ concentrations in intake water via a nondispersive infrared gas analyzer (Li-cor LI-840) per methods developed by Hunt et al. (2022). The HydroFIA system conducts hourly single-point titrations of filtered (pore size 0.2 μm) intake seawater with 0.1 N hydrochloric acid and utilizes bromocresol green as an indicator for spectrophotometric pH detection per methods by Li et al. (2013) and was re-calibrated within two-week periods via certified reference material (CRM) from Dickson et al. (2003).

To compensate for salinity no longer serving as a reliable proxy for total alkalinity, a model of total alkalinity calculated from a multiple linear regression of dissolved oxygen, pCO$_2$, and seawater temperature and parameterized against the collected total alkalinity data was developed, which encapsulated the entire study period. Individual variables were first tested as alternative proxies to salinity for total alkalinity, but the combination of dissolved oxygen, pCO$_2$, and seawater temperature produced the best retrieval of total alkalinity. Reduced freshwater-saltwater mixing during the drought period which overlapped with this study caused the
dominance of biogeochemical processes in the Gulf of Maine during the study period, as freshwater discharged from inland sources is the main driver of chemical cycling in coastal and estuarine environments (Hunt et al. 2022, McCarthy et al. 2023). Dissolved oxygen, pCO$_2$, and seawater temperature were measured by the CML flow-through system for the entire study period (Section 3.1). The following multiple linear regression was used for total alkalinity estimation:

$$TA = 4.47e3 + (-97.61T) + (-1.93C) + (-3.39O) + 0.0838TC + 0.115TO$$

where $TA$ is total alkalinity ($\mu$mol kg$^{-1}$), $T$ denotes temperature ($^\circ$C), $C$ is the partial pressure of pCO$_2$ (μatm), and $O$ represents dissolved oxygen ($\mu$mol kg$^{-1}$).

Figure S2.1: Total alkalinity ($\mu$mol kg$^{-1}$) measured by the Contros HydroFIA TA system of intake seawater at CML (blue) plotted alongside values for total alkalinity estimated from pCO$_2$ (μatm), dissolved oxygen (μmol kg$^{-1}$), and seawater temperature ($^\circ$C), measured from intake water at CML (red).
Supplementary Material 3: Validation Plots for Generalized Linear Mixed Effects Models of Crushing Force, Growth, and Mortality

Figure S3.1: Residuals of the generalized linear model of $F_c$ and shell length (mm) by size class. $F_c$ data was modelled as Gamma-distributed.

Figure S3.2: Residuals for the generalized linear mixed effects model of $F_c$ as a function of $\Omega$ and duration of time exposed to the acidification treatment. $F_c$ data was modelled as following a Gamma distribution.
Figure S3.3: Residuals for the generalized linear mixed effects model of growth as a function of $\Omega$ and length of exposure to treatment. The growth rates data was set as a gaussian distribution.

Figure S3.4: Residuals of the generalized linear mixed effects model of mortality as a function of $\Omega$ and length of exposure to treatment. Mortality counts were modelled with a poisson distribution.