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Using Biometrics, Behavioral Observations, and Multiple Molecular Techniques to Assess the Impacts of Changes in Temperature and Salinity on the Common Bay Mussel (*Mytilus trossulus*)

Casey Martin
Portland State University

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by

Casey Martin

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Thesis Committee:
Bradley A. Buckley, Chair
Deborah A. Duffield
Anne W. Thompson

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Abstract

The intertidal zone is a place of rapid and frequent change that is home to a variety of creatures who are essential to the integrity of the habitat. Mussels are robust sessile bivalves that anchor to the rocks of the intertidal. The prominent species on the Oregon Coast, the Common Bay Mussel (*Mytilus trossulus*), plays an essential role as a coastal food source, water column filter, and barrier to prevent erosion due to wave action. *Mytilus trossulus* withstands daily shifts in temperature, salinity, and tide, as well as seasonal changes. Global climate change due to excess carbon emissions is expected to increase temperature, decrease salinity, and cause shifts in sea level beyond what intertidal organisms such as *M. trossulus* experience. Observing the response of *M. trossulus* to these changing conditions can reveal the limits of essential intertidal invertebrates leading to its potential as a biological indicator for climate change. To gain a comprehensive understanding of the impacts of climate change, we must understand seasonal impacts and navigate various ways of testing impacts on this species.

The aim of this work was to: 1) characterize seasonal weight variability in the gametes, gills, and adductor muscles of *M. trossulus*, 2) use protein assay and flow cytometry to further understand the impact of seasonal and climate changes, and 3) understand the influence of tidal fluctuation on a mussels survivability in response to short-term changes in temperature and salinity.

Approximately 190 mussels were collected from the Boiler Bay Intertidal Research Reserve in the Fall (November 15, 2020), Winter (February 26, 2021), and Spring (May 15, 2021). Mussel body size varied greatly (5-30 g), but during the Spring individuals could reach 45 g (Figure 2.4). Fulton's Body Condition Index (K) was

calculated to assess nutritional condition between the seasons. The average seasonal weight of adductor muscles (Fall= 0.177 g, Winter= 0.153 g, Spring= 0.259 g), gametes (Fall= 0.14 g, Winter= 0.206 g, Spring= 0.655 g), and gills (Fall= 0.345 g, Winter= 0.314 g, Spring= 0.576 g) were measured and revealed an increase in gamete weight in the Winter and Spring and gill weight in the Spring.

Mussels (n= 15 from each season) were placed in a treatment group that was based on temperature (12.8°C, 15.5°C, and 18.3°C) and salinity (0 ppt, 15 ppt, and 35 ppt). Following those treatments, n= 5 mussels were measured and dissected, leading to the performance of a Bradford Protein Assay to assess protein concentration in adductor muscles and flow cytometry to assess the cell cycle within gills. A decrease in the protein concentration of adductor muscles in response to increases in temperature and decreases in salinity could impact muscle contraction resulting in a decreased ability to protect internal organs. There was no significant impact on the concentration of protein within the adductor muscles in response to these treatments.

In this study, flow cytometry was used to understand the impact of seasonal changes and climate changes on distribution of gill cells in the cell cycle. Changes in the cell cycle of gill tissue could lead to cellular arrest or death (apoptosis). Flow cytometry revealed over 50 % of cells were in G1 phase in the Fall and Winter, while in the Spring 46.9 % percent of cells were in G1 phase. This led to an increase in potentially apoptotic/non-phase content and an increased sensitivity to increases in temperature and decreases in salinity during the Spring.

Following the temperature and salinity treatments, n= 5 mussels were placed in an aerial exposure at a specified temperature (12.8°C, 15.5°C, and 18.3°C) and n= 5 mussels

were placed underwater at 12.8°C and 35 ppt. These exposures revealed that the mussels collected and treated during the Spring experienced mortality more frequently and rapidly than mussels from other seasons. Additionally, nearly all mussels that were submerged post treatment survived past 300 hours while during the aerial exposures mortality occurred more rapidly as temperatures were increased and salinities decreased.

Efforts to understand seasonal impacts create a more accurate and comprehensive understanding of how environmental stressors are impacting an organism. This study demonstrates the impact of seasonality on the health of an organism, revealing that sensitivity to environmental stressors increased during the Spring which is spawning season. Temperature increases and salinity decreases were shown to have an impact on mussel survivability, but only after being pushed to the worst of conditions. *Mytilus trossulus* is a robust species, tough enough to withstand high wave action, solar radiation, and frequent environmental fluctuation. As changes to the environment increase, this species may experience a reduction in overall fitness and protective abilities. Comprehensive and long-term climate change policy will need to be implemented to prevent even the toughest of organisms from stress and mortality.

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Chapter One: The Natural History of the Common Bay Mussel, *Mytilus trossulus,* and Investigating the Effects of Climate Change

Introduction

Humans have released gigatonnes of carbon into the atmosphere (Brierly and Kingsford 2009, Fan et al. 2014, Dagtekin et al. 2020, Zhai et al. 2023). There are many consequences of increased carbon emissions that have direct impacts on marine systems such as rising sea level, increasing temperature, decreases in salinity due to glacier ablation, acidifying oceans, altered ocean circulation, and severe weather (Durack et al. 2018, Richards et al. 2021). As the primary heat reservoir in the climate system, the ocean has absorbed more than 90% of Earth's anthropogenically added heat since 1971 (Durack et al. 2018). Data collected by the US National Oceanic and Atmospheric Administration (NOAA) demonstrates that the ocean is warming from the surface to the abyss and has warmed approximately 1°C since the pre-industrial era (1880-1900). In the case of salinity, repeated observations reveal that both surface and sub-surface ocean water have experienced a sustained decrease in salinity since 1987 (Nan et al. 2015), likely due to glacier melt.

Temperature is linked to many important aspects of organismal fitness. For example, successful gamete development in freshwater mussels has been linked to temperature (Galbraith and Vaughn 2009). Therefore, global warming is likely to have significant consequences for animals that develop gametes during the winter and spawn in the spring in temperate northern latitudes due to the disruption of the relationship between temperature and photoperiod (Lawrence and Soame 2004). Species are

exhibiting shifts in ecological distribution, physiological performance, and behavioral strategy due to rising environmental temperatures (Nishizaki et al. 2015).

Salinity is a key factor influencing species distributions and community structure in aquatic animals (Lehtonen et al. 2016). For example, salinity levels have been observed to influence male nesting behaviors in sand gobies (Lehtonen et al. 2016). Brazilian flounder larvae do not survive in salinity lower than 20 parts per thousand (Sampaio et al. 2007). Salinities outside of 25.7 parts per thousand (ppt) have been seen to reduce the growth rate of *Mytilus* mussel species (Landes et al. 2015).

Many studies focus on the impact of one stressor such as temperature, salinity, or tidal fluctuations, where it might be more relevant to natural conditions to consider them together. In nature, organisms are exposed to and impacted by many stressors at once while still trying to maintain their normal function. To understand the full scope of the stress that marine organisms are enduring, we must investigate the combined impact of these changing conditions.

Intertidal organisms such as mussels, sea urchins. and sea stars experience vast changes on any given day due to tidal sequences. Other than climate change, humans have also released many toxins into the ocean such as industrial waste, sewage, and radioactive waste (Ilyas et al. 2019, Tuholske et al. 2021, Natarajan et al. 2020). Mussels provide an ideal system for studying anthropogenic (human) impacts on the intertidal zone as they are both sessile (fixed in one place) and filter feeders. Zebra mussels have been utilized as indicators of high concentrations of elements associated with anthropogenic activity (Benito et al. 2017). Since mussels are unable to escape from an area when conditions, biotic and abiotic, change and they consume directly from the

water column, they can accumulate toxins in their tissues among other proximate stressors.

Being sessile, filter feeders forces mussels to attempt tolerating conditions that may be outside of their limits. Mussels are facing the impact of climate change through various physiological perturbations including weakening of their shells due to decreases in pH and new levels of tidal variation due to sea level rise (Sui et al. 2017, Muis et al. 2020, Andrade et al. 2018). Mussel physiological parameters such as reproductive success, strength, ability to anchor to a substrate, and growth rate may also be negatively impacted by changes in temperature and salinity (Kamermans and Saurel 2022, Monaco et. 2019, Freitas et al. 2017). To assess potential changes in these physiological conditions, this study looks at body weight, tissue weight, changes in protein concentration, changes in cell cycle stages, and behavioral changes following temperature and salinity treatments.

Study System

Mussels are bivalve mollusks with hinged shells. They have adductor muscles which allow them to hold their shells tightly closed, helping them protect their internal organs from harsh UV rays, wave action, predators, dehydration during aerial exposure at low tide, and other environmental inputs and perturbations (Chantler 2006). Mussels are sessile due to the byssal threads they produce. These threads possess substantial tensile strength and allow mussels to attach to a substrate, helping to endure high tides and extreme wave action. Mussels have a high fecundity with an iteroparous life cycle (McKenzie 1986). Although, they have been found to experience a mass die off around 1 year of age, shortly after spawning (Heath et al. 1995). Mussels are filter feeders, using

an incurrent siphon to feed on plankton and microscopic organisms. The dominant species of mussel along the Oregon coast is the mussel *Mytilus trossulus* (Common Bay Mussel). *Mytilus trossulus* live in various environments (rocky coastlines, crevices, pier pilings, quiet bays), but are mostly restricted to the North Pacific (Riginos et al. 2004) within intertidal habitats (Braby and Somero 2005).

Mussels have been extensively utilized in the past as a biological indicator of pollution (Cunha et al. 2017; Li et al. 2019; Viarengo and Canesi 1991). As a sessile filter feeder, they are prone to accumulating pollutants in their tissues. Studies have revealed how the accumulation of pollutants can stress mussels, leading to deleterious effects on their rate of growth and production of gametes (Viarengo and Canesi 1991). Box et al. 2007 reported that mussels exposed to pollution caused by agriculture and tourism experienced oxidative stress - an imbalance between oxygen free radicals and antioxidants in the tissues. Oxidative stress can lead to cellular damage including denaturation of and damage to proteins and DNA (Braga 2020). These stressors could impact mussels health and abundance, becoming an issue for the environment and for the economy of harvesting mussels.

Mussels are natural filters which helps to purify the aquatic system and they are an important source of food to many predators, including marine invertebrates, waterfowl, and mammals such as otters, seals, sea lions and humans. Globally, ~3.2 billion humans rely on marine organisms for ~20% of their animal protein intake (Béné et al. 2015). Mussels experiencing stress, related to temperature and salinity changes, could become more weakly attached to their substrates, lose mass, enter cell cycle arrest, and

eventually undergo apoptosis which would reduce their populations and tremendously impact the many organisms that prey upon them.

Specific Aims:

My project focuses on the effect of temperature and salinity on the physiology of *M. trossulus* using a variety of approaches. The Specific Aims of my project are:

Aim1: To characterize seasonal weight variability in the gametes, gills, and adductor muscles of *Mytilus trossulus*. Here we can gain understanding about the impact of seasonal change vs. the impact of the temperature and salinity treatments.

Aim 2: To determine the impact that changes in temperature and salinity have on the protein concentration of adductor muscles and cellular growth of gills. This will give us insight into the impact of temperature and salinity treatments on organismal fitness.

Aim 3: To determine the impact that changes in temperature and salinity have on a mussel's ability to tolerate air exposure and prolonged submersion. Here we can gain further understanding of how the temperature and salinity treatments coupled with the harsh conditions of the intertidal zone impact the survivability of the organism.

Chapter Two: Variation in Tissue Weight and Body Condition in Response to Seasonal Changes, and Muscle Protein Concentration Change in Response to Seasonal Changes and Experimental Stressors

Introduction

The Common Bay Mussel (*Mytilus trossulus)* is the dominant species of mussel along the Eastern Pacific coast of North America. The Oregon Coast is marked by numerous basaltic monoliths that were pushed upward by volcanic activity in modern day Idaho hundreds of years ago and were thrust upward by the rise of the Cascade and Oregon Coastal Mountain ranges (Camp et al. 2003). These monoliths are modern day habitats for both sessile and mobile species. The Boiler Bay Intertidal Research Reserve is a rocky embayment located between Lincoln City and Depoe Bay, Oregon. To collect specimens from this site an Annual Shellfish Licenses must be purchased through the Oregon Department of Fish and Wildlife (ODFW). This research reserve is made up of basalt bluffs and ledges that are frequented by researchers collecting intertidal shellfish and invertebrates (Dahlhoff et al. 2002, Sotka 2007). This site has long been used in climate change-based studies (Helmuth et al. 2002, Menge et al. 2011).

The intertidal is a complex and harsh environment. The community structure of this environment in shaped by abiotic (waves, tides, and substrate) and biotic interactions (Chesson 1985, cited in Emery et al. 2022; Dayton 1971, cited in Emery et al. 2022; Paine 1974, cited in Emery et al. 2022). Organisms living in the intertidal experience daily and seasonal fluctuations in abiotic factors. Due to Earth's rotation, coastal communities' experiences two high and two low tides every 24 hours and 50 minutes and during the Spring, when the Earth, sun, and moon are aligned, these tides are much higher (Vellanoweth et al. 2022). Coupled with these tidal and wave action fluctuations,

aerial (wind, temperature, etc.…) and water conditions (salinity, oxygen availably, etc.…) also change daily and seasonally. Oregon intertidal zones vary in temperature (10-15°C) and salinity (20-35 ppt) due to daily and seasonal shifts, as well as onshore freshwater inputs. The long history of research and many stressors make this environment a unique place to study the impacts of climate change (Amstutz et al. 2021).

Intertidal organisms such as *Mytilus trossulus* (Common Bay Mussel) are an important source of food and a protective shield for the coastline against wave action (Mascorda Cabre et al. 2021, Gonzalez et al. 2021). The health and abundance of these organisms plays a significant role in the geological integrity of this area and in the lives of the organisms that prey upon them (Mascorda Cabre et al. 2021, Gonzalez et al. 2021). As climate continues to shift, mussels could become more susceptible to their daily and seasonal stressors. Specifically, temperatures above 15°C and salinity below 20 ppt could impact the health and growth of individual mussels and therefore the population. This study looks at the health and/or growth of specific tissues (gametes, adductor muscles, and gills) within the organism to gain further understanding of the impacts of seasonal change, temperature and salinity treatments, and tidal fluctuation on *M. trossulus* biometrics, body condition, muscle protein concentration, cell cycle phases, and survivorship.

The health of gametes is key to the growth and maintenance of a species. Gamete weight varies seasonally due to spawning (Hong et al. 2020). Changes in the climate have been found to impact the function of gametes. Temperatures outside of thermal tolerance limits, can cause negative effects on reproductive activity by inhibiting the expression of genes that control the synthesis of hormones and enzymes associated with gamete

development (Lema et al. 2022) and low salinity has been shown to affect fertilization and germination in marine plants (Kevekordes 2000).

A mussel's ability to keep their shells closed is essential to preventing UV damage and predation while they are exposed to air and to withstand wave action while submerged. The adductor muscle is responsible for opening and closing the shell. Hong et al. 2020 reported seasonal changes in the biochemical composition of adductor muscles during the spawning season of giant honeycomb oysters. These composition changes can also lead to weight changes (Hong et al. 2020). Climate related changes can also impact composition and function. Protein integrity and quantity are essential for the adductor muscles to perform the contractions necessary for proper opening and closing of their protective shells (Sugi et al. 2020). Elevated temperatures accelerate the degradation of normally stable proteins (Hofmann et al. 2000; Buckley et al. 2006, Buckley and Somero, 2009) potentially leading to muscle atrophy. Muscle atrophy involves the shrinkage of myofibers due to a net loss of proteins, organelles, and cytoplasm (Sandri 2013). Without properly functioning adductor muscles, *M. trossulus* would not be able to withstand the environmental stressors they encounter daily, such as predation, UV rays, and high wave action.

Gills are important for the consumption of dissolved oxygen making them necessary for the survival of aquatic organisms. This tissue is metabolically active and blood-rich making it vulnerable to changes in environmental conditions (Tsentalovich et al. 2019). Seasonally changes can impact the weight of this tissue by increasing or decreasing the levels of certain metabolomes, small-molecular-weight compounds (Clish 2015, Tsentalovich et al. 2019). Climate related changes have been found to impact the

capabilities of gills. Salinity and temperature can affect the regulation of osmotic pressure, while temperature can also affect metabolic oxygen consumption and ion regulation (Moreira 1980).

Gamete, gill, and adductor muscle weight can be used to understand the impacts of seasonal change on organism tissues and fitness. The body condition/physical status of the whole organism during each season can be used as well. The Fulton's Body Condition Index is broadly used in aquaculture and fisheries to assess organismal health and fitness. Fulton's Body Condition Index ($K= 100 \times (W/L^3)$) uses weight and length to estimate changes in nutritional condition (Jin et al. 2015). Body condition index calculations have been used to determine the impact of seasonal variation in freshwater fish (Alam et al. 2013), salinity on sea snakes (Brischoux 2012) and of temperature on wild rainbow trout (Meka et al. 2005).

In this Chapter, I address the questions: Does season impact the tissue masses, body condition, and muscle protein concentration of *Mytilus trossulus*? Do temperature and salinity treatments have an impact on adductor muscle protein concentration in *Mytilus trossulus*? This will provide insight into seasonal fluctuations in biometrics and protein concentration to establish these characteristics in a normal/untreated state and will further the understanding of the impacts of climate change at a molecular level.

Materials and Methods

Specimen Collection

Mussels were collected from the Boiler Bay Intertidal Research Reserve during the following seasons: Fall (November 15, 2020), Winter (February 26, 2021), and Spring (May 15, 2021). Prior to collections, the temperature and salinity of the seawater

was measured using an Infrared Digital Temperature Gun (Etekcity) and a Hydrometer (Instant Ocean). During each collection, approximately 190 mussels of various sizes were collected. The mussels were collected during low tide, so that mussels could be obtained from a variety of positions in the intertidal zone.

Mussels were placed in a 16-quart cooler that was filled with seawater obtained from adjacent tide pools. Two Fusion 200 air pumps (Petmate) were placed in the cooler to oxygenate the water. To improve the chance of mussel survival during the 2-hour transport from Boiler Bay to Portland State University, the temperature of the transport vehicle was kept at approximately 12.8°C, ±0.5°C. This temperature reflected the average water temperature at the collection site at the time of the collections. Once at the home institution, all but 10 mussels were placed in a re-circulating "common garden" tank, located in the aquatic facility in Portland State University's Center for Life in Extreme Environments (CLEE). The tank was maintained between 10°C-13°C and between 32 ppt-35 ppt. The remaining 10 mussels were prepared for immediate analyses (described below) to characterize starting condition.

Pre- and Post-Dissection Biometric Measurements

Prior to dissection, the wet weight (g) and the length (cm) of each mussel were measured. The position of the length measurement can be found in Figure 2.1. Fulton's condition index (K) was calculated as follows:

$$K = 100 \times W/L^3$$

Adductor muscles, gills, and gametes (Figure 2.1) were dissected out, weighed, and stored in 1.5 ml microcentrifuge tubes. The adductor muscles were stored dry at -

80°C, while the gills and gametes were put in artificial seawater (Instant Ocean at 35 ppt) and stored at 4°C. This process was performed on the n=10 control mussels not placed in the common garden tank upon return from collection, on n=5 mussels sampled before each set of treatments, and on n=15 mussels after each set of treatments. An additional n=5 mussels were sampled from three different treatments (total: n=15) to analyze the impact of temperature and salinity treatments on mussel survivability during tidal fluctuation (Chapter 4).

Gametes and gills were thawed on ice, then placed in a stainless-steel cell sieve (Sigma) fitted with one coarse (40 gauge) mesh and one fine (200 gauge) mesh. Artificial seawater was poured on the tissue and a glass pestle was used to pass the tissue through the mesh. The tissue slurries were centrifuged for 10 minutes at 100x g. Supernatants were removed and pellets preserved in 1 ml of 70% ethanol and then stored at 4°C. Cell cycle analysis was performed on the gill tissue using flow cytometry (Chapter 3).

Temperature and Salinity Treatments

Mussels were exposed to a range of temperatures (12.8°C, 15.5°C, and 18.3°C) and salinities (0 ppt, 15 ppt, and 35 ppt) (Figure 2.2). The average temperature at Boiler Bay throughout the year is 11°C, ±3°C dependent on the season. The lowest (control) temperature, 12.8°C, is within that range, while 15.5°C and 18.3°C were chosen as elevated exposure temperatures. The salinities chosen reflected those of freshwater (0 ppt), brackish water (15 ppt), and saltwater (35 ppt).

The treatments were conducted in a temperature-controlled room in 10-gallon, aerated tanks, fitted with over-the-side filter pumps (Marina) and maintained under

constant re-circulating flow. Each treatment was set up by adjusting the room temperature to one of the three experimental temperatures (12.8°C, 15.5°C, and 18.3°C), then three tanks were filled with either deionized freshwater, brackish water (deionized water at 15 ppt), or saltwater (deionized water at 35ppt). Fifteen mussels were placed in each of the tanks for a total of 45 individuals among the three salinity treatments. The treatments lasted 24 hours to test acute exposure effects.

After 24-h, n=5 mussels from each tank were prepared for immediate dissection. The 10 remaining mussels were put through either aerial exposure or water submersion (Chapter 4). Treatments were conducted on mussels collected in three different seasons, which allowed for the impacts of temperature and salinity exposures to be tested alongside changing seasonal conditions.

Quantification of Protein Concentration in Adductor Muscle

Adductor muscle tissue samples were removed from -80°C and placed on ice to thaw. The tissues were then weighed and homogenized in 200 µl of lysis buffer (32 $mmol^{-1}$ Tris-Cl, pH 6.8, 0.2% sodium dodecyl sulfide [SDS]) (Sleadd and Buckley 2013). The samples were boiled for 5 min at 100°C and then centrifuged for 10 min at 12,000 x g at room temperature. Supernatants were then processed for protein content using a Bradford Protein Assay according to the manufacturer's protocols (Sigma). The concentration of protein within the adductor muscles was compared to denote significance amongst all research specimens and all treatment parameters through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Significance was recorded as any change that produced a p-value less than or equal to 0.05.

Results

Seasonal Biometrics

The average seasonal weights of mussel adductor muscles, gametes, and gills are shown in Figure 2.3 (Table 2.1). During the Fall, the average weight of adductor muscles was 0.177 g, average gamete weight was 0.140 g, and average gill weight was 0.345 g. During the Winter, the average weight of adductor muscles was 0.153 g, average gamete weight was 0.206 g, and average gill weight was 0.314 g. Lastly, Spring average adductor muscle weight was 0.259 g, gametes 0.655 g, and gills 0.576 g. The body size of individuals increased in the Spring as well, being the only season to have multiple individuals weighing in at 30 g or more (Figure 2.4, Table 2.1). The average Fulton's Body Condition Index was K=13.33 in the Fall, K=13.64 in the Winter, and K=11.55 in the Spring (Figure 2.5, Table 2.1).

Seasonal Adductor Muscle Protein Concentration as Percent of Tissue Mass

Mussels collected in the Fall (11/15/20) had on average 2% protein that made up their overall adductor muscle weight (Figure 2.6, Table 2.2). Mussels collected in the Winter (2/25/21) had on average 0.5% protein that made up their overall adductor muscle weight (Figure 2.6, Table 2.2). Mussels collected in the Spring (5/15/21) had on average 1.3% protein that made up their overall adductor muscle weight (Figure 2.6, Table 2.2).

Effect of decreasing salinity on adductor muscle protein concentration

The average protein concentration of Fall adductor muscles significantly (p-value<0.001, Appendix A, Table 1) increased in *M. trossulus* individuals treated at 18.3°C x 15 ppt (4.44%), when compared against the individuals treated at 18.3°C x 0 ppt

(1.07%) and 18.3°C x 35 ppt (0.95%) (Figure 2.7A, Table 2.3). No other significant change in protein concentration occurred due to salinity during the Fall, Winter, and Spring (Figure 2.8A and 2.9A, Table 2.4 and 2.5).

Effect of increasing temperature on adductor muscle protein concentration

During the Fall, the average protein concentration within the adductor muscles significantly (p-value= 0.030, Appendix A, Table 1) decreased when the temperature increased from 12.8°C (2.47%) to 18.3°C (1.07%) at 0 ppt (Figure 2.7B, Table 2.3). During the Fall at 15 ppt, the average protein concentration significantly ($p<0.001$, Appendix A, Table 1) increased when temperature increased from 12.8°C (1.78%) and 15.5°C (2.14%) to 18.3°C (4.44%) (Figure 2.7B, Table 2.3). The Fall mussels treated at 35 ppt, experienced a significant (p= 0.035, Appendix A, Table 1) decrease in the average protein concentration of adductor muscles when temperature increased from 12.8°C (2.39%) to 18.3°C (0.95%) (Figure 2.7B, Table 2.3). No significant change in protein concentration occurred due to temperature during the Winter and Spring (Figure 2.8A and 2.9A, Table 2.4 and 2.5).

Effect of temperature and salinity treatments on adductor muscle protein concentration

During the Fall, mussels treated at 18.3°C x 15 ppt had a significantly (p-values range from 0.003 to <0.001, Appendix A, Table 1) higher average protein concentration than all other treatments (4.44%) (Figure 2.7C, Table 2.3). The 18.3°C x 0 ppt (1.07%) treated mussels had significantly (p-value= 0.030, Appendix A, Table 1) less protein on average within the adductor muscles than the individuals treated at 12.8°C x 0 ppt (2.47%) (Figure 2.7C, Table 2.3). The 18.3°C x 35 ppt (0.95%) treated mussels had

significantly (p= 0.019 and p= 0.035, Appendix A, Table 1) less protein on average within the adductor muscles than the individuals treated at 12.8°C x 0 ppt (2.47%) and the 12.8°C x 35 ppt (2.39%) (Figure 2.7C, Table 2.3). No other significant change in protein concentration occurred due to the temperature and salinity treatments during the Winter and Spring (Figure 2.8A and 2.9A, Table 2.4 and 2.5).

Discussion

In this Chapter, I first addressed the question: Does season impact the tissue masses, body condition, and muscle protein concentration of *Mytilus trossulus*? Season was found to influence *M. trossulus* biometrics.

During the Fall and Winter, gills (Fall=0.345 g, Winter=0.314 g) weighed more than the gametes (Fall=0.140 g, Winter=0.206 g) and adductor muscles (Fall=0.177 g, Winter=0.153 g) (Figure 2.3, Table 2.1). The gametes increased in weight from Fall (0.140 g) to Winter (0.206g) and then Winter (0.206g) to Spring (0.655g) (Figure 2.3, Table 2.1). During the Spring, the weight for all three tissue types increased, but the gametes surpassed the gills (0.576 g) and adductors (0.259 g) in weight (Figure 2.3, Table 2.1). The increase in gamete weight in the Winter is likely due to the beginning of gamete development and the increase during the Spring is likely due to preparing to spawn. Gills also increased in size, during the Spring, potentially due to an increase in metabolic rate to support oxygen delivery to gametes or a general increase in metabolic demand. The increase in mass and overall body size from Fall to Spring is also likely due to the spawning process (Figure 2.4, Table 2.2).

Fulton's Body Condition Index showed a decrease in the K-value during the Spring (K=11.55) (Figure 2.5, Table 2.1) suggesting a change in nutritional condition.

However, to consider this as an indication of seasonal impact on condition further studies utilizing Fulton's Body Condition Index will need to be performed on shelled organisms. This index is primarily used in fisheries science (Jin et al. 2015) and has not been utilized on an organism that's weight is primarily made up by its shell, such as *Mytilus trossulus*.

Seasonal variation did not impact protein concentration prior to treatments (Figure 2.6, Table 2.2). Protein concentration did not vary significantly during the Spring, however significant changes did occur as a result of treatment at 18.3°C during the Fall (Figure 2.7, Table 2.3). In similar study systems, changes in the composition of adductor muscles occurred during the spawning season (Hong et al. 2020). Further studies on the protein concentration and composition of adductor muscles should be performed to gain further understanding on this occurrence and rule out anomalous data.

Mytilus trossulus experiences an increase in gamete, gill, and body mass during the Spring due to spawning. Studies have shown, organisms that develop gametes in the Winter and spawn in the Spring in northern temperate latitudes, such as Oregon coast *M. trossulus*, experience the consequences of global warming more significantly due disruption of the relationship between temperature and photoperiod (Lawrence and Soame 2004). In Chapter 3 and 4, we will further explore the impacts of season on *M. trossulus* fitness.

In this Chapter, I also addressed the question: Do temperature and salinity treatments impact on adductor muscle protein concentration in *Mytilus trossulus*? Protein concentration experienced significant change due to increases in temperature during the Fall.

Treatment 18.3°C x 15 ppt on average had significantly (p-values range from 0.003 to <0.001, Appendix A, Table 1) higher protein than all other treatment groups (4.44%) (Figure 2.7, Table 2.3). This result was likely an anomaly, due to this relationship not being shared amongst the other seasonal groups or 18.3°C treatments. The *Mytilus trossulus* individuals treated at 18.3°C x 0ppt (1.07%) and 18.3°C x 35 ppt (0.95%) had significantly (p-value= 0.030, p= 0.019, and p= 0.035, Appendix A, Table 1) less protein than the 12.8°C x 0 ppt (2.47%) and 12.8°C x 35 ppt (2.39%) treatments (Figure 2.7, Table 2.3). During the Fall, protein concentration decreased due to an increase in temperature to 18.3°C. Increasing temperature has been shown to not only denature protein but cause significant decreases in protein concentration (Qixing et al. 2014).

This study shows that season influences tissue weight and bodyweight due to Spring spawning. Further studies should be performed to prove the validity of the use of Fulton's Body Condition Index on this study system before stating that season impacted body condition. Increases in temperature were shown to cause decreases in protein in most cases during the Fall. As the Earth continues to warm, *Mytilus trossulus* will experience increased sensitivity during the Spring spawning season and may also experience decreases in protein concentration. This may lead to population decreases and muscle weakening. The gametes of *M. trossulus* were not analyzed further than weight in this study. Analysis of the impact of the experimental variables used in this study on the gametes of *M. trossulus* should be performed to expand knowledge of this organism in a changing climate.

Chapter Three: Using Flow Cytometry to Assess Cell Cycle Stage as a Metric of Growth and/or Stress

Introduction

An organ that is necessary for survival of mussels and is an indicator of healthy growth are the gills (Kádár et al. 2010). When looking at gill health, cell cycle proliferation is necessary for the gills to remain in good condition and be capable of taking up the appropriate amount of dissolved oxygen necessary for survival (Zhang et al. 2020). Cellular division is the most fundamental developmental process in unicellular or multicellular organisms, and the cell cycle describes the entire process of cell division from the end of the last mitosis to the completion of the next mitosis (Jacobs 1992).

Flow cytometry is a technology that rapidly analyzes single cells or particles as they flow past single or multiple lasers while suspended in a buffered salt-based solution (McKinnon 2019). It is a frequently used tool in immunology, virology, molecular biology, cancer biology, and infectious disease monitoring (Cossarizza et al. 2021, Georvasili et al. 2022, Robinson 2022). In the field of molecular biology, it can be used for cell cycle analysis (Yang et al. 2020). Cell cycle analysis assays consist of fixing cells in 70% ethanol solution, staining which permeabilizes the cells, and then saturating the DNA with a DNA binding dye (McKinnon 2019). Propidium Iodide (PI) is a fluorescent DNA-binding dye that binds to nucleic acids so that fluorescence emission is proportional to the DNA content of a cell (Riccardi and Nicoletti 2006). PI stain can permeate the membrane of dead or dying cells and living cells when fixed in ethanol (Riccardi and Nicoletti 2006).

This can allow for the quantification of cells in each phase of the cell cycle and the detection of fragmented DNA to determine when a cell has reached apoptosis (Fedr et al. 2023). In my experimental process, flow cytometry was used to identify the quantity of cells in each phase of the cell cycle and the identification of fragmented DNA was attempted during each season and after each treatment. This will provide further insight into the impacts of seasonal change and climate change on gill cell proliferation, therefore the health and growth of *Mytilus trossulus*.

G1, growth phase cells have two chromosome copies and a low fluorescence signal, while cells in G2, growth and preparation for mitosis, phase have double the chromosomes and a high fluorescence signal (Fedr et al. 2023). Cells in S, DNA synthesis, phase will have DNA content and a fluorescence signal between G1 and G2, due to synthesizing DNA on their way to G2 (Fedr et al. 2023). Apoptotic cells contain a fractional DNA content relative to viable cells that can be readily distinguished by flow cytometry (Henry et al. 2013). Cells experiencing apoptosis can be identified as cells with ‘‘sub-G1’’ content (Sleadd et al. 2014). In this study, cells outside of the phases of the cell cycle are referred to as non-phase content/potentially apoptotic when a clear apoptotic signal, fluorescence signal lower than G1, was absent. Though the *Mytilus* species has long been studied in ecological and physiological contexts (Kennedy et al. 2020, Riginos et al. 2005, Hofmann and Somero 1995), flow cytometry has yet to be used for the cellular analysis of these organisms. Will using flow cytometry show an impact of temperature and salinity treatments during the three field seasons on the cell cycle of gills?

Materials and Methods

Cell Cycle Analysis via Flow Cytometry

The flow cytometry protocol used was adapted from Sleadd et al. 2014. Gill samples were removed from 4°C, centrifuged for 1 min at 100x g, and the 70% ethanol was removed from the centrifuge tube. The samples were washed twice by adding 1 x PBS to the sample, centrifuging the sample for 1 min at 100 x g, and then removing the 1 x PBS. The pellets were suspended in 500 µl of 1 x PBS and pipetted through 50 µm micro filters into 5 ml round bottom polystyrene test tubes. In a dark room, 2.5 µl of Propidium Iodide (PI) working solution (100 µl PI stock solution [100 mg dry PI into 200 µl DI H2O] into 900 µl of 1 x PBS) and 10 µl RNase A was added to the test tubes.

A BD Influx™ (BD Biosciences) cytometer was the model of flow cytometer utilized in this study. A 488nm laser was utilized to produce scatter and fluorescent light signals (McKinnon 2019). Data collection was triggered on forward scatter, measuring the relative size of cells (McKinnon 2019). The small particle detector option was implemented to improve forward scatter detection (BD Biosciences). The flow cytometer was calibrated using 4 µm beads for 30 seconds prior to the first sample and after each 5 samples. Each sample was run through the flow cytometer for 2 minutes or less depending on the cell concentration of the sample. The flow cytometer stopped counting cells when the cell count reached 1 million cells.

Data Analysis

Data from the Flow Cytometer was saved on an external hard drive and analyzed with FlowJo™ Software (BD Biosciences). Cell populations were isolated and then analyzed (Figure 3.1). This revealed the quantity of cells overall, in each phase of the cell

cycle, and potentially apoptotic/non-phase content. The quantity of cells within each phase of the cell cycle was compared to denote significance amongst all research specimens and all treatment parameters through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Significance was recorded as any change that produced a p-value less than or equal to 0.05.

Results

Seasonal variation in cell cycle

The variation in cell cycle percent breakdown of the phases between the Fall (mussels collected on 11/15/20), Winter (mussels collected on 2/25/21), and Spring (mussels collected on 5/15/21) is displayed in Figure 3.2 (Table 3.1). On average, G1 had the highest number of cells in any given season. In the Fall and Winter, G1 represented an average of more than 60% of the overall cell count, with S phase representing between 10-20% and G2 representing between 5-10% of overall cell count. In the Fall and winter, between an average of 0-10% was unaccounted for, potentially apoptotic/non-phase content. In the Spring, an average of 46.9% of total cells were in G1 phase, 19.8% in S phase, and 14.8% in G2 phase. On average, approximately 20% of total cells were unaccounted for, potentially apoptotic/non-phase content, during the Spring Season.

Effect of temperature and salinity treatments on overall cell cycle percentages

In the Fall, as the temperature increased the quantity of cells in each phase decreased, increasing the amount of potentially apoptotic/non-phase content. At 12.8°C, the average quantity of cells in G1 phase was between 70-80%, S phase was between 10-20%, and G2 phase was 5-10% (Figure 3.3, Table 3.2). The quantity of non-phase

content in the 12.8°C treatments was between 0-5% (Figure 3.3). At 15.5°C, the average quantity of cells in G1 was between approximately 40-60%, S phase was between 5-25%, and G2 was between approximately 5-15% (Figure 3.3, Table 3.2). Non-phase content in the 15.5°C treatments was between 15-30% (Figure 3.3). At 18.3°C, the average quantity of cells in G1 was between approximately 20-25%, S phase was between 5-20%, and G2 phase was between 10-20% (Figure 3.3, Table 3.2). The non-phase content of 18.3°C treatments ranged between 45-55% (Figure 3.3).

Compared to Fall mussels, Winter mussels showed more potentially apoptotic/non-phase content in the 12.8°C and 15.5°C than in the 18.3°C treatments. The average quantity of cells in each phase of the cell cycle experienced a higher level of variation among each treatment. At 12.8°C, the average quantity of cells in G1 phase was between 40-70%, S phase was between 5-20%, and G2 phase was between 5-15% (Figure 3.4, Table 3.3). At 15.5°C, the average quantity of cells in G1 phase was between 35-80%, S phase was between approximately 10-25%, and G2 phase was between 5-20% (Figure 3.4, Table 3.3). At 18.3°C, the average quantity of cells in G1 phase was between 45-65%, S phase was between 15-25%, and G2 was between approximately 10-20% (Figure 3.4, Table 3.3). The highest levels of non-phase content were found in the 12.8°C x 0 ppt (~45%), 12.8°C x 35 ppt (30%), 15.5°C x 0 ppt (~20%), and 15.5°C x 15 ppt treatments (~20%) (Figure 3.4). The phase content percent is relatively normal when compared to average cell cycle phase content from mussels that have not been treated.

Spring mussels had the lowest average quantity of potentially apoptotic/non-phase content amongst all the seasons mussels were collected (0-15%) (Figure 3.5). The 12.8°C

x 15 ppt treatment stood out amongst the rest as having nearly equal average G1 (42%) and average S (30.76%) phase content and the highest level of average non-phase content (~15%) (Figure 3.5, Table 3.4). Mussels treated at all three salinities and 18.3°C had a majority G1 phase content, between 80-95% (Figure 3.5). When compared against other seasons, the Spring collected mussels that were treated at 18.3°C had the highest G1 phase content.

Effect of salinity on cell cycle phases

In the Fall, Salinity had no significant impact on G1 phase content (Figure 3.6A). A significant (p-value= 0.026, Appendix A, Table 2) decrease in average S phase content occurred when salinity decreased from 15 ppt (22.29%) to 0 ppt (6.336%) under 15.5°C (Figure 3.6B, Table 3.2) Salinity had no significant impact on G2 phase content (Figure 3.6C).

During the Winter, a significant (p-value= 0.021, Appendix A, Table 3) decrease in average G1 phase content occurred when salinity decreased from 35 ppt (79.44%) to 0 ppt (38.74%) under 15.5°C (Figure 3.7A, Table 3.3). No significant impact of salinity on S phase content occurred (Figure 3.7B). Salinity had no significant impact on G2 phase content (Figure 3.7C).

Spring collected mussels experienced a significant (p-value= 0.029, Appendix A, Table 4) increase in average G1 phase content when salinity decreased from 15 ppt (42%) to 0 ppt (80.44%) at 12.8°C (Figure 3.8A, Table 3.4). A significant (p-value= 0.007 and 0.011, Appendix A, Table 4) increase in average S phase content occurred when the mussels were treated at 12.8°C x 15 ppt (30.76%), when compared to the 12.8°C x 0 ppt

(11.238%) and 12.8°C x 35 ppt (12.556%) treatments (Figure 3.8B, Table 3.4). There was no significant impact of salinity on G2 phase content (Figure 3.8C).

Effect of temperature on cell cycle phases

The average G1 phase content of Fall collected mussels significantly (p-value= 0.007 and 0.045, Appendix A, Table 2) decreased when temperature increased from 12.8°C (74.44%) and 15.5°C (61.74%) to 18.3°C (26.62%), at 0ppt (Figure 3.6D, Table 3.2). Average G1 phase content significantly (p-value= 0.029 and <0.001, Appendix A, Table 2) decreased when temperature increased from 12.8°C (77.48%) to 15.5°C (39.18%) and 18.3°C (18.60%), at 15 ppt (Figure 3.6D, Table 3.2). Average G1 phase content significantly (p-value= 0.010, Appendix A, Table 2) decreased when temperature increased from 12.8°C (71.75%) to 18.3°C (23.49%), at 35 ppt (Figure 3.6D, Table 3.2). Temperature had no significant impact on S phase content (Figure 3.6E). A significant (p-value= 0.016, Appendix A, Table 2) increase in average G2 phase content occurred when temperature increased from 15.5°C (4.58%) to 18.3°C (18.83%), at 0 ppt (Figure 3.6F, Table 3.2).

Winter collected mussels experienced a significant (p-value= 0.035, Appendix A, Table 3) increase in average G1 phase content when temperature increased from 12.8°C (42.51%) to 15.5°C (79.44%), at 35 ppt (Figure 3.7D, Table 3.3). Temperature had no significant impact on S phase content (Figure 3.7E). A significant (p-value= 0.042, Appendix A, Table 3) increase in average G2 phase content occurred when temperature increased from 12.8°C (5.74%) to 18.3°C (17.75%), at 15 ppt (Figure 3.7F, Table 3.3).

In the Spring, a significant (p-value= 0.015 and 0.003, Appendix A, Table 4) increase in average G1 phase content occurred when temperature increased from 12.8°C (42%) to 15.5°C (84.80%) and 18.3°C (94.12%), at 15 ppt (Figure 3.8D, Table 3.4). A significant (p-value= 0.005 and <0.001, Appendix A, Table 4) decrease in average S phase content occurred when temperature increased from 12.8°C (30.76%) to 15.5°C (10.61%) and 18.3°C (4.08%), at 15 ppt (Figure 3.8E, Table 3.4). No significant impact of temperature on G2 phase content occurred (Figure 3.8F).

Discussion

In this Chapter, I addressed the question: Will using flow cytometry show an impact of temperature and salinity treatments during the three field seasons on the cell cycle of gills? Utilizing flow cytometry to analyze the cell cycle of *Mytilus trossulus* gill cells revealed changes in the cell cycle during all three seasons in response to rising temperatures (15.5°C and 18.3°C) and decreasing salinities (0 ppt).

Cell cycle analysis was first performed on untreated mussels to set a baseline for the distribution of cells in each phase of the cell cycle during the Fall, Winter, and Spring (Figure 3.2, Table 3.1). This revealed that the largest proportion of cells should be in G1 phase in any given season (45-75%). Additionally, mussels collected and analyzed during the Spring had the highest quantity of non-phase/potentially apoptotic cells. This suggested an increased sensitivity to stressors may occur in the Spring. Other studies, have observed shifts in cell proliferation due to seasonal changes in temperature, photoperiod, and metabolic demand in fish and seasonally breeding mammals (Dunlap et al. 2011, Helfer et al. 2019).

Following the temperature and salinity treatments, Fall and Spring collected mussels experienced a shift in cell production. During the Fall, as temperature increased to 18.3°C the quantity of cells in G1 phase decreased (15-25%), increasing the quantity of non-phase/potentially apoptotic content (45-55%) (Figure 3.3). During the Spring, as temperature increased to 18.3°C the quantity of cells in G1 phase increased (80-95%) (Figure 3.5). This suggests an increased chances of apoptosis during the Fall and G1 cellular arrest, a pause in the cell cycle, during the Spring. A clear apoptotic signal, fluorescence signal lower than G1, was not located using the methods described previously, further studies using flow cytometry should be performed to clarify the occurrence of apoptosis, ruling out tissue clumping, in *M. trossulus*. However, Spring cellular arrest is likely due to the metabolic demand of Spring spawning increasing *M. trossulus* temperature sensitivity (Lawrence and Soame 2004). Similar response in cell proliferation during the spawning season of scorpionfish have been identified (Andreyeva et al. 2019).

Salinity was also shown to have a significant (Appendix A, Table 2-4) impact on the cell cycle of gill tissue, during all three seasons. During the Fall (Figure 3.6B, Table 3.2) and Winter (Figure 3.7B, Table 3.3), decreasing salinity to 0 ppt either caused decreases in S phase (22.29% (15 ppt) to 6.336% (0 ppt)) or G1 phase (79.44% (35 ppt) to 38.74% (0 ppt)) content under 15.5°C. During the Spring, decreasing salinity to 0 ppt caused an increase in G1 phase content (42% (15 ppt) to 80.44% (0 ppt)) at 12.8°C (Figure 3.8A, Table 3.4). Salinity fluctuations have been found to invoke a cellular stress response such as apoptosis and cellular arrest in fish (Evans and Kültz 2020). Decreases in S phase and G1 phase content during the Fall and Winter in response to decreasing

salinity may be connected to apoptosis, however a clear apoptotic signal, fluorescence signal lower than G1, was absent. The increase in G1 phase content in response to decreasing salinity during the Spring is likely G1 phase cellular arrest due to spawning (Lawrence and Soame 2004, Andreyeva et al. 2019)

Increases in temperature had a significant (Appendix A, Table 2-4) impact on the cell cycle of *M. trossulus* gill cells. During the Fall, as temperature increased to 18.3°C the quantity of G1 phase cells decreased at all salinity levels (70-75% (12.8°C) to 20-25% (18.3°C)). During the Winter as temperature increased to 15.5°C, G1 phase (42.51% (12.8°C) to 79.44%(15.5°C)) (Figure 3.7D, Table 3.3) at 35 ppt increased. Lastly, in the Spring increasing temperature to 15.5°C and 18.3°C increased G1 phase content (42% (12.8°C) to 84.80% (15.5°C) to 94.12% (18.3°C)), at 15 ppt (Figure 3.8D, Table 3.4). This correlates with a decrease in G2 phase content following the same treatments (30.76% (12.8°C) to 10.61% (15.5°C) to 14.08% (18.3°C)) (Figure 3.8E, Table 3.4). Apoptosis linked G1 phase content loss may be occurring in the Fall in response to heat stress, however a clear apoptotic signal, fluorescence signal lower than G1, was absent. During the Winter and Spring, G1 experienced an increase as temperatures increased suggesting G1 phase arrest in response to heat stress. Heat stress has been found to induce apoptosis and G1 cellular arrest (Sun et al. 2020, Trotter 2001)

In the Fall, *Mytilus trossulus* has increased sensitivity to decreases in salinity (0 ppt) and increases in temperature (15.5°C and 18.3°C), potentially leading to apoptosis. During the Spring, *M. trossulus* has an increased sensitivity to decrease in salinity (0 ppt) and increasing temperature (15.5°C and 18.3°C), leading to G1 phase cellular arrest (Trotter 2001). G1 phase arrest could be occurring during the Spring in relation to

increased climate change sensitivity during the spawning process ((Lawrence and Soame 2004, Andreyeva et al. 2019). As the Earth continues to warm, organisms such as *M. trossulus* may have difficulty spawning or surviving to the spawning stage during the warmer months.

Chapter Four: Pre-Exposure to Elevated Temperature and Low Salinity Impacts on Survivorship During Subsequent Aerial Exposure and Water Submersion in the Common Bay Mussel (*Mytilus trossulus)*

Introduction

While determining the impact of temperature and salinity on Bay Mussels, it is important to take into consideration the shifts in the environment that *Mytilus trossulus* experiences regularly. Coastal systems are characterized by natural fluctuations in abiotic conditions occurring on a daily, seasonal, and annual basis (Shim 2007, Wootton 2008). Being an intertidal organism, *M. trossulus* experiences long periods of aerial exposure during low tide and long periods of being fully submerged during high tide. During submersion mussels have high food availability, dissolved oxygen, and a relatively stable body temperature (Mangan 2019). During aerial exposures mussels are unable to feed, have limited access to oxygen, and body temperature is dependent on air temperature, solar irradiance, evaporation, and wind speed (Helmuth 1998). These factors make aerial exposures more stress inducing than being fully submerged.

When considering the impacts of climate change on an intertidal organism, it is necessary to include the impacts of tidal variation to begin to understand the full scope of stressors these organism encounter. Intertidal organisms, such as *M. trossulus*, experience high levels of variation in conditions such as temperature and salinity daily due to the tidal cycle (Collins et al. 2020). The body temperature of an intertidal organism is dependent on the sea water during high tide and the air during low tide. The tidal cycle has been found to impact an intertidal organism's response to stressors such temperature by increasing heart rate and metabolic activity when exposed to air (Collins et al. 2020).

Considering the influence of tidal variation on the study system's response to seasonal and climate related changes allows for an expanded understanding on how these organisms may respond to similar changes in nature. Does seasonal variation and temperature and salinity treatments have an impact on the survivability of mussels exposed to air or fully submerged?

Materials and Methods

Temperature and Salinity Treatments

As described in Chapter 2, mussels were exposed to a range of temperatures (12.8°C, 15.5°C, and 18.3°C) and salinities (0 ppt, 15 ppt, and 35 ppt). The treatments were conducted in a walk-in temperature-controlled room in 10-gallon tanks. The room temperature was set to three experimental temperatures, then three tanks were filled with either deionized freshwater (0 ppt), brackish water (deionized water at 15 ppt), or saltwater (deionized water at 35 ppt). Fifteen mussels were placed in each of the tanks for 24 hours.

Post-treatment Aerial and Submerged Exposures

Following the temperature and salinity treatments, n=5 mussels were removed from the tanks and placed on wet paper towels in the same walk-in temperature-controlled room. Those individuals were observed daily to determine length of survivorship during an aerial exposure. Mortality versus survival was observed by tapping on the shell of the individual to cause adductor muscle reflexive contraction. If an individual did not respond, mortality was recorded. Additionally, an individual producing a strong odor was determined to have experienced mortality.

Alongside the aerial exposures, a separate full water submersion was being conducted on n=5 mussel in a 10-gallon tank under control parameters (12.8°C and 35 ppt). Identical to the aerial exposure observations, mussels were observed for mortality. Daily observations ceased after 300 hours in both post treatment exposures.

Results

Seasonal variation in post treatment exposure survivorship

During the aerial exposures, Fall (Table 4.1) collected mussels persisted the longest, compared to the Winter (Table 4.2) and Spring (Table 4.3) collected mussels, regardless of prior exposure to various temperatures and salinities (Figure 4.1).

During the water submersions, Spring collected mussels experienced one mortality during each temperature treatment. While the mussels collected in the Fall and Winter experienced full survivorship (Figure 4.2, Table 4.4).

Effect of pre-exposure temperatures on subsequent aerial exposure survivorship.

During all seasons, when mussels were exposed to increasing temperatures, mortality increased at a more rapid rate. Post 12.8°C aerial exposures lasted approximately 200 to 275 hours dependent upon the salinity and season. Post 15.5°C aerial exposures lasted approximately 125 to 250 hours dependent upon the salinity and season. Post 18.3°C aerial exposures lasted approximately 50 to 200 hours dependent upon the salinity and season (Figure 4.1, Table 4.1, 4.2, and 4.3).

Effect of pre-exposure salinities on subsequent aerial exposure survivorship.

Across the seasons and temperatures, the mussels that were exposed to 35 ppt (Saltwater) survived the aerial exposure the longest. The first salinity group to experience

complete loss of individuals switched, throughout the temperatures and seasons, between the 0 ppt (Freshwater) and 15 ppt (Brackish) salinity groups (Figure 4.1, Table 4.1, 4.2, and 4.3).

Effect of pre-exposure to temperature and salinity treatments on subsequent water submersion.

The Mussels collected in the Fall and Winter all survived the temperature and salinity exposures before entering the Water Submersion. All Fall and Winter mussels survived once placed in the water submersion regardless of the temperature or salinity they were exposed to in the 24-h treatment. One spring collected mussel that was exposed to 12.8°C x 15 ppt was recorded as having experienced mortality at 27.5 hours in the water submersion. One spring collected mussel that was exposed to 15.5°C x 0 ppt did not survive the initial treatment before entering the water submersion. One spring collected mussels from the 18.3°C x 0 ppt treatment and one from the 18.3°C x 15 ppt treatment did not survive those initial treatments before entering the water submersion. All other mussels in the 15.5°C and 18.3°C survived the water submersion regardless of salinity treatment (Figure 4.2, Table 4.4).

Discussion

In this Chapter, I addressed the question: Does seasonal variation and temperature and salinity treatments have an impact on the survivability of mussels exposed to air or fully submerged? Seasonal metabolic demand, increasing temperature, and decreasing salinity increased mortality during both aerial exposure and water submersion, with greater impact in the aerial exposure.

Increased sensitivity and vulnerability to Spring seasonal changes continued in this Chapter. Spring mussels experienced the most rapid rates of mortality among the seasons. Most likely due to mussels being under high environmental and physiological stress making them more sensitive to increases in temperature, salinity, and prolonged aerial exposure. As temperature increased and salinity decreased, simultaneously or separately, survivability decreased over time during long periods of aerial exposure in our target species (Figure 4.1). As these conditions exceeded *Mytilus trossulus* optimal ranges for temperature and salinity in an aerial environment, metabolic activity and heart rate may have increased eventually causing death (Collins et al. 2020).

In the water submersion exposures, mussels collected in the Spring were more sensitive to the temperature and salinity treatments than mussels collected in other seasons, likely related to the metabolic demand of spawning. At least one mortality occurred at each temperature, suggesting minimal temperature influence on water submersion survivability (Table 4.4). Spring mussels were sensitive to 0 ppt and 15 ppt salinity, with two mortalities occurring at both salinities (Figure 4.2).

These findings may be explained by the fact that mussels in the Winter and Spring were accumulating gametes in preparation for spawning. When a mussel is exposed to air during low tide, they are unable to feed, take up oxygen, and their body temperature varies greatly (Helmuth 1998) rendering them more sensitive to shifts in temperature and salinity (Lawrence and Soame 2004). Minimal mortality was experienced during the water submersion this is likely due to having high food availability, dissolved oxygen, and a relatively stable body temperature (Mangan 2019). As climate change progresses, mussels exposed to air for prolonged periods of time during their spawning season will

experience more mortality. However, sea level rise is a factor of climate change that may mitigate the impacts of planetary warming by flooding large areas of the intertidal. Further studies on the potential for sea level rise to have a positive impact on the intertidal would contribute greatly to intertidal and climate research.

Chapter Five: Summary

During the years 2020-2021, 190 Common Bay Mussels (*Mytilus trossulus*) were collected from the Boiler Bay Research Reserve and placed in various temperature and salinity treatments and aerial or water exposures. Biometrics, protein assay's, flow cytometry, and behavioral observations were used to assess the impact of sublethal treatments of temperature and salinity and exposures on the gills, gametes, and adductor muscles of *M. trossulus*. Weight and length measurements prior to treatments were used to establish an understanding of the seasonal impacts on body condition, body size, and tissue mass (Chapter 2). Protein concentration within adductor muscles was used to analyze seasonal variation and the impact of stressors on tissue health (Chapter 2). The impact of temperature and salinity treatments and seasonality on the cell growth within gills was analyzed using flow cytometry, which to our knowledge has never been done within this Genus (Chapter 3). Lastly, survivability was observed post temperature and salinity treatments within prolonged aerial exposures and water submersions (Chapter 4). These procedures were utilized to create a comprehensive assessment of the impacts of temperature and salinity and seasonality on cellular health and physiology of *M. trossulus*.

In Chapter 2, I addressed the questions: Does season impact the tissue masses, body condition, and muscle protein concentration of *Mytilus trossulus*? Do temperature and salinity treatments impact on adductor muscle protein concentration in *Mytilus trossulus*? Biometrics showed that *M. trossulus* experiences an increase in body mass and therefore overall body size during the Spring months (March-May). This increase in body mass correlated with an increase in gamete and gill weight, related to

spawning. The nutritional condition, Fulton's Body Condition Index, decreased in the Spring. This shows that seasonal changes do impact the overall body size, but to make that claim for nutritional condition of *M. trossulus* further studies using Fulton's Body Condition Index must be performed on shelled organisms. Protein assay data revealed no substantial evidence for seasonal changes in adductor muscle protein concentration prior to treatment, but post temperature and salinity treatment Fall mussels experienced a decrease in protein concentration when they were treated with 18.3°C at 0 ppt and 35 ppt. Protein has been shown to decrease due to heat stress (Qixing et al. 2014), but further studies are needed to understand why this decrease only occurred in the Fall.

In Chapter 3, I addressed the question: Will using flow cytometry show an impact of temperature and salinity treatments during the three field seasons on the cell cycle of gills? Flow cytometry allowed for the quantification of cells in each phase of the cell cycle, but fractional DNA content was not observed leading to the lack of a clear apoptotic signal, fluorescence signal lower than G1. The cell cycle of *M. trossulus* gills, prior to treatment, during the Fall and Winter have ≥60% of cells in G1 phase, ≤30% in S phase, and ≤30% in G2 phase. During the Spring, cell production shifts to <50% in G1 phase and between 10-20% in S and G2 phase. This result as well as post treatment results indicate Spring mussels are more likely to experience cell cycle changes and G1 phase arrest caused by exposure to temperature and salinity stressors, due to Spring spawning being a metabolically demanding time (Lawrence and Soame 2004, Andreyeva et al. 2019, Trotter 2001).

Temperatures above 15°C and salinities below 20 ppt had an impact on cell cycle of *M. trossulus* gills. Flow cytometry revealed that mussels collected in the Spring, when

exposed to 15.5°C or 18.3°C, experienced an increase in G1 phase percent. During the Fall, G1 decreased when mussels were treated at 18.3°C. Mussels exposed to 18.3°C were particularly sensitive to G1 phase arrest during the Spring (Trotter 2001) and potentially apoptosis in the Fall, suggested by the loss of G1 phase content. During the Spring, decreasing salinity to 0 ppt (Freshwater) increased the percentage of cells in the G1 phase. This further corroborates that G1 phase arrest may be occurring in the Spring as result of increased stressors during the metabolically demanding spawning season

Lastly in Chapter 4, I addressed the question: Does seasonal variation and temperature and salinity treatments have an impact on the survivability of mussels exposed to air or fully submerged? During the aerial survivability trials, increases in temperature and decrease in salinity were paired with increasing and rapid mortality across all seasons, with the most rapid occurring during the Winter and Spring months. The metabolic demands of Spring spawning make *M. trossulus* more sensitive to temperatures greater than 15°C and salinities less than 20 ppt. Mussels that were fully submerged following temperature and salinity treatments were able to fully recover from the stress inducing treatments in most cases. One Spring mussel experienced mortality during the submersions. Aerial exposures push mussels to their limits daily during low tide and water submersion return mussels to a relatively stable environment during high tide (Helmuth 1998). The addition of increased temperature and salinity stressors make long periods of aerial exposure more physiologically straining.

Overall, as climate change progresses due to increased carbon emissions intertidal species such as *Mytilus trossulus* we need to cope with increased temperatures and decreased salinities (Durack et al. 2018, Richards et al. 2021) on top of the daily and

seasonal stressors they experience. Due to spawning, *M. trossulus* will be the most vulnerable to climate changes during the Spring. This species may experience an increase in G1 phase arrest and aerial exposure mortality during the spawning season. Temperatures higher than 15°C and salinities lower than 20 ppt were shown to have the greatest impact on gill cell proliferation and organismal survival. Additionally, temperatures exceeding 18°C may lead to decreases in protein concentration of adductor muscles, but further studies are needed. The impacts of climate change that were observed in this study could pose a threat to the *Mytilus trossulus* species, and therefore the intertidal ecosystem. This species of mussel is the primary species along the Oregon coast and is essential in protecting against coastal erosion, filtering the water column, and is a widely consumed prey item (Mascorda Cabre et al. 2021, Gonzalez et al. 2021).

Study Limitations and Future Studies

It should be acknowledged that gametes were not analyzed further than weight, this paper cannot be considered an assessment for reproductive health. A closer look at gamete integrity post temperature and salinity treatments would add further knowledge to the threats posed to *M. trossulus* in a changing climate. Additionally, the 24-h length of the treatments limits this assessment to short-term climate events. A long-term project that looks at the impacts of multiple stressors would yield the closest replication to the true environment *M. trossulus* has waiting for them in a changing world. Flow cytometry offered insight into the cell cycle of *M. trossulus,* but the lack of a clear apoptosis requires further investigation. Lastly, prior to temperature and salinity treatment mussels were keep fully submerged in a common garden tank. This does not perfectly replicate the experience of *M. trossulus* in the intertidal, where they would be experiencing two

high tides and two low tides daily. An *in-situ* investigation would allow for comprehensive understanding of the implications of daily change in the intertidal.

Tables

Table 2.1: Biometrics and Fulton's Body Condition Index for all *Mytilus trossulus* collected during the Fall, Winter, and Spring

Season	wet weight (g)	length (cm)	adductor(g)	gamete(g)	gill(g)	Fulton's Body Condition Index (K)
Fall	14.485	5.5	0.368	0.270	0.599	8.706235913
	13.216	4.3	0.173	0.174	0.390	16.622435760
	15.903	4.4	0.311	-	0.465	18.668998870
	18.317	5.2	0.177	0.169	0.756	13.026997040
	8.973	5.5	0.010	0.110	0.061	5.248784212
	15.952	4.4	0.368	0.110	1.206	18.726521410
	16.768	5.6	0.133	0.313	0.256	9.548104956
	11.293	5.2	0.093	0.129	0.478	8.031548703
	8.141	5.3	0.162	0.163	0.590	5.468272467
	17.377	5.3	0.220	0.470	0.487	11.672051430
	31.009	5.8	0.180	0.161	0.371	15.892923040
	13.682	4.7	0.161	0.340	0.231	13.178197510
	11.748	4.5	0.200	0.074	0.310	12.892181070
	6.344	4.4	-	-	-	7.447407964
	14.087	4.5	0.154	0.154	0.145	15.458984910
	16.508	5.0	0.117	0.192	0.486	13.206400000
	14.132	4.6	0.074	0.045	0.292	14.518780310
	11.822	4.7	0.199	0.087	0.363	11.386686960
	10.305	4.2	0.156	0.040	0.231	13.909135080
	9.231	4.1	0.128	0.028	0.372	13.393595570
	12.897	5.0	0.128	0.063	0.281	10.317600000
	10.887	4.0	0.215	0.087	0.286	17.010937500
	9.825	4.2	0.134	0.050	0.470	13.261256880
	7.249	3.8	0.096	0.022	0.157	13.210745010
	8.571	4.0	0.093	0.083	0.320	13.392187500
	25.454	5.65	0.300	0.141	0.560	14.112719060
	16.556	4.9	0.263	0.221	0.293	14.072367810
	9.755	4.0	0.137	0.087	0.318	15.242187500
	8.082	3.9	0.153	0.090	0.224	13.624639660
	7.359	3.5	0.083	0.029	0.206	17.163848400
	20.192	5.3	0.508	0.250	0.638	13.562874050
	17.361	5.3	0.190	0.026	0.514	11.661304300
	13.363	4.0	0.119	0.838	0.448	20.879687500

	11.173	4.2	0.710	0.006	0.440	15.080714830
	12.890	4.4	0.180	0.091	0.232	15.131949660
	13.104	4.4	0.294	0.142	0.308	15.383170550
	13.403	5.4	0.100	0.091	0.387	8.511786821
	8.257	4.1	0.185	0.084	0.270	11.980383340
	12.288	4.3	0.135	0.171	0.323	15.455242930
	15.007	4.7	0.173	0.075	0.402	14.454407980
	12.660	4.3	0.105	0.032	0.248	15.923126270
	3.596	2.7	0.066	0.012	0.129	18.269572730
	13.154	4.6	0.359	0.178	0.333	13.514013310
	4.285	3.3	0.081	0.048	0.116	11.923644160
	8.956	4.6	0.205	1.000	0.294	9.201117778
	21.793	6.0	0.422	0.271	0.570	10.089351850
	14.625	4.8	0.097	0.072	0.395	13.224283850
	12.042	4.7	0.114	0.108	0.475	11.598586060
	5.606	3.5	0.047	0.039	0.173	13.075218660
	5.708	3.5	0.054	0.028	0.055	13.313119530
	19.191	5.3	0.270	0.210	0.770	12.890506930
	13.264	4.5	0.220	0.150	0.130	14.555829900
	8.309	4.5	0.050	0.030	0.030	9.118244170
	10.599	3.6	0.110	0.040	0.250	22.717335390
	3.659	3.5	0.010	-	0.070	8.534110787
	12.743	4.4	0.136	0.355	0.411	14.959382040
	9.112	4.2	0.103	0.032	0.210	12.298887810
	10.594	4.1	0.276	0.093	0.182	15.371222120
	12.210	4.5	0.082	0.039	0.212	13.399176950
	3.709	2.9	0.060	0.014	0.161	15.207675590
Average			0.177067797	0.142824561	0.34542373	13.328311010
Standard Deviation			0.12331469	0.15230446	0.20247343	3.351717560

Season	**wet weight (g)**	**Length (cm)**	**adductor(g)**	**gamete(g)**	**gill(g)**	**Fulton's Body Condition Index (K)**
Winter	14.123	4.8	0.235	0.301	0.486	12.770363140
	3.462	3.0	0.072	0.008	0.176	12.822222220
	8.421	4.9	0.146	0.133	0.128	7.157731897
	18.523	5.6	0.327	0.302	0.516	10.547444420
	14.494	5.1	0.101	0.050	0.611	10.926415930
	5.710	3.6	0.091	0.170	0.227	12.238511660
	19.755	5.4	0.371	0.446	0.597	12.545724740
	8.594	4.1	0.164	0.132	0.302	12.469348960

13.748	4.7	0.288	0.218	0.423	13.241767240
5.784	3.3	0.074	0.014	0.208	16.094832620
3.117	2.8	0.039	0.027	0.094	14.199161810
14.153	4.8	0.178	0.276	0.328	12.797489870
23.654	5.4	0.263	0.196	0.405	15.021846260
5.845	3.4	0.049	0.063	0.096	14.871259920
15.218	5.0	0.214	0.398	0.351	12.174400000
20.853	5.5	0.210	0.200	0.320	12.533734030
18.519	5.6	0.090	0.220	0.720	10.545166730
3.186	2.9	0.043	-	0.148	13.063266230
8.041	3.7	0.137	0.203	0.168	15.874676720
19.767	5.2	-	-	-	14.058232820
17.609	5.3	0.340	0.346	0.543	11.827884760
5.679	3.4	0.036	0.049	0.166	14.448911050
17.488	5.3	0.237	0.445	0.477	11.746609620
12.049	4.3	0.257	0.414	0.349	15.154640470
19.788	5.4	0.185	0.399	0.412	12.566681910
23.977	5.9	0.227	0.266	0.454	11.674513950
9.290	4.0	0.115	0.130	0.146	14.515625000
9.186	4.3	0.164	0.253	0.258	11.553699670
14.826	4.9	0.193	0.221	0.435	12.601892070
7.296	3.9	0.082	0.048	0.179	12.299600470
19.057	5.4	0.198	0.312	0.438	12.102448810
3.747	3.2	0.055	0.058	0.059	11.434936520
15.216	4.5	0.142	0.473	0.382	16.697942390
7.612	3.7	0.065	0.046	0.174	15.027737750
10.577	4.2	0.056	0.020	0.167	14.276266060
7.595	3.9	0.066	0.062	0.197	12.803654820
9.516	4.2	0.156	0.113	0.253	12.844185290
13.032	4.4	0.094	0.062	0.394	15.298647630
6.970	4.0	0.119	0.131	0.197	10.890625000
16.751	5.4	0.123	0.340	0.540	10.637987100
7.320	3.4	0.069	0.108	0.245	18.624058620
16.756	5.0	0.232	0.337	0.477	13.404800000
15.728	4.8	0.205	0.300	0.434	14.221643520
3.563	3.2	0.035	0.044	0.138	10.873413090
11.704	4.5	0.223	0.408	0.331	12.843895750
9.818	4.0	0.117	0.204	0.145	15.340625000
29.036	5.5	0.329	0.612	0.674	17.452141250

	6.682	3.9	0.027	0.017	0.164	11.264518960
	6.050	3.2	0.075	0.033	0.099	18.463134770
	6.968	3.2	0.106	0.018	0.127	21.264648440
	7.189	4.1	0.033	0.048	0.212	10.430783070
	11.058	4.1	0.140	0.211	0.201	16.044456700
	9.705	4.4	0.058	0.046	0.220	11.392984600
	19.289	5.0	0.204	0.233	0.584	15.431200000
	9.231	4.3	0.109	0.138	0.248	11.610298460
	14.064	4.7	0.183	0.333	0.374	13.546131400
	6.437	3.5	0.085	0.081	0.247	15.013411080
	23.961	5.5	0.267	0.531	0.472	14.401803160
	9.548	4.1	0.074	0.080	0.179	13.853542460
	16.385	5.0	0.354	0.391	0.444	13.108000000
	15.740	4.6	0.199	0.463	0.382	16.170789840
	18.697	5.0	0.370	0.631	0.528	14.957600000
	9.922	4.0	0.099	0.129	0.203	15.503125000
	9.841	4.0	0.174	0.238	0.221	15.376562500
	11.145	4.5	0.142	0.101	0.299	12.230452670
	17.247	4.8	0.129	0.122	0.463	15.595160590
	10.379	3.8	0.092	0.128	0.185	18.914929290
	10.467	4.3	0.132	0.175	0.240	13.164878560
	7.058	3.6	0.109	0.135	0.184	15.127743480
	21.138	5.5	0.173	0.186	0.430	12.705033810
Average			0.15284058	0.20626471	0.31411594	13.638426480
Standard Deviation			0.08990757	0.15627011	0.15883904	2.338096282

Season	**wet weight (g)**	**Length (cm)**	**adductor(g)**	**gamete(g)**	**gill(g)**	**Fulton's Body Condition Index (K)**
Spring	19.137	5.5	0.190	0.588	0.637	11.502329080
	27.083	6.1	0.196	1.223	0.637	11.931835700
	12.312	5.3	0.202	0.461	0.532	8.269914090
	21.845	5.9	0.268	0.815	0.620	10.636433130
	6.925	4.4	0.130	0.209	0.318	8.129460932
	12.402	4.8	0.144	0.399	0.417	11.214192710
	10.795	4.5	0.260	0.360	0.377	11.846364880
	24.420	6.0	0.400	0.891	0.738	11.305555560
	11.584	4.5	0.153	0.258	0.397	12.712208500
	20.370	5.6	0.337	0.807	0.571	11.599170920
	28.081	5.9	0.253	0.885	0.537	13.672770830
	25.847	5.9	0.280	1.149	0.712	12.585025730

34.386	7.0	0.265	1.368	1.077	10.025072890
20.241	5.7	0.230	0.788	0.500	10.929678770
29.464	6.4	0.302	0.535	0.727	11.239624020
13.517	4.8	0.134	0.275	0.347	12.222403070
24.474	5.6	0.256	0.612	0.519	13.936087830
26.830	6.4	0.223	0.812	0.430	10.234832760
36.922	7.1	0.481	1.402	1.168	10.315972410
30.265	6.6	0.387	0.989	0.792	10.527102990
42.680	7.5	0.369	1.007	1.248	10.116740740
11.412	4.6	0.165	0.403	0.455	11.724336320
26.995	6.1	0.528	1.251	0.523	11.893065940
13.631	4.7	0.184	0.283	0.382	13.129075450
20.928	6.0	0.208	0.999	0.534	9.688888889
25.564	6.0	0.266	0.570	0.806	11.835185190
20.382	5.4	0.263	0.657	-	12.943910990
14.466	4.3	0.172	0.154	0.314	18.194624370
15.120	5.3	0.181	0.570	0.557	10.156034850
13.050	4.7	0.210	0.405	0.425	12.569469190
20.982	5.6	0.147	0.317	0.454	11.947658530
21.820	5.5	0.281	0.852	0.528	13.114951160
15.055	4.7	0.190	0.593	0.403	14.500640510
22.012	5.7	0.390	0.825	0.737	11.885978410
27.774	6.5	0.249	1.435	0.773	10.113427400
16.532	5.2	0.324	0.713	0.515	11.757510240
15.952	5.1	0.263	0.371	0.578	12.025540700
21.860	5.9	0.231	0.581	0.646	10.643736700
17.525	4.8	0.171	0.285	0.464	15.846535010
20.574	5.5	0.289	0.697	0.551	12.366040570
16.095	5.0	0.317	0.405	0.494	12.876000000
28.022	6.0	0.424	0.318	0.604	12.973148150
18.711	5.5	0.321	0.436	0.448	11.246280990
21.493	6.0	0.215	0.493	0.513	9.950462963
15.038	5.1	0.316	0.508	0.291	11.336514610
44.564	7.5	0.565	1.336	1.232	10.563318520
10.322	4.4	0.088	0.296	0.315	12.117299020
26.022	6.1	0.388	0.492	0.562	11.464395700
23.339	6.0	0.172	0.523	0.686	10.805092590
26.432	6.0	0.246	0.700	0.541	12.237037040
29.652	6.2	0.197	1.111	1.014	12.441677020

16.794	4.9	0.266	0.222	0.416	14.274664470
24.075	6.5	0.381	0.733	0.777	8.766499772
16.670	5.2	0.211	0.686	0.436	11.855655440
12.502	5.2	0.230	0.301	0.337	8.891385981
15.455	5.3	0.132	0.600	0.644	10.381052820
21.983	6.3	0.412	0.901	0.727	8.791547189
16.951	5.0	0.299	0.727	0.490	13.560800000
18.028	6.0	0.280	0.714	0.690	8.346296296
20.713	5.7	0.289	0.519	0.637	11.184548010
13.219	4.4	0.196	0.384	0.358	15.518172430
12.389	5.2	0.157	0.364	0.374	8.811020710
13.175	4.4	0.157	0.177	0.371	15.466519530
24.873	5.8	0.369	0.638	0.578	12.748062650
21.774	6.1	0.288	0.878	0.461	9.592873412
23.145	6.4	0.340	0.554	0.946	8.829116821
12.543	5.0	0.135	0.358	0.355	10.034400000
17.051	5.1	0.212	0.696	0.399	12.854030500
13.856	5.0	0.170	0.852	0.501	11.084800000
20.063	6.2	0.206	1.132	0.728	8.418230338
Average		0.2593	0.65497143	0.57611429	11.553004160
Standard Deviation		0.09744212	0.32163153	0.21441802	1.924440200

Fulton's Body Condition Index ($K= 100 \times W/L^3$) was calculated using wet weight.

Table 2.2: Protein concentration within the adductor muscles of *Mytilus trossulus* during the Fall, Winter, and Spring collection seasons

Season	Protein weight per tissue wet weight (g)	Protein per tissue (%)
Fall	0.011487458	1.148745763
	0.018758530	1.875852961
	0.012313411	1.231341065
	0.014893384	1.489338436
	0.052711864	5.271186441
	0.008798409	0.879840924
	0.016880373	1.688037337
	0.026845574	2.684557439
	0.020671447	2.067144720
	0.015764913	1.576491305
Average		1.991253639
Standard Deviation		1.260340244
Winter	-0.000560127	-0.056012745

		0.004526972	0.452697208
		0.005572268	0.557226825
		0.001830688	0.183068829
		0.006805244	0.680524409
		0.005853890	0.585388994
		0.002747551	0.274755063
		0.006268557	0.626855676
		0.004063785	0.406378540
		0.010483871	1.048387097
Average			0.475926990
Standard Deviation			0.302859388
Spring		0.014633085	1.463308458
		0.012282904	1.228290366
		0.015739819	1.573981942
		0.010470564	1.047056385
		0.015676617	1.567661692
		0.020071073	2.007107321
		0.011093314	1.109331392
		0.009045583	0.904558290
		0.014208955	1.420895522
		0.010357800	1.035780004
Average			1.335797137
Standard Deviation			0.334472104

N=10 Mytilus trossulus individuals were dissected to collect the gills, gametes, and adductor muscles. The adductor muscle was then analyzed using Bradford Protein Assay to determine the percent of adductor muscle weight that is protein. This analysis was performed to understand the impact of seasonal changes on the protein concentration of the study system.

Table 2.3: Protein concentration within the adductor muscles of Fall collected *Mytilus trossulus* in response to temperature and salinity treatments

Temperature (°C)	Salinity (ppt)	Protein weight per tissue wet weight (g)	Protein per tissue (%)
12.8°C	**0 ppt**	0.019297381	1.929738059
		0.010111985	1.011198547
		0.021781097	2.178109739
		0.045617433	4.561743341
		0.026779661	2.677966102
Average			2.471751157
Standard Deviation			1.315656618
12.8°C	**15 ppt**	0.016561042	1.656104151

		0.026080058	2.608005770
		0.015789259	1.578925873
		0.014293070	1.429307016
		0.016246469	1.624646893
Average			1.779397941
Standard Deviation			0.471299503
12.8°C	**35 ppt**	0.019576271	1.957627119
		0.019304193	1.930419269
		0.025916061	2.591606134
		0.031025424	3.102542373
Average			2.395548724
Standard Deviation			0.561663977
15.5°C	**0 ppt**	0.007197534	0.719753358
		0.025713069	2.571306939
		0.014952611	1.495261141
		0.022150036	2.215003609
		0.011870491	1.187049118
Average			1.637674833
Standard Deviation			0.753475689
15.5°C	**15 ppt**	0.008291322	0.829132218
		0.029431673	2.943167305
		0.010347030	1.034702955
		0.038241197	3.824119686
		0.020785964	2.078596403
Average			2.141943713
Standard Deviation			1.267393413
15.5°C	**35 ppt**	0.016057123	1.605712295
		0.015104507	1.510450737
		0.026959248	2.695924765
Average			1.937362599
Standard Deviation			0.658658569
18.3°C	**0 ppt**	0.009041152	0.904115212
		0.012040933	1.204093294
		0.003223507	0.322350723
		0.007554287	0.755428737
		0.021809257	2.180925666
Average			1.073382727
Standard Deviation			0.695976278
18.3°C	**15 ppt**	0.011967225	1.196722522

		0.021742247	2.174224716
		0.076998597	7.699859748
		0.038892006	3.889200561
		0.072335203	7.233520337
Average			4.438705576
Standard Deviation			2.931995887
18.3°C	**35 ppt**	0.003463971	0.346397100
		0.007433380	0.743338008
		0.008813546	0.881354554
		0.018259116	1.825911641
		0.009653028	0.965302762
Average			0.952460813
Standard Deviation			0.542950950

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The adductor muscle was then analyzed using Bradford Protein Assay to determine the percent of adductor muscle weight that is protein. This analysis was performed to understand the impact of changes in temperature and salinity on the protein concentration of the study system. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 1).

Table 2.4: Protein concentration within the adductor muscles of Winter collected *Mytilus trossulus* in response to temperature and salinity treatments

Temperature (°C)	Salinity (ppt)	Protein weight per tissue wet weight(g)	Protein per tissue (%)
12.8°C	**0 ppt**	0.016703870	1.670386978
		0.020539829	2.053982883
		0.032029505	3.202950496
		0.014519269	1.451926901
		0.009994286	0.999428601
Average			1.875735172
Standard Deviation			0.834111398
12.8°C	**15 ppt**	0.009102181	0.910218103
		0.043660589	4.366058906
		0.008151904	0.815190420
		0.009657041	0.965704079
		0.022171337	2.217133698
Average			1.854861041
Standard Deviation			1.516675771
12.8°C	**35 ppt**	0.008960449	0.896044881
		0.019681140	1.968114034
		0.018949665	1.894966495

		0.013716053	1.371605253
		0.020500234	2.050023375
Average			1.636150808
Standard Deviation			0.491481632
15.5°C	**0 ppt**	0.032965425	3.296542472
		0.015930898	1.593089784
		0.018221599	1.822159888
		0.018364485	1.836448464
		0.017081580	1.708158018
Average			2.051279725
Standard Deviation			0.703028841
15.5°C	**15 ppt**	0.029991585	2.999158485
		0.008356929	0.835692927
		0.009349444	0.934944438
		0.043044753	4.304475328
		0.009732601	0.973260065
Average			2.009506249
Standard Deviation			1.569444295
15.5°C	**35 ppt**	0.016886396	1.688639551
		0.004905684	0.490568416
		0.052080411	5.208041141
		0.021239256	2.123925630
		0.027769986	2.776998597
Average			2.457634667
Standard Deviation			1.749199384
18.3°C	**0 ppt**	0.016839801	1.683980100
		0.026912804	2.691280440
		0.011182974	1.118297402
		0.027277128	2.727712787
		0.012469271	1.246927129
Average			1.893639572
Standard Deviation			0.773831158
18.3°C	**15 ppt**	0.013184572	1.318457219
		0.004954967	0.495496655
		0.025970149	2.597014925
		0.013366886	1.336688650
		0.014514925	1.451492537
Average			1.439829997
Standard Deviation			0.751057242

18.3°C	**35 ppt**	0.020877153	2.087715270
		0.039196326	3.919632606
		0.023074103	2.307410317
		0.021937769	2.193776878
		0.014026165	1.402616547
Average			2.382230324
Standard Deviation			0.928856929

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The adductor muscle was then analyzed using Bradford Protein Assay to determine the percent of adductor muscle weight that is protein. This analysis was performed to understand the impact of changes in temperature and salinity on the protein concentration of the study system. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 1).

Table 2.5: Protein concentration within the adductor muscles of Spring collected *Mytilus trossulus* in response to temperature and salinity treatments

Temperature (°C)	Salinity (ppt)	Protein weight per tissue wet weight(g)	Protein per tissue (%)
12.8°C	**0 ppt**	0.014222310	1.422230951
		0.007827965	0.782796544
		0.006529353	0.652935323
		0.005519770	0.551976957
		0.005673030	0.567303020
Average			0.795448559
Standard Deviation			0.362165387
12.8°C	**15 ppt**	0.005330880	0.533087985
		0.012429985	1.242998491
		0.003309250	0.330924993
		0.010352632	1.035263244
Average			0.785568678
Standard Deviation			0.425063443
12.8°C	**35 ppt**	0.012682220	1.268221997
		0.010823469	1.082346886
		0.016178410	1.617841027
		0.012816437	1.281643729
		0.011849175	1.184917518
Average			1.286994232
Standard Deviation			0.201333566
15.5°C	**0 ppt**	0.009906343	0.990634312
		0.008459451	0.845945083
		0.008024691	0.802469136

		0.013458286	1.345828587
		0.009094334	0.909433373
Average			0.978862098
Standard Deviation			0.217028078
15.5°C	**15 ppt**	0.012075571	1.207557141
		0.004850742	0.485074181
		0.009831843	0.983184334
		0.009674042	0.967404186
		0.010941560	1.094155987
Average			0.947475166
Standard Deviation			0.275973222
15.5°C	**35 ppt**	0.004429583	0.442958319
		0.016797421	1.679742140
		0.012371382	1.237138244
		0.013279243	1.327924273
		0.009586784	0.958678367
Average			1.129288269
Standard Deviation			0.462126574
18.3°C	**0 ppt**	0.010480962	1.048096221
		0.005277280	0.527727981
		0.009986772	0.998677249
		0.006366965	0.636696467
		0.008886845	0.888684547
Average			0.819976493
Standard Deviation			0.227867356
18.3°C	**15 ppt**	0.011711229	1.171122921
		0.012138717	1.213871697
		0.017059230	1.705922995
		0.007918263	0.791826309
		0.007521114	0.752111399
Average			1.126971064
Standard Deviation			0.386499748
18.3°C	**35 ppt**	0.006633384	0.663338364
		0.016135078	1.613507820
		0.011417918	1.141791812
		0.014638534	1.463853363
		0.009370870	0.937087012
Average			1.163915674
Standard Deviation			0.385556113

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The adductor muscle was then analyzed using Bradford Protein Assay to determine the percent of adductor muscle weight that is protein. This analysis was performed to understand the impact of changes in temperature and salinity on the protein concentration of the study system. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 1).

Table 3.1: Percent of *Mytilus trossulus* gill cells in each phase of the cell cycle during the Fall, Winter, and Spring collection seasons

Season	G1 Percent (%)	S Percent (%)	G2 Percent (%)
Fall	65.00	6.89	23.20
	85.00	2.57	10.60
	79.90	2.46	15.00
	98.60	0.23	0.70
	51.50	39.20	3.78
	39.20	10.80	41.70
	52.40	15.40	25.60
	14.50	38.90	16.00
	83.70	3.24	11.00
	66.40	5.23	22.80
Average	63.62	12.49	17.04
Standard Deviation	0.25034367	0.1468628	0.11918864
Winter	94.30	0.84	3.74
	86.50	2.23	9.75
	45.20	19.50	26.40
	97.60	0.11	2.01
	97.70	0.07	1.91
	8.65	18.60	42.30
	97.50	0.20	1.96
	93.10	0.77	5.40
	81.60	3.39	12.60
	30.50	27.90	24.00
Average	73.27	7.36	13.01
Standard Deviation	0.327426332	0.104383962	0.13658247
Spring	26.20	14.30	49.30
	95.20	0.38	3.86
	18.00	15.00	18.00
	36.70	29.40	17.80
	34.80	26.80	20.60
	66.30	5.22	23.90

	13.30	27.70	24.30
	9.59	24.90	18.40
	72.90	4.37	18.60
	96.10	0.18	3.20
Average	46.91	14.83	19.80
Standard Deviation	0.330673308	0.117761805	0.126852208

N=10 Mytilus trossulus individuals were dissected to collect the gills, gametes, and adductor muscles. The gill tissue was then analyzed using flow cytometry to determine the quantity of cells in each phase of the cell cycle. This analysis was performed to understand the impact of seasonal change on the cellular health of the study system. This method can be used to detect cellular arrest and apoptosis.

Table 3.2: Percent of Fall collected *Mytilus trossulus* gill cells in each phase of the cell cycle post temperature and salinity treatment

Temperature (°C)	Salinity (ppt)	G1 Percent (%)	S Percent (%)	G2 Percent (%)
12.8°C	**0 ppt**	91.60	6.22	1.31
		93.90	4.99	0.58
		96.10	2.91	0.50
		65.50	21.10	7.52
		25.10	25.30	29.90
Average		74.44	12.10	7.96
Standard Deviation		30.23521126	10.30561158	12.60848603
12.8°C	**15 ppt**	94.00	4.42	0.81
		87.50	9.12	1.94
		52.40	25.00	13.30
		79.10	13.30	4.63
		74.40	14.80	6.49
Average		77.48	13.33	5.43
Standard Deviation		15.92504317	7.672399885	4.930134887
12.8°C	**35 ppt**	77.20	13.60	4.89
		81.90	11.50	3.83
		59.70	26.80	6.73
		68.20	21.30	5.43
Average		71.75	18.30	5.22
Standard Deviation		9.840901043	7.061161378	1.206261442
15.5°C	**0 ppt**	11.10	5.12	7.42
		91.60	5.07	1.88
		94.70	3.79	0.84
		95.40	3.40	0.57
		15.90	14.30	12.20
Average		61.74	6.34	4.58
Standard Deviation		44.09277719	4.516882775	5.086444731
15.5°C	**15 ppt**	33.60	43.60	11.90
		23.50	32.10	27.60
		83.80	11.60	2.93
		30.30	5.95	7.08

		24.70	18.20	18.20
Average		39.18	22.29	13.54
Standard Deviation		25.28076344	15.39766216	9.700418548
15.5°C	**35 ppt**	91.80	4.59	1.87
		34.70	42.30	15.90
		26.20	22.90	33.10
		89.10	8.45	1.31
		11.80	10.50	26.30
Average		50.72	17.75	15.70
Standard Deviation		37.19283533	15.34163518	14.26096876
18.3°C	**0 ppt**	96.90	2.63	0.16
		6.12	9.64	24.90
		6.60	3.20	33.60
		8.10	10.20	22.40
		15.40	7.23	13.10
Average		26.62	6.58	18.83
Standard Deviation		39.46266438	3.532612348	12.73915696
18.3°C	**15 ppt**	8.31	3.93	5.62
		15.70	11.10	14.90
		25.00	9.09	4.55
		13.60	45.80	16.50
		30.40	14.90	13.80
Average		18.60	16.96	11.07
Standard Deviation		8.937198666	16.59854301	5.563710992
18.3°C	**35 ppt**	5.05	5.61	9.97
		9.50	16.00	24.60
		68.40	21.50	5.87
		5.89	2.75	6.88
		28.60	8.16	3.06
Average		23.49	10.80	10.08
Standard Deviation		26.87254119	7.749808385	8.48656173

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The gill tissue was then analyzed using flow cytometry to determine the quantity of cells in each phase of the cell cycle. This analysis was performed to understand the impact of changes in temperature and salinity on the cellular health of the study system. This method can be used to detect cellular arrest and apoptosis. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 2).

Table 3.3: Percent of Winter collected *Mytilus trossulus* gill cells in each phase of the cell cycle post temperature and salinity treatment

Temperature (°C)	Salinity (ppt)	G1 Percent (%)	S Percent (%)	G2 Percent (%)
12.8°C	**0 ppt**	4.42	1.43	3.93
		88.50	8.57	1.57
		17.10	7.89	21.70
		69.70	17.10	7.21
		18.50	9.11	10.40
Average		39.64	8.82	8.96

Standard Deviation		37.03479013	5.571848885	7.863788527
12.8°C	**15ppt**	91.30	6.45	1.33
		23.70	33.60	10.50
		68.80	21.00	5.95
		74.30	14.00	7.14
		78.40	14.60	3.77
Average		67.30	17.93	5.74
Standard Deviation		25.74597056	10.16486596	3.463765292
12.8°C	**35 ppt**	91.20	6.32	1.34
		7.25	5.40	11.30
		53.90	8.30	13.80
		51.80	31.20	9.23
		8.38	28.30	22.60
Average		42.51	15.90	11.65
Standard Deviation		35.33722089	12.72434989	7.694152325
15.5°C	**0 ppt**	34.50	17.40	22.10
		38.70	47.10	6.96
		6.40	8.51	22.00
		16.50	33.50	33.10
		97.60	2.05	0.19
Average		38.74	21.71	16.87
Standard Deviation		35.43646427	18.45933287	13.16731939
15.5°C	**15 ppt**	52.10	33.10	8.97
		42.70	10.70	7.90
		48.60	34.50	7.44
		53.30	24.60	11.60
		42.50	27.20	7.33
Average		47.84	26.02	8.65
Standard Deviation		5.086059378	9.488782851	1.772983361
15.5°C	**35 ppt**	95.00	4.24	0.17
		97.40	2.00	0.19
		84.90	10.20	2.57
		87.30	8.86	1.95
		32.60	23.00	27.30
Average		79.44	9.66	6.44
Standard Deviation		26.6938757	8.169137041	11.71166
18.3°C	**0 ppt**	82.60	12.40	3.15
		44.90	37.00	10.30
		33.00	14.40	19.10
		60.70	20.50	11.90
		89.30	8.60	1.08
Average		62.10	18.58	9.11
Standard Deviation		24.00364556	11.16073474	7.228463184
18.3°C	**15 ppt**	53.10	30.40	9.49
		94.60	4.24	0.57
		44.30	21.60	14.50
		35.50	26.60	24.50
		15.50	29.40	39.70

Average		48.60	22.45	17.75
Standard Deviation		29.24876066	10.73608495	15.01089171
18.3°C	**35 ppt**	59.40	28.70	6.95
		47.30	19.60	18.00
		85.00	10.40	2.45
		19.10	11.60	26.50
		24.60	41.50	22.60
Average		47.08	44.62	15.30
Standard Deviation		26.82344124	25.9607835	10.25749726

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The gill tissue was then analyzed using flow cytometry to determine the quantity of cells in each phase of the cell cycle. This analysis was performed to understand the impact of changes in temperature and salinity on the cellular health of the study system. This method can be used to detect cellular arrest and apoptosis. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 3).

Table 3.4: Percent of Spring collected *Mytilus trossulus* gill cells in each phase of the cell cycle post temperature and salinity treatment

Temperature (°C)	Salinity (ppt)	G1 Percent (%)	S Percent (%)	G2 Percent (%)
12.8°C	**0 ppt**	95.30	2.91	0.55
		68.40	15.80	8.23
		99.50	0.18	0.18
		63.00	22.70	7.25
		76.00	14.60	5.88
Average		80.44	11.24	4.42
Standard Deviation		16.22445685	9.42241052	3.795098154
12.8°C	**15ppt**	20.80	22.60	19.00
		63.80	25.80	5.77
		31.20	35.50	18.20
		53.20	32.30	8.24
		41.00	37.60	10.40
Average		42.00	30.76	12.32
Standard Deviation		17.08625178	6.379890281	5.967245596
12.8°C	**35 ppt**	99.40	0.52	0.038
		24.40	19.50	27.60
		39.90	37.80	13.10
		99.70	0.25	0.08
		94.40	4.71	0.28
Average		71.56	12.56	8.22
Standard Deviation		36.45206441	16.15039721	12.20227457
15.5°C	**0 ppt**	93.70	5.18	0.48
		47.60	29.60	13.00
		94.50	2.83	1.30
		51.10	28.90	11.50
		33.60	26.50	27.30
Average		64.10	18.60	10.72
Standard Deviation		28.15945667	13.40045969	10.88918179
15.5°C	**15 ppt**	92.20	6.24	0.77

		63.20	22.70	8.04
		91.40	6.46	1.20
		92.90	5.45	0.74
		84.30	12.20	1.95
Average		84.80	10.61	2.54
Standard Deviation		12.55726881	7.273946659	3.1131415
15.5°C	**35 ppt**	88.80	8.17	1.52
		39.80	40.60	12.10
		87.20	8.92	2.42
		68.10	16.70	8.02
		98.80	0.97	0.00
Average		76.54	15.07	4.81
Standard Deviation		23.34797636	15.31894481	5.077412727
18.3°C	**0 ppt**	13.30	31.40	38.70
		96.80	2.51	0.35
		99.40	0.44	0.09
		99.10	0.79	0.04
		97.90	1.82	0.12
Average		81.30	7.39	7.86
Standard Deviation		38.02716135	13.4460206	17.24083396
18.3°C	**15 ppt**	99.60	0.26	0.07
		80.30	13.00	4.28
		99.40	0.49	0.00
		92.80	5.18	0.81
		98.50	1.47	0.00
Average		94.12	4.08	1.03
Standard Deviation		8.213221049	5.363510977	1.848236998
18.3°C	**35 ppt**	98.10	1.62	0.03
		85.50	10.20	2.34
		66.80	13.80	10.30
		97.80	1.47	0.27
		93.70	5.06	0.60
Average		88.38	6.43	2.71
Standard Deviation		13.09110385	5.434712504	4.340348972

Mytilus trossulus individuals that were exposed to each temperature and salinity treatment were dissected to collect the gills, gametes, and adductor muscles. The gill tissue was then analyzed using flow cytometry to determine the quantity of cells in each phase of the cell cycle. This analysis was performed to understand the impact of changes in temperature and salinity on the cellular health of the study system. This method can be used to detect cellular arrest and apoptosis. Two-way ANOVA and LSD post hoc testing was performed to find statistical significance (Appendix A, Table 4).

Table 4.1: Aerial Exposure Related Mortality Observations post temperature and salinity treatments of Fall collected *Mytilus trossulus*

Temperature (°C)	Hours in Exposure	0 ppt Mortality	15 ppt Mortality	35 ppt Mortality
12.8°C	126.00	2	0	0
	150.00	1	0	0
	220.50	2	3	2

	244.00	0	1	2
	269.00	0	1	1
Complete Mortality (Hrs.)		220.50	269.00	269.00
15.5°C	102.75	0	1	0
	128.00	4	1	0
	150.00	0	0	1
	171.00	1	1	0
	197.00	0	2	1
	242.50	0	0	3
Complete Mortality (Hrs.)		171.00	197.00	242.50
18.3°C	71.15	1	1	0
	97.00	3	4	0
	123.00	1	0	1
	147.75	0	0	1
	172.00	0	0	1
	195.75	0	0	2
Complete Mortality (Hrs.)		123.00	97.00	195.75

N= 5 mussels from each temperature and salinity treatment were placed in a temperature control room (set at the temperature they were previously treated with) for an aerial exposure. Mussels were observed daily for mortality vs. survival. Mortality was recorded after a lack of adductor muscle contraction occurred in response to tapping on its shell. This table lists observations where a mortality was recorded. Mussels treated with higher temperatures and lower salinities experienced mortality at a more rapid rate than mussels treated at 12.8°C and 35 ppt.

Table 4.2: Aerial Exposure Related Mortality Observations post temperature and salinity treatments of Winter collected *Mytilus trossulus*

Temperature (°C)	Hours in Exposure	0 ppt Mortality	15 ppt Mortality	35 ppt Mortality
12.8°C	0.00	0	2	0
	98.50	1	0	0
	170.00	3	0	1
	192.00	1	3	3
	219.00	0	0	1
Complete Mortality (Hrs.)		192.00	192.00	219.00
15.5°C	50.00	1	1	0
	73.00	0	2	0
	92.00	3	1	0
	123.00	1	1	1
	168.00	0	0	3
	215.50	0	0	1

Complete Mortality (Hrs.)		123.00	123.00	215.50
18.3°C	0.00	2	0	0
	49.50	1	3	1
	75.00	1	1	2
	97.50	1	1	2
Complete Mortality (Hrs.)		97.50	97.50	97.50

N= 5 mussels from each temperature and salinity treatment were placed in a temperature control room (set at the temperature they were previously treated with) for an aerial exposure. Mussels were observed daily for mortality vs. survival. Mortality was recorded after a lack of adductor muscle contraction occurred in response to tapping on its shell. This table lists observations where a mortality was recorded. Mussels treated with higher temperatures and lower salinities experienced mortality at a more rapid rate than mussels treated at 12.8°C and 35 ppt.

Table 4.3: Aerial Exposure Related Mortality Observations post temperature and salinity treatments of Spring collected *Mytilus trossulus*

Temperature (°C)	Hours in Exposure	0 ppt Mortality	15 ppt Mortality	35 ppt Mortality
12.8°C	71.00	1	0	0
	97.50	2	1	2
	124.00	0	1	0
	146.50	1	0	1
	168.00	0	2	0
	194.00	0	1	1
	217.00	1	0	0
	265.00	0	0	1
Complete Mortality (Hrs.)		217.00	194.00	265.00
15.5°C	0.00	1	0	0
	80.00	2	0	0
	103.00	1	0	2
	123.00	0	2	0
	148.00	1	2	2
	171.00	0	1	0
	195.50	0	0	1
Complete Mortality (Hrs.)		148.00	171.00	195.50
18.3°C	0.00	1	1	0
	26.00	0	1	0
	54.00	0	3	0
	76.00	3	0	2
	103.00	1	0	3
Complete Mortality (Hrs.)		103.00	54.00	103.00

N= 5 mussels from each temperature and salinity treatment were placed in a temperature control room (set at the temperature they were previously treated with) for an aerial exposure. Mussels were

observed daily for mortality vs. survival. Mortality was recorded after a lack of adductor muscle contraction occurred in response to tapping on its shell. This table lists observations where a mortality was recorded. Mussels treated with higher temperatures and lower salinities experienced mortality at a more rapid rate than mussels treated at 12.8°C and 35 ppt.

Table 4.4: Water Submersion Related Mortality Observations post temperature and salinity treatments of Spring collected *Mytilus trossulus*

Temperature (°C)	Hours in Exposure	0 ppt Mortality	15 ppt Mortality	35 ppt Mortality
12.8°C	27.50	0	1	0
15.5°C	0.00	1	0	0
18.3°C	0.00	1	1	0

N= 5 mussels from each temperature and salinity treatment were submerged in a 10-gallon tank with water set to 12.8°C and 35 ppt. Mussels were observed daily for mortality vs. survival. Mortality was recorded after a lack of adductor muscle contraction occurred in response to tapping on its shell. This table lists observations where a mortality was recorded. One mortality was experienced during the submersion at 27.5 hrs. The mortalities reported at 0 hrs. in the exposures occurred at some point during the initial temperature and salinity treatments. No other mortalities occurred during the Fall, Winter, and Spring submersions.

Figures

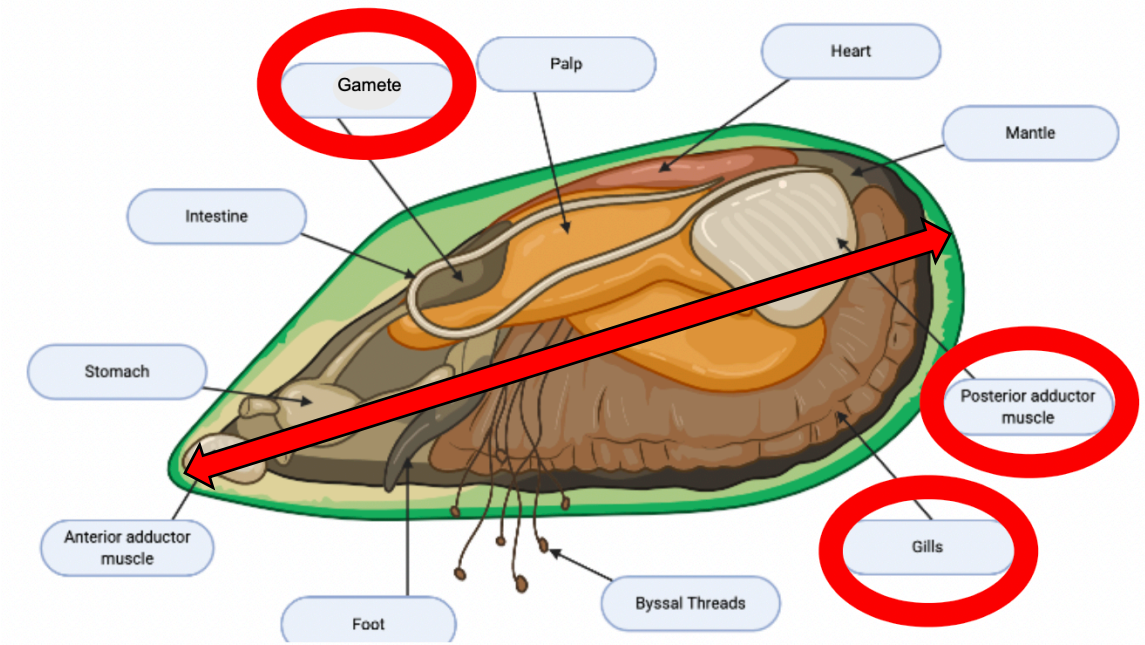

Figure 2.1: Mussel anatomy. *Experimental tissues are encircled. Position of length measurement indicated by red arrow (Created in BioRender).*

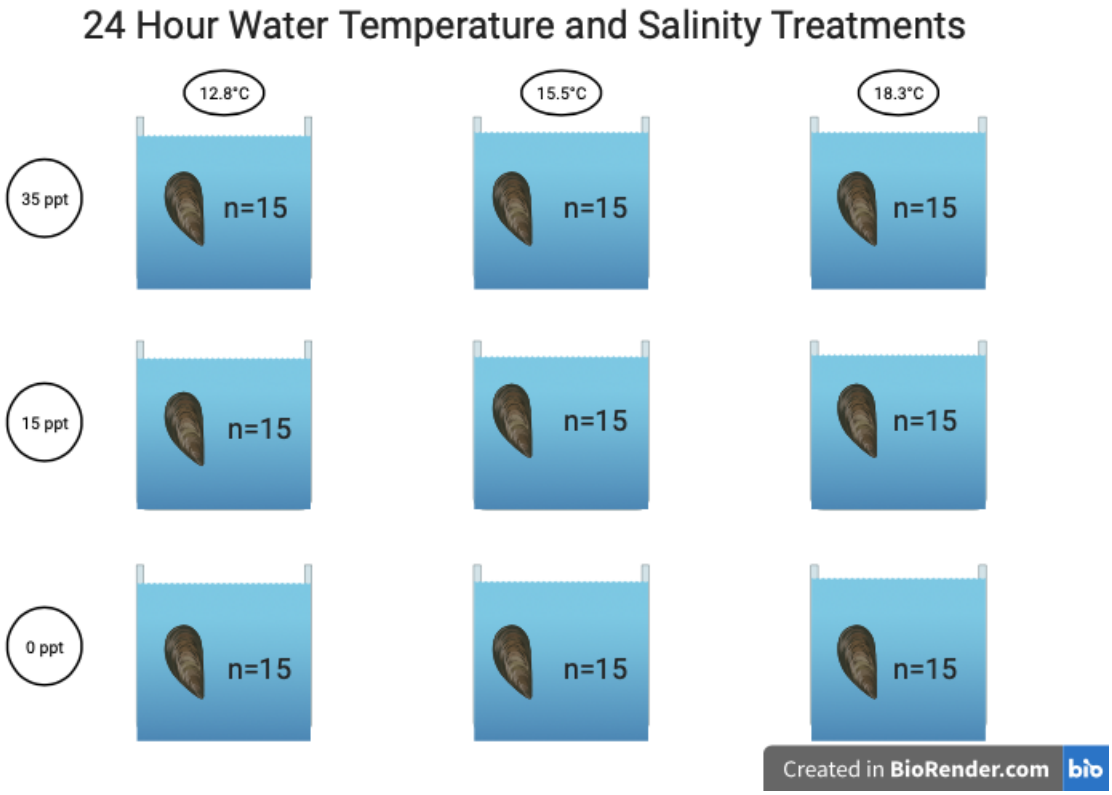

Figure 2.2: Visual representation of temperature and salinity treatments. *Treatments include various combinations of salinities (0 ppt, 15 ppt, or 35 ppt) and temperature (12.8 °C, 15.5 °C, or 35 °C). Rows represent salinity variables and Columns represent temperature variables. Fifteen Mytilus trossulus individuals were placed in each tank for 24 hours (Created in BioRender).*

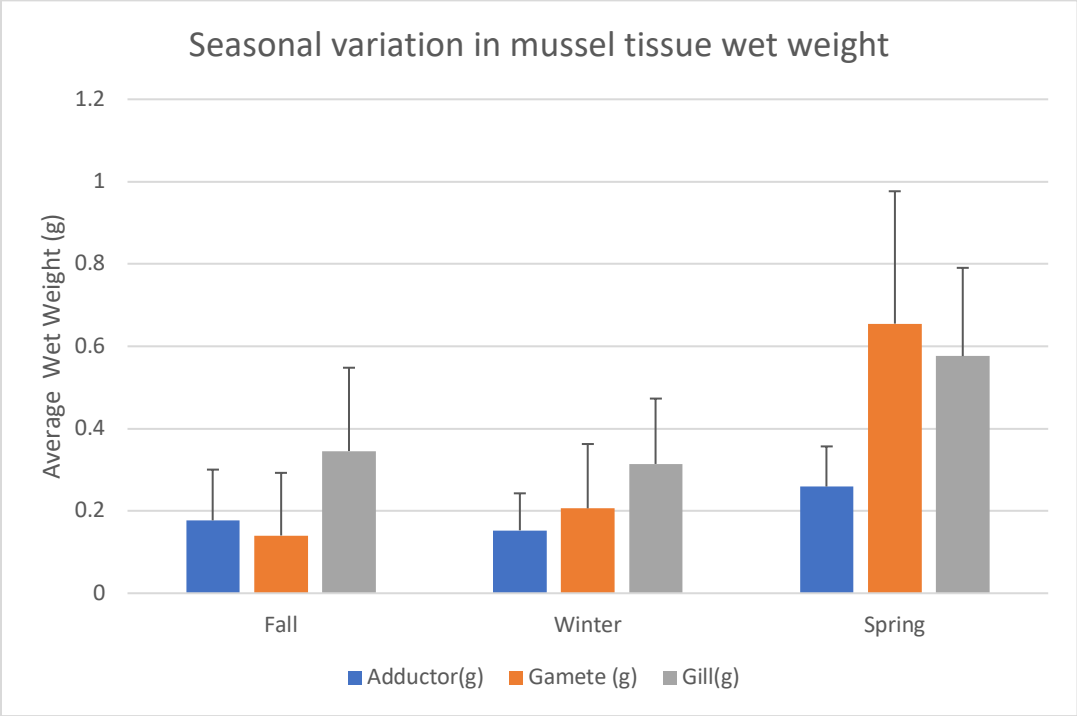

Figure 2.3: Seasonal variation of adductor, gamete, and gill weight (g). *Average wet weights collected from all Mytilus trossulus individuals during the Fall, Winter, and Spring collection seasons.*

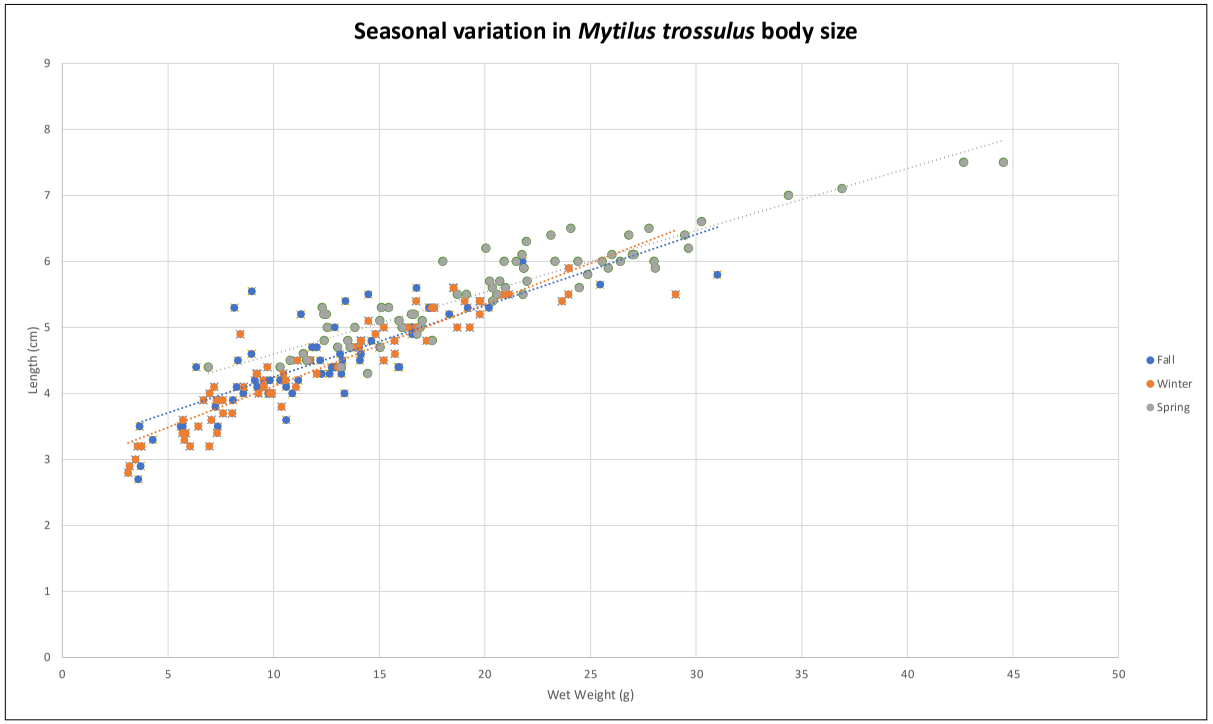

Figure 2.4: Seasonal variation of Mytilus trossulus body size. *Weight/length of all individual mussels collected in the Fall, Winter, and Spring.*

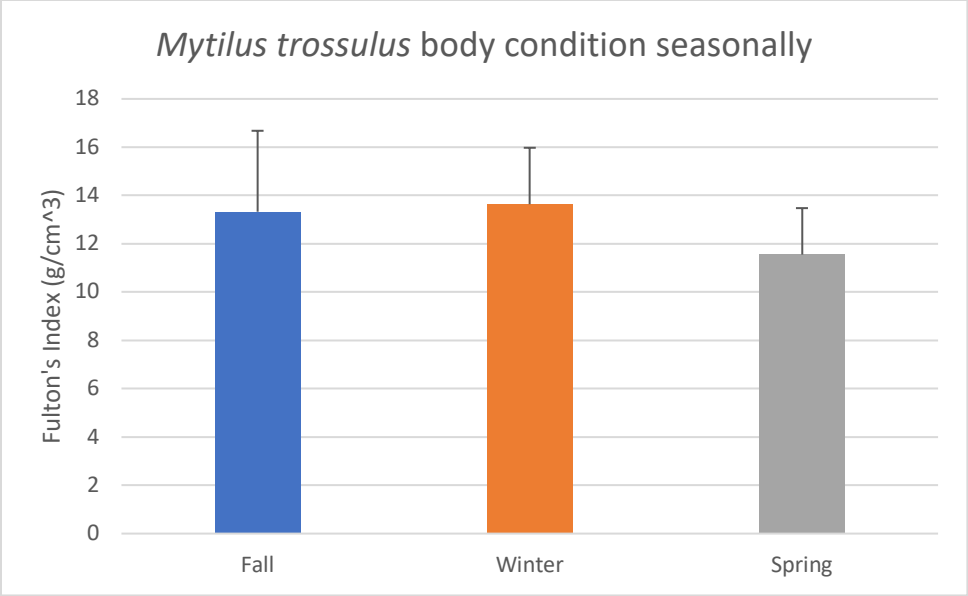

Figure 2.5: Fulton's Body Condition Index (K) for experimental individuals. *Data are average values for all specimens; therefore, they reflect an omnibus assessment of the mussels' size and condition. Fulton's Body Condition Index ($K= 100 \times W/L^3$) was calculated using wet weight.*

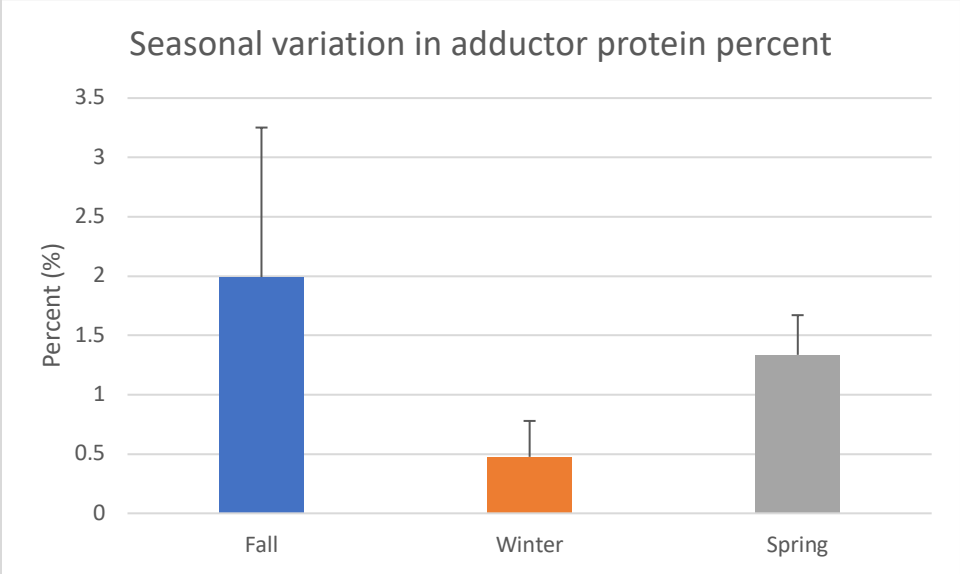

Figure 2.6: Seasonal variation in adductor muscle protein concentration. *This figure represents the average percent of protein within the adductor muscles or Mytilus trossulus during the Fall, Winter, and Spring collection seasons. Protein concentration was calculated using wet weight.*

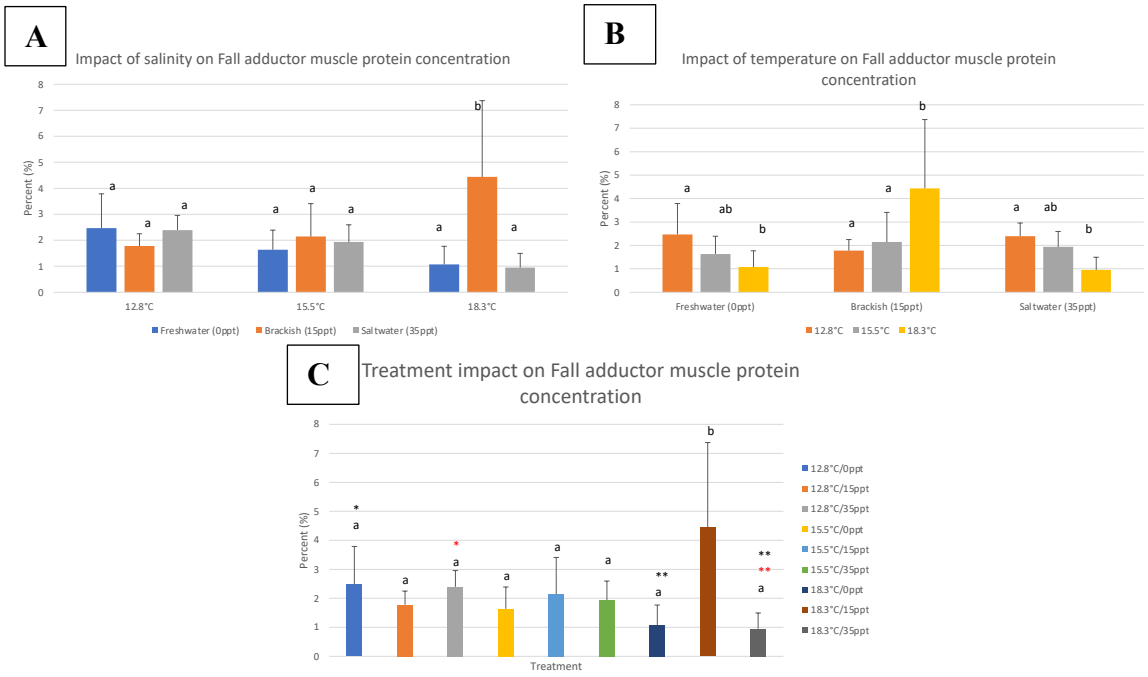

Figure 2.7: Variation in protein percent of adductor muscle tissue from Fall collected individuals post temperature and salinity treatments. *(A.) shows the impact of salinity on the average percent of protein in adductor muscles among temperature groups, (B.) show the impact of temperature on the average percent of protein in adductor muscles among salinity groups, and (C.) compares the average percent of protein from mussels exposed to various treatment groups. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. Protein concentration was calculated using wet weight. The p-values can be found in Appendix A, Table 1.*

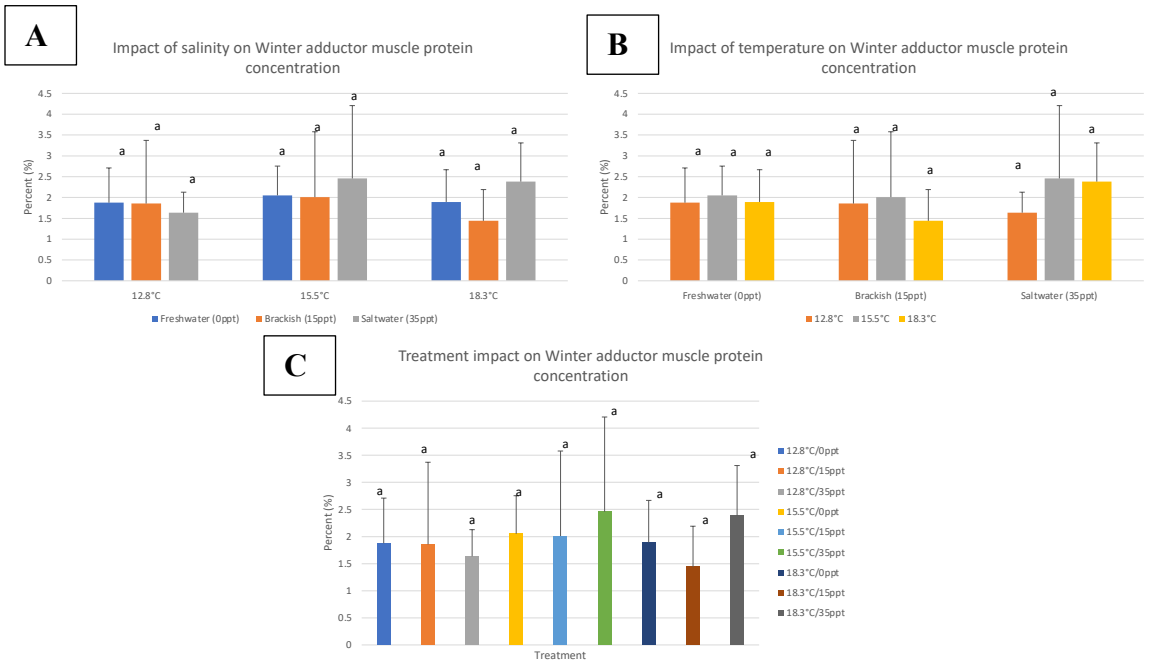

Figure 2.8: Variation in protein percent of adductor muscle tissue from Winter collected individuals post temperature and salinity treatments. *(A.) shows the impact of salinity on the average percent of protein in adductor muscles among temperature groups, (B.) show the impact of temperature on the average percent of protein in adductor muscles among salinity groups, and (C.) compares the average percent of protein from mussels exposed to various treatment groups. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. Protein concentration was calculated using wet weight. The p-values can be found in Appendix A, Table 1.*

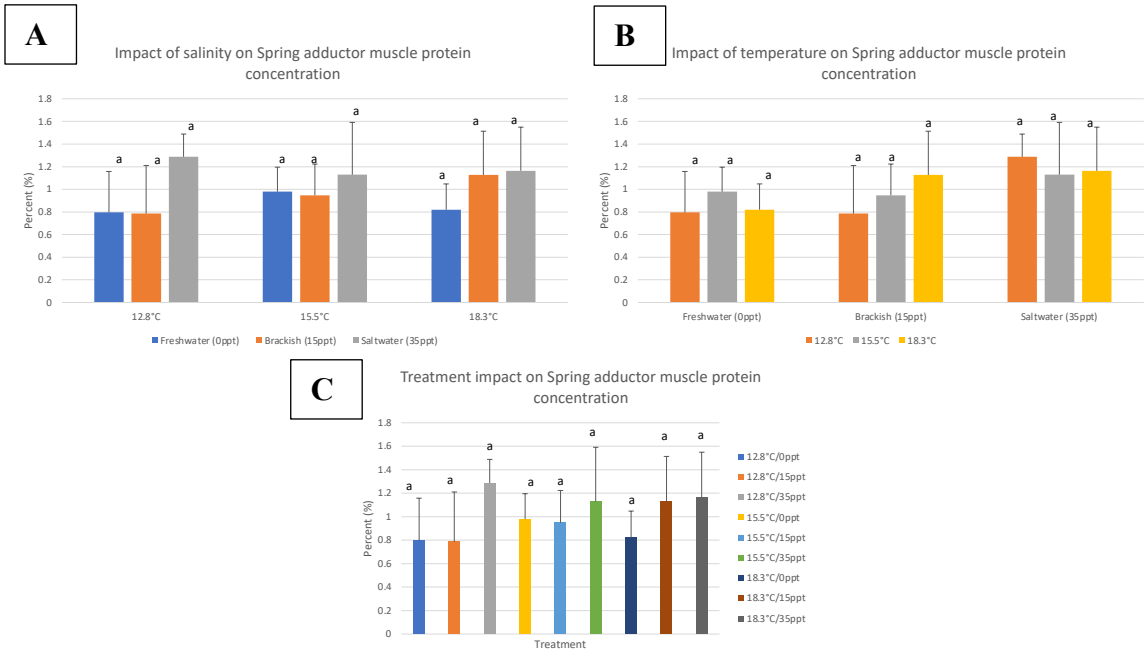

Figure 2.9: Variation in protein percent of adductor muscle tissue from Spring collected individuals post temperature and salinity treatments. *(A.) shows the impact of salinity on the average percent of protein in adductor muscles among temperature groups, (B.) show the impact of temperature on the average percent of protein in adductor muscles among salinity groups, and (C.) compares the average percent of protein from mussels exposed to various treatment groups. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. Protein concentration was calculated using wet weight. The p-values can be found in Appendix A, Table 1.*

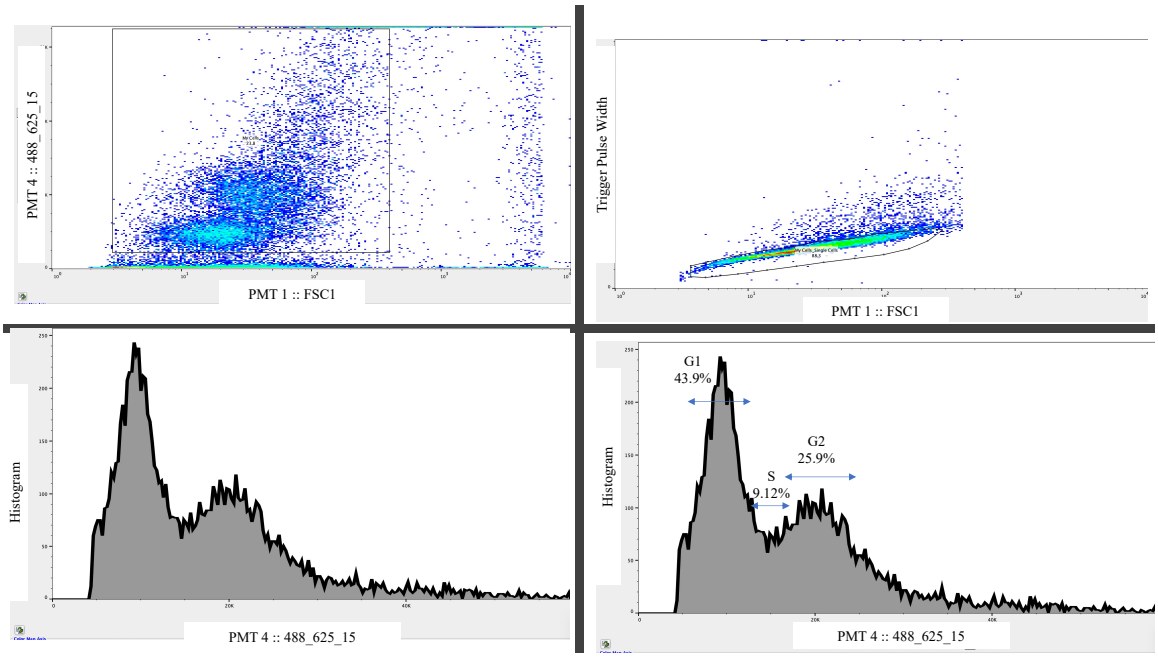

Figure 3.1: Example of flow cytometry cell population gating and histogram cell cycle phase gating.

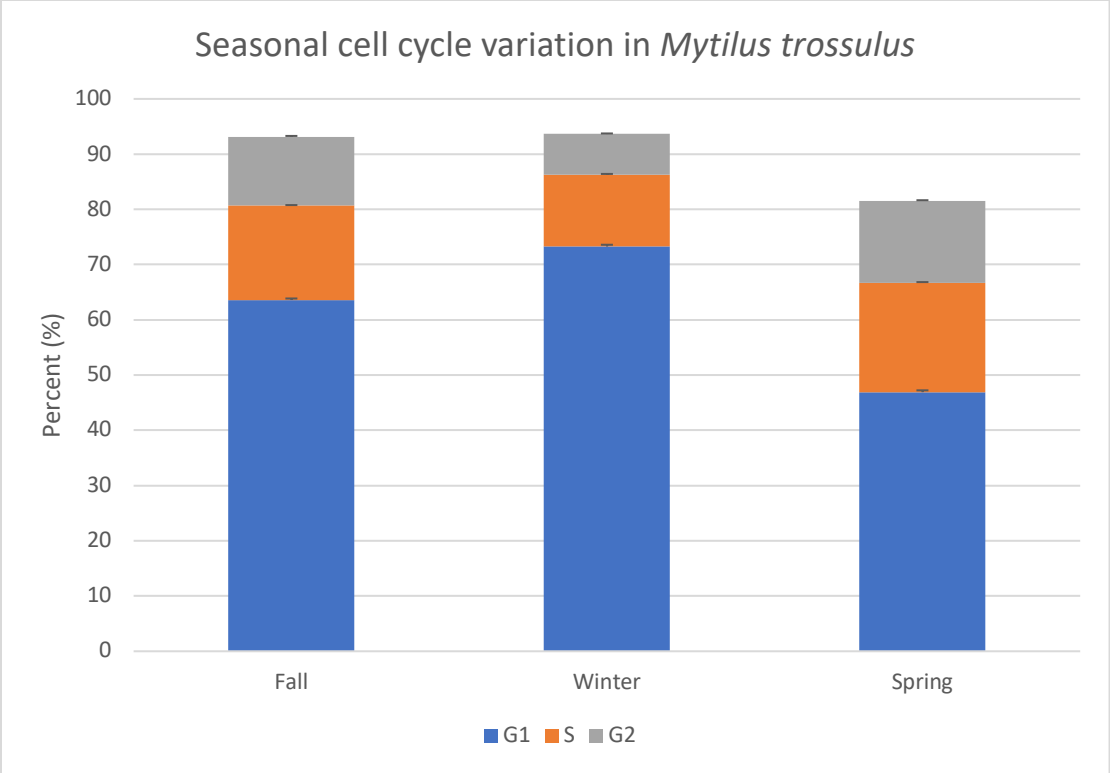

Figure 3.2: Seasonal variation of cell cycle phase percentages. *This figure shows the average percent of cells within each phase of the cell cycle for Mytilus trossulus individuals (n=10) that were collected during the Fall, Winter, and Spring collection seasons.*

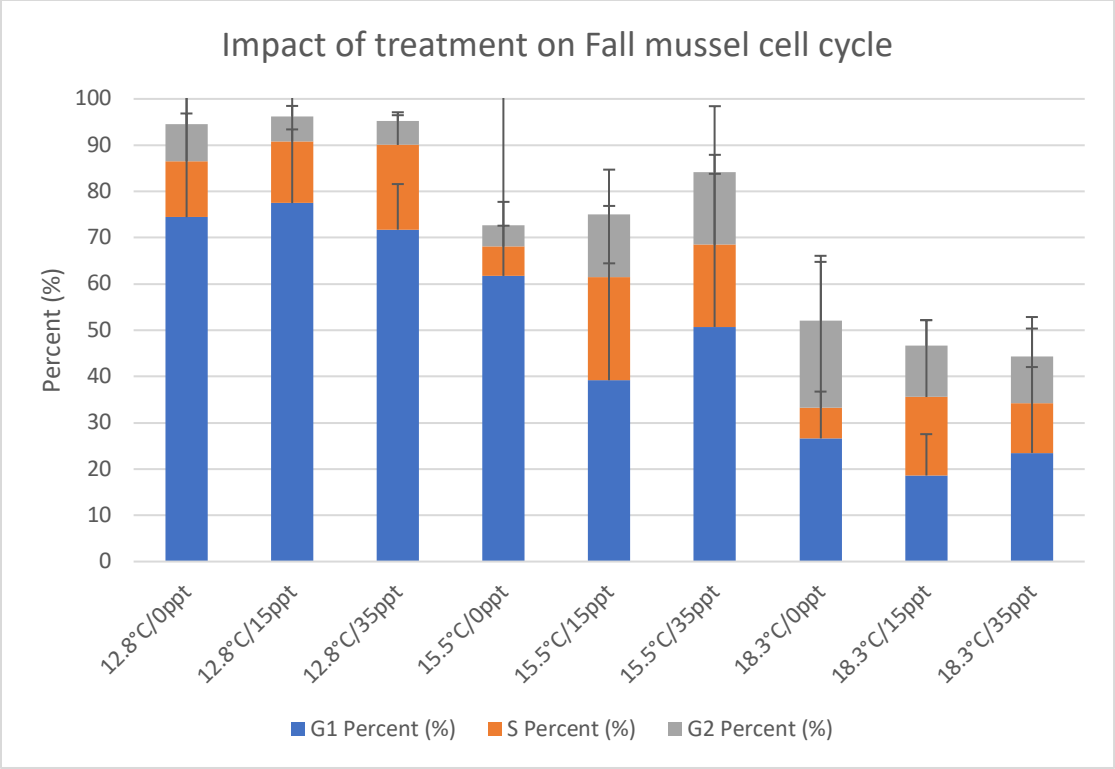

Figure 3.3: Variation of cell cycle percentages of Fall collected individuals post temperature and salinity treatments. *This represents the averages from each cell cycle phase. As the temperature variable was increased the quantity of cells in the sample that were part of a specific phase of the cell cycle decreased. In other words, the quantity of cells that were not assigned to a specific phase increased. These unaccounted-for cells are potentially apoptotic.*

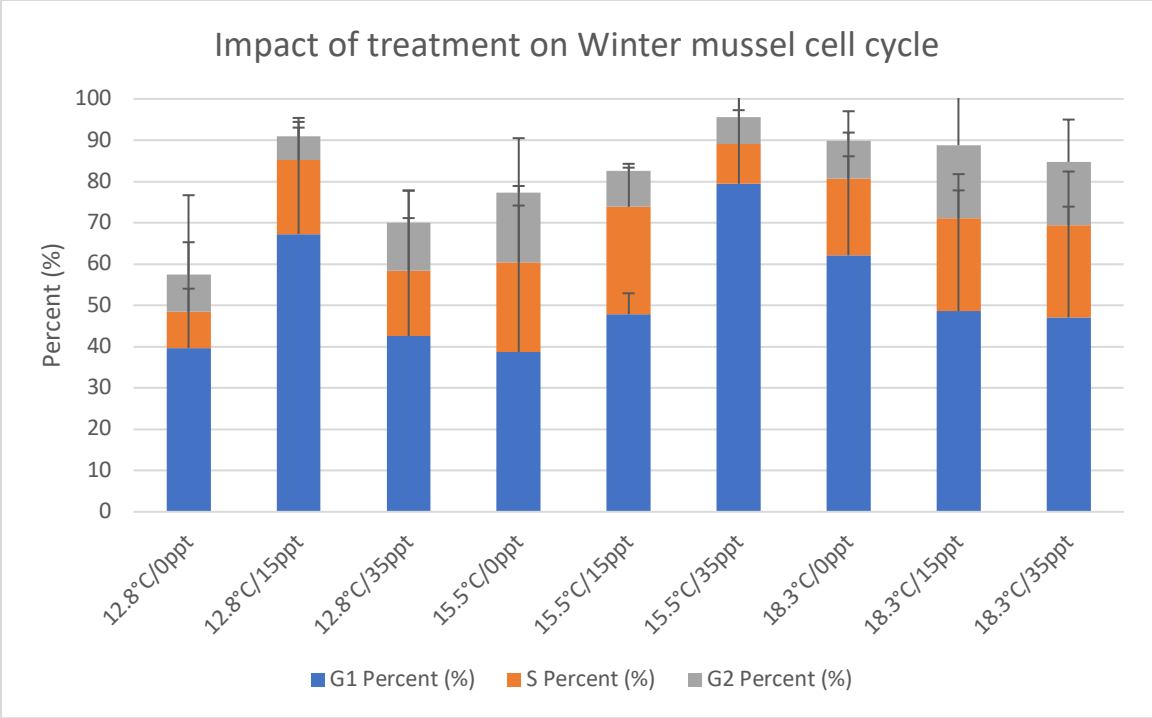

Figure 3.4: Variation of cell cycle percentages of Winter collected individuals post temperature and salinity treatments. *This represents the averages from each cell cycle phase. The remainder of cells which are unaccounted for on this figure are not assigned to a specific phase of the cell cycle. This non-phase content is potentially apoptotic.*

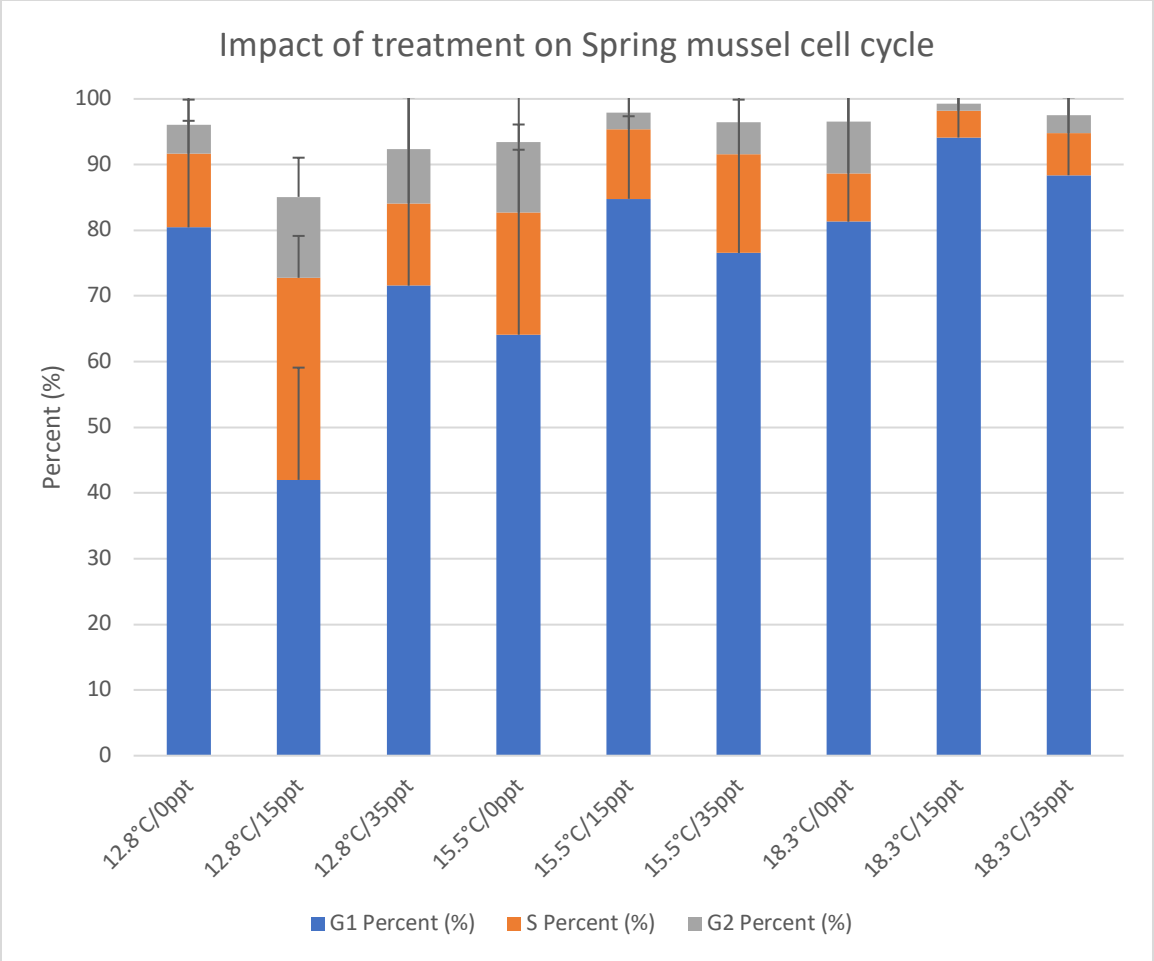

Figure 3.5: Variation of cell cycle percentages of Spring collected individuals post temperature and salinity treatments. *This represents the averages from each cell cycle phase. The remainder of cells which are unaccounted for on this figure are not assigned to a specific phase of the cell cycle. This non-phase content is potentially apoptotic.*

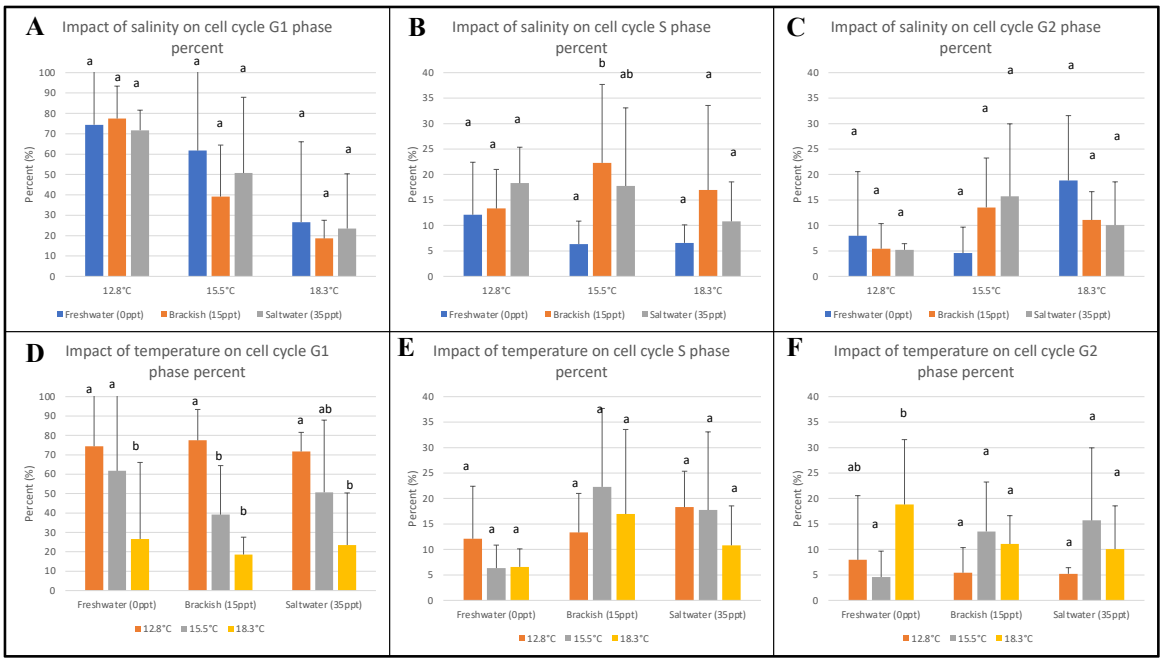

Figure 3.6: Impact of temperature vs. salinity on the cell cycle phases of Fall collected individuals. *The sections of this figure are (A.) impact of Salinity on the average percent of cells in G1 phase, (B.) impact of Salinity on the average percent of cells in S phase, (C.) impact of Salinity on the average percent of cells in G2 phase, (D.) impact of Temperature on the average percent of cells in G1 phase, (E.) impact of Temperature on the average percent of cells in S phase, and (F.) impact of Temperature on the average percent of cells in G2 phase. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. The p-values can be found in Appendix A, Table 2.*

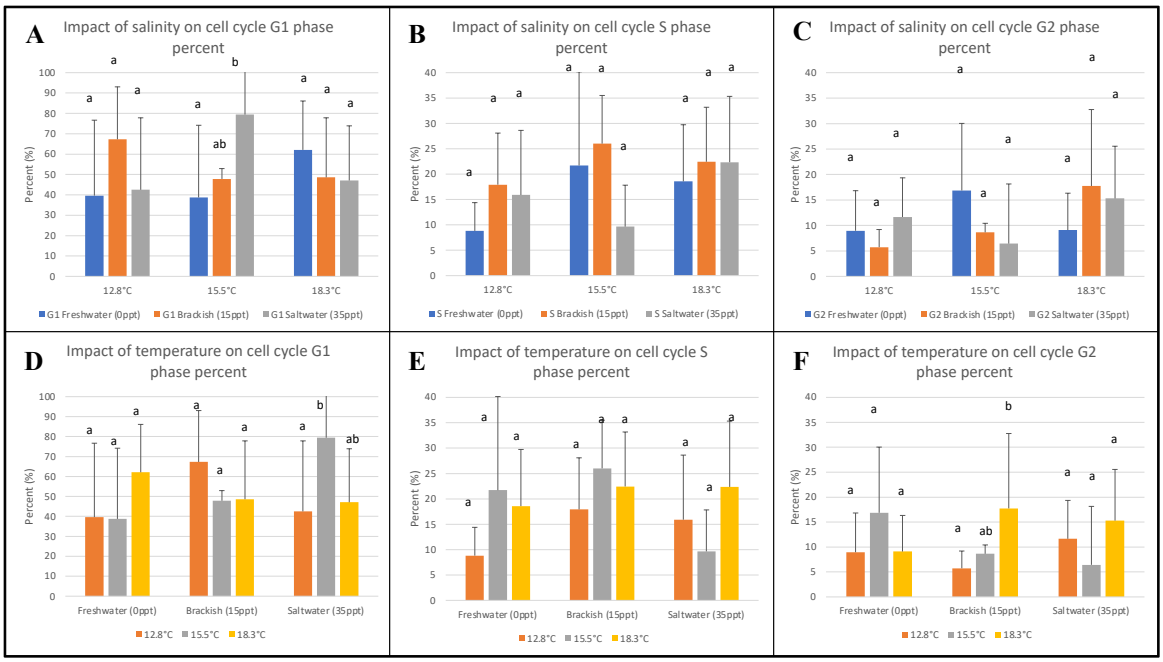

Figure 3.7: Impact of temperature vs. salinity on the cell cycle phases of Winter collected individuals. *The sections of this figure are (A.) impact of Salinity on the average percent of cells in G1 phase, (B.) impact of Salinity on the average percent of cells in S phase, (C.) impact of Salinity on the average percent of cells in G2 phase, (D.) impact of Temperature on the average percent of cells in G1 phase, (E.) impact of Temperature on the average percent of cells in S phase, and (F.) impact of Temperature on the average percent of cells in G2 phase. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. The p-values can be found in Appendix A, Table 3.*

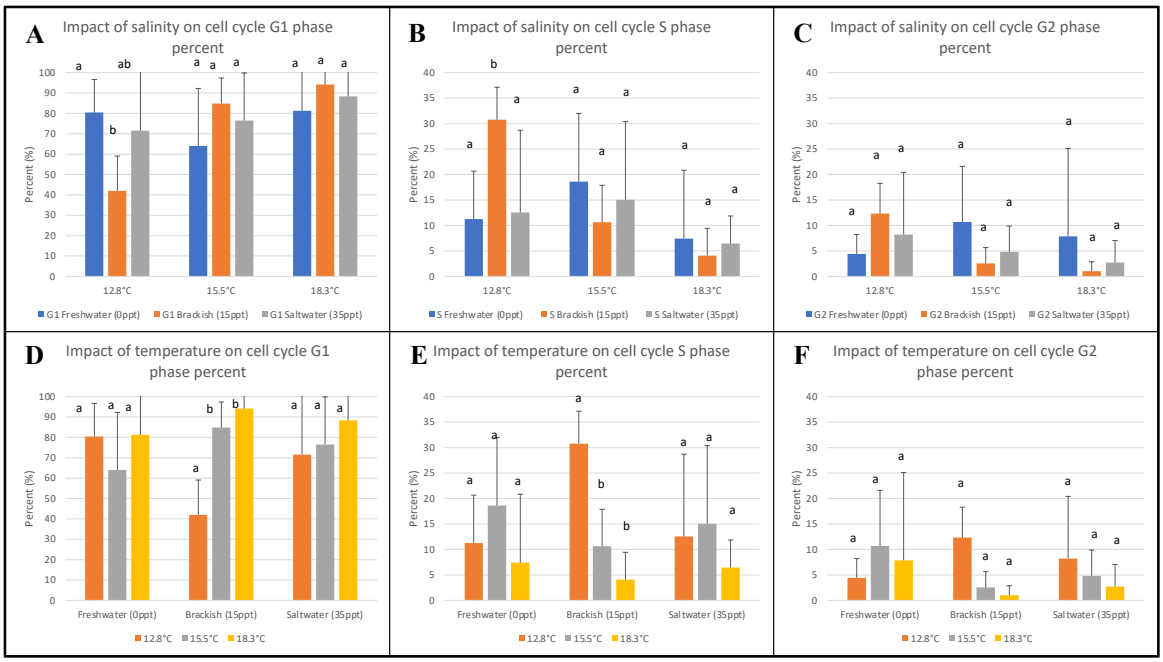

Figure 3.8: Impact of temperature vs. salinity on the cell cycle phases of Spring collected individuals. *The sections of this figure are (A.) impact of Salinity on the average percent of cells in G1 phase, (B.) impact of Salinity on the average percent of cells in S phase, (C.) impact of Salinity on the average percent of cells in G2 phase, (D.) impact of Temperature on the average percent of cells in G1 phase, (E.) impact of Temperature on the average percent of cells in S phase, and (F.) impact of Temperature on the average percent of cells in G2 phase. Significance ($p \leq 0.05$) was determined through SPSS two-way ANOVA and LSD pair-wise post hoc testing. Different letters and/or different symbol (*) quantities indicate statistically significant differences. The p-values can be found in Appendix A, Table 4.*

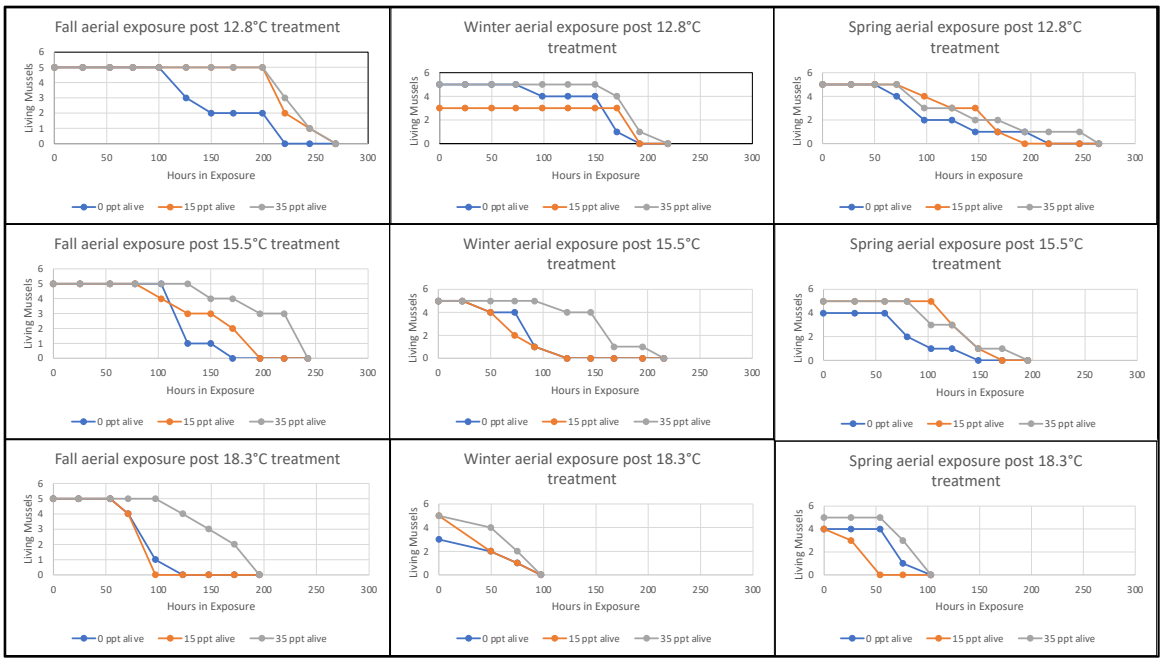

Figure 4.1: The effect of pre-exposure to a range of temperatures and salinities on subsequent survivorship of aerial exposure, across three seasons. *Mussels collected in Fall, Winter or Spring were exposed to 24-h treatments of either 12.8°C, 15.5°C, or 18.3°C and either 0 ppt, 15 ppt, or 35 ppt salinities. Survivorship values of subsequent aerial exposure are depicted.*

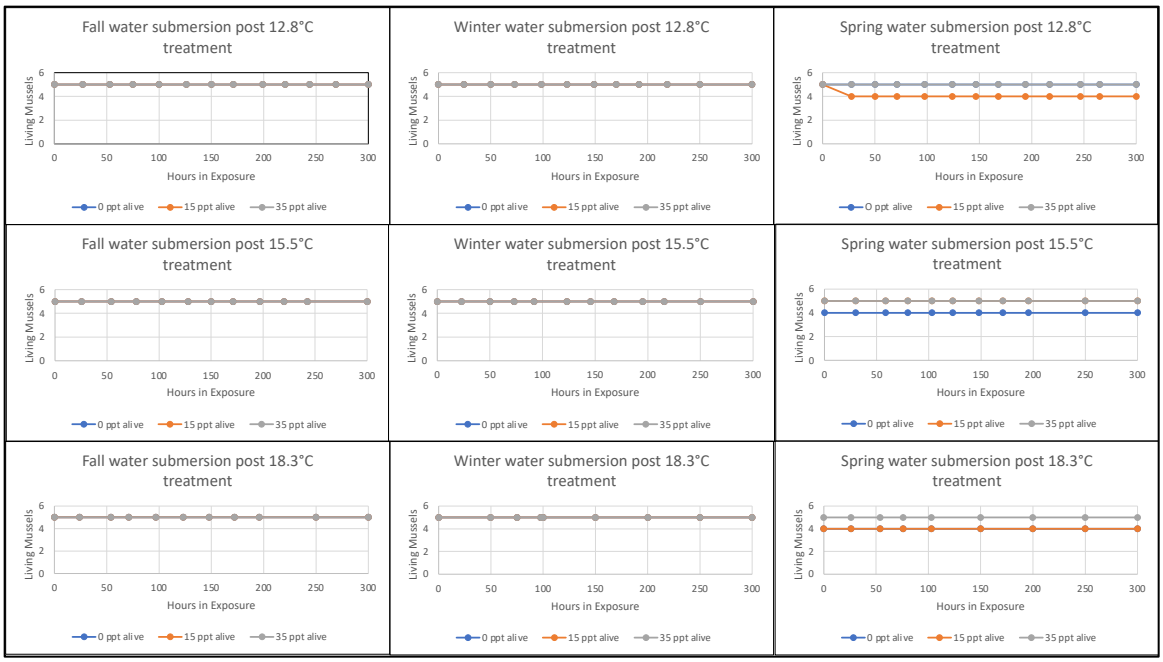

Figure 4.2: The effect of pre-exposure to a range of temperatures and salinities on subsequent survivorship of water submersion, across three seasons. *Mussels collected in Fall, Winter or Spring were exposed to 24-h treatments of either 12.8°C, 15.5°C, or 18.3°C and either 0, 15, or 35ppt salinities. Survivorship values of subsequent water submersion are depicted.*

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Appendix A. Significance Tables

Table 1: List of all p-values generated through two-way ANOVA and LSD post hoc testing from comparing proportions of Fall, Winter, and Spring collected Common Bay Mussel (Mytilus trossulus) adductor muscles in protein concentration from each treatment to all other treatments. Highlighted values indicate statistically significant values ($p<0.05$). Grey boxes indicate repetitive comparisons.

Fall	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.278	0.91	0.192	0.605	0.468	0.03	0.003	0.019
12.8°C/15ppt		0.363	0.824	0.569	0.83	0.269	<.001	0.196
12.8°C/35ppt			0.263	0.707	0.552	0.052	0.003	0.035
15.5°C/0ppt				0.429	0.684	0.376	<.001	0.283
15.5°C/15ppt					0.781	0.096	<.001	0.064
15.5°C/35ppt						0.242	<.001	0.182
18.3°C/0ppt							<.001	0.849
18.3°C/15ppt								<.001
18.3°C/35ppt								
Winter	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.974	0.707	0.783	0.834	0.362	0.978	0.494	0.427
12.8°C/15ppt		0.731	0.758	0.808	0.345	0.951	0.515	0.408
12.8°C/35ppt			0.515	0.558	0.199	0.686	0.758	0.243
15.5°C/0ppt				0.948	0.524	0.805	0.338	0.603
15.5°C/15ppt					0.482	0.856	0.372	0.559
15.5°C/35ppt						0.377	0.112	0.906
18.3°C/0ppt							0.477	0.444
18.3°C/15ppt								0.141
18.3°C/35ppt								
Spring	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.988	0.441	0.773	0.811	0.6	0.969	0.603	0.563
12.8°C/15ppt		0.458	0.775	0.811	0.611	0.959	0.613	0.576
12.8°C/35ppt			0.629	0.594	0.804	0.464	0.802	0.847
15.5°C/0ppt				0.961	0.813	0.803	0.816	0.771
15.5°C/15ppt					0.775	0.841	0.778	0.734
15.5°C/35ppt						0.627	0.997	0.957
18.3°C/0ppt							0.63	0.589
18.3°C/15ppt								0.954
18.3°C/35ppt								

Table 2: List of all p-values generated through two-way ANOVA and LSD post hoc testing from comparing proportions of Fall collected Common Bay Mussel (Mytilus trossulus) gill cells in G1, G2, and S phase from each treatment to all other treatments. Highlighted values indicate statistically significant values ($p<0.05$). Grey boxes indicate repetitive comparisons.

G1 Phase Fall	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.861	0.884	0.465	0.044	0.174	0.007	0.002	0.004
12.8°C/15ppt		0.756	0.365	0.029	0.125	0.004	<.001	0.002
12.8°C/35ppt			0.587	0.079	0.255	0.016	0.005	0.01
15.5°C/0ppt				0.196	0.526	0.045	0.014	0.029
15.5°C/15ppt					0.507	0.47	0.237	0.367
15.5°C/35ppt						0.167	0.066	0.119
18.3°C/0ppt							0.644	0.857
18.3°C/15ppt								0.778
18.3°C/35ppt								
G2 Phase Fall	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.666	0.658	0.563	0.341	0.188	0.065	0.595	0.718
12.8°C/15ppt		0.972	0.884	0.167	0.081	0.024	0.336	0.428
12.8°C/35ppt			0.918	0.181	0.093	0.03	0.346	0.434
15.5°C/0ppt				0.127	0.059	0.016	0.268	0.348
15.5°C/15ppt					0.713	0.366	0.673	0.554
15.5°C/35ppt						0.592	0.43	0.337
18.3°C/0ppt							0.186	0.136
18.3°C/15ppt								0.864
18.3°C/35ppt								
S Phase Fall	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.863	0.41	0.416	0.153	0.426	0.436	0.493	0.854
12.8°C/15ppt		0.509	0.325	0.208	0.533	0.342	0.608	0.722
12.8°C/35ppt			0.114	0.596	0.941	0.121	0.859	0.32
15.5°C/0ppt				0.026	0.109	0.973	0.136	0.529
15.5°C/15ppt					0.522	0.028	0.453	0.107
15.5°C/35ppt						0.117	0.912	0.328
18.3°C/0ppt							0.145	0.551
18.3°C/15ppt								0.385
18.3°C/35ppt								

Table 3: List of all p-values generated through two-way ANOVA and LSD post hoc testing from comparing proportions of Winter collected Common Bay Mussel (Mytilus trossulus) gill cells in G1, G2, and S phase from each treatment to all other treatments. Highlighted values indicate statistically significant values ($p<0.05$). Grey boxes indicate repetitive comparisons.

G1 Phase Winter	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.113	0.869	0.958	0.637	0.024	0.198	0.606	0.669
12.8°C/15ppt		0.155	0.102	0.264	0.485	0.765	0.283	0.246
12.8°C/35ppt			0.828	0.759	0.035	0.26	0.726	0.792
15.5°C/0ppt				0.6	0.021	0.18	0.57	0.631
15.5°C/15ppt					0.071	0.412	0.965	0.965
15.5°C/35ppt						0.319	0.078	0.064
18.3°C/0ppt							0.437	0.388
18.3°C/15ppt								0.93
18.3°C/35ppt								
G2 Phase Winter	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.582	0.645	0.178	0.957	0.666	0.98	0.135	0.28
12.8°C/15ppt		0.313	0.059	0.619	0.905	0.565	0.042	0.104
12.8°C/35ppt			0.373	0.607	0.373	0.663	0.298	0.533
15.5°C/0ppt				0.162	0.076	0.186	0.88	0.788
15.5°C/15ppt					0.705	0.938	0.121	0.257
15.5°C/35ppt						0.648	0.055	0.132
18.3°C/0ppt							0.141	0.291
18.3°C/15ppt								0.675
18.3°C/35ppt								
S Phase Winter	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.2	0.319	0.071	0.017	0.906	0.17	0.057	0.058
12.8°C/15ppt		0.775	0.594	0.255	0.245	0.927	0.524	0.532
12.8°C/35ppt			0.413	0.155	0.379	0.706	0.357	0.363
15.5°C/0ppt				0.544	0.091	0.659	0.917	0.927
15.5°C/15ppt					0.023	0.295	0.614	0.606
15.5°C/35ppt						0.21	0.073	0.075
18.3°C/0ppt							0.585	0.594
18.3°C/15ppt								0.99
18.3°C/35ppt								

Table 4: List of all p-values generated through two-way ANOVA and LSD post hoc testing from comparing proportions of Spring collected Common Bay Mussel (Mytilus trossulus) gill cells in G1, G2, and S phase from each treatment to all other treatments. Highlighted values indicate statistically significant values ($p<0.05$). Grey boxes indicate repetitive comparisons.

G1 Phase Spring	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.029	0.609	0.348	0.802	0.822	0.96	0.431	0.648
12.8°C/15ppt		0.091	0.205	0.015	0.049	0.025	0.003	0.009
12.8°C/35ppt			0.668	0.446	0.774	0.575	0.196	0.334
15.5°C/0ppt				0.235	0.474	0.323	0.086	0.164
15.5°C/15ppt					0.634	0.84	0.592	0.837
15.5°C/35ppt						0.784	0.312	0.496
18.3°C/0ppt							0.461	0.684
18.3°C/15ppt								0.741
18.3°C/35ppt								

G2 Phase Spring	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.178	0.516	0.283	0.748	0.946	0.556	0.563	0.77
12.8°C/15ppt		0.483	0.784	0.096	0.201	0.446	0.056	0.102
12.8°C/35ppt			0.67	0.332	0.56	0.951	0.22	0.347
15.5°C/0ppt				0.164	0.314	0.625	0.1	0.173
15.5°C/15ppt					0.698	0.364	0.796	0.977
15.5°C/35ppt						0.602	0.518	0.719
18.3°C/0ppt							0.244	0.379
18.3°C/15ppt								0.774
18.3°C/35ppt								

S Phase Spring	12.8°C/15ppt	12.8°C/35ppt	15.5°C/0ppt	15.5°C/15ppt	15.5°C/35ppt	18.3°C/0ppt	18.3°C/15ppt	18.3°C/35ppt
12.8°C/0ppt	0.007	0.852	0.3	0.929	0.589	0.588	0.314	0.498
12.8°C/15ppt		0.011	0.088	0.005	0.029	0.001	<.001	<.001
12.8°C/35ppt			0.394	0.784	0.723	0.467	0.233	0.388
15.5°C/0ppt				0.261	0.619	0.116	0.042	0.088
15.5°C/15ppt					0.529	0.65	0.358	0.556
15.5°C/35ppt						0.28	0.123	0.224
18.3°C/0ppt							0.64	0.892
18.3°C/15ppt								0.74
18.3°C/35ppt								