ABSTRACT

Title of Document: IMPLICATIONS OF OCEAN ACIDIFICATION FOR THREE PACIFIC ARCTIC BIVALVE SPECIES

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Directed by: Dr. Jacqueline M. Grebmeier
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Sea ice retreat, seawater warming, and now ocean acidification are recognized as physical stressors impacting the productive benthic communities on the shallow continental shelves of the northern Bering and Chukchi Seas, particularly calcifying organisms like bivalves that are prey items for benthivorous predators including walruses, eiders, and bearded seals. Using time-series benthic faunal collections and laboratory experiments, my research: 1) evaluates the abundance and dominant size class of *Macoma calcarea* in the northern Bering Sea and the southeastern Chukchi Sea during summer months from 1998-2014, and 2) investigates the effects of ocean acidification on growth and oxygen consumption of two size classes of three dominant bivalve species, *M. calcarea, Astarte montagui,* and *Astarte borealis.* Results suggest a northward shift in bivalve distribution

($p < 0.01$) and a recent size reduction at both sites. Experimental results suggest that one dominant size class (2.1-3 cm) will be more susceptible to ocean acidification.

IMPLICATIONS OF OCEAN ACIDIFICATION FOR THREE PACIFIC ARCTIC BIVAVLE SPECIES

by

Christina Leigh Goethel

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Advisory Committee:

Research Professor Dr. Jacqueline Grebmeier, UMCES CBL Chair
Research Professor Dr. Lee Cooper, UMCES CBL
Professor Dr. Thomas Miller, UMCES CBL

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Chapter 1: Introduction

Ocean Acidification Research Interest in the Arctic

Atmospheric carbon dioxide concentrations are rising and have been steadily increasing in recent decades from activities like burning of fossil fuels, deforestation and other land use changes, and cement production (e.g. Ciais et al. 2013, Le Quéré et al. 2015). The ocean acts as a sink, absorbing about 30% of the anthropogenic produced CO_2 (Sabine and Feely 2007), and it has been suggested that on a multiple thousand year time scale, about 90% of the anthropogenically produced CO_2 will be absorbed by the ocean (Sabine et al. 2004). Changes to ocean chemistry, caused by increases of dissolved CO_2 into the oceans, termed ocean acidification, are expected to disrupt the balance of the chemical equilibria and change the carbonate chemistry and speciation of carbon in the oceans (e.g. Caldeira and Wickett 2003, 2005, Feely et al. 2004, Orr et al. 2005). The changing ocean chemistry around the world has potentially strong impacts for calcifying marine organisms (e.g. Kleypas et al. 1999, Riebesell et al. 2000). Decreases in pH and the subsequent decrease in the saturation state of calcium carbonate in seawater can affect organisms in two primary ways: changes to calcification rate and disturbances to acid-base (metabolic) physiology (Fabry et al. 2008, Feely et al. 2009, Waldbusser et al. 2014), but it may also cause changes to physiology, development, and behavior (Melatunan et al. 2013).

Undersaturation of calcium carbonate is expected to occur in the high latitude seas, and the Arctic and Southern Oceans, earlier than other regions because these marine waters often have naturally lower pH values, inherently low concentrations of carbonate ions, and cold water temperatures that lead to enhanced solubility of gases, including CO_2

(Orr et al. 2005, Bates and Mathis 2009, Fabry et al. 2009, Steinacher et al. 2009). These high latitude systems, therefore, can act as bellwethers for more temperate and tropical ecosystems (Fabry et al. 2009). The saturation state (Ω), is the value that determines if calcium carbonate, the material many shells and tests are comprised from, is in equilibrium ($\Omega=1$) with seawater, or if it will precipitate ($\Omega>1$) or dissolve ($\Omega<1$) based upon exchange with the carbonate system in seawater. These Ω values are already lower in the high latitude regions because the solubility of calcium carbonate increases with decreasing temperatures. The saturation horizon, the depth at which carbonates are saturated above, but undersaturated below, is expected to shoal, (i.e. become shallower), as CO_2 concentrations increase (Fabry et al. 2008). Because the saturation states in the high latitude regions are already relatively low, increasing CO_2 concentrations will drive further shoaling of the saturation horizon (Fabry et al. 2008). Model predictions suggest aragonite (the more soluble form of calcium carbonate) will be undersaturated in the surface waters of the Southern Ocean as early as 2050 (Orr et al. 2005), while the surface waters of the Arctic were predicted to see signs of ocean acidification as early as 2016 (Steinacher et al. 2009). Cases of seasonal undersaturations in bottom waters of the Arctic were reported as early as 2002 (e.g. Bates et al. 2009, Mathis et al. 2011a, Mathis and Questal 2013). Several studies have documented the changing chemistry of the Pacific Arctic in regard to ocean acidification (pH and pCO_2 measurements) and changing saturation states (e.g. Bates et al. 2009, Mathis et al. 2011a, Mathis and Questal 2013, Mathis et al. 2015); however, currently very little is known about how the organisms of this area will respond to the chemical changes.

This study aims to determine the potential biological effects of decreased pH, which are already observed seasonally in the Chukchi Sea (Mathis and Questal 2013, Yamamoto-Kawai et al. 2016), on three common Pacific Arctic bivalve species, *Macoma calcarea*, dominant in the SE Chukchi Sea, and *Astarte borealis* and *Astarte montagui*, both dominant in the NE Chukchi Sea. Additionally, I place observed changes from laboratory ocean acidification experiments (Chapter 2) into context with a time-series data set of changing abundance and dominant size class of *M. calcarea* at six other locations, five in the northern Bering Sea and one in the SE Chukchi Sea (Chapter 3) (Table 1.1)

Ocean Acidification and the Calcium Carbonate System

Concentrations of atmospheric CO_2 during the pre-industrial period (based upon the 800,000 year ice core record) fluctuated between 200 and 280 ppm (Sabine et al. 2004, Lüthi et al. 2008). By 2004, concentrations averaged around 380 ppm, and today the global average atmospheric concentration of CO_2 exceeds 400 ppm ("Trends in Atmospheric Carbon Dioxide" 2016). Because the rate at which the concentration of atmospheric CO_2 is rising faster than it has in the last 50 million years (Caldeira and Wickett 2003, Hönisch et al. 2012), the role of the ocean as a potential sink for the increased atmospheric releases of CO_2 has become increasingly important. Without the ocean as a sink, atmospheric concentrations of CO_2 would be 55% higher (Sabine et al. 2004).

The net effect of adding CO_2 to seawater is to increase the concentration of carbonic acid (H_2CO_3, *Eq.1*), bicarbonate (HCO_3^-, and H^+ (*Eq. 2*), and to decrease concentrations of the carbonate ion (CO_3^{2-}, *Eq. 3*) (see formulas below):

$CO_2 + H_2O \leftrightarrow H_2CO_3$ *(carbonic acid)* *(Eq. 1)*

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ *(bicarbonate)* *(Eq. 2)*

$H^+ + CO_3^{2-}$ *(carbonate)* $\leftrightarrow HCO_3^-$ *(Eq. 3)*

The carbonate ions in the water bond with the additional H^+ ions favoring the product (HCO_3^-) in *Eq. 3*, thus causing carbonate ions to become unavailable for organisms to use in shell and test creation. Under these conditions of ocean acidification, pH decreases. Importantly the higher acidity makes the water corrosive to carbonate structures. The lowest emission scenarios from the Intergovernmental Panel on Climate Change (IPCC) project a decrease of 0.3 in open ocean surface pH by the end of this century, while the highest emission scenarios project decreases in surface pH by as much as 0.5 (Caldeira and Wickett 2005). As of 2005, the global average of surface pH had decreased by 0.1 (Caldeira and Wickett 2005).

Many marine organisms, e.g. molluscs and corals, precipitate carbonate ions into solid calcium carbonate structures (*Eq. 4*).

$Ca^{2+} + 2HCO_3^- \leftrightarrow CO_2 + H_2O + CaCO_3$ *(Eq. 4)*

The saturation state (*Eq. 5*) dictates whether the precipitated calcium carbonate will remain stable as a precipitate or will be subject to dissolution.

$\Omega = [Ca^{2+}]\,[CO_3^{2-}] / K_{sp}$ *(Eq. 5)*

Dissolution of calcium carbonate structures is favored when the water becomes undersaturated in carbonate ion, (i.e. when $\Omega < 1$), but organisms have shown varying responses, and precipitation can occur at apparently undersaturated conditions (e.g.

Barton et al. 2012, Waldbusser et al. 2014). Calcium ions in the oceans are conservatively distributed and co-vary with salinity, so changes in the carbonate ion directly relate to changes in the carbonate saturation state (Fabry et al. 2008). Modeling indicates that doubling of atmospheric CO_2 concentrations will lead to a decrease of 30% in carbonate ion concentration and a 60% increase of H^+ ions (Sabine et al. 2004).

The two common forms of calcium carbonate that organisms utilize in mollusc shell production are aragonite and calcite, which are precipitated in saturated environments. Aragonite is more soluble, and is therefore more susceptible to dissolution in undersaturated waters. Calcite and aragonite global saturation (Ω) states, when atmospheric CO_2 concentrations were around 280 ppm, averaged around 5.2 and 3.4 respectively (Caldeira and Wickett 2005). In 2000, after atmospheric CO_2 concentrations rose to about 380 ppm and the global surface pH dropped about 0.1 units, the global saturation state of calcite was approximately 4.4 and the global aragonite saturation state was about 2.9 (Caldeira and Wickett 2005).

Ocean Acidification in the Pacific Arctic

Under the IPPC's A2 business as usual scenario (meaning no changes in mitigating atmospheric carbon dioxide emissions will be undertaken), the atmospheric CO_2 concentration increases to 552 ppm by 2054, and at that concentration, 50% of the Arctic Ocean's surface is expected to be undersaturated in aragonite for the entire year. Predictions indicate that the entire water column will be undersaturated when atmospheric carbon dioxide concentrations increase to 765 ppm (Fabry et al. 2009). Although carbon dioxide concentrations are currently not that high, seasonal ocean acidification events are already being documented in the Pacific Arctic, in both the

Bering and Chukchi Seas (Bates and Mathis 2009, Bates et al. 2009, Mathis et al. 2011a, Mathis et al. 2011b, Cross et al. 2013, Yamamoto-Kawai et al. 2016).

In addition to a low carbonate ion concentration and cold waters, the Pacific influenced Arctic possesses other characteristics that will exacerbate ocean acidification events. The primary system that drives undersaturations in this region is the biological pump and the Phytoplankton Carbonate Saturation State (PhyCaSS) (Bates and Mathis 2009, Mathis et al. 2011a, Mathis et al. 2011b). Phytoplankton blooms at the surface utilize dissolved inorganic carbon (DIC), including CO_2, in the mixed layer. This uptake of CO_2 decreases the H^+ ion concentration (*Eq. 1-3*), which leads to an increase in the pH, which is the opposite of the sequence described above. The carbonate ion (*Eq. 3*) is also more bioavailable, thus increasing the saturation state of calcium carbonate in the surface waters (e.g. Cross et al. 2012). However, production during a bloom is uncoupled from grazers (Bates and Mathis et al. 2009, Mathis et al. 2011b), so organic material from the bloom sinks to the bottom, where it is remineralized by benthic organisms through respiration. This produces CO_2, thus driving the pH and the calcium carbonate saturation state down in bottom waters (Bates and Mathis 2009).

A second mechanism leading to enhanced ocean acidification events is the changes in sea ice extent and retreat as well as other sources of freshwater input. Seasonal sea ice extent is declining and retreat of the sea ice is occurring earlier in the season (Frey et al. 2014). Freshwater input from sea ice melt decreases total alkalinity, which reduces carbonate buffering capacity, and therefore can lead to undersaturations of aragonite in the surface waters (Steinacher et al. 2009, Bates et al. 2014). Additionally, as sea ice declines, more open water is exposed creating a greater surface area for sea-air exchange of gases, including anthropogenic CO_2, as well as a greater area for a potential increase of primary production (e.g. Cross et al. 2014). An increase in primary production could presumably intensify the PhyCaSS processes with additional uptake of inorganic carbon. This exposure of more open water has also led to an increase of carbon uptake by the Arctic Ocean from 24 to 66 Tg C per year over three decades (Bates et al. 2006).

Biological Consequences of Ocean Acidification

Changing ocean chemistry around the world has potentially strong impacts upon calcifying marine organisms (e.g. Kleypas et al. 1999, Riebesell et al. 2000). Changes to calcification, physiology, and behavior have been documented in molluscs due to decreased pH (Fabry et al. 2008, Feely et al. 2009, Melatunan et al. 2013, Waldbusser et al. 2014). Shells and hard structures that provide many benefits, including protection from predation, for the organisms that produce them are often affected by these changes. Therefore, threats, such as dissolution from carbonate undersaturations and stresses from decreased pH to shell composition, may lead to reduced fitness in these organisms, thus potentially giving a competitive advantage to non-calcifying organisms that are not facing the same stressors in how to allocate energy for maintenance of a calcium carbonate shell (Fabry et al. 2008). Biological studies in the high latitudes are needed because these regions, as documented above, are expected to be impacted sooner under high CO_2 condition and how organisms will respond is poorly understood.

Description of Study Area

Bivalves for the study were collected from seven different sites, one in the SE Chukchi Sea (station UTN 2, 67.050 N, 168.728 W), one in the NE Chukchi Sea (station ML 5-10, 71.603 N, 162.202 W), and five in the northern Bering Sea (Table 1.1, Figure

1.1). Animals were collected from station ML 5-10 aboard the RV *Norsemen II* as part of the Arctic Marine Biodiversity Observing Network (AMBON) project in 2015 and used for ocean acidification experiments presented in Chapter 2. Collections for bivalve growth characterizations were made from station UTN 2 aboard the Canadian Coast Guard Ship (CCGS) *Sir Wilfrid Laurier* (SWL) in 2014 and 2015 as part of the Distributed Biological Observatory (DBO) effort that undertakes repeated observations of biological variables at ecologically productive locations in the Pacific Arctic. The growth data collected from the SE Chukchi Sea 2014 and 2015 samples (SWL UTN 2) were used for comparison to the growth data collected from the NE Chukchi Sea station (AMBON ML 5-10). Additionally, these data are added to a time-series of abundance and size (1998-2012) of *M. calcarea* from the five sites in the northern Bering Sea and the SE Chukchi Sea site, which are presented in Chapter 3.

The general area where these samples were collected, the northern Bering Sea, and the SE and NE Chukchi Sea are recognized as some of the most seasonally productive areas in the world, while also acting as a carbon sink particularly in May and June, when large phytoplankton blooms associated with melting sea ice occur (Springer et al. 1996, Lee et al. 2007, Gradinger 2009, Cooper et al. 2012). Ice melt during the spring permits greater light penetration and increased stratification allowing for nutrients in the surface waters to be utilized by phytoplankton (Grebmeier 2012). The sea ice dynamics and the variability of the ice cover, affects the length and intensity of the phytoplankton production in this region (Arrigo et al. 2008). Pacific waters advected into the area bring nutrients, heat, and organic carbon to the shelf, adding to the organic material already produced there (Grebmeier et al. 2015). Because of reduced zooplankton grazing, this large amount of organic matter is deposited to the sediments below (Grebmeier et al. 2006b, Nelson et al. 2014) creating localized and regionally high biomass in the benthos, which have been termed hotspots (Grebmeier et al. 2006a, Grebmeier et al. 2015). The location of these hotspots coincides with high levels of chlorophyll-a in the water column (e.g. Lee et al. 2007, Brown et al. 2011, Cooper et al. 2012, 2013). These areas of high benthic biomass provide productive foraging grounds for higher trophic predators such as walrus, gray whales, and diving sea ducks (Lovvorn et al. 2009, Jay et al. 2012, Moore et al. 2014)

The northern Bering Sea, which includes the St. Lawrence Island Polynya (SLIP) sites, has sediments comprised mainly of fine-grained silt and clay (~71-73%) with high total organic carbon (TOC) concentrations due to low current speeds (Grebmeier et al. 2015). Nutrient concentrations in the bottom water are often high as well due to the proximity to nutrient-rich upwelled Anadyr waters (Figure 1.2). Bottom temperatures, however, tend to be colder, with mean values even in summer averaging around -1.6 °C (Grebmeier et al. 2015). The ice dynamics in this area have been relatively stable over the last several years (Grebmeier et al. 2015). The dominant benthic organisms in this particular hotspot are bivalves and polychaetes that provide food for spectacled eiders, bearded seals, and walruses (Lovvorn et al. 2014, Moore et al. 2014, Jay et al. 2014, Figure 1.3). Feeding by these benthivores generally occurs during the winter and early spring, while there is still sea ice (Grebmeier et al. 2015). The persistence of this hotspot is likely due to early primary production that settles because of slower currents into consistently cold bottom waters prior to significant grazing (Grebmeier and Barry 2007, Cooper et al. 2012, 2013).

Station UTN 2 lies within the southeast Chukchi Sea hotspot (SECS) (Figure 1.3). Water advected into this region comes from three sources: salty, cold Anadyr water, warmer and fresher Alaska Coastal Water, and an intermediate Bering Sea water that is a mixture of the two (Spall 2007, Figure 1.2). The sediments of this region are very similar to that of the northern Bering Sea, with ~71-73% of the sediment comprised of silt and clay, and these sediments have a high total organic carbon (TOC) content (Grebmeier et al. 2015). While similar in sediment composition and TOC concentration, the average bottom water temperature here is much warmer in summer (2.2 °C from July-September) due to variable stratification and stronger mixing (Grebmeier et al. 2015). Sea ice persistence in this hotspot has changed in the last several decades. Annually, sea ice extent is declining by 9-12 days per decade, break-up of ice in the spring is occurring 3-5 days earlier per decade, and re-freezing in the fall is happening 7-8 days later per decade (Grebmeier et al. 2015). These changes in ice conditions have implications for the phytoplankton blooms that ultimately feed and maintain the benthic biomass. Arrigo et al. (2011) hypothesized that primary production may initially increase as sea ice declines because more exposed open water allows more light penetration. However, with warming and increases of freshwater input, stratification of the water column may increase causing nutrient depletion, thus ultimately reducing primary production (Grebmeier et al. 2006b). These changes in primary production may not only affect the benthos, but also have implications for ocean acidification events. Similar to the northern Bering Sea, this hotspot is dominated by bivalves, specifically *Macoma*, *Nuculanidae,* and, *Nuculidae*, as well as polychaetes, and it also provides foraging grounds for upper trophic organisms (Grebmeier 2012, Figure 1.3). Telemetry studies indicate that walruses forage here during spring and the fall, and the latter season has been when ocean acidification events have been documented (Jay et al. 2012, Mathis and Questal 2013). High annual primary production, mixing of several water masses, including Anadyr water with high nutrients, and varying sediment composition helps to sustain this hotspot (Grebmeier et al. 2006a).

Finally, collections were made at the ML 5-10 site that occurs within the northeastern Chukchi Sea hotspot (NECS) (Figure 1.3). While silt and clay are still the primary sediment type here, it makes up a lower percentage (~52%) in the more heterogeneous sediment types than it does at the other two hotspots (Grebmeier et al. 2015). Bottom temperatures here lie between the means for the northern Bering Sea and the SECS, averaging -0.7 °C from May to September (Grebmeier et al. 2015). Annual sea ice changes here are the most prominent, but show similar trends as at the SECS site. Persistence of sea ice decreased by approximately 30 days per decade and freeze up occurred about 15 days later per decade (Grebmeier et al. 2015). Again these changes will have consequences for primary production in the region (Grebmeier et al. 2006b, Arrigo et al. 2011), which may lead to changes in seasonal ocean acidification and associated consequences. The offshore locations in this hotspot are dominated by bivalves, polychaetes, and sipunculids, while at the inshore stations amphipods dominate (Grebmeier et al. 2015). As is the case in the SECS, walruses use this area as a foraging ground, and they are present during the summer months (Jay et al. 2012). Maintenance of this hotspot is due to variable organic carbon content associated with variable productivity and steering of cold bottom waters with high nutrient content through the complex bottom topography (Blanchard and Feder 2014, Grebmeier et al. 2015).

Overall, among these three sites, benthic biomass (measured as both g wet weight per square meter and dry weight g C per square meter) increases northward, but overall, biomass decreased during 2000-2012. Benthic biomass (measured same as above) in the northern Bering Sea averaged 16 g organic C m^{-2}, the SECS averaged 32 g organic C m^{-2}, and the NECS hotspot declined to 13 g organic C m^{-2} (Grebmeier et al. 2015). These sites provide study areas for examining biological responses in a shallow shelf system to changes in sea ice, other physical factors like temperature, and phenomenon like ocean acidification.

Description of Study Organisms

Benthic macrofanual communities in the Arctic provide good indicators of changing environmental conditions because of long and sessile life spans that allow them to be easily and consistently sampled (Warwick 1993, Grebmeier et al. 2010, Iken et al. 2013). Bivalves were chosen as the study organism because of their prevalence in the Pacific Arctic, calcium carbonate shells, and their role as an important prey base for higher trophic animals including walrus (Sheffield and Grebmeier 2009) and diving sea ducks (Lovvorn et al. 2009).

I used three species for the ocean acidification experiments in Chapter 2, *Macoma calcarea, Astarte montagui,* and *Astarte borealis.* Chapter 3 focused on historical abundance and size data for *M. calcarea* based upon collections made in 1998-2012. *M. calcarea* generally reach up to about 45 mm, and those used in the ocean acidification experiments ranged from 15-45 mm. They are both deposit and suspension feeders that live in muddy/gravelly benthos, and have free swimming pelagic larvae. Their shells are comprised of aragonite (Wassenaar et al. 1988) adding to the value of studying them under conditions of ocean acidification.

The two *Astarte* species have similar characteristics. Both are suspension feeders, benthic as adults, with free swimming pelagic larvae. *A. montagui* usually reach about 12.5-20 mm in length (Gofas 2004a) and *A. borealis* can reach up to 25-50 mm (Gofas 2004b). The length of organisms used in the experiments ranged from 14-23 mm and 25-35 mm, respectively. As is the case with *M. calcarea*, both produce an aragonite shell (Majewske 1974, Simstich et al. 2005), making them suitable for studies of ocean acidification vulnerability.

Rationale for Study

The Pacific Arctic ecosystem is sustained by high production of large phytoplankton species, such as diatoms (e.g. Grebmeier et al. 2008) and a large proportion of water column organic matter is exported to the bottom waters because this production is largely uncoupled from water column grazing (Mathis and Questal 2013). Up to 75% of the production from the phytoplankton bloom can be exported to the bottom because of delays in the initiation of grazing (Mathis et al. 2007). These high rates of carbon export to the bottom from surface waters supply food for the benthos, but it also makes this region a strong seasonal sink for atmospheric CO_2 (Mathis and Questal 2013). Annual uptake estimates for CO_2 in the Chukchi Sea are as high as 53 Tg C/year (Bates 2006, Bates et al. 2011), and because of shallow bathymetry (~ 50 meters), anthropogenic CO_2 inputs can immediately infiltrate bottom waters (Yamamoto-Kawai et al. 2016). The remineralization of organic matter and production of CO_2 from respiration results in decreased pH, increased partial pressure of CO_2, and suppressed aragonite saturation states (Bates and Mathis 2009), with the strongest suppression to date occurring at the head of Barrow Canyon (Bates et al. 2009). In 2010, pH in some bottom waters of the Chukchi Sea declined to as low as 7.75 and aragonite undersaturations were present in bottom water throughout September and October (Mathis and Questal 2013). Similar patterns were observed at Hope Valley in the SE Chukchi Sea, near station UTN 2, in 2012 (Yamamoto-Kawai 2016). In the end, this export production that provides the food for a successful and biologically diverse benthic community, which in turn supports higher trophic levels like diving ducks, whales, seals, and walruses (Grebmeier et al. 2006a), also leads to elevated rates of respiration and remineralization of organic matter (Grebmeier and McRoy 1989, Grebmeier et al. 2006a) causing reductions in pH and saturation states that may affect the production of calcium carbonate shells and tests of these benthic organisms (Mathis et al. 2011a).

Based upon documentation of ocean acidification events and decreased pH in the bottom waters in the Chukchi Sea during the summer and fall months (Bates et al. 2009, 2013, Mathis et al. 2014, Bates 2015, Yamamoto-Kawai et al. 2016), it is crucial that biological studies complement the chemical and physical observations. These studies are necessary to understand and forecast the biological impacts this region will undergo. However, these impacts are uncertain because different species can be expected to respond differently. Arctic pteropods (*Limacina helicina*) and juvenile red king (*Paralithodes camtschaticus*) and tanner crabs (*Chionoecetes bairdi*) demonstrated negative responses to carbonate undersaturations (Comeau et al. 2009, Long et al. 2013), whereas studies of walleye pollock (*Theragra chalcogramma*) uncovered no negative effects, but the effects on their calcifying prey are unknown (Hurst et al. 2012). The saturation state in the Chukchi Sea is seasonally variable, but it is expected to shift out of the historical range (Ω= 1.2 ± 0.1) of variability for most of the year as early as 2027 (Mathis et al. 2015), posing an environmental challenge to shell production and maintenance. Therefore, there is an urgency to understanding what impacts this will have on benthic organisms, which are currently experiencing undersaturations in the summer and fall in the Chukchi Sea (Mathis and Questal 2013).

Bivalves are an important component of the Chukchi Sea benthos (e.g. Grebmeier et al. 2015). Molluscs, in other studies and ecosystems, have exhibited reductions in growth, decreased calcification rates, and reductions in metabolic activity (e.g. Feely et al. 2004, Orr et al. 2005, Gazeau et al. 2007). Some of the higher trophic animals that feed on bivalves are a source of subsistence food in in local villages. These trophic connections make it crucial to understand how these sustained, seasonal ocean acidification events will affect the biology of lower trophic organisms in the region.

My study aims to evaluate the potential effects of decreased pH on the growth and oxygen consumption of three Pacific Arctic bivalve species representative of the lower trophic food base in the Chukchi Sea. Laboratory experiments aim to characterize these changes on *M. calcarea*, *A. borealis*, and *A. montagui.* Additionally, use of time-series data (length and size of *M. calcarea)* will allow me to try and place any observed changes from laboratory ocean acidification experiments into a larger context.

Statement of Hypotheses and Thesis Structure

Six objectives were identified and explored in two subsequent chapters to help understand the effects of ocean acidification and decreased pH on the three bivalve species specified above:

2.1 Do elevated CO_2 concentrations and therefore subsequent decreased pH in bottom waters alter growth and oxygen consumption in the three Pacific Arctic bivalve species?

2.2 Will food availability play the same role in the vulnerability from effects associated with decreased pH and ocean acidification of the three Pacific Arctic bivalves?

2.3 Does the size of the bivalve affect the growth or oxygen consumption response to decreased pH?

3.1 I frame these studies of abundance and size of *M. calcarea* into hypotheses to test for changes over the past fifteen years at the six study sites in the northern Bering Sea and the southeastern Chukchi Sea.

3.2 Does total abundance and distribution of size classes differ among the six sites (SLIP 1-5, and UTN 2) throughout the fifteen-year time-series?

3.3 Do allometric growth equations of recently collected bivalves differ between sampling sites?

In trying to address these six objectives, I develop hypotheses and future experiments to test whether a sustained decrease in pH values could cause changes in dominant size of bivalves in the northern Bering Sea and the SE Chukchi Sea.

The hypotheses described above are tested and explored in two of the following chapters. In Chapter 2, results from two sets of experiments investigating the effects of decreased pH and controlled food availability are presented, testing hypotheses 2.1, 2.2, and 2.3. The first set of experiments was undertaken for seven weeks from October-December 2015 and consisted of two separate pH treatments. The second set of experiments was undertaken for eleven weeks from January- April 2016 and consisted of four treatments, altering both pH and food availability. Statistical tests, including t-test comparisons, Kruskal Wallis one way analysis of variance, and two-way analysis of variance (ANOVA) were used to analyze differences in several parameters, including % change in length (mm) and wet weight (g) and oxygen consumption (mg O_2/L/hour).

Material in Chapter 3 tests hypotheses 3.1, 3.2, and 3.3 using time-series data from 1998-2012 of changing abundance and dominant size class of select bivalves at sites in the northern Bering Sea and the southeastern Chukchi Sea (Table 1.1). I utilized dynamic factor analysis and additional clustering techniques to explore how abundance has changed at these sites in the fifteen year time frame and to make inferences as to how it will continue to change in the future. Results from these analyses were used to make predictions about the role ocean acidification may have played in smaller bivalves and shifting abundance.

Chapter 4 frames the role ocean acidification is potentially playing in driving changes in bivalve abundance and dominant size classes in the Pacific Arctic. This chapter also contains concluding remarks and recommendations for future work.

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Tables

Table 1.1 Latitude and longitude for the six stations used in abundance and size time-series analysis.

Station	Latitude (N)	Longitude (W)
SLIP 1	62.011	-175.059
SLIP 2	62.049	-175.209
SLIP 3	62.390	-174.570
SLIP 5	62.560	-173.549
SLIP 4	63.030	-173.460
UTN 2	67.049	-168.728

Figures

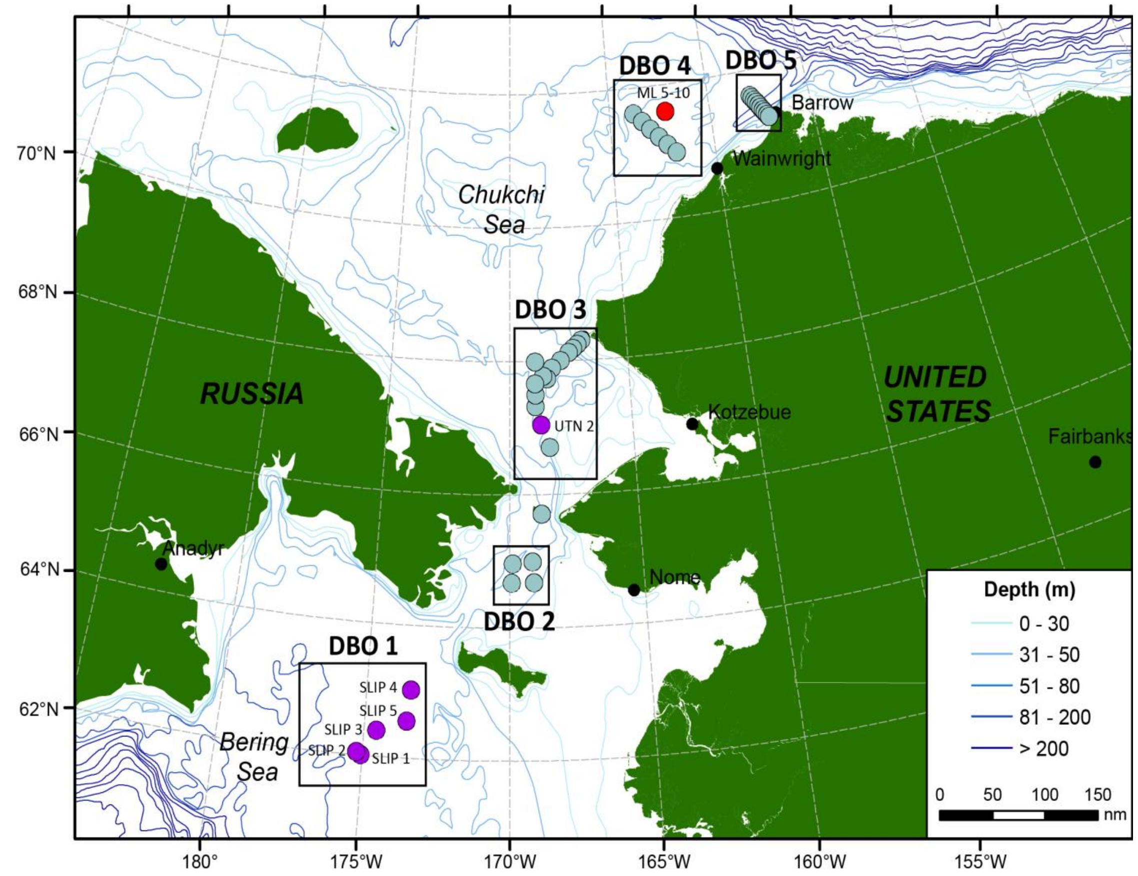

Figure 1.1 Map of stations with bivalve collections used in this study. Collections from the red station (ML 5-10) were part of the Arctic Marine Biodiversity Observing Network (AMBON) in 2015 and used for ocean acidification experiments (Chapter 2). Collections from purple stations (SLIP 1-5, UTN 2) were part of the Distributed Biological Observatory (DBO) cruises from 1998-2015 and were used to characterize growth relationships and changes in abundance and dominant size class (Chapter 3).

Figure 1.2 Map of the currents in the study region. Figure from Grebmeier 2012.

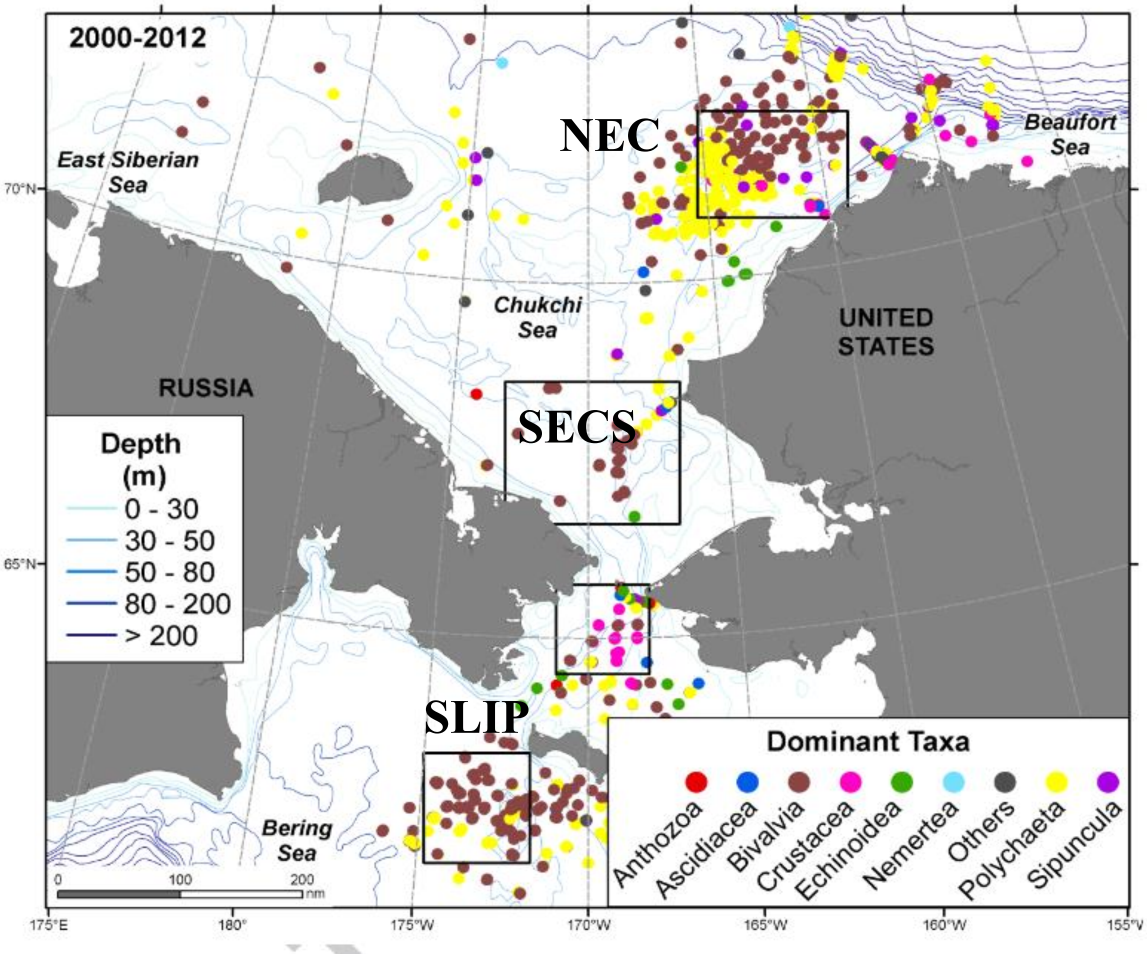

Figure 1.3 Map of the benthic hotspots and dominant taxa, highlighting the three used in this study. Figure modified from Grebmeier et al. 2015. Key: SLIP=St. Lawrence Island Polynya region, SECS=Southeast Chukchi Sea, NECS=Northeast Chukchi Sea.

Chapter 2: Implications of Ocean Acidification in the Pacific Arctic: Experimental Responses of three Arctic Bivalves to Decreased pH and Food Availability

Abstract

Recent sea ice retreat and seawater warming in the Pacific Arctic are physical changes that are impacting arctic biological communities. Recently ocean acidification from increases in anthropogenic CO_2 has been identified as an additional stressor, particularly to calcifying organisms such as bivalves, which are common prey items for walruses, bearded seals, and diving seaducks (Moore et al. 2014). I investigated the effects of decreased pH and food availability on both growth (% change in length and wet weight and allometric growth characterizations) and oxygen consumption (mg O_2/L/hour) of three common Arctic bivalves, *Macoma calcarea, Astarte montagui,* and *Astarte borealis.* Two sets of experiments were run for seven and eleven weeks, exposing the bivalves to ambient (8.05 ± 0.02 and 8.19 ± 0.003, respectively) and acidified (7.76 ± 0.01 and 7.86 ± 0.01 respectively) treatments. Although length, weight, and oxygen consumption were not significantly different among treatments, particularly in the seven-week exposure, negative effects were observed by the end of the eleven-week exposure. Specifically, shells of *A. borealis* displayed a decrease in length in response to decreased pH and *M. calcarea* showed a decrease in length in response to limited food. While these negative effects were small, and species responses varied, these observations suggest that with sufficiently long exposure, growth may be affected by decreased pH in some of these species.

1. Introduction

1.1 Ocean Acidification in the Pacific Arctic

Atmospheric CO_2 continues to increase due to human activities such as burning of fossil fuels and deforestation. The oceans act as a sink for this anthropogenic $CO_{2,}$ absorbing about 30% of the anthropogenic contributions (Sabine and Feely 2007). Increases of dissolved CO_2 into the oceans are expected to alter the balance of chemical equilibria for the inorganic carbon system, changing carbonate chemistry and speciation of carbon in the oceans (Caldeira and Wickett 2003, 2005, Feely et al. 2004, Orr et al. 2005). The net effect of adding CO_2 to seawater is an increase in the concentration of carbonic acid, bicarbonate, and H^+, and a decrease in the carbonate ion. The carbonate ions in the water bond with the additional H^+, causing carbonate ions to become less available for calcifying organisms to use in shell and test creation. Under these conditions, pH decreases, making the water more acidic and corrosive to carbonate structures.

The changing ocean chemistry around the world has potentially strong impacts for calcifying marine organisms (e.g. Kleypas et al. 1999, Riebesell et al. 2000). The saturation state (Ω), describes whether calcium carbonate, the material that comprises many shells and tests, is in equilibrium (Ω=1) with seawater or if the concentration of H^+ ions is low enough to favor precipitation (Ω>1) or high enough to favor dissolution (Ω<1). Ω values for both aragonite and calcite are already relative low at high latitudes because the solubility of calcium carbonate increases with decreasing water temperatures. The carbonate saturation horizon, the depth at which Ω=1, and waters are saturated above, but undersaturated below with respect to calcium carbonate, is expected to become shallower as CO_2 concentrations increase (Fabry et al. 2008). Organisms primarily use one of two forms of calcium carbonate, aragonite, which is the more soluble of the two, and calcite.

Decreases in pH and the subsequent decrease in the saturation state of aragonite or calcite can affect organisms in two primary ways: changes to calcification rate and disturbances to acid-base (metabolic) physiology (Fabry et al. 2008, Feely et al. 2009, Waldbusser et al. 2014), but changes may also occur to physiology, development, and behavior (Melatunan et al. 2013). Additionally, shells and hard structures provide many benefits, including protection from predators for the organisms that produce them. Therefore, threats, such as dissolution of shell structure from carbonate undersaturations, may lead to reduced fitness, thus potentially giving a competitive advantage to non-calcifying organisms (Fabry et al. 2008).

Both the Bering and Chukchi Seas in the Pacific Arctic are already showing signs of intensified seasonal ocean acidification events in the surface and bottom waters (Bates and Mathis 2009, Bates et al. 2009, Mathis et al. 2011a, Mathis et al. 2011b, Cross et al. 2013, Yamamoto-Kawai et al. 2016). The natural variability in the carbonate system and pH of the Pacific Arctic are partly controlled by the local seasonal oceanography. The Bering and Chukchi Seas are affected by freshwater input from both river runoff, including runoff that is entrained within the Bering Sea inflow to the Arctic Ocean (Mathis et al. 2011a), along with sea ice melt. Freshwater input from sea ice melt decreases total alkalinity, which reduces the carbonate buffering capacity, and therefore can lead to undersaturation of aragonite in the surface waters (Steinacher et al. 2009, Bates et al. 2014). Additionally, seasonal sea ice extent is declining and retreat of the sea ice is occurring earlier in the season (Frey et al. 2014). As seasonal sea ice diminishes, a greater extent of open water is exposed, which creates a larger surface area for sea-air exchange of gases, including CO_2 (e.g. Cross et al. 2014). This exposure of more open water because of sea ice retreat has led to an estimated increase of carbon uptake by the Arctic Ocean from 24 to 66 Tg C per year in the last three decades (Bates et al. 2006).

However, the primary driver decreasing pH in the two systems is a biologically driven “Phytoplankton Carbonate Saturation State” (PhyCaSS) that is based upon uptake and respiration in the water column (Bates et al. 2009, Mathis et al. 2011a, Mathis et al. 2011b, Cross et al. 2012). Phytoplankton blooms result in uptake of dissolved inorganic carbon (DIC) in the mixed layer, creating an increase in pH and saturation state in surface waters (e.g. Cross et al. 2012). However, due to a decoupling from grazers (Bates and Mathis et al. 2009, Mathis et al. 2011b), phytodetritus sinks to the seafloor and results in elevated rates of respiration and remineralization of organic matter (Grebmeier and McRoy 1989), which decrease the pH and increase pCO_2 in the bottom waters (Bates and Mathis 2009, Mathis et al. 2014). Therefore, the export of surface production that provides the food for a successful and biologically diverse benthic community can also lead to reductions in pH and saturation states that may affect production of calcium carbonate for shells and other structures (Mathis et al. 2011a). The PhyCaSS system could be intensified with the potential increases in primary production as greater open water areas become available due to earlier sea ice retreat (Cross et al. 2014).

The Pacific Arctic region has naturally variable carbonate chemistry; however, exchange of CO_2 from anthropogenic sources provides enough extra CO_2 to facilitate the persistent seasonal undersaturations that are now being observed (Mathis et al. 2011a).

Early calculations indicate that without anthropogenic CO_2 input, both calcite and aragonite would be supersaturated (Ω>1) in the spring and the summer in the Bering Sea, and aragonite undersaturations would not be present at depths <100 meters, with no evidence for calcite undersaturation (Mathis et al. 2011a, 2011b).

Carbonate undersaturation is present in bottom waters of the Chukchi Sea during the summer and fall months (Bates et al. 2009, 2013, Mathis et al. 2014, Bates 2015, Yamamoto-Kawai et al. 2016). Up to 75% of the production from the phytoplankton bloom can be exported to the bottom because of limited seasonal grazing in the water column (Mathis et al. 2007). The high benthic biomass supported by this organic carbon export (Dunton et al. 2005, Grebmeier et al. 2006a) allows these areas to also support higher trophic levels including diving ducks, whales, seals, and walruses (Grebmeier et al. 2006a).

However, these high rates of carbon export to the bottom from the surface also make this region a strong seasonal sink for atmospheric CO_2 (Mathis and Questal 2013). Annual uptake estimates for CO_2 in the Chukchi Sea are as high as 53 Tg C/year (Bates 2006, Bates et al. 2011), and because of shallow bottom depths of around 50 meters, anthropogenic CO_2 inputs can immediately infiltrate bottom waters (Yamamoto-Kawai et al. 2016). The remineralization of organic matter results in decreased pH, increased partial pressure of CO_2, and suppressed aragonite saturation states (Bates and Mathis 2009), with the strongest suppression to date observed at the head of Barrow Canyon (Bates et al. 2009). Bates et al. (2009) noted that the aragonite saturation state dropped below the saturation horizon at depths ranging from 40-150 meters over the northern shelf of the Chukchi Sea. In 2010, some areas of the Chukchi Sea had pH values as low as 7.75 with bottom water $\Omega_{aragonite}$ <1 in September and October (Mathis and Questal 2013). Similar patterns were observed at Hope Valley (Distributed Biological Observatory (DBO) 3 transect), a known biological hotspot, in 2012 (Yamamoto-Kawai 2016).

1.2 Biological Consequences of Decreased pH in the Chukchi Sea

Although the changing carbonate chemistry in the Chukchi Sea is relatively well documented, it is not known how biological organisms will be affected. Many effects of decreased pH are expected to be species specific. For example, pteropods and juvenile red king (*Paralithodes camtschaticus*) and tanner crabs (*Chionoecetes bairdi*) have shown negative effects to carbonate undersaturation (Comeau et al. 2009, Long et al. 2013), but on the other hand, no specific impacts on walleye pollock (*Theragra chalcogramma)* were detected (Hurst et al. 2012), even if there are probable effects on the calcified prey of these fish (Hurst et al. 2012).

Beyond seasonal aragonite undersaturation, the Bering Sea is projected to experience lower Ω values than those currently observed for many organisms by 2044, while the Chukchi Sea is expected to exhibit similar lower omega values as early as 2027 (Mathis et al. 2015). Therefore, there is an urgency to understand the impacts of ocean acidification on the calcifying animals, particularly benthic organisms where undersaturation will be greatest, of the Bering and Chukchi Seas. The benthic community of the Chukchi, in particular, is dominated by carbonate producing bivalves and molluscs (Grebmeier 2012, Grebmeier et al. 2015). In other experimental studies and ecosystems, these taxa have exhibited reductions in growth, decreased calcification rates, and reductions in metabolic activity (e.g. Feely et al. 2004, Orr et al. 2005, Gazeau et al.

2007). I developed this study to follow-up on these earlier efforts and to further understanding of how these persistent, and seasonal ocean acidification events in the Pacific Arctic will affect the specific species found in this region and how such changes may impact the biology of upper trophic level animals.

1.3 Objectives of Study

The goal of these experiments was to test the effects of decreased pH and food availability on the growth and oxygen consumption of three common Pacific Arctic bivalve species. The three bivalves include *Macoma calcarea*, dominant in the southeast Chukchi Sea, and *Astarte borealis* and *Astarte montagui*, both dominant in the northeast Chukchi Sea. Additionally, I compared the allometric growth relationships between length and weight of the three species from the collection site ML 5-10 (Figure 2.1) in the NE Chukchi Sea and in the six treatment conditions from both the 2015 and 2016 experiments. I conducted two sets of laboratory experiments on these three Pacific Arctic bivalve species; one was undertaken for seven weeks in the fall of 2015 and one was run for eleven weeks in the spring of 2016 to determine the potential effects of decreased pH on these organisms.

1.4 Statement of Hypotheses

2.1 Do elevated CO_2 concentrations and therefore subsequent decreased pH in bottom waters alter growth and oxygen consumption in the three Pacific Arctic bivalve species?

Hypothesis 2.1 There is no significant change in growth, measured as % change in both length (mm) and wet weight (g), or in oxygen consumption (mg O_2/L/hour) between treatments for each species.

2.2 Will food availability play the same role in vulnerability from effects associated with decreased pH and ocean acidification of the three Pacific Arctic bivalves?

Hypothesis 2.2 No significant difference exists in growth (% change in length (mm) and wet weight (g) or oxygen consumption (mg O_2/L/hour) among bivalves of each species from four feeding and acidification treatments: fed/ambient, fed/acidified, non-fed/ambient and, non-fed/acidified.

2.3 Does the size of the bivalve affect the growth or oxygen consumption response to decreased pH?

Hypothesis 2.3 Size does not affect how the growth of oxygen consumption of the bivalve responds to decreased pH.

2. Material and Methods

2.1 Sample Collection

Bivalves used in this study were collected using a 0.1 m^2 van Veen grab, with 32 kg weights, in August 2015 from the RV *Norseman II*. A total of 408 bivalves, including *M. calcarea* (n=87), *A. borealis* (n=116), and *A. montagui* (n=205), were collected at station ML5-10 (71.603 N, 162.202 W, Figure 2.1).

Bivalves collected at sea were maintained in groups of 15 individuals in sealed 950 mL high density polyethylene containers at approximately 3°C for the remainder of the cruise. Daily maintenance for the bivalves included alternating water changes, in which half the volume of water (approximately 500 mL) was removed every other day and replaced with fresh bottom water (~32.5 salinity) collected from the CTD rosette as needed, together with gentle rotation of the container for approximately ten seconds to introduce more oxygen. At the end of the cruise, the containers were sealed with electrical tape, packed in insulated containers with ice packs, and flown from Wainwright, Alaska, back to the Chesapeake Biological Laboratory (CBL), Solomons, Maryland.

At CBL, the clams were stored in a walk-in cold room set to 2.5° C. Four 75-liter tanks were set up with approximately 150 clams in each tank. Artificial seawater mixed with Instant Ocean^{TM} (Spectrum Brands, Inc., Blacksburg, Virginia) to salinity (32.5) similar to the water from which the bivalves were collected from and mixed in over time to season the new seawater. To reduce stress on the animals, temperatures were held between 2 - 3°C to mimic natural conditions (based on *in situ* CTD measurements at station ML 5-10 (-0.31°C, 32.19 salinity, Bering Sea winter water). The cold room was kept dark, except for short periods necessary for tank maintenance and feeding, consistent with the natural conditions in the Chukchi Sea at the depth of collection (<0.1% light level, Frey et al. 2011). In the first couple weeks following tank set up, I also monitored nitrate + nitrite and ammonia concentrations and pH using aquarium test kits from Aquarium Pharmaceuticals^{TM} (API Mars, Inc., McLean, Virginia). Tank maintenance after the initial set-up included checking salinity and temperature every other day using a YSI 85^{TM} multi parameter probe (YSI, Yellow Springs, Ohio) and feeding each tank with 1 mL of Shellfish Diet 1800^{TM} (Reed Mariculture, Campbell, California).

2.2 Experimental Set up

2.2. a 2015 Experiments

In 2015 (hereafter year 1), experiments were undertaken from October 28 to December 16 (seven-week exposure). All three species of clams were randomly assigned to one of fourteen 20-liter experimental tanks. Seven tanks were held at ambient pH levels (8.05 ± 0.02) and seven tanks were held at a reduced pH level (7.76 ± 0.02). Within each experimental tank, three 475 mL high-density polyethylene (HDPE) containers were established, with representatives from each species of bivalve. Each container was filled to the rim with clean sand collected from a local Chesapeake Bay beach (Cove Point, Maryland), which had been rinsed with deionized water three times. The sand was soaked for 72 hours in Instant Ocean^{TM} seawater to acclimate it to cold room conditions and to allow for development of microbial flora associated with the incubated bivalves. Each tank held six *M. calcarea*, eight *A. montagui,* and one *A. borealis.* Tanks were covered with polycarbonate plastic covers.

I used carbon dioxide (Airgas RD300) additions to manipulate the pH in two of the four 75-liter experimental tanks. Each experimental tank was monitored with a Cole-Parmer pH electrode (Cole-Parmer model 27003-12. Cole-Parmer, Vernon Hills, Illinois) coupled to an Alpha pH 190 pH/ORP controller (Omega Engineering Inc., Stamford, Connecticut) to continuously measure the conductivity (mV) of the water within the tank. pH electrodes were checked for Nernstian behavior (i.e. consistent with the Nernst equation) before each experiment. Millivolt readings were later converted to pH using equation (*Eq. 1*), the Nernst equation for a pH electrode at 2.5°C.

$$pH = \frac{mV - constant}{-54.7} \qquad (Eq.\ 1)$$

Once a week, the electrodes were calibrated with a pH 3 solution using 1 L of 2.5°C deionized water, well-mixed with 43.83 grams of NaCl, and 1 mL of 1N HCL. Individual electrodes varied in performance, so a constant was determined for each electrode using equation (*Eq. 2*).

$$mV\ from\ electrode = (-54.7 * 3) + constant \qquad (Eq.\ 2)$$

Once the constant was calculated, I determined a set point for the electrode in the acidified stock tank to ensure the water stayed at the appropriate pH based upon equation (*Eq. 3*).

$$set\ point = (-54.7 * 7.8) + calculated\ constant \qquad (Eq.\ 3)$$

If the water pH went above the set point, a solenoid, wired to the controller and electrode, opened, allowing CO_2 to flow through the one-way valve into a glass air stone to disperse the gas into the seawater tank until the set point was reached. Voltages for each stock tank were recorded once a day when water changes occurred.

Over the seven-week exposure period, approximately 2000 mL of seawater were removed from each of the fourteen 20-L experimental tanks holding the clams at the same time each day. New seawater, from either the control or acidified stock tanks, was then added back to the small experimental tanks to maintain a clean water supply, as well as the target treatment conditions. Once the water was changed, an Oakton General-Purpose sealed, double-junction, epoxy body, handheld pH electrode (calibrated with the methods described above for the stock tank electrodes) linked to a VWR Scientific

(VWR, Radnor, Pennsylvania) handheld pH meter model 2000 was used to measure pH, by conversion of measured mV values to pH using *Eq. 1*. In addition to daily water changes and pH measurements, temperature and salinity measurements were made daily using the YSI85 conductivity meter. Alkalinity was later calculated using an equation that derived estimates from salinity (Table 2.1, Table 2.2). Clams were fed every other day in each tank with 0.5 mL of Shellfish Diet 1800^{TM}.

2.2.b 2016 Experiments

In 2016 (hereafter year 2), experiments were run from January 19 to April 4, (eleven-week duration). All three species of clams were randomly assigned to one of twelve tanks and a 2 x 2 factorial design with pH and food availability variation was used. Of the twelve 20-L tanks, three were held at ambient pH levels (8.19 ± 0.004) and fed 0.5 mL of Shellfish Diet 1800^{TM} every other day; three were held at ambient pH levels (8.19 ± 0.003) and not fed over the course of the experiment; three were held at the experimental pH level (7.86 ± 0.01) and fed 0.5 mL of Shellfish Diet 1800^{TM} every other day; and the final three tanks were held at the experimental pH level (7.86 ± 0.02) and not fed over the course of the experiment. Within each tank, there were three 475 mL HDPE containers filled to the rim with sand collected from a local Chesapeake Bay beach as described for the previous experiment. Each tank held three *M. calcarea,* seven *A. montagui,* and two *A. borealis.* Monitoring of this experiment followed the same procedures as in the 2015 experiments.

2.3 Net Body Growth and Shell Measurements

Differences in growth were assessed by determining both wet weight and length of each clam before and directly after both sets of experiments (2015 and 2016).

Specifically, following the procedure used by Schram et al. (2016) for Antarctic gastropods, each clam was patted dry with a paper towel and then weighed to the nearest hundredth of a gram. Percent change (%Δ) in wet weight over the course of the experiment(s) was calculated using equation (*Eq. 4*).

$$\%\ change\ (\Delta) = \frac{Final\ Wet\ Weight - Initial\ Wet\ Weight}{Initial\ Wet\ Weight} * 100 \qquad (Eq.\ 4)$$

Similar steps were taken to document changes in clam shell length using Capri 15-cm stainless steel digital calipers to measure the length of individual clam shells to the nearest hundredth of a mm [maximum distance along the anterior-posterior axis as described by Gaspar et al. (2001)] before and directly after each experiment (Figure 2.2). A similar percent change (%Δ) formula to (*Eq. 4*) was applied to length measurements.

2.4 Oxygen Consumption

Changes in bivalve oxygen consumption were used as an indicator for metabolic activity. Measurements were made using four clams each day for the last nine days (December 8 - December 16) of the experiment in 2015, and four clams a day for the last twelve days (March 20 - April 1) of the experiment in 2016. Oxygen consumption was measured using a Pyroscience FireStingO_2 Optical Oxygen Meter (Pyro Science, Aachen, Germany) using the vendor-sourced software for data collection. The meter had attachments for four probes, each of which I inserted into individual jars that held one clam each. In 2015, oxygen consumption was measured in six, randomly chosen clams from each species/pH treatment combination (n=36), and in 2016 oxygen consumption in four, randomly chosen clams from each species/pH/food treatment combination (n=48) were measured.

These oxygen respiration measurements were prepared as follows: Individual clams were placed into 100 mL jars filled to the top with seawater from either the control or treatment pH tank and were capped tightly to ensure they were airtight. The probes measured dissolved oxygen in mg/L. Because measurements were for dissolved oxygen in water, the probes were calibrated at the beginning of each day to the "1 point in humid air/water" setting, as well as a set temperature of 2.5°C, measured by an attached temperature sensor, and a salinity of 32.5. Once calibrated, each of the four oxygen probes was inserted into small holes drilled into each jar's lid. One jar lid had two drill holes, one for the oxygen probe, and one for the external temperature probe. Dissolved oxygen concentrations (mg/L) were recorded every two minutes over a 24-hour period and the volume of water in each jar was used to convert to an oxygen utilization rate (mg O_2/L/hour). These measurements, beginning with calibration, were repeated every day for either nine or twelve days.

Oxygen use over each 24-hour period was plotted as a graph of linear decay (of dissolved oxygen); a linear regression and slope using RStudio® (https://www.rstudio.com/) was calculated (Figure 2.3). In several cases (14% in 2015 and 10% in 2016), there was not a linear decay of dissolved oxygen, and those data were not used in further analyses because of the potential for sampling artifacts. The slope of the regression was multiplied by the volume of water in the 100 mL jars and divided by the time the clams were held in the jar to determine the rate of oxygen consumption (O_2 mg/L/hour). Differences in rates among species and treatments were analyzed using statistical methods described below.

2.5 Length Weight Relationships and Growth Characterizations

The wet weight and length measurements determined before each experiment were used to determine allometric equations for the three species. Both the before and after length (L)-weight (W) relationships were described first with *Eq. 5* and then expressed in a linear form using *Eq. 6*, where a = intercept and b = slope.

$$W = aL^b \qquad (Eq.\ 5)$$

$$Log\ W = log\ a + b\ log\ L \qquad (Eq.\ 6)$$

An isometric relationship was defined as b=3, while slopes less than three were considered to be negatively allometric and slopes greater than three were considered to be positively allometric.

2.6 Statistical Analyses

All statistical analyses used RStudio® statistical software (https://www.rstudio.com/). Data (individual 20-L tanks, not individual clams) were tested for normality using the Shapiro-Wilk test. In 2015, the t-test and Kruskal-Wallis rank sum test were used to assess differences between pH treatments for percent change in length and wet weight, as well as oxygen consumption. In 2016, when food availability was added as a variable, all measured parameters were analyzed using a two-way analysis of variance (two-way ANOVA). The residuals of each two-way ANOVA were tested for normality using the Shapiro-Wilk test.

Allometric growth relationships were determined using linear regressions of the log transformed length and wet weight data in order to calculate a slope and a 95% confidence interval (CI) of the slope for each experimental treatment and species (e.g. *M.*

calcarea pre-experiment, post experiment control, post experiment acidified, etc.). If a slope value (b) of three fell between the upper and lower bound of the 95% confidence interval, the clam was classified as showing isometric growth (b=3, see section 2.5). If the confidence interval bounds for the slope fell below three (e.g. 2.4-2.9), the growth was classified as negatively allometric; and if the confidence interval bounds fell above three (e.g. 3.1-3.4), the growth was classified as positively allometric.

3. Results

3.1 Treatment conditions

Average tank conditions that were measured daily during the seven and eleven week exposure time, including pH, salinity, and temperature, were calculated for both control and acidified tanks from the 2015 experiments (Table 2.1) and the 2016 experiments (Table 2.2). Because salinity data was readily measurable, I used salinity and estimated total alkalinity (TA) obtained from the Chukchi Sea salinity-TA regression equation from Yamamoto-Kawai et al. (2016) in order to determine saturation of the carbonate system. Although I used Instant Ocean®, and not water from the Chukchi Sea, some water collected in the Chukchi Sea was mixed into the experimental containers and the temperature and salinity were comparable to natural conditions in the Chukchi Sea. I used the salinity, temperature, pH, and TA values estimated from the published regression equation, and inserted these values into the CO_2sys spreadsheet (Pierrot et al. 2006) to determine pCO_2 and the saturation states for both calcite and aragonite.

3.2 Net Body Growth and Shell Measurements

3.2a 2015 experiments

Twelve clams in the control and acidified treatments died, including five *M. calcarea* from both control and acidified treatments, and two *A. montagui* held in the control treatment.

A decrease in length was observed in *M. calcarea* held in both control (-0.72 mm ± 1.42) and acidified (-2.13 mm ± 3.42) treatments. However, statistical analysis did not indicate that these differences between control and experimental treatments were significant (t-test, p=0.341, Figure 2.4). In contrast, with the exception of *A. montagui* kept in the control treatment, the two *Astarte* species, showed an average increase in length after the seven-week exposure. *A. montagui* from the acidified treatment averaged an increase in length of 0.38 mm ± 1.67, whereas *A. borealis* from the control treatment averaged an increase in length of 0.10 mm ± 1.41. *A. borealis* maintained in the acidified treatments averaged a length increase of 0.30 mm ± 0.60 over the course of the experiment. No significant differences between treatments were observed in the *Astarte* species (*A. montagui*: Kruskal-Wallis, p=0.57; *A. borealis*: t-test, p= 0.74, Figure 2.4, Table 2.3).

In addition to the aggregate analysis present above, I separated clams by size classes (Figure 2.5). *M. calcarea* and *A. montagui* were split into two groups, 10-19.99 mm and 20-29.99 mm, while *A. borealis* was split into two groups of 20-29.99 mm and 30-39.99 mm. In the control treatment, the smaller sized *M. calcarea* averaged a -2.11 mm ± 3.67 decrease in length, and those held in the acidified treatment averaged a -1.43

mm ± 3.55 decrease in length. The larger individual category, while not showing significant differences in length changes between the control and experimental treatments (two-way ANOVA, F= 2.8646, p= 0.1035), displayed an increase (control: 0.31mm ± 1.77) and a lesser degree of decline (acidified: -0.35 mm ± 3.55) in length, providing potential evidence of dissolution.

The *A. montagui* smaller size class showed a decrease in length (control: -0.35 mm ± 2.28; acidified: -0.25 mm ± 1.74), while the larger organisms had a lesser degree of decline (control: -0.05 mm ± 0.68) and an increase in length (acidified: 0.77 mm ± 2.12). Again, there was no significant difference between size classes (two-way ANOVA, F=0.9323, p=0.34) or treatments (F=0.4526, p=0.51). *A. borealis* followed the opposite trend as the other two species. The small size class (20-29.99) displayed an increase in growth in both the control (1.24 mm ± 0.91) and acidified treatment (0.81 mm ± 0.26), while the larger clams (30-39.99 mm) had a decrease in length in both treatments (control: -0.75 mm ± 1.07; acidified: -0.09 mm ± 0.46). Differences in length were not significant between treatments (two-way ANOVA, F=0.2289, p=0.64); however, length changes between the size classes were significantly different (F=12.1472, p=0.006).

Changes in wet weight generally followed the same patterns as changes in length. *M. calcarea* decreased in wet weights after seven weeks (Control: -6.39 g ± 6.25, Acidified: -8.12 g ± 6.29), and all *Astarte* species showing an increase in wet weight (*A. montagui:* Control: 0.44 g ± 4.58, Acidified: 3.44 g ± 8.37; *A. borealis*: Control: 0.68 g ± 0.88, Acidified: 1.00 g ± 0.91). No significant differences were found between treatments in wet weight for any of the species (t-test, all p-values > 0.05, Table 2.3, Figure 2.4).

3.2b 2016 experiments

Only three clams died during the 2016 experiments; all of them were *M. calcarea*. One was held in the AF treatment, one in the ANF treatment, and one in the CNF treatment.

The experimental and control treatments are summarized in Table 2.3. *M. calcarea* only showed an increase in length in the CF treatment (0.55 mm ± 0.34), but showed a decrease in length after eleven weeks in CNF (-0.98 mm ± 0.58), AF (-0.52 mm ± 0.68), and ANF (-0.25 mm ± 0.31). Two-way ANOVAs indicated the interaction of food and pH generated one significant effect (F=9.65, p=0.02). *M. calcarea* held in CF tanks had a higher percentage change in length than those held in control tanks that were not fed (Figure 2.6). Only animals held in the CNF treatment averaged a decrease in wet weight of -0.45 g ± 4.76. All other treatments showed an increase in wet weight (CF: 2.15 g ± 0.62; AF: 4.12 g ± 3.53; ANF: 2.25 g ± 0.28). No significant difference was found for two-way ANOVA tests among any of the treatments for wet weight of *M. calcarea* (Figure 2.6, Table 2.3).

A. montagui length and wet weight changes were not significantly different for treatment or control (Figure 2.6, Table 2.3). All treatments, for both variables, except one, showed an increase. The average length measurement in the AF treatment was the only one to show a decrease (-0.04 g ± 0.53). Average lengths for CF, CNF, and ANF treatments were 0.30 mm ± 0.14, 0.50 mm ± 0.34, and 0.13 mm ± 0.16, respectively. Wet weight increased in all four treatments (CF: 0.94 g ± 0.29, CNF: 0.45 g ± 0.27, AF: 1.54 g ± 3.31, ANF: 1.61 g ± 1.30).

A significant effect of pH was observed for the change in length of *A. borealis* (two-way ANOVA, F= 7.9032, p=0.02, Figure 2.6, Table 2.3). All animals held in control treatments regardless of food treatment displayed an increase in growth (CF: 0.56 mm ± 0.30; CNF: 1.06 mm ± 0.66), while those held in acidified treatments had a decrease in growth (AF: -0.14 mm ± 0.85; ANF: -0.23 mm ± 0.51) after the eleven-week exposure. All treatments demonstrated a decrease in average wet weight at the end of the experiments, but no significant difference was noted (CF: -0.11 g ± 0.56; CNF: -0.33 g ± 0.33; AF: -0.21 g ± 0.09; ANF: -0.75 g ± 0.84) (Figure 2.6, Table 2.3).

I ran the same size class analysis as described in the 2015 experiments (Figure 2.7). Because there were more treatments, a few clams did not have any representatives in specific size classes. For example, there were no *M. calcarea* specimens in the 20-29.99 mm size class in the CNF treatment, and no *A. borealis* in the same size class in the CF treatment. Additionally *A. borealis* in the smaller size class only had one representative in both the AF and ANF treatments. Just as in 2015, *M. calcarea* and *A. montagui* were split into two groups, 10-19.99 mm and 20-29.99 mm, while *A. borealis* was split into two groups of 20-29.99 mm and 30-39.99 mm. In the control treatment *M. calcarea* of the smaller size averaged a decrease of -0.005 mm ± 0.79 in the fed treatment, and -0.98 mm ± 0.58 in the non-fed treatment. Individuals held in the acidified treatment averaged a decrease in length of -0.90 mm ± 1.88 in the fed treatment, and an average decrease of -0.17 mm ± 0.32 in the non-fed treatment. Larger individuals displayed a similar pattern as those from 2015, in that the changes in length of the larger individuals had a larger range than the smaller clams (CF: 1.06 mm ± 0.65; CNF: no representatives; AF: -0.36 mm ± 0.75; ANF: -0.26 mm ± 0.40), but overall there was not a significant difference among treatments (two-way ANOVA, F=1.7156, p=0.22) or size classes (F=0.8578, p=0.37).

In the 2016 experiment, *A. montagui* showed an increase in length in all but two treatments. The smaller and larger individuals held in the AF treatment (small: -0.09 mm ± 0.64; large: -0.10 mm ± 0.31) were the only two where a decrease in length was observed. The rest of the treatments in both size classes showed positive growth (small: CF: 0.51 mm ± 0.19, CNF: 0.55 mm ± 0.60, ANF: 0.07 mm ± 0.15; large: CF: 0.13 mm ± 0.11, CNF: 0.20 mm ± 0.19, ANF: 0.22 mm ± 0.55). There was no significant difference among treatments (two-way ANOVA, F=1.7132, p= 0.20) or size classes (F=0.7995, p= 0.38).

A. borealis had no representatives from the CF treatment in the small size class (20-29.99 mm) and only one representative from the AF (1.44 mm) and ANF (-0.44 mm) treatment in the same size class. The CNF treatment in the small size class averaged an increase in length of 1.74 mm ± 1.11. The larger size class showed an increase in length in the two control treatments (CF: 0.56 mm ± 0.30; CNF: 0.84 mm ± 0.60) and a decrease in growth in the two acidified treatments (AF: -0.34 mm ± 0.50; ANF: -0.25 mm ± 0.52). Both treatment (two-way ANOVA, F= 5.2515, p=0.02) and size (F= 5.4151, p=0.04) differences were significant. The treatment significance matches the significance noted in the change in length in *A. borealis* with all the size classes combined. The larger specimens had smaller overall increases in changes in length than the smaller representatives. While differences between treatment and size classes were not significant in the 2015 data, there was a similar trend as was the case with the 2016 data. Smaller individuals had a larger percent increase in growth, while the larger individuals showed a smaller percent increase in growth. These results suggest that the smaller *A. borealis* may be less vulnerable to acidified waters than larger individuals.

3.3 Oxygen Consumption

3.3a 2015 experiments

For all species, oxygen consumption (mg/L/hour) was not significantly different between treatments (t-test, all p-values > 0.05, Table 2.3). *M. calcarea* held in the control treatment averaged $5.59 x 10^{-7}$ ± $4.51 x 10^{-7}$ mg/L/hour, while those kept in the acidified treatment averaged $3.99 x 10^{-7}$ mg/L/hour ± $4.32 x 10^{-7}$ (Figure 2.8). *A. montagui* maintained in both the control and acidified treatments had similar average oxygen consumption rates, $1.90 x 10^{-7}$ mg/L/hour ± $1.05 x 10^{-7}$ and $2.02 x 10^{-7}$ mg/L/hour ± $9.28 x 10^{-8}$, respectively (Figure 2.8). *A. borealis* kept in the control treatment averaged $2.74 x 10^{-7}$ mg/L/hour ± 2.30, and those maintained in the acidified treatment averaged $2.89 x 10^{-7}$ mg/L/hour ± 1.96 (Figure 2.8).

Oxygen consumption and weight data were log transformed. Oxygen concentrations were modified so oxygen consumption was a positive number before transformation in order to complete the log transformation. All species in all treatments show similar linear patterns in oxygen utilization (Figure 2.9). The *A. borealis* in the acidified treatment were slightly, but not significantly larger than the rest of the species used, but had similar oxygen consumption rates.

3.3b 2016 experiments

Average oxygen consumption for *M. calcarea* varied from $4.26 x 10^{-8}$ to $2.28 x 10^{-7}$ mg/L/hour, and showed no significant difference among the four treatments (two-way

ANOVA, F= 2.78, p>0.05, Table 2.3, Figure 2.10). The two *Astarte* species also showed no significant difference in oxygen consumption among pH and feeding treatments (two-way ANOVA, p>0.05, Table 2.3). Rates averaged between 3.00×10^{-8} to 1.86×10^{-7} mg/L/hour for *A. montagui*, and between 1.27×10^{-7} and 2.81×10^{-7} mg/L/hour for *A. borealis* (Figure 2.10).

Oxygen consumption and weight were again log transformed as described above (i.e. the absolute values of oxygen consumption rates were used) and plotted (Figure 2.11). Again, all species in all treatments show similar linear oxygen utilization rates. As in 2015, the *A. borealis* specimens were larger than the other two species, and clustered away from the other species consumption rates. However, this species still appears to follow the same general pattern of increasing oxygen consumption with increasing size as was observed in the other two species.

3.4 Growth allometry

3.4a 2015 experiments

Allometric growth equations were calculated for all species based upon both pre-experimental and post experimental measurements (Table 2.4). The *M. calcarea* collected from station ML 5-10 collectively exhibited negative allometric growth (pre-experiment). *M. calcarea* held in both the control and ambient treatment also displayed negative allometric growth (post-experiment) (Figure 2.12). Both of the *Astarte* species showed positive allometric growth in measurements directly after collection (pre-experiment) (Figure 2.13, 2.14). Those kept in the control treatment displayed isometric growth, differing from what was observed in the field. *Astarte* maintained in the acidified treatment also demonstrated positive allometric growth, matching the pre-experiment observations.

3.4b 2016 experiments

Bivalves used in the 2016 experiments were part of the same collection at ML 5-10 as those used in the 2015 experiments; therefore, the pre-experiment relationships are the same as described above. The post experiment characterizations showed isometric growth in all species and all treatments (Table 2.4, Figure 2.12-2.14).

4. Discussion

Ocean acidification events in bottom waters in the Pacific Arctic shelf waters result from loss of ice cover that in turn can result in primary production blooms. These factors influence the supply of organic matter to the benthos. Uncertainties concerning how primary production and zooplankton grazing are changing introduce uncertainties as to how ocean acidification events will change and influence this region and its associated biology. Changes in physical factors and seasonal ice cover have the potential to reorganize the Pacific Arctic ecosystem (e.g. Grebmeier and McRoy 1989, Grebmeier, 2006a, 2006b). If the system becomes a pelagic-dominated system (Grebmeier et al. 2006a), the question arises whether natural variability of pH in the bottom waters will stabilize, or if the increases in anthropogenic CO_2 in the atmosphere will eventually cause undersaturation in the entire water column.

The results of this study indicate that Arctic bivalves are likely not strongly and directly affected by the episodic levels of ocean acidification expected in Arctic bottom waters over the shelves in the coming years. In experiments conducted in two separate years, over time scales relevant to the expected exposure, I document only marginal effects of ocean acidification on growth in three species of bivalves. One species *Astarte borealis* showed a decrease in percentage change of length under acidified conditions, but only after eleven weeks of exposure, suggesting that longer exposure times may be required for negative effects to be apparent. Some comparisons can be made to other polar systems. For example, Schram et al. (2016) found very minimal negative effects of ocean acidification on two Antarctic gastropod species. Despite using two different molluscs, the Schram et al. (2016) study is comparable to ours because they used a similar exposure time, seven and six weeks, respectively, a short amount of time in the long life spans of the organisms.

I examined the effects of decreased pH on two size classes of adults in each species. Results from both experiments showed no significant difference in response between the two size classes of *M. calcarea* or *A. montagui*, however the smaller individuals did have a greater percentage decrease in length than the larger ones, suggesting that younger and smaller individuals may be more negatively affected by acidified waters. However, larger *A. borealis* individuals had a larger percent decrease in growth than the smaller individuals, suggesting that the larger individuals of this species may be more vulnerable than smaller individuals, the opposite case of the other two species. Ultimately, there may be an optimal shell size for maintenance in undersaturated seawater that differs among species. Although there was only one significant difference noted in the size class analysis (*A. borealis* 2016 experiments), it does appear that size may play a role in determining vulnerability to acidification. Therefore, future experiments would be warranted to address effects on multiple size classes of the same species of bivalves in order to determine the most vulnerable stage in growth.

I used allometric growth relationships as an additional metric for further evaluating the effects of acidified seawater on the health of the three species. The slope of the linearized allometric relationship varies by species, and can also fluctuate among different conditions a given species may be exposed to throughout the year. Therefore, the relationship should be determined empirically for each species and system of interest, and not universally applied (Glazier 2005, Seibel 2007).

The experiments conducted in 2015 showed negative allometric growth, meaning length increased faster than weight, in the pre- and post-experiment measurements for *M. calcarea*, both in the control and acidified treatments. However, the two *Astarte* species held in control treatments displayed isometric growth only following the experiment, while pre-experiment measurements and measurements from the acidified treatment exhibited positive allometric growth, where weight increases faster than length. I collected these organisms in the late summer (August 2015), when acidification has been observed in the Chukchi Sea; therefore, the *Astarte* I collected could have already been exposed to lower pH conditions pre-collection, potentially explaining why the initial growth equations and the equations from those individuals held in the acidified aquaria displayed the same positive allometric growth pattern. *Astarte* held in the control aquaria, however, may have had sufficient resources to allocate more energy to growth that was isometric in nature, whereas those in the stressed, lower pH aquaria increased weight faster than length.

In 2016 post- experiment allometric determinations, all species from all four treatments showed isometric growth. These determinations all differ from the pre-experiment values in which *M. calcarea* displayed negative allometric growth, and the two *Astarte* species displayed positive allometric growth. The replication of each species in each aquaria during this set of experiments was low (three *M. calcarea*, seven *A. montagui*, and two *A. borealis*). With little replication, the confidence intervals generated by the slope calculations were wide and therefore all of them overlapped with a slope value of three (Table 2.4). The low replication and subsequent wide confidence intervals are likely the reason why all of the relationships were classified as isometric in the 2016 post-experiment analysis. Additionally, clams used in 2016 experiments had been stored in stock aquaria in the cold room since collection in August of 2015, so they may have acclimated to the cold room conditions, including the constant food supplied while 2015 experiments were conducted.

M. calcarea demonstrated negative allometric growth in the pre and post 2015 experiment calculations, but those held in the acidified treatment displayed a much shallower slope (b value) than those from the field and those kept in control conditions. The length of these clams increase quickly, with very little change to weight. This may be consistent with a negative effect of acidified seawater because length measurements were calculated by measuring the longest point of each shell; therefore, faster increases in length than weight could suggest allocation of resources and energy to shell production, but at a cost to maintaining growth of internal structures that add to the overall weight of the organism. While there was no significant difference between treatments in the percent change in length or weight, the relationship between the two parameters clearly differs between treatments.

This study used only adult bivalves, but life history stage responses are likely to vary (Byrne 2011). The larval stage is thought to be the most vulnerable for many species (Kurihara et al. 2004, 2004b, Dupont et al. 2008, Kurihara 2008, Brennand et al. 2010, Byrne 2011). Negative effects, including increased mortality, have been observed in the larvae of several organisms including sea urchins, copepods, Pacific grass shrimp, and brittle stars (Kurihara et al. 2004, 2004b, Dupont et al. 2008, Kurihara 2008, Brennand et al. 2010). All three bivalve species used in this study have pelagic larvae. Therefore, when discussing adults versus larvae, it is noteworthy that lower pH values and undersaturations are observed primarily in bottom waters, while at sites of high primary production, surface pH values are higher in the summer as DIC is removed. As a result, larvae may not be exposed to acidified conditions for as many months as the juveniles and adults are exposed to undersaturation on the bottom (Bates and Mathis 2009, Mathis et al. 2011a, Mathis et al. 2011b, Cross et al. 2012). In addition to studying the direct effects to the larvae, few studies, particularly in the polar regions, have investigated the effects of ocean acidification on larvae produced by adults that have been exposed to acidified conditions (Suckling et al. 2014). Studies that test larval survival under undersaturation in the spring, when spawning occurs, could determine if these conditions are detrimental.

Several studies have shown ocean acidification has synergistic effects with the other main, contemporary threat to marine organisms, warming temperatures, but the interaction between the two is widely unknown (Walther et al. 2010, Harvey et al. 2013).

Harvey et al. (2013) conducted a meta-analysis of 107 peer reviewed articles examining the collective effects of acidification and warming and found that the combination of the two stressors led to a stronger response, both positive and negative, than exposure to only one of these stressors. Warming alone has been shown to lead to increased metabolic costs (O'Connor et al. 2009), as well as increased prey consumption (Sanford 1999). These measurements of oxygen consumption presented here did not result in significant responses to decreased pH or food manipulations, but warming water temperatures, which were not independently tested in this study, may introduce responses synergistically with changes in food availability and pH. If warming waters increase food consumption, the projected potential implications for decreases in phytoplankton blooms include an additional stressor to the system in the form of food availability. The Harvey et al. (2013) meta-analysis noted that mollusc growth was affected negatively when both warming and acidification co-occur. Therefore, the negative effects observed here from acidification alone to growth in *A. borealis*, suggest that future studies could profitably examine the combined effects of warmer temperatures, decreased pH, and limited food supply; especially because the temperatures at the sediment water interface affect metabolic rates, which in turn affects growth and remineralization intensity (Grebmeier et al. 2015). Because these organisms are not solely exposed to one changing condition, it is important to look at the interactions among all of the likely changes.

Decreased pH in the bottom waters in the Chukchi Sea currently occurs in the summer and fall (Mathis and Questal 2013, Yamamoto-Kawai et al. 2016). With changing sea ice dynamics, the timing and intensity of phytoplankton blooms will likely change (Grebmeier et al. 2015), with both increases and decreases in primary production potentially possible. Primary production may increase because there will be more open surface area for penetration of sunlight (Arrigo et al. 2008, 2011). Such an increase in primary production could lead to more organic carbon exported to the benthos, potentially leading to more remineralization and additional seasonal decreases in pH in bottom waters.

Scenarios for decreases in primary production due to timing changes for ice edge blooms are also possible (Grebmeier et al. 2006a). While more surface area will be exposed with less ice extent and earlier retreating ice, warming and freshening of the upper seawater layers may increase stratification, and prevent a ventilation of surface waters with nutrient resupply from bottom waters. A reduction in primary production will decrease the organic carbon deposition to the benthos and thus decrease benthic remineralization, potentially limiting how low pH can drop in the bottom waters (Grebmeier et al. 2006a). However, because the shelf system is shallow, bottom waters could still remain undersaturated with respect to carbonate for much of the year as described previously, despite potential decreases in remineralization. A decreased primary production scenario could also cause surface pH to decline as uptake by smaller phytoplankton blooms may not be able to keep up with the increased air-sea fluxes of atmospheric CO_2.

These potential changes to light and nutrient availability, as well as the ongoing warming and decreases in sea ice extent, not only affects what will happen to primary production, but also has implications for zooplankton. Earlier warming would increase zooplankton growth, abundance, and grazing (Coyle et al. 2007), limiting the quality and quantity of organic matter exported to the benthos.

Changes in the timing of the phytoplankton blooms could also potentially change the timing of ocean acidification events. For example, if the occurrence of fall blooms increase due to wind mixing of open water when sea ice was formerly present, there is the potential for ocean acidification events to extend into the winter months, when there is no newly produced food. As demonstrated in this current study, food availability can affect the growth of bivalves. *M. calcarea* displayed a significant difference in percent change in length in aquaria with food additions versus aquaria where food was not added, with those fed showing an increase in length, and those not fed showing a decrease in length. Therefore, if exposure to acidified conditions expands into a time when food is limited, the negative effects of limited food could be intensified by the presence of low pH values, thus adding to the complexity of understanding how these organisms will respond to changing acidity. Future research efforts should include experimentation on bivalves collected at different times of the year, i.e. spring, summer, fall, and winter, to test how pre-conditioning to acidified conditions may alter responses.

While most of the results from both sets of experiments did not indicate significant effects from pH and food treatments, there were a few significant changes or differences observed that confirm that changes to organisms in response to decreased pH and ocean acidification will be species specific, and that some negative effects are being observed on bivalves in the Pacific Arctic. *A. borealis* demonstrated decreased length in response to an eleven-week exposure in acidified treatments regardless of food availability. *M. calcarea* displayed decreased length in aquaria with food limitation versus aquaria in which food was available, suggesting that food availability plays a role in their shell growth, and if bloom timing and location changes, *M. calcarea* may be more susceptible to acidification impacts even though no effects were noted in these experiments. I assume here that length of exposure to decreased pH is likely to be one of the factors controlling negative effects. These organisms are exposed to seasonally variable conditions, but if pH values decline below their natural range and exposure time increases due to changes in phytoplankton blooms and ice conditions as described above, it is likely the minimal effects observed in these experiments will increase in more prominent ways.

5. Conclusions

As anthropogenic emission of CO_2 continues to increase, the effects of decreased pH and other subsequent changes to ocean chemistry from increased CO_2 may become more pronounced and critical. Both the Bering Sea and the Chukchi Sea display naturally varying pH conditions because of mechanisms such as the PhyCaSS interactions and remineralization at depth (e.g. Bates et al. 2009, Mathis et al. 2011a, Mathis et al. 2011b, Mathis and Questal 2013, Yamamoto-Kawai et al. 2016). However, estimates based upon current atmospheric concentrations of CO_2 indicate that without anthropogenic contributions both calcite and aragonite would be supersaturated in the spring and the summer in the Bering Sea, and aragonite undersaturation would not be present until a depth of 100 meters at the shelf break, with no sign of calcite undersaturation (Mathis et al. 2011a, 2011b). As human induced changes continue to occur, monitoring of responses by calcifying organisms should continue because the system is expected to move out of the natural range of the carbonate system parameters [i.e. saturation state ($\Omega_{aragonite}= 1.2 \pm 0.1$), pH, etc.] as early as 2027 (Mathis et al. 2015), and once this happens, the responses by organisms will likely become more significant and obvious.

I quantified how bivalves common in the Chukchi Sea would respond to these documented decreases in pH (Mathis and Questal 2013, Yamamoto-Kawai et al. 2016). Several determinations were made of bivalve growth, including percent change in length and wet weight, as well as determination of allometric growth classifications. These measurements were coupled with oxygen consumption rates to assess the vulnerabilities of these organisms under acidified treatments. Since bivalves are important prey for higher trophic benthivores, including walrus, negative impacts on the prey base could impact larger portions of the food chain. As increases in atmospheric CO_2 are expected to continue, understanding the consequences of changing carbonate chemistry is crucial, especially in regions like the Pacific Arctic, where duration and intensity of such events are expected to continue to increase in the coming decades and have impacts much sooner than other regions of the world.

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Tables

Table 2.1 Water column parameters (mean±1 SD) measured from the CTD in 2015 in the NE Chukchi Sea as part of the AMBON cruise (temperature and salinity) as well as control and treatment pH used in the experiments. *Total alkalinity (TA) was determined from salinity with the regression equation from Yamamoto-Kawai et al. 2016. Salinity, temperature, pH, and total alkalinity were inserted into the CO_2sys spread sheet to calculate $pCO_{2,}$, calcite saturation state (Ω), and aragonite saturation state (Ω). Key: Control Fed=Control conditions; Acidified Fed=Experimental, lower pH conditions.

Parameter	CTD Bottom Water	Control Fed (mean ±SD)	Acidified Fed (mean ±SD)
pH		8.05 ± 0.02	7.76 ± 0.02
Temperature (°C)	-0.31	2.51 ± 0.09	2.53 ± 0.06
Salinity (psu)	32.19	32.51 ± 0.11	32.64 ± 0.08
TA* (µmol/kg SW)		2295.91	2303.61
pCO_2* (µatm)		388.9	801.56
Calcite Ω*		2.37	1.49
Aragonite Ω*		1.29	0.81

Table 2.2 Water column parameters (mean ±1 SD) measured from the CTD in 2015 in the NE Chukchi Sea as part of the AMBON cruise(temperature and salinity) as well as control and treatment pH used in the experiments. *Total alkalinity (TA) was determined from salinity with the regression equation from Yamamoto-Kawai et al. 2016. Salinity, temperature, pH, and total alkalinity were inserted into the CO_2sys spread sheet to calculate pCO_2, calcite saturation state (Ω), and aragonite saturation state (Ω). Key: Control F= Control conditions fed during the course of the experiment; Control NF= Control conditions not fed over the course of the experiment; Acidified F= Experimental, lower pH conditions fed over the course of the experiment; Acidified NF= Experimental, lower pH conditions not fed over the course of the experiment.

Parameter	CTD Bottom Water	Control F (mean ±SD)	Control NF (mean ±SD)	Acidified F (mean ±SD)	Acidified NF (mean ±SD)
pH		8.19 ± 0.004	8.19 ± 0.003	7.86 ± 0.01	7.86 ± 0.02
Temperature (°C)	-0.31	2.59 ± 0.11	2.55 ± 0.05	2.56 ± 0.05	2.50 ± 0.08
Salinity (psu)	32.19	32.61 ± 0.26	32.78 ± 0.13	32.60 ± 0.08	32.53 ± 0.06
TA* (µmol/kg SW)		2301.83	2311.9	2301.24	2297.09
pCO2* (µatm)		271.44	272.28	627.66	626.55
Calcite Ω*		3.17	3.19	1.6	1.59
Aragonite Ω*		1.99	2	1	1

Table 2.3 Results of statistical testing (t-test, Kruskal Wallis, two-way ANOVA) of the effects of decreased pH and controlled food supply for *M. calcarea*, *A. montagui*, and *A. borealis.* Significant differences are shown in bold type.

Trait	Year	Species	Source	d.f.	Mean Square	F or equivalent	p	Test
Length (% Change)	2015	*M. calcarea*	pH	1			0.341	T test
		A. montagui				11.637	0.5653	Kruskal Wallis
		A. borealis				8.1059	0.7432	T test
Weight (% Change)	2015	*M. calcarea*	pH			12	0.6144	T test
		A. montagui				9.3027	0.4272	T test
		A. borealis				11.99	0.5269	T test
Oxygen Consumption (mg/L/hour)	2015	*M. calcarea*	pH			8.77	0.5656	T test
		A. montagui				9.84	0.8477	T test
		A. borealis				3.76	0.9285	T test
Length (% Change)	2016	*M. calcarea*	pH	1	0.09365	0.3713	0.55922	2-way ANOVA
		A. montagui		1	0.36207	3.2662	0.1083	2-way ANOVA
		A. borealis		**1**	**2.96043**	**7.9032**	**0.0228**	**2-way ANOVA**
		M. calcarea	**Food**	**1**	**1.19164**	**4.4242**	**0.06148**	**2-way ANOVA**
		A. montagui		1	0.10238	0.9236	0.3647	2-way ANOVA
		A. borealis		1	0.11807	0.3152	0.5899	2-way ANOVA
		M. calcarea	**pH x food**	**1**	**2.43414**	**9.65**	**0.01452**	**2-way ANOVA**
		A. montagui		1	0.00079	0.0071	0.9349	2-way ANOVA

	A. borealis		1	0.25725	0.6868	0.4313	2-way ANOVA
Weight (% Change)	*M. calcarea*	pH	1	16.4314	1.8446	0.2115	2-way ANOVA
	A. montagui		1	2.3282	0.7275	0.4185	2-way ANOVA
	A. borealis		1	0.1991	0.7014	0.4266	2-way ANOVA
	M. calcarea	Food	1	15.0258	1.6868	0.2302	2-way ANOVA
	A. montagui		1	0.1352	0.0422	0.8423	2-way ANOVA
	A. borealis		1	0.4254	1.4986	0.2557	2-way ANOVA
	M. calcarea	pH x food	1	0.4038	0.0543	0.8367	2-way ANOVA
	A. montagui		1	0.2321	0.0725	0.7945	2-way ANOVA
	A. borealis		1	0.07831	0.2759	0.6137	2-way ANOVA
Oxygen Consumption (mg/L/hour)	*M. calcarea*	pH	1	6.455x10^{-14}	2.78	0.1213	2-way ANOVA
	A. montagui		1	3.7549 x10^{-14}	1.3358	0.2723	2-way ANOVA
	A. borealis		1	1.761 x10^{-14}	0.3854	0.552	2-way ANOVA
	M. calcarea	Food	1	1.0095 x10^{-14}	0.4368	0.5212	2-way ANOVA
	A. montagui		1	1.2267 x10^{-14}	0.4364	0.5225	2-way ANOVA
	A. borealis		1	8.042 x10^{-15}	0.176	0.6859	2-way ANOVA
	M. calcarea	pH x food	1	1.3989 x10^{-14}	0.6053	0.4516	2-way ANOVA
	A. montagui		1	4.572 x10^{-15}	0.1626	0.6945	2-way ANOVA
	A. borealis		1	1.2576 x10^{-14}	0.2752	0.2752	2-way ANOVA

Table 2.4 Allometric growth equations and characterizations for *M. calcarea*, *A. montagui*, and *A. borealis* for in situ and all treatment conditions in 2015, and 2016. W= weight in grams; L= length in mm.

Species	N	Station/Year	Pre/Post Experiment/Treatment	Mean Length ± 1SD	Allometric equation	R^2	95% CI of b	Relationship
Macoma calcarea	**120**	**ML5-10/2015**	**Pre experiment**	**20.51 ± 3.47**	$\mathbf{W=-8.2283L^{2.8059}}$	**0.9312**	**2.667-2.944**	**(-) allometry**
Astarte montagui	195	ML5-10/2015	Pre experiment	19.47 ± 2.71	$W=-9.40138L^{3.33077}$	0.8935	3.168-3.494	(+) allometry
Astarte borealis	38	ML5-10/2015	Pre experiment	31.62 ± 4.14	$W=-10.7297L^{3.6723}$	0.9121	3.293-4.0520	(+) allometry
Macoma calcarea	37	ML5-10/2015	Post experiment Control 2015	20.86 ± 2.97	$W=-7.4891L^{2.360313}$	0.9505	2.360-2.755	(-) allometry
Astarte montagui	**54**	**ML5-10/2015**	**Post experiment Control 2015**	**19.17 ± 2.89**	$\mathbf{W=-8.8063L^{3.1381}}$	**0.9231**	**2.889-3.387**	**isometric**
Astarte borealis	**7**	**ML5-10/2015**	**Post experiment Control 2015**	**31.51 ± 5.68**	$\mathbf{W=-10.7454L^{3.6909}}$	**0.8872**	**2.324-5.058**	**isometric**
Macoma calcarea	37	ML5-10/2015	Post experiment Acidified 2015	20.83 ± 3.49	$W=-8.01063L^{2.71697}$	0.9641	2.540-2.894	(-) allometry
Astarte montagui	55	ML5-10/2015	Post experiment Acidified 2015	19.58 ± 2.70	$W=-9.3989L^{3.3230}$	0.9148	3.047-3.600	(+) allometry
Astarte borealis	7	ML5-10/2015	Post experiment Acidified 2015	31.51 ± 5.68	$W=-12.4849L^{4.1759}$	0.9676	3.376-4.976	(+) allometry
Macoma calcarea		ML5-10/2015	Post experiment Control Non Fed 2016	16.43 ± 1.88	$W=-8.6526L^{2.9307}$	0.9411	2.256-3.606	isometric
Macoma calcarea		ML5-10/2015	Post experiment Control Fed 2016	22.11 ± 5.78	$W=-8.3092L^{2.8174}$	0.9835	2.573-3.062	isometric
Astarte montagui		ML5-10/2015	Post experiment Control Non 2016	19.85 ± 2.26	$W=-9.3373L^{3.2970}$	0.9227	2.851-3.743	isometric
Astarte montagui		ML5-10/2015	Post experiment Control Fed 2016	20.10 ± 2.83	$W=-9.70737L^{3.4315}$	0.922	2.965-3.898	isometric
Astarte borealis		ML5-10/2015	Post experiment Control Non Fed 2016	30.64 ± 3.69	$W=-10.0131L^{3.4766}$	0.905	2.092-4.861	isometric
Astarte borealis		ML5-10/2015	Post experiment Control Fed 2016	33.63 ± 1.69	$W=-15.9726L^{5.1390}$	0.8703	2.711-7.567	isometric
Macoma calcarea		ML5-10/2015	Post experiment Non Fed Acidified 2016	19.09 ± 2.13	$W=-8.1164L^{2.7587}$	0.9251	2.037-3.480	isometric
Macoma calcarea		ML5-10/2015	Post experiment Acidified Fed 2016	19.06 ± 1.86	$W=-7.4936L^{2.5315}$	0.919	1.841-3.222	isometric
Astarte montagui		ML5-10/2015	Post experiment Acidified Non Fed 2016	19.15± 2.47	$W=-9.2231L^{3.2770}$	0.9207	2.828-3.726	isometric
Astarte montagui		ML5-10/2015	Post experiment Acidified Fed 2016	19.33 ± 2.60	$W=-9.3645L^{3.3137}$	0.9005	2.800-3.828	isometric
Astarte borealis		ML5-10/2015	Post experiment Acidified Non Fed 2016	31.74 ± 1.54	$W=-9.015L^{3.177}$	0.4341	(-)0.834-7.189	isometric
Astarte borealis		ML5-10/2015	Post experiment Acidified Fed 2016	31.67 ± 2.69	$W=-10.1014L^{3.4797}$	0.8599	1.764-5.196	isometric

Figures

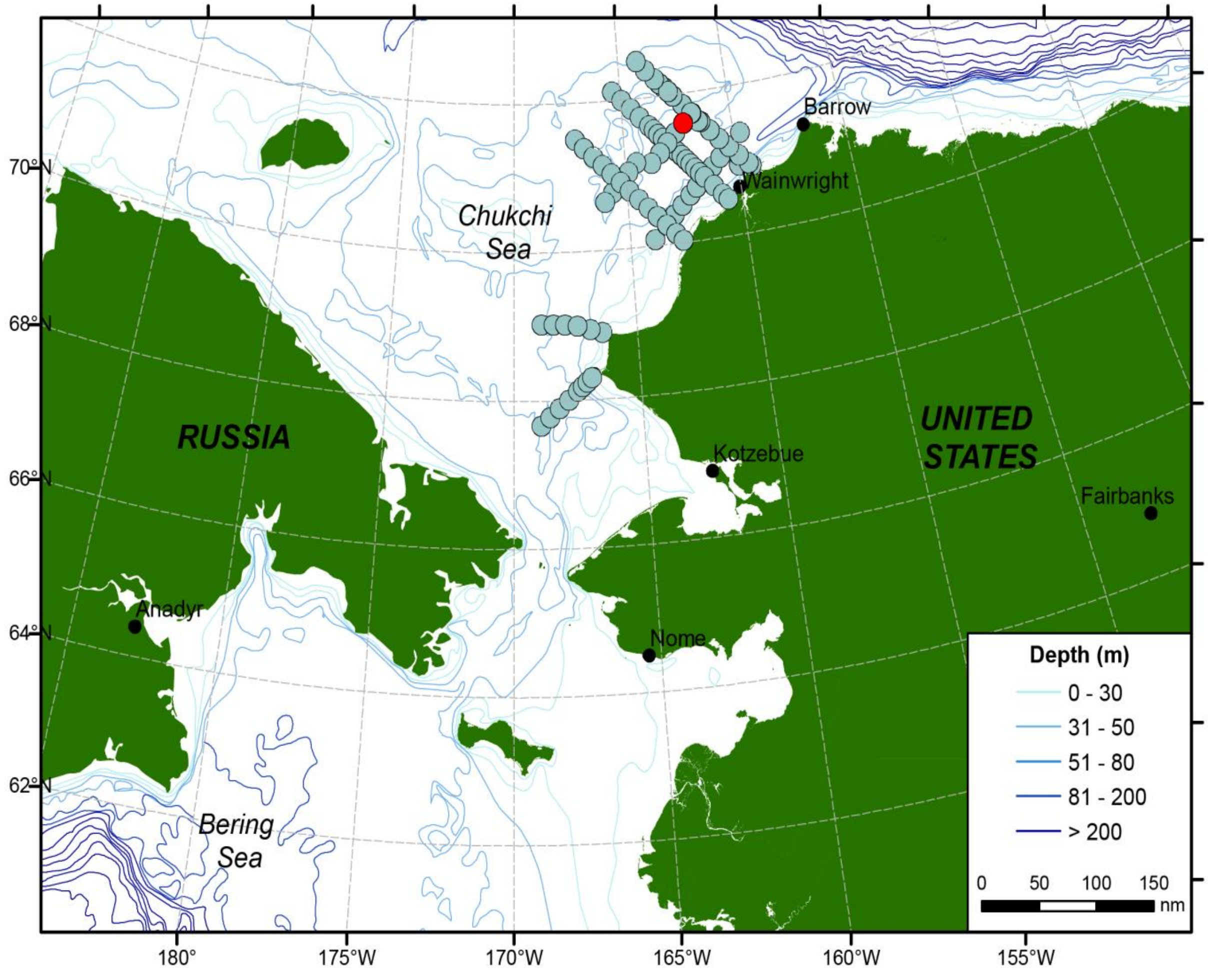

Figure 2.1 Station map of AMBON cruise in 2015. Bivalves used in experiments were collected from station ML 5-10 (highlighted in red).

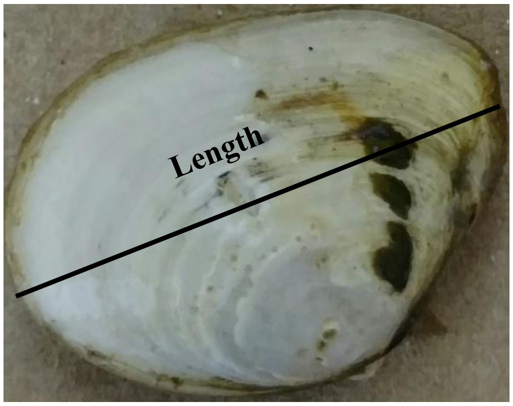

Figure 2.2 Location of length measurement on all bivalve shells.

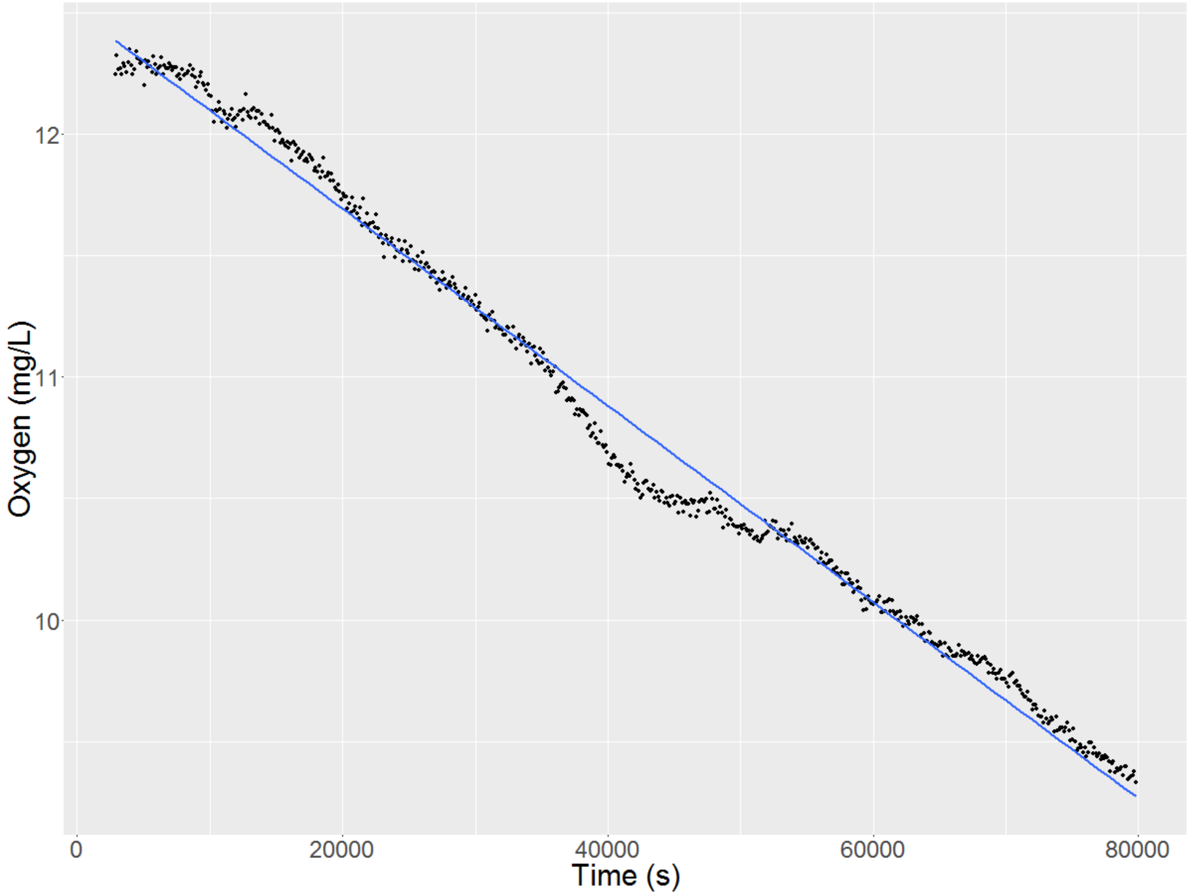

Figure 2.3 An example of the regression analysis (*A. montagui* from an acidified tank, 2015) to determine oxygen consumption (mg O_2/L/hour) of each individual clam during experiments. The slope from the regression was multiplied by the volume of water to calculate oxygen consumption (mg O_2/L/hour).

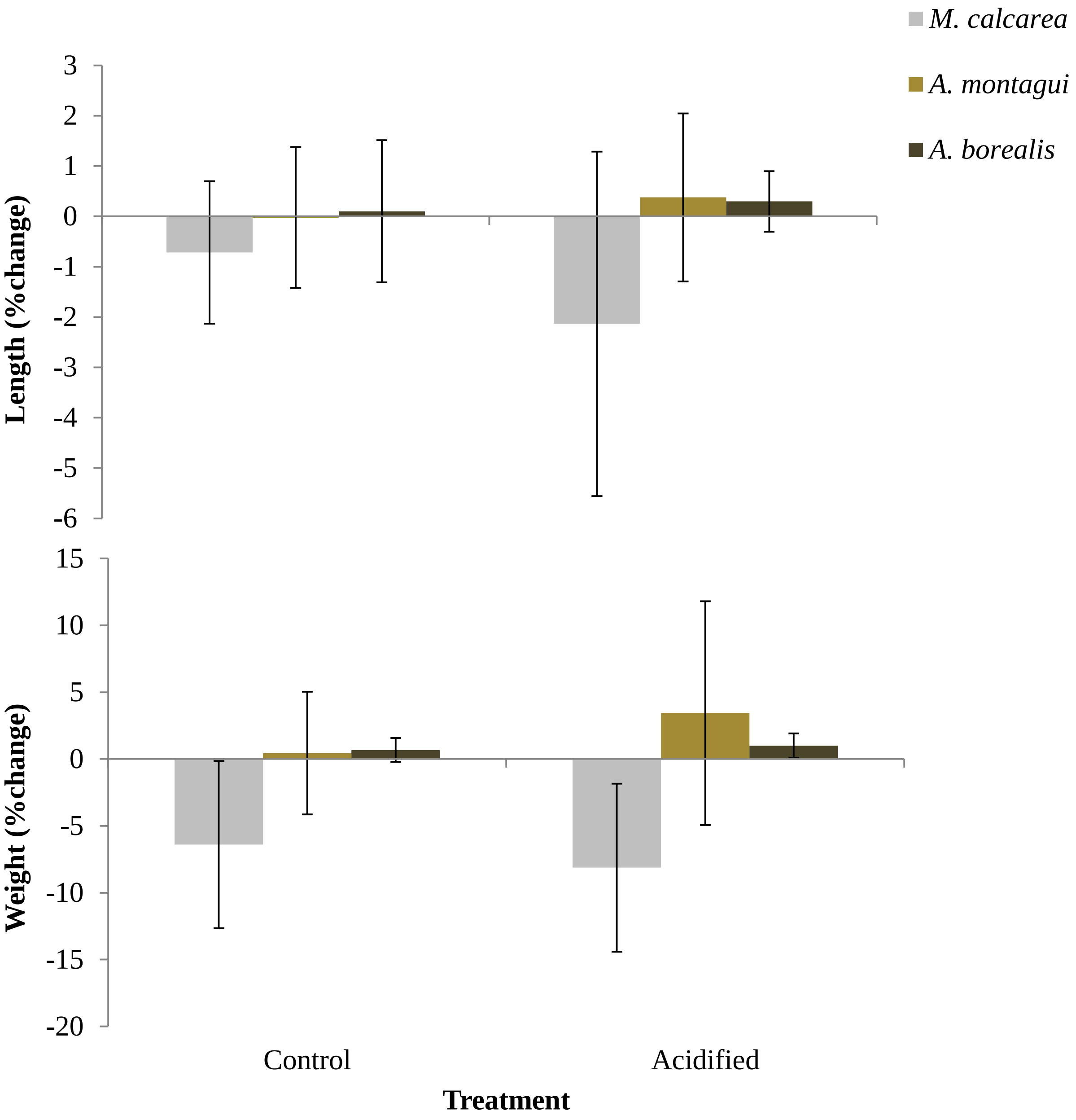

Figure 2.4 Mean percent change in length (mm) (top) and weight (g) (bottom) (± 1 SD) of *M. calcarea*, *A. montagui*, and *A. borealis* from the control treatment (8.05 ± 0.02) and acidified treatment (7.76 ± 0.02) in 2015.

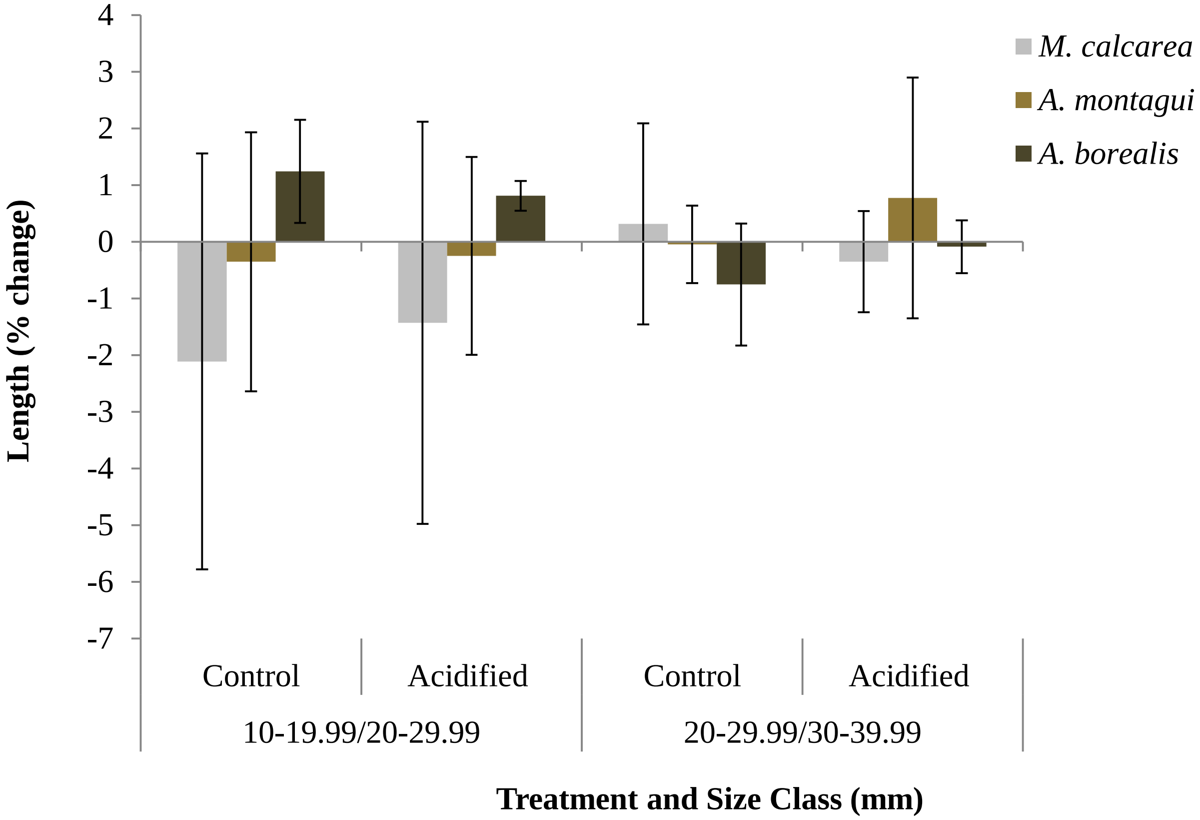

Figure 2.5 Mean (±1SD) percent change in length (mm) by species and size class in 2015.

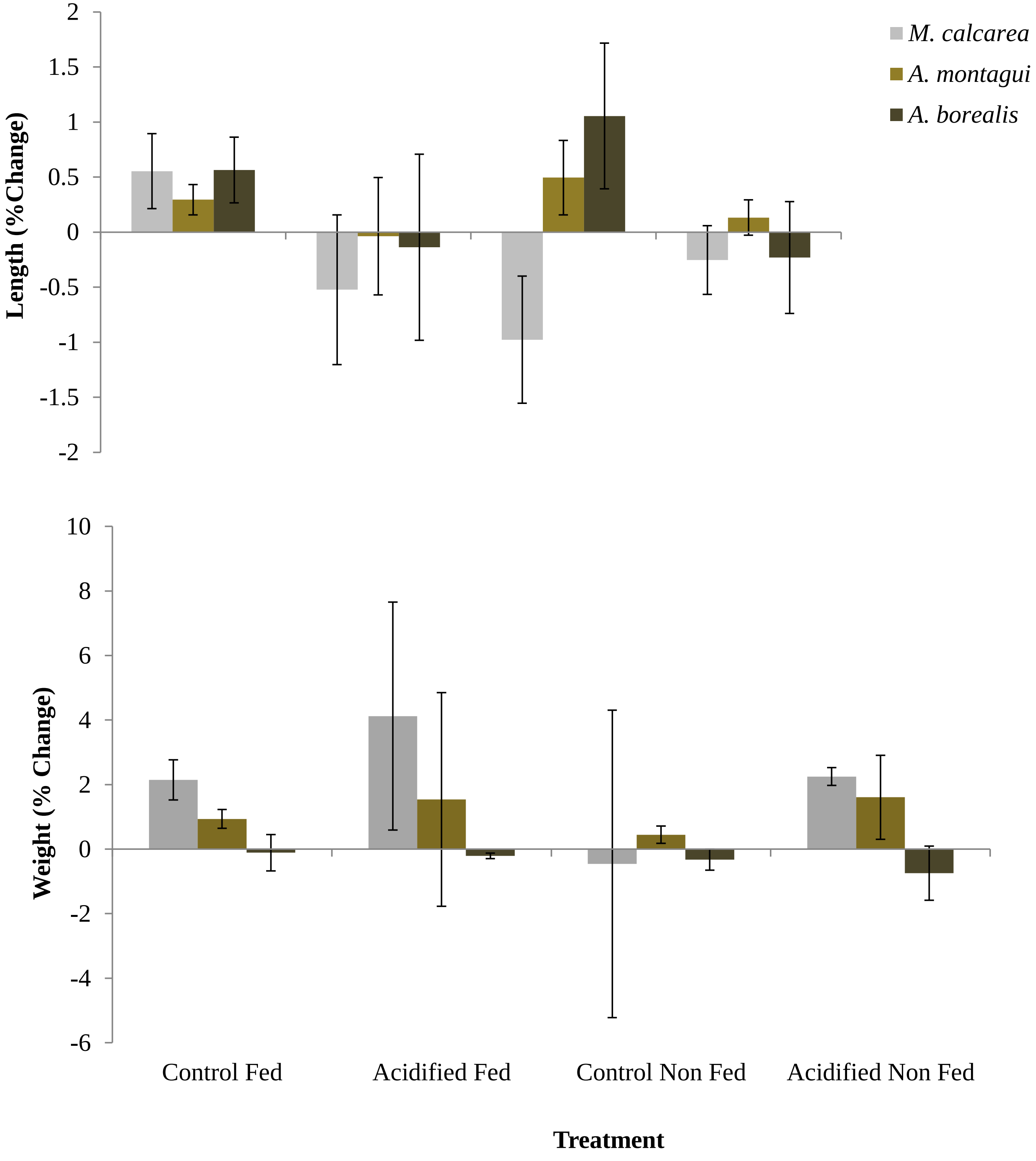

Figure 2.6 Mean percent change in length (mm) (top) and weight (g) (bottom) (±1SD) in the four treatments of 2016 experiments.

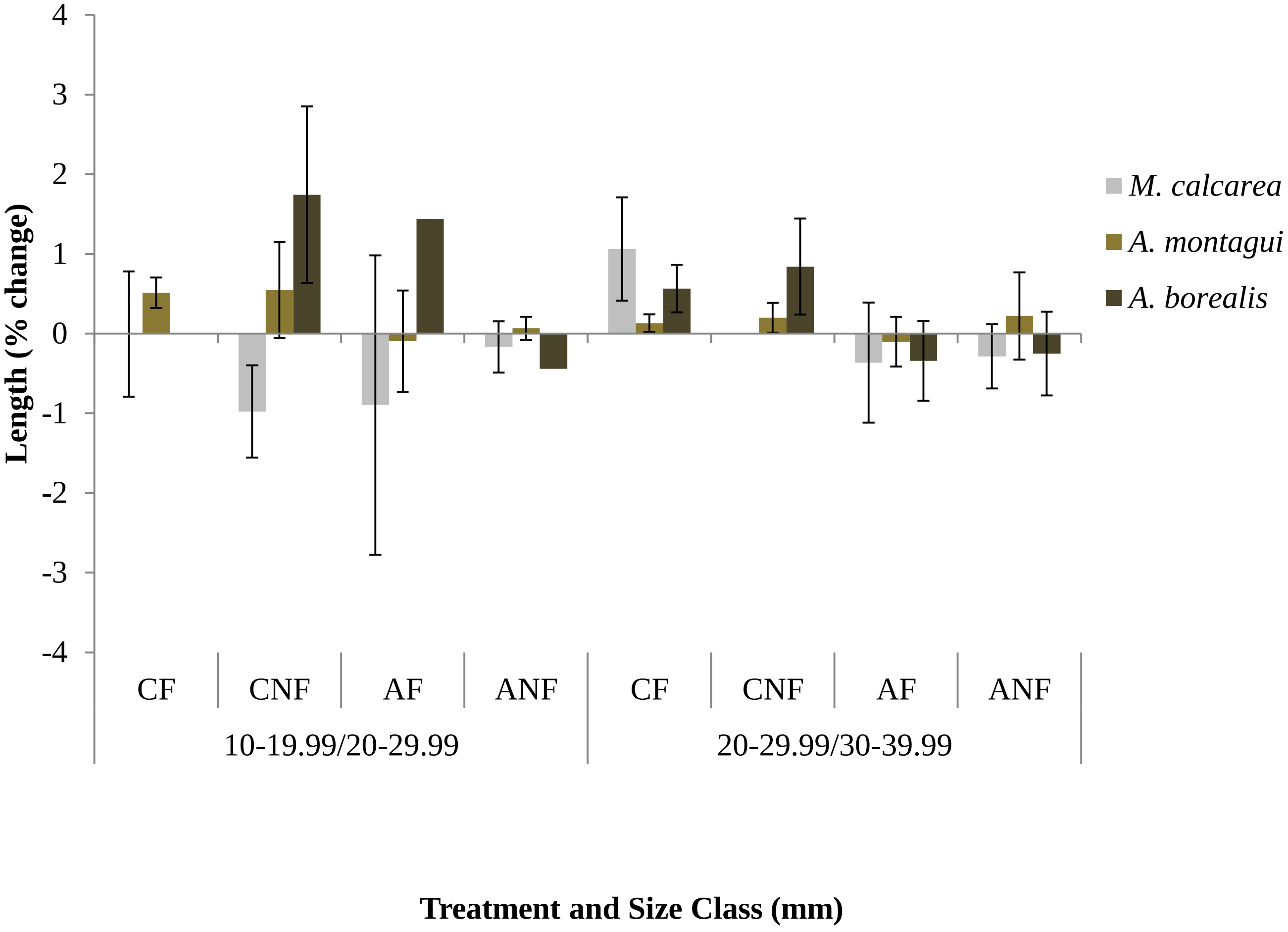

Figure 2.7 Mean (±1SD) percent change in length (mm) by species and size class in 2016. Key: CF= Control Fed, CNF= Control non-fed, AF: Acidified Fed, ANF: Acidified non-fed. Note: bars with no error bars only had one representative.

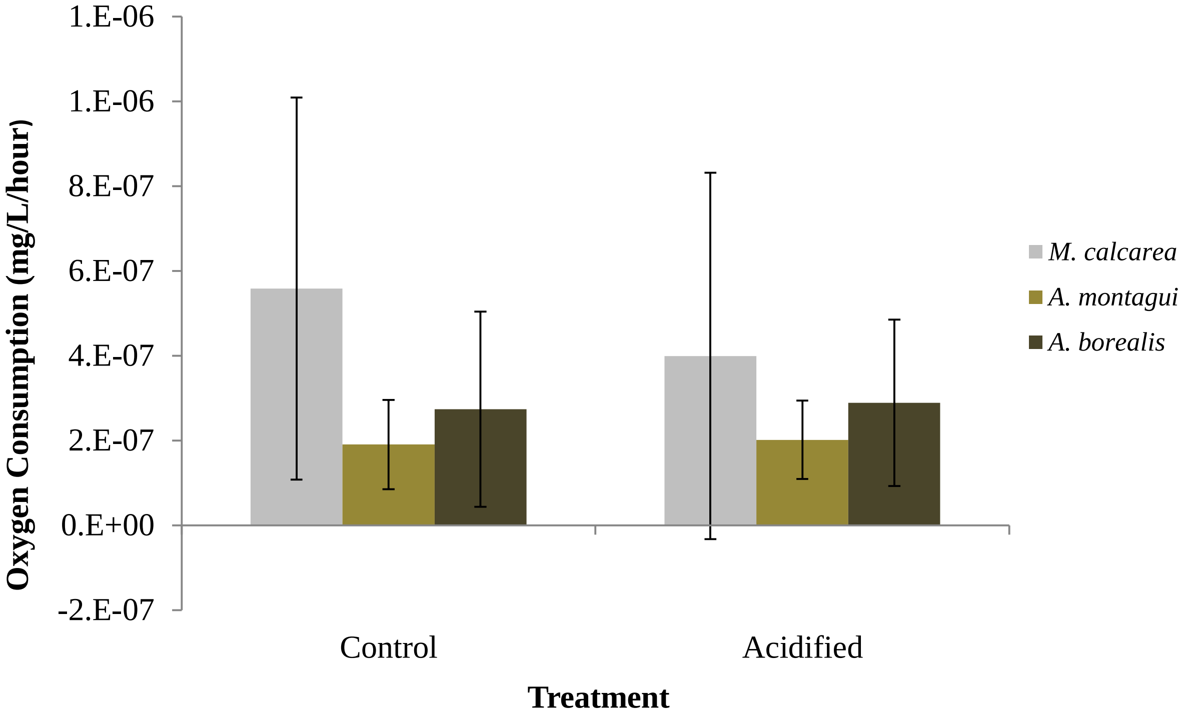

Figure 2.8 Mean oxygen consumption (mg/L/hour) ± 1SD, by treatment and species for 2015 experiments.

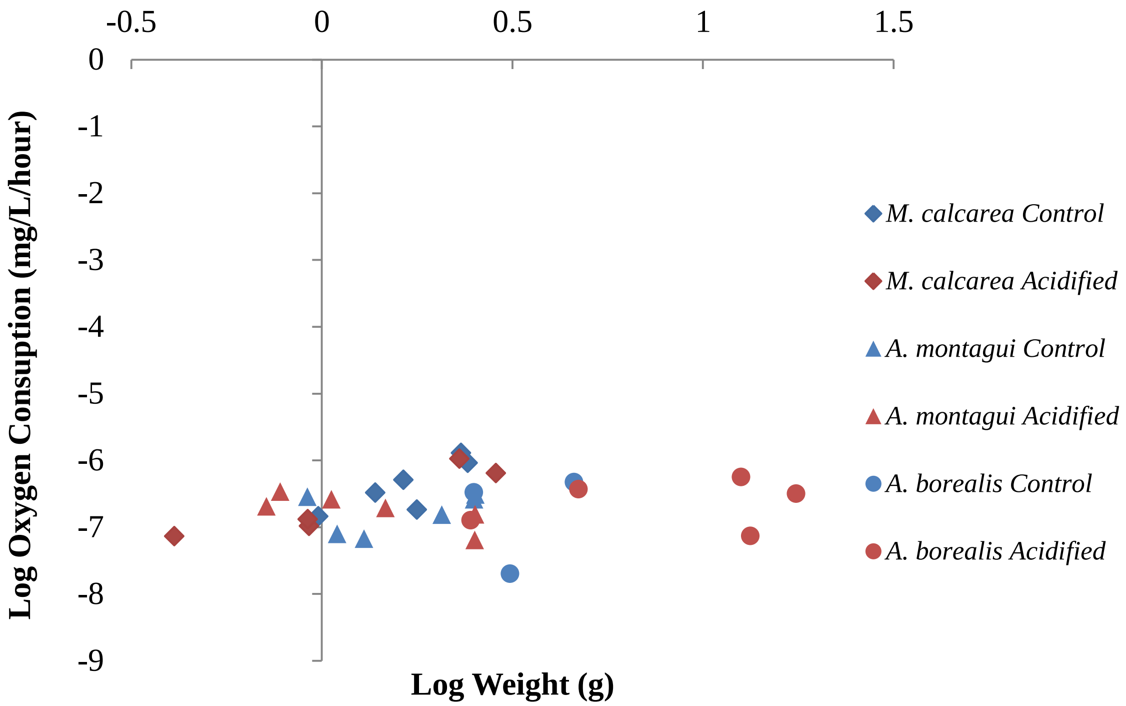

Figure 2.9 Log transformed oxygen consumption vs log transformed weight of all species and both treatments from 2015 experiments.

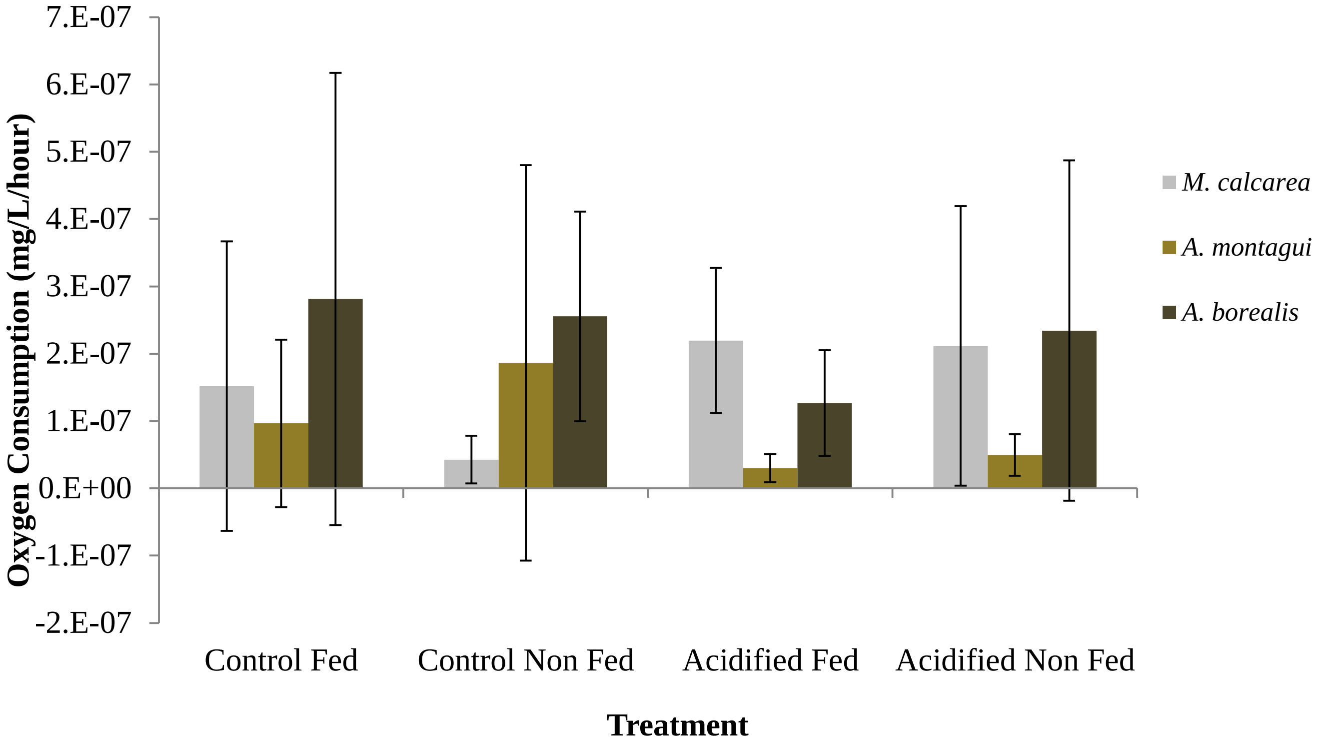

Figure 2.10 Mean oxygen consumption (mg/L/hour) ± 1SD, by treatment and species for 2016 experiments.

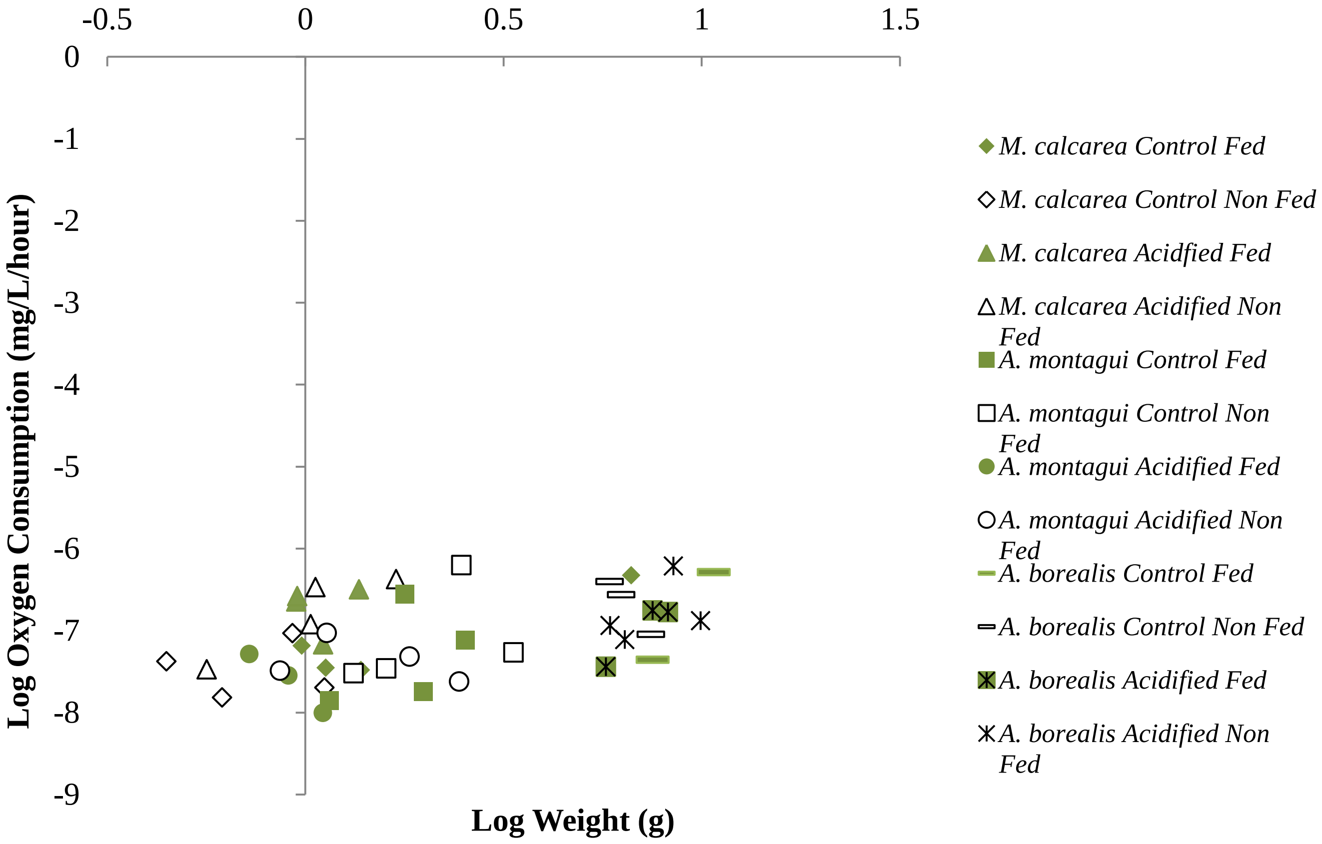

Figure 2.11 Log transformed oxygen consumption vs log transformed weight of all species and treatments from 2016 experiments.

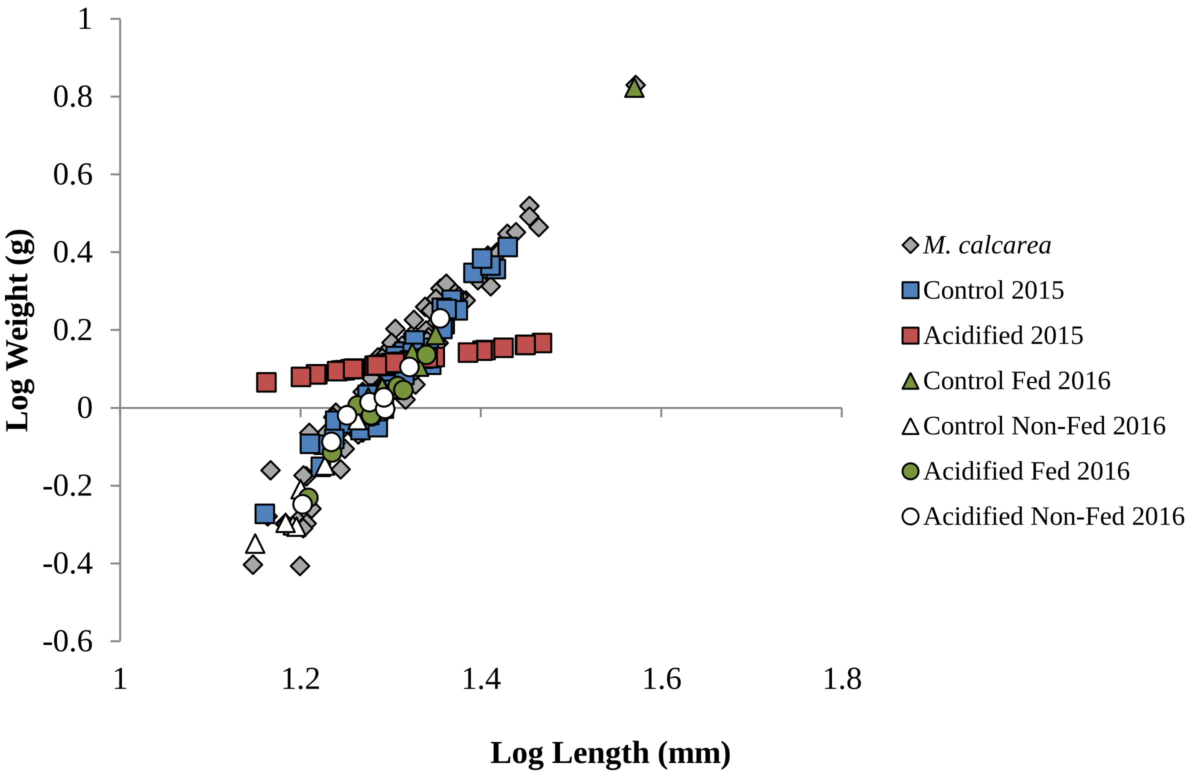

Figure 2.12 Allometric growth for *M. calcarea* from in situ measurements and treatments from both 2015 and 2016 experiments.

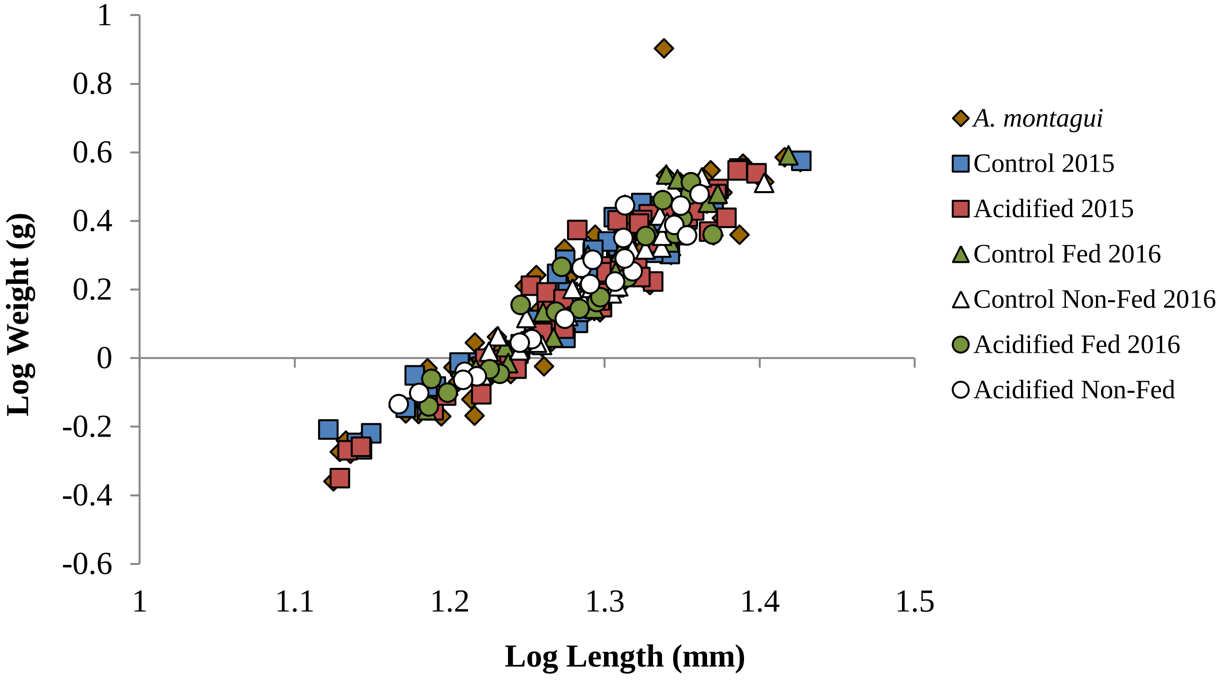

Figure 2.13 Allometric growth for *A. montagui* from in situ measurements and treatments from both 2015 and 2016 experiments.

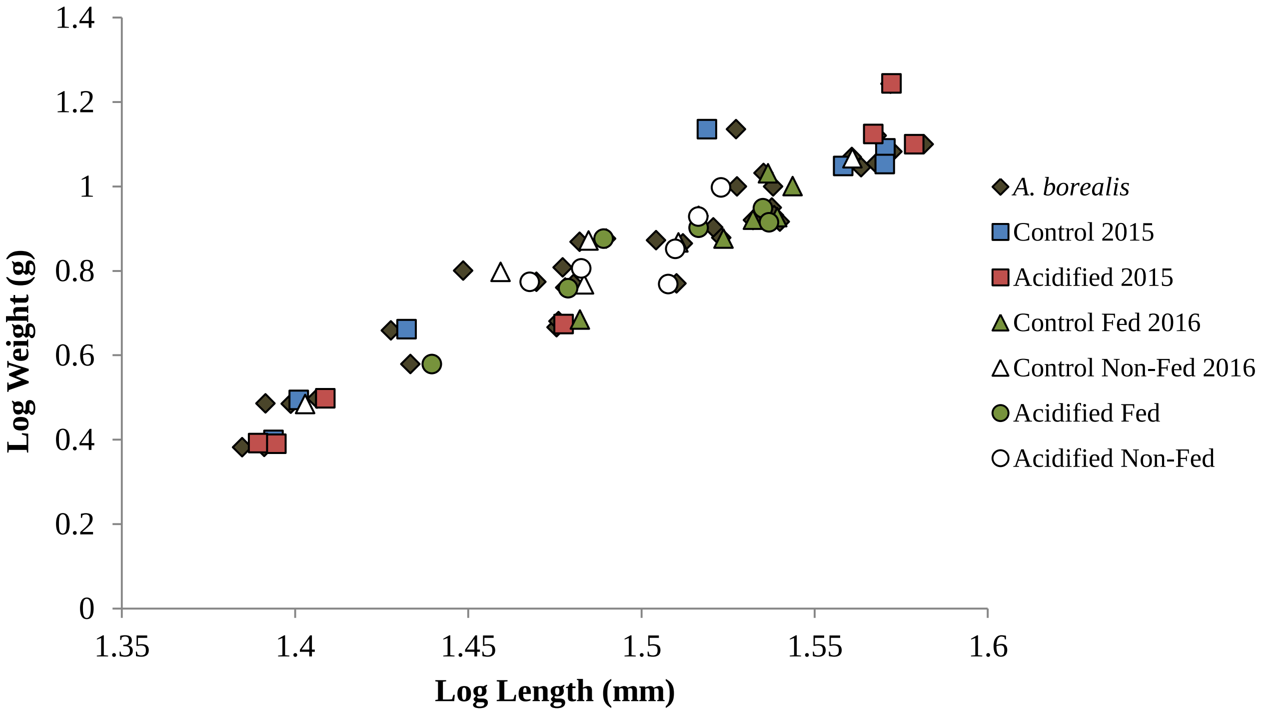

Figure 2.14 Allometric growth for *A. borealis* from in situ measurements and treatments from both 2015 and 2016 experiments.

Chapter 3: Tracking changes in abundance and dominant size class of the bivalve *Macoma calcarea* in the northern Bering Sea and the southeastern Chukchi Sea

Abstract

Changing sea ice extent, warming seawater temperatures, and shifting sediment composition are known drivers for benthic macrofaunal community composition and biomass in the Pacific Arctic. I tracked the abundance and dominant size class of a common bivalve, *Macoma calcarea*, and compared abundances among six sites in the Distributed Biological Observatory (DBO); five in the northern Bering Sea (DBO-SLIP1-5) and one in the southeastern Chukchi Sea (DBO-UTN2). The northern Bering Sea is the overwintering feeding area for the federally listed spectacled eider, so tracking prey abundance is crucial for understanding changes to these seaduck populations. Bivalve abundance was analyzed from 1998-2012, and associated size class abundance was analyzed for clams collected between 1999 and 2006. Results from dynamic factor and time-series cluster analyses indicate that two trends influence abundance at these sites, with station DBO-SLIP 5 identified as being the transition between stations to the south and the north. Notably, smaller clams were more dominant in abundance than larger clams at all six of the sites. The study concluded that there were spatial differences in a declining bivalve prey base that have implications for higher trophic benthivores.

1. Introduction

1.1 Changing Bivalve Abundance in the Pacific Arctic

Recent sea ice retreat, seawater warming, and shifts in sediment composition in the Pacific Arctic are physical changes that impact benthic macrofaunal community composition and biomass (Grebmeier 2012, Grebmeier et al. 2015). Benthic macrofanual communities in the Arctic provide indicators of changing environmental conditions because of their long and sessile life spans that allow them to be easily and consistently sampled (Warwick 1993, Grebmeier et al. 2010, Iken et al. 2013). Bivalves, a significant component of this benthic community, are common prey items for benthivorous predators such as walruses, bearded seals, and diving seaducks (Sheffield and Grebmeier 2009, Lovvorn et al. 2009, Moore et al. 2014). Because shell size (Lovvorn et al. 2003) and availability (Grebmeier 2012, Lovvorn et al. 2014, Jay et al. 2014) of prey items can affect benthivore feeding success, knowledge of the recruitment and size distributions of the benthic food base allows for a better understanding of potential feeding behaviors and geographic shifts of apex predators in response to biological and physical changes.

Here I document and assess recruitment and size patterns of a common Pacific Arctic bivalve species, *Macoma calcarea,* from 1998-2012 using length-weight (L/W) data at known biological hotspots, which are areas of regionally high benthic biomass, in the SE Chukchi Sea and in the region south of St. Lawrence Island (Figure 3.1). *M. calcarea* bivalves size class distributions were documented for eight of the years of the time series (1999-2006) at the five locations south of St. Lawrence Island. Additionally, the allometric growth characteristics of *M. calcarea* were determined for one additional site (repeated sampling station DBO-UTN 2) in the SE Chukchi Sea and compared to a site in the NE Chukchi Sea (repeated sampling site ML 5-10) in 2014 and 2015.

1.2 Benthic Hotspots

The study sites used in the northern Bering Sea and SE Chukchi Seas are recognized as some of the most seasonally productive areas in the world, while also acting as focus points for organic carbon settling, especially in May and June, when large phytoplankton blooms associated with sea ice occur (Springer et al. 1996, Lee et al. 2007, Gradinger 2009, Cooper et al. 2012). Ice melt during the spring permits greater light penetration and increased stratification allowing for nutrients to become trapped in the surface layer for utilization by phytoplankton (Grebmeier 2012). The sea ice dynamics and the variability of the sea ice cover and thickness affects the length and intensity of the phytoplankton production in this region (Arrigo et al. 2008). Pacific waters advected into the area bring nutrients, heat, and organic carbon to the shelf, adding to the organic material already produced there (Grebmeier et al. 2015). Because of reduced zooplankton grazing, this large amount of organic material is deposited to the underlying sediments (Grebmeier et al. 2006b, Nelson et al. 2014) creating concentrations of regionally high biomass in the benthos, which have been termed hotspots (Grebmeier et al. 2006a, Grebmeier et al. 2015). The location of these hotspots coincides with high levels of chlorophyll-a in the water column (e.g. Lee et al. 2007, Brown et al. 2011, Cooper et al. 2012, 2013). These high benthic biomass sites, provide higher trophic animals, such as walrus, gray whales, and diving sea ducks with productive foraging grounds (Lovvorn et al. 2009, Jay et al. 2012, Moore et al. 2014).

1.3 Description of Study Area

The primary focus region in this study was the benthic hotspot south of St. Lawrence Island (SLI; Grebmeier and Cooper, 2016) (Figure 3.1). I also compare abundance data from south of SLI with a station in the SE Chukchi Sea (UTN2) (Figure 3.1). Both of these regions are identified as benthic hotspots and are part of the DBO program that monitors latitudinal changes in benthic communities (Grebmeier et al. 2010, 2015, Grebmeier 2012). The benthic hotspot in the northern Bering Sea that was studied south of SLI, is influenced by a reoccurring polynya, which is affected mainly by wind direction and speed (Cooper et al. 2002), key parameters also important for ecological processes during the spring and summer open water period (Cooper et al. 2012, 2013). Some of the highest benthic biomass in the Pacific Arctic lies beneath this seasonal polynya (Grebmeier and Cooper 1995). This region has very cold bottom water throughout most of the year (less than -1°C) that restricts benthic fish and epibenthic predators from the south, helping to maintain the high infaunal biomass (Grebmeier 2012). The sediments here are comprised mainly of fine-grained silt and clay (~71-73%) with high total organic carbon (TOC) concentrations due to low current speeds (Grebmeier et al. 2015). Nutrient concentrations in the bottom water are often high as well due to the proximity to nutrient-rich upwelled Anadyr waters. The seasonal ice dynamics in this area have been relatively stable over the last several years, and unlike other portions of the Arctic, numbers of days with ice cover annually have not significantly declined (Grebmeier et al. 2015) (Figure 3.2).

The dominant benthic organisms in this particular hotspot are bivalves and polychaetes that provide food for spectacled eiders, bearded seals, and walruses (Lovvorn et al. 2014, Moore et al. 2014, Jay et al. 2014). Feeding by these benthiovores, particularly walrus and spectacled eiders, generally occurs during the winter and early spring, while there is still sea ice present in the northern Bering Sea (Grebmeier et al. 2015). Walrus use the area south of the island for both feeding, reproduction, and resting in the winter (Jay et al. 2012, 2014). The persistence of this hotspot is likely due to early primary production that settles because of slower currents into consistently cold bottom waters prior to significant zooplankton grazing (Grebmeier and Barry 2007, Cooper et al. 2012, 2013).

Station UTN 2 lies within the southeast Chukchi Sea hotspot (SECS) in the DBO3 region (Figure 3.1). Water advected northward into this region comes from three sources: salty, cold Anadyr water in the west, warmer and fresher Alaska Coastal Water in the east, and an intermediate Bering Sea water that is a mixture of the two (Spall 2007). The sediments of this region are very similar to that of the northern Bering Sea, with ~71-73% of the sediment comprised of silt and clay, also with a high TOC content (Grebmeier et al. 2015). While similar in sediment composition and TOC concentration, the average bottom water temperature here in the SECS is much warmer in summer (2.2 °C from July-September) due to variable stratification and stronger mixing (Grebmeier et al. 2015). Sea ice persistence in this hotspot has changed in the last several decades (Frey et al. 2015). Annually, sea ice extent is declining by 9-12 days per decade, break-up of ice is occurring 3-5 days earlier per decade, and re-freezing is happening 7-8 days later per decade (Frey et al. 2015, Grebmeier et al. 2015) (Figure 3.2). This hotspot also is dominated by bivalves, specifically from the families Tellinidae, Nuculanidae, and Nuculidae, as well as polychaetes, and it is also a foraging ground for upper trophic organisms (Grebmeier 2012). High annual primary production and export production to the benthos, mixing of several water masses, including Anadyr water with high nutrients, and total organic carbon content in varying sediment types in the region facilitate the persistence of this hotspot (Grebmeier et al. 2006a, 2015).

I also collected *M. calcarea* (F. Tellinidae) during the 2015 Arctic Marine Biodiversity Observing Network (AMBON) project at a relatively high biomass benthic hotspot site in the northeast Chukchi Sea (NECS, DBO4 region; see http://www.arctic.noaa.gov/dbo/ for locations), and evaluated growth characterizations to compare to *M. calcarea* collected from the SECS site (UTN 2). While silt and clay are the primary sediment type at this location, this region has a lower percentage of the silt and clay content (~52%) within the more heterogeneous sediment type compared to the other two hotspots further south (Grebmeier et al. 2015). Bottom temperatures here lie between the means for the northern Bering Sea and the SECS, averaging -0.7 °C from May to September (Grebmeier et al. 2015). Annual sea ice changes are most prominent at this site, although trends are similar to the SECS. Persistence of sea ice decreased by approximately 30 days per decade and freeze up occurred about 15 days later per decade in the last three decades (Frey et al. 2015, Grebmeier et al. 2015) (Figure 3.2). The offshore stations in this hotspot are dominated by bivalves, polychaetes, and sipunculids, while at the inshore stations amphipods dominate (Grebmeier et al. 2015). As is the case in the SECS, walruses use this area as a foraging ground, and are present during the summer months (Jay et al. 2012). Key environmental drivers for this benthic hotspot include variable water column production, sediment carbon content, and hydrographic steering of cold bottom waters by a complex bottom topography (Grebmeier et al. 2015, Blanchard and Feder 2014).

1.4 Declines in Bivalve Abundance and Subsequent Consequences

Decreases in benthic biomass, changes to predation patterns, and shifts northward of the benthic hotspots are all changes that could occur because of diminishing sea ice and warming seawater (Grebmeier 2012, Grebmeier et al. 2015, Lovvorn et al. 2009, 2014). Declines in overall benthic biomass have already been observed, with the largest declines in the region south of SLI (Grebmeier 2012). Historically, the region to the southwest of SLI has had high biomass of three families of bivalves: *Macoma calcarea* (Tellinidae), *Ennucula tenuis* (Nuclidae), and *Nuculana radiata* (*Nuculanidae*) (Grebmeier and Cooper 1995, 2016, Grebmeier et al. 2006a, 2015, Grebmeier 2012). Data from the five time-series sites (SLIP 1-5) that make up the core DBO1 (Figure 3.1) stations have shown long term declines in benthic communities, particularly in the bivalve *N. radiata* (Figure 3.3) (Grebmeier et al. 2006b, Grebmeier 2012), a once common prey item for the spectacled eider (Lovvorn et al. 2003). Although populations of *N. radiata* are decreasing (Figure 3.3), *E. tenuis* is increasing in abundance, and this species is not utilized efficiently by diving seaducks (Lovvorn et al. 2003, 2014, Grebmeier 2012). The location of seasonal sea ice plays a large role in whether spectacled eiders can access optimal foraging locations (Lovvorn et al. 2009, 2014), and with the recent variability in sea ice, the decreases in clam populations have coincided with declines in spectacled eiders (Lovvorn et al. 2009, Grebmeier 2012).

1.5 *Macoma calcarea*

I focused in this study on the third commonly found bivalve south of SLI, the tellinid *Macoma calcarea*, which is dominant in the SE Chukchi Sea (UTN2). I chose this bivalve to take advantage of its broad distribution across a latitudinal range from the northern Bering Sea to the NE Chukchi Sea. On average the length of *M. calcarea* shells ranges from 35-50 mm (Gofas 2004c). It is both a deposit and suspension feeder that lives in muddy-to-sand/gravel sediments, and it has free swimming pelagic larvae.

1.6 Objectives of Study

Observations have already been made about the decline of *N. radiata* and the recent increases in biomass of *E. tenuis* (Grebmeier 2012, Figure 3.3). Here I document some of the same changes in another metric, abundance, and size of *M. calcarea* from the years 1998-2012. In the 1950s and early 1960s, *M. calcarea* was the dominant species in the region, but *N. radiata* replaced it in the late 1980s (Sirenko and Koltun 1992). Around the same time, in the mid-1980s, studies started documenting declines in overall bivalve biomass, as well as a decrease in the average size of the bivalves (Grebmeier and Dunton 2000). In a study conducted by Lovvorn et al. (2003), spectacled eiders were found to prey predominantly upon *N. radiata* in the 18-24 mm size class. Despite the fact that both *E. tenuis* and *M. calcarea* of similar size provide more energy than *N. radiata* (44% and 62%, respectively, Lovvorn et al. 2003), each has characteristics that likely make it less ideal prey for the spectacled eider. *E. tenuis* has a thicker shell and is believed to take more energy to digest in the gizzard, whereas *M. calcarea* live in deeper sediments (generally >10 cm deep) and are therefore harder for the ducks to obtain (Lovvorn et al. 2003).

It is unknown if eiders can use *E. tenuis* as a viable food source (Lovvorn et al. 2003, 2014, Grebmeier 2012), so the abundance of *M. calcarea* and changes in its dominant size classes could inform whether that species can serve as a practical primary food source for spectacled eiders, and other apex predators. Using the time series data from 1998-2012 at the SLIP and UTN 2 stations, I sought to quantify the current state and recent trends in abundance and dominant size classes of *M. calcarea* in the Pacific Arctic. The ultimate goal is to put these trends into perspective with the larger context of physical changes, such as shifting ice dynamics and ocean acidification presented in Chapter 2, as well as biological consequences including changing prey bases for many predators.

1.7 Statement of Hypothesis

3.1 I frame these studies of abundance and size of *M. calcarea* into hypotheses to test for changes over the past fifteen years at the six study sites in the northern Bering Sea and the southeastern Chukchi Sea.

> *Hypothesis 3.1*: There has been no significant change in abundance or size of *Macoma calcarea* in the last fifteen years at the six study sites.

3.2 Does total abundance and abundance of different size classes differ among the six sites (SLIP 1-5, UTN 2) throughout the fifteen-year time series? If yes, how?

> *Hypothesis 3.2*: There are no differences in the abundance and dominant size class among the six sites.

3.3 Do allometric growth equations of recently collected bivalves differ between sampling sites?

Hypothesis 3.3 The equations and growth style will differ at the two different sampling sites.

2. Materials and Methods

2.1 Sample Collection

Bivalves for this study were collected at six different sites, one in the SECS (station UTN 2, 67.050 N, 168.728 W), and five in the northern Bering Sea (station SLIP 1-5) (Table 3.1, Figure 3.1). Collections for growth characterizations and abundance analysis were made aboard the Canadian Coast Guard Ship (CGGS) *Sir Wilfrid Laurier* (SWL) in 2014 (n=84) and 2015 (n=284) as part of the interdisciplinary Distributed Biological Observatory (DBO) effort that undertakes repeated observations at biologically productive locations in the Pacific Arctic. The bivalve growth data collected from UTN 2 in 2014 and 2015 as part of the DBO project were also used for comparison to the bivalve growth data collected from a NE Chukchi Sea site (ML 5-10, 71.603 N, 162.202 W, Figure 3.1) that was sampled as part of the Arctic Marine Biodiversity Observing Network (AMBON) project in 2015. Bivalve abundance from both these recent cruises and additional data sets from previous cruises over the past fourteen year years were included in the analysis. Data from five SLIP stations (DBO1) and the UTN 2 station (DBO2) were collected during summer cruises (HX214, HX224, HLY0601 and SWL 2000-2015) between 1998-2012 using a 0.1 m^2 van Veen grab with 32 kg weight on the grab. Bivalve specimens collected in 2014 and 2015 were later used for allometric growth determinations, but these were not determined for clams collected prior to 2014. The clams collected in 2014 and 2015 were drawn from specimens that were used in live laboratory ocean acidification experiments (see Chapter 2).

2.2 Abundance and Size Class Classifications

Abundance data for all years (1998-2012) were calculated by first counting the number of individuals in each grab. Four grabs were collected from each site and faunal abundance counted. The count from each grab was multiplied by 2.5 and then those four values were added together in order to scale up to a per meter squared basis (number/m^2) for each site in each year. Sampled clams at each site were also separated into a size distribution representing the five dominant size classes seen among all the cruises (0-1 cm, 1.1-2 cm, 2.1-3 cm, 3.1-4 cm, and 4.1-5 cm). However some larger individuals of several different size classes (>5 cm) (n=212) were observed only during the HLY0601 cruise (2006), 197 of these were at SLIP 5 and SLIP 4, but these data are not included here because they were only found on one cruise. No other cruise reported individuals larger than 5 cm. The retrospective (pre-2014) length data were generated using a scaled ruler measured to the nearest mm, and preserved samples were weighed on a calibrated digital scale (see methods in Lovvorn et al. 2003). By comparison, bivalves collected in 2014 and 2015 were measured for length with a set of Capri 15 cm stainless steel digital calipers that measures to the nearest hundredth of a mm [maximum distance along the anterior-posterior axis as described by Gaspar et al. (2001)] (Figure 3.4). Later these values were converted to the nearest mm to match the retrospective data.

2.3 Length Weight Relationships and Growth Characterizations

I measured length and wet weight of individuals in order to determine growth patterns exhibited by *M. calcarea* collected in recent years (2014 and 2015) in the SECS versus the NECS. For wet weight measurements, each live clam was patted dry with a paper towel and then weighed to the nearest hundredth of a gram. Length/weight relationships were described first using equation (*Eq. 1*) and then expressed in a linear form using equation (*Eq. 2*), where a=intercept and b=slope.

$$W = aL^b \qquad (Eq.\ 1)$$

$$Log\, W = log\, a + b\, log\, L \qquad (Eq.\ 2)$$

An isometric relationship between length and weight was defined by a slope (b) equal to 3, considered a critical threshold based on the relationship between length and weight (Gaspar et al. 2001). Slopes less than three were classified as negatively allometric and slopes greater than three were considered to be positively allometric.

2.4 Statistical Analyses

All statistical analyses were undertaken using RStudio® (https://www.rstudio.com/, R version 3.2.3) statistical software. Sections 2.4a, 2.4b, and 2.4c describe analyses run on the retrospective data (1998-2012), and section 2.4d describes analyses run on the recently collected clams (2014, 2015).

2.4a Kruskal-Wallis Abundance and Size Class Comparisons

Total abundance for all fourteen years (1998-2012) was compared using the Kruskal-Wallis rank sum test. Similar analyses for abundance of dominant size class were also undertaken. Abundance of dominant size class was compared from 1999-2006, as size data was not readily available from 1998 and 2007-2012, using the Kruskal-Wallis rank sum test or a one-way ANOVA. Follow up tests included Dunn's Test of Multiple Comparisons Using Rank Sums for the Kruskal-Wallis rank sum test and Tukey’s test was used for ANOVA. Using the same tests, I determined which size class, if any, was most abundant at each site.

2.4b Time-series Clustering

For this clustering analysis, I standardized the data from the six sites and then used the CSlideCluster function from the ‘funtimes’ package (Lyubchich et al. 2016) in RStudio®. This function conducts clustering of time series data at a slide level based on algorithms from Ciampi et al. (2010) and is used to determine if the values at each site behave in the same way. If they are clustered separately then they behave differently from one another, and if they cluster together they behave similarly.

2.4c Dynamic Factor Analysis

Dynamic factor analysis (DFA) is a tool used to identify common patterns within a group of time series relationships. Here I use the ‘MARSS’ package (Holmes et al. 2013) in RStudio® to look for common patterns in *M. calcarea* abundance from 1998-2012 at the six sites previously described. The data for this analysis was scaled to z-scores.

2.4d Allometric Growth

Allometric growth relationships were determined using linear regressions of the log transformed length and wet weight data in order to calculate a slope and a 95% confidence interval (CI) of the slope for each site (UTN 2, ML5-10). If a slope value (b) of three fell between the upper and lower bound of the 95% confidence interval, the clam was classified as having isometric growth (b=3, as explained in section 2.5). If the confidence interval bounds for the slope fell below three (e.g. 2.4-2.9), the growth was classified as negatively allometric; and if the confidence interval bounds fell above three (e.g. 3.1-3.4), the growth was classified as positively allometric.

3. Results

3.1 Abundance: Kruskal-Wallis

Using the Kruskal Wallis test, I compared the total mean abundance over the fourteen years at each site (Figure 3.5). Results were significantly different ($p < 0.05$), leading to use of the Dunn's Test of Multiple Comparisons Using Rank Sums to make additional comparisons. This analysis showed several significant differences in abundance among sites, primarily between the more northern sites in both the northern Bering and SE Chukchi Sea and the more southern sites in the SLIP region. The three southern-most stations, SLIP 1, 2, and 3, were not significantly different from one another and the more northern sites, SLIP 4 and 5 and UTN 2, were not significantly different from one another. However, all individual southern sites (SLIP 1, 2, 3) were significantly different from all individual northern sites (SLIP 4, 5, UTN 2) (Table 3.2, Figure 3.6).

3.2 Dominant Size Class

The abundance of the three smallest size classes (0-1 cm, 1.1-2 cm, and 2.1-3 cm) among the six stations varied significantly (Kruskal-Wallis, $p = 0.004$; one way ANOVA, $p <0.01$; one way ANOVA, $p <0.01$, respectively, Table 3.3; Figure 3.7, Figure 3.8, Figure 3.9). However, the two larger size classes (3.1-4 cm and 4.1-5 cm) showed no significant difference in abundance among the stations (Kruskal-Wallis, $p = 0.09$; and Kruskal-Wallis, $p = 0.52$, Figure 3.10, Figure 3.11).

Following the Kruskal-Wallis analysis, I used Dunn's Test of Multiple Comparisons Using Rank Sums to determine which stations were significantly different from one another. Significant differences are reported in Table 3.4. SLIP 4 and 5 typically had the highest abundance of all of the size classes and differed from SLIP 1-3, which is the same pattern seen in overall abundance in the larger data set (1998-2012). While abundance of the 3.1-4 cm size class was not statistically different among sites ($p = 0.09$), when the Dunn’s test was run results indicated that abundance of this size class at the three southern stations (SLIP 1, 2, 3) were significantly different from the abundance at the two northern stations (SLIP 4, 5) ($p = 0.02$ for all three southern stations when compared to SLIP 4 and $p= 0.04$ for all three southern stations when compared to SLIP 5). The higher fractions of smaller size classes documented at SLIP 4 and 5 indicate a recruitment event that coincides with the high abundance and biomass observed in the northern sites.

Significant differences existed among many of the size classes within each site (Table 3.3, Figure 3.12). Overall, the two smallest size classes were generally the most abundant. The 2.1-3 cm size class was also more abundant than the two larger size classes at the majority of the sites. Comparisons of size class within each site are tabulated in Table 3.4.

3.3 Time-series Clustering Analysis: Abundance and Size Classes

Cluster analysis indicated that the total abundance of bivalves at SLIP 1, 2, 3, 4, and UTN 2 were similar and distinct from the total abundance at SLIP 5, which clustered individually (Figure 3.13). This analysis does not test for significant differences, and only describes how abundances at different stations are behaving; therefore, the fact that UTN 2 and SLIP 1-3 behave similarly does not mean that behavior occurs at the same rate or in the same pattern.

Cluster analysis was also applied to the abundance of each of the five size classes at all of the stations. This analysis indicated that the total abundance of the 0-1 and 2.1-3 cm size class at all of the stations clustered together. The total abundance of the 1.1-2 cm size class at SLIP 1 and SLIP 3 clustered individually from the other stations. Abundance of the 3.1-4 cm size class at SLIP 1 and SLIP 2 clustered together, while the abundance of this size class at all of the other stations clustered separately from each other (Figure 3.14). Abundance of the 4.1-5 cm size class clustered together at SLIP 1, 3, and 4, while the two remaining stations (SLIP 2 and SLIP 5) clustered separately from one another.

3.4 Dynamic Factor Analysis: Abundance

Several models of total abundance from each site were tested with varying numbers of trends, the best model, chosen by lowest Akaike information criterion (AIC) value which gives a measure of the quality of the models produced, displayed two trends in the abundance data (Figure 3.15). Based on loadings, SLIP 1, 2, 3, and 4 clustered and were consistent with one trend, UTN 2 clustered separately and consistently with a second trend, and SLIP 5 was influenced by both trends (Figure 3.16). Both trend one and two show an increase in the early years of the time-series; however, around 2006/2007, the first trend decreases and then increases again around 2010, while the second trend continues to steadily rise through 2012.

This analysis, therefore, in conjunction with the time-series clustering approach, supports that SLIP 5 is the transition zone between the southern and northern sites, as it is the sole station that clusters away from the other stations in the time-series clustering and is the only site influenced by both trends in the DFA.

3.5 Bivalve Allometric Growth of Recent Field Collections

Allometric growth relations were determined for *M. calcarea* from UTN 2 using live clams collected in 2014 and 2015 in the SECS, and compared to equations for live *M. calcarea* collected from site ML 5-10 in 2015 in the NECS. Both sets of clams (2014 and 2015) collected from the UTN 2 site showed isometric growth, while the clams collected from the ML 5-10 site had negative allometric growth (Table 3.5, Figure 3.17).

4. Discussion

Tracking abundance of organisms through time provides insights on ecosystem change and potential consequences that result from these changes. Abundance data from the current study indicate that abundance of the common bivalve *M. calcarea* has risen in recent years (2006-2012) at sites to the north, while this species has declined at sites further south, supporting a northward shift in the maximum abundance of this species that could help explain in part the reduction in the biomass in the northern Bering Sea sites (Grebmeier and Cooper 2016).

Time-series cluster analyses and DFA were used to try and understand the common patterns and behaviors of the abundance of *M. calcarea* at the studied sites. The time-series clustering (detailed in Sections 2.4b and 3.3) clusters stations together based on the behavior of the abundance values at the individual stations, but does not provide information on the specifics of the behavior, i.e. is it increasing, decreasing, etc., or at what rate does this increase or decrease occur over time. The DFA (described in Sections 2.4c and 3.4) provides more information on the specifics of the behavior by describing the trends that influence the data and then clustering locations based on those trends.

Results from both cluster analyses, imply that SLIP 5 is the transition point for *M. calcarea* abundance moving from south to north. The time-series clustering indicated that SLIP 5 behaved differently from the other five stations, suggesting that it might be the site at which patterns in abundance begin to change. However, because SLIP 1-3 (southern sites) and SLIP 4 and UTN 2 (northern sites) all clustered together, these results could also indicate that the behavior of abundance at SLIP 5 is simply an outlier from the rest of the stations. Therefore, I proceeded with the DFA to examine if there were differences within the similar behaviors at SLIP 1-4 and UTN 2. The best-fit model produced by the DFA showed two underlying trends that influenced and described how abundance at these sites was behaving. Stations SLIP 1-4 were influenced by the first trend, UTN 2 clustered alone under the second trend, and the abundance at SLIP 5 was influenced by both of the trends (Figure 3.15). Both trends produced by the DFA show increases in abundance, a similar overall behavior, which helps explain why most of the stations clustered together in the time-series method. While both trends increase, the increasing nature of the trends differs from one another. Trend one starts with a pattern of increasing abundance from 1998 to about 2006 where it begins to decrease until about 2010, at which point it begins to increase again. Trend 2, decreases until about 2003 where it begins to steadily increase until 2012. The steadily increasing pattern in trend 2 continues to support the northward shift in distribution of *M. calcarea*, because only the northern most station (UTN 2) was described strictly by the continuous increasing of trend 2. These differences between the trends suggest that although SLIP 1-4 and UTN 2 clustered together in the time-series clustering, the details of that behavior are different as they clustered under different trends in the DFA. SLIP 5 was the only station to be influenced by both trends, further indicating it is likely a transition station for abundance of *M. calcarea*.

It might be expected that SLIP 4 would be the transition zone between the Bering Sea and the Chukchi Sea as it is the northern most station studied in the Bering Sea. Abundance values at SLIP 4 are higher than they are at the more southern SLIP sites (Figure 3.6), which supports a northward shift; however, the abundance values there are still not as high as they are at UTN 2 (Figure 3.6). UTN 2 was influenced by trend 2, which displays a steady continuing increase in abundance, whereas SLIP 4 was described by trend 1, so in comparison abundance is likely increasing faster at UTN 2 than SLIP 4, potentially showing differences between the northern Bering and southern Chukchi Seas. Adding years to the time-series data set, as well as more stations in the southern Chukchi Sea, would provide information as to if the increasing and decreasing pattern of trend 1 continues through time, and if other sites in the Chukchi Sea are influenced by trend 2, or if a third pattern of changing abundance emerges, i.e. a third trend. This extra information could strengthen the argument that SLIP 5 is the current transition site.

Over the last 25 years, there have been other changes to the area, including both increasing air and water temperatures and sea ice loss (Grebmeier 2012, Figure 3.18, Figure 3.2). As temperatures have risen, some sub-Arctic species have been able to extend their range northward, and are likely competing with Arctic species for resources (Grebmeier et al. 2006a, Mueter and Litzow 2008). Mueter and Litzow (2008) hypothesized a northward migration of subarctic and arctic species due primarily to retreat of sea ice that controls the cold pool in the SLIP region. Their time series study concluded that bottom temperature played the largest role and that warmer temperatures in the Bering Sea are the probable cause of the distribution shifts northward of various fish and epibenthic species (Mueter and Litzow 2008). Additionally, their results show a ~230 km northward retreat of the cold pool since the 1980s (Mueter and Litzow 2008) (Figure 3.19). In addition to sub-Arctic species moving in to the northern Bering Sea, there has been a shift from a dominance of bivalves to polychaetes starting around 2009 at the SLIP locations (Grebmeier and Cooper 2016). Changes to sediment grain size, an indicator of current speed, have been occurring in this region with a switch to finer sediments (~71-73% silt and clay), which favors polychaetes (Grebmeier et al. 2015, Grebmeier and Cooper 2016). DFA has the capability to identify explanatory variables and define the relationships and patterns between the time series and the variables. While it is beyond the scope of this study, the changes in air and seawater temperatures, sea ice extent, the extent of the cold pool, and benthic macrofaunal community composition could be added to the DFA to see if these results are primarily affected by bottom temperatures and extent of the cold pool as observed by Mueter and Litzow (2008).

It has been hypothesized that benthic hotspots that persist in colder temperatures could be more sensitive to future warming that would continue to allow sub-Arctic species to expand northward (Grebmeier et al. 2015, Figure 3.18). While this is likely the case for the biological hotspot at the southerly SLIP sites, where bottom water throughout the summer currently averages -1.6°C, UTN 2, is not influenced by persistent cold pool waters through the summer, so susceptibility to changing bottom temperatures and subsequent northward shifts may not be as prevalent at this site. The pre-existing warmer waters in the Chukchi versus the warming of very cold waters, and contraction of the cold pool in the Bering Sea, may help explain why the distributions of bivalve populations are shifting northward away from the SLIP sites towards sites like UTN 2, although sites like UTN 2 are not impervious to invasive species.

In addition to observed declines in biomass (Grebmeier et al. 2006a, Grebmeier 2012) and changes in abundance documented in this study, there have been changes in species composition at the sites (Grebmeier 2012). Beginning in the 1980s, *Nuculana radiata* became the prominent species in this region (Sirenko and Koltun 1992), although recently numbers have started to decline (Grebmeier 2012). Spectacled eiders feed primarily on *N. radiata* in the 18-24 mm length range (Lovvorn et al. 2003). The two most abundant size classes of *M. calcarea* found at these sites were the 0-1 cm and 1.1-2 cm, which encompasses the 18-24 mm size classes (1.8-2.4 cm). However, while *M. calcarea* in this size range is abundant and provides 62% more energy than *N. radiata*, it is still unknown if it is a viable prey item because *M. calcarea* live deeper in the sediment (up to 10 cm deep), making this species more difficult for eiders to obtain during feeding (Lovvorn et al. 2003).

The dominance of smaller individuals of *M. calcarea* at these sites could be a product of annual variation in spawning times. This species is a broadcast spawner, producing copious numbers of larvae that produce small first year class bivalves. A similar decline in size at these sites appears to have continued from 2012-2015 (Caradine-Taber 2016), suggesting that the declining bivalve size over the past decade is more likely a response to environmental changes and ensuing ecosystem re-structuring, and not simply a result of spawning time variations.

In order to continue characterizing growth features of communities with *M. calcarea* in the Pacific Arctic, I also examined allometric growth relationships for clams collected in 2014 and 2015 from two different sites, UTN 2 in the southeast Chukchi Sea and ML 5-10 in the northeast Chukchi Sea. The slope of the linearized allometric relationship between length and weight varies for species, and can also fluctuate among different environmental conditions a given species may be exposed to throughout the year. Therefore, the relationship should be determined empirically for each species and system of interest, and not universally applied (Glazier 2005, Seibel 2007), which is why I determined relationships from both of these sites. Organisms collected at UTN 2 had isometric growth in both 2014 and 2015, meaning length and weight increased similarly in magnitude, while those collected at ML 5-10 in 2015 had negative allometric growth, meaning that length increased faster than weight.

Water advected in the area of UTN 2 comes from three sources: salty, cold, nutrient-rich Anadyr water, warmer and fresher Alaska Coastal Water, and an intermediate Bering Sea water that is a mixture of the two (Spall 2007), whereas the water masses in the ML 5-10 area consist of the Alaska Coastal Water and the Bering Shelf Water (Figure 3.20). Sediments in the UTN 2 benthic hotspot are ~71-73% silt and clay, with a high TOC content (Grebmeier et al. 2015), while in the hotspot area of ML 5-10, silt and clay are still the primary sediment type, but it makes up a lower percentage (~52%) in the more heterogeneous sediment types. Bottom water temperatures at the southeast site average 2.2 °C in the summer months. The northeast site has average bottom water temperatures of -0.7 °C (Grebmeier et al. 2015). Annually sea ice extent at the southern site is declining by 9-12 days per decade, break-up of ice is occurring 3-5 days earlier per decade, and re-freezing is happening 7-8 days later per decade (Grebmeier et al. 2015). Annual sea ice changes in the northern hotspot area are the most prominent. Persistence of sea ice decreased by approximately 30 days per decade and freeze up occurred about 15 days later per decade (Grebmeier et al. 2015). Because, the relationship can fluctuate among different environmental conditions the above differences in the two areas could explain the different growth patterns in the same bivalve species, *M. calcarea*. It would be helpful to also calculate this relationship for individuals collected at the SLIP stations to increase spatial sampling and to compare these sites in the Chukchi Sea to help evaluate the drivers for different growth patterns (detailed in Sections 1.2 and 1.3).

Shifts in distribution of abundance, documentation of dominant size class, and tracking allometric growth relationships, with the addition of biomass information, will allow future work to tease out if these observed changes are due to environmental changes or a product of natural variability within a population. Additionally this work examined only one species, so applying analyses such as these to an increased number of species, as Mueter and Litzow (2008) did, will provide more information about community changes. Overall, my study suggests three conclusions: 1) recruitment events of *M. calcarea* have been more prominent to the north, 2) smaller individuals are dominant, and 3) growth relationships differ in different regions of the Chukchi Sea. While any specific site is likely to have fewer large individuals because of increased mortality with age due to predation, size class distributions and year classes may not necessarily align with expectations. For example, a clam that is four years old could have been exposed to acidified waters for three out of those four years. If that individual clam has been exposed to acidified waters (as explored in Chapter 2), it may not be able to maintain the growth rate it could have in non-acidified water. Therefore this clam could be smaller because of the acidified waters, a changing environmental parameter, not because it is a young individual. One of the conclusions from Chapter 2 is that *M. calcarea* held in acidified treatments had a much lower slope in allometric growth than those held under more neutral pH (ambient) conditions. So while the differences in the systems at UTN 2 and ML 5-10 could be due to natural differences in the system, these sites could be affected over time by changes like ocean acidification.

5. Conclusions

This study, using a subset of the data from a 25-year dataset, documented a northward shift of bivalve abundance from 1998-2012 away from a critical feeding habitat of the spectacled eider. However some individual sites in that winter feeding area (SLIP 2 and SLIP 4) are showing increases in bivalve abundance, specifically *M. calcarea* although it is not clear if the clams are available for feeding due to a deeper displacement into the sediments. Additionally, between the years 1999 and 2006 smaller clams were more abundant than larger clams at the stations south of St. Lawrence Island, including the size range the eiders prefer. Future work can evaluate bivalve abundance data for other years not evaluated in this study. It would also be useful to integrate data on temperature and sea ice extent with the ultimate goal of developing a predictive model to understand how these factors contribute to the changing benthic ecosystem that is the food supply of many apex predators, which may already be suffering from loss of sea ice and diminishing prey. This study contributes to this effort through identification of the changes in abundance of clams of the preferred size class so that we can better understand how populations of these spectacled eiders may change in the future in response to food supply constraints.

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Tables

Table 3.1 Latitude and longitude of the five St. Lawrence Island Polynya (SLIP=DBO1) stations in the northern Bering Sea.

Station	Latitude (N)	Longitude (W)
SLIP 1	62.011	-175.059
SLIP 2	62.050	-175.209
SLIP 3	62.390	-174.570
SLIP 5	62.560	-173.549
SLIP 4	63.030	-173.460
UTN 2	67.050	-168.728

Table 3.2 Dunn's test results comparing the northern benthic sites (SLIP4,5 and UTN2) with the southern benthic sites (SLIP1-3). The northern sites are highlighted in green and southern sites are highlighted in blue.

Site	p-value
SLIP 1 vs. SLIP 4	**<0.01**
SLIP 2 vs. SLIP 4	**<0.01**
SLIP 3 vs. SLIP 4	**<0.01**
SLIP 1 vs. SLIP 5	**<0.01**
SLIP 2 vs. SLIP 5	**0.01**
SLIP 3 vs. SLIP 5	**0.04**
SLIP 1 vs. UTN 2	**<0.01**
SLIP 2 vs. UTN 2	**<0.01**
SLIP 3 vs. UTN 2	**<0.01**

Table 3.3 Statistical results from the abundance and size class analyses of the bivalves used in the study. Bolded denotes statistically significant results at alpha=0.05

Trait	Year(s)	Source	d.f.	Mean Square	t value or equivalent	p	Test
Size Class	1999-2006	**0-1 cm**	**4**		**15.37**	**<0.01**	**Kruskal Wallis**
		1.1-2 cm	**4**	**313.52**	**24.41**	**<0.01**	**One way ANOVA**
		2.1-3 cm	**4**	**24.916**	**4.578**	**<0.01**	**One way ANOVA**
		3.1-4 cm	4		8.0755	0.09	Kruskal Wallis
		4.1-5 cm	4		3.2161	0.52	Kruskal Wallis
Size Class/Site	1999-2006	**SLIP 1: size class**	**4**		**15.395**	**<0.01**	**Kruskal Wallis**
		SLIP 2: size class	**4**		**16.18**	**<0.01**	**Kruskal Wallis**
		SLIP 3: size class	**4**		**23.646**	**< 0.01**	**Kruskal Wallis**
		SLIP 5: size class	**4**		**29.426**	**< 0.01**	**Kruskal Wallis**
		SLIP 4: size class	**4**		**19.31**	**<0.01**	**Kruskal Wallis**

Table 3.4 Statistically significant results from analyses examining differences in size class among sites and differences among size classes within sites. Sites or size classes that are bolded had a higher abundance of *M. calcarea* then the non-bolded counterpart.

Site	Size Class (cm)	p value
SLIP 1 vs. **SLIP 5**	0-1	<0.01
SLIP 1 vs. **SLIP 4**		0.04
SLIP 2 vs. **SLIP 3**		0.02
SLIP 2 vs. **SLIP 5**		<0.01
SLIP 2 vs. **SLIP 4**		0.01
SLIP 1 vs. **SLIP 4**	1.1-2	0.02
SLIP 1 vs. **SLIP 5**		<0.01
SLIP 2 vs. **SLIP 5**		<0.01
SLIP 3 vs. **SLIP 5**		<0.01
SLIP 4 vs. **SLIP 5**		<0.01
SLIP 1 vs. **SLIP 5**	2.1-3	0.02
SLIP 2 vs. **SLIP 5**		0.02
SLIP 1	**0-1** vs. 3.1-4	<0.01
	0-1 vs. 4.1-5	<0.01
	1.1-2 vs. 3.1-4	0.04
	1.1-2 vs. 4.1-5	0.01
	2.1-3 vs. 4.1-5	0.02
SLIP 2	**0-1** vs. 3.1-4	0.03
	0-1 vs. 4.1-5	0.01
	1.1-2 vs. 3.1-4	<0.01
	1.1-2 vs. 4.1-5	<0.01
	2.1-3 vs. 4.1-5	0.01
SLIP 3	**0-1** vs. 1.1-2	0.06
	0-1 vs. 2.1-3	0.03
	0-1 vs. 3.1-4	<0.01
	1.1-2 vs. 3.1-4	0.01
	1.1-2 vs. 4.1-5	0.02
	2.1-3 vs 3.1-4	0.02
	2.1-3 vs. 4.1-5	0.01
		0.02

SLIP 5	**0-1** vs. 2.1-3	
	0-1 vs. 3.1-4	<0.01
	0-1 vs. 4.1-5	<0.01
	1.1-2 vs. 2.1-3	0.05
	1.1-2 vs. 3.1-4	<0.01
	1.1-2 vs. 4.1-5	<0.01
	2.1-3 vs. 4.1-5	0.01
SLIP 4	**0-1** vs. 3.1-4	<0.01
	0-1 vs. 4.1-5	<0.01
	1.1-2 vs. 3.1-4	<0.01
	1.1-2 vs. 4.1-5	<0.01
	2.1-3 vs. 4.1-5	0.01

Table 3.5 Allometric growth equations and categorizations for *M. calcarea* collected from station UTN 2 in the southern Chukchi Sea and station ML 5-10 in the NE Chukchi Sea in 2014 and 2015.

Species	N	Station/Year	Mean Length ± 1SD	Allometric equation	R^2	95% CI of b	Relationship
Macoma calcarea	34	UTN2/2014	23.56 ± 7.62	$W=-9.26034L^{3.10339}$	0.9875	2.980-3.227	isometric
Macoma calcarea	284	UTN2/2015	21.35 ± 3.74	$W=-8.81181L^{2.96914}$	0.8595	2.829-3.110	isometric
Macoma calcarea	120	ML5-10/2015	20.51 ± 3.47	$W=-8.2283L^{2.8059}$	0.9312	2.667-2.944	(-) allometry

Figures

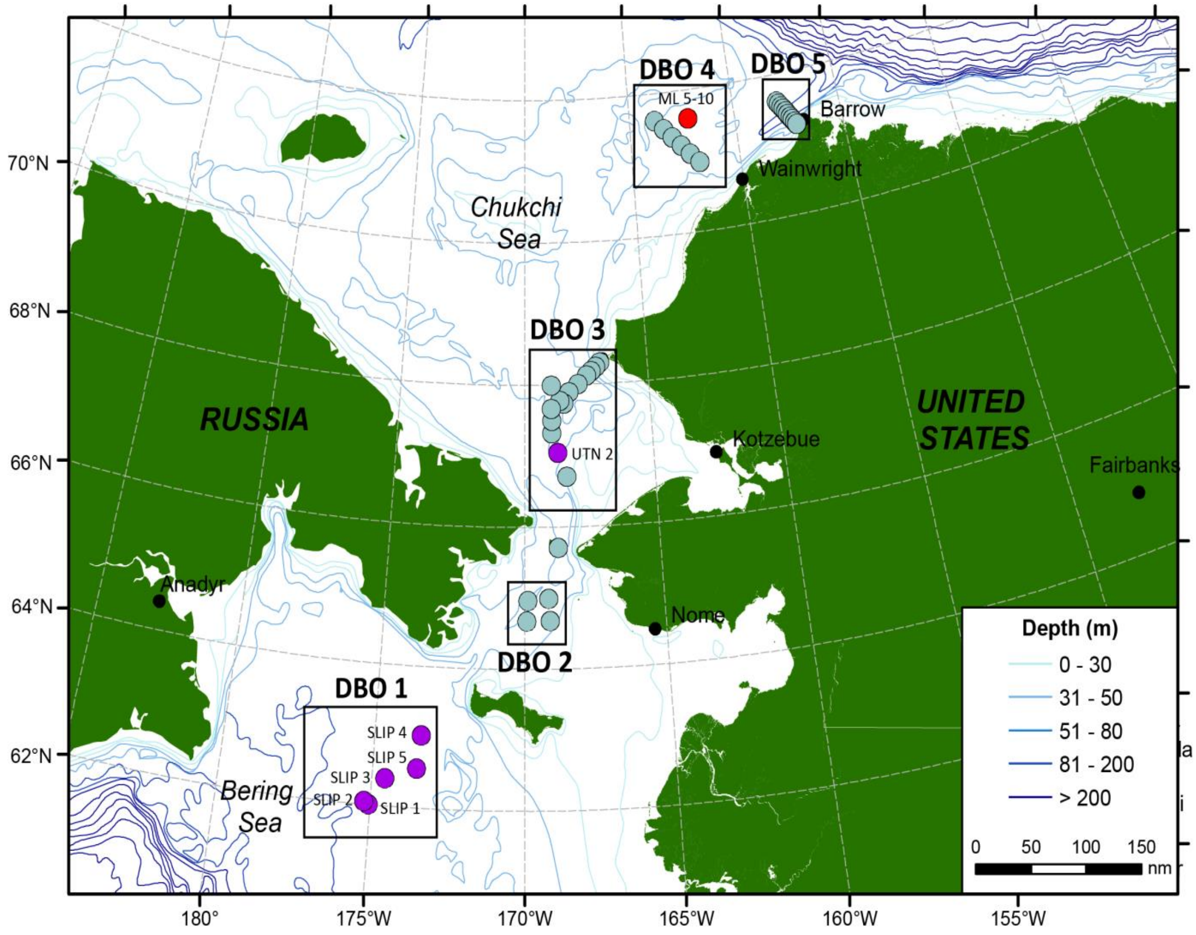


Figure 3.1 Station map of CGCS Sir Wilfrid Laurier (SWL) cruises. Bivalves used in experiments were collected from station SLIP 1-5 (DBO1) in the northern Bering Sea and UTN 2 (DBO2 region) in the southern Chukchi Sea (all highlighted in purple). Allometric growth equations were also determined for bivalves collected at station ML 5-10 in the NE Chukchi Sea in 2015 (highlighted in red). Key: DBO= Distributed Biological Observatory

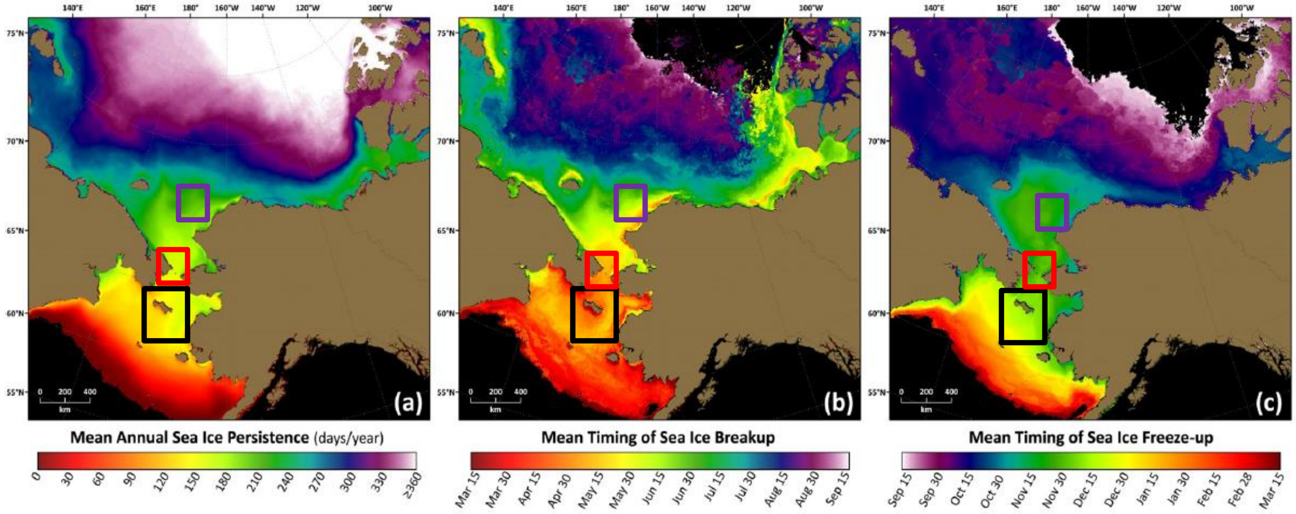


Figure 3.2 Average ice persistence from 2003-2010 (a), average time of sea ice breakup from 2003-2011 (b), and (c) average sea ice freeze up timing from 2002/2003- 2010/2011 for the Pacific Arctic. These values cover about nine years of the fourteen year time series (1998-2012). Black box in each panel denotes the area of the SLIP sites, the red box in each panel shows the approximate location of UTN 2, and the purple box marks the approximate location of ML 5-10. Figure modified from Frey et al. 2015.

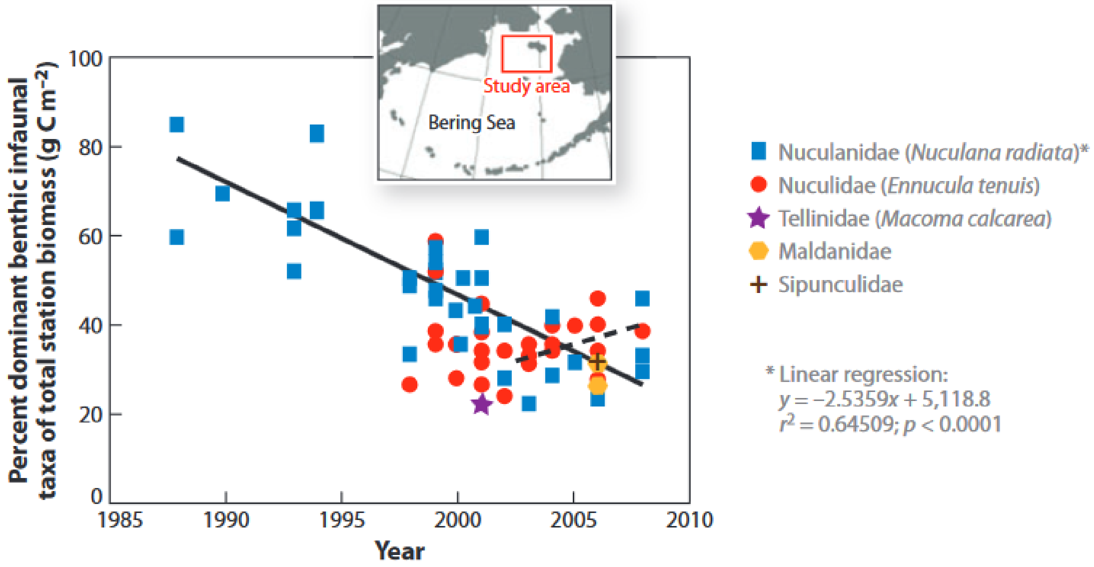


Figure 3.3 The decline of the previously dominant *Nuculana radiata*, preferred prey for spectacled eiders, at the five sites in the northern Bering Sea (SLIP 1-5). Coincidently, there is also an upward trend of *Ennucula tenuis* at these sites, but only one time point for *Macoma calcarea*. Figure from Grebmeier 2012.

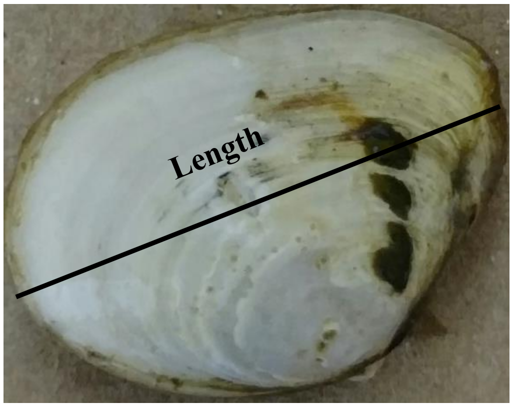


Figure 3.4 Location of length measurement on all bivalve shells for length-weight analysis.

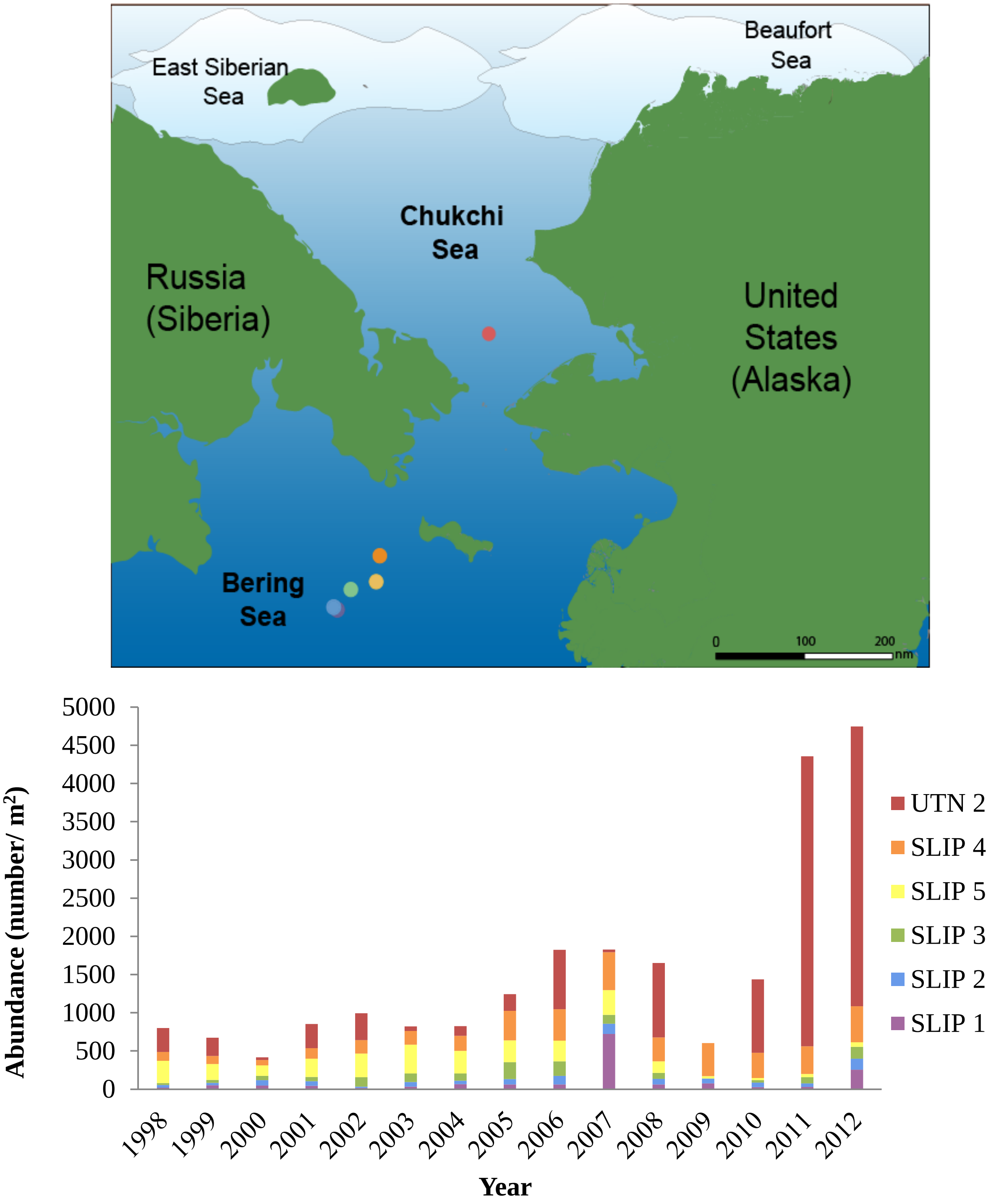


Figure 3.5 Mean abundance of *M. calcarea* for 1998-2012 at the five SLIP stations in the northern Bering Sea (SLIP1-5) and one station in the SE Chukchi Sea (UTN 2).

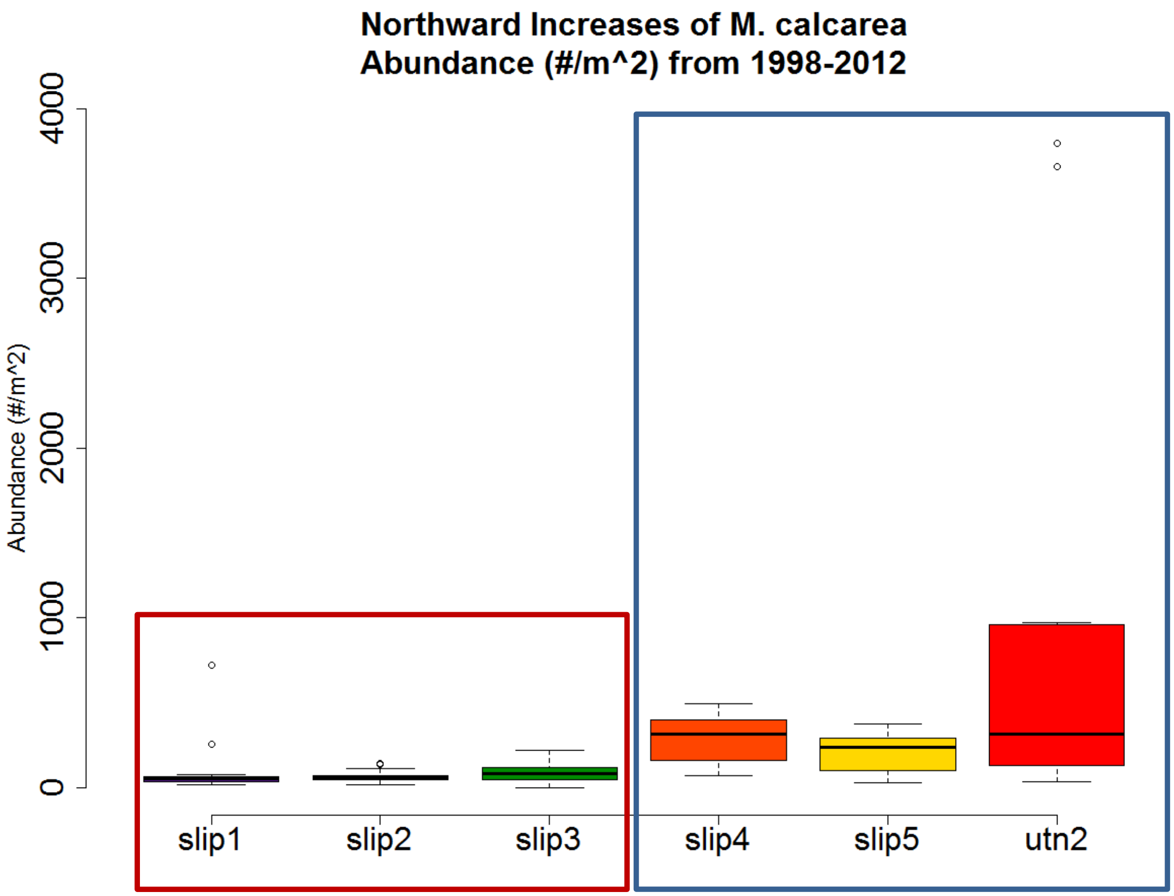


Figure 3.6 Mean abundances within stations sampled showing the significantly higher abundance in the northern sites in both the northern Bering Sea and SE Chukchi Sea. Southern sites in the northern Bering Sea are marked with the red box, and northern sites are marked in the blue box. Note that from south to north the northern stations are SLIP 5 and SLIP4 (northern Bering Sea), and UTN 2 (SE Chukchi Sea).

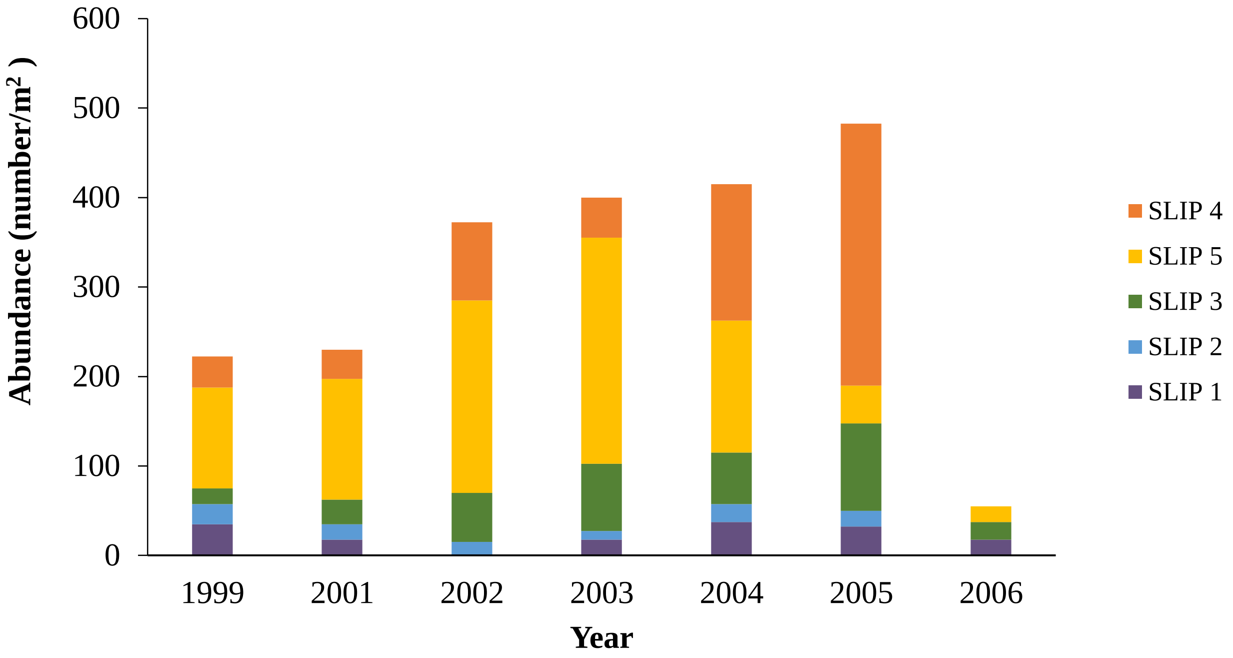


Figure 3.7 Mean abundance of the size class 0-1 cm at the SLIP stations in the northern Bering Sea from 1999-2006.

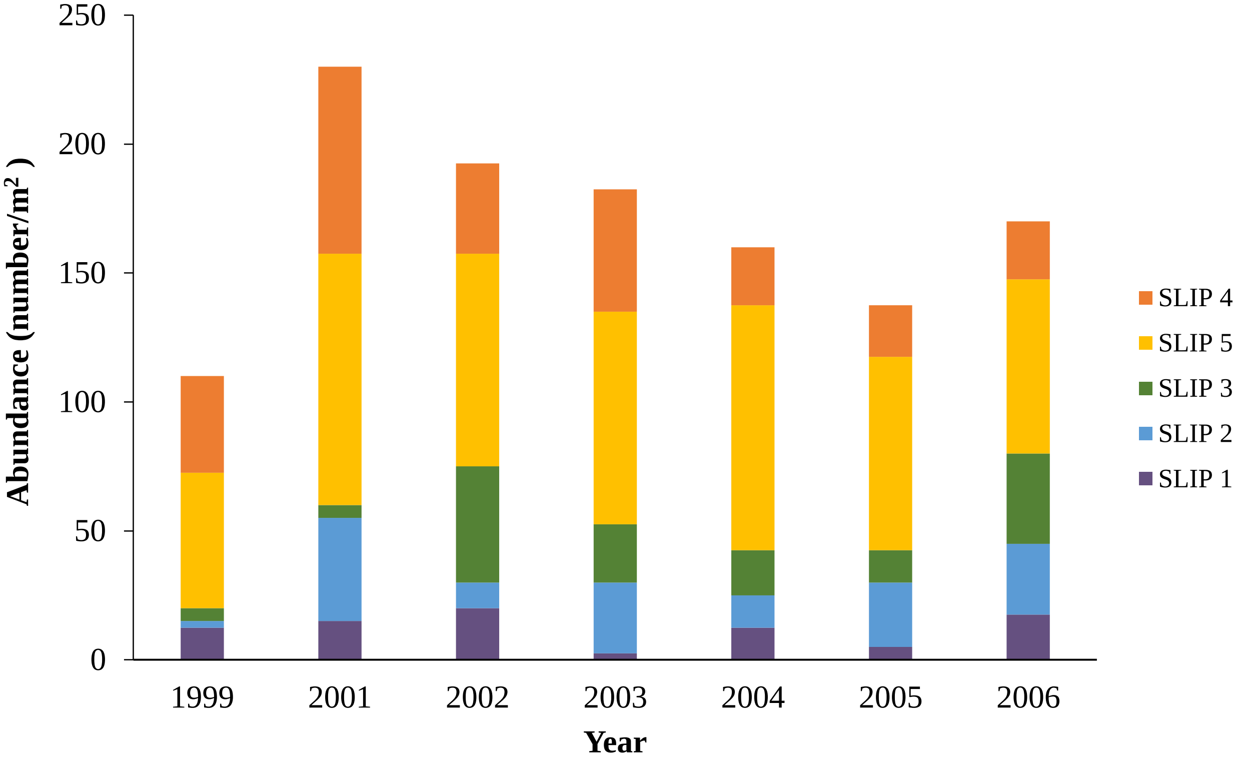


Figure 3.8 Mean abundance of the size class 1.1-2 cm at the SLIP stations in the northern Bering Sea from 1999-2006.

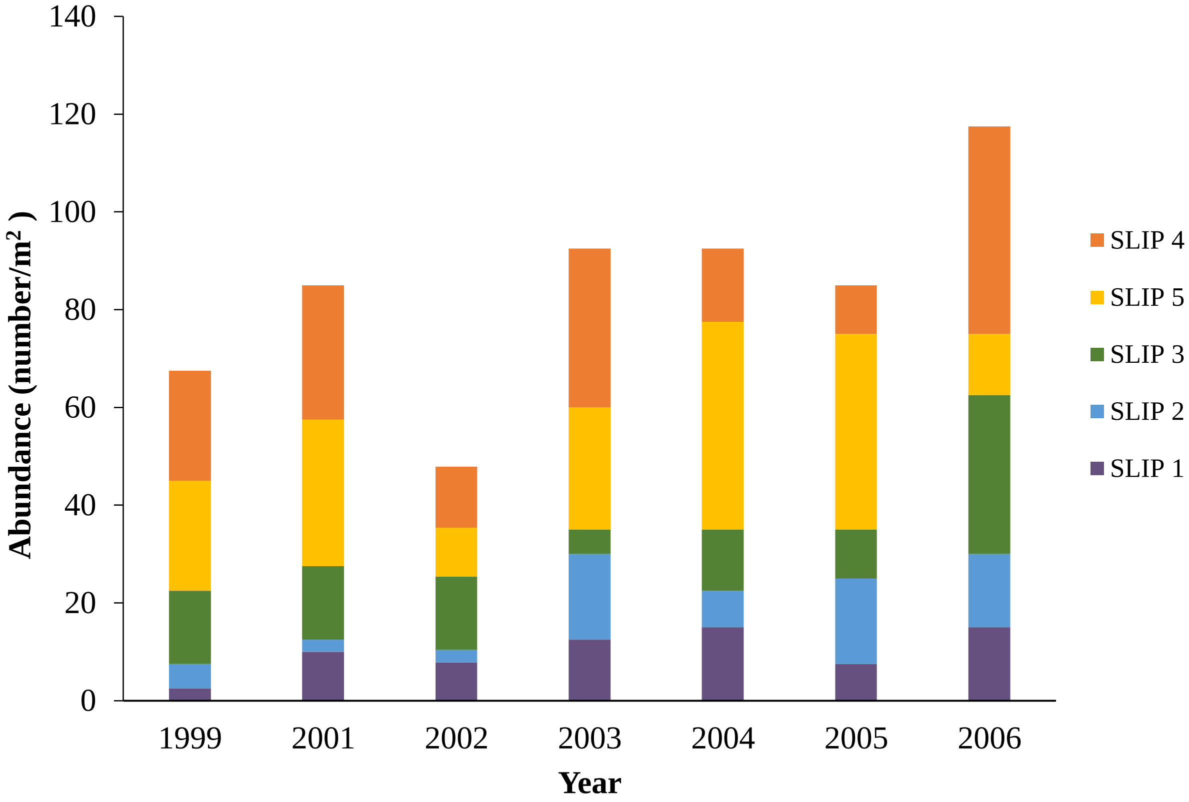


Figure 3.9 Mean abundance of the size class 2.1-3 cm at the SLIP stations in the northern Bering Sea from 1999-2006.

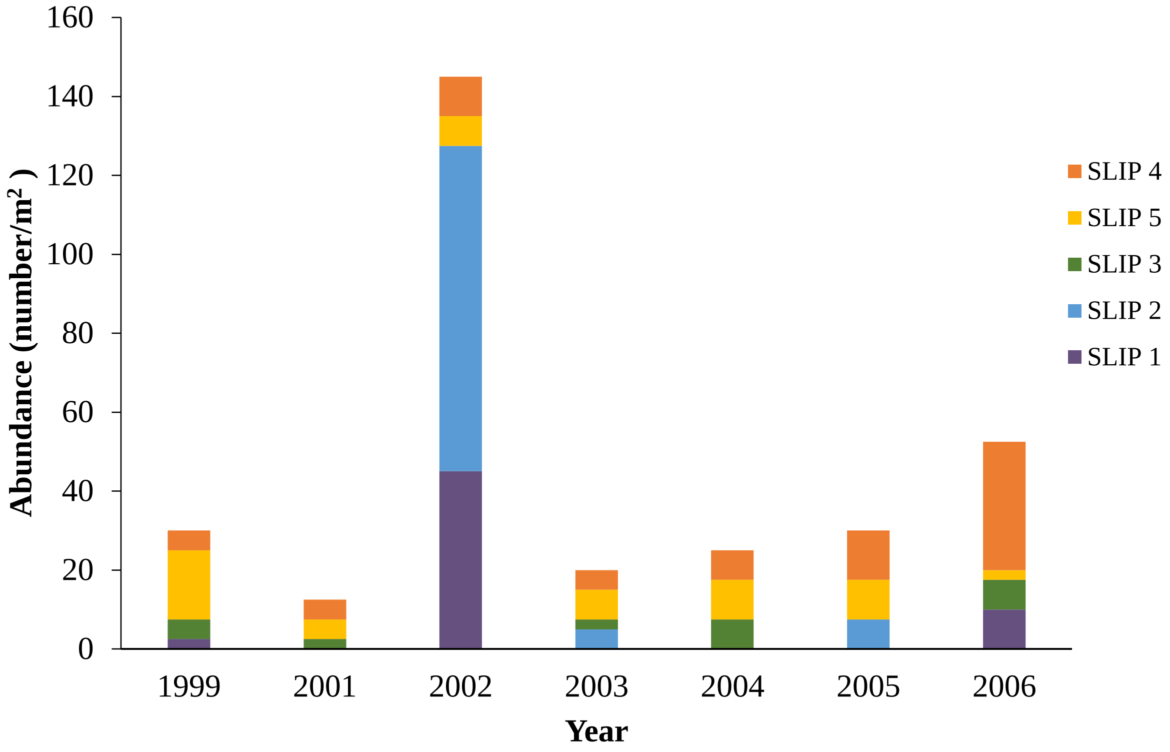


Figure 3.10 Mean abundance of the size class 3.1-4 cm at the SLIP stations in the northern Bering Sea from 1999-2006.

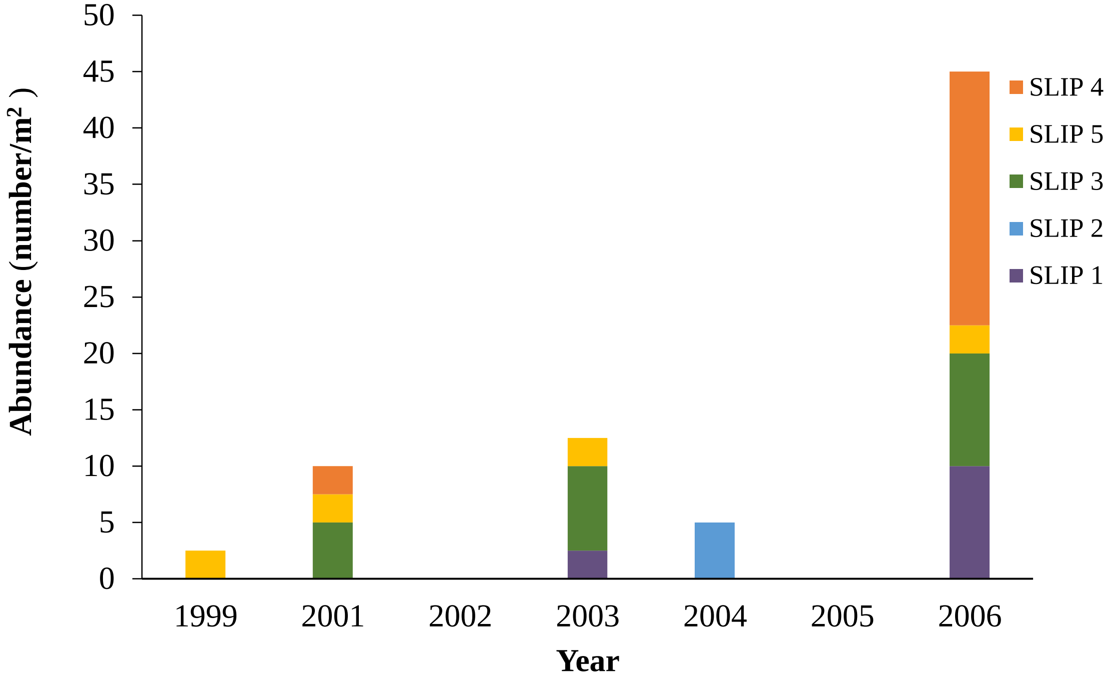
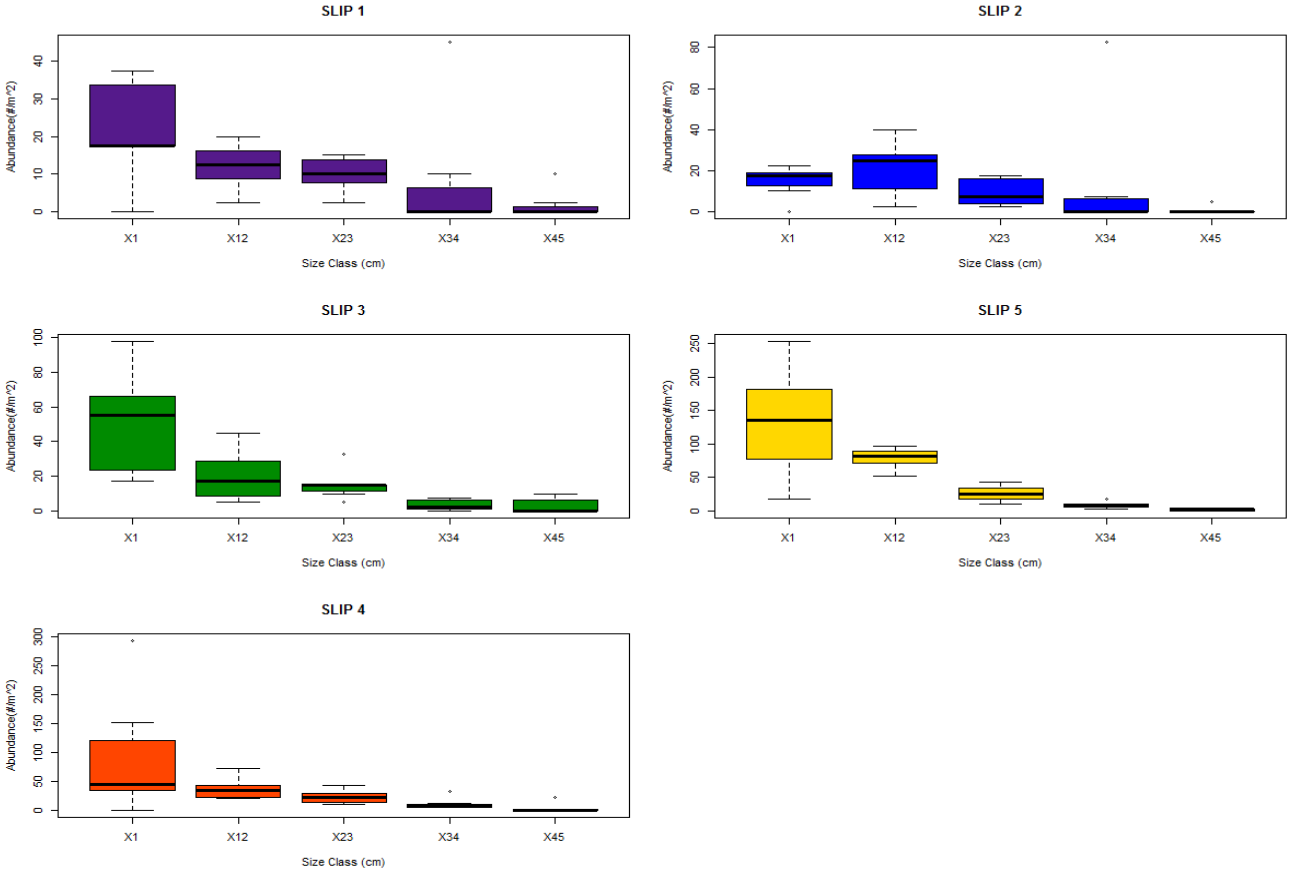


Figure 3.11 Mean abundance of the size class 4.1-5 cm at the SLIP stations in the northern Bering Sea from 1999-2006.



SLIP 1

SLIP 2

SLIP 3

SLIP 5

SLIP 4

Figure 3.12 Comparison of size class dominance within each of the five SLIP stations in the northern Bering Sea.

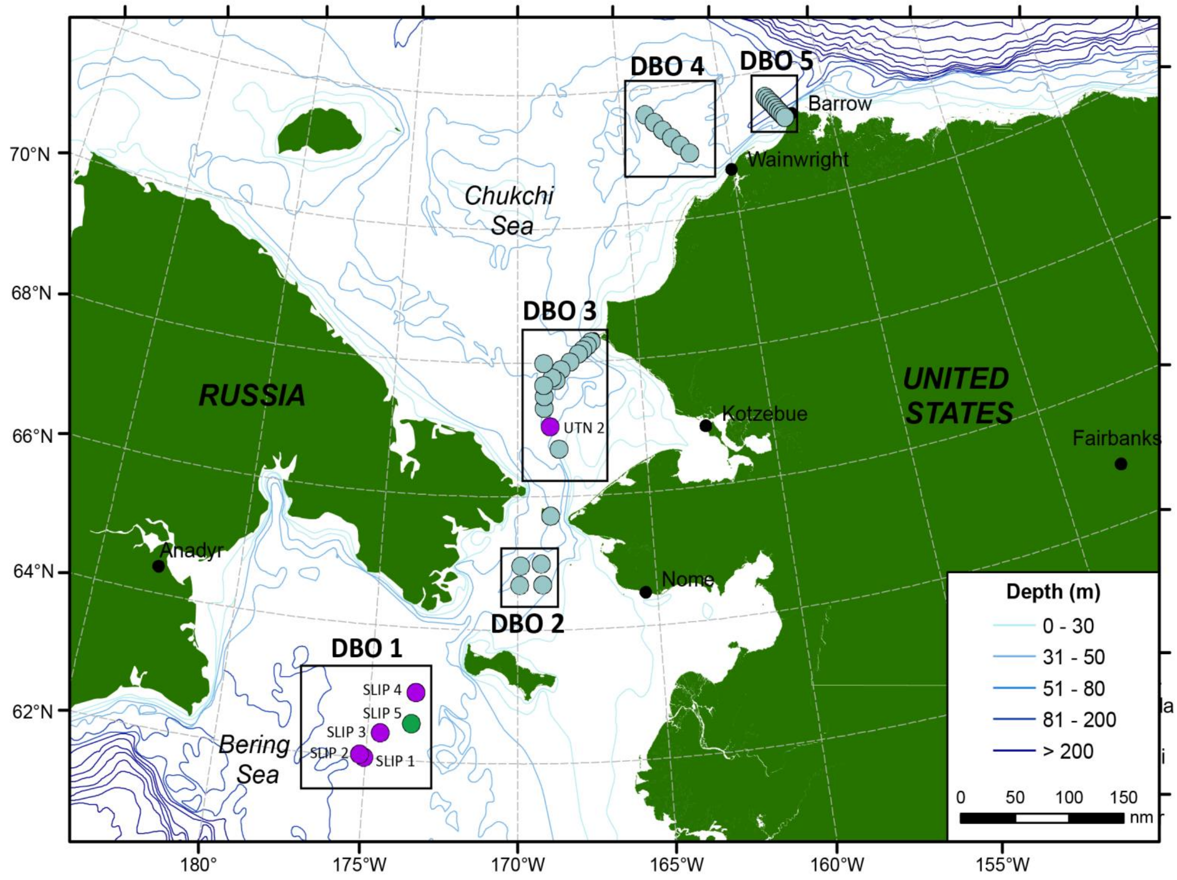


Figure 3.13 Station map with results from the cluster analysis of the trends in total abundance in the stations from the northern Bering Sea (SLIP) and SE Chukchi Sea (UTN 2). Stations highlighted in the same color clustered together.

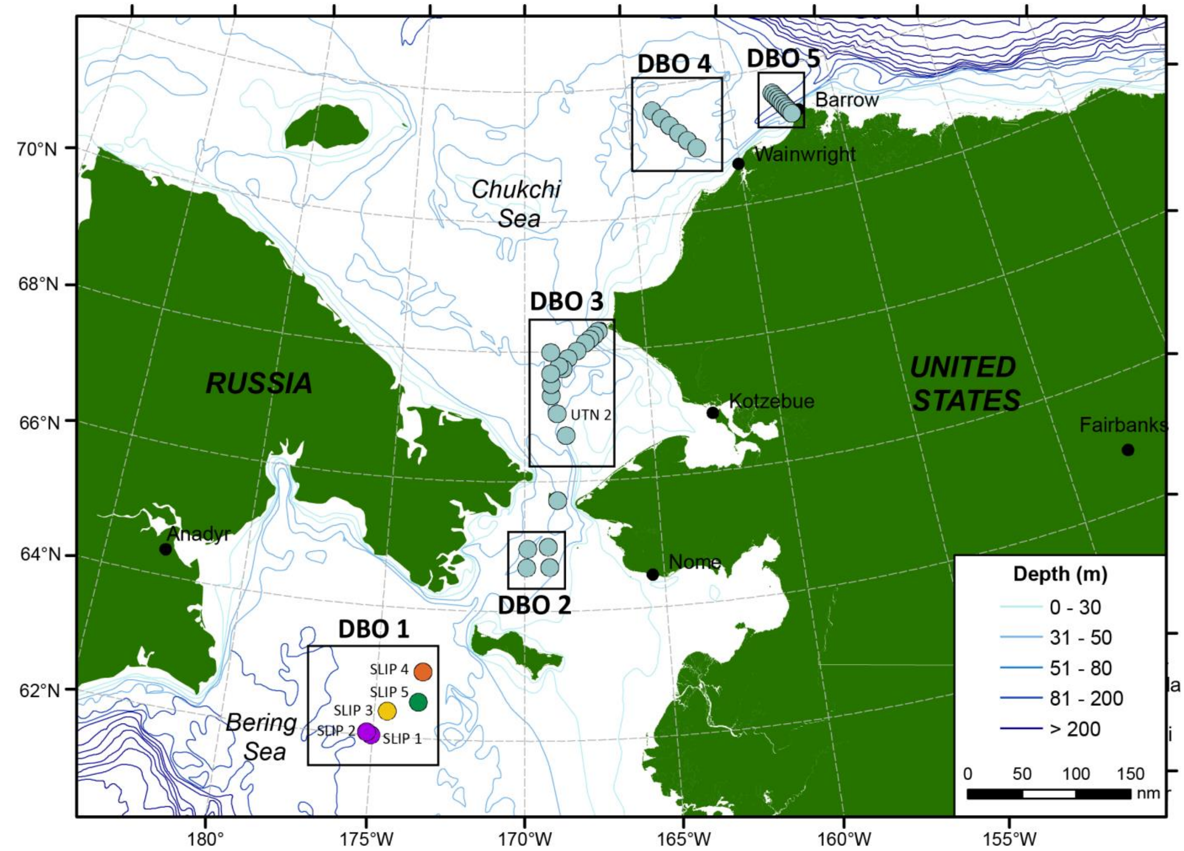


Figure 3.14 Station map with results from the cluster analysis of the trends in the abundance of the 3.1-4 cm bivalve *M. calcarea* size class. Stations highlighted in the same color clustered together.

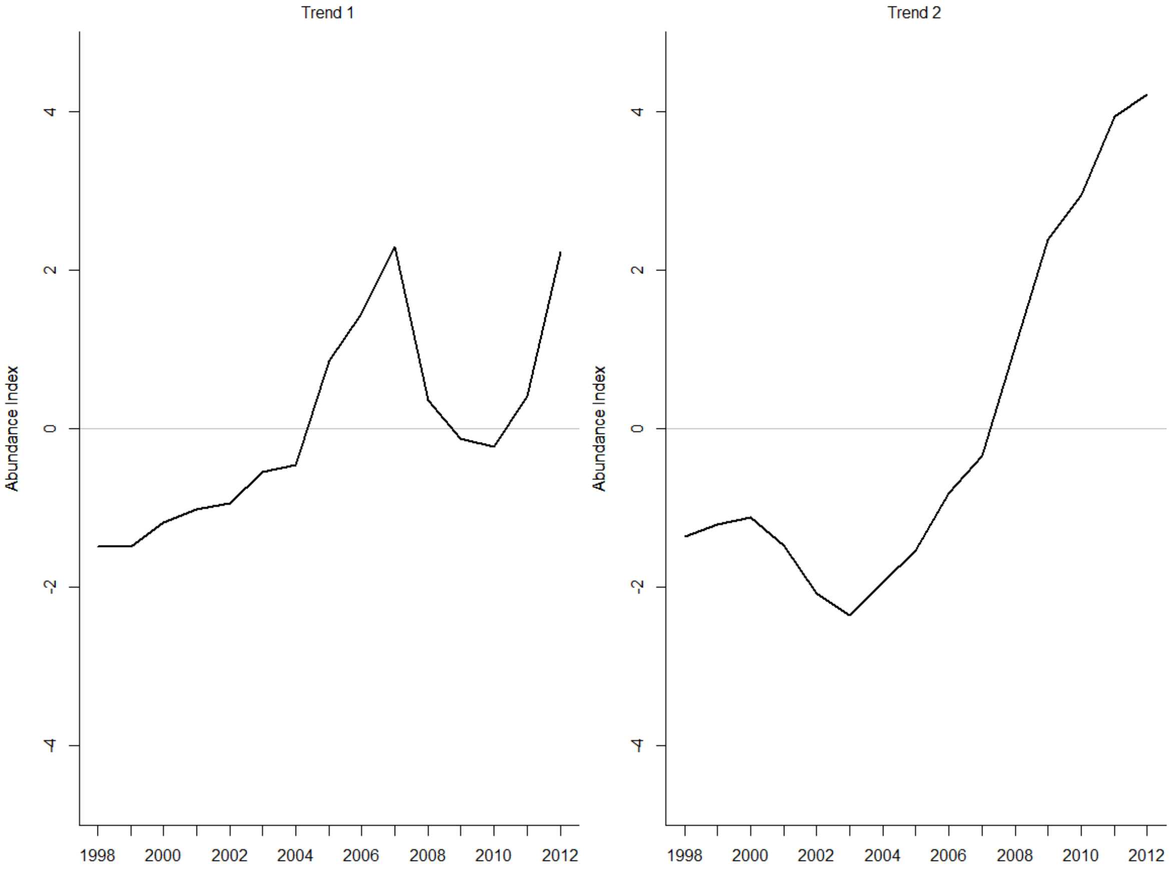


Figure 3.15 Two trends produced from the dynamic factor analysis model describing common patterns in abundance data of *M. calcarea* in the northern Bering and SE Chukchi Sea. Abundance at SLIP 1-4 was influenced by Trend 1 (left), UTN 2 was influenced by trend 2 (right), and SLIP 5 was influenced by both trends.

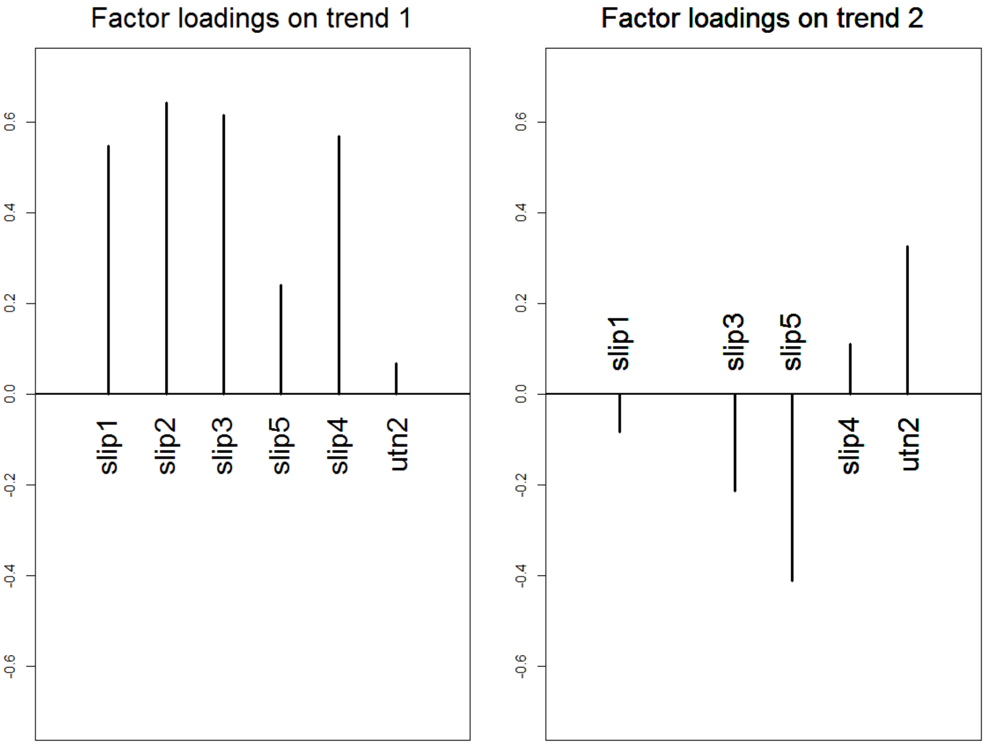


Figure 3.16 Loadings from DFA model output. Loadings show the separation of stations influenced by trend 1 vs. trend 2.

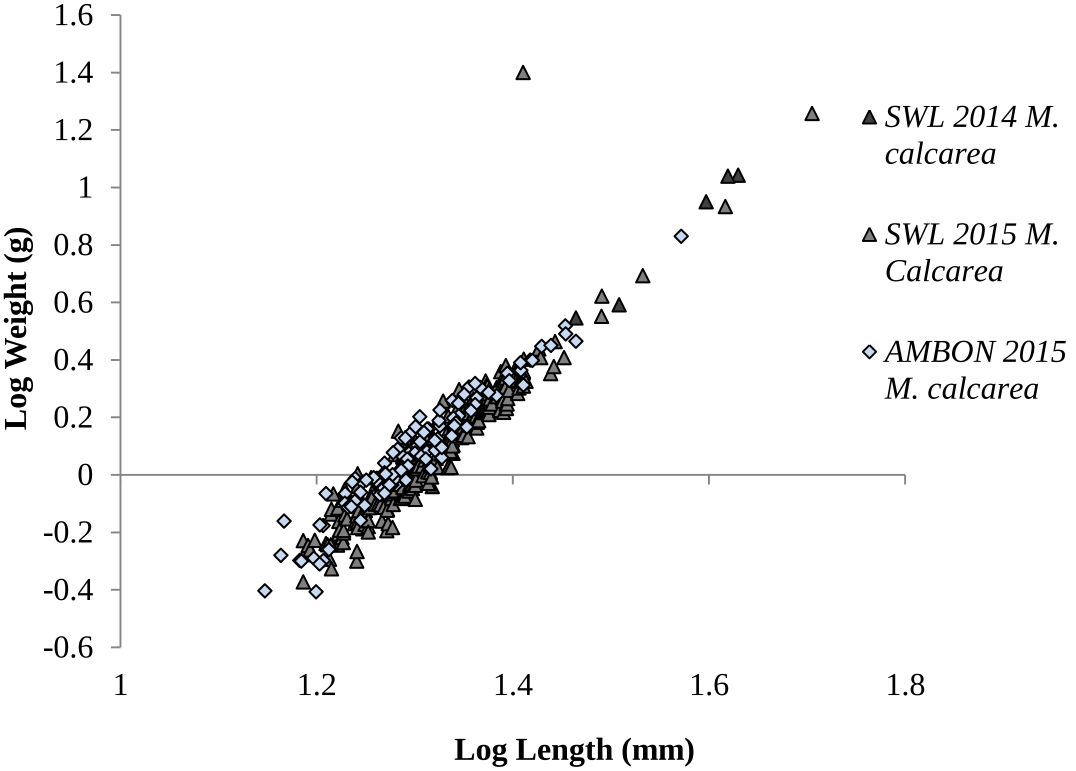


Figure 3.17 Allometric growth calculations for *M. calcarea* collected from SWL 2014, 2015 and AMBON 2015 collections. Length and weight were measured after collection.

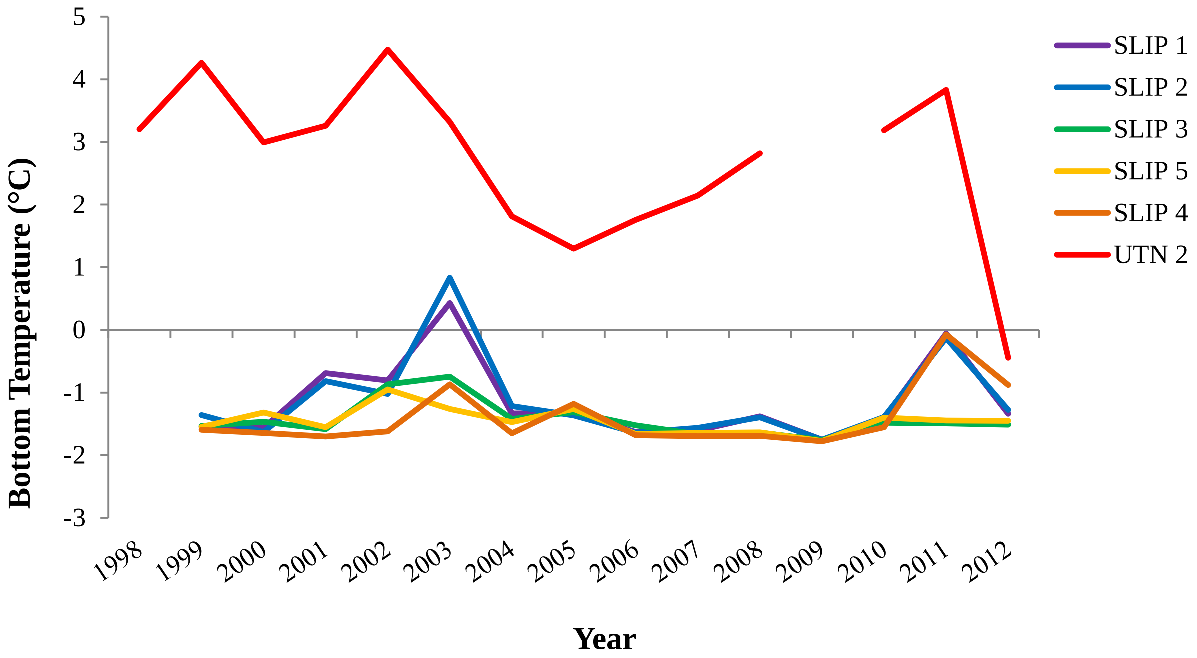


Figure 3.18 Bottom water temperature measured with a CTD deployed during the CCGS *Sir Wilfrid Laurier* (SWL) cruises at the six study sites from 1998-2012.

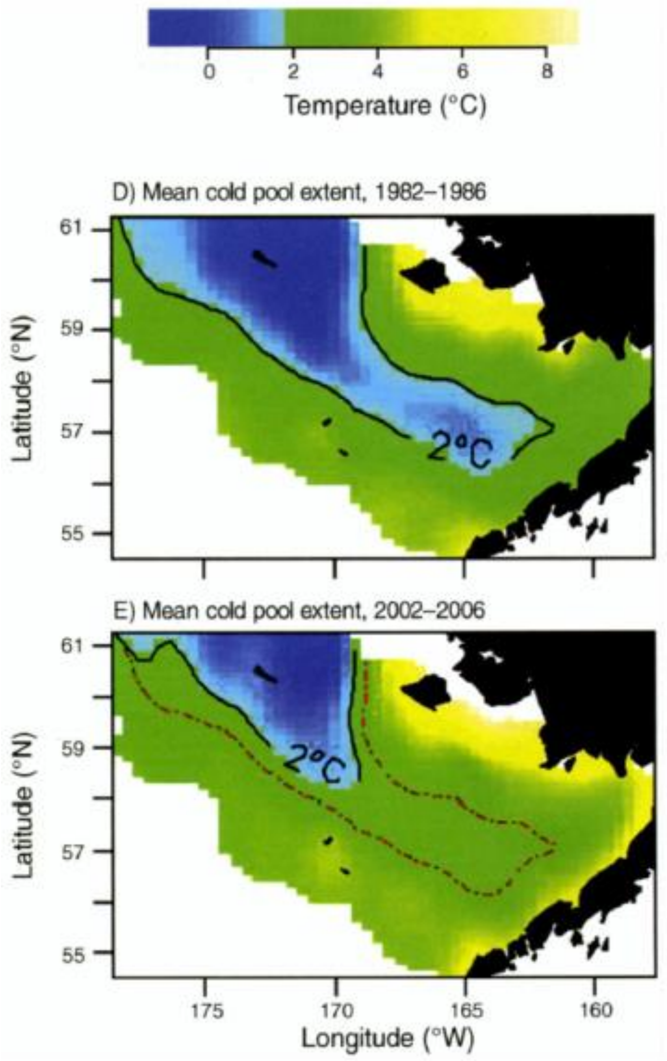


Figure 3.19 Retraction of the mean extent of the cold pool in the Bering Sea from 1982-1986 (top) to the 2002-2006 (bottom). Figure modified from Mueter and Litzow 2008.

Figure 3.20 Map of the currents in the study region. Figure from Grebmeier 2012.

Chapter 4: Conclusions

The Role of Acidification and Recruitment Studies in Today's Arctic Research

As carbon dioxide levels continue to rise, sea ice extent continues to decrease (exposing a larger area of open water), and changes to primary production occur, long-term monitoring projects that assess the changing conditions of the Pacific Arctic Region (PAR) can provide valuable information that facilitates our understanding of the changing ecosystem, such as those presented in chapter 2 and 3 of this thesis. Data used in these two chapters were collected during two observational projects: the Arctic Marine Biodiversity Observing Network (AMBON) (http://ambon-us.org/) and the Distributed Biological Observatory (DBO) (http://www.arctic.noaa.gov/dbo/about). Clams for the acidification experiments, presented in chapter 2, were collected from the first field season of the AMBON project (2015). The main goal of AMBON is to understand and monitor the biodiversity of the continental shelf of the Chukchi Sea. *Macoma calcarea* examined in the third chapter were all part of collections made in the Chukchi Sea at hotspots within the DBO. The DBO is an ongoing long-term observational project that collects data in the water column and benthos along several transects that cross biologically productive sites in the northern Bering and Chukchi Seas.

The studies presented here augment existing observations by using laboratory experiments and measurements to document changes over time that may be due to environmental pressures on dominant bivalves in the region. In addition to understanding the effects of diminishing ice extent and warming temperatures, studies examining calcifying organisms in the PAR are particularly important as atmospheric CO_2 continues to increase and as acidification events in bottom waters become stronger and last longer throughout the seasons (Mathis et al. 2011a, Mathis et al. 2011b, Mathis and Questal 2013). Results from chapter 2 indicate that there will be detrimental effects to some calcifying organisms, as observed in the significant decrease in percent change of length in *Astarte borealis* during an eleven-week exposure to acidified conditions. These effects are likely to be time-dependent because they were not observed in a shorter seven-week exposure.

Tracking the abundance and dominant size class of a common bivalve can provide an indicator of benthic macrofaunal health and availability of the quality of food for upper trophic levels. Both the composition and the biomass of the organisms living in the sediments play a key role in assessing the availability of an adequate food base for benthivores. For example, spectacled eiders (*Somateria fischeri*), feed in the benthic hotspot south of St. Lawrence Island (SLI), the region examined in chapter 3 for *M. calcarea* abundance. Historically, spectacled eiders have fed primarily on the bivalve *Nuculana radiata*; however, the abundance of this bivalve species has declined over the past couple decades (Grebmeier et al. 2006b, Grebmeier 2012), coinciding with decreases in the seaduck population (Grebmeier et al. 2015, Lovvorn et al. 2016). The seaducks forage for these clams on the seafloor and the optimal size range preferred is 18-24 mm in length (Lovvorn et al. 2003, 2015, 2016). Therefore, the results from chapter 3 provide valuable information about another commonly found bivalve that could be a viable food source for higher trophic levels.

Review of Project Goals and Key Findings

In chapter 2, effects of decreased pH and decreased food on three commonly found bivalves, *Macoma calcarea*, *Astarte montagui*, and *Astarte borealis*, were reported as the results of two different experiments, one seven weeks long and one eleven weeks long. ANOVA and Kruskal-Wallis tests were run to examine how acidification and food limitation affected the percent change in length, percent change in weight, and oxygen consumption (mg/L/hour) of the individual species. The most notable result from these two experiments was the percent change in length of one of the species, *A. borealis*. Individuals held in the acidified treatments showed a decrease in length after eleven weeks of exposure, while individuals of the same species held in the control treatments showed a positive increase in length. During food limiting experiments, *M. calcarea* exhibited a larger percent increase in growth in aquaria that received food than in aquaria that did not receive food.

Additionally, different size classes of each bivalve were examined to determine if smaller individuals were more susceptible to acidified waters. *M. calcarea* and *A. montagui* were split into two size classes (10-19.99 mm and 20-29.99 mm). *A. montagui* displayed no clear effect of size in response to acidification. However, larger *M. calcarea* held in the control treatment had an increase in length and those held in the acidified conditions had a decrease in length, a result documented in both the 2015 and 2016 experiments. *A. borealis* was also split into two size classes (20-29.99 mm and 30-39.99 mm). Size responses were significantly different between treatments, with the larger size class showing a decrease in length in the acidified treatments versus the control, whereas the same result was not observed in the smaller individuals.

In chapter 3, total abundance and abundance of individual size classes were tracked for *M. calcarea* at six different sites, five in the northern Bering Sea (SLIP 1-5) and one in the southeastern Chukchi Sea (UTN 2) using dynamic factor analysis (DFA) and time-series clustering over fifteen years. More detailed size class analyses were also undertaken for the five stations in the northeastern Bering Sea. Results showed a northward movement of abundance (number/m^2) over time and a higher abundance of the smaller size classes at all five sites. All five stations had mainly clams in the 0-1 cm size class followed by either the 1.1-2 cm size class or the 2.1-3 cm size class.

Ultimately, combining these two studies may help determine if effects from ocean acidification events in the bottom waters are already visible in populations of bivalves in the Pacific Arctic. The ocean acidification experiment results suggest that size of the bivalves will affect how they respond to decreasing pH. Because both chapter 2 and 3 examined *M. calcarea*, I will make inferences for that species only. The seemingly more vulnerable size class of *M. calcarea* was 2.1-3 cm. The 2.1-3 cm size class was usually the second or third most abundant size class at the sites in the northern Bering Sea, suggesting that significant fractions of the most vulnerable sized clams are present.

Through the acidification experiments conducted using clams collected in the Chukchi Sea, and the size class analyses that were run in the northern Bering Sea, these studies provide a start to understanding how acidification will affect the benthos. These studies can also provide some initial information about how multiple stressors are working synergistically, possibly exacerbating the problems, as ocean acidification is likely not the only factor potentially contributing to higher abundances of smaller clams. It is important to remember that smaller clams will always be present in the biological community and life cycle. Moreover, individual clams can live 5-20 years, allowing for overlapping clam populations of different year classes.

Initial data of size classes from station UTN 2 in the SE Chukchi Sea suggests that the most abundant size classes are the 1.1-2 cm and 2.1-3 cm clams. If the dominant size class at these sites is the same size class affected most by decreased pH, the effects of ocean acidification could be even more detrimental. A second question that arises from these two studies is the role ocean acidification plays in the recent declines in bivalve size, over more broadly distributed size classes. *A. borealis* displayed a decrease in length under acidified conditions, and although *M. calcarea* showed no significant evidence of a decrease in growth, longer exposure to acidified conditions may be a contributing factor to the observed declining sizes. However, size class and year class of individuals should not be expected to always align. In other words, the size of an individual bivalve may be due to young age, but it also could be an older individual whose size has been affected by environmental factors, including ocean acidification. While this study cannot definitely tease apart which scenario is dominant in this species, future studies could aim to age individuals in addition to measuring size, in order to increase the value of time-series data on abundance of different size classes.

Additionally, it was observed that food played a significant role in growth for *M. calcarea*. As seawater temperatures have risen, sub-Arctic species have been able to extend their range northward (Grebmeier 2012). As they expand, they can compete with Arctic species for resources. If native Arctic species are outcompeted for food, growth and dominant size classes may change. If growth is slowed because of fewer resources, then smaller clams may become more prevalent, and therefore fall within the size class most susceptible to ocean acidification. Although no significant differences were observed between the combined acidified and no food treatments, different allometric growth allocations as seen in *M. calcarea* from the 2015 acidified treatment might be expected.

Results from both of these studies begin to answer questions revolving around how decreased pH and ongoing ocean acidification events will affect an already changing PAR benthos. Both of these studies provide information on the availability and quality of the food source available for the upper trophic organisms, such as seaducks that forage south of St. Lawrence Island. Consistent reductions in bivalve size classes would be expected to have a negative impact on the quality and quantity of food of prey items.

Future Work

Although this work provides a means for answering several questions regarding the changing benthos and impacts from ocean acidification in the Pacific Arctic, my work examining effects on acidification of size class can also be greatly expanded upon. For example, more representatives from each size class of clams used is my acidification experiments need to be added into an experimental set-up, to avoid low representation as seen in the 2016 experiments. Additionally, clams from larger size classes, (e.g. >3 cm), need to be studied experimentally in order to better understand how acidification is dependent upon organism size because the largest *M. calcarea* studied here was in the 2.1-3 cm size class. While the larger clams are present at a lower abundance at the sites studied, it is still unknown how they will respond to acidification. As we gain a better understanding of how size classes are changing and how each of these are affected by decreasing pH in the bottom waters, these two approaches (experimental exposure to acidified conditions and time-series changes in size distribution) can be understood in context. In addition to expanding upon the size class analyses, there are several other steps that can be taken in continuing this work, including studies of larvae to see if there are differences in how the larval and adult stages react to declining pH values. The three bivalve species examined in my work are of course not the only calcifying organisms in the Pacific Arctic, and experiments should be undertaken to study effects to other bivalve species, crabs, and gastropods.

The time-series data presented here are relatively short and part of the DBO project and its 25-year dataset for benthic biomass. Future work should provide data from other years not analyzed here in order to develop a longer time-series and to continue collecting data and filling in the gaps for other years. This proposed future work will facilitate a stronger understanding of the effects of ocean acidification in the Pacific Arctic and the changes observed to community composition and biomass of the benthos of this shelf system.

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