Implications of ocean acidification in the Pacific Arctic: Experimental responses of three Arctic bivalves to decreased pH and food availability

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**A R T I C L E   I N F O**

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**A B S T R A C T**

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 like bivalves. These bivalves are common prey items for benthivorous predators such as Pacific walruses (Odobenus rosmarus divergens), bearded seals (Erignathus barbatus), and diving seaducks, such as Spectacled Eiders (Somateria fischeri). We investigated the effects of decreased pH and food availability on growth (% change in length and wet weight and allometric growth characteristics) and oxygen consumption (mg/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 control (8.05 ± 0.02 and 8.19 ± 0.003, respectively) and acidified (7.76 ± 0.01 and 7.86 ± 0.01, respectively) pH treatments. Length, weight, and oxygen consumption were not significantly different among the varying treatments after the seven-week exposure and only one significant effect of decreased pH and one significant effect of decreased food availability were observed after 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. The negative effects of pH observed in the experiments on growth and oxygen consumption were small, suggesting that at least two of these species are generally resilient to decreasing pH.

1. Introduction

Atmospheric CO$_2$ continues to increase due to human activities such as the burning of fossil fuels and deforestation (Pejlejero et al., 2005; IPCC, 2014; Meinschaeusen et al., 2017). 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 changing the balance of chemical equilibria for the inorganic carbon system, affecting carbonate chemistry and speciation of carbon in the oceans, and resulting in ocean acidification (Caldeira and Wickett, 2003, 2005; Feely et al., 2004; Orr et al., 2005).

High latitudes will be subjected to the effects of decreased pH and ocean acidification earlier because of pre-existing natural conditions that magnify ocean acidification, including cold water temperatures (increasing dissolved gas capacities) and low concentrations of carbonate ions (Orr et al., 2005; Bates and Mathis, 2009; Fabry et al., 2009; Steinacher et al., 2009). The biological pump associated with high seasonal production and respiration also results in seasonal CO$_2$ accumulation that increase the vulnerability of organisms to ocean acidification (Bates et al., 2009; Mathis et al., 2011a, 2011b; Cross et al., 2012). The proportionally high contribution of freshwater at high latitudes, including from both sea ice melt and runoff (Steinacher et al., 2009; Mathis et al., 2011a; Bates et al., 2014), is an additional factor increasing vulnerability to anthropogenic inputs of carbon dioxide. These mechanisms of natural vulnerability, in combination with anthropogenic CO$_2$, facilitates the persistent undersaturations (defined as $\Omega < 1$, where $\Omega = [\text{Ca}^{2+}] \times [\text{CO}_3^{2-}] / ([\text{CaCO}_3])$), which are observed during the summer and fall in both the surface and bottom waters of the Bering and Chukchi Seas (Bates and Mathis, 2009; Bates et al., 2009; Mathis et al., 2011a, 2011b; Cross et al., 2013; Yamamoto-Kawai et al., 2016). In the Chukchi Sea, the shallow shelf system allows anthropogenic CO$_2$ inputs to immediately infiltrate bottom waters (Yamamoto-Kawai et al., 2016) and high benthic carbon metabolism allows for a seasonal efflux of CO$_2$ to bottom waters over the continental shelf (Mathis et al., 2014). In 2010, some bottom waters of the Chukchi Sea had pH values as low as 7.75, with bottom water aragonite undersaturations lowest in September and October (Mathis and Questel, 2013).

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Strong impacts on organisms due to changing ocean chemistry have been widely demonstrated (e.g. Kleypas et al., 1999, Riebesell et al., 2000). Decreases in pH and the subsequent decrease in Ω values of aragonite or calcite can affect organisms in two primary ways: changes to calcification rates and disturbances to acid-base (metabolic) physiology (Fabry et al., 2008; Feely et al., 2009; Waldbusser et al., 2014). Additional changes to physiology, development, morphology (phenotypic plasticity), and behavior may also occur (Melatunen et al., 2013). Because shells and hard structures provide many benefits for the organisms that produce them, including protection from predators, dissolution of shell structure from carbonate undersaturations may lead to reduced fitness (Fabry et al., 2008).

Although the changing carbonate system in the Chukchi Sea is relatively well documented, it remains comparatively unknown how these changes will affect organisms. The high benthic biomass in the Chukchi Sea, supported by large exports of pelagic organic carbon (Dunton et al., 2005; Grebmeier et al., 2006), includes prey items that support higher trophic level organisms such as spectacular eiders (Somateria fischeri), gray whales (Eschrichtius robustus), bearded seals (Eringnathus barbatus) and Pacific walruses (Odobenus rosmarus divergens) (Grebmeier et al., 2006; Moore et al. 2014). Some of these prey items include bivalves that produce aragonitic shells, although no direct studies of acidification impacts have been accomplished on the dominant bivalves in the Chukchi Sea. Elsewhere, other species of bivalves such as Mytilus edulis and Crassostrea gigas collected from estuaries in the Netherlands, have exhibited reductions in growth, decreased calcification rates, and reductions in metabolic activity in response to decreased pH (e.g. Gazeau et al., 2007). In another study, Wood et al. (2008) found an increase in calcification and metabolic activity, but at a cost to the muscle mass of the arms of the brittle star Amphiura filiformis. These existing studies suggest effects of decreased pH on bivalve physiology and ecology will be species-specific (e.g. Dupont et al., 2008, Fabry et al., 2008).

The goal of this study was to test the effects of decreased pH and decreased food availability on the growth (percent change and allometric growth equations), metabolic activity (oxygen consumption), and potential tradeoffs associated with metabolic activity (allometric growth classifications) of three common Pacific Arctic bivalve species. The three bivalves used were Macoma calcarea (Gmelin, 1791), dominant in the southern Chukchi Sea, and Astarte borealis (Schumacher, 1817) and Astarte montagui (Dillwyn, 1817), both dominant on the continental shelves of the northern Chukchi Sea. We expect decreased pH and limited food to slow growth rates as the species allocates more energy to counteract the additional stressors. Additionally, we would expect oxygen consumption to increase in the stressed environment, as was observed by Wood et al. (2008). In a food limited situation we also expect limited growth. Finally, based on the meta-analysis of Ramajo et al. (2016), we expect that if sufficient food is available, the energy generated will be used to counteract the effects of the depressed pH, but in a multiple stressor scenario (limited food and acidified conditions) the effects will exceed the additive effects of each individual effect.

2. Material and methods

We performed two sets of experiments, one undertaken for seven weeks in the fall of 2015 and one undertaken for eleven weeks in spring of 2016, to test for impacts associated with decreasing pH and variable food supply.

2.1. Sample collection and pre-experimental holding conditions

Bivalves used in this study were collected using a 3.05 m plumb- staff beam trawl (PSBT) with 7 mm mesh and an Abookire modification (PSBT-A) (Abookire and Rose, 2005), in August 2015 from aboard 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 MLS-10 (71.6028 N, 162.2022 W, Fig. 1).

Bivalves collected at sea were maintained in groups of 15 individuals in 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 psu) 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 transported from Wainwright,
Alaska, back to the Chesapeake Biological Laboratory (CBL) in Solomons, Maryland.

Once at CBL, and before the experiments were set up, the clams were stored in a walk-in cold room maintained at 2.5 °C and held in four-75 L tanks with approximately 150 clams per tank. Artificial seawater, made from Instant Ocean™ (Spectrum Brands, Inc., Blacksburg, Virginia) sea salt, was mixed to a similar salinity (32.5) as the water in which the clams were collected. In addition, the artificial seawater was inoculated with some of the water the bivalves were collected from at sea in order to “season” the new artificial seawater. Temperatures were held between 2 and 3 °C based on the average seasonal range of bottom water temperatures known for the area (Grebmeier et al., 2015), including in situ CTD measurements at station ML 5–10 (−0.31 °C, 32.19 salinity, classified as Bering Sea winter water) to mimic natural conditions as best as possible and to reduce stress on the animals. The cold room was kept dark, except for approximately 20 min every other day for tank maintenance and feeding, consistent with the natural conditions at the depth of collection (<0.1% light level, Frey et al., 2011). In the first two weeks following tank set up, we also monitored nitrate/nitrite and ammonia levels and pH using aquarium test kits from Aquarium Pharmaceuticals™ (API Mars, Inc., McLean, Virginia). After the first two weeks these values normalized as conditions in each tank stabilized. Maintenance of these four tanks, before the experiments began in October, included checking salinity and temperature every other day using a YSI 850 conductivity meter (YSI, Yellow Springs, Ohio) probe and feeding each tank with 1 mL of Shellfish Diet 1800™ (Reed Mariculture, Campbell, California).

2.2. Experimental set up

2.2.1. 2015 experiments

In 2015 we conducted a factorial experiment involving exposure of three species of bivalves to water of two different pH values: pH 8.05 (control) and pH 7.76 (acidiﬁed treatment). The experimental pH value selected was based on the lowest value observed by Mathis and Questel (2013), and the acidiﬁed pH value used by Schram et al. (2016). Similarly, Wood et al. (2008) and Melatunan et al. (2013) observed growth and metabolic effects at pH maintained at 7.70. Experiments were undertaken from October 28 to December 16 (seven-week exposure). Two 75 L stock tanks held Instant Ocean™ seawater, in which carbon dioxide (Airgas Research Grade Size 300 cylinder) additions were used to manipulate the pH of these two stock tanks (Fig. 2), a similar set up to the methods described in Schram et al. (2016). The pH of each stock tank was monitored with a pH electrode (Cole-Parmer model 27003-12, Vernon Hills, Illinois) coupled to an Alpha pH 190 pH/ORP controller (Omega Engineering Inc., Stamford, Connecticut) to continuously measure the conductivity (in mV units) of the water within the tank (Fig. 2). Conductivity readings were converted to pH using Eq. (1), the Nernst equation for a pH electrode at 2.5 °C.

\[
\text{Conductivity (mV)} = (-54.77 \times \text{pH}) + \text{constant} \\
\]  
(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 g of NaCl, and 1 mL of 1 N HCl. Individual electrodes varied in performance, so a constant was determined for each electrode using Eq. (2).

\[
\text{mV reading from electrode} = (-54.7 \times \text{reading}) + \text{constant} \\
\]  
(2)

Once the constant was established, we determined a set point for the electrode in the acidiﬁed stock tank to ensure the water stayed at the appropriate pH using Eq. (3).

\[
\text{setpoint} = (-54.777.8) + \text{calculated constant} \\
\]  
(3)

If the water pH went above the set point, a solenoid, wired to the controller and electrode, opened, allowing CO2 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. The conductivity values (in mV) for each stock tank were recorded once a day when water changes occurred.

All three species of clams were randomly assigned to one of fourteen experimental 20 L tanks (Fig. 2). Seven tanks were ﬁlled with the control treatment water (pH 8.05 ± 0.02) and the other seven tanks were ﬁlled with acidiﬁed treatment water (pH 7.76 ± 0.02). Within each tank, three 475 mL high-density polyethylene containers ﬁlled to the rim with sand collected from a local Chesapeake Bay beach were established. Prior to use the sand was rinsed with deionized water three times and then it was soaked for 72 h in Instant Ocean™ seawater to acclimate it to cold room conditions and to allow for development of microbial flora associated with the incubated bivalves. Each one of the fourteen experimental tanks held six M. calcarea for a total of 84 individuals (6 individuals per tank * 14 tanks = 84), eight A. montagui (8 individuals * 14 tanks = 112), and one A. borealis (1 individual * 14 tanks = 14). Tanks were covered with polycarbonate covers to reduce evaporation.

Over the seven-week exposure period, approximately 2000 mL of seawater were removed from each of the fourteen experimental tanks at the same time each day. New seawater, from the appropriate 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 to the best of our ability. This general technique was also used in Schram et al. (2016). Once the water was changed, an Oakton General-Purpose sealed, double-junction, epoxy body, handheld pH electrode (calibrated using the methods described above for the stock tank electrodes) connected to a pH meter (VWR Scientiﬁc Model 2000, Radnor, Pennsylvania) 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 (Wood et al., 2008) were made using the YSI850 conductivity meter.

Clams were fed every other day in each tank with 0.5 mL of Shellfish Diet 1800™.

2.2.2. 2016 experiments

In 2016, we conducted three 2 × 2 factorial experiments. Each experiment used one of three species and two experimental factors, pH level (control and acidiﬁed) and food availability (fed and unfed). The experiment was run from January 19 to April 4 (eleven-week duration). All of the stock tank procedures were the same as described for the 2015 experiments. All three species of clams were randomly assigned to one of the twelve experimental 20 L tanks, each provided with the same style of sand ﬁlled containers as 2015. Three experimental tanks were held at control pH levels (8.19 ± 0.02) and not fed over the course of the experiment (Control Not-fed = CNF); three experimental tanks were held at control pH levels (8.19 ± 0.003) and fed 0.5 mL of Shellﬁsh Diet 1800™ every other day (Control Fed = CF); three experimental tanks were held at control pH levels (8.19 ± 0.003) and not fed over the course of the experiment (Control Not-fed = CNF); three experimental tanks were held at the experimental pH level (7.86 ± 0.01) and fed 0.5 mL of Shellﬁsh Diet 1800™ every other day (Acidiﬁed Fed = AF); and the final three experimental tanks were held at the experimental pH level (7.86 ± 0.02) and not fed over the course of the experiment (Acidiﬁed Not-fed = ANF). Each tank held three M. calcarea (3 individuals * 12 tanks = 36), seven A. montagui (7 individuals * 12 tanks = 84), and two A. borealis (2 individuals * 12 tanks = 24). Monitoring of this experiment followed the same procedures as in the 2015 experiments.

2.3. Net body growth and shell measurements

Changes in growth were assessed by determining both wet weight (g) and length (mm) 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 hundredd of a gram. We tested analytical variability in the scale by measuring an individual from each species ﬁve times during one day. The
largest difference in weight for the same animal was 0.07 g. Percent change (\%Δ) in wet weight over the course of the experiment(s) was calculated using Eq. (4).

Changes in clam shell length were documented using Capri 15-cm

\[
\text{% change (Δ)} = \left( \frac{\text{Final Wet Weight} - \text{Initial Wet Weight}}{\text{Initial Wet Weight}} \right) \times 100
\] (4)
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. We ran the same precision test on the calipers. An individual from each species was measured five times during one day and the largest difference was 0.15 mm. A similar percent change (%Δ) formula to (4) was applied to length measurements.

2.4. Oxygen consumption

Changes in oxygen consumption were measured to serve as an indicator of metabolic activity. Oxygen consumption was measured using a Pyroscience FireSting O2 Optical Oxygen Meter (Pyro Science, Aachen, Germany) and vendor-sourced proprietary software for data collection. The meter handles data from four probes, each of which we inserted into individual jars that held one clam each. Measurements were made on four randomly selected clams each day for the last nine days of the experiment in 2015 (December 8, 2015–December 16, 2015) (4 clams/day × 9 days = 36), and four randomly selected clams a day for the last twelve weeks of the 2016 experiments (March 20, 2016–April 1, 2016) (4 clams/day × 12 days = 48). Each species was equally represented in the total number of clams measured. In 2015 oxygen consumption was measured in six, randomly chosen clams from each species/pH treatment combination, and in 2016 oxygen consumption in four, randomly chosen clams from each species/pH/food treatment combination was measured.

For the oxygen consumption measurements, individual clams were placed into 100 mL jars filled to the top with seawater from either the control or treatment pH stock tank and these containers were capped tightly to ensure they were airtight. Data from the probes is reported as dissolved oxygen in mg/L. The probes were calibrated at the beginning of each day to the recommended “1 point in humid air/water” setting (to be used when placed in water), 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 were recorded every two minutes over a 24-h period and the volume of water in each jar was used to convert to an oxygen utilization rate (mg O2/L/hour). These measurements, beginning with calibration, were repeated every day for either nine or twelve days.

Oxygen use over each 24-h period was plotted as a graph of linear decay (of dissolved oxygen); a linear regression and slope using RStudio® (Version 3.3.2) was calculated. In several cases (14% in 2015 and 10% in 2016), no linear decay of dissolved oxygen was observed, and those data were not used in further analyses. The absence of a linear decay of dissolved oxygen indicated that the probe wasn’t functioning properly. 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 (O2 mg/L/hour). Differences in rates between species and among treatments were analyzed using statistical methods described below.

Oxygen consumption and weight were later log transformed and plotted to account for differences in weight of the organisms [the measured changes in dissolved oxygen concentrations were converted to positive before transformation (Fig. 5)].

2.5. Length weight relationships and growth characterizations

The wet weight and length measurements determined before each experiment were then used to determine allometric slopes and classifications for the three species. 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.

\[ W = aL^b \]
\[ \log W = \log a + b\log L \]

Slopes of the equations were compared using statistical analyses described in Section 2.6. Additionally, three classifications were used to describe the type of growth: isometric, positive (+) allometric, and negative (-) allometric growth. These classifications provide insights for how and where these animals might be allocating their available energy. Here isometry is defined when length and weight increase through time at approximately the same rate. In negative allometric growth, length increases faster than weight and in positive allometric growth weight increases faster than length, both suggesting some sort of potential tradeoff between the two parameters that could change in a stressed environment. Numerical justification for assigning these classifications is explained in Section 2.6. We also determined this relationship for M. calcarea collected using a 0.1 m³ van Veen grab at a southeastern Chukchi Sea site (Station UTN2, 67.050 N, 168.728 W) for comparison to the northern site where the experimental clams were collected (ML5-10) (Fig. 1).

2.6. Statistical analyses

All statistical analyses used RStudio® statistical software (Version 3.3.2, https://www.rstudio.com/). Data 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, food availability was added as a variable and 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.). For slopes (b) = 3 after accounting for the upper and lower bound of the 95% confidence interval, the class was calculated as having isometric growth. If the confidence interval bounds for the slope fell below three (e.g. 2.4–2.9), the growth was characterized as negatively allometric; and if the confidence interval bounds fell above three (e.g. 3.1–3.4), the growth was characterized as positively allometric. Slopes were compared using the Kruskal-Wallis test.

All results were compared only within the year the experiment was conducted, not between years, despite the clams all being collected at the same time (August 2015). The primary reason for this was that clams used in the fall of 2015 and clams used in the 2016 experiments were acclimated to cold room settings (described in Section 2.1) for different amounts of time, two and four months, respectively.

3. Results

3.1. Treatment conditions

Average tank pH, salinity, and temperature were measured daily during the seven and eleven week exposure time for all tanks from the 2015 experiments (Table 1) and the 2016 experiments (Table 2). We calculated total alkalinity (TA) based on Instant Ocean™ ion concentrations. TA provides an estimate of a second parameter for the carbonate system, which we then used in conjunction with pH to solve for the remaining parameters. We used the salinity, temperature, pH, and estimated TA values and entered these values into the CO2sys spreadsheet (Pierrot et al., 2006) to determine pCO2 and the saturation state for aragonite, the carbonate form used by the three species in this study.
3.2. Net body growth and shell measurements

3.2.1. 2015 experiments

No statistically significant differences between treatments were noted in percent change of length in the three species in the 2015 experiments. A percent decrease in length was observed in M. calcarea held in both control (−0.72% ± 1.42%) and acidified (−2.13% ± 3.42%) treatments, with no significant difference (t-test, p = 0.341) between the two treatments (Fig. 3). The two Astarte species showed an average increase in percent change in length after the seven-week exposure with the exception of A. montagui kept in the control treatment (−0.023% ± 1.40%). A. montagui from the acidified treatment averaged an increase in length of 0.38% ± 1.67%, while A. borealis from the control treatment averaged a percent increase of 0.10% ± 0.41%. The A. borealis maintained in the acidified treatment averaged a 0.30% ± 0.60% increase in length over the course of the experiments. There were no significant differences between treatments in length of the Astarte species (A. montagui: Kruskal-Wallis, p = 0.57; A. borealis: t-test, p = 0.74, Fig. 3).

Changes in wet weight followed the same patterns as changes in length. M. calcarea decreased in mean percent wet weight after the seven weeks (Control: −6.39% ± 6.25%; Acidified: −8.12% ± 6.29%), and all Astarte species showed a percent increase in mean wet weight (A. montagui: Control: 0.44% ± 4.58%, Acidified: 3.44% ± 8.37%; A. borealis: Control: 0.68% ± 0.88%, Acidified: 1.00% ± 0.91%). However, there were no significant differences in wet weights between treatments in any of the species (t-test, all p-values > 0.05, Fig. 3).

In 2015, twelve clams in the control and acidified treatments died, including five M. calcarea each from both control and acidified treatments, and two A. montagui held in the control treatment.

3.2.2. 2016 experiments

Similar to results in 2015, the majority of the comparisons made in 2016 were not significantly different; however, there were two statistically significant results relating to changes in shell length. The first significant difference noted was in the shell length of M. calcarea due to the interaction between pH and food (two-way ANOVA, p = 0.02). Differences were seen between the control fed (CF) treatment where M. calcarea displayed an increase in percent change in length (0.55% ± 0.34%), and the control not-fed (CNF) (−0.98% ± 0.58%), acidified fed (AF) (−0.52% ± 0.68%), and acidified not-fed (ANF) (−0.25% ± 0.31%) treatments where M. calcarea showed a percent decrease in length (Fig. 4). The average length of A. montagui in the AF treatment showed a negative percent change (−0.04% ± 0.53%). Average lengths for CF, CNF, and ANF treatments increased by 0.30% ± 0.14%, 0.50% ± 0.34%, and 0.13% ± 0.16%, respectively; however, these changes in length were not significantly different from one another. The second significant effect noted in length measurements was the effect of pH on the length of A. borealis (two-way ANOVA, p = 0.02, Fig. 4). All A. borealis held in control treatments regardless of food treatment displayed a percent increase in growth (CF: 0.56% ± 0.30%; CNF: 1.06% ± 0.66%), while those held in acidified treatments had a percent decrease in growth (AF: −0.14% ± 0.85%; ANF: −0.23% ± 0.51%) after the eleven-week exposure.

Wet weights did not vary significantly among treatment effects in any of the three examined species. M. calcarea held in the CNF treatment averaged a percent change in wet weight of −0.45% ± 4.76%. However, M. calcarea held in the other three treatments showed a percent increase in wet weight (CF: 2.15% ± 0.62%; AF: 4.12% ± 3.53%; ANF: 2.25% ± 0.28%), but these differences were not significantly different from each other (Fig. 4). Wet weight of A. montagui increased in all four treatments (CF: 0.94% ± 0.29%, CNF: 0.45% ± 0.27%, AF: 1.54% ± 3.31%, ANF: 1.61% ± 1.30%), but none of these results were significantly different from one another (Fig. 4). Wet weight of A. borealis showed no significant difference within all treatments, although there was a consistent percent decrease in average wet weight at the end of the experiments (CF: −0.11% ± 0.56%; CNF: −0.33% ± 0.33%; AF: −0.21% ± 0.09%; ANF: −0.75% ± 0.84%) (Fig. 4).

Only three M. calcarea died during the 2016 experiments: one was held in the AF treatment, one in the ANF treatment, and one in the CNF treatment.

3.3. Oxygen consumption

3.3.1. 2015 experiments

For all species, oxygen consumption (mg/L/hour) was not significantly different among treatments (t-test, all p-values > 0.05). Oxygen consumption in M. calcarea held in the control treatment averaged 5.59 × 10⁻⁷ mg/L/h ± 4.51 × 10⁻⁷, clams kept in the acidified treatment averaged oxygen consumption of 3.99 × 10⁻⁷ mg/L/h ± 4.32 × 10⁻⁷. A. montagui maintained in both the control and acidified

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**Table 1**

Table 1. Water column data (mean ± 1 SD) that were measured from the conductivity, temperature and depth (CTD) sensor in 2015 in the NE Chukchi Sea as part of the Arctic Marine Biodiversity Observing Network (AMBN) cruise (temperature and salinity) and treatment conditions (pH, temperature, and salinity) for the 2015 experiments are listed in the table. Parameters measured with an asterisk (total alkalinity (TA), pCO₂, and aragonite saturation (Ω)) were not directly measured during the experiment, but estimated at a later date. TA was estimated from the known alkalinity of Instant Ocean®. Salinity, temperature, pH, and total alkalinity were then inserted into the CO₂SYS spread sheet (Pierron et al., 2006) to calculate pCO₂ and aragonite saturation state (Ω). Control and acidified pH values were significantly different (t-test, p < 0.01).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTD Bottom Water</th>
<th>Control (mean ± SD)</th>
<th>Acidified (mean ± S D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>−0.31</td>
<td>8.05 ± 0.02</td>
<td>7.76 ± 0.02</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>32.19</td>
<td>2.51 ± 0.09</td>
<td>2.53 ± 0.06</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>32.19</td>
<td>32.51 ± 0.11</td>
<td>32.64 ± 0.08</td>
</tr>
<tr>
<td>TA (µmol/kg SW)*</td>
<td>637.09</td>
<td>2195</td>
<td>2195</td>
</tr>
<tr>
<td>pCO₂ (µatm)*</td>
<td>2.50</td>
<td>1.52</td>
<td>0.85</td>
</tr>
</tbody>
</table>

**Table 2**

Table 2. Water column parameters (mean ± 1 SD) that were measured from the CTD in 2015 in the NE Chukchi Sea as part of the Arctic Marine Biodiversity Observing Network (AMBN) cruise (temperature and salinity) and treatment conditions (pH, temperature, and salinity) for the 2016 experiments are listed in the table. Parameters measured with an asterisk (total alkalinity (TA), pCO₂, and aragonite saturation (Ω)) were not directly measured during the experiment, but estimated at a later date. TA was estimated from the known alkalinity of Instant Ocean®. Salinity, temperature, pH, and total alkalinity were then inserted into the CO₂SYS spread sheet (Pierron et al., 2006) to calculate pCO₂ and aragonite saturation state (Ω). Control and acidified pH values were significantly different (t-test, p < 0.01). Key: CF = Control conditions; animals fed during the course of the experiment; CNF = Control conditions; animals not fed over the course of the experiment; AF = Experimental, lower pH conditions; animals fed over the course of the experiment; ANF = Experimental, lower pH conditions; animals not fed over the course of the experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTD Bottom Water</th>
<th>CF (mean ± SD)</th>
<th>CNF (mean ± SD)</th>
<th>AF (mean ± SD)</th>
<th>ANF (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>−0.31</td>
<td>8.19 ± 0.004</td>
<td>8.19 ± 0.003</td>
<td>7.86 ± 0.01</td>
<td>7.86 ± 0.02</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>32.19</td>
<td>2.59 ± 0.11</td>
<td>2.55 ± 0.05</td>
<td>2.56 ± 0.05</td>
<td>2.50 ± 0.08</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>32.61 ± 0.26</td>
<td>32.78 ± 0.13</td>
<td>32.60 ± 0.08</td>
<td>32.53 ± 0.06</td>
<td>2195</td>
</tr>
<tr>
<td>TA (µmol/kg SW)*</td>
<td>445.83</td>
<td>445.98</td>
<td>1003.51</td>
<td>1606.11</td>
<td>1.04</td>
</tr>
<tr>
<td>pCO₂ (µatm)*</td>
<td>2.01</td>
<td>2.01</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aragonite Ω*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3. Mean percent change in length (mm) (± 1 SD) (top row) of *Macoma calcarea* (left), *Astarte montagui* (middle), and *Astarte borealis* (right) (all collected from the Chukchi Sea in August 2015) from the 2015 experiments in which all treatments were fed. The control treatment (pH 8.05 ± 0.02, blue) and acidified treatment (pH 7.76 ± 0.02, red) percent change in length were not significantly different from one another within any of the species. Mean percent change in wet weight (g) (± 1 SD) (bottom row) of *M. calcarea* (left), *A. montagui* (middle), and *A. borealis* (right) from the control treatment (pH 8.05 ± 0.02, blue) and acidified treatment (pH 7.76 ± 0.02, red) in 2015 also did not differ significantly between treatments.

Fig. 4. Mean percent change in length (mm) (± 1 SD) (top row) in the four treatments (CF = control/fed (dark green); CNF = control/not fed (dark gray); AF = acidified/fed (light green); ANF = acidified/not fed (light gray)) for the three species (*Macoma calcarea* (left), *Astarte montagui* (middle), and *Astarte borealis* (right)) in the 2016 experiments ran on clams collected from the Chukchi Sea in 2015. a and b denote significant difference in the *A. borealis* graphic. Mean percent change in weight (g) (± 1 SD) (bottom row) of all species in all treatments from 2016 experiments (same identifiers as length). Wet weights did not differ significantly among treatments.
treatments had similar average oxygen consumption, $1.90 \times 10^{-7}$ mg/L/h ± $1.05 \times 10^{-7}$ and $2.02 \times 10^{-7}$ mg/L/h ± $9.28 \times 10^{-8}$, respectively. *A. borealis* kept in the control treatment averaged oxygen consumption of $2.74 \times 10^{-7}$ mg/L/h ± 2.30, and those from the acidified treatment averaged consumption of $2.89 \times 10^{-7}$ mg/L/h ± 1.96. All species in all treatments showed similar patterns in oxygen consumption per unit mass (Fig. 5). The *A. borealis* in the acidified treatment were slightly, but not significantly larger than the rest of the animals used, and had similar consumption values.

### Table 3

Allometric growth equation dynamics including intercepts (a) and slopes (b) for *M. calcarea*, *A. montagui*, and *A. borealis*. Organisms marked in blue ($n = 34$) and 2015 ($n = 284$) from another site (UTN2; 67.050 N, 168.728 W) for comparison. All organisms in black were collected in 2015 from site ML 5–10 in the Chukchi Sea. Measurements for in situ (Pre experiment) and all treatment conditions in 2015 and 2016 are listed. Bolded values indicate a significant difference in length values. These significant differences are also denoted in figures three and four.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Treatment</th>
<th>Mean Length (mm) ± 1 SD</th>
<th>a</th>
<th>b</th>
<th>95% CI of b</th>
<th>Determination coefficient</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. calcarea</em></td>
<td>34</td>
<td>Pre experiment</td>
<td>23.56 ± 7.62</td>
<td>9.260</td>
<td>3.103</td>
<td>2.980–3.327</td>
<td>0.9875</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>284</td>
<td>Pre experiment</td>
<td>21.35 ± 3.74</td>
<td>8.812</td>
<td>2.969</td>
<td>2.829–3.310</td>
<td>0.8995</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>120</td>
<td>Pre experiment</td>
<td>20.51 ± 3.47</td>
<td>8.228</td>
<td>2.806</td>
<td>2.667–2.944</td>
<td>0.9312</td>
<td>(+) allometry</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>195</td>
<td>Pre experiment</td>
<td>19.47 ± 2.71</td>
<td>9.401</td>
<td>3.331</td>
<td>3.168–3.494</td>
<td>0.8935</td>
<td>(+) allometry</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>38</td>
<td>Pre experiment</td>
<td>31.62 ± 4.14</td>
<td>10.730</td>
<td>3.367</td>
<td>3.293–4.052</td>
<td>0.9121</td>
<td>(+) allometry</td>
</tr>
</tbody>
</table>

#### 2015

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Treatment</th>
<th>Mean Length (mm) ± 1 SD</th>
<th>a</th>
<th>b</th>
<th>95% CI of b</th>
<th>Determination coefficient</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. calcarea</em></td>
<td>37</td>
<td>Control</td>
<td>20.86 ± 2.97</td>
<td>7.849</td>
<td>2.360</td>
<td>2.360–2.755</td>
<td>0.9505</td>
<td>(-) allometry</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>37</td>
<td>Acidified</td>
<td>20.83 ± 3.49</td>
<td>8.011</td>
<td>2.717</td>
<td>2.540–2.894</td>
<td>0.9641</td>
<td>(-) allometry</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>54</td>
<td>Control</td>
<td>19.17 ± 2.89</td>
<td>8.806</td>
<td>3.138</td>
<td>2.889–3.387</td>
<td>0.9231</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>55</td>
<td>Acidified</td>
<td>19.58 ± 2.70</td>
<td>9.399</td>
<td>3.323</td>
<td>3.047–3.600</td>
<td>0.9148</td>
<td>(+) allometry</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>7</td>
<td>Control</td>
<td>31.51 ± 5.68</td>
<td>10.730</td>
<td>3.367</td>
<td>3.293–4.052</td>
<td>0.9121</td>
<td>(+) allometry</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>7</td>
<td>Acidified</td>
<td>30.96 ± 6.39</td>
<td>12.485</td>
<td>4.176</td>
<td>3.376–4.976</td>
<td>0.9676</td>
<td>(+) allometry</td>
</tr>
</tbody>
</table>

#### 2016

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Treatment</th>
<th>Mean Length (mm) ± 1 SD</th>
<th>a</th>
<th>b</th>
<th>95% CI of b</th>
<th>Determination coefficient</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. calcarea</em></td>
<td>8</td>
<td>CNF</td>
<td>16.43 ± 1.88</td>
<td>8.653</td>
<td>2.931</td>
<td>2.256–3.606</td>
<td>0.9411</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>9</td>
<td>CF</td>
<td>22.11 ± 5.78</td>
<td>8.309</td>
<td>2.814</td>
<td>2.573–3.062</td>
<td>0.9835</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>8</td>
<td>ANF</td>
<td>19.09 ± 2.13</td>
<td>8.116</td>
<td>2.759</td>
<td>2.037–3.480</td>
<td>0.9251</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>M. calcarea</em></td>
<td>8</td>
<td>AF</td>
<td>19.06 ± 1.86</td>
<td>7.494</td>
<td>2.532</td>
<td>1.841–3.222</td>
<td>0.9190</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>21</td>
<td>CNF</td>
<td>19.85 ± 2.26</td>
<td>9.337</td>
<td>3.297</td>
<td>2.851–3.743</td>
<td>0.9227</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>21</td>
<td>CF</td>
<td>20.10 ± 2.83</td>
<td>9.707</td>
<td>3.432</td>
<td>2.965–3.898</td>
<td>0.9220</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>21</td>
<td>ANF</td>
<td>19.15 ± 2.47</td>
<td>9.223</td>
<td>3.277</td>
<td>2.828–3.726</td>
<td>0.9207</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. montagui</em></td>
<td>21</td>
<td>AF</td>
<td>19.33 ± 2.60</td>
<td>9.365</td>
<td>3.314</td>
<td>2.800–3.828</td>
<td>0.9095</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>6</td>
<td>CNF</td>
<td>30.64 ± 3.69</td>
<td>10.013</td>
<td>3.477</td>
<td>2.092–4.861</td>
<td>0.9050</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>6</td>
<td>CF</td>
<td>33.63 ± 1.69</td>
<td>15.973</td>
<td>5.139</td>
<td>2.711–7.567</td>
<td>0.8703</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>6</td>
<td>ANF</td>
<td>31.74 ± 1.54</td>
<td>9.015</td>
<td>3.177</td>
<td>(&lt;10.834–7.189)</td>
<td>0.4341</td>
<td>Isometric</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>6</td>
<td>AF</td>
<td>31.67 ± 2.69</td>
<td>10.101</td>
<td>3.480</td>
<td>1.764–5.196</td>
<td>0.8599</td>
<td>Isometric</td>
</tr>
</tbody>
</table>

Fig. 5. Log oxygen (mg/L/hour) vs. log weight (g) for each species in 2015 in control and acidified treatments, all of which were fed (top), and the four treatments in 2016. Key: CF = Control Fed, CNF = Control Not-fed, AF = Acidified Fed, and ANF = Acidified Not-fed (bottom).
3.3.2. 2016 experiments

As in the 2015 experiments, oxygen consumption did not vary significantly among treatments in any of the species. Average oxygen consumption for *M. calcarea* varied from $4.26 \times 10^{-8}$ to $2.28 \times 10^{-7}$ mg/L/h. Values for the two *Astarte* species averaged between $3.00 \times 10^{-8}$ to $1.86 \times 10^{-7}$ mg/L/h for *A. montagui*, and between $1.27 \times 10^{-7}$ and $2.81 \times 10^{-7}$ mg/L/h in *A. borealis*.

All species in all treatments show a similar relationship with oxygen utilization scaled to biomass (Fig. 5). As in 2015, the *A. borealis* specimens were larger than the other two species, and clustered away from the other species consumption values.

4. Allometric growth

4.1. 2015 experiments

Allometric growth equations were calculated for all species both before and after the exposure to experimental conditions (Table 3). *M. calcarea* collected from the ML 5–10 station exhibited negative allometric growth (pre-experiment). *M. calcarea* held in both the control and acidified treatment also displayed negative allometric growth (post-experiment) (Fig. 6). Both of the *Astarte* species showed positive allometric growth in measurements directly after collection (pre-experiment) (Figs. 7 and 8). Those kept in the control treatment displayed isometric growth, differing from the pre-experiment classification of positive allometric growth. *Astarte* maintained in the acidified treatment also demonstrated positive allometric growth, matching the pre-experiment observations. Allometric slopes were not statistically different among treatments in 2015 (Kruskal-Wallis, $p = 0.51$).

4.2. 2016 experiments

Bivalves used in the 2016 experiments were part of the same collection made at station ML 5–10 as those used in the 2015 experiments; therefore, the pre-experiment allometric relationships are the same as described above. The post-experiment characterizations indicated isotropic growth in all species and all treatments (Table 3, Figs. 6–8). Allometric slopes in 2016 were also not significantly different among treatments (Kruskal-Wallis, $p = 0.64$).

However, when the 2015 and 2016 data are combined, the individual species displayed significantly different allometric slopes (Kruskal-Wallis, $p < 0.01$).

4. Discussion

The common Chukchi Sea bivalve species we studied were generally resilient to contemporary levels of acidification even with the addition of food limitations. Only one of the three species, *A. borealis*, showed a significant response in length to decreased pH. These results support the principle that response to declining pH will be species-specific, and that at least two of the species here are generally resilient to current levels of ocean acidification. Because the experiments were undertaken under laboratory conditions, factors such as seasonal variation in food supply were not practical to control for, although it is thought that many benthic invertebrates in this Arctic region take advantage of organic matter that is re-worked by bacteria and available throughout the year (Lovvorn et al., 2005).

*M. calcarea* displayed no significant responses that we measured to decreased pH in 2015, and in 2016 showed a percent decrease in length...
in the unfed condition, but demonstrated no significant effects in response to decreased pH alone. In 2015, *M. calcarea* displayed a negative percent change in length in both the control and acidified treatments, but in 2016 the individuals held in the control, fed treatment (planned as identical to the 2015 control treatments) displayed a positive percent change in growth. Two factors could help explain this difference. First, the differences in acclimation time as described in Section 2.6 could have an effect, as *M. calcarea* used in the 2015 experiments were held for less time in controlled cold room conditions. Second, the discrepancies between years could be due to the differences in pH between the control treatments each year. The control pH in each experiment was targeted to be similar a (8.1–8.2), but in 2015 the average control pH was 8.05 and in 2016 it was 8.19. The difference in averages could be due to varying batches of Instant Ocean™ used in the two different years, as well as acquisition of experience in the best ways to manipulate the pH in the small experimental tanks through water additions from the large stock tanks. Care was taken to ensure that control and acidified tanks stayed at significantly different pH values. The 8.05 value could have been a little too low to accurately reflect control growth treatments for *M. calcarea*, although in the study reported by Fabry et al. (2008), this pH is considered the present average ocean pH. However, we did not observe a significant difference in weight or length changes between the two pH treatments in either 2015 or 2016, suggesting that *M. calcarea* is likely resilient to all of these pH values over seven and eleven week exposure intervals. Allometrically, *M. calcarea* (Fig. 6) showed negative allometric growth in 2015, meaning length increased faster than weight, in both the pre- and all post-experiment measurements. In 2016, post-experiment allometric determinations for *M. calcarea* in all four treatments showed isometric growth.

These determinations all differ from the pre-experiment values in which *M. calcarea* displayed negative allometric growth. The replication for each species in each aquaria during this set of experiments was low (three *M. calcarea*). With this limitation associated with replication, the confidence intervals generated by the slope calculations were large; all slopes overlapped with a value of three (Table 3). Additionally, clams used in the 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 controlled cold room conditions, including the constant food supplied prior to the experimentation.

*A. montagui* displayed no significant effects to decreased pH or limited food in either of the experiments for length, weight, respiration, or allometric slope. Allometrically, the control treatment and the pre-experiment measurements differed in classification. Individuals held in the control treatments displayed isometric growth following the experiment, while the pre-experiment measurements and measurements from the acidified treatment exhibited positive allometric growth, where the weight increased faster than length (Figs. 7 and 8). We collected these organisms in the late summer (August 2015), when acidification has been observed in the Chukchi Sea; therefore, the *Astarte* we 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. Because clams were acclimated to cold room settings for two months before the start of experiments, the conditions observed in the acidified aquaria were not likely products of the growth patterns at collection. *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 (e.g. maintenance of internal organisms) faster than length. Again in 2016, *A. montagui* from all four treatments displayed isometric growth, likely due to low replication as described in the *M. calcarea* paragraph above (seven individuals per treatment).

*A. borealis* displayed no significant changes in response to decreased pH after 7 weeks in 2015, but did display a significant negative effect in percent change in length to decreased pH (∼0.23 Δ = 1.06 Δ) in 2016, suggesting that the longer experiment was necessary to detect an influence of acidified conditions on shell lengths. It is just conjecture on our part, but in this species three months may be the time frame in which negative effects begin to appear because of the difference in results between the 2015 and 2016 experiments and since the 2016 experiments approached 3 months in length. For comparison, Schram et al. (2016) found very minimal negative effects of ocean acidification (pH 7.8) on two Antarctic gastropod *Nacella concinna* (2–12% Δ in shell length) and the snail *Margarella antarctica* (1–4% Δ in shell length). The specimens after six weeks, which is a similar time interval to our 2015 experiment. However, these six and seven week intervals are a short amount of time in the long life spans and slow growth patterns of these organisms. The % Δ in *A. borealis* was smaller than Schram et al. (2016) observed in Antarctic bivalves, so while our 2015 differences are not significant, the individuals held in the acidified treatment both showed negative percent changes while those held in the ambient pH both showed a positive percent change, potentially suggesting that the differences are due to declining pH values. In bottom waters of the Chukchi Sea, Mathis and Questel (2013) found decreased pH (7.7–7.9) in October and Δμ\text{\textit{aq}} < 1 for September and October, an acidification event arising from the interaction of seasonal bottom water organic carbon mineralization and anthropogenic CO2. If those trends persist into winter months, aragonite undersaturations could persist longer than eleven weeks. Allometrically, *A. borealis* showed the same trends as *A. montagui*.

The three species showed different responses to decreased pH and diminished food, suggesting a species-specific response. *M. calcarea* generally reach up to about 45 mm in length and have shells comprised of aragonite (Wassenaar et al., 1988). They are both deposit and
suspension feeders that live in muddy/gravelly benthos, and have free swimming pelagic larvae. The two *Astarte* species have similar characteristics. Both are benthic suspension feeders, with 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). As is the case with *M. calcarea*, both astartid bivalves produce an aragonite shell (Majewski, 1974; Simstich et al., 2005). The three species are very similar, suggesting the difference in response lies in an internal mechanism, genetic makeup, or energy allocation that should be explored further in future work.

Several studies have shown ocean acidification has a synergistic effect with increasing water temperatures, although not tested here. Warming alone has been shown to lead to increased metabolic costs (O’Connor et al., 2009), as well as increased prey consumption (Sanford, 1999). We measured oxygen consumption as a proxy for metabolic costs, but saw no significant difference among food or pH treatments in any of the species, which suggests, in general, the organisms were not metabolically stressed. This could be due to the increased food availability compensating for stress from the decreased pH, which was suggested in Ramajo et al. (2016). For those organisms that were not fed, the duration without food may not have been long enough to cause metabolic stress, as these animals naturally survive where food is limited over the winter months, a condition that they would have adapted mechanisms through time to overcome.

Our oxygen consumption results appear to be consistent with other studies in that the effect of long-term, moderate CO₂ exposure has been found to have a minimal effect on oxygen consumption (Pörtner, 2008). However, Pörtner (2008) suggested that minor changes in oxygen consumption, while not significant, could still affect the organism’s performance as it is tied closely with other activities including growth, neural functioning, regulation of body fluid pH, and intracellular pH. In order to evaluate these potential tradeoffs, we categorized allometric growth as isometric, positive, or negative. The slope of the linearized allometric relationship varies for species, and can also fluctuate among different conditions a given species is exposed to throughout the year. Therefore, the relationship must be determined empirically for each species and system of interest, and not universally applied (Glatzer, 2005; Seibel, 2007). The value of determining the relationship for each system of interest is shown in the differences in allometric relationships of *M. calcarea* between those collected at Station UTN 2 (southeastern Chukchi Sea), and those collected at Station ML 5–10 (northeastern Chukchi Sea) (Table 3). The allometric slopes among the species were significantly different, suggesting they were allocating energy in different ways. The apparently different allocations of energy could explain why *A. borealis* responded negatively to acidified conditions after eleven weeks, while the other species did not. Studies investigating further details of calcification and ion regulation within each of these species may provide a better understanding of what tradeoffs and negative responses may occur under acidified conditions.

Decreased pH in the bottom waters in the Chukchi Sea currently occurs in the summer and fall (Mathis and Questel, 2013; Yamamoto-Kawai et al., 2016) well after early season phytoplankton settles to the seafloor (Mathis and Questel, 2013). This carbon addition stimulates benthic carbon cycling, leading to an influx of inorganic carbon in bottom water following organic carbon mineralization (Mathis et al., 2014). In a meta-analysis Ramajo et al. (2016) showed that a steady food supply can reduce the effects of ocean acidification to growth and calcification. Given changing sea ice dynamics and warming temperatures in the Arctic, the timing and intensity of phytoplankton blooms, the food supply for the three species (Grebmeier et al., 2015), and zooplankton grazing behavior (Coyle et al., 2007) will all likely change.

As these physical changes occur, both increases and decreases in primary production are possible, and increased zooplankton grazing could occur. As seasonal sea ice extent decreases (Frey et al., 2014), more open surface area is exposed, allowing for increased sunlight penetration that could increase primary production (Arrigo et al., 2008, 2011). An increase in primary production could lead to more organic carbon exported to the seafloor, i.e., a larger food source, potentially leading to more remineralization and an intensification of seasonal decreases of pH in bottom waters. However, under high food and low pH scenarios, the growth rate of animals tends to increase, although an upper limit is approached when pH decreases by 0.6 units or more (Ramajo et al., 2016). In this study, *A. borealis*, however, showed a percent decrease in growth in both acidified conditions (fed and non-fed).

A decrease in primary production is also possible due to changes in the timing of ice edge blooms, and warming and freshening of the upper seaway waters that may increase stratification. This could cause a depletion in nutrient resupply from bottom waters that could decrease overall primary production (Grebmeier et al., 2006). A reduction in primary production will decrease the organic carbon deposition to the seafloor and thus decrease benthic remineralization, potentially limiting how low pH will drop due to natural recycling processes in bottom waters (Grebmeier et al., 2006). Additionally, organic deposition to the seafloor could decrease in both quality and quantity from an increase in zooplankton growth, abundance, and grazing (Coyle et al., 2007) due to increasing temperatures and earlier warming. Organisms exposed to a low food supply, but not as extreme a pH decrease, generally saw reduced growth rates (Ramajo et al., 2016), which held true in this study for all species, except *A. montagui* which showed an average increase in length in the no food, acidified treatment.

Timing of the phytoplankton blooms may also change (Grebmeier, 2012; Grebmeier et al., 2015) and this could change when ocean acidification events occur. For example, if the likelihood of a fall bloom increases due to wind mixing of open water when sea ice was formerly present, there is the potential for ocean acidification events to extend over the winter months, when there is no newly produced food, but continued benthic carbon cycling. 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 the fed versus unfed treatments, with those fed showing an increase percent change in length, and those not fed showing a percent 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 lowered pH, leading to the low food, low pH growth conditions described by Ramajo et al. (2016). Although there was no indication of a multiple stressor scenario (combined effects of low pH and decreased food) observed here, winter conditions persist longer than the eleven weeks of this experiment.

While this study used only adult bivalves, life history stage responses to declining pH are likely to vary (Byrne, 2011). All three bivalve species used in this study have pelagic larvae. Therefore, when discussing adults vs. 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 aragonite undersaturation on the bottom (Bates and Mathis, 2009; Mathis et al., 2011a, 2011b; Cross et al., 2012). However, in general the larval stage is believed to be the most vulnerable for many species (Kurihara and Shirayama, 2004a; Kurihara et al., 2004b; Dupont et al., 2008; Kurihara, 2008; Brennand et al., 2010; Byrne, 2011); therefore studies of larvae are needed. Studies that are able to test larval survival under carbonate undersaturation in the spring and early summer, when spawning occurs, could determine if these conditions are detrimental.

The interaction between ocean acidification and warming is still widely uncertain (Walther et al., 2010; Harvey et al., 2013; Glandon and Miller, 2016), although more data are available on this subject than for the combined effects of ocean acidification and food availability. For example, Harvey et al. (2013) conducted a meta-analysis of 107 studies on ocean acidification and water warming, compared to the 12 studies in the Ramajo et al. (2016) review on ocean acidification and food
availability. Harvey et al. (2013) found that the combination of the two stressors, ocean acidification and water warming, led to a stronger response, both positive and negative, than exposure to only one of these stressors. Examining all three of these parameters (warming, acidification, and food limitation) will add to the complexities of evaluating the impacts of ocean acidification, although these factors are all interrelated. Changes to one parameter tend to have effects on the others (e.g. production of usable food may become diminished as warming increases zooplankton grazing). If warming waters increase food consumption, an even higher food supply might be necessary to combat the stress from declining pH. However, if production in the region declines, as outlined earlier, then food supply would diminish, despite the demand for food increasing. 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. 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 to evaluate organismal response. While most of the results from both sets of experiments indicated non-significant responses, there were a few significant changes or differences observed. These differences corroborate other indications that organismal responses to decreased pH and ocean acidification will be species-specific, and that while there are some minor negative effects on Pacific Arctic bivalves, the species examined here are generally resilient. Kroeber et al. (2013) noted that species-specific responses were elevated when acidification scenarios were applied to multi species assemblages, as used here, helping to explain why the results varied among the three species. These organisms are exposed to seasonally variable conditions, but if pH values decline below their natural range and exposure time increases, it is likely the minimal effects observed in these experiments may change in more prominent ways.

Although we did not evaluate biochemical mechanisms that could be impacted by ocean acidification and associated species physiological response (e.g., changes in calcification rates, osmotic changes), some implications for these other types of responses arise from the results here, which could be further explored. In the 2015 experiment, we tested for traditional acidification stress (ionoregulatory and calcification stress) decreased pH and aragonite undersaturation; whereas in 2016 aragonite was not undersaturated so only ionoregulatory acidification stress was tested with a metabolic stress (reduced food). We saw metabolic starvation stress in 2016 with the significant reduction in length in M. calcarea between the CF and CNF treatments. We observed an ionoregulatory acidification stress (pH reduction without undersaturation) in A. borealis between the ambient (CF, CNF) and acidified treatments (AF, ANF) in 2016. These observations indicate that there is value to be gained by additional, focused laboratory studies that are essential to further our understanding of ocean acidification impacts on key benthic prey that are critical to large benthivores in the Pacific Arctic continental shelves, such as walrus. The bivalves used in our study are common for the continental shelf benthic systems in the Pacific Arctic and may well be more resilient to perturbations via multiple stressors. Results here indicate that A. borealis is likely the most susceptible species. M. calcarea may also be potentially susceptible, but the results of our experiments do not lead to definitive conclusions. Finally, A. montagui is the most resilient to these changes. Notably, the density of Macoma calcarea is a key indicator of food availability for foraging walrus in both the northern Bering Sea (Jay et al., 2012) and northern Chukchi Sea (Beatty et al., 2016; Wilt et al., 2014; Young et al., 2017). Thus if it becomes more vulnerable in the future due to further declines in pH, this species could emerge as a critical link impacting walrus foraging. Focused experimental studies on physiological responses of sentinel species, such as bivalves, are needed to forecast further acidification impacts and biological response (positive and negative) to these stressors. Such experimental work would enable the evaluation of mechanisms for adapting to any changing phenology of factors influencing benthic populations, including food supply, sea ice persistence, and seawater temperatures.

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