Macroalgal-associated amphipod assemblages exhibit short term resistance to ocean acidification (#110325)

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Macroalgal-associated amphipod assemblages exhibit short term resistance to ocean acidification

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The pH of the world's oceans has decreased since the Industrial Revolution due to the oceanic uptake of increased atmospheric CO_2 in a process called ocean acidification. Low pH has been linked to negative impacts on the calcification, growth, and survival of calcifying invertebrates. Along the Western Antarctic Peninsula, dominant brown macroalgae often shelter large numbers of diverse invertebrate mesograzers, many of which are calcified. To assess how acidification may influence the survival of different members in these assemblages, mesograzers associated with the brown alga *Desmarestia menziesii* were collected from the immediate vicinity of Palmer Station, Antarctica (64°46′S, 64°03′W) in January 2020 and maintained under three different pH treatments simulating ambient conditions (approximately pH 8.1), near-future conditions for 2100 (pH 7.7), and distant future conditions (pH 7.3) for 52 days then enumerated. Total assemblage number and the relative proportion of each species in the assemblage were found to be similar across the pH treatments. These results suggest that amphipod assemblages associated with *D. menziesii* may be resistant to short term exposure to decreased pH.

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Abstract

The pH of the world's oceans has decreased since the Industrial Revolution due to the oceanic uptake of increased atmospheric CO_2 in a process called ocean acidification. Low pH has been linked to negative impacts on the calcification, growth, and survival of calcifying invertebrates. Along the Western Antarctic Peninsula, dominant brown macroalgae often shelter large numbers of diverse invertebrate mesograzers, many of which are calcified. To assess how acidification may influence the survival of different members in these assemblages, mesograzers associated with the brown alga *Desmarestia menziesii* were collected from the immediate vicinity of Palmer Station, Antarctica (64°46′S, 64°03′W) in January 2020 and maintained under three different pH treatments simulating ambient conditions (approximately pH 8.1), near-future conditions for 2100 (pH 7.7), and distant future conditions (pH 7.3) for 52 days then enumerated. Total assemblage number and the relative proportion of each species in the assemblage were found to be similar across the pH treatments. These results suggest that amphipod assemblages associated with *D. menziesii* may be resistant to short term exposure to decreased pH.

Introduction

Atmospheric CO_2 concentrations have increased by approximately 50% since the Industrial Revolution (Joos & Spahni, 2008; NOAA, Lan & Keeling, 2024) from human-derived emissions such as the combustion of fossil fuels, the production of cement, and deforestation (Malhi, Meir & Brown, 2002; Heede, 2014; Paraschiv & Paraschiv, 2020). Approximately a third of atmospheric CO_2 enters the ocean (Sabine et al., 2004; Gruber et al., 2019) where it reacts with seawater and ultimately releases free hydrogen ions, thereby lowering pH in a process called ocean acidification (OA). As a result of this process and increasing CO_2 emissions, the average ocean surface pH has decreased by 0.1 pH units and is predicted to decrease a further 0.4 pH units over the next eighty years (IPCC, 2022).

Decreased ocean pH can have a direct impact on the physiology and behavior of marine organisms, especially those that calcify (Kroeker et al., 2013; Kindinger, Toy & Kroeker, 2022). OA decreases the availability of carbonate and alters seawater chemistry, both of which can reduce an organism's ability to calcify (Hurd et al., 2009; Doney et al., 2020). For this reason, benthic ecosystems are likely to favor non-calcifying organisms as pH decreases (Andersson, Mackenzie & Gattuso, 2011). However, the transition to a non-calcified dominant community will not be immediate. Many organisms vary not only in their ability to tolerate decreased pH but also in their ability to maintain reproductive output if they survive (Doney et al., 2009; Lopes et al., 2019; Mardones et al., 2022). Variation in responses to pH changes will likely result in ecosystems undergoing successive changes of organisms, resulting in an eventual reduction in structural complexity and decreased biodiversity in benthic communities (Andersson, Mackenzie & Gattuso, 2011; Doney et al., 2020). Such communities have already been observed in regions along a natural pH gradient (e.g., Hall-Spencer et al. 2008). For instance, communities located

around volcanic CO_2 vents are dominated by of seagrasses and fleshy macroalgae rather than calcified invertebrates and coralline algae in areas closer to the vents where seawater pH is lower (Hall-Spencer et al., 2008; Koch et al., 2013) . Reduced biodiversity from OA exposure has also been observed in microbial communities (Maas et al., 2013; Nelson et al., 2020). This loss of microbial biodiversity can impact macroorganisms by reducing the settlement rate of invertebrate larvae from a likely decrease in settlement cues (Nelson et al., 2020).

Many invertebrates are sensitive to OA because they rely on calcification processes to create important external body structures like exoskeletons, spicules, feeding structures, etc. OA can also lower calcification (Anand et al., 2021; Ramaekers et al., 2023), increase mortality (Park et al., 2020), hinder growth (Sheppard Brennand et al., 2010; Bhuiyan et al., 2022), increase tissue damage (Anand et al., 2021), decrease fertilization (Kurihara & Shirayama, 2004; Ericson et al., 2010; Borges et al., 2018), and lead to developmental abnormalities (Ericson et al., 2010). Marine invertebrates, are also susceptible to internal acidification of body fluids and the dissolution of shells to compensate for internal acidification (Doney et al., 2009).

Most invertebrates can compensate for increased internal proton concentrations by either consuming more food or diverting energy toward compensatory behaviors. For example, larvae of the sea urchin *Strongylocentrotus purpuratus* diverts over 40% of its total ATP to protein synthesis and ion transport under OA conditions (Pan, Applebaum & Manahan, 2015). Krill, scallops, mussels, and hard corals have all been found to increase their feeding rates to meet higher metabolic rates and to mitigate decreases in growth and calcification under decreased pH conditions (Melzner et al., 2011; Saba et al., 2012; Towle, Enochs & Langdon, 2015; Ramajo et al., 2016). However, merely consuming more food may not provide enough energy for compensatory behaviors, especially if their food source is also impacted by OA. This may result

in invertebrates reallocating energy away from growth and reproduction potential to be invested in compensatory behaviors (Whiteley, 2011; Mardones et al., 2022).

Most OA work has focused on single species making current understanding of single organism responses to OA better known than community or ecosystem level responses (Doney et al., 2009, 2020). However, the response of the community can buffer the impacts of OA on a particular species (Hendriks, Duarte & Álvarez, 2010) if biodiversity is high. The insurance effect describes a situation where a community is protected and stabilized by high biodiversity that ensures that a subsection of the community can maintain its functional roles even if another subsection of the community fails (Yachi & Loreau, 1999). In practice, high biodiversity has been shown to lower the negative impacts of OA on vulnerable organisms in hard bottom communities by 50-90% depending on the species (Rastelli et al., 2020). However, the insurance effect can only be effective if biodiversity remains high. Changes to the environment can reduce the relative efficiency of resistant species and weaken the insurance effect of biodiversity (Eklöf et al., 2012).

Marine environments in high latitudes, such as the waters around the Western Antarctic Peninsula (WAP), are notably vulnerable to decreases in pH from CO_2 absorption. Cold, high latitude waters have a higher Revelle factor (Revelle & Suess, 1957; Sabine et al., 2004) and, therefore, lower buffering capacity (Jiang et al., 2019). Furthermore, saturation states of aragonite and calcite tend to be lower in polar regions due to a higher K_{sp} from lower water temperatures (Jiang et al., 2015; Cai et al., 2021).

Macroalgae often dominate shallow, hard bottom communities along the WAP (Wiencke, Amsler & Clayton, 2014). These macroalgal forests shelter large numbers of mesograzers (Huang et al., 2007; Aumack et al., 2011b; Amsler et al., 2022) in assemblages that mostly

consist of amphipods with large numbers of gastropods and smaller numbers of isopods, copepods, and ostracods (Iken, 1999; Huang et al., 2007; Schram et al., 2016a; Amsler et al., 2022). The density of amphipods on macroalgae in this region can be extremely high. Stands of *Desmarestia menziesii*, a dominant brown alga, can shelter an estimated 300,000 individuals m^2 of the benthos and an estimated 26,000 individuals m^2 on the red alga *Plocamium* sp. (Huang et al., 2007; Amsler, McClintock & Baker, 2008). These densities of amphipods are one to three times higher than those typically reported in tropical and temperature regions (Amsler, McClintock & Baker, 2014).

Mesograzer assemblages, particularly amphipods, have a mutualistic relationship with the macroalgae they shelter on (Amsler, McClintock & Baker, 2014). Most of the macroalgal community is chemically defended against herbivory, including all of the large browns and many of the common reds with the notable exception of *Palmaria decipiens* (Amsler et al., 2005; Aumack et al., 2010; Amsler, McClintock & Baker, 2020). Instead of consuming their macroalgal hosts, amphipods consume epiphytic algae and emergent filamentous endophytes from their hosts (Aumack et al., 2011a) and gain refuge from predatory fish in return (Zamzow et al., 2010). These amphipods are so effective at removing epiphytes that macroalgae in the WAP usually lack visible epiphytic filaments in areas with amphipod grazers (Peters, 2003; Amsler et al., 2009). The removal of filamentous algae improves the overall health of the macroalga by removing competition for light that arises when the filaments grow out of the algal thallus (Aumack et al., 2011a).

A significant shift in the amphipod-macroalgal dynamic could have major consequences on the structure of the benthic community (Rodriguez & Saravia, 2024). Changes to the environment, such as OA, could impact the relationship between macroalgae and amphipods.

Crustaceans located in polar regions are likely sensitive to OA due to these species having historically low metabolic activity and low temperature habitats (Clarke, 1998; Whiteley, 2011). Amphipod communities in polar regions have shown assemblage reorganization in lowered pH waters by increasing the relative abundances of copepods and ostracods and decreasing the relative abundance of the amphipods *Metaleptamphopus pectinatus* and *Oraderea* spp. (Schram et al., 2016a). Part of this reorganization may be occurring due to physiological challenges faced by amphipods. A similar OA study by Schram et al. (2016b) found the most noticeable spikes in mortality among the amphipods *Gondogeneia antarctica* and *Paradexamine fissicauda* maintained under OA conditions corresponded with the time of highest molt frequency.

More information is needed to understand why some amphipod species in these assemblages are more resistant to OA than others. In the present study, we assessed how the survival of different members of a macroalgal-associated amphipod assemblage were impacted by OA conditions. Assemblages were maintained under three different pH treatments simulating ambient conditions (approximately pH 8.1), near-future conditions for 2100 (pH 7.7, IPCC 2019), and distant future conditions (pH 7.3, IPCC 2019) for 52 days then enumerated. This assessment was used to determine which amphipod species were comparatively more resistant to OA.

Materials & Methods

Collection of macroalgae and amphipod assemblage

The brown macroalga *Desmarestia menziesii* and its associated mesograzer assemblage were collected from five sites near the U.S. Antarctic Research Program's Palmer Station on Anvers Island on the western Antarctic Peninsula. *Desmarestia menziesii* was collected by scuba from Palmer Station pier (S64°46.477′, W64°03.274′), Amsler Island (S64°45.629',

W64°05.879), Litchfield Island (S64°46.095′, W64°03.025′), Stepping Stones (S64°47.031′, W63°59.453′), and Christine Island (S64°47.479′, W64°01.024′) between 4 and 11m on 21-23 January 2020. *Palmaria decipiens* was collected on small rocks from the Palmer Station pier on 20 and 21 January 2020 and from Hero Inlet (S64°46.555′, W64°02.944′) on 23 January 2020.

Amphipods for the experiment were collected using the methods described by Huang et al. (2007). Briefly, *D. menziesii* thalli were cut with a knife then gently floated into a fine mesh bag to avoid disturbing the associated grazers. Collection bags were immediately transported to Palmer Station in 19-L buckets filled with seawater. Grazers were removed from *D. menziesii* by repeatedly rinsing the alga with seawater in a series of fine mesh bags submerged in a tank of seawater. After the final rinse, any remaining grazers were removed by hand.

The collected fauna from *D. menziesii* was pooled to create a sample representative of the local grazer assemblages around Palmer Station. Individuals of the carnivorous amphipod *Bovallia gigantea* larger than approximately 1.5 cm were removed from the pool to prevent predation on other species during the experiment. The sample was divided into thirty-two equal aliquots with a Folsom plankton splitter to generate an approximate grazer number and diversity representative of that associated with 85g of *D. menziesii* in the original collections. Aliquots were randomly assigned and placed in either one of the twenty-four experimental buckets or one of the eight initial samples preserved for later identification and enumeration of species.

Experimental setup

Two adjacent aquarium tanks (2.5 m diameter and 1 m depth; 3800 L) in the aquarium facility at Palmer Station were equipped with twenty-four 19-L white plastic buckets. The experimental setup was maintained on a 24-h light cycle consistent with light availability at the time of collection. An ambient flow-through seawater bath was plumbed for both tanks to

maintain an ambient temperature in all experimental buckets. Nylon mesh (75 μm) was fixed to the outflow pipes in the buckets to prevent grazers from flowing out of the experiment.

An aliquot of the grazer assemblage and an approximately 85g wet weight of *D. menziesii* thallus was placed in each bucket. To provide a food supply for the grazers, *D. menziesii* were seeded with diatoms and held in outdoor tanks with unfiltered seawater for over three weeks to allow the growth of an epiphytic diatom assemblage on the thalli. Plankton mesh (63 μm, regularly cleaned) was placed over the water inlets and standpipe drains to prevent additional mesograzers entering buckets with the unfiltered water. The epiphytes were grown in higher abundance than observed *in situ* to ensure a consistent food supply over the experiment. In addition to *D. menziesii*, a *P. decipiens* blade was placed into each bucket as an alternative food source for the assemblage as some, but not all, of the amphipod species consume this non-chemically defended red alga (Amsler, McClintock & Baker, 2020).

A common head tank dispersed filtered seawater into a water distributor located in the center of each aquarium tank. Water flowed into twelve mixing reservoirs from a distributor located in the center of each aquarium tank. In addition to the water inflow pipe, each mixing reservoir also contained a pH probe, a tube with mixed air and compressed CO_2, and a tube with compressed air alone. Seawater pH was monitored and adjusted to the desired pH in the mixing reservoirs then flowed into the experimental buckets before flowing out of the buckets and into the seawater bath.

Eights buckets were assigned for each pH treatment [ambient (8.1), 7.7, and 7.3] based on the average pH recorded for Palmer Station during collection (approximately 8.1) and the predicted pH levels for the near and distant future (IPCC, 2022). Replicates in the decreased pH treatments were individually regulated with an automated pH monitoring system (Schoenrock et

192 al., 2016; Schram et al., 2016a). Bucket pH was lowered and maintained by bubbling an air-CO_2
193 mixture or air alone into the mixing reservoirs as needed. A Multi-tube Gas Proportioning
194 rotameter (Omega Engineering, Inc.,Stamford, CT, USA) connected to multiple air pumps and a
195 CO_2 cylinder combined ratios of air and CO_2 to create the air-CO_2 mixture. Half of the replicates
196 for each treatment were randomly assigned to each water bath to control for differences in light
197 availability due to the location of the water baths. Gas exchange with the atmosphere was
198 reduced by fixing a clear Plexiglass cover to each bucket (Schram et al., 2016a).
199 A quarter of the buckets had seawater samples collected daily for pH (determined
200 spectrophotometrically) and seawater total alkalinity (TA) measurements (determined by
201 potentiometric titration; Dickson et al. 2007). This resulted in all buckets being tested over a
202 four-day cycle. Additionally, the pH of every bucket was monitored daily with a hand-held pH
203 probe.
204 After the grazers and macroalgae were placed in the buckets, the pH was slowly
205 decreased in the lower pH treatments over a 28-hour period. Following a 52-day exposure, *D.*
206 *menziesii* were removed from the buckets. Each alga was successively rinsed in 19-L buckets of
207 seawater to remove the grazers and subjected a visual inspection to ensure grazers were fully
208 removed. The collected mesograzer assemblages were preserved in 10% formalin and later
209 identified to the lowest taxon possible using a dissecting microscope and enumerated.
210 **Carbonate chemistry determination**
211 Seawater pH was determined using a Perkin Elmer UV/VIS Spectrometer Lambda 40P
212 on the total hydrogen scale (pH_T) after the addition of m-cresol purple, a pH sensitive indicator,
213 (Dickson et al., 2007). TA was measured using open cell potentiometric titration (SOP 3b,
214 Dickson et al. 2007). Seawater samples were maintained at 20°C using a Neslab RTE-7

Circulating Bath (Thermo Scientific, USA). Titrations were completed utilizing a Mettler-Toldeo T50 open cell titrator equipped with a pH probe (Model DGi 115-SC) while Mettler-Toledo LabX® software recorded titrant volumes in real time. Temperature, salinity, pH, and TA data were used to calculate carbonate chemistry parameters. CO2calc software (Robbins et al., 2010) with CO_2 constants from Roy et al. (1993) and a $KHSO_4$ acidity constant from Dickson (1990) were used for carbonate calculations. Salinity was measured with a Seabird 45 MicroTSG from the Palmer Station Waterwall (Palmer Station Instrument Technician, 2023) and temperature was recorded during seawater sample collection.

Statistical Analyses

Univariate statistical analyses were performed with R v4.4.0 (R Core Team, 2024) using the *vegan* (Oksanen et al., 2024) and *stats* package (R Core Team, 2024). Normality and homogeneity of variance were tested using the Shapiro-Wilks test and Levene's test, respectively. A one-way analysis of similarity (ANOSIM) quantified similarities of the amphipod assemblages across the pH treatments. Metric multidimensional scaling (mMDS) was used to compare and visualize the similarity of the amphipod assemblage compositions. A one-way analysis of variance (ANOVA) was performed to examine the abundance of taxa between the experimental groups. The cutoff for statistical significance was set to $\alpha \leq 0.05$. A post-hoc power analysis was conducted using the observed effect size from the ANOVA and Kruskal-Wallis tests (ranging from 0.0132 to 0.116) with the *pwr* package (Champely, 2020).

Nonparametric, multivariate analyses using PRIMER-e v. 7 (Quest Research Limited) were performed to compare macroalgal and overall species assemblages across sample sites with statistical methodology following recommendations of Clarke et al. (2014). Because species numbers varied by several orders of magnitude across samples, these data were square-root

transformed to down-weight the influence of the most abundant species. To visually compare similarity among initial and experimental amphipod assemblages, non-metric multidimensional scaling (nMDS) ordination plots were created based on Bray-Curtis similarity matrices. Statistical differences were determined using CLUSTER analysis with similarity profile (SIMPROF) tests ($\alpha = 0.05$). To further visually compare amphipod assemblages in the pH treatments, bootstrap averages and bootstrap regions were calculated within a metric multidimensional scaling (mMDS) ordination plot created on Bray-Curtis similarity matrices.

Results

Seawater parameters during the experiment are summarized in Table I. Mean (± SD) pH values of the lowered pH treatments were close to their target pH. Data for each parameter (pH, total alkalinity, salinity, DIC, etc.) on each day of the experiment are available at the US Antarctic Program Data Center (see Details of Data Deposit statement, below). Total alkalinity, temperature, and salinity remained similar between the pH treatments.

No significant differences between the amphipod assemblages at different pH was revealed by an ANOSIM. This type of test produces an R-statistic that ranges from -1 and 1 with values close to 0 indicating no difference between the groups. The data in the present study produced an R-statistic of -0.02, demonstrating that assemblages between the pH treatments were very similar to each other. The nMDS analyses also did not show a significant difference in the pH treated assemblages but did show a significant difference (SIMPROF test) between the initial samples and treatment groups (Figure 1). However, the bootstrap analysis of the mMDS data (Figure 2, supplementary video) did reveal that the amphipod assemblages were beginning to separate into distinct pH groups when analyzed at the completion of the 52-day experiment. The

total abundance of all mesograzers and total abundance of amphipods both generally decreased with decreased pH but neither trend was significant (Figure 3).

The relative proportions of each taxon across the pH treatments were not found to be significantly different (Figure 4). However, some general, nonsignificant trends were observed. *Djerboa furcipes* and *Bovallia gigantea* both showed resilience to decreased pH. *Bovallia gigantea* had a similar abundance across the pH treatments while *D. furcipes* generally increased in proportion with decreasing pH. *Gondogeneia antarctica* and *Metaleptamphopus pectinatus* both fell into an intermediate group. *Gondogeneia antarctica* experienced a decrease in its relative abundance from ambient pH to pH 7.7. However, its relative abundance was similar between the pH 7.7 and pH 7.3 treatments. *Metaleptamphopus pectinatus*, in comparison, initially increased in relative abundance as pH decreased to 7.7, but its relative abundance decreased to a similar level as in the ambient treatment at pH 7.3. Finally, both *Oradarea* spp. and *Prostebbingia gracilis* slightly decreased in abundance with decreased pH. A post-hoc power analysis indicated that the study only had an average of 5.84% power to detect a statistically significant effect at $\alpha \leq 0.05$.

Discussion

Macroalgal-associated mesograzer assemblages were exposed to three pH treatments simulating current ambient conditions (pH 8.1), end of the century conditions (pH 7.7, IPCC 2019), and distant future conditions (pH 7.3, IPCC 2019) for 52 days. Although all experimental treatments were significantly different from the initial assemblages, there was no significant difference in total abundance or species proportion between any of the pH treatments. These non-significant results could be explained by the experiment being underpowered to detect a meaningful result with an average 5.84% power. It should be noted that these post-hoc

calculations should be interpreted with caution since they were computed with observed data and do not provide a true effect size. However, since the amphipod assemblages among the treatments had become somewhat divergent in a bootstrap analysis, it is possible that they could have become significantly different had logistical constraints allowed a longer experimental duration.

Some trends in relative amphipod abundances were noted, but many of these trends were not consistent with the findings of a similar study by Schram et al. (2016a). Both our study and Schram et al. (2016a) found that the abundance of *B. gigantea* stayed consistent across the pH treatments. A decrease in the abundance of *Oradarea* spp. between the ambient and pH 7.3 treatment was also found in both studies. The proportion of *M. pectinatus* in this study increased in the pH 7.7 treatment but was similar between the ambient and pH 7.3 treatments. Schram et al. (2016a), however, found a significant decrease of *M. pectinatus* with decreased pH. Opposite trends were found in *P. gracilis* with the species decreasing with decreased pH in our study but increasing with decreased pH in Schram et al. (2016a).

One possible explanation for the discrepancy between the two studies is the starting assemblage organization. Although both studies sampled in similar locations using the same methods, species compositions varied. For example, *D. furcipes* constituted a large proportion of our assemblages, approximately 11% in the ambient treatment, but was not present in Schram et al. (2016a). Furthermore, *M. pectinatus* and *Oradarea* spp. abundances were found to be ten-fold and two-fold higher, respectively, in the assemblages of Schram et al. (2016a) compared to this study. The starting assemblage composition could have had an impact on the final assemblage composition in the experimental treatments. Collection times varied between the two studies with collections occurring in January 2020 in the present study and in March 2013 in Schram et

306 al. (2016a). Seasonal or interannual differences in species abundances could explain why the
307 starting assemblage composition varied between the two studies.

308 Another possible explanation for these varied results could be the length of the
309 experiment. The experiment in Schram et al. (2016a) ran for 30 days while our experiment ran
310 for 52 days. This difference in experiment time could explain why some species, like *M.*
311 *pectinatus*, were so comparatively low in our experiment. Our initial samples contained, on
312 average, over 300 *M. pectinatus*. However, most of the final assemblages across each of the
313 experimental treatments contained less than fifteen *M. pectinatus*, demonstrating a massive
314 amount of mortality from being held in the experiment. Part of this mortality could be due to the
315 size of *M. pectinatus*. This species is generally small in size, making it vulnerable to predation by
316 the small number of *B. gigantea* in the experiment. Final assemblage composition could have
317 been impacted by increased mortality from being held in the experiment for a longer period of
318 time. This reduction in *M. pectinatus* could also partially explain why our results differed from
319 Schram et al. (2016a) since a majority of the dissimilarity of the pH 7.3 assemblage in their
320 experiment was driven by low *M. pectinatus* abundance.

321 Our results indicate that amphipod assemblages associated with *D. menziesii* exhibit
322 resistance to short-term exposure to near future and distant future OA conditions. These results
323 are in contrast to the reduction in species richness and abundance of crustacean assemblages
324 with decreased pH (Hale et al., 2011; Kroeker et al., 2011; Fabricius et al., 2014). However,
325 mesocosm experiments have been found to be less sensitive in detecting species replacements,
326 community reshuffling, or biodiversity changes in response to OA compared to natural systems
327 (Nagelkerken & Connell, 2022). A longer experiment is likely necessary to gain a better
328 understanding of how OA impacts macroalgal-associated amphipod assemblages. Originally, we

planned on having a longer exposure period for the amphipod assemblages. The unexpected COVID-19 pandemic forced us to end the experiment prematurely. Even with this shortened experiment time, bootstrap nMDS data (Figure 2) shows that the assemblages were beginning to separate into separate groups, even if this result was not significant. A significant increase in mortality of the amphipods *G. antarctica* and *Paradexamine fissicauda* has been found after a three-month exposure to OA conditions (Schram et al., 2016b). The mortality of *G. antarctica* in this longer experiment was higher than the mortality reported in Schram et al. (2016a), demonstrating that exposure time has an impact of the severity of OA affects for these assemblages. Furthermore, peaks in mortality in Schram et al. (2016b) coincided with peaks in molt frequency which could indicate that OA impacts on other physiological processes, like molting, likely impact overall survival.

While the results of this experiment show that amphipod assemblages were not impacted in relative mortality, this does not mean that the assemblages were completely unaffected to OA conditions. For example, adult Antarctic krill can maintain their survival, growth, and respiration rate under 2000 μatm pCO_2 exposure (Ericson et al., 2018). However, hatch rates and embryo survival of Antarctic krill decreases by over 90% under the same CO_2 conditions (Kawaguchi et al., 2011), demonstrating there may be unforeseen long-term impacts on species that are identified as more resistant to OA in shorter studies. In some cases, the severity of OA effects is reliant on the amount of time an organism has to acclimate. The sea urchin *Strongylocentrotus droebachiensis* experiences a 4.5-fold decrease in fecundity and a 5-9-fold decrease in offspring reaching the juvenile stage when exposed to four months of decreased pH. However, there was no difference in either fecundity or offspring survival when the sea urchins are exposed to decreased pH for sixteen months compared to ambient conditions (Dupont et al., 2013).

In addition to longer exposure across one individual's life, transgenerational exposure can have positive and negative effects on a species. Lopes et al. (2019) found that exposure to OA conditions decreased the amphipod *Gammarus locusta* parental generation's survivability and caused DNA damage in their offspring. Furthermore, the offspring that could survive OA were incapable of returning to ambient conditions without experiencing an increase in lipid damage and death. In some cases, transgenerational exposure increases an organism's ability to withstand decreased pH. Parker et al. (2015) exposed the oyster *Saccostrea glomerata* to lowered pH conditions for three generations. They found that adults that were descended from a parental generation exposed to lowered pH had better extracellular pH regulation compared to controls. Furthermore, the transgenerational exposed oysters had more resilient offspring with lower percentages of abnormalities, faster development, and faster shell growth compared to oysters that were naïve to decreased pH. Second generation clams of *Ruditapes philippinarum* exposed to decreased pH could regulate the carbonate chemistry of their calcifying fluids more efficiently than controls by switching the type of carbon they extracted from their environments (Zhao et al., 2018). The mussel *Musculista senhousia* produces larger eggs when exposed to decreased pH which in turn increases larval growth, survival, and metamorphosis in offspring exposed to the same conditions (Zhao et al., 2019).

Mesograzer assemblages along the WAP may be preconditioned to tolerate short term decreases in pH. Seawater pH along the WAP can fluctuate up to 0.6 pH units annually (Schram et al., 2015). There is a growing theoretical framework for how organisms will respond to climate change based on the magnitude and predictability of environmental fluctuations. Phenotypic plasticity and bet-hedging are the two most common adaptations that arise from fluctuating selection, but the type of adaptation that evolves is dependent on the timescale of

fluctuations (Tufto, 2015). Frequent and predictable environmental change supports the development of plastic adaptations (Botero et al., 2015). This concept has also been seen experimentally. Bitter et al. (2021) found that plasticity in response to lowered pH was higher in populations of the mussel *Mytilus galloprovincialis* which experienced more predictable pH fluctuations compared to populations in unpredictable environments. Similar findings have been observed in Antarctic mites as a response to temperature fluctuations. Deere et al. (2006) examined five mite species from terrestrial and marine environments. Mites from more stable marine environments had higher thermal tolerances compared to mites from more unpredictable terrestrial environments. The amphipod assemblages examined in the present study may have high tolerances to pH fluctuations because they are found in environments that are known to have large pH fluctuations throughout the year (Schram et al., 2015).

Amphipods may be benefiting from their close relationship with macroalgae. Seaweeds have boundary layers that can range from 0.1 to 10.2 mm thick depending on the species and surrounding water flow (Raven & Hurd, 2012). These boundary layers can serve as a refuge for calcifying species during the day by buffering seawater pH (Hurd et al., 2011). The pH within the boundary layers is controlled by seaweed metabolism. During the day, pH tends to increase in the boundary layer as photosynthesis occurs. At night, pH decreases as algae continue to undergo respiration (Hurd, 2015). The pH within the boundary layers of macroalgae and seagrasses can be 0.07 to 1.2 pH units higher than surrounding seawater during the day (Jones, Eaton & Hardwick, 2000; Krause-Jensen et al., 2015; Hendriks et al., 2017). Experimentally, macroalgae have been found to mitigate some negative effects of OA on associated calcifiers. The addition of *Ulva* in high CO_2 treatments was found to increase saturation states of aragonite and calcite and increased the growth rates of clams, scallops, and oysters (Young & Gobler,

2018). Wahl et al. (2018) found that the brown alga *Fucus vesiculosus* can act as a temporal refuge from OA conditions to the mussel *Mytilus edulis*. The mussels were able to maintain high calcification in low pH treatments by shifting a majority of their calcification process to the daytime when algal photosynthesis was occurring, and pH and calcite saturation were higher. In the present experiment, *D. menziesii* could have been acting as a refuge for the amphipods. Live *D. menziesii* thalli were maintained on a 24-hour light cycle consistent with the time of collection throughout the experiment. Photosynthesis should have been occurring continuously throughout the entire exposure period. The amphipod assemblages could have been benefiting from a possible increase in pH in the alga's boundary layer, possibly explaining why no significant difference in mortality was found for the total assemblage or within a species between the different pH treatments.

Conclusions

The results of the present study show that invertebrate mortality of a macroalgal-associated assemblage is not negatively impacted by OA. The assemblages between the pH treatments were similar in total assemblage number and assemblage composition. These results differ from previous studies (Schram et al., 2016a) and demonstrate that starting assemblage composition or exposure time could impact assemblage resistance to OA. Furthermore, the close association with these assemblages to *D. menziesii* could be mitigating some of the direct negative impacts of OA. Overall, our results suggest that *D. menziesii*-associated amphipod assemblages may be resistant to short term OA exposure.

Data Availability

Data are available at the United States Antarctic Program Data Center: https://www.usap-dc.org/view/project/p0010193.

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Figure Captions

Figure 1. Nonmetric multidimensional scaling (nMDS) plot based on Bray–Curtis similarity matrix for mesograzer assemblages without copepods or ostracods maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period and initial samples. The green circles represent groups that are not significantly different ($p > 0.5$) in a SIMPROF test.

Figure 2. Three different 3-D perspectives of a metric multidimensional scaling (mMDS) bootstrap analysis for amphipod assemblages maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period. The black markers indicate average values for each pH treatment. The colored markers do not represent data points but rather bootstrap regions, analogous to error bars on plots of univariate data. See also the supplementary video file of the plot being rotated in space.

Figure 3: Total assemblage and amphipod counts (mean ± SE) maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period ($n = 8$).

Figure 4: Relative abundance of amphipods maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period ($n = 8$). Species that consistently made up less than 1% of the total assemblage were combined and graphed as the group 'other.' This group included *Jassa* spp., *Paraphimedia integricauda*, *Gnathiphimedia* sp., and unidentifiable amphipods.

Table 1 (on next page)

Carbonate chemistry of the ambient and lowered pH treatments (mean ± SD).

Seawater parameters (n = 8) calculated from TA (µmol kg^{-1} SW), spectrophotometric pH_T (mean ± SD), temperature (°C), and salinity (ppt). Calculated parameters included pCO_2 (µatm) and saturation states of aragonite (Ω_{arg}) and calcite (Ω_{cal}).

Table 1. Carbonate chemistry of the ambient and lowered pH treatments (mean ± SD). Seawater parameters (n = 8) calculated from TA (μmol kg^{-1} SW), spectrophotometric pH_T (mean ± SD), temperature (°C), and salinity (ppt). Calculated parameters included pCO_2 (μatm) and saturation states of aragonite (Ω_{arg}) and calcite (Ω_{cal}).

	Ambient	pH 7.7	pH 7.3
pH_T	8.07 ± 0.07	7.69 ± 0.11	7.32 ± 0.08
TA	2284 ± 78	2261 ± 42	2245 ± 64
Temp	2.45 ± 0.34	2.46 ± 0.34	2.46 ± 0.33
Salinity	32.97 ± 0.29	32.96 ± 0.29	32.96 ± 0.29
pCO_2	367 ± 48	947 ± 221	2245 ± 409
DIC	2145 ± 56	2243 ± 41	2343 ± 72
Ω_{arg}	1.62 ± 0.37	0.73 ± 0.21	0.32 ± 0.07
Ω_{cal}	2.59 ± 0.58	1.17 ± 0.33	0.50 ± 0.11

Figure 1

Nonmetric multidimensional scaling (nMDS) plot based on Bray–Curtis similarity matrix for mesograzer assemblages without copepods or ostracods maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period and initial samples.

The green circles represent groups that are not significantly different ($p > 0.5$) in a SIMPROF test.

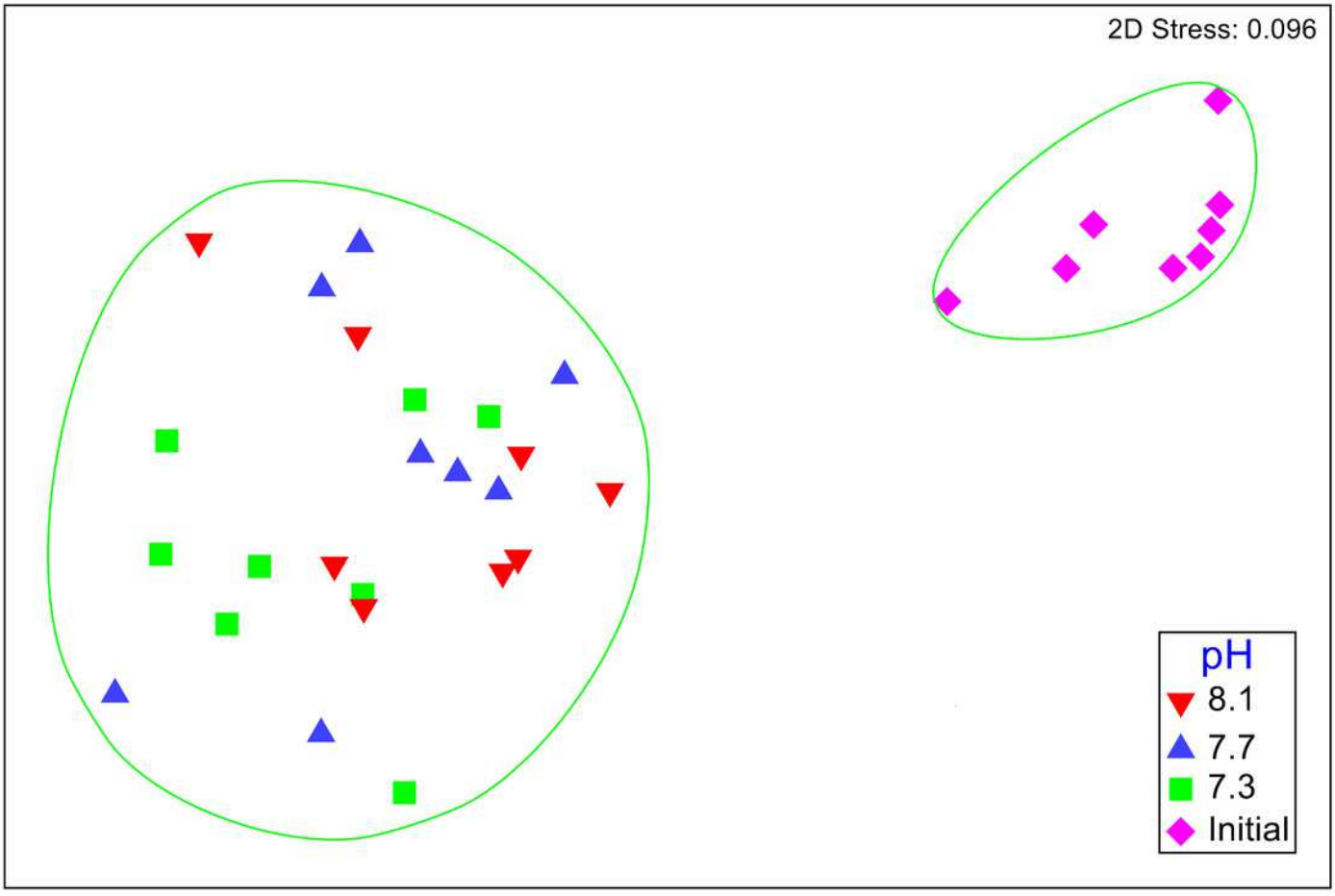


Figure 2

Three different 3-D perspectives of a metric multidimensional scaling (mMDS) bootstrap analysis for amphipod assemblages maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period.

The black markers indicate average values for each pH treatment. The colored markers do not represent data points but rather bootstrap regions, analogous to error bars on plots of univariate data. See also the supplementary video file of the plot being rotated in space.

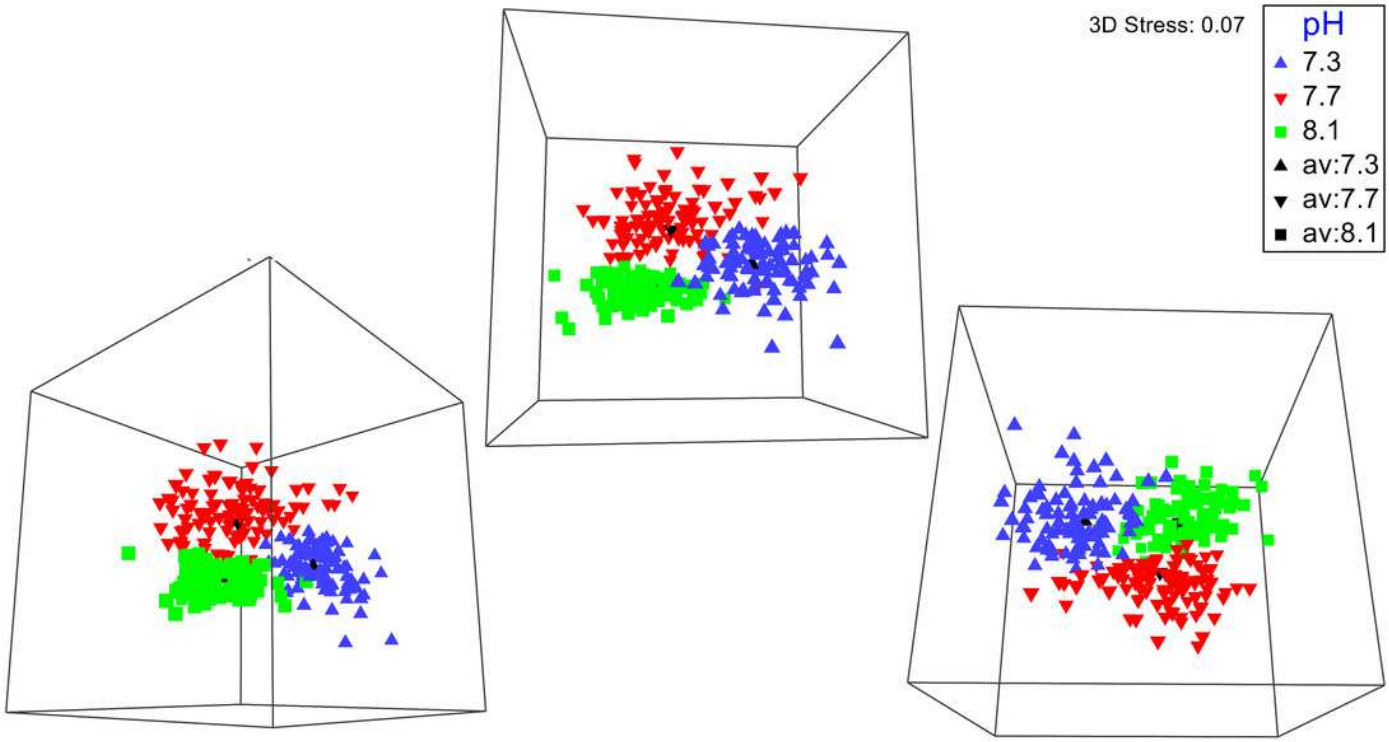
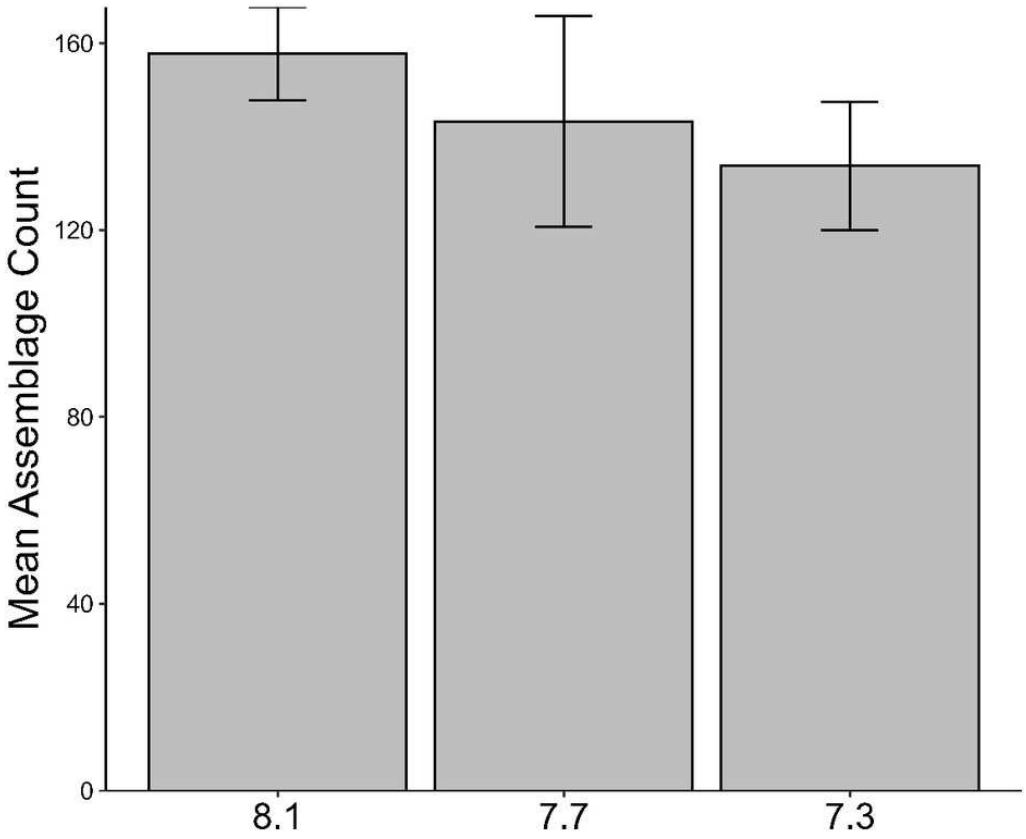


Figure 3

Total assemblage and amphipod counts (mean ± SE) maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period (n = 8).


Mean Assemblage Count
160
120
80
40
0
8.1
7.7
7.3

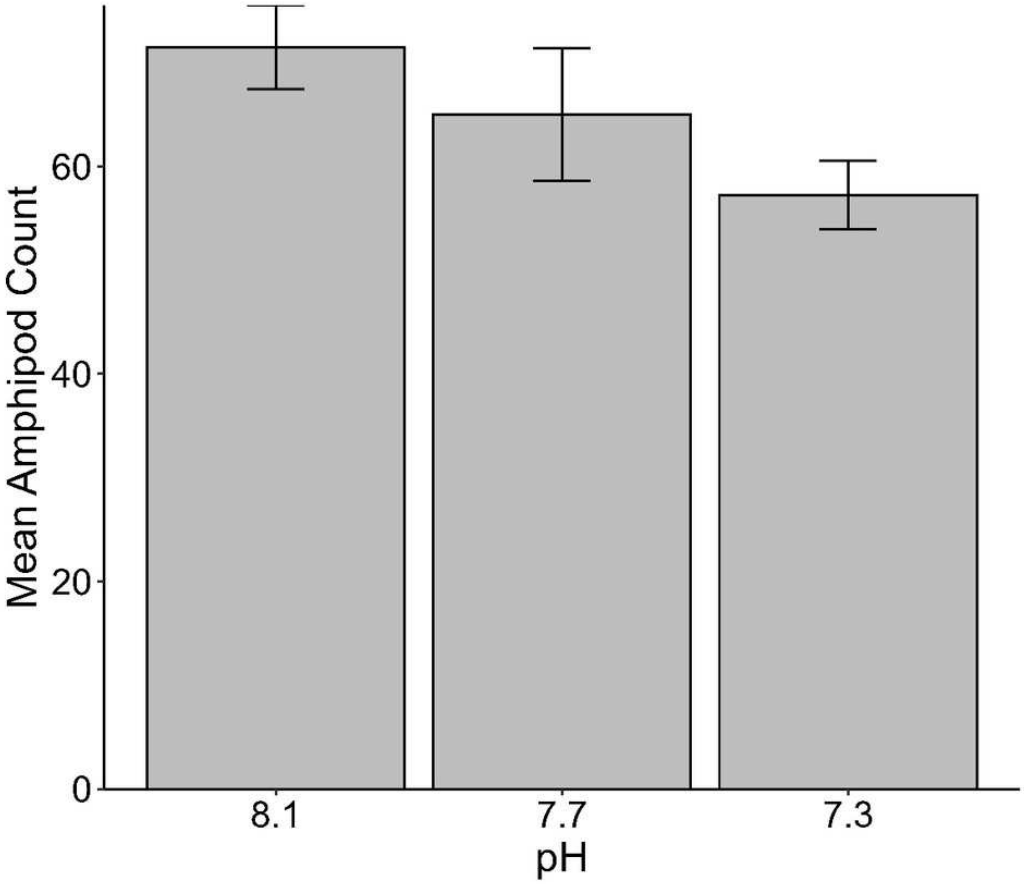

Mean Amphipod Count
60
40
20
0
8.1
7.7
7.3
pH

Figure 4

Relative abundance of amphipods maintained at pH 8.1, pH 7.7, and pH 7.3 for a 52-day exposure period (n = 8).

Species that consistently made up less than 1% of the total assemblage were combined and graphed as the group 'other.' This group included *Jassa* spp., *Paraphimedia integricauda*, *Gnathiphimedia* sp., and unidentifiable amphipods.