CLIMATE CHANGE ALTERS TROPHIC INTERACTIONS IN COASTAL ECOSYSTEMS

by

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Understanding the effects of multiple anthropogenic changes on local ecosystems is important for understanding community interactions. Because they lie at the interface between the land and sea coastal ecosystems are often heavily impacted by anthropogenic stressors and environmental change. For example, approximately one third of the anthropogenic $CO_2$ released into the atmosphere is taken up by the ocean, causing reductions in pH and in the amount of bio-available carbonate ions. Simultaneously, we are experiencing increases in sea surface temperatures. These two stressors are impacting coastal ecosystems by altering biodiversity, species phenology and distribution, community composition, and biological invasions. These changes in individual species will undoubtedly affect their trophic interactions, which might be especially important for ecological communities centered around foundation species, which stabilize and provide habitat for a multitude species. Therefore, I asked if ocean acidification and increased sea surface temperatures would impact growth and survival of the foundation species, the eastern oyster (*Crassostrea virginica*), change the nature of the trophic interactions between juvenile eastern oysters and predatory mud crabs (*Panopeus spp.*), and alter coastal community compositions. To examine these questions I setup a 2x4 experimental design where oysters were grown in one of two levels of $CO_2$ (ambient and elevated) and one of four different temperature treatments (0, 1, 2, and 3°C above ambient). Oysters alone showed decreased survival, shell height, and filtration with increasing temperature. In the presence of mud crabs, more oysters were consumed when grown in elevated $CO_2$ and increased temperature. Elevated $CO_2$ environments increased soft bodied organisms, such as *Molgula manhattensis* which can compete with oysters for food and settling space, and decreased the presence of organisms that rely on calcium ions. These results illustrate the importance of investigating trophic interactions in multiple stressor environments. These types of studies are an important step for managers attempting to understand and predict the impacts of climate change on important and in some cases economically valuable ecosystems.

# CLIMATE CHANGE ALTERS TROPHIC INTERACTIONS IN COASTAL ECOSYSTEMS

A Thesis

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Master of Science in Biology

by

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# INTRODUCTION

Anthropogenic climate change is dramatically impacting natural ecosystems (Hoegh-Guldberg & Bruno, 2010; IPCC, 2014; Walther et al., 2002). Global warming is exacerbating the release of greenhouse gasses (e.g. $CO_2$) (Raynaud et al., 1993), and these temperature increases are leading to the loss of ice sheets which are contributing to sea level rise and salt water intrusions into coastal plains (Nicholls & Cazenave, 2010). By the end of the 21$^{st}$ century, mean ocean water surface temperature is expected to have increased anywhere from 0.3 to 4.8°C (IPCC, 2014), while dissolution of elevated atmospheric $CO_2$ by the oceans is decreasing ocean pH by approximately -0.0014 to -0.0024 per year (Rhein et al., 2013). Such dramatic changes to earth systems are expected to significantly impact biodiversity and the normal functioning of ecosystems (Doney et al., 2012), but we are only beginning to understand which species will be impacted and the magnitude of those changes (Moritz & Agudo, 2013). Changes in species phenology, community composition, biological invasions, and range shifts are altering species distributions and the interaction networks experienced by many species (Walther et al., 2002). While numerous studies have examined how the effects of climate drivers such as temperature, salinity, and pH affect the autecology of individual species (Crain, Kroeker, & Halpern, 2008; Parmesan, 2006), relatively fewer studies have attempted to elucidate how climate change associated environmental impacts are affecting the strength and nature of complex trophic interactions (Rosenblatt & Schmitz, 2014).

Trophic interactions describe the relationships between consumers and resources. Interactions occurring at higher trophic levels can cascade down food chains and indirectly influence interactions occurring at lower trophic levels, and vice versa (Paine, 1980). The indirect effects of trophic interactions on higher or lower trophic levels are often described as top-down or bottom-up control (respectively). While top-down and bottom-up population control has been studied in a wide variety of taxa and systems (Elmhagen & Rushton, 2007; Menge, 2000), we know little about how environmental changes via climate change will affect these relationships. Climate change associated changes involved in species interactions might be especially important for ecological communities that are organized around foundation species, which stabilize and provide habitat for a multitude species (Doney et al., 2012; Ellison et al., 2005; Osland, Enwright, Day, & Doyle, 2013). For example, marine foundation species, such as oysters and corals, might be negatively impacted by rising sea temperatures and decreasing ocean pH due to the decreased availability of carbonate ions for shell deposition (Doney et al., 2012), while their predators may be neutrally or positively affected creating combined impacts of climate and predators on foundation species' survival.

For foundation species, these combined effects will also impact the species that benefit from the foundational habitats they provide. A recent meta-analysis revealed 328 studies have dealt with at least one climate change variable on trophic interactions, and they showed that multiple stressors commonly created antagonistic effects relative to single stressor manipulations (Rosenblatt & Schmitz, 2014). Only 34 of these studies examined the simultaneous effects of multiple stressors, of which five were marine and focused on plant-herbivore interactions. Indeed, much more research is needed to understand how multiple climate change stressors will affect trophic interactions, especially for foundation species in marine and coastal ecosystems which are currently under-studied (Rosenblatt & Schmitz, 2014).

Coastal and marine ecosystems are dynamic environments with multiple foundation species that provide a variety of ecosystem services such as food resources (fisheries), water purification, and coastal protection (Liquete et al., 2013). Unfortunately, these ecosystems have been directly impacted by anthropogenic stressors, such as habitat loss, over-exploitation, pollution, species invasions, and climate change (Crain et al., 2008; Doney et al., 2012). While some future ecological perturbations are unknowable, some are more predictable. For example, we are confident that coastal ecosystems are going to be increasingly affected by stressors such as ocean acidification, increased sea surface temperatures, and increased salinization (IPCC, 2014). Approximately one third of the anthropogenic $CO_2$ released into the atmosphere (Sabine et al., 2004) is taken up by the ocean, causing reductions in pH and in the amount of bio-available carbonate ions (Doney, Fabry, Feely, & Kleypas, 2009). While the magnitude of the effects of these environmental stressors can differ among marine organisms, ocean acidification is expected to disproportionately negatively impact shelled aquatic organisms that use carbonate ions such as aragonite or calcite for substrate production that for foundation species leads to habitat formation (Kroeker, Kordas, Crim, & Singh, 2010).

For coastal and marine organisms, increases to sea surface temperatures can increase metabolic demands, reduce survivorship, and change the distribution and abundances of species (Hoegh-Guldberg & Bruno, 2010). For example, higher temperatures should aid shell deposition for calcifying organisms due to the lower solubility of calcium carbonate (Lord & Whitlatch, 2014), but may also facilitate biological invasions and fouling of marine communities (Dukes & Mooney, 1999; Sorte, Williams, & Zerebecki, 2010). Fouling is a natural process where organisms colonize solid surfaces or stratum such as the shells of living and dead organisms, and can sometimes result in biological invasions (Wahl, 1989). The diversity of fouling organisms has been shown to increase with

increasing salinity (Ortega & Sutherland, 1992), and colonization by non-native fouling organisms changes species interactions in marine ecosystems (Barnes, Luckenbach, & Kingsley-Smith, 2010; Schmitt, Osenberg, & Bercovitch, 1983) sometimes with severe socioeconomic consequences (Pimentel, Lach, Zuniga, & Morrison, 2000; Pimentel, Zuniga, & Morrison, 2005). Combined, shifts in pH and temperature are likely to impact the structure and diversity of coastal communities, especially those formed around foundation species (e.g. oysters).

Oysters are autogenic foundation species, which means they modify their environment via biogenically created structures that form the dominant physical characteristics of their habitats (Dayton, 1973; Ellison et al., 2005; Jones, Lawton, & Shachak, 1994). The accumulation of oyster shells and other hard bodied organisms creates a structurally complex reef that facilitates other species by providing resources, refugia, and settlement space for sessile individuals (Gutiérrez, Jones, Strayer, & Iribarne, 2003). By serving as a barrier between the coast and the shoreline in many systems, oyster reefs reduce coastal erosion (Meyer, Townsend, & Thayer, 1997), provide water filtration, help reduce eutrophication (Newell, 2004), sequester carbon dioxide, function as important nutrient (Smyth, Geraldi, & Piehler, 2013) and carbon sinks (Granek, Compton, & Phillips, 2009; Volety, Haynes, Goodman, & Gorman, 2014; Wingard & Lorenz, 2014), and they are a valuable fishery. Indeed, in North Carolina, oyster harvest generates between \$12.80-\$32.00 per 10 $m^2$. Unfortunately, in many bays (37% of 144 globally evaluated) approximately 99% of remaining oyster reefs are functionally extinct, or serving no ecosystem role (Beck et al., 2011). In North Carolina, for example, tens of millions of dollars has been spent trying to recover the eastern oyster fisheries (Beck et al., 2011) and their biogenically created habitat. However, such efforts to restore habitats for long-term sustainability may be futile if managers do not understand how multiple climate change stressors will impact trophic interactions in addition to the health of the oysters themselves.

The trophic interactions of oysters have been well documented in the literature. For example, oysters are common prey to many species of gastropod predators and crustaceans. One such predator of the oysters, mud crabs (*Panopeus spp.*), while small in size, have been documented consuming 320 oysters/$m^2$/day (Rindone & Eggleston, 2011). This number can be compared to the larger crustacean predators of oysters, stone crabs and blue crabs, that consume on average 10.2 and 2.4 oysters/$m^2$/day (Rindone & Eggleston, 2011). This trend can be attributed to oysters having a size refuge (less than 25mm left valve length) from larger crustacean predators, but these smaller sizes make them susceptible to predation by mud crabs (Rindone & Eggleston, 2011). Alternatively, oysters are predators (e.g. filter feeders) of many different planktonic species.

One of the main ecosystem functions of oysters is water purification through filtration, which can reduce eutrophication is coastal systems (Newell, 2004). Therefore changes in oyster consumption by crabs, could indirectly affect filtration by oysters and consequently increase eutrophication. However, crabs are not the only organisms that can potentially impact oysters and their ability to filter feed. As a foundation species, oysters also provide habitat to many organisms. Many marine invertebrates settle and live on oyster reefs and other hard substrates. Some of these can negatively impact oysters and harm the oyster reef community by reducing the amount of settling space for oyster larvae, providing competition for available food in the water column, or bioeroding the shells of oysters making them more susceptible to predation (Fitridge, Dempster, Guenther, & de Nys, 2012; Peck et al., 2015). Therefore, it is vital we understand how oyster and the coastal communities supported by oyster reefs will shift in response to climate change.

The effects of ocean acidification and sea surface temperatures on eastern oysters has been the focus of several recent studies (Amaral, Cabral, & Bishop, 2011; Ekstrom et al., 2015; Ivanina et al., 2013; Matoo, Ivanina, Ullstad, Beniash, & Sokolova, 2013; Talmage & Gobler, 2009; Waldbusser et al., 2014), however many questions remain. Adult oysters exposed to different combinations of $CO_2$ and temperature experienced decreased energy reserves and increased mortality due to increased temperature, and decreased shell hardness due to both elevated $CO_2$ and temperature (Ivanina et al., 2013; Matoo et al., 2013). In contrasts to these findings, another study showed no negative impacts of temperature on juvenile eastern oysters (Talmage & Gobler, 2009). To our knowledge, few studies have looked at the combined effects of $CO_2$ and temperature on the strength of interactions between calcifying organisms and their predators. One such study found that while green crabs and periwinkle snails individually responded to changes in their environment, their interaction was not impacted (Landes & Zimmer, 2012). Increased acidification has been shown to cause increased predation on oysters, where oysters reared at elevated $CO_2$ levels had smaller shell areas (Sanford et al., 2014). Acidification has also been shown to weaken mollusk shells, but not the shell strength of the carapaces of their crab predators (Amaral et al., 2011). Moreover, gastropod predators drilled through acidified oysters faster than oysters from non-acidified sites (Amaral et al., 2011), suggesting impacts of acidification on the strength of the their species interactions. In addition to potential predation impacts, elevated $CO_2$ has been shown to negatively impact the clearance and ingestion rates (e.g. filter feeding capabilities) of juvenile bivalves (Vargas et al., 2015). Finally, recent studies focused solely on the larval stage of oysters, found mineral saturation state conditions to have the largest impact on oyster shell formation;

however, in nature decoupling mineral and ion concentrations from pH is unrealistic (Waldbusser et al., 2014). While these studies suggest acidification can affect shell formation and oyster health in some situations, and predation risk in other situations, we still do not have a complete picture of how biotic interactions and multiple impacts from climate change can combine to affect oyster survival.

The main objective of this study is to evaluate how trophic interactions in coastal ecosystems are impacted by multiple climate change impacts. Specifically, I ask if ocean acidification and increased sea surface temperatures will: 1.) impact juvenile eastern oyster, *Crassostrea virginica*, growth and survival. 2) change the nature of trophic interactions between eastern oysters and predatory mud crabs, *Panopeus spp.* 3.) affect rates of filtration by oysters, one of their primary ecosystem functions. 4.) influence the formation of coastal communities.

# METHODS

*Experimental Setup*

This experiment was conducted at the Duke Marine Laboratory in Beaufort, North Carolina (Fig. 1). An 18.9 liter bucket was placed in the center of each of eight 1.22 x 0.61 meter tanks (four on a bottom row, and four on a top row) to receive inputs of unfiltered sea water from a flow through system. Each bucket was equipped with two or three submersible aquarium heaters (three heaters were needed in the highest temperature treatment). Two holes were drilled into each bucket, on opposite sides, and outfitted with adjustable valves. From each valve, water flowed into a 5.68 liter plastic containers (34.3 x 21.0 x 12.1 cm) containing the oysters (containers were given a specific tank ID for statistical analyses). Eight ten pound $CO_2$ tanks were used to simulate ocean acidification. Each tank was outfitted with a dual regulator equipped with a solenoid valve (purchased from GreenLeafAquariums). The solenoid valve (which allowed for the $CO_2$ to flow or not flow), was regulated in real time by a pH probe attached to a digital pH monitor. The probe detected the pH of the water and when the level reached a pH reading of 7.8 (the 2081-2100 year RCP8.5 prediction for ocean acidification) (IPCC, 2014), the solenoid valve was opened allowing the $CO_2$ to enter the water through a diffuser. This setup allowed simultaneous manipulation of the temperature and pH of continuously flowing unfiltered seawater. Water temperature naturally varied from the source, but each temperature treatment was maintained at approximately 0, 1, 2, and 3°C above ambient temperature. Ambient temperature varied from 18.5° to 30.0°C throughout the duration of the experiment. pH probes were calibrated monthly (or on an as needed basis). Temperature and pH were measured twice a day using secondary handheld probes to ensure the system was functioning properly.

In May 2015, 1000 oyster spat (*Crassostrea virginica*) that had settled on shell were obtained from Millpoint Aquaculture in Sea Level, NC. Individual oysters were pooled into groups of 10 and placed into 24" mesh mariculture bags. For each group of ten, I quantified initial wet weight (g) using an electronic balance (Ohaus Valor 3000) with a readability of 0.01 g, and photographed (Cannon T5, 55mm lens) each group for acquiring oyster height (distance from umbo to dorsal edge) measurements using Image J software. A ruler was placed in each photograph to standardize the magnification in each frame. The groups were then randomly assigned to a specific $CO_2$/Temperature treatment. Six bags of oysters were placed into each experimental arena (total of 60 oysters per container, 120 per treatment). Throughout the duration of the experiment, the oysters were wet weighed on a weekly basis. After approximately two months, oyster tanks were supplemented with 21.5 mL of a 1/10 dilution of Shellfish

Diet 1800 (Reed Mariculture, Inc.). This was due to a lack of growth seen across all treatments. To add the supplemental diet, water flow was briefly stopped (one hour each day) and oxygen was bubbled into the tanks to keep acceptable DO (dissolved oxygen) levels. After five months, all oysters were photographed and the number of dead oysters counted and separated from their treatment bags.

*Mud Crab Feeding Trial*

In October 2015, eight mud crabs (26.5±3.9mm) (*Panopeus spp*.) were obtained from the banks of the Duke Marine Laboratory in Beaufort, North Carolina. Each mud crab was wet weighed (g) using a digital balance, and the length of their carapace (mm) was obtained using digital calipers. Each mud crab was assigned to a random container that corresponded to a specific $CO_2$/Temperature treatment. A piece of PVC pipe was included in each of the containers to provide cover for the mud crabs. The crabs were placed into treatment tanks at approximately 10am and were starved and allowed to acclimate to their environment for 24 hours. After 24 hours, 10 oysters, selected randomly from a pooled stock corresponding to the crab's specific $CO_2$/Temperature treatment, were added into the crab container and the number of oysters eaten after six hours was quantified. The crabs were left in their tanks for the remainder of the second day. On the third day, the same crabs were randomized and placed into their new treatments and the experiment was replicated. There were a total of four experimental replicates using the same eight crabs randomized for each trial.

*Oyster Crushing Experiment*

At the conclusion of the mud crab feeding trial, all remaining oysters were bagged and brought back to East Carolina University laboratories. For this experiment, oyster bags were taken out of their 20°C holding freezer and ten individuals from each treatment were randomly chosen for testing crush resistance. I measured height (distance from umbo to dorsal edge) (mm), length (distance between anterior and posterior margin) (mm), and whole oyster shell thickness (largest distance between the outsides of the closed vales) (mm) with digital calipers before crushing. To determine the relative force needed to crush an oyster, each oyster was individually placed under a flat metal surface beneath the outer edge of an 18.9 liter bucket. Sand was added to the bucket at a slow but continuous rate until the oyster was crushed. The bucket along with the sand was weighed (kg) and recorded.

*Filtration Experiment*

Approximately three months into the experiment, I randomly selected five oysters from each of the $CO_2$/Temperature treatments. Each group of oysters was wet weighed (g) and photographed for standardization

across treatments. Each group of five oysters was placed into a 50mL GeneMate tube. A tube with no oysters was used as a control for this experiment. The tube was filled with 25 mL of water from each individual tank and 3mL of Shellfish Diet 1800 (1/10 dilution). The lids were left off to allow oxygen into the tubes. The experiment ran for 12 hours. After 1.5 hours, the tubes were shaken in order to assist in re-oxygenation of the water and re-suspension of shellfish diet; after six hours, 10mL of water for each tank was added to the respective tube, and after 7.5 hours the tubes were shaken a second time. After 12 hours, the oysters were returned to their treatments; the tubes were sealed and placed into a freezer. To determine the amount of feces produced by oysters, a proxy for oyster filtration, the samples from each tube were run through vacuum filtration using 47 mm glass microfiber filters. After each sample was filtered all equipment was rinsed in water followed by a 70% ethanol solution. Each sample was run through filtration for five minutes and afterwards placed in a 60°C oven for one week. Each filter was weighed on an electronic balance before filtration and after the drying oven. This experiment was replicated three times in each of two time blocks separated by ~ 1.5 months.

*Coastal Community Composition Analysis*

After 3 months, I replaced the 5.68 liter plastic containers (34.3 x 21.0 x 12.1 cm) containing the oysters. After removal, I sieved the accumulated sediments through a 2mm mesh catch net. All organisms that remained in the net were placed in a plastic container of water. In the water, I identified unique invertebrate organisms and counted their abundance. After sifting through sediment, I counted the number of organisms that settled on the container and on a standardized slate plate (20.32 x 20.32 cm) present in each container. Representatives of each species were preserved in 70% ethanol and later identified using a microscope to the lowest possible taxonomic level. Due to low numbers of individuals in each area (sediment, container, plate), I combined the areas abundances for each tank. There were a total of two replications for each unique $CO_2$/Temperature treatment.

*Statistics*

All data were analyzed in the R statistical programing environment. Because temperatures fluctuated overtime, I used median temperature as a continuous covariate in all analyses. In contrast, $CO_2$ was treated as a two level factor, elevated or ambient $CO_2$. To analyze oyster height (mm) and wet weight (g), I used linear mixed effects models (LMM), where $CO_2$ and temperature were fixed effects and tank ID was treated as a random effect to account for auto correlated errors among individuals reared in the same tank. To analyze oyster survival, I used a generalized linear mixed effects model (GLMM) with binomial family error distribution. $CO_2$, temperature, and tank

ID were again treated as fixed and random effects, and then an additional individual level random effect was added to account for mild overdispersion in the data (effectively fitting a beta binomial error distribution). Mortality from mud crab predation and relative crush force data were analyzed using a GLMM with binomial family error distribution and a linear regression, respectively. For these two analyses, oysters were pooled by treatment and then randomly selected for experimentation; any error due to individuals being reared in a common environment was redistributed into the overall model error. Filtration data were analyzed using a LMM, where $CO_2$ and temperature were fixed effects, and tank ID and time block (one or two) were treated as random effects. Community composition data were analyzed using NMDS (non-metric multidimensional scaling) plots and ANOSIM (analysis of similarities). In addition to whole community data, I investigated how temperature and $CO_2$ affected colonization by organisms requiring calcium ions using generalized linear models (GLM) with Poisson error distributions, and due to nonlinearity fit a 3rd order polynomial for the temperature effect. To test for the effects of $CO_2$ on the most abundant colonizing species (sea squirts), I performed a paired t-test. Inferences from GLMs, LMMs and GLMMs are based on likelihood ratio tests comparing models with and without target fixed effects. Model assumptions were evaluated visually using QQ plots, residual plots and likelihood profiles, as appropriate.

## RESULTS

*Effects of* $CO_2$ *and Temperature on Oysters*

Oysters height (mm) decreased with increasing temperature over the course of the experiment (df=1, Chisq=4.4798, P=0.0343; Fig. 2). There was also a negative effect of $CO_2$ on oyster height but this difference was not statistically significantly different. There was no relationship between wet weight for oysters as a function of either temperature or $CO_2$ (Fig. 3). Finally, there was a significant reduction in oyster survivorship (df=1, Chisq=9.584, P=0.001; Fig. 4) with increasing temperature. As with shell height, oyster survival was negatively impacted by elevated $CO_2$ but this effect was not statistically significant.

*Effects of* $CO_2$ *and Temperature on Mud Crabs + Oysters*

Oyster consumption by mud crabs significantly increased with temperature and increased $CO_2$ (df=1, Chisq=20.568, P<0.001; df=1, Chisq=8.529, P=0.003; Fig. 5). Interestingly, oysters grown in elevated $CO_2$ environments also required significantly less crushing force than oysters in ambient $CO_2$ conditions (F=6.96, P=0.008; Fig. 6). While whole oyster shell thickness affected the amount of weight to crush oysters (F=38.688, P<0.001; Fig. 6), there was no relationship between temperature and crushing force, or temperature and oyster shell thickness.

*Effects of* $CO_2$ *and Temperature on Algae + Oysters*

Oysters appeared to filter less from the water (as measured by fecal production) as temperature increased (df=1, Chisq=3.8295, P=0.05; Fig. 7). There was also a negative effect of $CO_2$ on oyster filtration but this difference was not statistically significantly different.

*Effects of* $CO_2$ *and Temperature on Coastal Community Composition*

Other marine invertebrates, which can compete with oysters for food and space, shifted community structure over the course of the experiment (Fig. 8 and 9). $CO_2$ groups appeared to have separate community compositions (ANOSIM, P=0.08), and temperature groups had significantly different community structures (ANOSIM, P=0.006). The changes in the fouling communities were largely driven by two groups. First, organisms that rely on calcium ions driving $CO_2$ treatment separations (Fig. 8) were significantly less abundant in increased $CO_2$ environments (df=1, Chisq=23.723, P<0.001; Fig. 10) and changed nonlinearly in response to changes in temperatures. A large driver of the separation in temperature treatments over time (Fig. 9), appeared to be the

presence of a local tunicate, *Molgula manhattensis*. However, a paired t-test revealed no significant in tunicate abundance between $CO_2$ environments (P=0.29; Fig. 11).

# DISCUSSION

In marine systems, previous research has focused heavily on the impacts of single climate stressors in relation to individual species (Crain et al., 2008; Rosenblatt & Schmitz, 2014). However, more recent analyses have shown that the combined effects of multiple stressors leads to direct antagonistic impacts to individuals (Crain et al., 2008), as well as their trophic interactions (Rosenblatt & Schmitz, 2014). In this study I found that oysters grown in higher temperatures had decreased survival and decreased growth, and both increased temperature and acidification affected trophic interactions which resulted in increased predation on oysters by 50%. Moreover, oysters grown in elevated $CO_2$ environments were crushed with less weight, suggesting they had weaker shells, which may explain observed increases in predation by mud crabs. Oysters in elevated $CO_2$ environments were also less efficient filter feeders. Finally, in addition to changing trophic interactions, $CO_2$ and temperature influenced community assembly leading to differences in the structure and composition of colonizing communities. By quantifying the effects of temperature and $CO_2$ on the interactions between oysters and other species, I uncovered impacts of multiple stressors on these organisms which went undetected when solely focused on single species level impacts.

For example, I saw a significant decrease in the overall height of oysters in higher temperature environments. However, there were no significant differences in oyster shell thickness with increasing temperature. This may suggest differential deposition of carbonate ions on shells. Indeed, laboratory studies have shown no differences in shell height, but increases in shell thickness as a function of temperature (Lord & Whitlatch, 2014). In addition to a decrease in oyster height with increasing temperature, there was also a significant decline in oyster survival with increasing temperatures. This result contrasts with studies such as Talmage and Gobler (2011), which found no significant decline in juvenile oyster survivorship with an increase in temperature of 4°C. However, their short duration study (45 days) did begin to see difference in survival 97±6% to 93±6%, and had they continued their study they may have observed significant decreases in oyster survivorship with increasing temperature. In this study, low food resources for the oysters in the flow through water during the first two months may have contributed to the decline in survival, however other studies have documented similar declines in survival with increased temperature. Indeed, Ivanina et al. (2013) also observed increased mortality in adult oysters with a 5°C increase in temperature. While not statistically significantly different, oysters also appeared to have lower survival in elevated $CO_2$ which is consistent with previous studies that have shown decreases in oyster survival with an increase in carbon dioxide (Talmage & Gobler, 2009).

Moreover, oysters decreased filtration (e.g. feces production) with increasing temperature. While decreased filtration (e.g. concentration of food particles removed over time) with decreasing temperatures has been shown, the effects were comparing changes in temperature across seasons (Walne, 1972). In contrasts, I increased temperatures above ambient during the warm summer months, which may have been sufficiently stressful to lower average fecal production (e.g. proxy for filtration). In addition, filtration has been shown to increase with shell height (Walne, 1972). Therefore the observed decrease in oyster filtration with increasing temperature may stem from the significant decrease in oyster shell height with increasing temperature (Fig. 2). While not statistically significant, oysters in elevated $CO_2$ showed increased filtration at ambient temperatures and decreased filtration in higher temperatures, which is inconsistent with negative impacts of increased $CO_2$ on a filter feeding of a bivalve in cooler temperatures (Vargas et al., 2015). That study did not separate the effects of $CO_2$ and temperature, so it is unclear if their bivalves would have experienced decreased filtration with increased $CO_2$ and higher temperatures.

The significant increase in the consumption of oysters by mud crabs in the higher temperatures and elevated $CO_2$ treatments, may have resulted from these oysters having weaker shells (Fig. 4 & 5). Many previous studies have found weaker mollusk shells in decreased pH environments (Amaral et al., 2011; Ivanina et al., 2013; Matoo et al., 2013). While this study indicated that oysters had reduced crushing resistance in elevated temperature and $CO_2$ treatments, I cannot rule out other explanations for observed patterns of mortality (Kroeker, Sanford, Jellison, & Gaylord, 2014). In this study I was only able to acclimate the predators to the specific $CO_2$ and temperature environments and so was not able to isolate impacts of these environments on the mud crabs themselves versus differences in oysters. So, it is possible that the higher temperature and $CO_2$ environments enhanced mud crab foraging behavior. While mud crab metabolism and developmental rates are known to increase with temperature (Costlow, Bookhout, & Monroe, 1962), there is no reason to expect the effect of temperature on the crabs metabolic rate and feeding rates to be stronger with elevated $CO_2$. Although I did not record individual claw size or sex in this study, I used early juvenile size classes and previous work has shown that in mud crabs "grip strength" does not differ between species or with crab size (Milke & Kennedy, 2001). Therefore the observed increases in mortality from mud crab predation was likely due to the effects of $CO_2$ on oysters rather than due to any effects of $CO_2$ acclimation on the crabs. Interestingly, while I found no significant impacts of $CO_2$ or temperature on many oyster endpoints (such as wet weight and filtration), $CO_2$ and temperature strongly affected the strength of trophic interactions with mud crabs.

Overall, I saw decreased growth and survival in oysters grown in elevated temperature environments. Additionally, oysters appeared to produce less fecal matter (e.g. filter less), in elevated temperatures, which suggest that oysters in natural communities may see similar fitness declines with increasing temperatures which may cause increased turbidity and eutrophication in coastal communities. However, oysters do not live in isolation, and with the addition of an important oyster predator that inhabits oyster reefs, mud crabs, I saw that oysters in increased $CO_2$ /temperature environments experienced increased mortality. This could lead to unforeseen top-down effects, magnifying the potential affects of reduced filtration of plankton from the water column, and increase rates of eutrophication in these systems.

In this study I also documented changes in marine invertebrate community composition between experimental environments. While the community composition was not strongly influenced by $CO_2$, there were differences in both $CO_2$ and temperature treatments. The most abundant species, *Molgula manhattensis*, were more prevalent in higher $CO_2$ environments, while organisms relying on calcium ions were less abundant in higher $CO_2$ environments (Fig. 10 & 11). These data suggest that in increasing $CO_2$ environments, oysters might suffer increased competition for settling space as well as food due to increases in species such as *Molgula manhattensis*. Marine invertebrate species such as the tunicate, *Molgula manhattensis*, can reduce settling space for newly settling oysters and compete with oysters for food (Zajac, Osman, & Whitlatch, 1989). This increase in soft bodied organisms such as ascidians in elevated $CO_2$ environments is consistent with another recent study that found decreases in organisms with hard exoskeletons in increased $CO_2$ environments (Peck et al., 2015). Changes in community composition can lead to increases in bioeroding organisms, compromise shellfish valve openings, and decrease the appearance and marketability of shellfish (Fitridge et al., 2012). For marine aquaculture, identifying changes to future fouling community composition could provide important insights for antifouling techniques (Fitridge et al., 2012).

In concert, these data support the hypothesis that changes in temperature and $CO_2$ predicted from global climate change can influence marine communities both via direct effects on individual species, and by changing the nature of their trophic interactions. This study highlights the importance of investigating trophic interactions in multiple stressor environments because the combined climate change effects may not be predictable from single species analyses. Future research should focus on understanding the integrated effects of multiple stressors on

multiple trophic interactions. Such data will be invaluable to ecologist and managers attempting to understand and predict the impacts of climate change on important and in some cases economically valuable ecosystems.

FIGURES

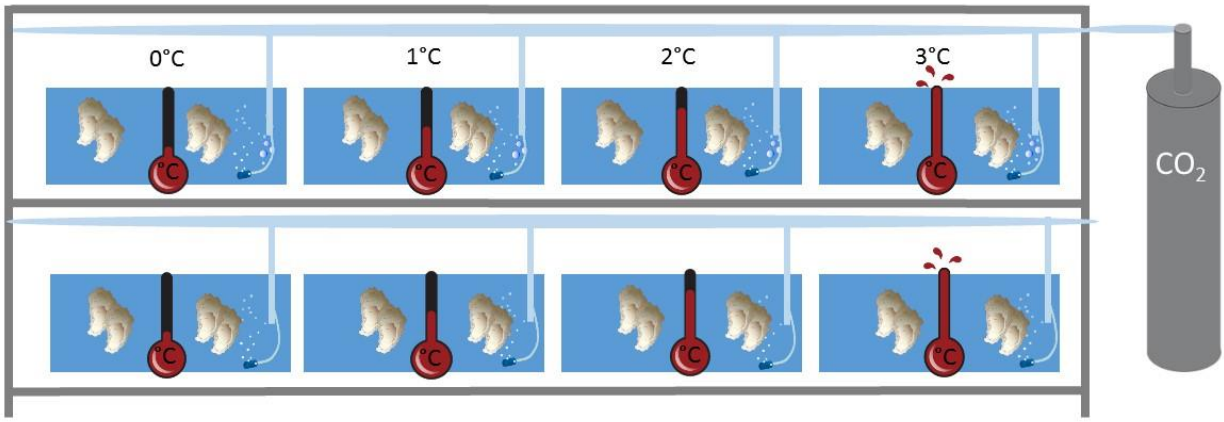


Figure 1. Experimental Setup. Oysters were placed in one of eight possible treatments. There were four temperature treatments (0°,1°,2°,3°C above ambient temperature) that heated two containers each, and one of the two containers received an input of $CO_2$ (NOTE: each $CO_2$ treatment had its own $CO_2$ tank) The treatments were each replicated once (top row and bottom row). A total of 60 oysters were placed into each container, totaling 120 oysters for each $CO_2$/Temperature treatment.

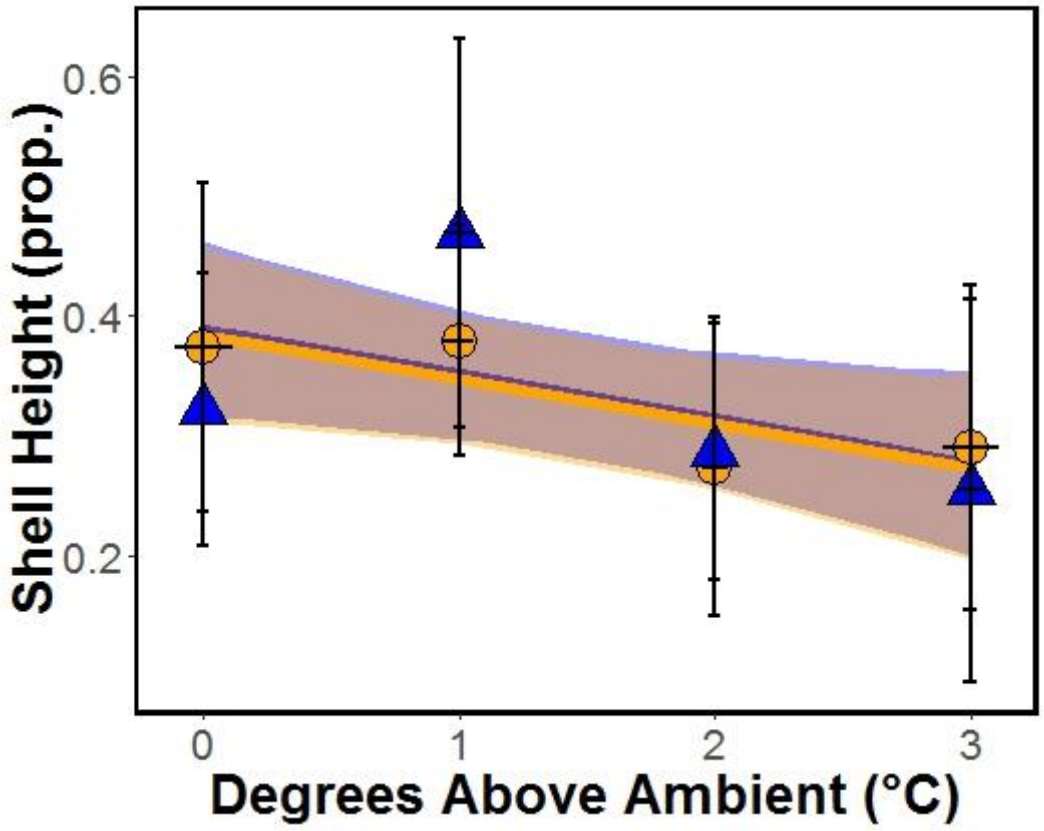


Figure 2. Shell height. Temperature is displayed as degrees Celsius above ambient, and growth is a measure of the log difference between final and initial oyster height. Lines represent predicted values of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the average growth using the raw data elevated (O) or ambient (Δ) $CO_2$ with horizontal and vertical error bars representing the standard deviations.

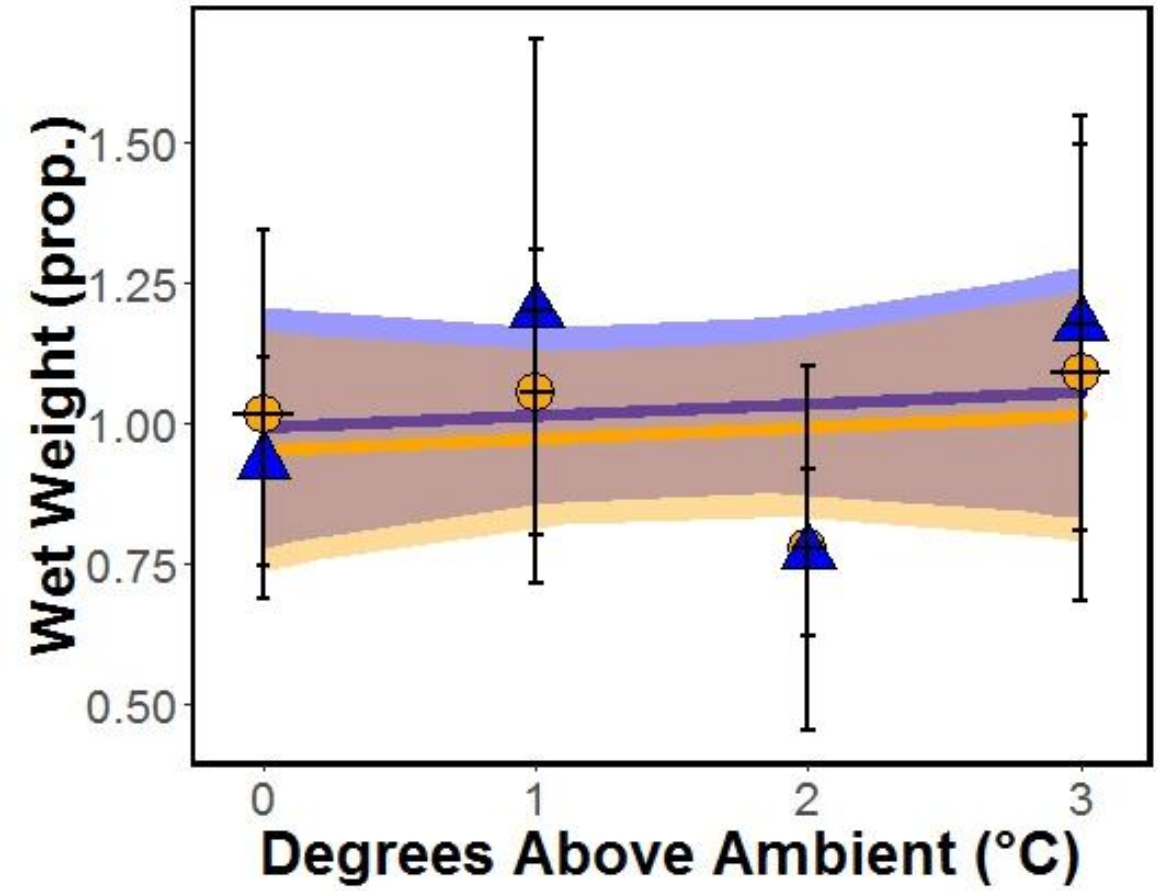


Figure 3. Wet weight. Temperature is displayed as degrees Celsius above ambient, and wet weight is a measure of the log difference between final and initial oyster wet weight. Lines represent predicted values of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the average growth using the raw data elevated (O) or ambient (Δ) $CO_2$ with horizontal and vertical error bars representing the standard deviations.

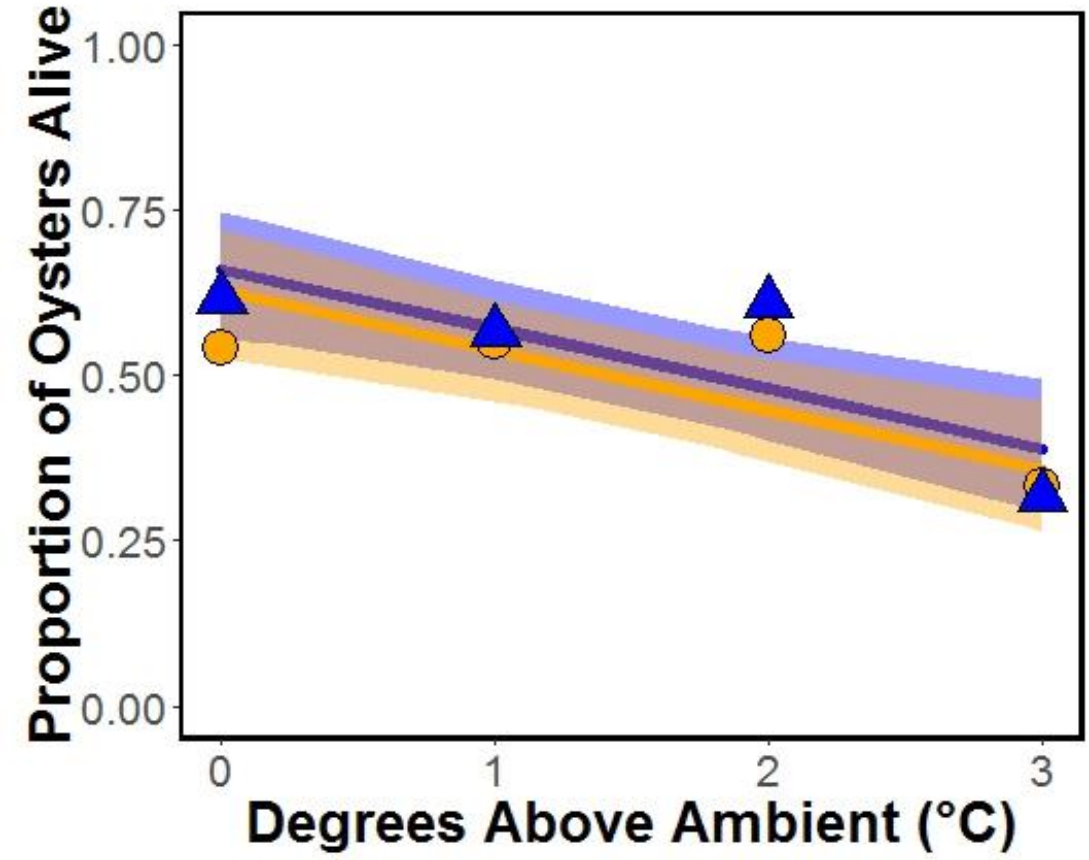


Figure 4. Proportion of oysters alive (before the mud crab predator trials). Temperature is displayed as degrees Celsius above ambient, and survival is the final divided by initial number of oysters alive. Lines represent predicted values (binomial error distribution) of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the average proportion alive using the raw data for addition (O) or no addition (Δ) of $CO_2$.

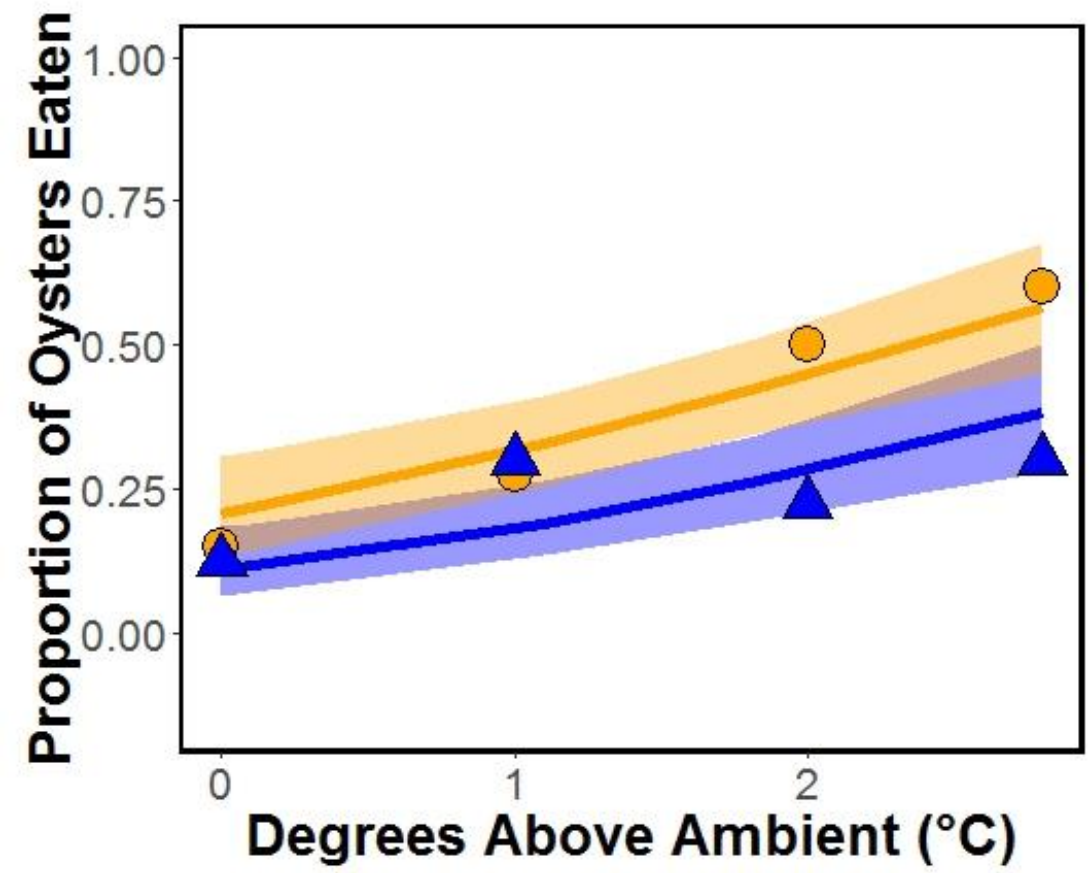


Figure 5. Proportion of oysters eaten. Temperature is displayed as degrees Celsius above ambient, and proportion eaten is the number eaten divided by the initial amount of oysters supplied. Lines represent predicted values (binomial error distribution) of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the average proportion alive using the raw data for elevated (O) or ambient (Δ) $CO_2$.

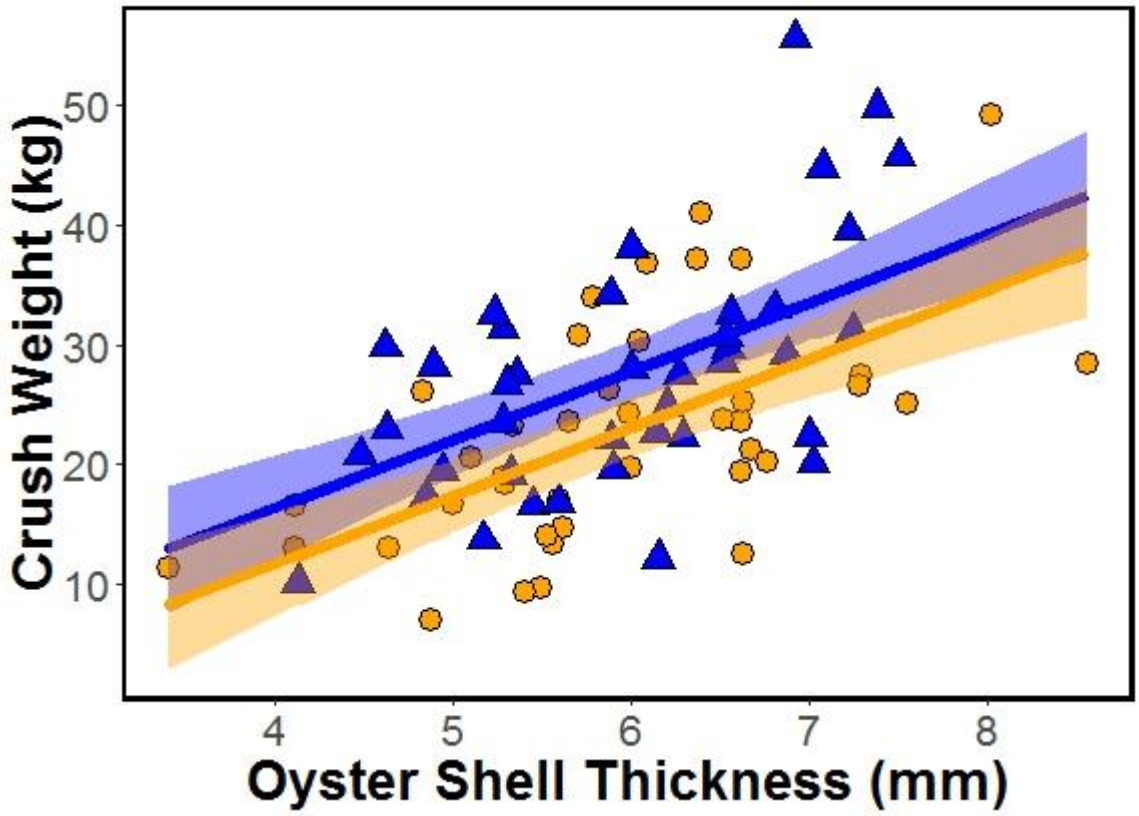


Figure 6. Crush weight (proxy for shell strength). Sand plus bucket weight is represented as crush weight (kg). Lines represent predicted values of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the raw data for elevated (O) or ambient (Δ) $CO_2$.

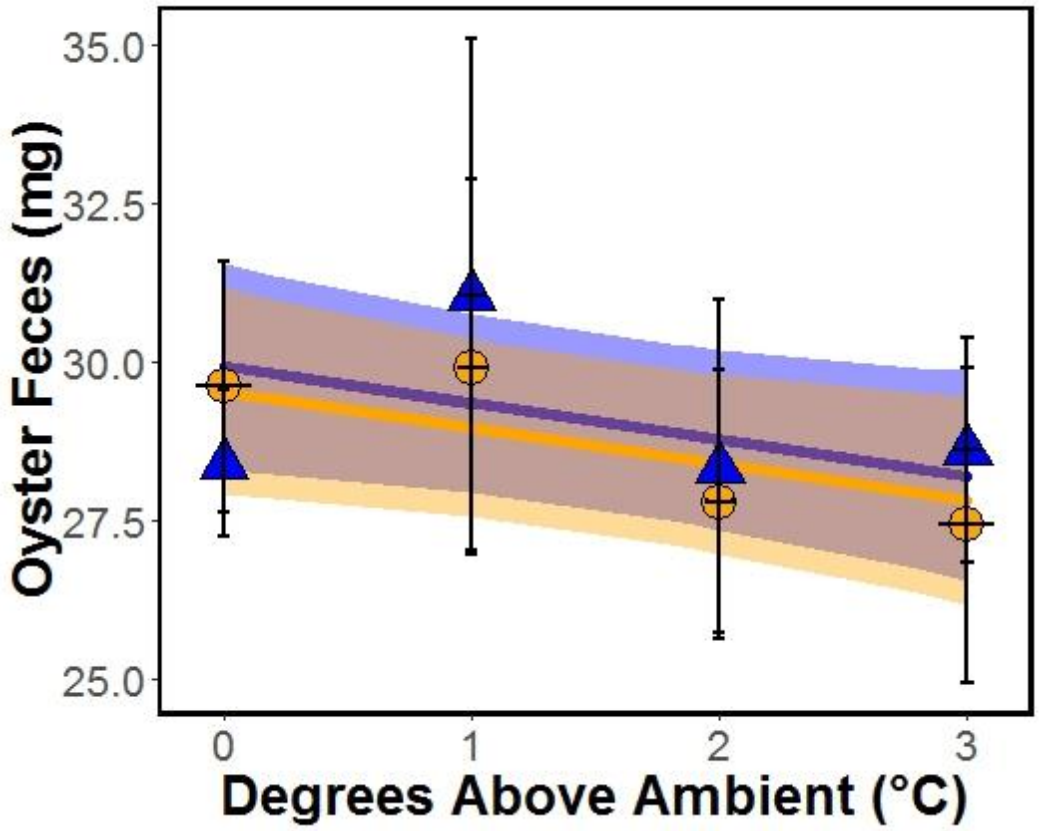


Figure 7. Oyster feces (proxy for filtration). Temperature is displayed as degrees Celsius above ambient, and oyster feces is a measure of the log difference between final and initial filter weight. Lines represent predicted values of either elevated (orange) or ambient (blue) $CO_2$, and the corresponding envelopes represent 95% confidence intervals. Individual points represent the average growth using the raw data elevated (O) or ambient (Δ) $CO_2$ with horizontal and vertical error bars representing the standard deviations.

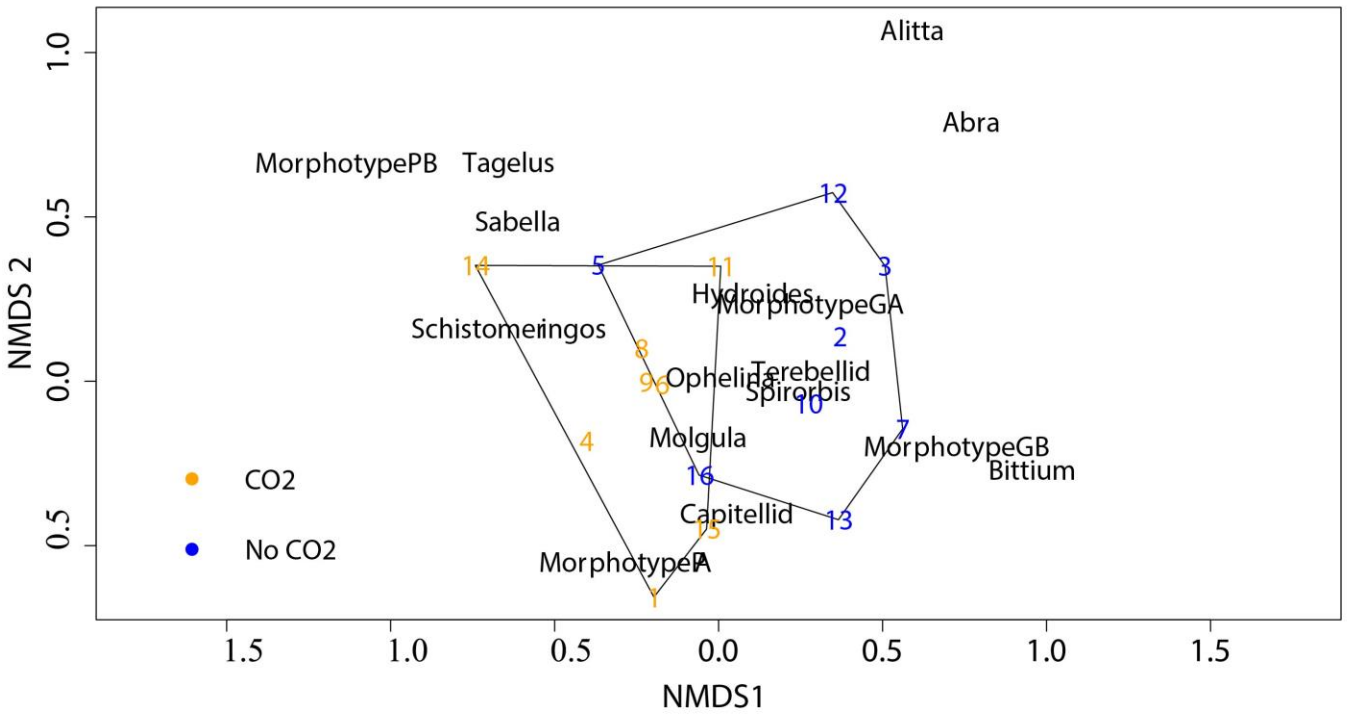


Figure 8. NMDS plot with tanks weighted by the relative abundance of different marine invertebrates. Tanks circled in orange represent those that experienced elevated $CO_2$ (1,4,6,8,9,11,14,15) and blue represent tanks with ambient $CO_2$ (2,3,5,7,10,12,13,16).

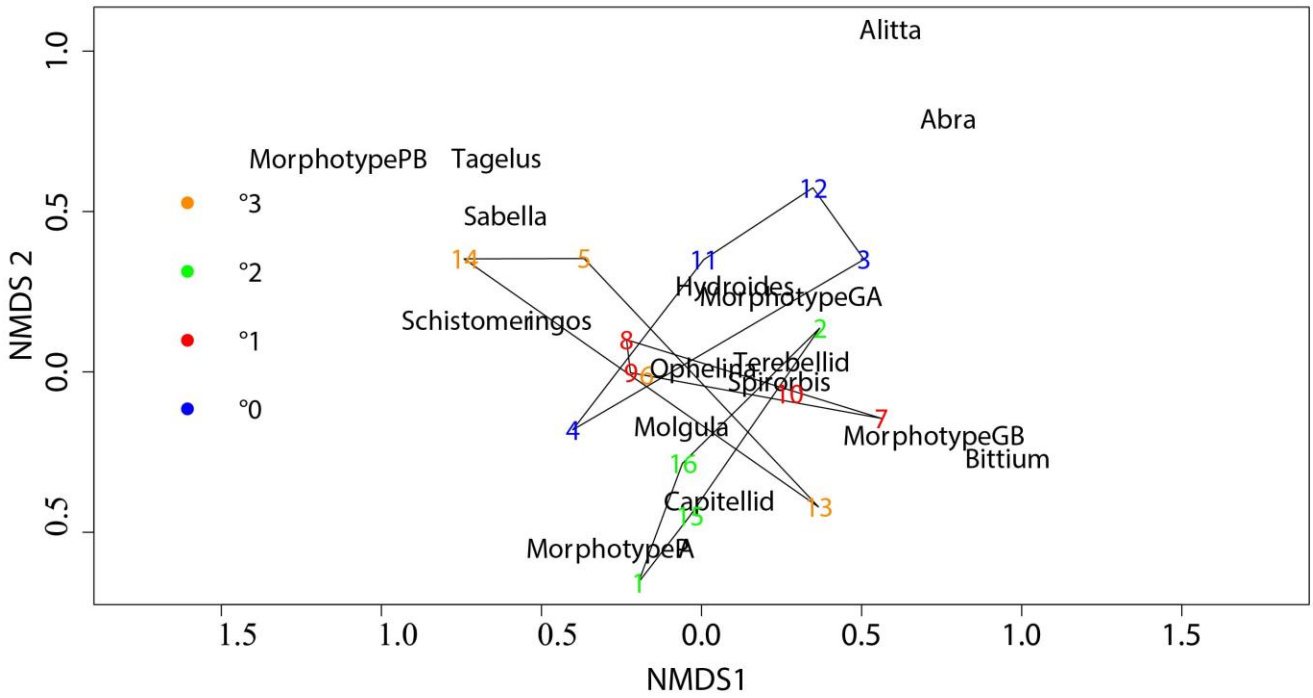


Figure 9. NMDS plot with tanks weighted by the relative abundance of different marine invertebrates. Tanks circled in orange represent those that experienced 3°C above ambient temperature(5,6,13,14), green represents 2°C above ambient temperature (1,2,15,16), red represents 2°C above ambient temperature (7-10), and blue represent 0°C above ambient temperature (3,4,11,12).

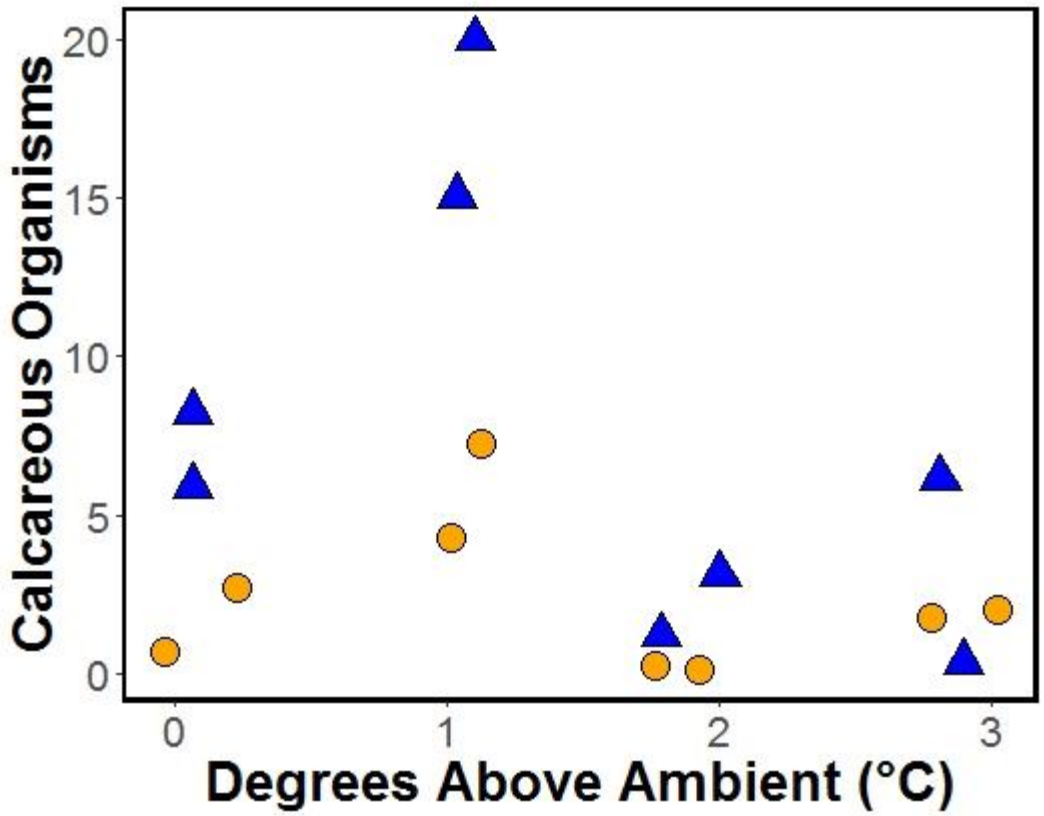


Figure 10. Calcareous organisms. Temperature is displayed as degrees Celsius above ambient, and the y-axis represents the abundance of calcareous organisms in each treatment. Individual points are shaped to represent elevated (O) and ambient (Δ) $CO_2$ treatments.

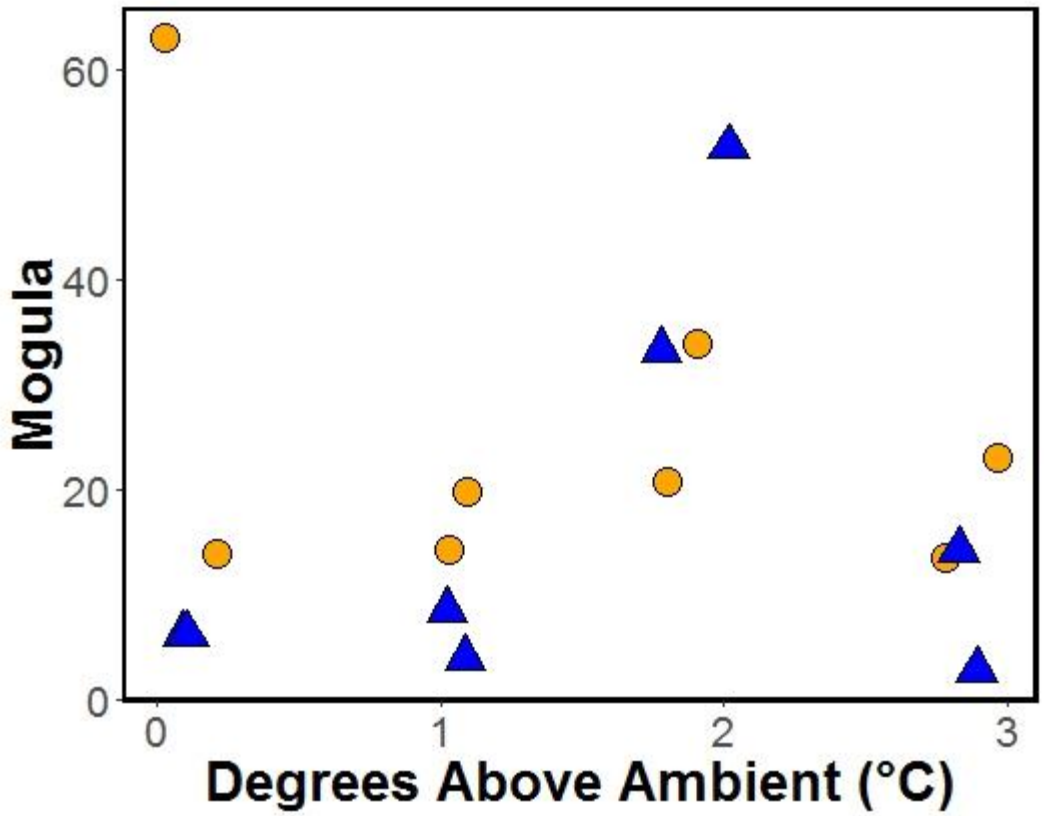


Figure 11. *Molgula*. Temperature is displayed as degrees Celsius above ambient, and the y-axis represents the abundance of *Molgula* in each treatment. Individual points are shaped to represent elevated (O) and ambient (Δ) $CO_2$ treatments.

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