

The Effects of Ocean Warming and Acidification on Seaweed Growth and Urchin Grazing

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SIGNATURE PAGE

THESIS: THE EFFECTS OF OCEAN WARMING
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GROWTH AND URCHIN GRAZING

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Abstract

Human produced carbon dioxide concentrations in the atmosphere are currently higher than previously recorded and are continuing to rise at alarming rates. This greenhouse gas is the primary driver for changing climate scenarios highlighted by an approximate 1°C increase in sea surface temperatures. In addition to driving global warming, CO₂ is readily absorbed by the oceans, resulting in changes in seawater chemistry and a decrease in seawater pH (acidification). The singular effects of ocean warming and acidification are known to impact marine organisms; lesser known, however, are the combined effects of these stressors, particularly on biotic interactions. This study aimed to expand on the knowledge of how these abiotic stressors affect seaweed and seaweed-herbivore interactions by comparing seaweed growth and herbivore feeding rate and selectivity under combinations of current and modelled future temperature (18°C and 21°C) and pH (8.1 and 7.8) conditions. Growth rates of two seaweed species, a calcified red alga (*Lithothrix aspergillum*) and a non-calcified brown alga (giant kelp *Macrocystis pyrifera*), were compared among manipulated seawater conditions. In addition, the feeding rates and feeding selectivity of a common sea urchin herbivore (*Strongylocentrotus purpuratus*) for these two seaweeds were compared among water conditions. *Lithothrix* was not affected by the singular effects of pH or temperature but under combined future temperature and pH conditions, the seaweed performed poorly. While acidification is known to affect the ability of calcifying species to deposit calcium carbonate, *Lithothrix* appeared to only be impacted by acidification under temperature stress. *Macrocystis*, on the other hand, performed significantly better under future acidic conditions, regardless of temperature, as it likely experienced an increase in photosynthetic rate driven by high CO₂ concentrations. Urchin herbivory rates were elevated for both seaweeds grown under acidification scenarios, possibly due to increased grazing susceptibility of *Lithothrix* during poor calcification/decalcification conditions and *Macrocystis* during new growth conditions. Feeding preference trials were inconsistent with feeding rate patterns as urchins

exhibited low overall consumption and no selectivity for either seaweed under any water condition. Although the impacts of warming and acidification on growth of seaweeds and susceptibility to grazing by urchins are variable among taxa, potential future stressors are likely to alter seaweed population and seaweed-herbivore dynamics.

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1. Introduction

Carbon dioxide concentrations in the atmosphere are currently higher than the earth has experienced over the past 650,000 years (Seigenthaler et al. 2005). Atmospheric carbon dioxide, released from the burning of fossil fuels and deforestation (Doney et al. 2009), have increased at a rate approximately 100 times faster than previously seen (Fabry et al. 2008) and is expected to continue to increase on a global scale at a rate of ~0.5% per year (Forster et al. 2007). This greenhouse gas is the primary driver for changing climate scenarios, highlighted by an approximate 1°C increase in global sea surface temperatures (SST) over the past 150 years, with projections of continued increases, under best case scenarios, of 2-4.5 °C by year 2100 (IPPC 2007). In addition to being a primary driver of climate change and increased SSTs, CO₂ is readily absorbed by the ocean, resulting in changes in seawater chemistry highlighted by a decrease in oceanic pH (ocean acidification). As atmospheric carbon dioxide combines and dissolves into sea water, it forms carbonic acid (H₂CO₃) which further breaks down to form higher concentrations of bicarbonate (HCO₃⁻), carbonate (CO₃⁻²), and hydrogen (H⁺). The free hydrogen ion that is produced is responsible for the more acidic oceanic conditions.

Increased SSTs and ocean acidification can have a number of impacts on the physical components of the ocean. Ocean warming can reduce the extent of sea ice, alter salinity conditions, cause level rise, increase stratification, and change circulation patterns (Harley et al. 2006, Aral and Guan 2016, Capotondi et al. 2012). For example, sea level has risen 15-20cm over the past century (Aral and Guan 2016) as a result of seawater expansion and melting of ice caps with warming. In addition, changing climate scenarios can increase the occurrence and intensity of upwelling as atmospheric pressure increases coastal wind stress (Harley et al. 2006, Snyder et al. 2003). Under high oceanic CO₂ conditions due to ocean acidification, the upwelling of the

CO₂ rich water can lead to hypoxia (Feely et al. 2008). In coastal ecosystems, this phenomenon is occurring more frequently than previously observed (Steckbauer et al. 2015).

Ocean warming and acidification also can have large impacts on the biology and ecology of marine species. Increased SSTs have been shown to affect organism physiology, chemistry, and behavior and cause shifts in species ranges, community structure, and biodiversity patterns. With increased warming, many species may be exposed to their upper thermal tolerances, which may reduce their fitness, such as a decline in growth or productivity, or cause mortality. For example, intertidal snails exhibit reduced growth and declines in abundances due to the physiological stress of warming (Somero 2002). Temperature-sensitive corals are particularly vulnerable to warming with large bleaching events and overall population declines observed globally (Donner et al. 2005, Berkelmans 2002, Hoegh-Gulberg 1999). Numerous species have shifted their geographic ranges, following their required temperature regime, including seaweeds (Lima et al. 2007; Wernberg et al. 2011) and fish (Neuheimer et al. 2011). For example, tropical macroalgae assemblages have been found to shift ~3200km from where they typically reside near the Indian Ocean (Wernberg et al. 2011).

Similar to ocean warming, ocean acidification can affect the fitness of organisms through physiological changes and result in alterations of community composition. This is particularly evident for those species that deposit calcium carbonate to form shells and skeletons. Seawater is naturally saturated with carbonate; the H⁺ ions that are elevated because of increase atmospheric CO₂ have a higher attraction to carbonate than it does to calcium, saturating the seawater with more bicarbonate. Consequently, carbonate is no longer available to form the calcium carbonate (CaCO₃) required for calcifying organisms. When exposed to high CO₂ conditions for an extended period of time, growth of shells of mussels (Michaelidis et al. 2005), snails (Bibby et al. 2007), and pteropods (Fabry et al. 2008, Feely et al. 2004) was greatly reduced thus having direct effects on fitness and survival. Additionally, corals that have been exposed to high CO₂ treatments have experienced nearly 50% bleaching compared to ≤10% bleaching when exposed

to intermediate to low levels of CO₂ (Anthony et al. 2008). Ocean acidification can also enhance primary productivity in fleshy algae; eelgrasses and brown seaweeds that were exposed to high CO₂ treatments displayed an increase in productivity (Zimmerman et al. 1997, Nunes et al. 2016). Increases in primary production will likely lead to shifts in macroalgae community assemblages that allow greater abundance of seaweeds that can utilize the increase in CO₂ (Harley et al 2006).

On a global scale, ocean warming and acidification can have negative impacts on marine communities that rely on fisheries as a primary source of economics. In the United States \$5.1 billion in revenue comes from shellfish fisheries (Cooley and Doney 2009). Mollusks, for example, make up 19% of those harvested in 2007 (Cooley and Doney 2009) but saw a ~25% decline in their calcification rate and an increase in mortality under more acidic conditions (Farby et al. 2008). It is anticipated that with this decline in mollusk abundance fisheries revenue will decrease \$75-187 million each year during the upcoming years (Cooley and Doney 2009). Commercial fisheries are likely to be impacted both positive and negatively by ocean warming as fish species are shifting their distribution and abundance, body sizes are reduced, and mortality increases. As oceans warm, areas where predominately cold-water species reside will begin to become inhabited with warmer water species, increasing fish abundance (Griffith et al. 2012). However, cold-water fish species that cannot adjust their thermo-tolerance will likely exhibit a decrease in their body size and higher mortality rates (Neuheimer et al. 2011).

The singular effects of ocean warming and ocean acidification are known to have major repercussions on the biology and ecology of marine organisms. However, most studies only focus on the direct effects of one environmental stress on a given species (Poore et al. 2013) and minimal experimentation has been done (Wernberg et al. 2012). There is a gap in understanding of the combined effect of these environmental stressors, although a small number of studies have been conducted. When combined, ocean warming and acidification are known to severely impact marine organism's growth and survival rates. During early germination stages of kelp, future temperature and pH scenarios reduced the rate of spore germination (Gaitán-Espitia et al. 2014).

Sea urchins grown under the combined climate stressors displayed stunted growth compared to sea urchins grown under current temperature and pH conditions (Brown et al. 2016).

Additionally, biodiversity can be reduced under ocean warming and reduced pH scenarios as mollusks and arthropods exhibited shell deterioration, leading to increased mortality (Hale et al. 2011).

In addition to there being a gap in understanding the combined effects of warming and acidification, there is also a gap in studying both the singular and combined effect of these stressors on species interactions. Some modeling has been conducted using meta-analysis from peer-reviewed literature to statistically analyze species interactions, but this approach is limiting in that models are not able to capture all ecosystem functions (ie. nutrient availability, seasonal dispersal of top predators, abiotic factors) and most researchers recognize the need for experimental approaches (Fabry et al. 2008, Wernberg et al. 2012, Harvey et al. 2013, Poore et al. 2013). When applied independently, ocean warming has been shown to strengthen plant-herbivore interactions and shift trophic dynamics. O'Connor (2009) found that seaweeds exposed to increased temperature did not have an effect on herbivore feeding rate. Another study found herbivores to shift feeding preference to new food sources after their typical food had been exposed to elevated temperature (Sotka and Giddens 2009). On the other hand, ocean acidification alone has been shown to increase herbivore feeding of calcifying seaweeds as calcification is reduced (Kayma et al. 2016). A limited number of studies on the combined effects of both stressors suggest that changes in trophic dynamics may be variable. Sea urchin consumption of *Macrocystis* increased after it had been grown under both future temperature and pH conditions (Brown et al. 2016). Similarly, another study found herbivores to consume more seaweed when it was grown under low temperature and reduced pH; however, when temperature was increased, there was a significant decline in consumption (Poore et al. 2013).

Given the current gaps in knowledge about the combined effects of ocean warming and acidification on organism growth and species interactions, this study aimed to expand on the

knowledge of how these abiotic stressors affect seaweed and seaweed-herbivore interactions. More specifically, I compared growth rates of a calcified red alga, *Lithothrix aspergillum* J.E. Gray, and a non-calcified brown alga, *Macrocystis pyrifera* (Linnaeus) C. Agardh, under combination of ocean warming and acidification conditions, as they were likely to respond differently given that reduced pH is known to reduce calcification rate yet increase photosynthetic rates. I predicted that under reduced pH scenarios, *Lithothrix* would experience stunted growth as its calcification becomes depleted, while *Macrocystis* would have increased growth, as the increased CO₂ would allow the seaweed to maximize its photosynthetic capability. In addition, I aimed to examine how herbivory on these seaweeds by the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857), are altered by comparing feeding rates and feeding selectivity for seaweeds grown under different oceanic conditions. If future conditions reduced the ability for *Lithothrix* to deposit calcium carbonate effectively, I predicted that under future climate stressors sea urchins would shift their diet away from their known selectivity for *Macrocystis* to the, more vulnerable, calcified seaweed. This study elucidates how combined climate stressors can impact different primary producers and trophic dynamics along the west coast and, thus, give us the ability to predict the outcomes of climate change on ecosystem functioning and provide insight on the need to management of coastal marine ecosystems.

2. Methods

2.1 Target Species and Collection Sites

Two common coastal intertidal seaweed species were used in this study. The geniculate red alga, *Lithothrix aspergillum*, is a Rhodophyte (Florideae, Corallinales) commonly found in the intertidal and subtidal zones from British Columbia to Baja California, Mexico (Abbot and Hollenberg, 1976) where the turfs are home to an abundance of marine invertebrates (Kelaher et al. 2001). This articulated seaweed deposits calcium carbonate in its intergenicula segments and, thus, is likely to be affected by changes in pH. In addition to a calcium carbonate depositor, a non-calcifying brown alga (Phaeophyceae, Laminariales), *Macrocystis pyrifera* (Linnaeus), also known as giant kelp, was examined. The giant kelp is typically found in the subtidal zone from Alaska to Baja California, Mexico (Abbot and Hollenberg, 1976), in dense kelp forests where they provide habitat for a variety of marine species and act as a major food source for a suite of herbivores (Leighton 1966, Kenner 1992). The common coastal purple sea urchin, *Strongylocentrotus purpuratus* (Echinodermata, Echinoidea), was used as an herbivore in this experiment. Purple sea urchins can be found in the low intertidal and subtidal zone, typically on or under rock surfaces, from Alaska to Baja California, Mexico (Rogers-Bennett, 2007). This species is primary major herbivore in the local marine coastal ecosystem and is known to consume a variety of algal foods, particularly geniculate corallines and Laminarian kelps. While some studies suggest that geniculate coralline algae are the dominant food source (Kenner 1992, Thornber et al. 2008), others note a clear preference and consumption of kelps (Tegner and Dayton 1981, Springer et al. 2006, Basch and Tegner 2007). This variability may possibly be attributed to spatial and temporal variation in food availability, particularly in the availability of drift kelp (Kenner 1992).

Target species were collected prior to the start of each trial from White Point, also known as Royal Palms County Beach, and Point Fermin State Marine Park, in San Pedro, CA. All three species were common at both sites and collected through snorkeling and/or during low tide where

they were then transported back to the laboratory in coolers with seawater. Urchins were placed in a holding tank at 18°, 8.1 pH where they were fed fresh *Macrocystis* until experimental use.

2.2 Treatments

To examine the effects of increased oceanic temperatures and reduced ocean pH, seaweed growth and sea urchin feeding experiments were performed in a laboratory controlled experiment. Experiments were conducted within an interconnected running seawater system (~500 Liters), consisting of 12 individual aquaria (38 Liters) with overflow onto an open aquaria table. Individual aquaria were additionally partitioned with a divider into four sections for growth and feeding rate experiments and two sections for feeding preference experiments using a divider. Light was provided using Finnex Ray II LED lights with diffuse light (400-700 nm) (Beer et al, 1983) provided on an ~12:12h light-dark cycle. Given the aquaria set-up was a flow through system with all aquaria/replicates subjected to the same water, I recognize that samples are pseudoreplicated within one water condition treatment. However, it is not uncommon for climate change studies to be conducted in this manner (Havenhand et al. 2010, Cornwall and Hurd 2016), especially given the large amount of resources needed to maintain separate water conditions for each replicate.

Treatments consisted of four combinations of ambient and future seawater temperature and pH levels, with two temperature (18°C ambient and 21°C future) and two pH (8.1 ambient and 7.8 future) conditions. Sea surface temperatures in local shallow waters of southern California typically range annually from 14-19°C, with peaks to 21°C not being uncommon in summer months during anomalously warm years. For this study, 18°C was used as the ambient temperature, as this was the SST for southern California for most of the collection period (spring and summer 2016). Global sea surface temperatures with climate change have been predicted to increase up to 4°C by 2100 (IPCC 2007) thus would expect the normal range of southern

California to increase to 18-23°. I used a conservative 21°C as the elevated temperature as this would be the more mid to higher range temperature under future climate change scenarios and is also a temperature observed in southern California during anomalously warm years. In addition, previous studies show that when temperature exceeds 22°C, sea urchins display increased mortality rates (Farmanfarmaian and Giese 196). An ambient pH of 8.1 was chosen as this was the current pH of southern California waters. A future pH of 7.8 (Yanovsky 2012; Poore et al. 2013) was chosen based off of 0.3-0.4 units of expected global decline (IPCC 2007).

Seawater temperatures were constantly monitored and regulated using an Arctica DA-5008 commercial series titanium aquarium chiller and automatic controller. The pH levels were modified by injecting carbon dioxide into the seawater system as a fine mist using a glass diffuser with a ceramic plate and was maintained using a Milwaukee MC122 pH control unit, which automatically added CO₂ if pH shifted ± 0.2 units above the desired level. Salinity (~35 ppt) and other water conditions were held constant across samples and were similar to natural seawater conditions. Because nitrogen and phosphorous are limiting nutrients in marine systems, I added 2.5 mL Flourish Nitrogen for every 160L sweater and 2.5 mL Flourish Phosphorous for every 80L seawater, as instructed by the manufacturer Seachem.

2.3 Growth

Algal growth was assessed by measuring the change in the wet weight during exposure to each of the 4 water treatment conditions for a 2- week period during two separate, replicated trials for each water treatment. Each of the 12 aquariums was divided into four compartments for 12 replicates of growth measures for both species run simultaneously. At the start of each trial, excess water was removed from each algal species using a salad spinner or blot dried with paper towels before recording an initial standardized wet weight and then placed in their randomly assigned treatment aquaria. Initial wet weights for *Lithothrix* ranged from ~ 11 to 14 g while

Macrocystis wet weights ranged from ~ 6 to 9 g. At the end of the 2-week trial, seaweeds were removed and the wet-weight of each seaweed species was again recorded. Trials were conducted from May to July, 2016 to reduce temporal variation in water condition responses for seaweed species. Changes in wet weights (g) were then converted to percent wet weight change as initial weights varied among samples. Percent wet weight change data were analyzed using a generalized linear mixed model (GLMM) with tank as a random factor, and temperature and pH as fixed factors. In cases where normality was not met, I used a cube root transformation on the percent change in weight data.

Prior to exposure to manipulated seawater conditions, a portion of each algal replicate sample was removed and the wet weight determined using methods described previously. Samples were then dried at 60°C for 48 hours (Noisette et al. 2013) to determine dry weight for the seaweed samples. Following each experimental trial, portions of the 24 seaweed replicates were again removed to determine wet and dry weights; the entire seaweed sample could not be used for dry weight determination as most of the seaweed sample was needed for subsequent feeding experiments. To determine if the algae's water content changed over the length of the trial, a paired sample t-test was performed comparing a calculated wet weight to dry weight ratio for samples before (initial wet weight/ initial dry weight) and after (final wet weight/ final dry weight) for each seaweed species per trial.

For several of the trials, there was a significant shift in wet weight to dry weight ratio over the 2 week period. Therefore, change in dry weight was also compared among treatments. Here, the mean initial and mean final wet weight to dry weight ratios were used to convert initial and final wet weights of experimental algae into dry weights (Ex. mean initial wet weight/ initial wet:dry = dry weight) . Similar to wet weight data, I converted the change in dry weight into a percent weight change over the experimental period and analyzed the data similarly (GLMM, tank random factor, temperature and pH fixed factors).

2.4 Feeding rates and feeding preference trials

Immediately following each of the two replicated growth experiments, seaweeds that had been exposed to manipulated seawater conditions were removed of any excess water, an initial wet-weight determined, and placed back in their assigned aquaria in manipulated water conditions to begin feeding trials. Feeding trials consisted of two separate trials: single-food feeding rate trials and two-choice feeding preference trials. Each experimental trial for each of the 4 water treatments consisted of new urchins collected two weeks prior during seaweed collection; urchins were continually replaced to eliminate bias from previous feeding experiments.

In the single-food feeding trials, sea urchins (~ 40 to 50 mm in diameter) were placed in a randomly assigned treatment aquarium, where they were offered either *Lithothrix* or *Macrocystis* and allowed to feed freely. Each of the 12 aquaria was divided into four compartments to simultaneously run 12 replicate urchins feeding trials for each species, and 12 controls without urchins. Within isolated compartments for each aquaria, controls were established to measure autogenic weight change of seaweeds without the herbivore present. Feeding lasted a maximum of 48 hours, or until ~50% of the algal food source was consumed. At the end of the trial, the final wet weight was determined. The percent change in wet weight consumed by the sea urchins were determined, standardized for a 24 hour period, and converted to a percent change in seaweed wet weight consumed per day ($\text{g consumed day}^{-1}$), accounting for percent weight change in the controls with the herbivore absent. Single choice feeding trials were analyzed with a GLMM, with the tank each sea urchin was assigned to as a random factor, and temperature and pH as fixed factors. When normality was not met, I transformed the percent change in algae wet weight using a cube root transformation.

In two-choice feeding trials, 12 sea urchins were offered both algal food sources at one time and allowed to choose between both seaweeds. Aquaria were divided into two compartments with one compartment containing only the seaweeds to account for autogenic weight change.

Similar to feeding rate trials, feed selectivity trials were conducted for a maximum of 48 hours, or until ~50% of one of the algal food source was consumed, so that sea urchins did not shift their diet preference due to a lack of food available. To test whether sea urchins selectively consumed one species of the other, a paired sample t-test was performed, comparing the difference in percent wet weight change of *Macrocystis* minus *Lithothrix* in treatments with herbivores versus controls without herbivores (see Peterson and Renaud 1989). This was conducted separately for each water condition treatment but did not compare among water treatments. In cases where the data were not normally distributed, I used the non-parametric Wilcoxon-signed rank test to determine feeding preference.

All statistics were analyzed with R program (R Core Team, 2016). The lme4 package was used in order to perform all GLMMs.

3. Results

3.1 Growth

For all trials conducted, seaweeds consistently lost weight over the 2 week period with no pattern of positive growth; however, the difference in weight loss revealed significant patterns of performance for species among the water treatments. The percent wet weight change of *Lithothrix* varied significantly for temperature, pH, and the interaction term (GLMM, Table 1). *Lithothrix* performed best at 21°C and an ambient pH of 8.1 and worst at 21°C under a reduced pH of 7.8 (Fig. 1). At 18°C, percent changes in weight wets were similar between to the pH treatments while there were large differences at 21°C. For *Macrocystis*, the percent change in wet weights was similar among the different factors with no significant interaction term (GLMM, Table 1). *Macrocystis* exhibited weak patterns of performing best at 18°C and 7.8 pH and worst at 21°C and 7.8 pH (Fig. 2). Although the temperature factor was not significant ($p=0.067$), *Macrocystis* exhibited the most tissue loss in the higher water temperature treatments when comparing within similar pH levels. The effect of pH on *Macrocystis* varied at the two temperatures, with less weight loss at lower pH at 18°C and more weight loss at the lower pH at 21°C.

The wet weight to dry weight ratio compared before and after each trial varied among the different water treatments for both seaweed species. For *Lithothrix*, the ratios before and after were similar for the ambient pH of 8.1, regardless of temperature (18 or 21°C) (Fig. 3, Paired T-test Table 2). However, at both temperatures, *Lithothrix* wet weight to dry weight ratio increased at the future 7.8 pH level, suggesting that the low pH resulted in water retention. All combinations of ambient and future temperature and pH conditions resulted in significant shifts in the wet weight to dry weight ratio for *Macrocystis* but with no clear patterns (Fig. 4, Paired T-test Table 2). At 21°C and 8.1 pH, the ratio for *Macrocystis* increased but exhibited the opposite pattern in the remaining water conditions.

Given significant shifts in the wet weight to dry weight ratios, and thus water retention, I also examined the percent change in dry weight for each species under each water condition scenario as a better indicator of growth responses. For *Lithothrix*, the percent change in dry weight varied significantly for temperature, pH, and the interaction term, driven mostly by the large amount of weight loss at the future temperature (21°C) and pH (7.8) treatment (Fig 5, GLMM Table 3); *Lithothrix* dry weight changes were similar among the remaining water conditions. *Macrocystis* dry weight change was significantly impacted by pH only (GLMM Table 3) with less weight loss under future pH conditions (7.8), regardless of temperature (Fig. 6).

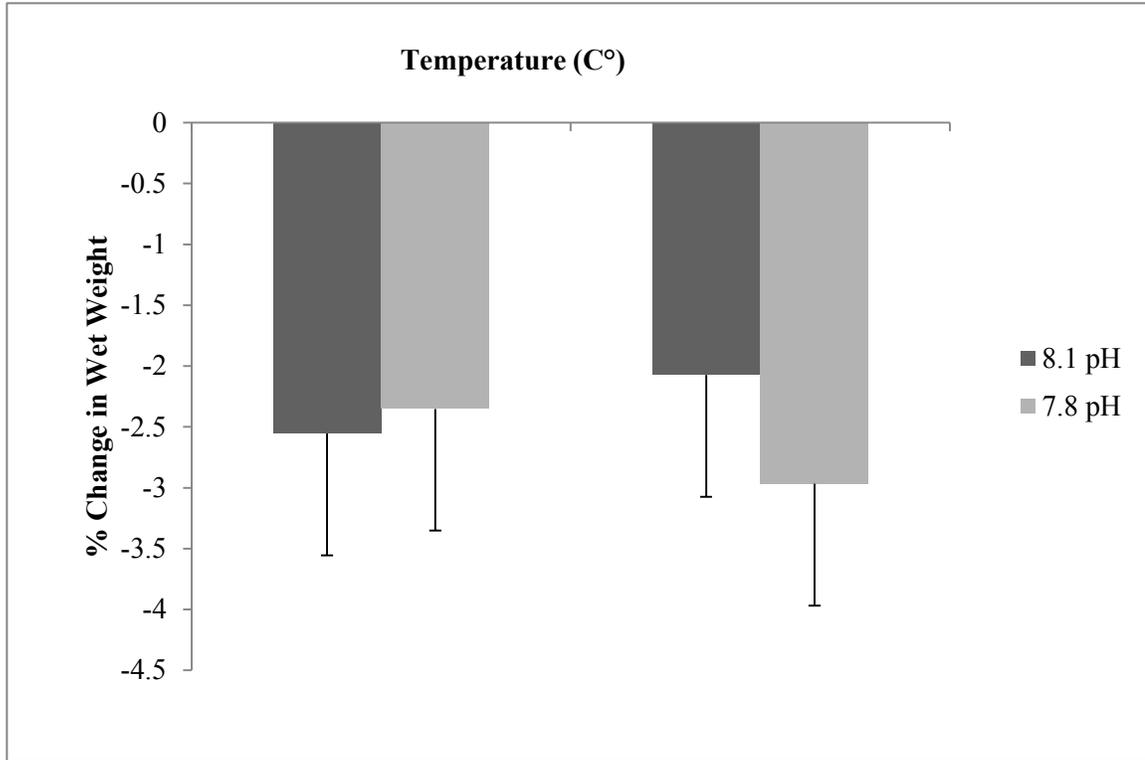


Fig. 1. The percent change in wet weight (\pm SE) of *Lithothrix* grown under combinations of ambient and future temperature and pH conditions. GLMM statistical analysis found significant patterns for temperature, pH, and the interaction term (Table 1).

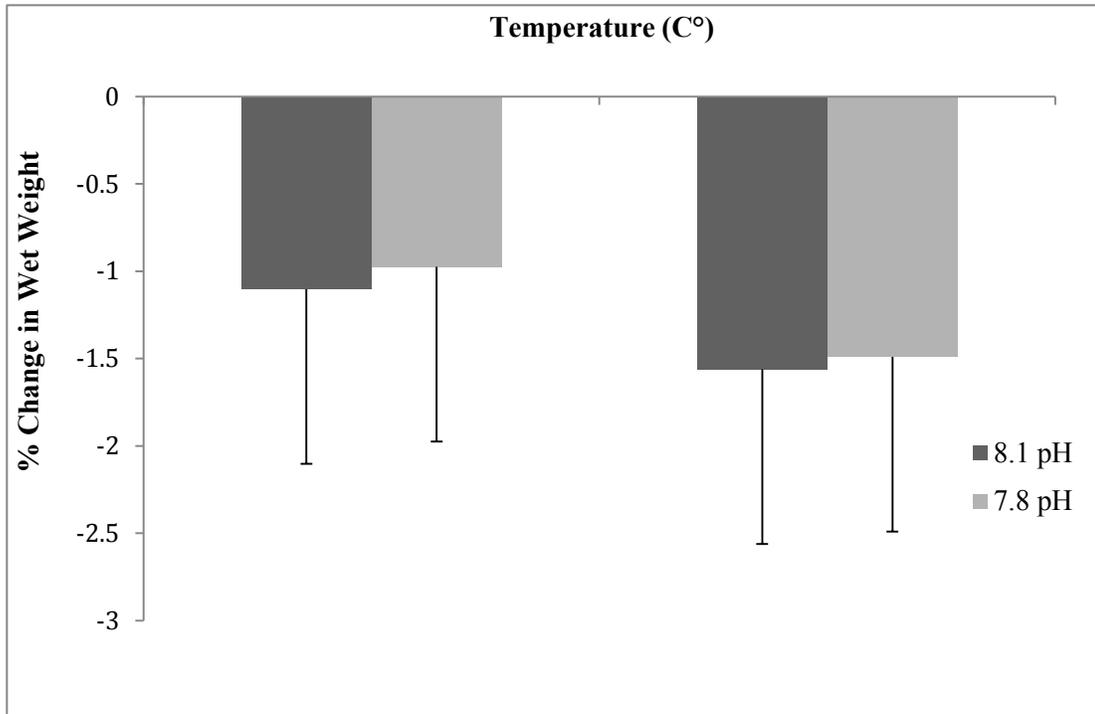


Fig. 2. The percent change in wet weight (\pm SE) of *Macrocyctis* grown under combinations of ambient and future temperature and pH conditions. GLMM analysis found no significant patterns for any factor (Table 1).

Table 1. Results of GLMM statistical analysis comparing the percent change in wet weight of *Lithothrix* and *Macrocystis* grown under combinations of ambient and future temperature and pH conditions. * P< 0.05.

Species	Factor	Num DF	Den DF	MS	F-value	P-value
<i>Lithothrix</i>						
	Temperature	1	81	364.3	5.13	0.026 *
	pH	1	81	1610.3	22.69	<0.001 *
	Interaction	1	81	3210.5	45.24	<0.001 *
<i>Macrocystis</i>						
	Temperature	1	81	2134.0	3.47	0.066
	pH	1	81	9.1	0.01	0.903
	Interaction	1	81	1586.5	2.58	0.112

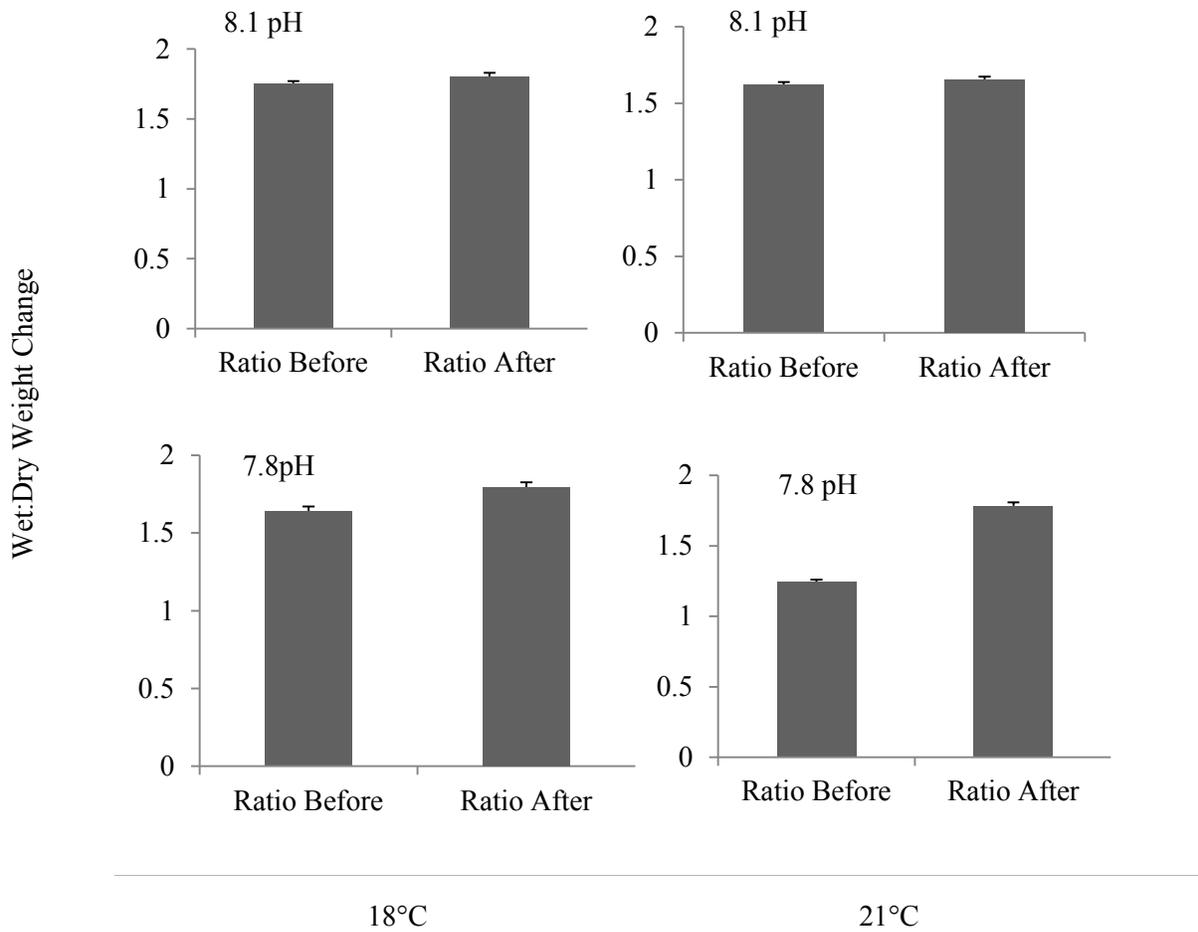


Fig. 3. Wet weight to dry weight ratio (\pm SE) before and after 2 week trials under combinations of ambient and future temperature and pH conditions for *Lithothrix*. Paired T-tests reveal significant differences for 18°C/7.8 pH and 21°C/7.8 pH (Table 2).

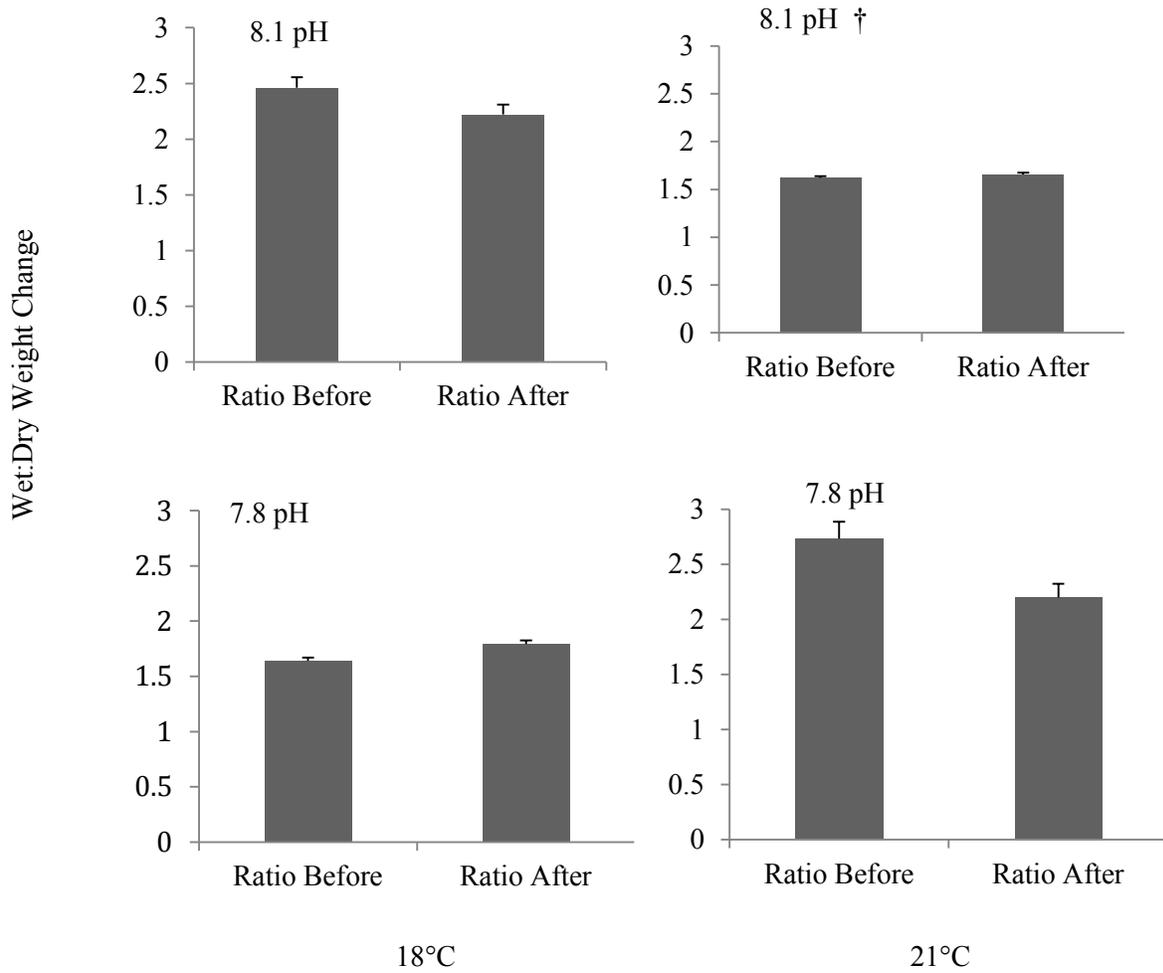


Fig. 5. Wet weight to dry weight ratio (\pm SE) before and after 2 week trials under combinations of ambient and future temperature and pH conditions for *Macrocyctis*. Paired T-tests and a non-parametric, Wilcoxon (indicated by †) reveal significant differences for all conditions (Table 2).

Table 2. Results of the paired sample t-test comparing the wet weight to dry weight ratio before and after 2 week trials under combinations of ambient and future temperature and pH conditions for *Lithothrix* and *Macrocystis*. † indicates the non-parametric, Wilcoxon. * P< 0.05.

Species	Treatment	t-value	SE Ratio Before, Ratio After	P-value
<i>Lithothrix</i>				
	18°C, 8.1 pH	1.76	0.016, 0.029	0.091
	18°C, 7.8 pH	-3.96	0.027, 0.033	0.002 *
	21°C, 8.1 pH	-1.46	0.017, 0.021	0.157
	21°C, 7.8 pH	-17.88	0.014, 0.026	<0.001 *
<i>Macrocystis</i>				
	18°C, 8.1 pH	2.72	0.094, 0.088	0.012 *
	18°C, 7.8 pH	3.14	0.070, 0.086	0.009 *
	21°C, 8.1 pH †	na	0.053, 0.110	0.022 *
	21°C, 7.8 pH	2.51	0.154, 0.124	0.029 *

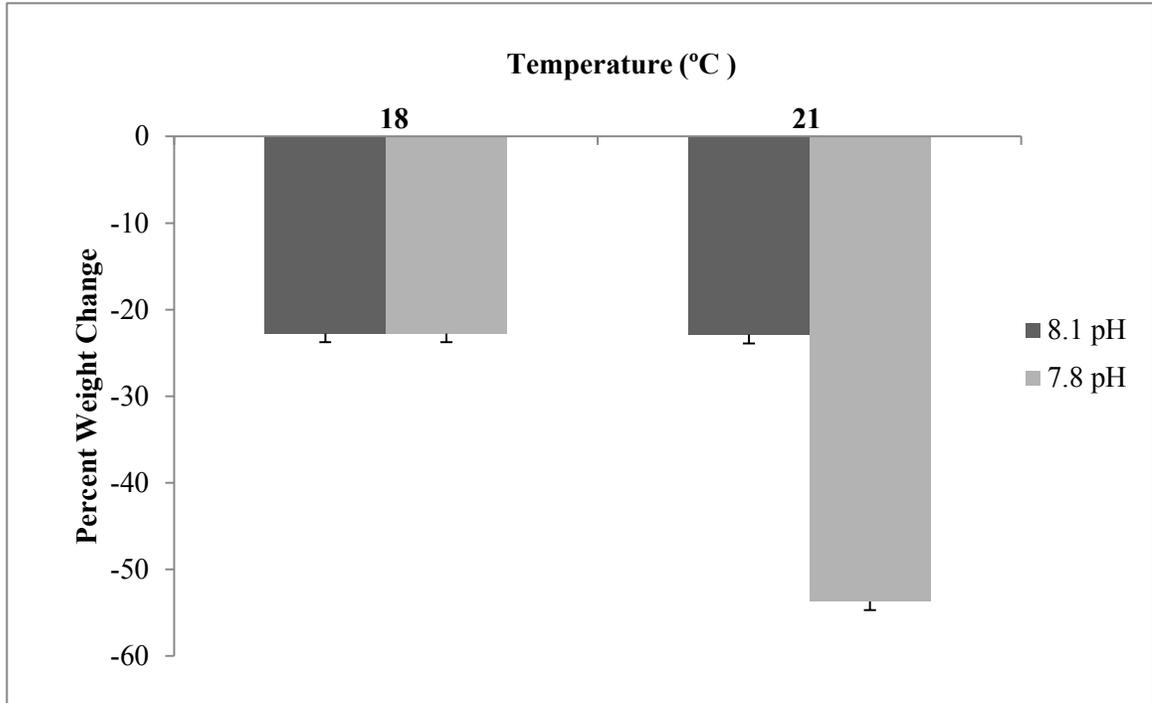


Fig. 5. The percent change in dry weight (\pm SE) of *Lithothrix* grown under combinations of ambient and future temperature and pH conditions. GLMM statistical analysis found significant patterns for temperature, pH, and the interaction term (Table 3).

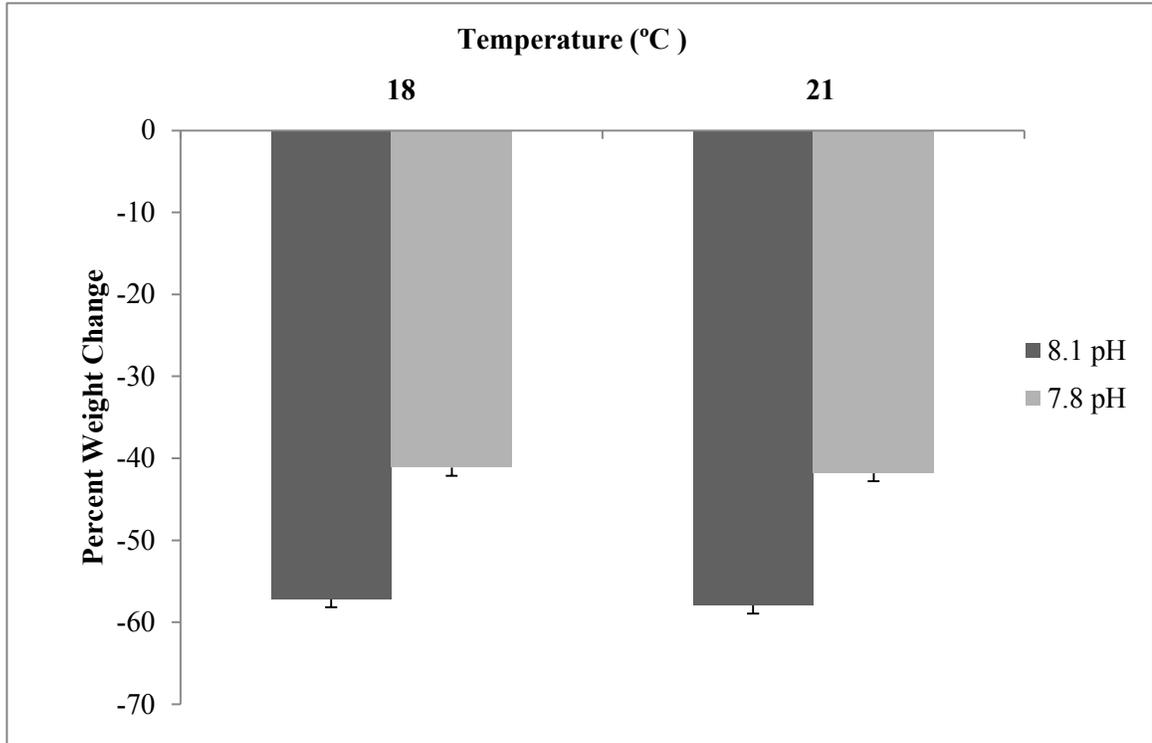


Fig. 6. The percent change in dry weight (\pm SE) of *Macrocystis* grown under combinations of ambient and future temperature and pH conditions. GLMM statistical analysis found significant patterns for pH only (Table 3).

Table 3. Results of GLMM statistical analysis comparing the percent change in dry weight of *Lithothrix* and *Macrocystis* grown under combinations of ambient and future temperature and pH conditions. * indicates significance.

Species	Factor	Num DF	Den DF	MS	F-value	p-value
<i>Lithothrix</i>						
	Temperature	1	33	2867.6	160.19	<0.001 *
	pH	1	33	2867.6	160.19	<0.001 *
	Interaction	1	33	2867.6	160.19	<0.001 *
<i>Macrocystis</i>						
	Temperature	1	33	9.3	0.24	0.630
	pH	1	33	3170.5	80.68	<0.001 *
	Interaction	1	33	0.5	0.01	0.908

3.2 Feeding Preference

In the single choice feeding trials, the feeding rates (% wet weight consumed day⁻¹), of the urchin *Strongylocentrotus purpuratus* on *Lithothrix* varied significantly for each treatment and interaction term (GLMM, Table 4) with magnitudes higher consumption occurring after the seaweed was exposed to an ambient temperature of 18°C and reduced pH of 7.8 (Fig. 7). Urchin feeding rates on *Lithothrix* for the remaining water conditions were relatively similar. Urchin consumption of *Macrocystis* was significantly higher when the seaweed was subjected to low future pH levels but neither temperature nor the interaction term were found to be significant (Fig. 8, GLMM Table 4).

Feeding preference experiments suggest that there is no preference of the urchin between *Lithothrix* and *Macrocystis*, regardless of water treatment, as the difference in the percent change of seaweeds (*Macrocystis* minus *Lithothrix*) was the same between treatments with herbivores and controls without herbivores (Paired T-tests, Table 5). Under both temperatures at ambient pH (8.1) conditions, urchins generally did not eat much of either species (Fig. 9). However, at both temperatures and a future pH of 7.8, both seaweeds were eaten but at similar amounts. There was a slight trend of more consumption of both species at 21°C.

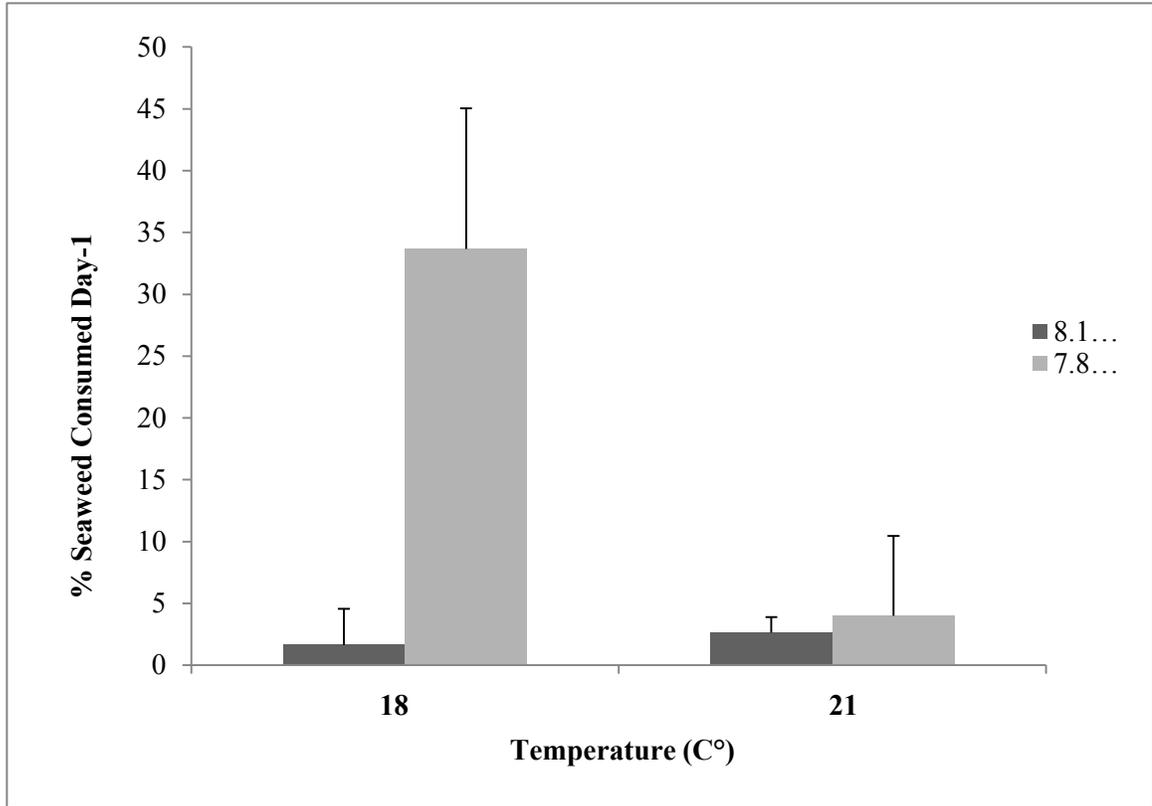


Fig. 7. Consumption rates (% change in wet weight per day, \pm SE) of the urchin *Strongylocentrotus purpuratus* during single-food feeding trials on *Lithothrix*, grown for 2 weeks under combinations of ambient and future temperature and pH conditions. GLMM statistical analysis found significant patterns for temperature, pH, and the interaction term (Table 4).

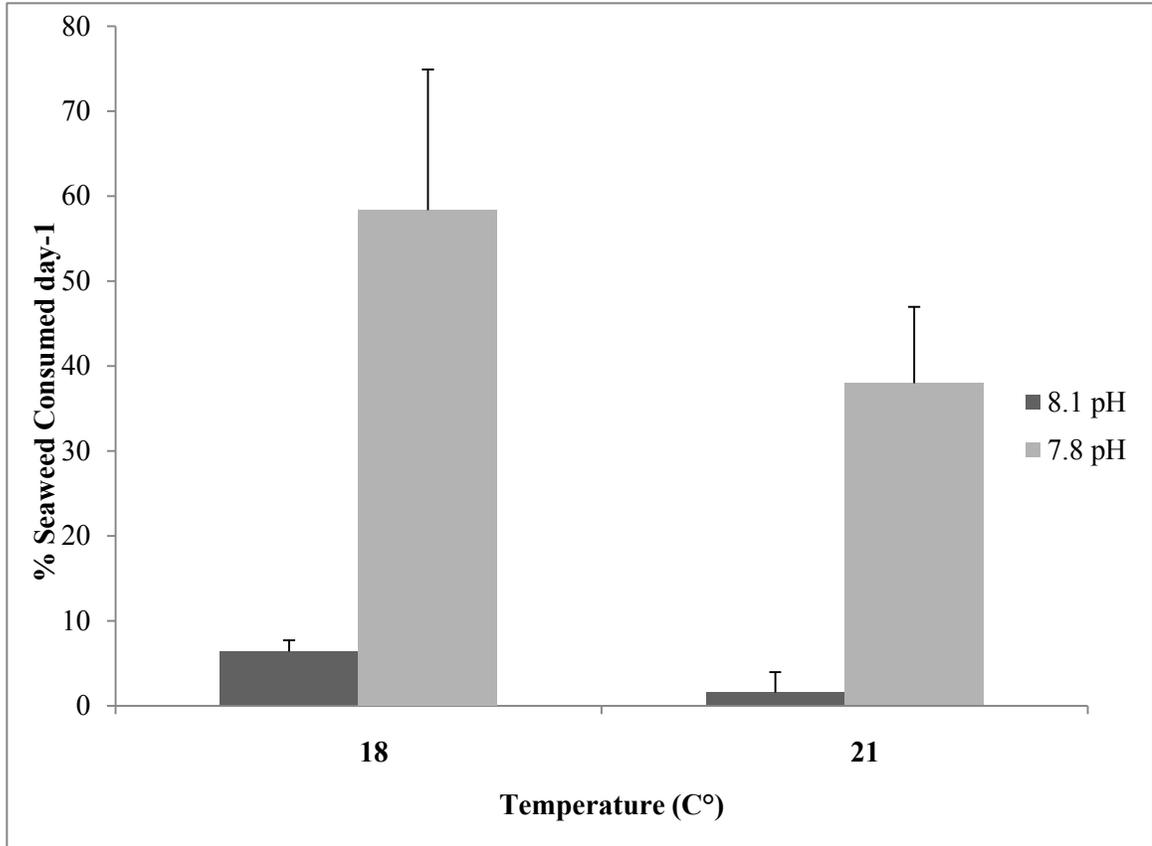


Fig. 8. Consumption rates (% change in wet weight per day, \pm SE) of the urchin *Strongylocentrotus purpuratus* during single-food feeding trials on *Macrocystis*, grown for 2 weeks under combinations of ambient and future temperature and pH conditions. GLMM statistical analysis found significant patterns for pH only (Table 4).

Table 4. Results of GLMM statistical analysis comparing the consumption rate (% change in wet weight per day) of the urchin *Strongylocentrotus purpuratus* during single food feeding trials on *Lithothrix* and *Macrocystis*, grown for 2 weeks under combinations of ambient and future temperature and pH conditions. * indicates significance.

Species	Factor	df	MS	F-value	p-value
<i>Lithothrix</i>					
	Temperature	33	2290.9	4.41	0.033 *
	pH	33	2541.6	4.90	0.014 *
	Interaction	33	3012.4	5.80	0.023 *
<i>Macrocystis</i>					
	Temperature	33	2703.6	2.41	0.184
	pH	33	19385.9	17.31	<0.001 *
	Interaction	33	345.9	0.31	0.409

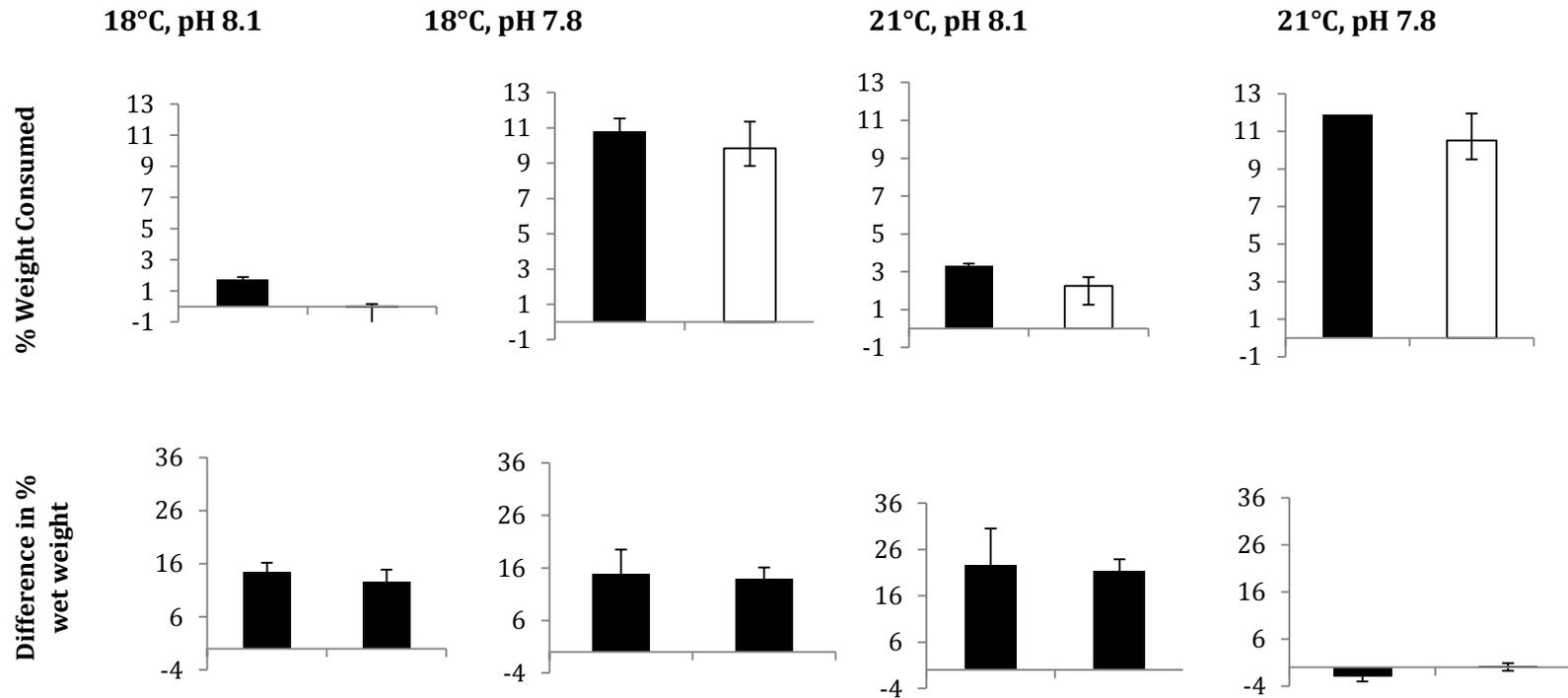


Fig. 9. Percent change in weight consumed (\pm SE; top figures) of *Lithothrix* (black) and *Macrocystis* (white) over 48 hours in treatments with the urchin *Strongylocentrotus purpuratus* present and controls without the herbivore (autogenic weight change). Seaweeds were grown prior to feeding experiments for 2 weeks under combinations of ambient and future temperature and pH conditions. Also indicated (bottom figures) is the difference in the percent wet weight change (*Macrocystis* minus *Lithothrix*) in treatments (black) and controls (white). A paired sample T-test, conducted separately for each water condition, comparing difference in % weight change between treatments and controls yielded no significant differences (Table 5). In general, consumption by the urchin was similar for both algal species for all trials.

Table 5. Results of the paired sample t-test comparing the difference in the percent wet weight change (*Macrocystis* minus *Lithothrix*) in treatments with the herbivore present and controls without herbivores. While a significant value would indicate a feeding preference for one algal species over the other, all trials conducted under combinations of ambient and future temperature and pH conditions exhibited no selective feeding.

Treatment	df	t-value	SE <small>Treatments, Controls</small>	p-value
18°C, 8.1 pH	11	0.529	0.144, 0.174	0.911
18°C, 7.8 pH	11	0.168	0.724, 1.507	0.869
21°C, 8.1 pH	11	0.310	0.119, 0.471649004	0.762
21°C, 7.8 pH	11	0.163	1.523, 1.447	0.873

4. Discussion

Ocean warming and acidification have impacts on a wide variety of marine organisms that can ultimately alter species abundances and diversity. Increased temperature alone is known to increase mortality of sea urchins and cause bleaching in corals (Farmanfarmaian and Giese 1963, Donner et al. 2005, Berkelmans 2002, Hoegh-Gulberg 1999), while increased CO₂ reduces the calcification rate of shells and skeletons of calcifying organisms (Noiset et al. 2013, Anthony et al. 2008, Thomsen et al. 2013). While researchers recognize the need to further study and understand the effects these climate stressors have on marine ecosystems, gaps in knowledge remain on the combined effects of ocean warming and acidification. Approximately 65% of marine climate change studies focus on only ocean warming or acidification on single species rather than species interactions, with only ~35% of studies concentrating on the combination of factors (Wernberg et al. 2012). These two climate stressors are not mutually exclusive and future research needs to continue to focus on both factors, particularly on species interactions. To contribute to this knowledge gap, this study examining the combined effect of increased oceanic temperature and reduced pH on seaweed growth and plant-herbivore interactions found variable and complex patterns. The impacts of warming and acidification on seaweed growth varied between species with a calcifying alga negatively impacted only by combined stressors while a non-calcified alga exhibited positive impacts by acidification only. Feeding rates on these seaweeds grown under different water conditions were generally higher under acidification conditions while feeding preference studies yielded no patterns.

4.1 Growth

Wet weight analysis revealed that *Lithothrix* performed poorly under combined future temperature and pH conditions while there were no significant changes in wet weight of

Macrocystis under manipulated water conditions. However, studies suggest that wet weight may not be an optimal measure of plant growth because water content can vary greatly (Mills and Lee, 1996) due to environmental factors such as sunlight exposure, heat stress, and wind. In this study, water content did vary under the different water conditions. Under future pH conditions *Lithothrix* held more water in its tissues, regardless of temperature. This suggests that as calcium carbonate is depleted from their cell walls with ocean acidification, more water is retained in its tissues. In contrast, *Macrocystis* likely experienced the most water retention at an ambient pH of 8.1, as cells walls may be more resistant to diffusion under ambient conditions, however, lost water content during all other treatment combinations as the tissues began to deteriorate. With shifts in wet weight to dry weight ratios after being treated with a variety of seawater conditions, growth rate is more appropriately measured as a percent change in dry weight for each species (Mills and Lee 1996). In marine growth studies, a vast majority of research has focused wet weight changes in seaweeds (Gao et al. 1993, Gutow et al. 2014, Brown et al. 2016), though dry weight measures are more common in terrestrial plant studies (Morison and Lawlor 1999, Mills and Lee 1996, Roberts 1897). This study highlights that future studies examining growth under manipulated temperature and/or pH should take into account the changes in water content.

The calcifying alga *Lithothrix* exhibited significantly higher dry weight loss when exposed to future oceanic temperature and pH conditions for two weeks. The loss of weight under low pH scenarios were expected, as acidification is known to decrease calcification rates, or in some cases driving decalcification, of species that deposit calcium carbonate in their tissue (Ries et al. 2009). With carbon having a higher attraction to bicarbonate than calcium, high levels of CO₂ in seawater reduce the availability to form calcium carbonate required for calcifying organisms. A number of studies globally have found similar patterns in other calcifying seaweed species grown under reduced pH scenarios, with seaweeds experiencing inhibited growth, increased bleaching, and higher mortality, as they cannot deposit calcium carbonate effectively (Kuffner et al. 2008, Gaitán-Espitia et al. 2014, Anthony et al. 2008, Noisette et al. 2013). The

impacts of temperature depend greatly on the alga's thermal tolerance. Coralline algae can withstand a wide range of temperature conditions, though, temperatures 2-5°C above the local average temperature can cause morphological changes (McCoy and Kamenos 2015) and increase their mortality rate (Martin and Gattuso 2009). The geographic range of *Lithothrix*, from Alaska to Baja California, suggests this species is a more cold-water adapted species with its warmer range tolerance in typical southern California waters. Thus, elevated temperatures above the southern California normal, such as that experienced in this study, are likely to result in physiological stress.

Although acidification and temperature independently are known to impact coralline algae, results from this study suggest that the interaction of combined stressors had the largest effect on *Lithothrix* growth. With performance generally being the same for the seaweed grown under ambient conditions, increased temperature only, and decreased pH only, studies focused on only a singular stressor would not have detected the combined effect, highlighting the importance of examining combined stressors. Similar effects were observed in Mediterranean coralline algae where combinations of elevated temperature and reduced ocean pH greatly reduced mortality and calcification rates magnitudes higher than the effects of one stressor alone (Martin and Gattuso 2009). Similar detrimental impacts were observed on other calcifying species, such as increased coral bleaching when exposed to combined abiotic stressors (Anthony et al. 2008).

Under future pH scenarios, *Macrocystis* displayed little tissue loss during both temperature treatments. The increased CO₂ in the water may have allowed for the brown alga to maximize its photosynthetic rate, allowing it to perform better under future pH conditions (Martin and Hall-Spencer 2017). This has been found to be consistent with other non-calcifying seaweeds. The increased CO₂ in seawater that creates the more acidic conditions is said to be beneficial for some marine alga, as it increases photosynthesis. Seagrasses and furoid species, for example, are known to grow faster under low pH conditions with an increase in photosynthetic rate (Gutow et al. 2014). Most brown, green, and red seaweeds, including kelps and coralline

algae, possess carbon-concentrating mechanisms (CCMs) (Hepburn et al. 2011, Poore et al 2013), which have allowed them to maintain photosynthetic efficiency by adapting to conditions of low or ambient CO₂ in the water. When carbon dioxide and bicarbonate are in excess in the water, CMMs allow seaweeds to convert the bicarbonate into a usable form of carbon dioxide for photosynthesis; which non-CCM algae do not have the ability to do (Nunes et al. 2016, Hepburn et al. 2011, Noisette et al. 2013).

Although temperature was not found to have a significant effect on *Macrocystis* growth, the most tissue loss occurred during an elevated temperature treatment likely due to their sensitivity to extreme temperatures. When exposed to elevated temperature, *Macrocystis* experienced severe tissue deterioration over the 2 week trial compared to when it was grown in low temperature treatments, which is likely due to the fact that *Macrocystis* has a preferred temperature range of 11°C -19°C (Graham et al. 2007, Gaitán-Espitia et al. 2014). When environmental stressors are combined, low temperature and low pH are known to promote growth during early stages of *Macrocystis* development (Gaitán-Espitia et al. 2014). Though when conditions are elevated to mimic future outcomes, *Macrocystis* recruitment may be compromised (Harley et al. 2012).

4.2 Feeding

During single choice feeding trials, sea urchins exhibited elevated consumption of *Lithothrix* grown under ambient temperature and reduced pH. *Lithothrix* deposits calcium carbonate as a physical defense, thus making it difficult for herbivores, such as sea urchins, to consume (Hay and Kappel 1994, Harley et al. 2012). Under reduced pH, *Lithothrix* calcium carbonate deposition was likely inhibited, thus making *Lithothrix* more vulnerable to herbivory. Additionally, some species exhibited decalcification under low pH conditions. For example, encrusting coralline algae experienced 190% reduction in calcification (Anthony et al. 2008) and

cold water corallines displayed decalcification after being exposed to high CO₂ treatments (Büdenbender et al. 2011). If this occurred in this experiment, the alga would potentially be even more susceptible to grazing. Unexpectedly, reduced pH at elevated temperatures did not appear to impact consumption rates of the sea urchin. From growth patterns, it was clear that the seaweed performed extremely poorly under these conditions, which may have affected consumption with mostly deteriorated tissue remaining which may not be ideal for sea urchins to consume. In another study, seaweeds that were considered high or low quality based on their chemical and physical characteristics were offered to marine snails; results showed snails had higher preference for the quality seaweed (Sotka and Giddens 2009).

Sea urchins consumed the most *Macrocystis* that had been grown under low pH conditions, though the varying levels of temperature did not have an impact on the rate of feeding. In support, another study found that sea urchins fed on more *Macrocystis* that was grown under elevated CO₂ despite seawater temperature, likely due to changes in the quality of the food (Brown et al. 2016). During growth trials, *Macrocystis* that was grown in high CO₂ treatments showed less tissue loss than under ambient pH conditions. The physiological changes that occurred under these reduced pH scenarios likely promoted a better quality food (ie. nutritional content, palatability) increasing urchin consumption. While this study did not expose urchins to treatment conditions prior to feeding trials, other studies that did found similar results in which sea urchins exposed to future water treatments consumed more *Macrocystis* that was also grown under future pH scenarios (Brown et al. 2016).

Contrary to original expectations that sea urchins would shift their selectivity for *Macrocystis* at ambient condition to *Lithothrix* with future water conditions as the calcifying species becomes more vulnerable to herbivory, sea urchins showed no preference for either seaweed species in all treatments during two-choice feeding trials. Generally, consumption of both seaweeds was low under ambient pH conditions with increased consumption of both seaweeds at reduced pH treatments. These patterns were somewhat consistent with single-food

feeding rate patterns. Regardless of consumption rates on the individual species, urchins exhibited similar consumption of both species under each water condition scenario, suggesting no selectivity. Research suggests that many herbivores that are known to consume a wide variety of food sources, preferring mixed diets (Kitting 1980, Underwood et al. 2004). When offered 2 or more food sources, limpets for example, showed no preference for any seaweed over the other but rather a mixed diet of all seaweeds (Kitting 1980). Kitting (1980) also suggests that herbivores will consume percentages of many types of seaweed in order to fulfill their nutritional requirements. However, there are a large number of studies that show clear patterns in selectivity (Cox and Murray 2006, Kennish and Williams 1997, Angell et al. 2012), although this might be driven by the types of seaweed food used in the experiment. While sea urchins have been known to prefer *Macrocystis*, higher growth rates were observed when feeding on mixed diet rather than a single food source (Foster et al. 2015).

4.3 Conclusions

Results suggest that the impacts of warming and acidification on growth of seaweeds were not consistent as the singular and additive effects of these stressors were variable between seaweed taxa. While a non-calcifying alga performed better under future pH scenarios, a calcifying alga exhibited reduced growth only under combined stressors with no clear effect of elevated temperature or decreased pH alone. Feeding rates of urchins on these seaweeds grown under different water conditions were also variable between seaweed taxa with consumption generally increasing with acidification but varying under combined acidification and warming. Results highlight the importance of examining the combined effects of potential future stressors.

The impacts of ocean warming and acidification on organisms can have direct and indirect effects on community composition, such as declines in certain species and alterations of biotic interactions. For example, shallow water rocky habitats that are typically dominated by filamentous and crusting algae are predicted to be replaced by fleshy red algae that benefit from

increase ocean CO₂ (Hepburn et al. 2011). Where calcified geniculate seaweeds, like *Lithothrix*, dominate, such as in southern California coastal waters, these algae maybe replaced by non-calcifying seaweeds that perform better under increased CO₂ condition or by species that are adapted to warmer water conditions. Coralline algal turfs are important in coastal ecosystems as they provide habitat to a variety of macro and microfauna (Kelaher et al. 2001). Therefore, declines in coralline turfs can results in shifts in species that rely on these species for food or habitat. Alternatively, increased CO₂ can increase *Macrocystis* growth, as observed in this study. Given *Macrocystis* is a well-documented ecosystem engineer, providing food and habitat for a diversity of flora and fauna, acidification may result in an increase in the abundance of this alga thus facilitating increases in the abundance of kelp forest associated organisms. Although temperature had minimal effects in this study, *Macrocystis* is typically a colder water species thus is likely to be impacted by warming, either under longer exposure to the 21°C used in this study or at higher temperatures modelled in the future (~4°C above *Macrocystis*' tolerance). In these cases, there will likely be a decline in the abundance of kelp with cascading impacts on kelp forest associated taxa. Herbivory on these seaweeds under future oceanographic conditions may also further intensify community changes. Currently, sea urchins primarily graze on *Macrocystis* drift; with the potential decline of *Macrocystis* availability, sea urchins may be forced to actively search for and find alternative food sources (Mattison et al. 1977, Graham et al. 2007). Given *Lithothrix* and other coralline algae may lose their ability to defend themselves through decreased deposition of calcium carbonate, sea urchins may increase feeding on these seaweeds thus causing further declines in coralline algae.

Ocean warming and acidification are expected to influence species and cause shifts in community assemblages and species interactions, thus management of these ecosystems needs to understand potential changes in the future. This study, and many others like this, investigated the effects of ocean warming and acidification on a small-scale level in a laboratory setting. However, many researchers recognize that species responses and alteration of species interactions

as a result of environmental stress would be more accurately measured in the natural habitat where realistic persistent environmental fluctuations and multiple and complex biotic interactions are taken into account.

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