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EFFECTS OF GLOBAL CHANGE ON ALGAL BIOMINERALIZATION AND
BENTHIC COMMUNITY INTERACTIONS ON CALIFORNIA'S TEMPERATE
ROCKY REEFS

A Thesis

Presented to

The Faculty of the Department of Marine Science

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Emily Donham

May 2016

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EFFECTS OF GLOBAL CHANGE ON ALGAL BIOMINERALIZATION AND
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ABSTRACT

EFFECTS OF GLOBAL CHANGE ON ALGAL BIOMINERALIZATION AND BENTHIC COMMUNITY INTERACTIONS ON CALIFORNIA'S TEMPERATE ROCKY REEFS

By Emily Donham

Marine ecosystems are threatened by CO₂-driven global change, such as ocean warming and acidification (OA). The primary objectives of this study were to: 1) assess the response of the coralline red alga, *Calliarthron cheilosporioides*, to global change; and 2) investigate the responses of California's kelp forest communities to OA and sea urchin grazing. Results indicated that *C. cheilosporioides* growth and calcification were reduced under both increased temperatures and increased *p*CO₂ despite increased photosynthetic rates in high *p*CO₂ conditions. Mineralogy did not differ among treatments as a function of warming or acidification. Differences were observed in Mg incorporation into calcified walls of different cell types. Impacts of OA and sea urchin grazing on community structure differed in central and southern CA. In central CA, community structure, calcification and juvenile kelp density showed strong effects of grazing, but no effect of *p*CO₂. Conversely, southern CA showed minor effects of grazing, but strong effects of *p*CO₂ on community structure and calcification, with the strength of response depending on the initial assemblage. These findings suggest that some species of coralline algae may be negatively affected by increased *p*CO₂ and temperature and that the emergent effects of ocean acidification may differ both within a reef as well as across broad spatial scales.

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This thesis is dedicated to my aunt, Julia Asher, who would have been terribly concerned about climate change.

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Introduction to Thesis

Atmospheric CO₂ concentrations are currently higher than at any time in the past 800,000 years (Lüthi et al. 2008; Doney et al. 2009), largely due to increased anthropogenic CO₂ emissions via the burning of fossil fuels, deforestation, and land use changes. The world's oceans have been shown to buffer these CO₂ emissions and are estimated to have absorbed nearly 30% of the total anthropogenic CO₂ since the beginning of the industrial revolution (Sabine et al. 2004). As dissolved concentrations of carbon dioxide (i.e., $p\text{CO}_2$) in the ocean's surface waters continue to increase, the physical and chemical properties of seawater are being altered at a global scale.

When CO₂ dissolves in seawater, it quickly transitions to bicarbonate, HCO₃⁻, releasing H⁺ ions and decreasing pH. This decrease in pH also alters the speciation of inorganic carbon by decreasing the availability of carbonate ions, CO₃⁻², thus lowering the saturation states (Ω) of carbonate polymorphs (Feely et al. 2004). This process has been termed ocean acidification (OA) and has already resulted in a decrease in pH of roughly 0.1 units and a decrease in carbonate ion concentration by ~30 $\mu\text{mol kg}^{-1}$ seawater (Caldeira and Wickett 2003; Hoegh-Guldberg et al. 2007). An additional drop in ocean pH of 0.3-0.5 units is expected by the year 2100 with a corresponding 50% decrease in carbonate ion concentrations compared to preindustrial levels (Feely et al. 2009).

In addition to ocean acidification, strong evidence exists linking increasing concentrations of atmospheric CO₂ to rising global temperatures on land and in the sea. The average global ocean temperature has increased by 0.74 °C since the beginning of the 20th century and is predicted to rise by an additional 1-4 °C by the year 2100 (Hoegh-Guldberg et al. 2007). Although OA and warming are perhaps the most well-known consequences of CO₂-induced climate change, other stressors such as sea-level rise, increased hypoxia events, significant changes to wave heights, upwelling intensity and duration, terrigenous nutrient runoff, and coastal salinity will also affect the near-shore marine landscape (Doney 2010; Gruber et al. 2012; Harley et al. 2012). Given the rapid oceanographic changes due to rising CO₂ concentrations, research assessing the biological, geochemical, and community responses of marine organisms is imperative in order to better understand the socioeconomic and ecological consequences of climate change.

Chapter I

Single-species consequences of warming and acidification on the temperate articulated coralline alga, *Calliarthron cheilosporioides*

Abstract

Global climate change stressors such as warming and acidification are predicted to have negative effects on calcifying organisms. Species that precipitate more soluble forms of calcium carbonate are thought to be at higher risk to the detrimental effects of ocean acidification. Coralline red algae are abundant and ecologically important species known to precipitate the most soluble form of carbonate, high Mg-calcite. I assessed the impacts of warming and acidification on the articulated coralline red alga, *Calliarthron cheilosporioides*, inhabiting a temperate kelp forest in San Diego, CA. I found calcification is significantly reduced under high temperature (70%) and further reduced by high $p\text{CO}_2$ or high $p\text{CO}_2$ x temperature (150%) conditions. Growth (as linear extension and surface area of new growth) was also significantly reduced by temperature (40% and 50% respectively), high $p\text{CO}_2$ (20% and 40% respectively) and high $p\text{CO}_2$ x temperature (50% and 75% respectively). The photophysiological parameter P_{max} increased by 100% under high $p\text{CO}_2$ conditions, but there was no effect of $p\text{CO}_2$ or temperature on photosynthetic efficiency. Mineralogy was not affected by temperature or $p\text{CO}_2$. However, variation in Mg incorporation in cell walls of different cell types (i.e., higher %Mg in cortical than medullary cells) was documented for the first time in this species. These results support findings from a growing body of literature suggesting that coralline red algae are likely to be negatively affected by increased acidification and warming associated with climate change.

Introduction

The majority of marine research assessing the threats of CO₂-driven global change has focused on the impacts of ocean acidification on marine calcifiers, such as commercially important bivalves, corals and tropical macroalgae, whose calcification has been shown to rely heavily on the saturation states of the biogenic carbonates precipitated (Kroeker et al. 2010; Ries 2011a). However, organisms inhabiting the lesser-studied subtropical and high latitude ecosystems are thought to be at higher risk for OA due to the naturally more corrosive, lower pH, and lower saturation states of the seawater in which they live (Andersson et al. 2008). Furthermore, not all calcifying organisms are equally as vulnerable, even within the same genus (Price et al. 2011).

Calcifying organisms can differ in the relative isolation of the calcifying space and mineral composition of their skeletal material. Carbonate skeletons consisting of high Mg-calcite (mol % Mg > 4%) are more soluble than those made of aragonite and calcite (Andersson et al. 2008). Organisms that secrete more soluble forms of calcium carbonate external to protective tissues may be even more vulnerable to the detrimental effects of OA (Ries et al. 2009). For these reasons, organisms that secrete high Mg-calcite and inhabit subtropical and high latitude ecosystems may be the first organisms to be impacted by ocean acidification.

CO₂-induced global warming is predicted to have large negative impacts on many marine organisms. Temperature alters biological processes due to

effects on metabolic rates and many physiological processes (Brown et al. 2004). Higher temperatures increase both photosynthetic (Yokohama 1973) and respiration rates, with the potential to increase calcification (Johnson and Carpenter 2012). These kinetic effects have been shown to result in higher growth rates in marine macrophytes in the field during seasonal warming (Keser et al. 2005). However, increases in temperature beyond a species' thermal optimum can also cause physiological stress and even mortality, as has been shown in temperate macroalgae (Schiel et al. 2004) or more commonly in scleractinian corals found on tropical coral reefs. Mass coral bleaching events due to warming have caused widespread mortality of corals and in some cases even complete collapse of coral reef ecosystems (Pandolfi et al. 2011). Therefore, ocean warming associated with climate change could provide benefits to organisms so long as temperatures remain within species-specific thermal tolerances, but could also have dire consequences to organisms with narrow thermal tolerances.

Coralline red algae (Corallinales, Rhodophyta) are calcifying macroalgae which secrete high Mg-calcite skeletons within their intracellular spaces (Borowitzka et al. 1974). They are ubiquitous members of coastal marine ecosystems around the world and provide structural support by acting as “cement” to bind reef substrate, refugia by providing habitat (articulated or branching forms), recruitment facilitation by producing settlement cues (e.g., Price 2010), and substrate for both intertidal and subtidal marine communities

(Steneck 1986). Furthermore, calcification plays an important role in growth and herbivore deterrence (Büdenbender et al. 2011), preventing overgrowth and fouling by tissue sloughing (Littler and Littler 1995), and protection from UV radiation (Gao and Zheng 2009).

Due to their ecological importance on reefs and their sensitivity to environmental conditions, many studies have investigated the impacts of OA and/or warming on coralline red algae. As predicted, responses of corallines to global change stressors have largely been negative. These responses have included reduced recruitment (Kuffner et al. 2007), decreased calcification or increased dissolution (Anthony et al. 2008; Jokiel et al. 2008; Martin et al. 2008; Gao and Zheng 2009; Martin and Gattuso 2009; Ries et al. 2009; Büdenbender et al. 2011; Hofmann et al. 2011; Diaz-pulido et al. 2012; Johnson and Carpenter 2012; Ragazzola et al. 2012; Johnson et al. 2014; Kram et al. 2015), decreased photosynthetic efficiency (Anthony et al. 2008; Gao and Zheng 2009; Hofmann et al. 2011), bleaching (Anthony et al. 2008), and weakening of the skeletal structure (Ragazzola et al. 2012).

Despite a convincing body of literature showing negative responses of coralline red algae to OA, a few studies have also found that some corallines may be more resilient than previously thought. Egilisdottir et al. (2012) found that respiration, gross primary production, and calcification were not significantly affected by elevated $p\text{CO}_2$ (up to 1000 μatm) in the intertidal alga, *Corallina elongata*. The authors suggest that this may be due, in part, to the dynamic

intertidal environment in which the alga has evolved to survive. Non-linear physiological responses to OA have also been found in some coralline red algae (Ries et al. 2009; Johnson and Carpenter, 2012). In these studies, growth and calcification increased in moderately elevated $p\text{CO}_2$ but declined at higher $p\text{CO}_2$ levels. These findings are consistent with work by Borowitzka (1981) who found that calcification rates in coralline algae are greatly influenced by external carbonate ion concentration and photosynthetic rates. At higher $p\text{CO}_2$, algae are able to increase photosynthetic rates due to the increased abundance of CO_2 and HCO_3^- ions. However, a threshold exists where the benefits of increased CO_2 for photosynthesis do not outweigh the decrease in carbonate ion availability for calcification. It's likely that species inhabiting naturally elevated or more variable $p\text{CO}_2$ seawater conditions may optimize photosynthesis and calcification at higher $p\text{CO}_2$.

Climate change stressors will likely have numerous effects on coralline red algal growth, calcification and mineralogy. Some species of corallines have also been documented to change the mineral composition of their skeletons, by reducing the mole % Mg incorporated into their Mg-calcite skeletons, under elevated $p\text{CO}_2$ (Ries 2011; Egilisdottir et al. 2012). Since the solubility of Mg-calcite is positively correlated with increasing mole % Mg (Andersson et al. 2008), species that exhibit mineralogical plasticity may be more resilient to OA. However, until we understand how this process affects overall growth and

survivorship, it is unclear whether it functions as a short-term coping mechanism or a longer-term solution to OA.

Positive correlations have been found between the Mg content of biominerals and temperature (Stanley and Hardie 1998; Andersson et al. 2008). Since some crustose coralline algae (CCA) produce annual banding patterns, analogous to rings on a tree (dendrochronology); variation with respect to the elemental composition of the skeletal material can be correlated with differences in the abiotic environment during the time of precipitation. Biomineral heterogeneity within long-living CCA has therefore been used to reconstruct paleoclimates using mole % MgCO_3 and SrCO_3 as proxies for sea surface temperatures (Kamenos et al. 2008; Kamenos and Law 2010). Far less is known about the interactive effects of warming and OA on mineral composition. Importantly, increased temperature may further stress organisms trying to maintain Mg-calcite skeletons under OA due to increased incorporation of Mg into the calcite lattice.

Additionally, recent work on tropical coralline red algae by Nash et al. (2011) discovered the presence of Mg-rich biogenic carbonates (protodolomite, magnesite), previously thought to form only during diagenetic processes (e.g., high heat and pressure). These mineral forms are more stable than calcite and therefore may make the skeletons of these organisms more resistant to dissolution (Nash et al. 2012). However, these minerals are yet to be found in any temperate algal species. It is uncertain whether this is due to the scarcity of

work using newly developed techniques, which offer higher resolution, or due to some other environmental or physiological mechanism preventing their precipitation. In general, little is known about fine scale variability in biominerals throughout the algal thallus, however, this variation may be critical for the survival of coralline red algae in a changing ocean.

Although many climate change stressors will occur simultaneously, fewer studies have measured their interactive effects on marine organisms compared with those that have measured the effects of a single variable alone. Since multiple stressors can act additively, synergistically and antagonistically, in order to accurately forecast the impacts of climate change on marine organisms it is important for future studies to incorporate a multi-stressor framework (Harley et al. 2012; Harvey et al. 2013; Kroeker et al. 2013). Given that OA and warming will most certainly affect basic physiological and biological processes, these two abiotic changes are an obvious starting point in testing the interactive effects of multiple climate change stressors on coastal marine organisms.

Studies that have measured the interactive effects of increasing temperature and OA suggest that the effects of both stressors may be multiplicative and therefore studies that focus on OA or warming alone may underestimate the impacts of future environmental conditions on organismal physiology and ecology (Hoegh-Guldberg et al. 2007; Anthony et al. 2008; Diaz-Pulido et al. 2012; Johnson and Carpenter 2012). A recent meta-analysis concluded that the combined effects of OA and warming had a significant

negative effect on calcification, survival and reproduction across a wide range of taxa with the magnitude of response greater than that observed for the stressors in isolation (Harvey et al. 2013). Furthermore, concurrent OA and warming had no effect on growth, but a significant positive effect on photosynthesis in autotrophs (Harvey et al. 2013).

The aim of this chapter is to address the following questions: 1) How will OA and warming affect the growth, calcification and mineralogy of the abundant coralline red alga, *Calliarthron cheilosporioides*, inhabiting California's temperate rocky reefs? I hypothesized that 1) high $p\text{CO}_2$ and warming would decrease growth and calcification rates with their interaction being multiplicative; 2) high $p\text{CO}_2$ will decrease the mol % Mg incorporated into biominerals, but high temperature will increase the mol % Mg, with an unknown interaction; 3) high $p\text{CO}_2$ and warming will increase photosynthetic efficiency, with their interaction being multiplicative.

Materials and Methods

Study Site and Collections

The articulated coralline red alga, *Calliarthron cheilosporioides*, was collected subtidally (~13 m depth) using SCUBA at Mia's Reef in San Diego, CA (N 32° 51' 14.8", W 117° 16' 52.4") due to proximity of the experimental rearing facilities at Scripps Institution of Oceanography (SIO). *C. cheilosporioides* individuals were gently dislodged from the substrate with holdfasts intact. This

articulated coralline, abundant on subtidal reefs from southern to central California, has been found to release chemical cues for the settlement of invertebrate larvae and create habitat for juvenile purple sea urchins (Harrold et al. 1991). Unlike slower growing coralline crusts, *C. cheilosporioides*, has been shown to have fast growth rates up to 1.8 cm² per month (Martone 2010). *C. cheilosporioides* was chosen for this study due to its importance for temperate reefs, wide spatial distribution, and fast growth rates which provided adequate new growth over the duration of our experiment.

An autonomous pH and temperature logger, SeaFET (Martz et al. 2010), was deployed at Mia's Reef to record continuous high-resolution (every 15 min) pH and temperature measurements to improve understanding of natural fluctuations at my site and aid in the interpretation of experimental results. Photosynthetically active radiation (PAR) was also measured *in situ* (every half hour) within the kelp forest at ~13 m depth using a LICOR 4- π quantum sensor (Biospherical, Inc.).

Seawater Bubbling System

Ocean acidification studies to date have mostly been conducted using pre-equilibrated seawater to simulate stable changes in pH and carbonate chemistry predicted to occur by the end of the century. However, relatively recent technological advances have made it possible to better understand fluctuations in pH in nearshore coastal environments. Unlike open-ocean environments, which

have been used to model predicted future ocean conditions, near-shore environments show much more variable pH conditions due to biological activity (i.e., photosynthesis, respiration, calcification) and terrestrial influences (Frieder et al. 2012; Hofmann et al. 2011; Price et al. 2012). These studies have shown that some organisms in nearshore regions are already periodically exposed to pH conditions predicted by the year 2100 in the global ocean surface waters. Therefore, predictions made on open-ocean conditions may not be indicative of future conditions that will occur in nearshore environments. In order to better simulate the possible impacts of OA on nearshore ecosystems, I used a state-of-the-art seawater bubbling system that manipulates $p\text{CO}_2$ and temperature on top of natural coastal variability. By maintaining a constant bubbling rate and seawater flow, $p\text{CO}_2$ was increased by predicted magnitudes on top of naturally fluctuating seawater chemistry. This method more accurately mimics the potential effects of OA in the coastal environment by altering pH and carbonate chemistry on top of natural variability occurring over diel, semi-diurnal, and event (day-week) time scales.

The seawater bubbling system bubbles CO_2 into individual mesocosms supplied with flow-through seawater pumped directly from the SIO pier. Individual specimens of *C. cheilosporioides* (~1 g) were placed in 1-liter glass mason jars (mesocosms). Specimens ($n=7$ per treatment) were randomly allocated to one of four treatment levels. Treatment $p\text{CO}_2$ conditions were created by continuously bubbling a CO_2 -air gas blend into each individual

mesocosm at a rate that lowers the pH by 0.4 ± 0.05 units. This treatment pH was chosen to represent future ocean conditions by the year 2100 as specified by the IPCC's A2 emission scenario (IPCC 2007). Ambient mesocosms were also supplied with air originating from the same source as the CO₂-air gas blend used in the treatment conditions. Half of the aquaria were maintained at ambient temperature ($\sim 13^{\circ}\text{C}$) and half at 3°C above ambient ($\sim 16^{\circ}\text{C}$) for the duration of the experiment. Experimentally elevated temperatures were maintained in 3 replicate water baths, wherein mesocosms were immersed in seawater with submersible aquarium heaters. Ambient temperature mesocosms were placed in water baths (n=3) of ambient temperature water to control for the effects of the water bath. Photoperiod was set to 12:12 day:night cycle for the entirety of the experiment.

Temperature and pH data were collected daily at midday (13:00 PST $\pm$ 2 hours) in all aquaria using a HACH HQ40d handheld glass electrode pH probe calibrated each day with certified Tris buffer from the lab of Dr. Andrew Dickson at SIO. Minor adjustments were made to bubbling rates in individual aquaria if the experimental pH drifted above or below the desired 0.4 ± 0.05 units below ambient. Honeywell's Durafet non-glass pH probe logged pH and temperature every 15 minutes in one control mesocosm (i.e., no algal sample) at each treatment level for the duration of the experiment. Honeywell's Durafet Ion Sensitive Field Effect Transistor (ISFET) technology has been shown to remain stable for long periods of time (Martz et al. 2010), and therefore was not

calibrated daily. These pH sensors logged treatment conditions continuously in order to observe diurnal cycles in flow-through seawater conditions that may otherwise be missed from single point observations. To monitor carbonate chemistry parameters in the experiments (i.e., dissolved inorganic carbon, total alkalinity, and salinity), discrete water samples were collected in 500 mL Corning brand pyrex sample bottles at the beginning, middle and end of each experiment and immediately spiked with 120 μL of saturated HgCl_2 solution (1% headspace); these samples also served as calibration standards for the Durafet sensors. In order to assure that algal samples did not alter seawater chemistry within mesocosms, discrete water samples were taken from three control jars (without samples) and two randomly assigned specimen jars from each treatment at the beginning, middle, and end of the experiment. Total dissolved inorganic carbon (DIC_T) was measured using a Single Operator Multi-parameter Metabolic Analyzer (SOMMA) in the laboratory of Dr. Andrew Dickson (SIO). Total alkalinity (A_T) was measured via open cell acid titration using a Metrohm Dosimat Model 665 and Thermo Scientific Ross potentiometric pH probe and meter. Carbon species were calculated based on measured DIC_T and A_T using CO2SYS and stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987).

Physiological and Mineralogical Responses of Coralline Algae

To assess changes in physiology and mineralogy of coralline algae due to climate change, specimens were reared in the laboratory under two different $p\text{CO}_2$ levels (ambient = $\sim 450 \mu\text{atm}$, high = $\sim 1800 \mu\text{atm}$) and two different temperature levels (ambient = $\sim 13^\circ\text{C}$, high = $\sim 16^\circ\text{C}$) in a multifactorial design resulting in four different treatment conditions: (1) ambient temperature, ambient $p\text{CO}_2$, (2) high temperature, ambient $p\text{CO}_2$, (3) ambient temperature, high $p\text{CO}_2$, and (4) high temperature, high $p\text{CO}_2$, simulating a suite of possible future ocean conditions.

Growth

All laboratory experiments manipulating temperature and pH were conducted in the state-of-the-art flow-through seawater system described above. Samples were placed into holding tanks with flow-through seawater where they were acclimated to ambient laboratory conditions for two weeks. 24 hours before the start of the experiment, samples were placed in a 1 L beaker with 0.02% Calcofluor white (Fluorescent Brightener 28, Sigma-Aldrich) in seawater for 5 minutes in order to stain initial tips before skeletal material is deposited in response to treatment conditions. This method has been used by Martone (2010) to permanently incorporate the fluorescent dye into the calcium carbonate of growing tips and allow for future measurements of new growth.

To measure growth, linear extension and surface area were also calculated for each individual. Samples were visualized using a dissecting scope and illuminated with an ultraviolet lamp (315-400 nm) to visualize calcofluor stain. Calcofluor stained tips were randomly selected and removed at the closest genicula behind the leading edge of the stain using forceps. Photographs were taken of three replicate growing tips in Image Pro Plus Software (Media Cybernetics, Inc.) using a Leica microsystems camera fitted to a Leica MZ 12₅ dissecting scope. The field of view was calibrated using an objective micrometer (0-1mm/100) and the length from the apex of the stained region to the tip of the branch was measured to obtain linear extension (Fig. 1). From these same photographs, planar area was also calculated as the surface area of new growth after the calcofluor arch (Fig. 2). All three tip measurements per individual were for pooled for subsequent statistical analyses.

Figure 1. Calcofluor stained region is depicted as fluorescent blue arch on three growing tips from *C. cheilosporioides*. Yellow lines show linear extension measurements of new growth material during experimental rearing (L1, L2, L3 = linear extension in mm).

Figure 2. Surface area measurements of new growth material during experimental rearing. Yellow lines show planar surface area beyond the calcofluor white staining marker (PG1, PG3, and PG4+PG5 = surface area of new growth in mm²).

To measure calcification rates, all specimens were first cleaned of epiphytes by gentle removal using forceps and a soft-bristled brush and then were weighed using the buoyant weight method at the beginning and end of each experiment. Samples were placed in a metal basket completely submerged in seawater. The metal basket was attached to monofilament line and suspended from a weigh-below balance. The buoyant weight method was used since it has been found to be more accurate at measuring the weight of calcified algae than

other methods since it does not take into account the weight of fleshy material that are less dense than water (Davies 1989). Percent change in weight was calculated as, $\frac{W_f - W_i}{W_i}$, where W_i is the initial weight and W_f is the final weight.

Photophysiology

In order to compare differences in photosynthetic efficiency, photophysiological parameters were calculated experimentally. Samples from each treatment level ($n = 3$) were randomly selected and placed in individual 1.5 L polycarbonate containers and covered with a polycarbonate lid with an airtight rubber seal. One container with no sample (control) was also covered to control for changes in dissolved oxygen concentrations due to water column processes. $p\text{CO}_2$ conditions at the beginning of the incubations were obtained by bubbling either air or the same CO_2 -air gas blend used during the experiment into a 20 L carboy until the desired pH was obtained. Containers were incubated in the light (irradiance: 19, 36, 60, 103, 178, 198, 344, 392 and $500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and dark ($0 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 45 minutes. In between each light step, containers were opened and seawater was fully replenished. Temperature was held constant and similar to treatment conditions (i.e., ambient and high [ambient + 3°C]) for the duration of each incubation by submerging containers in a water bath maintained with flow-through seawater held at the desired temperature. Magnetic stir bars continuously stirred seawater within each container to disrupt diffusive boundary

layers. Dissolved oxygen was measured at the beginning and end of each incubation using a HACH 40D dissolved oxygen (DO) probe. Net production or respiration (NP (or R), $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$) was calculated by plotting DO versus time at time t_{0+10} to t_{0+20} , where t_0 indicates the sealing of the incubation chamber and each time step is one minute in duration. NP (or R) is the slope of the linear regression fit at each light level. To calculate photophysiological parameters NP vs Irradiance data were fit to the equation:

$$NP = P_{max} * (1 - e^{\frac{-\alpha * I}{P_{max}}} * e^{\frac{-\beta * x}{P_{max}}})$$

where P_{max} is the maximum photosynthetic rate, α is the photosynthetic efficiency, and β is the irradiance at which photoinhibition begins.

Mineralogy

A Hitachi 3400n Scanning Electron Microscope (SEM) with attached INCA Energy 250 Energy Dispersive X-ray Spectrometer (EDS) was used to examine the spatial and structural variability in biomineralization, and changes to the mol % Mg incorporated into biominerals. Using EDS, Mg^{2+} and Ca^{2+} concentrations were measured in the calcified cell walls surrounding both medullary and cortical cells (Fig. 3). Immediately following final calcification measurements (buoyant weighing), samples were placed in a drying oven at 60 °C for 48 hours. Each sample was visualized under a dissecting microscope with UV light projected onto the sample to visual the calcofluor white stain. Using forceps and a razor blade, thallus material deposited after the fluorescent dye marker was removed

from tips of two different branches and placed aside for subsequent SEM and EDS analysis. Each subsample was secured to an individual SEM stub with Epotek resin, polished and gold coated. Differences in the composition of Mg^{2+} and Ca^{2+} incorporated into biogenic carbonates was quantified by conducting spot analysis at nine points underlying a 3x3 grid matrix. At each spot the identity of the cell type (cortical versus medullary) was recorded. These analyses were conducted on two separate branch tips for each individual. To reduce error in measurements of Mg^{2+} and Ca^{2+} only spot analyses with a Wt % equal to 80-120 were considered. The mol % Mg at each spot analysis was calculated as $\frac{At\% Mg}{At\% Mg + At\% Ca} \times 100$, where *At % Mg* and *At % Ca* are determined from EDS. For each individual, mol % Mg from 3 spot analyses for each cell type, corresponding to those closest to a Wt % = 100, were pooled for subsequent statistical analyses.

Figure 3. Scanning electron microscopy (SEM) image at 1000x. Image depicts more circular cortical cells (A) and more elongate internal medullary cells (B).

Statistical Methods for Coralline Algae Experiments

Experimental results from coralline rearing experiments were analyzed using two-way ANOVAs with fixed factors of $p\text{CO}_2$ and temperature. The response variables tested were: calcification rate; linear extension; surface area; and mol % Mg. Shapiro-Wilkes (W) was calculated on the residuals to test for normality and homogeneity of variances (Shapiro and Wilk 1965). In the case where a response variable did not meet the assumptions of normality, it was square root transformed and analyses were rerun on transformed data. A two-sample t-test was used to compare differences between mol % Mg of cortical and

medullary cells pooled across treatments. A two-way ANOVA, with fixed factors of $p\text{CO}_2$ and temperature, was run on photophysiological parameters P_{max} and α .

Results

Experimental Conditions

Four distinct $p\text{CO}_2$ x temperature treatments were maintained for the duration of the 28-day experiment (Table 1-2; Fig. 4). Ambient $p\text{CO}_2$ treatments were approximately 0.40 pH units higher and $\sim 1200 \mu\text{atm}$ lower than the high $p\text{CO}_2$ treatments. Ambient temperature treatments were roughly 3°C lower than the high temperature treatments. *In situ* measurements of pH and temperature from autonomous SeaFET loggers at Mia's reef recorded a mean pH = 8.02 and mean temperature = 15.28°C from October 23, 2013 - April 12, 2014 (Table 1; Fig. 5).

Mean peak irradiance levels in treatment conditions were approximately $23 \mu\text{mol m}^{-2} \text{sec}^{-1}$, while *in situ* measurements of peak irradiance recorded at Mia's reef were $\sim 65 \mu\text{mol m}^{-2} \text{sec}^{-1}$ from October 6, 2013 to October 25, 2013 (Table 1; Fig. 6).

Table 1. Average environmental conditions in the field at Mia's Reef, San Diego, CA (collection site) and during the duration of the 28-day experiment

Treatment	Mean pH _{sw}	Min pH _{sw}	Max pH _{sw}	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Mean Peak Irradiance ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)
Field	8.02	7.57	8.15	15.28	11.40	17.71	64.98 $\pm$ 9.70
Ambient pCO ₂ , Ambient Temp	8.10	8.05	8.18	13.60	12.97	14.36	25.61 $\pm$ 1.66
Ambient pCO ₂ , High Temp	8.06	8.01	8.11	16.70	14.86	19.23	24.68 $\pm$ 2.34
High pCO ₂ , Ambient Temp	7.68	7.55	7.81	13.58	13.08	14.46	25.96 $\pm$ 1.80
High pCO ₂ , High Temp	7.66	7.51	7.81	16.53	14.67	18.44	21.11 $\pm$ 0.86

Table 2. Mean seawater chemistry for experimental mesocosms obtained at the beginning and end of 28-day experiment ($\pm$ SE). Discrete samples from mesocosms both with and without macroalgae were pooled within treatments.

Treatment	# Discrete Samples	Salinity	A_T ($\mu\text{mol kg}^{-1}$)	DIC_T ($\mu\text{mol kg}^{-1}$)	pH_{SW}	HCO_3^- ($\mu\text{mol kg}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	CO_2 ($\mu\text{mol kg}^{-1}$)	pCO_2 (μatm)	Ω_{Calcite}	$\Omega_{\text{Aragonite}}$
Ambient pCO_2 , Ambient temp	3	33.49 $\pm$ 0.019	2104 $\pm$ 7.107	2064 $\pm$ 7.086	7.96 $\pm$ 0.003	1924 $\pm$ 6.578	121 $\pm$ 1.088	19.0 $\pm$ 0.161	482 $\pm$ 3.644	2.9 $\pm$ 0.026	1.9 $\pm$ 0.017
Ambient pCO_2 , High temp	6	33.59 $\pm$ 0.040	2109 $\pm$ 4.551	2066 $\pm$ 3.814	7.95 $\pm$ 0.009	1919 $\pm$ 2.877	129 $\pm$ 1.616	18.3 $\pm$ 0.256	493 $\pm$ 10.980	3.1 $\pm$ 0.038	2.0 $\pm$ 0.024
High pCO_2 , Ambient temp	3	33.48 $\pm$ 0.066	2187 $\pm$ 6.832	2241 $\pm$ 7.019	7.42 $\pm$ 0.056	2128 $\pm$ 3.430	39 $\pm$ 5.042	73.7 $\pm$ 9.274	1874 $\pm$ 237.491	0.9 $\pm$ 0.121	0.6 $\pm$ 0.077
High pCO_2 , High temp	6	33.61 $\pm$ 0.0489	2190 $\pm$ 5.835	2235 $\pm$ 14.345	7.45 $\pm$ 0.053	2122 $\pm$ 10.666	46 $\pm$ 5.057	66.1 $\pm$ 8.628	1802 $\pm$ 240.003	1.1 $\pm$ 0.122	0.7 $\pm$ 0.078

Figure 4. *In situ* pH and temperature data collected from autonomous SeaFET sensor deployed at Mia's Reef, San Diego, CA. Instrument was deployed at 15 m and data were collected every 15 min. Panel A shows temperature (°C). Panel B shows pH_{sw}.

Figure 5. In situ irradiance measurements (PAR) at Mia's Reef, San Diego, CA from October 6, 2013 to October 25, 2013.

Figure 6. Daily experimental pH and temperature conditions in mesocosms for the duration of the 28-day experiment. Panel A shows pH_{sw}, with symbols representing average daily HACH pH glass electrode measurements $\pm$ s.e.m. Solid lines represent continuous pH measurements from Honeywell Durafet. Panel B shows average daily HACH temperature measurements $\pm$ s.e.m.

Growth

I observed a substantial reduction in buoyant weight of *C. cheilosporioides* in the high $p\text{CO}_2$ treatments over the course of the experiment, regardless of temperature (Fig. 7). Buoyant weight was also negatively affected by increasing temperature in the ambient CO_2 treatment (Table 3; Fig. 7), but the interaction was not significant (Table 3; Fig. 7). Under ambient $p\text{CO}_2$ conditions, increased temperature reduced calcification rates (i.e., change in buoyant weight) by roughly 70%, but still resulted in net positive growth of individuals. In contrast, high $p\text{CO}_2$ treatments resulted in net dissolution of CaCO_3 over the 28-day experiment, with no difference in dissolution rates between individuals reared in high $p\text{CO}_2$ and either temperature treatment (i.e. there was no additive effect on calcification). This corresponds to a ~150% reduction in CaCO_3 in high $p\text{CO}_2$ conditions in either temperature treatment compared to ambient $p\text{CO}_2$, ambient temperature conditions.

Table 3. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high) and temperature (ambient and high) on the change in buoyant weight of *C. cheilosporioides*

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
$p\text{CO}_2$	1	8.643	76.128	<0.0001
Temp	1	0.803	7.074	0.014
$p\text{CO}_2$ x Temp	1	0.209	1.840	0.188
Error	24	2.725

Figure 7. Percent change in buoyant weight of *C. cheilosporioides* in the four factorial $p\text{CO}_2$ and temperature treatments. Shared letters below error bars indicate mean change in buoyant weight did not differ between treatments. Error bars denote $\pm$ SE.

Significant decreases in linear extension of *C. cheilosporioides* branches were found in the elevated temperature and the high $p\text{CO}_2$ treatments, but there was no $p\text{CO}_2 \times$ temperature interaction (Table 4; Fig. 8A). Elevated temperature decreased linear extension by roughly 40% in ambient $p\text{CO}_2$ conditions. Under high temperature and high $p\text{CO}_2$, linear extension was further reduced by 50%. However, under ambient temperature and high $p\text{CO}_2$, linear extension was only reduced by 20% compared to ambient temperature and ambient $p\text{CO}_2$. I also found significant decreases in the surface area of new growth of branch tips in the increased temperature and the high $p\text{CO}_2$ treatments (Table 4; Fig. 8B).

Increased temperature decreased surface area by ~50% in ambient $p\text{CO}_2$ conditions compared with the controls. Under high temperature and high $p\text{CO}_2$, surface area was further reduced by 75%. Under ambient temperature and high $p\text{CO}_2$, linear extension declined by 40%. However, I did not find evidence for significant interactions between temperature and $p\text{CO}_2$ on either linear extension or surface area.

Table 4. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high) and temperature (ambient and high) on the growth of *C. cheilosporioides*

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
<i>Linear extension</i>				
$p\text{CO}_2$	1	0.003	4.335	0.048
Temp	1	0.017	24.463	<0.0001
$p\text{CO}_2$ x Temp	1	0.001	0.932	0.344
Error	24	0.017		
<i>Surface area</i>				
$p\text{CO}_2$	1	0.090	10.964	0.003
Temp	1	0.160	19.590	0.000
$p\text{CO}_2$ x Temp	1	0.001	0.081	0.778
Error	24	0.197

Figure 8. Growth rates as linear extension (A) and new growth surface area (B) for *C. cheilosporioides* in the four factorial $p\text{CO}_2$ and temperature treatments. Shared letters below error bars indicate mean change in buoyant weight did not differ between treatments. Error bars denote $\pm$ SE.

Photophysiology

Three individuals from each treatment were randomly selected for measurements of photosynthetic rates and calculation of photophysiological parameters. Photosynthesis-irradiance curves were fit to truncated data ($I = 0, 19, 36, 60, 103, 178$ and $198 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) from one individual in the high $p\text{CO}_2$, ambient temperature treatment and two individuals from the high $p\text{CO}_2$, high temperature due to unequal mixing at higher light levels during incubations. Photoinhibition (β) was only detected in the high $p\text{CO}_2$, high temperature treatment, and was therefore excluded from analysis of treatment effects. There were no significant effects of $p\text{CO}_2$, temperature, or the $p\text{CO}_2 \times$ temperature interaction on the photosynthetic efficiency, α (Table 5). However, I did find a significant effect of $p\text{CO}_2$ on P_{max} , with a roughly 100% increase in this parameter under high $p\text{CO}_2$ conditions (Table 5). There were no significant effects of temperature or the $p\text{CO}_2 \times$ temperature interaction on P_{max} (Table 5). High $p\text{CO}_2$ may increase the maximum photosynthetic rate (P_{max} ; Table 6; Fig. 9), while the non-significant trends (potentially due to low statistical power) suggest that under ambient $p\text{CO}_2$ conditions, increased temperature may decrease α and P_{max} . Conversely, under high $p\text{CO}_2$ conditions, increased temperature may increase α and decrease P_{max} (Table 6; Fig. 9).

Table 5. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high) and temperature (ambient and high) on the photophysiology of *C. cheilosporioides*

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
α				
$p\text{CO}_2$	1	0.036	2.204	0.176
Temp	1	0.000	0.025	0.877
$p\text{CO}_2 \times \text{Temp}$	1	0.045	2.734	0.137
Error	8	0.130		
$P_{\max}$				
$p\text{CO}_2$	1	1001.850	5.447	0.048
Temp	1	67.624	0.368	0.561
$p\text{CO}_2 \times \text{Temp}$	1	7.010	0.038	0.850
Error	8	1471.369		

Table 6. Mean photophysiological parameters of *C. cheilosporioides* reared under different treatments of $p\text{CO}_2$ (ambient and high) and temperature (ambient and high)

Treatment	$\alpha (\pm \text{SE})$	$P_{\max} (\pm \text{SE})$
Ambient $p\text{CO}_2$, Ambient temp	0.248 ± 0.080	17.737 ± 5.328
Ambient $p\text{CO}_2$, High temp	0.138 ± 0.059	14.517 ± 3.292
High $p\text{CO}_2$, Ambient temp	0.246 ± 0.035	37.539 ± 12.504
High $p\text{CO}_2$, High temp	0.369 ± 0.103	31.263 ± 7.047

Figure 9. Net photosynthesis versus irradiance curves for *C. cheilosporioides* after rearing in four factorial $p\text{CO}_2$ and temperature treatments for 28 days. Net photosynthesis was expressed in terms of oxygen production.

Mineralogy

There were no significant effects of $p\text{CO}_2$, temperature or their interaction on the mol% Mg incorporation into either cortical cells or medullary cells (Table 7; Fig. 10). Further analysis comparing differences between cell types did show a significantly higher mol % Mg in medullary cells (mean $\pm$ SE = 18.5 ± 0.39) versus cortical cells (mean $\pm$ SE = 14.81 ± 0.33 ; t -test: $t_{54} = 7.2788$, $P = <0.0001$).

Figure 10. Mol % Mg found in cortical and medullary cell walls for *C. cheilosporioides* in four factorial $p\text{CO}_2$ and temperature treatments. Error bars denote $\pm$ SE.

Table 7. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high) and temperature (ambient and high) on the mineralogy of *C. cheilosporioides* cortical and medullary cells

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
<i>Cortical</i>				
$p\text{CO}_2$	1	1.010	0.306	0.586
Temp	1	2.051	0.621	0.439
$p\text{CO}_2 \times \text{Temp}$	1	1.708	0.517	0.479
Error	24	79.313		
<i>Medullary</i>				
$p\text{CO}_2$	1	1.098	0.285	0.599
Temp	1	6.059	1.572	0.222
$p\text{CO}_2 \times \text{Temp}$	1	20.680	5.366	0.029
Error	24	92.489		

Discussion

Economically and ecologically important calcifying marine organisms have the potential to be negatively affected by global change. Recent work on temperate, tropical, and subtropical reefs has shown detrimental impacts on numerous physiological traits (e.g.; photosynthesis/respiration, calcification) in calcareous biota exposed to high $p\text{CO}_2$ and elevated temperature conditions (Kroeker et al. 2013). Despite the tight coupling of climate change stressors, such as OA and warming (IPCC 2007), far less is known about their combined or interactive effects compared to the effects of either in isolation. This study assessed the physiological responses of an ecologically important calcified red alga, *C. cheilosporioides*, to climate change within a multi-stressor framework.

Growth

In calcifying marine algae, calcification is necessary for growth and survival. The ability of an organism to calcify has been correlated with the saturation state (Ω) of seawater (Gattuso et al. 1998; Ries et al. 2009). Calcification is favored when $\Omega > 1$, whereas dissolution is favored $\Omega < 1$ (Morse et al. 2007). However, these properties describe carbonate mineral precipitation and dissolution kinetics in the absence of biology. In reality, biogenic carbonate precipitation (i.e.; calcification) slows substantially as Ω approaches 1, but can continue when $\Omega < 1$ (Ries et al. 2009; Büdenbender et al. 2011). Growing evidence suggests that species-specific controls on carbonate chemistry at the site of calcification may drive the calcification response to saturation state (e.g.; negative, parabolic; Holcomb et al. 2009; Ries et al. 2009) and could explain how some organisms continue to calcify in undersaturated conditions.

On temperate reefs, changes to Ω are driven mainly by changes in $p\text{CO}_2$ due to natural processes (e.g. benthic metabolism, upwelling) and increased anthropogenic carbon emissions. Undersaturation (i.e.; $\Omega < 1$) has been known to occur within the California Current System due to the upwelling of cold low pH seawater (Gruber et al. 2012). The frequency and intensity of upwelling events are predicted to increase in the future due to greenhouse-associated intensification of thermal low-pressure cells over coastal landmasses within upwelling regions (Bakun et al. 2010; Gruber et al. 2012; Hauri et al. 2013; Bakun et al. 2015). Therefore the extent to which saturation state affects growth

and calcification is important to our understanding of how these organisms will respond to future ocean conditions. In our experiment, ambient $p\text{CO}_2$ $\Omega_{\text{Ca}} = 3.0 \pm 0.032$ ($\mu \pm \text{SE}$), whereas, Ω_{Ca} in high $p\text{CO}_2$ treatments = 1.0 ± 0.12 ($\mu \pm \text{SE}$). I observed a reduction of calcification rates by up to 150% with a reduction in Ω_{Ca} of 2 units. Past studies have reported net reductions in calcification of tropical coralline algae under high $p\text{CO}_2$. For example, Johnson and Carpenter (2012) demonstrated a 24% reduction in calcification of *Hydrolithon onkodes* when Ω_{Ca} declined from 4.6-3.21 units, while Anthony et al. (2008) reported a 190% reduction in calcification of *Hydrolithon onkodes* when Ω_{Ca} declined from 3.3-1.7 units. In my system, natural dissolution of CaCO_3 due to seasonal undersaturation may be a common occurrence within kelp forests during the upwelling season; however, a reduction in baseline saturation states due to OA could tip the balance of net accretion to net dissolution within kelp forest communities.

Few studies have assessed how growth rates such as linear extension and surface area will be impacted in coralline algae due to climate change (but see: Ragazzola et al. 2012; McCoy et al. 2013; McCoy and Ragazzola 2014). However, this information is important to better understand how changes to physiology will alter growth and morphology. McCoy et al. (2014) found that thick-crustal crustose species decreased in thickness, but did not change their internal carbonate material in response to acidification. Conversely, thin-crustal species did not change their thickness, but did change the density of internal

carbonate material. Crustose coralline algal species exhibit both vertical and lateral growth potentially enabling them to be more plastic in response to acidification, whereas articulated species, such as *C. cheilosporioides*, grow primarily vertically. I observed a significantly greater reduction in linear extension and surface area due to increased temperature alone than elevated acidification alone, with no evidence of synergistic effects in response to the two stressors in combination. Negative relationships between temperature and calcite density have been documented previously in the arctic rhodolith, *Lithothamnion glaciale* (Kamenos and Law 2010). Higher temperatures affect stoichiometry and create higher saturation states; therefore the calcification process should be more favorable to species inhabiting warmer waters. However, if this is the case, current research suggests that corallines may allocate additional resources to linear extension instead of vertical accretion. Taken with my finding that high $p\text{CO}_2$ reduces calcification rates in *C. cheilosporioides*, this suggests that at ambient temperature, coralline algae may compensate for reductions in calcification due to increased $p\text{CO}_2$ by reducing the density of carbonate in skeletal materials while still maintaining growth. However, this reduction in carbonate material could have implications for structural integrity (Ragazzola et al. 2012). As storm frequency and intensity increase due to climate change, a reduction in standing kelp biomass may also reduce the wave buffering capacity within kelp beds (Byrnes et al. 2011). Reduced skeletal density (and decreased

structural integrity) coupled with increased physical disturbance could lead to reductions in articulated coralline biomass on rocky reefs.

Past studies suggest that increased temperature may modulate the $p\text{CO}_2$ response in coralline algae. Johnson and Carpenter (2012) found interactive effects of warming and $p\text{CO}_2$ on the tropical coralline alga *Hydrolithon onkodes* during a 21 day experiment. At low (420 μatm) and high (830 μatm) $p\text{CO}_2$ warming resulted in an increase in calcification rates relative to ambient temperatures, however, at moderate $p\text{CO}_2$ (530 μatm) this response was reversed. Diaz-Pulido et al. (2012) found that increased temperature also exacerbated the $p\text{CO}_2$ response (ambient = 400 μatm ; elevated = 1100 μatm) on *Hydrolithon onkodes* by increasing partial mortality from 9% (relative to ambient 1% mortality at ambient $p\text{CO}_2$ and temperature) to 15% over an 8-week experimental period. In this study, I did not find evidence for interactive effects of temperature and $p\text{CO}_2$ as predicted. This may be due in part to the length of our study (28 days) or due to local adaptation of the individuals in this study. On Mia's Reef, temperatures as high as 17°C were recorded during my deployments of SeaFET temperature and pH sensors (Fig. 4). During certain times of the year in San Diego, CA, depression of the thermocline can result in temperatures as high as 20°C at this site (personal observation). The frequency and duration of these warming events are unknown at this site. However, it is important to incorporate climate change stressors on top of the maximum range of

temperatures (and $p\text{CO}_2$) currently experienced by an organism in order to fully understand the potential threat they may impose.

Photophysiology

Changes in photophysiological performance in marine macrophytes can alter production on reefs. Researchers have suggested that the increase in CO_2 due to climate change could actually be beneficial for marine algae (Wu et al. 2008; Harley et al. 2012; Koch et al. 2013). Since CO_2 concentrations in seawater are low relative to other carbon species, most marine algae have evolved energetically costly carbon concentrating mechanisms to convert the more readily available bicarbonate ion, HCO_3^- , to CO_2 for use in photosynthetic pathways (Raven et al. 2011). If algal species are able to downregulate the use of carbon concentrating mechanisms in the presence of higher concentrations of CO_2 , it's possible that they may be able to utilize this extra energy for growth and reproduction (Cornwall et al. 2012). Although my study was preliminary in its efforts to assess photosynthetic performance, results suggest that the maximum photosynthetic rate could as much as double under high $p\text{CO}_2$ conditions. Johnson et al. (2014) also found that the maximum relative electron transport rate (rETR_{max}), a proxy for photosynthesis, increased under high $p\text{CO}_2$ conditions in the weakly calcified tropical red alga *Galaxaura rugosa*. Although metabolic theory predicts that increases in temperature should also increase physiological processes (Brown et al. 2004), my study lacked the power to detect a

temperature effect on photosynthetic performance. Coralline red algae are calcifying macroalgae and my study suggests that the potential benefits of increased photophysiological performance may not outweigh the costs of decreased calcification.

Mineralogy

The number of studies on biomineralization in coralline red algae has escalated in recent years due to increased concerns about the susceptibility of their high-Mg calcite skeletons to ocean acidification (Ries 2011; Fragoso et al. 2010; Nash et al. 2011, Nash et al. 2012; Nash et al. 2015). Numerous studies have assessed whether corallines are able to change the mol % Mg at reduced pH and saturation states using techniques which measure mol % Mg in bulk samples taken from whole thallus material (Ries 2011; Egilisdottir et al. 2012). One study by Ries (2011) found that the tropical crustose coralline alga, *Neogoniolithon* sp., reduced Mg/Ca ratios in calcite with decreasing pH after 60 days in experimental conditions. Conversely, the temperate intertidal articulated alga, *Corallina elongata*, did not change its mineralogy in response to acidification during a 3-week rearing experiment (Egilisdottir et al. 2012). My study did not find significant effects of $p\text{CO}_2$ or temperature on mineralogy. This could be because this species (and perhaps other articulated species) does not exhibit mineralogical plasticity in response to acidification or temperature or because of the relatively short experimental duration (28 days).

Techniques utilizing bulk tissue samples may mask important fine-scale variability, which could be crucial to how these organisms persist in dynamic ecosystems, such as tidepools and upwelling regions. Studies that have looked at higher resolution within the algal thallus have focused on either underlying skeletal material (i.e.; not containing living cells) or lack an understanding of the biology and ecology of the study species (Nash et al. 2011, 2012, 2015). My study found significantly higher mol % Mg in medullary cells than cortical cells. Medullary cells form the central core of filaments which run from geniculum to geniculum (Borowitzka and Vesik 1978). These internal cells had, on average, ~125% higher Mg/Ca ratios. The mechanism for this dramatic difference between different cell types remains unknown, but may be due to biology or abiotic conditions. For instance, relationships between growth rate and Mg/Ca ratios have been found, however, these measurements are confounded by temperature. At elevated temperatures, Mg/Ca ratios in CCA have been reported to increase, however, so do growth rates (Kamenos et al. 2008). The degree to which increased Mg incorporation is due to temperature versus growth rate remains unknown. It is possible that internal medullary cells simply grow faster than cortical cells, which could explain this pattern if growth rate is correlated with Mg incorporation. Medullary cells are responsible for increases in linear extension, whereas cortical cells thicken the alga. It's also possible that the reduced Mg incorporation in outer cortical cells may be a strategy to decrease solubility in cell types closer to the external environment.

Conclusion

C. cheilosporioides can maintain large expanses, referred to as the Calliarthron Zone (McLean 1962), inshore of kelp forests in shallow wave exposed environments (Graham 1997). The branching structure of *C. cheilosporioides* creates a complex 3-dimensional structure that creates habitat for numerous associated species (McLean 1962). Within kelp forests, *C. cheilosporioides* can also be found in high abundances and may act as a secondary foundational species, especially during times of high disturbance.

The present study found that *C. cheilosporioides* growth and calcification were reduced under acidification and warming. Reductions in net yearly calcification and changes to growth could have consequences for both the abundance and integrity of *C. cheilosporioides*. We found variation in the mol % Mg within carbonate skeletons of different cell types. A better understanding of the mechanism by which corallines regulate Mg incorporation will be crucial to predicting the impacts of global change stressors. Although *C. cheilosporioides* may be able to increase photosynthetic rates with increased availability of CO₂, the costs of increased temperature and acidification seem to counter any benefits. The changes to biology brought on by warming and acidification in this study could have cascading effects on species that rely on *C. cheilosporioides* for food, habitat, and other important ecological processes such as settlement cues.

My study was conducted on individuals collected from one location and lasted only 28 days in duration. Future research should investigate the potential

for acclimation over longer periods of time. Furthermore, variability in temperature and pH occurs spatially across the geographic range of *C. cheilosporioides*. Local adaptation may provide key insights into the mechanisms that coralline algae have evolved to persist in environments similar to those predicted to occur by the end of the century.

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Chapter II

Effects of ocean acidification and grazing on kelp forest communities from San Diego, CA and Carmel, CA

Abstract

Increases in dissolved CO₂ (i.e., $p\text{CO}_2$), as a consequence of anthropogenic CO₂ emissions, are altering seawater chemistry at an alarming rate. Studies have shown that increased $p\text{CO}_2$ and associated changes to the carbonate system will differentially affect calcifying and non-calcifying marine organisms. I investigated how multispecies assemblages from kelp forests in central and southern California respond to future $p\text{CO}_2$ conditions and sea urchin grazing in a multifactorial design. Settlement tiles were installed at each location to accrue natural mixed assemblages and pH sensors were used to record natural variability and estimate differences in pH exposure history. After one year, tiles were placed in a laboratory experiment for two months to simulate the effects of elevated CO₂ (~0.4 pH units below ambient) and grazing on subsequent community development. Community composition, growth, and calcification on tiles from central California showed a strong effect of grazing, but no effect of $p\text{CO}_2$. Conversely, species assemblages from southern California showed little effects of grazing, but a significant effect of $p\text{CO}_2$ on community composition, growth and calcification. In addition, block effects were detected, such that taxa-specific responses depended upon the initial community state, which differed among tiles cultivated on the separate racks from San Diego, CA. These findings suggest that the community-level responses to climate change stressors may depend on initial community composition, species interactions, and prior pH exposure.

Introduction

Numerous studies have addressed how individual taxa will respond to ocean acidification. In general, results from single-species experiments suggest that calcified taxa, such as corals and calcified macroalgae, will exhibit reduced growth and calcification due to lowered pH and saturation states (Kroeker et al. 2010; Harvey et al. 2013; Kroeker et al. 2013). Conversely, non-calcified photoautotrophs, such as fleshy macroalgae and seagrasses, may experience increased growth due to the increased availability of CO_2 and HCO_3^- for photosynthesis (Harley et al. 2012). These studies can be useful in order to understand how biological processes may be affected by climate change. However, as organisms interact on a reef, changes to biology (i.e., growth and metabolism) may alter their abilities to compete for resources and space. Since species interactions are complex, the effects of climate change on biodiversity and ecosystem functioning are challenging to predict from single-species experiments alone. Therefore, in order to better understand community level responses, it is necessary to focus on whole species assemblages.

Environmental stressors such as pH, temperature, and nutrients have been shown to dramatically change the structure and biodiversity in benthic communities (Schiel et al. 2004; Russell et al. 2009; Hale et al. 2011; Kroeker et al. 2011; Porzio et al. 2011; Hofmann et al. 2012; Kroeker et al. 2012; Price 2012; Enochs et al. 2015). Opposing responses of closely related species within the same assemblage suggest that compensatory dynamics may occur among

the most tolerant species (Kroeker et al. 2013). However, despite potential compensatory mechanisms, shifts from communities dominated by calcified taxa to communities dominated by fleshy taxa have been documented under OA-like conditions (Hoegh-Guldberg et al. 2007; Hall-Spencer et al. 2008; Russell et al. 2009; Fabricius et al. 2011; Porzio et al. 2011; Kroeker et al. 2012; Enochs et al. 2015) and even across natural present-day gradients in pH (Price et al. 2012). Taken together, these studies suggest that climate change stressors could have dramatic impacts on benthic communities and the subsequent organisms that depend on them for food, structure, and refuge.

Studies focusing on the impacts of climate change on temperate communities are lacking. Those that have been published suggest that reduced abundances of calcifying macroalgae and increased abundances of fleshy macroalgae and turfs will occur under OA (Russell et al. 2009; Hofmann et al. 2012). Although temperate communities are characteristically identified by the dominance of charismatic brown algae from the family Laminariales (i.e., kelps), they also maintain a high diversity of calcifying organisms such as crustose coralline red algae and shelled marine invertebrates. These calcified organisms are important to the structure and function of kelp forest communities as they cement reef structure and provide habitat, food, and settlement cues.

In addition to changes in numerous abiotic conditions (i.e., temperature, carbonate chemistry, etc.) in response to climate change, changes to ecological processes, such as grazing pressure, are also likely to affect benthic

communities. Grazers have been shown to structure community composition on reefs. Since many grazers, such as chitons and sea urchins, also build protective shells or skeletons by precipitating calcium carbonate, it is likely that they will also be negatively affected by OA. In addition, metabolic depression associated with a decrease in intra or extracellular pH has been observed in a number of marine invertebrates under hypercapnic (low-pH, high CO₂) conditions (Widdicombe and Spicer 2008). It is unclear how these physiological changes may affect grazing pressure; however, it is likely that numerous factors (i.e., changes to growth and survivorship, recruitment, grazing rates, etc.) will result in alterations to grazing pressure on reefs. More studies that directly measure changes to community structure across multiple ecosystems and that incorporate biotic stressors (such as grazing) are necessary to understand the emergent effects of climate change on marine systems.

Spatio-temporal variation in seawater chemistry along the California coast

The California coast is part of one of the four Eastern Boundary Current Systems (EBCS), which are highly productive and dynamic coastal environments driven by seasonal upwelling (Gruber et al. 2012). Southerly winds prevail during the spring and summer months causing upwelling of cold, low pH and nutrient-rich waters. During El Niño years, a depression of the thermocline results in reduced upwelling and warmer waters with decreased nutrients available to biological organisms. All of this seasonal and multi-year variation is

superimposed upon a larger climatic phenomenon known as the Pacific Decadal Oscillation (PDO), which occurs on a decadal time scale and oscillates between periods of cold, nutrient rich waters and warm, nutrient poor waters. For these reasons, California's rocky temperate reefs (hereafter, 'reefs') are among the most dynamic and productive ecosystems on the planet.

Due to the upwelling of high CO₂ (low pH) waters, the EBCS are naturally lower in pH and saturation states than other regions of the ocean (Feely et al. 2008). Therefore, calcifying organisms that inhabit these regions may already be near the physiological limits of what is necessary for growth and maintenance of calcium carbonate skeletons. Due to increasing anthropogenic CO₂ emissions, regional model projections have shown rapid acidification within the California current system (CCS) over the next 20-30 years with much of the nearshore region of the California coast experiencing summer-long undersaturation of carbonate ion with respect to aragonite (Ω_{Ar}) by 2050 (Gruber et al. 2012).

Furthermore, a large gradient in upwelling frequency and intensity exists latitudinally. North of Point Conception strong seasonal upwelling events and remineralization result in spring pH drops as low as 7.65 with Ω_{Ar} as low as 0.8 (Feely et al. 2008; Hauri et al. 2013). Upwelling is weaker, yet more persistent south of Point Conception (Hauri et al. 2013). Due to biological drawdown of CO₂ in warm subsurface waters, pH and saturation states reach their highest during the summer in southern California (Hauri et al. 2013). Spatial and temporal variability in pH and saturation states as well as the magnitude of that

variability differ greatly between northern and southern California. By measuring the responses of kelp forest communities from multiple locations along a natural gradient in pH, researchers may better understand how local adaptation to oceanographic conditions may alter the responses of similar taxa to ocean acidification.

The aim of this chapter is to address the following questions: 1) Will the differential responses of organisms to OA result in shifts in community composition? I hypothesize that the cover and/or biomass of calcified taxa will decrease in natural communities reared in high $p\text{CO}_2$, but increase in the presence of grazers. The magnitude of these responses will differ between central California and southern California, with central California sites being affected less than those from southern California because organisms in central California will be acclimatized to low pH conditions due to development in waters characterized by high levels of upwelling and thus lower pH. 2) How will ocean acidification alter grazer biology? I hypothesize that sea urchin growth and grazing rates will be reduced in high $p\text{CO}_2$ conditions, due to metabolic depression – and that these altered grazing rates can explain differences in macroalgae species assemblages?

Materials and Methods

Study Sites

In order to gain better insight into how different communities may respond to OA and warming along California's coast, two sites were chosen, one at Stillwater Cove in Monterey County (N 36° 36' 38.4" W 121° 56' 44.9") and one at Mia's Reef in San Diego County (N 32° 51' 14.8", W 117° 16' 52.4"), to install racks of settlement tiles (Fig. 1) for community-level experiments. Stillwater Cove is characterized by granite, sandstone and conglomerate terraces separated by cobble and sand channels (Edwards 1998). The dominant habitat-forming species include a surface canopy of *Macrocystis pyrifera* and a subsurface canopy of *Pterogophyera californica*. Fleshy red and calcareous algae and numerous genera of sessile invertebrates carpet the benthos (Edwards 1998). Stillwater Cove was chosen as a representative central California kelp forest subject to intense seasonal upwelling of low pH seawater and naturally lower mean pH and saturation states. Mia's Reef, similar to other southern California kelp forests, is characterized by limestone reef which can be fragmented into large boulders (Dayton et al. 1984). At Mia's Reef, a large terrace provided continuous bathymetry in which to deploy settlement tile arrays. The habitat-forming species is also *M. pyrifera* with an understory community dominated by low lying fleshy red and brown macroalgae, branching bryozoans and sponges. Mia's Reef was chosen as a representative southern California kelp forest with higher mean pH and weak seasonal upwelling events. Each site

was co-located with autonomous pH and temperature loggers, SeaFETs (Martz et al. 2010; Fig. 2). These instruments provide continuous high-resolution (every 15 min) pH and temperature measurements to improve my understanding of natural fluctuations at each site and aid in the interpretation of experimental results. SeaFET pH and temperature loggers were deployed at Stillwater Cove, Carmel, CA on November 12, 2012 and at Mia's Reef, La Jolla, CA on November 1, 2012. Water samples for carbonate chemistry were taken as frequently as possible at the SeaFET loggers using a custom Niskin to calibrate pH measurements. At the surface, water samples were transferred to 500 mL Corning brand Pyrex sample bottles and immediately spiked with 240 μ L of HgCl_2 solution. Carbonate chemistry and salinity were analyzed from water samples using water chemistry analysis methods outlined in chapter 1. In addition, *in situ* photosynthetically active radiation (PAR) was measured for one month at each site using a LICOR 4 π quantum sensor (Biospherical, Inc.) to characterize irradiances at each location.

Grazing pressure has been shown to alter community composition and structure on reefs (Paine and Vadas 1969; Breitburg 1984; Harrold and Reed 1985; Watanabe and Harrold 1991; Byrnes et al. 2013). In order to better understand how grazers may mediate competitive interactions under climate change stressors, the purple sea urchin (*Strongylocentrotus purpuratus*) was used in community level experiments. Sea urchins of the genus *Strongylocentrotus* alter algal species composition by removing algal biomass via

grazing (Paine and Vadas 1969). Therefore the presence of urchin grazers may reduce fleshy biomass and indirectly increase the biomass of calcified taxa.

Strongylocentrotus purpuratus are abundant in both central and southern California making them an ideal grazer for comparisons between both study sites.

Benthic Community Structure Responses to Ocean Acidification

To assess changes in growth and species interactions due to climate change at the community level, mixed communities of sessile organisms were cultured on settlement tiles outplanted to the field for one year. After a year, the tiles were retrieved and reared in the laboratory under a multi-stressor design. Experiments used two different $p\text{CO}_2$ levels (ambient $p\text{CO}_2 = \sim 450 \mu\text{atm}$ [pH = 8.07], high $p\text{CO}_2 = \sim 1800 \mu\text{atm}$ [pH = 7.69]). To determine whether benthic mesograzers may mediate interactions under OA, the presence or absence of sea urchin grazers, *S. purpuratus*, was cross-factored with the two $p\text{CO}_2$ treatments to result in a total of four different factorial treatment conditions: (1) ambient $p\text{CO}_2$, -grazer (2) ambient $p\text{CO}_2$, +grazer (3) high $p\text{CO}_2$, -grazer and (4) high $p\text{CO}_2$, +grazer. All laboratory experiments were conducted in a state-of-the-art flow through seawater system in the lab of Dr. Jennifer Smith at SIO.

Sets of settlement tiles were deployed at ~ 13 m within the two kelp forests to generate different initial communities of sessile invertebrates and algae to assess community-level responses to OA and grazing. Community tile racks

($n=3$) with 18 - 100 cm² tiles per rack were deployed at Stillwater Cove, Carmel, CA on January 16, 2013 and at Mia's Reef, La Jolla, CA on August 3, 2012 (Fig. 1). At each location, all three tile racks were installed within a 10x10 m cell. Holes were drilled into the reef and eyebolts were secured into holes using Z-Spar marine epoxy. Tile racks were secured to eyebolt attachment points via zip-ties. All settlement tiles remained on the reef for approximately 1 year to accumulate natural mixed communities of invertebrates and seaweeds, at which time $n=39$ and 40 individual tiles (Stillwater Cove and Mia's Reef respectively) were collected and transported to the laboratory for experimental rearing in the aforementioned treatment conditions for a two month period. Grazers were collected from the Point Loma kelp forest during the week of September 23, 2013. Prior to their use as experimental grazers, sea urchins were held in ambient flow-through aquaria and fed *M. pyrifera* fronds *ad libitum*.

Figure 1. Settlement tile array containing 18-100 cm² PVC settlement tiles deployed in the kelp forest at Stillwater Cove, Carmel, CA (A) upon deployment and (B) after ~ 9 months in the field.

Figure 2. Autonomous SeaFET temperature and pH sensor deployed in a kelp forest at Stillwater Cove, Carmel, CA.

All community tile experimental rearing was carried out in the same flow-through system as described for the coralline algae experiment explained in detail in Chapter 1 (Fig. 3). Briefly, the seawater bubbling system bubbled CO_2 into individual mesocosms supplied with flow-through seawater. Treatment $p\text{CO}_2$ conditions were created by continuously bubbling a CO_2 -air gas blend into each individual mesocosm at a rate that lowered the pH by 0.4 ± 0.05 units. This treatment pH was chosen to represent future ocean conditions by the year 2100

as specified by the IPCC's A2 emission scenario (IPCC 2007). Ambient mesocosms were also supplied with air originating from the same source as the CO₂-air gas blend used in the treatment conditions. Two distinct $p\text{CO}_2$ treatment conditions were maintained for the entirety of the experiment (ambient $p\text{CO}_2$ = ~450 μatm and treatment $p\text{CO}_2$ = ~1800 μatm). In addition to $p\text{CO}_2$, grazers were added to half of the mesocosms to yield a total of four factorial treatments (ambient $p\text{CO}_2$ -grazer, ambient $p\text{CO}_2$ +grazer, high $p\text{CO}_2$ -grazer, high $p\text{CO}_2$ +grazer). Three or four tiles from each rack (block) were cleaned of mesograzers using forceps and placed into individual square 1.5 L glass containers (mesocosms) maintained at each of the four factorial treatment conditions ($n = 10$ per treatment, except in the Stillwater Cove experiment ambient $p\text{CO}_2$, -grazer treatment where $n = 9$). Tiles remained free of all grazers and at ambient $p\text{CO}_2$ for one week in order to acclimatize to laboratory conditions. Following acclimatization, desired $p\text{CO}_2$ treatments were attained by bubbling either ambient air or an ambient air- CO₂ gas blend into mesocosms and individual sea urchins (one individual per mesocosm; test diameter ~1.5 cm) were placed on tiles specified as +grazer treatments. Tiles were exposed to experimental treatment conditions for 2 months to observe changes in space occupancy and survivorship as a result of differential growth and competitive abilities of various invertebrate and seaweed taxa in response to the experimental conditions.

Figure 3. Experimental laboratory mesocosm setup shows individual replicate glass aquaria containing a single settlement tile. Blue lines indicate gas lines bubbling either air or air-CO₂ mix. Black lines indicate flow-through seawater.

At the beginning and end of each experiment, all tiles were weighed using the buoyant weight technique to quantify calcification rates on tiles from each of the four treatments. This technique measures net community calcification, taking into account organisms inhabiting both the top and bottom of each tile. Before being placed in treatment conditions and at the end of the experiment, sea urchin grazers were also buoyant weighed and test diameter was measured to the nearest 0.01 mm using calipers. Percent change in buoyant weight of tiles and

percent change in buoyant weight of sea urchins and sea urchin test diameter were calculated as, $\frac{W_f - W_i}{W_i}$, where W_i is the initial weight and W_f is the final weight.

To examine changes in percent cover and community composition of taxa on the tiles in response to the treatment conditions, the top and bottom of each tile were photographed at the start of the experiment and was carefully removed from its mesocosm biweekly and photographed for the duration of the 2-month rearing experiments (Fig. 4, 5). Images were imported into Coral Point Count with Excel extensions (CPCe V4.1, National Coral Reef Institute) and a 10 x 10 point grid was overlaid over each photograph. Due to issues of overgrowth making it difficult to discern the space-occupying organism, only photographs from the start of the experiment were analyzed. The space-occupying organism underlying the crosshairs at each grid intersection was identified to the highest taxonomic resolution. These data were pooled to functional group and used to look at differences between tiles and blocks at the onset of the experiment. At the end of the experiment tiles were placed in 4 % formalin in seawater to preserve specimens for subsequent analysis of the emergent effects of $p\text{CO}_2$ and grazing on community composition. Fixed tiles were visualized under a dissecting scope and a 10 x 10 grid was placed over each tile. The space-occupying organism was identified under the crosshairs at each grid intersection. These data were pooled to functional group (i.e.; calcified invertebrates, non-

calcified invertebrates, calcified algae, fleshy algae, sediment, bare space) for subsequent statistical analyses.

Figure 4. Settlement tile top (A) and bottom (B) from Mia's Reef after 56 days in experimental rearing conditions in the lab. Numbers indicate key taxa (1 = calcified algae, 2 = bryozoan, 3 = fleshy macroalgae, 4 = tunicate, 5 = serpulid worm, 6 = bivalve, 7 = sponge).

Figure 5. Images of representative settlement tile tops from San Diego, CA after 56 days in experimental conditions. A) ambient $p\text{CO}_2$, -grazer; B) ambient $p\text{CO}_2$, +grazer; C) high $p\text{CO}_2$, -grazer; and D) high $p\text{CO}_2$, +grazer.

Because our analysis of community composition only took into account the space-occupying organism on the tile, the abundances of some macroalgae, such as the giant kelp *M. pyrifera*, may be underrepresented. Therefore, juvenile kelp were removed at the end of the experiment to quantify their density and biomass on community tiles. All kelp were gently removed using forceps, laid flat on waterproof paper, and photographed. After being photographed, juvenile sporophytes were placed in a drying oven at 60°C for 48 hours before being

weighed to calculate biomass as dry weight. Photographs were visualized using ImageJ software and all kelp sporophytes were counted to obtain a density as individuals per tile.

Sea urchin grazing responses to elevated $p\text{CO}_2$ were tested at the end of experiment. All sea urchins were removed from settlement tiles and held under treatment conditions (i.e., high or ambient $p\text{CO}_2$) in the same mesocosms used during the experiment. Sea urchins were starved for three days before being presented with a single pre-weighed (wet weight) kelp frond collected from the field the same day as the beginning of the grazing trial. Sea urchins were allowed to graze on kelp for 24 hours before the kelp were removed and reweighed (wet weight). The percent change in weight of kelp was calculated as, $\frac{W_f - W_i}{W_i}$, where W_i was the initial weight and W_f was the final weight. Urchin grazing rates were calculated as the percent change in wet weight of kelp per day.

Statistical Methods

Final percent cover of functional group taxa data were normalized and square-root transformed. Analysis of community structure was conducted separately for the top and bottom of the tiles. Bray-Curtis resemblance matrices were calculated from transformed community data. Multidimensional scaling (MDS) plots were constructed from resemblance matrices to visualize the

similarities between treatments in multidimensional space. Three-factor Permutational Multivariate Analysis of Variance's (PERMANOVA) were run on resemblance matrices using 9999 permutations with fixed factors $p\text{CO}_2$, sea urchin, and block. A factor of block was used to test for differences in the initial communities that developed on each settlement tile rack ($n=3$) and their subsequent composition, which could have resulted from micro-scale variability in environmental conditions (i.e., light and temperature), settlement behavior, or ecological processes (competition and mortality). Where a significant block effect was found in the PERMANOVA results ($P_{\text{perm}} < 0.05$), two-way PERMANOVA's were run on community structure data with fixed factors of $p\text{CO}_2$ and sea urchin grazing as separate blocks. Highly non-significant interactions ($P > 0.25$) were removed from statistical models.

Three-factor PERMANOVA's were conducted on univariate variables (functional groups) with fixed factors of $p\text{CO}_2$, sea urchin, and block to ascertain which group or groups explained the differences in community composition seen between treatments. In the case where a significant block effect was found in three-way PERMANOVA results, two-way PERMANOVA's were run on univariate variables with fixed factors of $p\text{CO}_2$ and urchin as separate blocks. Highly non-significant interactions ($P > 0.25$) were removed from statistical models.

Permutation-based approaches were run with 9999 permutations of square-root transformed data. A dummy variable = 1 was added to functional

groups in which two or more samples contained a value of zero thus precluding the calculation of Bray-Curtis dissimilarities. All permutation-based analyses were carried out in PRIMER v6 (PRIMER-E Ltd, Plymouth, UK). General linear models with fixed factors $p\text{CO}_2$, sea urchin, and block were conducted on square-root transformed variables of kelp biomass (dry weight) and kelp density on the tiles.

t-tests were used to compare differences between initial mean sea urchin buoyant weight and sea urchin test diameter between the ambient and high $p\text{CO}_2$ grazing assays. Sea urchin grazing rates, percent change in buoyant weight day^{-1} and percent change in sea urchin diameter day^{-1} were also compared using t-tests. Kruskal-Wallis tests were used when variables violated assumptions of normality. All tests were carried out in JMP Pro 12 v12.0.0.

Mean SeaFET pH and temperature were calculated at each site. By better understanding the natural variability in pH and temperature at each site I was able to better interpret the results of community tile experiments. Organisms that inhabit more variable or low pH environments may be more resilient to OA and warming and therefore may be less likely to show shifts in abundance and diversity.

Results

Mia's Reef, San Diego, CA

Experimental Conditions

Two distinct $p\text{CO}_2$ treatments were maintained for the duration of the 56-day experiment (Table 1; Fig. 6). Ambient $p\text{CO}_2$ treatments were 0.39 pH units higher than high $p\text{CO}_2$ treatments. Ambient mesocosm temperatures were approximately 13°C for the duration of the experiment. *In situ* measurements of pH and temperature from autonomous SeaFET loggers recorded a mean pH = 8.02 and mean temperature ~15°C from October 23, 2013 - April 12, 2014 (Table 1; Fig. 7).

Table 1. Average environmental conditions in the field and experiments for Mia's Reef, San Diego, CA

	Mean pH _{sw}	Min pH _{sw}	Max pH _{sw}	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Mean Peak Irradiance (μmol m ⁻² sec ⁻¹)
MIA (field)	8.02	7.57	8.15	15.28	11.40	17.71	64.98 ± 9.70
MIA high pCO ₂	7.69	7.50	8.02	13.15	12.53	13.54	30.6 ± 3.12
MIA ambient pCO ₂	8.08	7.97	8.41	13.16	12.88	13.67	30.47 ± 2.80

Figure 6. Daily experimental pH and temperature conditions in mesocosms for the duration of the 56-day experiment with tiles from Mia's Reef. pH_{sw} , with symbols representing average daily HACH pH glass electrode measurements $\pm$ SE. Solid lines represent continuous pH measurements from Honeywell Durafet.

Figure 7. *In situ* pH and temperature data collected from autonomous SeaFET sensor deployed at Mia's Reef, San Diego, CA. Instrument was deployed at 15m and data were collected every 15 min. Panel A shows temperature (°C). Panel B shows pH_{sw}.

Mean irradiance levels in treatment conditions were approximately $30 \mu\text{mol m}^{-2} \text{sec}^{-1}$. *In situ* measurements of irradiance were higher at $\sim 65 \mu\text{mol m}^{-2} \text{sec}^{-1}$ from October 6, 2013 to October 25, 2013 (Table 1; Fig. 8).

Figure 8. In situ irradiance measurements (PAR) at Mia's Reef, San Diego, CA from October 6, 2013 to October 25, 2013.

Buoyant Weight

Final buoyant weights of tiles from Mia's Reef, after 56 days in treatment conditions, were almost always lower than initial buoyant weights (i.e., a net loss of calcium carbonate) across all treatments during the duration of the experiment

(Fig. 9). I observed a significant reduction in the buoyant weight of organisms living on the tiles in high $p\text{CO}_2$ treatments and treatments that included urchin grazers, relative to the control $p\text{CO}_2$ tiles and those without grazers. High $p\text{CO}_2$ increased the loss in buoyant weight relative to the control (ambient $p\text{CO}_2$, - urchin) by 58% in the absence of grazers and 68% in the presence of grazers. Under ambient $p\text{CO}_2$, the presence of urchins increased the loss in buoyant weight by 24% relative to the control treatment. There was also a significant effect of block, with block A having a higher percent change in buoyant weight than blocks B and C. There was no effect of the interaction between $p\text{CO}_2$ and sea urchin grazing on buoyant weight (three-way ANOVA $F_{5,34} = 13.868$, $P = <0.0001$; Table 2: Fig. 9).

Table 2. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high), urchins (presence and absence), and block (A, B and C) on the change in buoyant weight of tile communities from Mia's Reef.

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
$p\text{CO}_2$	1	0.0217	40.7229	<0.0001*
Urchin	1	0.0033	6.1688	0.0181*
$p\text{CO}_2$ x Urchin	1	0.0002	0.4262	0.5183
Block	2	0.0109	10.2083	0.0003*
Error	34	0.0181

Figure 9. Percent change in buoyant weight of settlement tiles from Mia's Reef. Shared letters below error bars indicate mean change in buoyant weight did not differ between treatments. Error bars denote $\pm$ SE.

Community Composition

Six functional groups were identified on the tops and bottoms of tiles.

These groups included calcified invertebrates (bryozoans, barnacles and serpulid worms), non-calcified invertebrates (sponges, anemones and tunicates), calcified algae, fleshy algae, sediment, and bare space (Figs. 4, 5). Following two months exposure to the experimental treatments, I found a marginally significant effect of $p\text{CO}_2$ on community composition ($P = 0.059$), but no effect of sea urchin grazing or the interaction of $p\text{CO}_2 \times$ grazing (Table 3; Fig. 10D). However, there was also

a highly significant effect of block on community composition on the top of tiles (Table 3), indicating that the final community composition depended on the structure of the initial assemblage. Due to the significant block effect and marginally non-significant $p\text{CO}_2$ effect, separate PERMANOVAs were run on all three blocks. I found significant main effects of $p\text{CO}_2$ and sea urchin grazing on community structure in Block A (Table 3; Fig. 10A), but a highly non-significant interaction term that was dropped from the model. The species assemblages tended to cluster together in multivariate space as a function of the individual treatment they occurred in. There was a significant effect of $p\text{CO}_2$ on community composition in Block B (Table 3; Fig. 10B). Tiles from the high $p\text{CO}_2$ treatments clustered together in multivariate space, while tiles from the ambient $p\text{CO}_2$ treatments grouped separately. There were no significant differences in community structure in Block C when the interaction of $p\text{CO}_2 \times$ grazer was included in our model. Without the interaction, I found significant $p\text{CO}_2$ effects on community structure (Table 3; Fig. 10C), such that tiles from the ambient $p\text{CO}_2$ treatments clustered separately from the tiles in the high $p\text{CO}_2$ treatments.

Table 3. *PERMANOVA results of the effects of pCO₂ (ambient and high), sea urchins (presence and absence), and block (A, B and C) on community composition on the tops of settlement tiles from Mia's Reef.*

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>P</i> (perm)
<i>All</i>				
pCO ₂	1	286.310	2.258	0.059
Grazer	1	118.150	0.932	0.460
pCO ₂ x grazer	1	123.780	0.976	0.430
Block	2	1049.400	8.276	0.001
Residuals	34	126.790		
<i>Block A</i>				
pCO ₂	1	311.120	2.493	0.041
Grazer	1	337.250	2.703	0.043
Residuals	10	124.790		
<i>Block B</i>				
pCO ₂	1	306.120	3.265	0.021
Grazer	1	25.150	0.268	0.911
pCO ₂ x grazer	1	81.582	0.870	0.499
Residuals	10	93.759		
<i>Block C</i>				
pCO ₂	1	289.600	2.352	0.034
Grazer	1	64.809	0.526	0.724
Residuals	10	123.150

Figure 10. nMDS plots showing dissimilarities in community composition between settlement tile benthic assemblages from Mia's Reef reared in experimental $p\text{CO}_2$ and urchin treatments. Points (tiles) closer together indicate communities more similar than points further apart. A-C show nMDS plots of community data from the top of tiles separated by block, D shows all community data from the top of tiles together.

Further analyses conducted on the responses of individual functional groups helped to explain which group or groups contributed to differences in

community composition observed among treatments. In Block A (top), we found significant effects of both $p\text{CO}_2$ and grazing (Table 4; Fig. 11A). At ambient $p\text{CO}_2$, grazing reduced fleshy algal cover by 35%; however, at high $p\text{CO}_2$, fleshy algal cover was 26% in the absence of grazing. In Block B (top), I found a ~35% lower percent cover of sediment on tiles reared in high $p\text{CO}_2$, but observed ~27% higher cover of bare space in the high $p\text{CO}_2$ conditions (Table 5; Fig. 11B). On average I found a 45% higher percent cover of calcified sessile invertebrates at ambient $p\text{CO}_2$ than in high $p\text{CO}_2$ treatments on Block C (top) (Table 6; Fig. 11C).

Table 4. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the top of Block A tiles from Mia's Reef.*

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>P</i> (perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	31.513	0.137	0.839
Grazer	1	104.610	0.455	0.563
pCO ₂ x grazer	1	62.399	0.271	0.691
Residuals	9	230.020		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	517.540	0.452	0.768
Grazer	1	1519.500	1.327	0.285
pCO ₂ x grazer	1	493.630	0.431	0.784
Residuals	9	1144.800		
<i>Calcified algae</i>				
pCO ₂	1	409.230	1.104	0.327
Grazer	1	1518.200	4.094	0.069
pCO ₂ x grazer	1	416.090	1.122	0.327
Residuals	9	370.810		
<i>Fleshy algae</i>				
pCO ₂	1	253.430	6.089	0.034
Grazer	1	242.000	5.815	0.036
Residuals	10	416.180		
<i>Sediment</i>				
pCO ₂	1	76.406	1.036	0.324
Grazer	1	262.410	3.558	0.088
pCO ₂ x grazer	1	53.146	0.721	0.417
Residuals	9	73.758		
<i>Bare space</i>				
pCO ₂	1	1123.300	3.699	0.067
Grazer	1	458.950	1.511	0.235
pCO ₂ x grazer	1	253.240	0.834	0.408
Residuals	9	303.680		

Table 5. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the top of Block B tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	57.743	0.267	0.695
Grazer	1	26.032	0.121	0.848
pCO ₂ x grazer	1	467.750	2.166	0.173
Residuals	10	215.970		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	5.465	0.014	0.980
Grazer	1	199.950	0.499	0.532
pCO ₂ x grazer	1	323.960	0.809	0.397
Residuals	10	400.390		
<i>Calcified algae</i>				
pCO ₂	1	927.310	1.105	0.389
Grazer	1	1031.400	1.229	0.301
pCO ₂ x grazer	1	1044.400	1.244	0.292
Residuals	10	839.480		
<i>Fleshy algae</i>				
pCO ₂	1	0.537	0.011	0.941
Grazer	1	2.776	0.056	0.831
pCO ₂ x grazer	1	3.246	0.066	0.817
Residuals	10	49.464		
<i>Sediment</i>				
pCO ₂	1	852.480	6.919	0.027
Grazer	1	31.682	0.257	0.663
pCO ₂ x grazer	1	61.068	0.496	0.510
Residuals	10	123.200		
<i>Bare space</i>				
pCO ₂	1	390.790	10.870	0.008
Grazer	1	38.579	1.073	0.318
pCO ₂ x grazer	1	120.390	3.349	0.098
Residuals	10	35.950		

Table 6. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the top of Block C tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	779.740	5.089	0.045
Grazer	1	101.660	0.663	0.481
pCO ₂ x grazer	1	69.638	0.455	0.576
Residuals	9	153.210		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	1349.400	2.505	0.139
Grazer	1	162.540	0.302	0.684
pCO ₂ x grazer	1	2.673	0.005	0.993
Residuals	9	538.710		
<i>Calcified algae</i>				
pCO ₂	1	1322.200	1.529	0.249
Grazer	1	957.250	1.107	0.404
pCO ₂ x grazer	1	947.300	1.095	0.412
Residuals	9	864.860		
<i>Fleshy algae</i>				
pCO ₂	1	334.150	0.988	0.363
Grazer	1	226.360	0.670	0.471
pCO ₂ x grazer	1	582.730	1.724	0.223
Residuals	9	338.100		
<i>Sediment</i>				
pCO ₂	1	67.310	0.856	0.371
Grazer	1	4.838	0.062	0.839
pCO ₂ x grazer	1	23.392	0.298	0.603
Residuals	9	78.590		
<i>Bare space</i>				
pCO ₂	1	1.739	0.025	0.905
Grazer	1	25.955	0.379	0.553
pCO ₂ x grazer	1	50.262	0.733	0.414
Residuals	9	68.571

Figure 11. Percent cover of functional group taxa on the top of tiles by block from Mia's Reef. *Note:* *, indicates a significant difference in percent cover between factor grazer; **, a significant difference in percent cover between $p\text{CO}_2$ treatments; ***, a significant difference in percent cover by block ($\alpha = 0.05$). Error bars denote $\pm$ SE.

I found a significant effect of block on community composition on the bottoms of tiles as well, but no effect of $p\text{CO}_2$, sea urchin grazing, or the interaction of $p\text{CO}_2$ x grazing (Table 7; Fig. 12D). These results indicate that the final community assemblage on the bottom of the tiles depended on the settlement tile rack on which they were cultivated. Due to the significant block effect, separate PERMANOVAs were run on all three blocks. No significant differences in community structure were found in Block A or Block B (Table 7; Fig. 12A-B). In Block C, I found significant grazing effects on community structure (Table 7; Fig. 12C), with the tiles in the +grazer treatments clustering separately in multivariate space from the tiles in the –grazer treatments.

Table 7. *PERMANOVA results of the effects of pCO₂ (ambient and high), grazers (presence and absence), and block (A, B and C) on community composition on the bottoms of settlement tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>All</i>				
pCO ₂	1	76.997	0.590	0.618
Grazer	1	365.210	2.799	0.063
pCO ₂ x grazer	1	63.752	0.489	0.686
Block	2	987.640	7.570	0.001
Residuals	34	130.470		
<i>Block A</i>				
pCO ₂	1	177.950	1.426	0.274
Grazer	1	28.154	0.226	0.828
pCO ₂ x grazer	1	248.720	1.993	0.144
Residuals	9	124.830		
<i>Block B</i>				
pCO ₂	1	73.040	0.609	0.640
Grazer	1	245.270	2.044	0.123
pCO ₂ x grazer	1	-4.895	Negative	
Residuals	10	120.020		
<i>Block C</i>				
pCO ₂	1	49.665	0.435	0.730
Grazer	1	476.860	4.176	0.021
pCO ₂ x grazer	1	283.850	2.486	0.086
Residuals	9	114.180

Figure 12. nMDS plots showing dissimilarities in community composition between settlement tile benthic assemblages reared in experimental $p\text{CO}_2$ and grazer treatments. Points (tiles) closer together indicate communities more similar than points further apart. A-C show nMDS plots of community data from the bottom of tiles separated by block, D shows all community data from the bottom of tiles together.

On the bottom of tiles, there was approximately 50% lower percent cover of non-calcified invertebrates in the presence of sea urchin grazers in Block B (Table 8-10; Figure 13A-C). There was a 60% and 15% higher percent cover of calcified invertebrates in the absence of grazing within $p\text{CO}_2$ treatments (in ambient and high $p\text{CO}_2$ respectively) in block C. In addition, block C had ~50% higher percent cover of bare space in the presence of sea urchins. I also found a significant interaction between $p\text{CO}_2$ and grazing on non-calcified taxa in block C, indicating that at ambient $p\text{CO}_2$, sea urchin grazing increased the abundance of non-calcified taxa. However, at high $p\text{CO}_2$, sea urchin grazing negatively affected the abundance of non-calcified taxa. There were no effects of $p\text{CO}_2$, grazing, or the interaction on the bottom of block A.

Table 8. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the bottom of Block A tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	0.001	0.000	0.999
Grazer	1	7.990	0.156	0.705
pCO ₂ x grazer	1	2.775	0.054	0.825
Residuals	9	51.076		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	897.480	0.731	0.602
Grazer	1	447.260	0.364	0.846
pCO ₂ x grazer	1	2101.300	1.711	0.169
Residuals	9	1228.200		
<i>Fleshy algae</i>				
pCO ₂	1	102.290	0.303	0.628
Grazer	1	88.125	0.261	0.667
pCO ₂ x grazer	1	23.687	0.070	0.876
Residuals	9	337.310		
<i>Sediment</i>				
pCO ₂	1	57.083	0.600	0.582
Grazer	1	57.083	0.600	0.575
pCO ₂ x grazer	1	57.083	0.600	0.590
Residuals	9	95.138		
<i>Bare space</i>				
pCO ₂	1	185.100	1.047	0.328
Grazer	1	46.193	0.261	0.644
pCO ₂ x grazer	1	339.950	1.923	0.192
Residuals	9	176.750		

Table 9. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the bottom of Block B tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	25.082	0.338	0.570
Grazer	1	56.791	0.764	0.392
pCO ₂ x grazer	1	1.538	0.021	0.926
Residuals	9	74.307		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	28.599	0.183	0.715
Grazer	1	828.390	5.315	0.041
pCO ₂ x grazer	1	65.334	0.419	0.557
Residuals	10	155.870		
<i>Fleshy algae</i>				
pCO ₂	1	957.250	2.694	0.132
Grazer	1	367.970	1.036	0.340
pCO ₂ x grazer	1	239.950	0.675	0.458
Residuals	10	355.350		
<i>Sediment</i>				
pCO ₂	1	61.427	0.262	0.646
Grazer	1	167.590	0.714	0.416
pCO ₂ x grazer	1	59.245	0.253	0.672
Residuals	10	234.620		
<i>Bare space</i>				
pCO ₂	1	89.883	0.842	0.398
Grazer	1	25.863	0.242	0.640
pCO ₂ x grazer	1	2.331	0.022	0.921
Residuals	10	106.780		

Table 10. *PERMANOVA results of the effects of pCO₂ (ambient and high) and grazers (presence and absence) on community composition on the bottom of Block C tiles from Mia's Reef.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	2.971	0.088	0.793
Grazer	1	258.440	7.624	0.019
pCO ₂ x grazer	1	77.699	2.292	0.159
Residuals	9	33.897		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	461.050	2.633	0.124
Grazer	1	115.990	0.662	0.481
pCO ₂ x grazer	1	1610.800	9.199	0.014
Residuals	9	175.110		
<i>Fleshy algae</i>				
pCO ₂	1	164.830	0.272	0.618
Grazer	1	109.910	0.181	0.692
pCO ₂ x grazer	1	55.437	0.091	0.772
Residuals	9	606.270		
<i>Sediment</i>				
pCO ₂	1	504.130	2.365	0.177
Grazer	1	7.473	0.035	0.854
pCO ₂ x grazer	1	7.473	0.035	0.847
Residuals	9	213.170		
<i>Bare space</i>				
pCO ₂	1	113.340	1.023	0.338
Grazer	1	874.300	7.889	0.022
pCO ₂ x grazer	1	3.570	0.032	0.924
Residuals	9	110.830

Figure 13. Percent cover of functional group taxa on the bottom of tiles by block from Mia's Reef. *Note:* *, indicates a significant difference in percent cover between factor grazer; **, a significant difference in percent cover between $p\text{CO}_2$ treatments; ***, a significant difference in percent cover by block ($\alpha = 0.05$). Error bars denote $\pm$ SE.

Juvenile kelp sporophytes were present on 22 of 40 tiles at the end of the experiment. Sea urchin grazing significantly reduced the density of kelp on tiles, but not the total biomass (ANOVA: kelp density $F_{5,34} = 2.2967$, $P = 0.0669$; kelp biomass $F_{5,34} = 1.193$, $P = 0.3333$; Table 11; Fig. 14). Tiles without urchin grazers present had 50% and 75% higher kelp densities on ambient and high $p\text{CO}_2$ tiles, respectively. Although not significant, kelp biomass data did follow a similar trend with increased biomass of juvenile kelp on tiles that excluded urchin grazers.

Table 11. ANOVA results of the effects of $p\text{CO}_2$ (ambient and high) and urchin grazers (presence and absence) on the density and biomass of juvenile kelp on settlement tiles from Mia's Reef.

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
<i>Kelp density</i>				
$p\text{CO}_2$	1	1.374	1.148	0.292
Urchin	1	8.182	6.837	0.013
$p\text{CO}_2 \times \text{Urchin}$	1	2.649	2.213	0.146
Block	2	2.192	0.916	0.410
Error	34	54.431		
<i>Kelp biomass</i>				
$p\text{CO}_2$	1	0.083	0.738	0.396
Urchin	1	0.323	2.863	0.100
$p\text{CO}_2 \times \text{Urchin}$	1	0.203	1.800	0.189
Block	2	0.058	0.259	0.774
Error	34	3.835

Figure 14. Density (A) and biomass (B) of juvenile kelp on settlement tiles from Mia's Reef at the end of 56 days reared under different $p\text{CO}_2$ and urchin grazing treatments. Shared letters below error bars indicate mean density did not differ between treatments. Error bars denote $\pm$ SE.

Sea Urchin Growth and Grazing

Initial mean sea urchin buoyant weight and sea urchin test diameter did not differ between treatments (t-test buoyant weight, $t_{18} = 0.2635$, $P = 0.7952$; t-test test diameter, $t_{18} = 0.1683$, $P = 0.8683$). However, sea urchin growth measured as buoyant weight was 60% higher in ambient $p\text{CO}_2$ than at high $p\text{CO}_2$, while there was no detectable difference in change in test size between treatments, suggesting that sea urchin tests became thinner under high $p\text{CO}_2$ conditions (t-test percent change in buoyant weight, $t_{16} = -2.8562$, $P = 0.0114$; t-test percent change in test diameter, $t_{16} = -1.4441$, $P = 0.1680$; Figure 15).

Figure 15. Growth rates of sea urchins reared on settlement tiles from Mia's Reef in ambient and high $p\text{CO}_2$ conditions standardized to initial size after 56 days in experimental rearing. Error bars denote $\pm$ SE.

Nine sea urchins from ambient and high $p\text{CO}_2$ treatments were removed from tiles at the end of the experiment for use in a grazing assay. One replicate from my high $p\text{CO}_2$ assay was removed from analysis due to inaccurate reweighing of kelp frond at the end of the experiment. Results from my grazing trial showed significantly higher grazing rates (lower % change in wet weight of kelp) in sea urchins exposed to ambient $p\text{CO}_2$ than high $p\text{CO}_2$ treatments (t-test, $t_{15} = 4.410192$, $P = 0.0005$; Fig. 16). Kelp in the ambient CO_2 treatments lost mass as a result of sea urchin grazing, whereas kelp in the high CO_2 treatments gained mass, even in the presence of grazers.

Figure 16. Results from grazing assay with sea urchins from settlement tiles accrued at Mia's reef after 56 days of rearing in ambient or high $p\text{CO}_2$. A decrease in g day^{-1} indicates a removal of algal biomass via grazing. Error bars denote $\pm$ SE.

Stillwater Cove, Carmel, CA

Experimental Conditions

Two distinct $p\text{CO}_2$ treatments were maintained for the duration of the 56-day experiment using tiles from Stillwater Cove (Table 12; Fig. 17). Ambient $p\text{CO}_2$ treatments ($\text{pH} = 8.06$) were 0.36 pH units higher than high $p\text{CO}_2$ treatments ($\text{pH} = 7.70$), while mesocosm temperatures were roughly 13°C for the duration of the experiment. *In situ* measurements of pH and temperature from autonomous SeaFET loggers recorded a mean $\text{pH} = 7.75$ and mean temperature $\sim 12^\circ\text{C}$ from October 23, 2013 - April 12, 2014) from Stillwater Cove, Carmel, CA (Table 12; Fig. 18).

Table 12. Average environmental conditions in the field and experiments for Stillwater Cove, Carmel, CA.

	Mean pH _{sw}	Min pH _{sw}	Max pH _{sw}	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Mean Peak Irradiance ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)
SWC (field)	7.75	7.39	8.23	11.97	8.74	14.83	74.66 $\pm$ 10.40
SWC high pCO ₂	7.70	7.57	8.06	12.66	12.21	13.38	28.37 $\pm$ 1.75
SWC ambient pCO ₂	8.06	7.97	8.34	12.69	12.24	13.34	28.70 $\pm$ 1.69

Figure 17. Daily experimental pH and temperature conditions in mesocosms for the duration of the 56-day experiment. pH_{sw} , with symbols representing average daily HACH pH glass electrode measurements $\pm$ SE. Solid lines represent continuous pH measurements from Honeywell Durafet.

Figure 18. *In situ* pH and temperature data collected from autonomous SeaFET sensor deployed at Stillwater Cove, Carmel, CA. Instrument was deployed at 15m and data were collected every 15 min. Panel A shows temperature (°C). Panel B shows pH_{sw}.

Mean irradiance levels in treatment conditions were approximately 28 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. *In situ* measurements of irradiance were $\sim 75 \mu\text{mol m}^{-2} \text{sec}^{-1}$ from October 6, 2013 to October 25, 2013 (Table 12; Fig. 19).

Figure 19. In situ irradiance measurements (PAR) at Stillwater Cove, Carmel, CA from March 7, 2013 to March 26, 2013.

Buoyant Weight

Final buoyant weights after 56 days in treatment conditions were almost always lower than initial buoyant weights (i.e., a net loss of calcium carbonate) across all treatments during the duration of the experiment. I observed a

significant reduction in buoyant weight of the tiles from treatments with sea urchin grazing present, but did not detect any significant effects of $p\text{CO}_2$, block, or the interaction of $p\text{CO}_2 \times$ grazing (Table 13; Fig. 20). Buoyant weights of tiles were approximately 90% and 85% (ambient and high $p\text{CO}_2$ respectively) lower in the presence of sea urchins relative to similar $p\text{CO}_2$ conditions in the absence of grazing.

Table 13. ANOVA results for the effects of $p\text{CO}_2$ (ambient and high), grazers (presence and absence), and block (A, B and C) on the change in buoyant weight of tile communities from Stillwater Cove.

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
$p\text{CO}_2$	1	2.3354	1.9815	0.1686
Grazer	1	0.0304	152.3239	<0.0001
$p\text{CO}_2 \times$ grazer	1	0.0051	0.4262	0.5676
Block	2	0.0632	2.0619	0.1433
Error	33	0.5060

Figure 20. Percent change in buoyant weight of settlement tiles from Stillwater Cove, Carmel, CA. Shared letters below error bars indicate mean change in buoyant weight did not differ between treatments. Error bars denote $\pm$ SE.

Community Composition

Six functional groups were identified on the tops and bottoms of tiles.

These groups included calcified invertebrates (bryozoans, barnacles and serpulid worms), non-calcified invertebrates (sponges and tunicates), calcified algae, fleshy algae, sediment, and bare space (Figs. 21, 22). Communities on the tops of the tiles from Stillwater Cove differed significantly as a function of sea urchin grazing (Table 14; Figure 23A), but did not exhibit any significant effects of pCO₂,

block, or the interaction of $p\text{CO}_2$ x grazing. The tiles from the treatments with sea urchin grazers cluster together in multivariate space, while the tiles from the treatments without sea urchins cluster separately. There was also a significant effect of grazing on community composition on the bottom of tiles, but no effect of $p\text{CO}_2$, block, or the interaction of $p\text{CO}_2$ x grazing (Table 15; Fig. 23B). Similarly to the top of the tiles, the species assemblages from the +grazer treatments on the bottom of the tiles cluster together in multivariate space, while the tiles from the –grazer treatments cluster separately, regardless of the $p\text{CO}_2$ treatment.

Figure 21. Settlement tile top (A) and bottom (B) from Stillwater Cove after 56 days in experimental rearing. Numbers indicate key taxa (1 = calcified algae, 2 = fleshy macroalgae, 3 = serpulid worm, 4 = bivalve, 5 = barnacle).

Figure 22. Images of representative settlement tile tops from Stillwater Cove, CA after 56 days in experimental conditions. A) ambient $p\text{CO}_2$, -grazer; B) ambient $p\text{CO}_2$, +grazer; C) high $p\text{CO}_2$, -grazer; and D) high $p\text{CO}_2$, +grazer.

Table 14. *PERMANOVA results of the effects of pCO₂ (ambient and high), grazing (presence and absence), and block on community composition on the tops of settlement tiles from Stillwater Cove.*

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>P</i> (perm)
<i>All</i>				
pCO ₂	1	40.5020	0.3615	0.7953
Grazer	1	2154.4000	19.2260	0.0001
Block	2	190.5400	1.7004	0.1237
pCO ₂ x grazer	1	44.5770	0.3978	0.7685
Residuals	33	112.0600		

Table 15. *PERMANOVA results of the effects of pCO₂ (ambient and high), grazing (presence and absence), and block on community composition on the bottoms of settlement tiles from Stillwater Cove.*

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>P</i> (perm)
<i>All</i>				
pCO ₂	1	186.1200	1.2262	0.2951
Grazer	1	928.7800	6.1193	0.0063
Block	2	124.7900	0.8222	0.5112
pCO ₂ x grazer	1	50.5670	0.3332	0.7430
Residuals	33	151.7800

Figure 23. nMDS plots showing dissimilarities in community composition between settlement tile benthic assemblages from Stillwater Cove, CA reared in experimental $p\text{CO}_2$ and grazer treatments. Points (tiles) closer together indicate communities more similar than points further apart. A, top of tiles; B, bottom of tiles.

Significantly lower percent cover of calcified invertebrates and calcified algae occurred in assemblages on the tops of tiles with grazers present (Table 16, Fig. 24A). Calcified invertebrate percent cover declined by 34% and 42% in ambient and high $p\text{CO}_2$ treatments with grazers relative to my controls (ambient $p\text{CO}_2$, -grazer). Calcified algae abundance was even further reduced by 43% and 56% in ambient and high $p\text{CO}_2$ treatments with urchins relative to my controls (ambient $p\text{CO}_2$, -grazer). I also found an approximately 60% increase in the percent cover of bare space in the presence of urchin grazers, likely due to the loss of barnacles (the most abundant calcified invertebrate) and calcified algae. There were no effects of $p\text{CO}_2$, block, or $p\text{CO}_2 \times$ grazer interaction on the communities on the bottom of the tiles from Stillwater Cove.

Table 16. *PERMANOVA results of the effects of pCO₂ (ambient and high), grazing (presence and absence) and block on functional group taxa on the tops of settlement tiles from Stillwater Cove.*

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>P</i> (perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	10.9900	0.1748	0.7430
Grazer	1	1795.4000	28.5600	0.0001
Block	2	33.0440	0.5257	0.6421
pCO ₂ x grazer	1	52.2310	0.8309	0.3758
Residuals	33	62.8620		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	69.1640	0.2434	0.6304
Grazer	1	156.1400	0.5495	0.4474
Block	2	748.8900	2.6357	0.0951
pCO ₂ x grazer	1	13.9220	0.0490	0.8351
Residuals	33	284.1400		
<i>Calcified algae</i>				
pCO ₂	1	192.8200	0.7080	0.4173
Grazer	1	3284.4000	12.0600	0.0010
Block	2	248.6100	0.9129	0.4108
pCO ₂ x grazer	1	275.7200	1.0124	0.3265
Residuals	33	272.3400		
<i>Fleshy algae</i>				
pCO ₂	1	532.3900	1.6270	0.2105
Grazer	1	1070.8000	3.2725	0.0622
Block	2	686.1700	2.0970	0.1152
pCO ₂ x grazer	1	100.8700	0.3083	0.6824
Residuals	33	327.2200		
<i>Sediment</i>				
pCO ₂	1	124.9900	0.2922	0.6816
Grazer	1	1114.2000	2.6052	0.1010
Block	2	608.3300	1.4224	0.2415
pCO ₂ x grazer	1	151.2300	0.3536	0.6255
Residuals	33	427.6900		
<i>Bare space</i>				
pCO ₂	1	22.3810	0.6746	0.4285
Grazer	1	1799.6000	54.2460	0.0001
Block	2	80.9400	2.4398	0.0960
pCO ₂ x grazer	1	46.2700	1.3947	0.2385
Residuals	33	33.1750

Figure 24. Percent cover of functional group taxa on the top (A) and bottom (B) of tiles from Stillwater Cove, Carmel, CA. *Note:* *, indicates a significant difference in percent cover between factor grazer; **, a significant difference in percent cover between $p\text{CO}_2$ treatments; ***, a significant difference in percent cover by block ($\alpha = 0.05$). Error bars denote $\pm$ SE.

Table 17. *PERMANOVA results of the effects of pCO₂ (ambient and high), grazing (presence and absence) and block on functional group taxa on the bottoms of settlement tiles from Stillwater Cove.*

Source of variation	df	Mean squares	Pseudo-F	P(perm)
<i>Calcified invertebrates</i>				
pCO ₂	1	15.2990	0.0659	0.8919
Grazer	1	1629.9000	7.0169	0.0088
Block	2	38.8640	0.1673	0.8948
pCO ₂ x grazer	1	9.4505	0.0407	0.9295
Residuals	33	232.2900		
<i>Non-calcified invertebrates</i>				
pCO ₂	1	516.4400	1.3078	0.2481
Grazer	1	1849.4000	4.6835	0.0389
Block	2	307.0300	0.7775	0.4665
pCO ₂ x grazer	1	639.5500	1.6196	0.2098
Residuals	33	394.8900		
<i>Fleshy algae</i>				
pCO ₂	1	1265.1000	4.3110	0.0410
Grazer	1	53.8710	0.1836	0.6833
Block	2	1200.8000	4.0921	0.0189
pCO ₂ x grazer	1	151.3700	0.5158	0.4816
Residuals	33	293.4500		
<i>Sediment</i>				
pCO ₂	1	124.7500	1.1647	0.5153
Grazer	1	92.2920	0.8617	0.3462
Block	2	120.3100	1.1233	0.3403
pCO ₂ x grazer	1	114.5000	1.0691	0.3897
Residuals	33	107.1000		
<i>Bare space</i>				
pCO ₂	1	6.3976	0.1669	0.6941
Grazer	1	313.0500	8.1666	0.0079
Block	2	10.2490	0.2674	0.7770
pCO ₂ x grazer	1	0.4330	0.0113	0.9456
Residuals	33	38.3330		

On the bottom of tiles, there was a significant negative effect of grazing on the percent cover of calcified invertebrates and a significant positive effect on non-calcified invertebrates, and bare space (Table 17, Fig. 24B). Percent cover of calcified invertebrates was reduced by ~40% in the presence of grazers under both ambient and high $p\text{CO}_2$ conditions. Conversely, the percent cover of non-calcified invertebrates increased 125% and ~300% in ambient and high $p\text{CO}_2$ treatments in the presence of sea urchin grazers. However, it is important to note that non-calcified invertebrate abundances were around 1% cover versus the much higher 20-30% of calcified invertebrates. Bare space also increased in the presence of sea urchins (~25% in both $p\text{CO}_2$ treatments). In addition, there was a significant negative effect of $p\text{CO}_2$ and a significant effect of block on fleshy algal abundance (Table 17, Fig. 24B). Fleshy algae were only found on one of the three blocks, therefore further analyses by block were not conducted.

Juvenile kelp sporophytes were present on 24 of 39 tiles at the end of the experiment. ANOVAs with fixed factors of $p\text{CO}_2$, grazers, and block were conducted on square-root transformed variables of kelp biomass (dry weight) and kelp density. Sea urchin grazers significantly reduced both biomass and density of kelp, but there was no effect of $p\text{CO}_2$, block, or $p\text{CO}_2 \times$ grazer interaction (Table 18; Fig. 25A-B). Under ambient $p\text{CO}_2$ conditions, the presence of sea urchins reduced both density and biomass of kelp by ~98%. At high $p\text{CO}_2$ conditions, the presence of sea urchins reduced kelp density and biomass by 93% and 76% relative to our ambient $p\text{CO}_2$, -grazer control.

Table 18. ANOVA results of the effects of $p\text{CO}_2$ (ambient and high) and grazing (presence and absence) on the density and biomass of juvenile kelp on settlement tiles from Stillwater Cove.

Source of variation	<i>df</i>	Sum of squares	<i>F</i> -statistic	<i>P</i> -value
<i>Kelp density</i>				
$p\text{CO}_2$	1	14.1334	1.8251	0.1859
Grazer	1	125.3992	16.1931	0.0003
$p\text{CO}_2$ x grazer	1	19.1288	2.4701	0.1256
Block	2	5.4663	0.3529	0.7052
Error	33	255.5521		
<i>Kelp biomass</i>				
$p\text{CO}_2$	1	0.0165	0.5185	0.4765
Grazer	1	0.5396	16.9583	0.0002
$p\text{CO}_2$ x grazer	1	0.0715	2.2482	0.1433
Block	2	0.0437	0.6865	0.5104
Error	33	1.0501

Figure 25. Density (A) and biomass (B) of juvenile kelp on settlement tiles in different $p\text{CO}_2$ and grazer treatments. Error bars denote $\pm$ SE.

Sea Urchin Growth and Grazing

Initial mean sea urchin buoyant weight and sea urchin diameter did not differ between treatments (t-test buoyant weight, $t_{18} = -0.574$, $P = 0.5731$; Kruskal-Wallis test diameter, $X^2 = 0.0702$, $df = 1$, $P = 0.7911$). Sea urchin growth rates (i.e., change in buoyant weight and test diameter) did not differ between $p\text{CO}_2$ treatments (Kruskal-Wallis percent change in buoyant weight, $X^2 = 2.2857$, $df = 1$, $P = 0.1306$, t-test percent change in test diameter, $t_{18} = -0.302$, $P = 0.7663$; Fig. 26A-B).

Figure 26. Growth rates of sea urchins reared on settlement tiles from Stillwater Cove in ambient and high $p\text{CO}_2$ conditions standardized to initial size after 56 days in experimental rearing $\pm$ SE.

Ten sea urchins were removed from settlement tiles exposed to ambient and high $p\text{CO}_2$ conditions at the end of the experiment for use in grazing assays. In contrast to the results of experiments from Mia's Reef, results from my grazing trial on tiles from Stillwater Cove found no significant differences in grazing rates of sea urchins subjected to ambient and high $p\text{CO}_2$ treatments following 60 days of exposure (t-test, $t_{18} = -0.061$, $P = 0.952$; Fig. 27).

Figure 27. Results from grazing assay with urchins from settlement tiles accrued at Stillwater Cove after 56 days of rearing in ambient or high $p\text{CO}_2$. A decrease in g day^{-1} indicates a removal of algal biomass via grazing. Error bars denote $\pm \text{s.e.m.}$

Discussion

Global change will almost certainly impact marine ecosystems due to species-specific changes to biology, physiology and ecology. However, it is challenging to predict how these species-specific responses will manifest at the ecosystem level without controlled studies conducted on species assemblages as a whole. This study focused on the emergent effects of ocean acidification at two kelp forest locations acclimated to different pH and temperature regimes. In addition, I addressed how mobile grazers will aggravate or ameliorate the impacts of ocean acidification on benthic community composition.

Calcification and Community Structure

Climate change stressors such as decreased pH (OA) and/or increased temperature have been shown to dramatically change the landscape scale patterns seen on tropical coral reefs and at natural CO₂ vent locations. The present study was conducted using species assemblages accrued at two kelp forest locations along the California coast, where the main habitat-forming kelp, *Macrocystis pyrifera*, dominates. The initial species assemblages differed greatly between sites. In Carmel, CA, barnacles were the dominant living taxa on tiles, covering nearly 50% and 30% of tile surfaces (top and bottom respectively). However, in San Diego, the top surface of tiles was nearly 40% fleshy macroalgae with the bottom surface dominated by bryozoans (~50%). The community level responses varied greatly both between sites (Carmel versus

San Diego) as well as within a site (San Diego) likely due to geographic differences in the initial structure of the assemblages inhabiting the settlement tiles, as well as in prior exposure history to low pH conditions during the year in which the tiles were deployed in the field.

In San Diego, CA, where natural variability in pH is more static and mean pH values are on average 8.06, I found that OA could affect net ecosystem calcification on the tiles. Negative changes in the percent buoyant weight of calcifying organisms throughout all treatments could suggest an experimental artifact or could just be due to seasonal differences in calcification rates of organisms on the tiles. However, despite an overall decrease in calcium carbonate across treatments, I found a significantly greater reduction in calcium carbonate on tiles reared under high $p\text{CO}_2$ conditions. Net loss of calcium carbonate was also found in a subtropical coral reef community reared under high $p\text{CO}_2$ conditions for ~8 months (ambient $p\text{CO}_2 = 568 \mu\text{atm}$, high $p\text{CO}_2 = 1147 \mu\text{atm}$) (Andersson et al., 2009). High $p\text{CO}_2$ or low pH conditions are known to reduce the calcification rates of numerous taxa in single species experiments (Kroeker et al., 2010). This is largely assumed to be due to a decrease in the availability of CO_3^{2-} , a necessary building block of biogenic carbonates. Coral reef communities have been shown to exhibit decreased net accretion rates of corals and coralline red algae under elevated $p\text{CO}_2$ (Hoegh-Guldberg et al., 2007; Jokiel et al., 2008). It's likely that the $p\text{CO}_2$ effect seen here is due to both reduced calcification, via decreased growth and thinner calcium carbonate

skeletons, as well as increased dissolution. Furthermore, high $p\text{CO}_2$ had a greater effect than sea urchin grazing in San Diego. However, the reduction in calcified biomass, or net dissolution in the presence of sea urchins, may be due in part to both consumption of calcareous organisms or erosive processes as mobile sea urchins grazed on fleshy species.

I found that the $p\text{CO}_2$ effects on community structure were dependent on the initial communities in our experiment from San Diego, CA. Since functional group taxa responded differently between blocks, overall effects were masked when blocks were pooled together. Interestingly, each block only exhibited minor differences in the abundances of functional group taxa at the onset of my experiment. These species assemblages were obtained from the same reef outcrop (spaced five meters away from each other and differing in depth by less than one meter) and experienced similar environmental conditions (e.g., temperature, pH, grazing) before being exposed to ocean acidification and grazing. This finding is surprising as such small-scale heterogeneity on reefs has largely been unstudied in the context of climate change, yet it may play an important role in the persistence of marine ecosystems. Recent work by Cornwall et al. (2014) suggests that diffusive boundary layers around algal assemblages could alter the effects of OA by decreasing flow and allowing pH to increase within the algal assemblage. Therefore, minor variability in not just the abundance, but also the spatial arrangement of species within a community could change the response to acidification. It's possible that minor differences in

flow and light levels within the kelp forests at Mia's Reef could alter assembly processes and therefore lead to different species arrangements and subsequent responses to both OA and grazing between blocks.

Although the differential responses of taxa between blocks may be driven by spatial arrangement, it's also possible that the experimental duration was not sufficient to detect landscape level impacts on community structure. Past studies subjecting intact assemblages to acidification vary greatly in the length of study, but the general pattern of detrimental impacts to calcifying organisms and positive effects on non-calcifying taxa are supported. Temperate intertidal communities comprised of articulated coralline red algae and fleshy red macroalgae subjected to high $p\text{CO}_2$ (1486 μatm) for 86 days found decreased calcification in the articulated coralline red alga, *Corallina officinalis*, and increased cover of the fleshy red alga, *Chondrus crispus* (Hofmann et al. 2012). James et al. (2014) found reduced growth of crustose coralline red algae in early successional communities reared at a pH of 7.60 (compared to ambient pH = 8.05) for only 6 weeks. My experiment lasted 56 days, which is within the range of past studies on community impacts of OA in temperate systems. However, intact communities dominated by adults and persistent taxa may be more resilient to OA and therefore in order to detect changes in community structure within dynamic ecosystems should be conducted over longer time scales.

Species assemblages from Carmel, CA responded differently to OA and grazing than assemblages from San Diego, CA. This is likely due to differences

in community composition, but also potentially due to local adaptation or acclimation to different pH regimes. Local adaptation to environmental stressors such as temperature and pH have been documented in numerous species both in the field as well as in laboratory experiments (Sanford and Kelly 2011). In addition to adaptation in a single species, geographic mosaics of coevolution can result in variability in species interactions due to differences in selection pressures driven by abiotic stressors, such as temperature (Sanford 2003). Mean pH conditions in the field in Carmel, CA (pH = 7.75), where the tiles were deployed for a year to accrue mixed assemblages, were only 0.05 pH units higher than mean pH in our high $p\text{CO}_2$ treatment. Carmel Bay is a location of strong and persistent upwelling of deep water from the nearby Carmel Canyon and organisms living in this area are exposed to pH levels significantly lower than in San Diego or the global average. Therefore, it's not surprising that there was a lack of $p\text{CO}_2$ effect on most of the response variables reported in this experiment. This also stresses the need to manipulate $p\text{CO}_2$ treatment conditions specific to the site (or region) of study as ocean acidification in one region may be indicative of natural conditions in another. Unfortunately, long term monitoring of pH is not available in all locations and I did not have the opportunity to process the *in situ* sensor data from my two study locations prior to the start of the laboratory experiment.

I did find strong impacts of sea urchin grazing on both calcification rates as well as community structure in Carmel, CA. Sea urchins of the genus

Strongylocentrotus are known to alter algal species composition by preferentially removing algal biomass (Paine and Vadas 1969). One study by Breitburg (1985) found that in Santa Barbara kelp forests only grazer-resistant algal crusts (i.e. crustose coralline algae), diatom films and ephemeral filamentous algae were abundant in the presence of sea urchin and sea star grazers. However, when grazers were excluded communities consisted of filamentous red algae, encrusting bryozoans, barnacles and tubicolous amphipods. My study found a significant reduction in the percent cover of both calcified invertebrates (mainly barnacles) and coralline red algae in the presence of grazers. However, the coralline algae in my study were small crusts and articulated individuals (less than ~3cm tall) and therefore may have been more susceptible to grazing than the grazer-resistant crusts in Breitburg's study. Sea urchins have also been implicated in phase shifts from high diversity fleshy macroalgae dominated kelp forests to low diversity coralline algae dominated sea urchin barrens (Pearse 2006). The dramatic reductions in calcification seen in grazer treatments from tiles from Carmel, CA were mainly caused by removal of barnacles by sea urchins. Sea urchins likely both consumed barnacles directly and caused their removal as sea urchins fed on fleshy macroalgae. This is also mirrored in the dramatic increase in bare space after the removal of barnacles on tiles.

Impacts of OA and Grazing on Juvenile Kelp

Interest in the consequences of climate change for kelp forest ecosystems has increased since recent studies have shown that early life history stages of kelp may be particularly vulnerable to high $p\text{CO}_2$ and temperature (Gaitán-Espitia et al. 2014). Early life history stages are likely to become increasingly important to the recovery of kelp forests in future oceans as disturbances that act to remove kelp plants become more frequent and intense (Byrnes et al. 2011). Although it was not my initial intention, this study provided an interesting opportunity to preliminarily assess the emergent effects of climate change and grazing on giant kelp, *Macrocystis pyrifera*. At the outset of both lab experiments, no kelp were visible to the naked eye on tile surfaces, suggesting that they were present at some unknown microscopic stage upon removal from the reef. No differences in density or biomass were seen as a result of $p\text{CO}_2$ at either site, suggesting that the impacts of OA on kelp growth and survival may not be impacted at this early stage, as has been shown in previous studies when only $p\text{CO}_2$ was manipulated to simulate OA (Roleda et al. 2012). I have no way of knowing what stage of the lifecycle *M. pyrifera* individuals were in when they were placed in my experiment or whether densities were similar across treatments. However, this is the first study to assess how juvenile kelp sporophytes may respond to ocean acidification. I found no apparent effect of OA at the juvenile sporophyte stage as evidenced by my finding that there was no significant difference between the density and biomass of juvenile kelp

between ambient and high $p\text{CO}_2$ treatments. However, since cryptic microstages have been shown to be susceptible to environmental stressors (Gaitán-Espitia et al. 2014) it will be important to directly assess how impacts at various microscopic stages (i.e., settlement, germination, sporophyte production) may alter the landscape scale patterns of kelp forest recovery dynamics.

Although grazing did not alter community structure in San Diego, CA, I did find a reduction in the density of juvenile kelp sporophytes in the presence of sea urchin grazers. Changes to sporophyte density in response to grazing would likely alter other physical properties of the environment. One study by Alsterberg et al. (2013) found both direct and indirect effects of OA, warming, and grazers on benthic microalgae in a seagrass community. In the presence of grazers, the direct and indirect effects of OA and warming did not significantly affect benthic microalgae. However, in the absence of grazers, benthic microalgae were indirectly negatively affected by sediment-associated microalgal detritus and macroalgal shading, yet directly positively affected by acidification and warming. Although few direct effects of grazing on community structure were evident on tiles from San Diego, CA for the duration of our experiment, it is likely that the indirect effects of macroalgal shading and changes to flow regimes could alter the CO_2 response of benthic communities over longer timescales.

In my first experiment with tiles and sea urchins collected from San Diego, CA, I found that sea urchin growth and grazing rates were significantly depressed in high $p\text{CO}_2$ treatments. Russell et al. (2013) also found that the herbivorous

gastropod, *Littorina littorea*, showed decreased consumption rates in high $p\text{CO}_2$ conditions. However, in my second experiment with tiles from Carmel, CA and sea urchins collected from San Diego, CA we found no $p\text{CO}_2$ effect on growth or grazing rates. Sea urchins were collected once and used in both experiments. In between experiments sea urchins were held in a large aquarium and fed giant kelp *ad libitum*. During this time sea urchins doubled in size and may have become acclimatized to laboratory conditions or more resistant to the effects of $p\text{CO}_2$. This may also explain the strong grazing effects on both community composition and density and biomass of kelp on tiles from Carmel, CA. Therefore, any comparisons of grazing impacts between sites should be made with caution. However, the differential responses of OA on grazing are important to understand as changes to the strengths of algal-grazer interactions could have major consequences for marine ecosystems, such as favoring shifts from kelp forests to sea urchin barrens.

Conclusion

Climate change impacts on kelp forest ecosystems are likely to vary spatially both at local and regional scales. Here I show differences in the susceptibility of kelp forest benthic communities to ocean acidification and the interactive effects of grazing. In San Diego, CA, ocean acidification reduced calcification and altered community structure whereas sea urchin grazing showed few emergent effects. However, in Monterey, CA, ocean acidification had no

effect, but sea urchin grazing had a strong impact on community composition. These findings support predictions that local adaption may play a role in species susceptibility to acidification. Those inhabiting naturally more corrosive seawaters may have evolved mechanisms to tolerate low pH. Findings like this can be encouraging; however, our understanding of how these mechanisms have evolved limits our ability to predict whether some species will be able to keep up with the rate of accelerating global change.

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Conclusion to Thesis

Marine ecosystems worldwide are threatened by CO₂-driven global changes, such as ocean warming and acidification. How these changes will impact marine ecosystems and the services these ecosystems provide is still unclear. In Chapter 1, I assessed the response of an ecologically important and high-risk coralline algal species, *Calliarthron cheilosporioides*, to warming and acidification. My findings suggest that this species is likely to be negatively impacted by global change stressors. I found that growth and calcification were reduced in high $p\text{CO}_2$ and elevated temperature treatments, yet the two stressors did not act synergistically. I also found evidence that high $p\text{CO}_2$ can affect photophysiology of coralline algae by increasing the maximum photosynthetic rate (P_{max}). This study did not find evidence for mineralogical plasticity, but did document variation in Mg incorporation in different cell types of coralline red algae. Together these results suggest that unless *C. cheilosporioides* is capable of acclimating to ocean acidification and warming over time scales longer than my current experiment, I expect to see reductions in both the abundance and distribution of this species.

In Chapter 2, I scaled up to examine the emergent effects of ocean acidification and grazing on benthic communities from two different geographic regions of the California Current, central and southern California, that differ in the intensity of upwelling and thus the level of pH naturally experienced by the

benthos. I found that the community responses differed greatly between sites and even within a single site (San Diego). San Diego, CA exhibited decreased calcification rates and changes to the abundances of key functional group taxa in response to acidification, but few responses to sea urchin grazing. Monterey, CA, on the other hand, expressed decreased calcification and a reduction in the abundances of calcified invertebrates and algae due to sea urchin grazing, but no response to acidification. Although my study only contained two locations (one in central and one in southern CA), the changes to community structure due to OA at both sites are in line with predictions based on prior exposure and the potential for local adaptation.

Together these chapters suggest that temperate kelp forest ecosystems may be negatively impacted by climate change. Future research is necessary to understand the potential for populations inhabiting higher pH conditions to acclimate to acidification over longer time scales as well as the thresholds beyond which low-pH adapted populations also feel the effects of OA.

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