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The Effect of Ocean Acidification on the Ecology of Two Tropical Crustose Coralline Algae (Phylum Rhodophyta)

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biology

By

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DEDICATION

I would like to dedicate this thesis to Adrian, Linda, and Joseph, also known as pop, mom, and broseph. They have always supported and encouraged my passion for research, and I hope they know how much it has meant to me. I also dedicate this thesis to my grandmother Doreen for encouraging my interest in science and the ocean at a very young age.

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ABSTRACT

The Effect of Ocean Acidification on the Ecology of Two Tropical Crustose Coralline Algae (Phylum Rhodophyta)

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Crustose coralline algae (CCA) are important members of coral reef communities. They accrete and consolidate the calcium carbonate framework of coral reefs, and some species are an important settlement substratum for coral larvae. CCA community composition is shaped, at least in part, by herbivory and competition. However, ocean acidification (OA) is negatively affecting CCA, with potential to affect CCA responses to herbivory (wounding) and their ability to compete for space. Changes in seawater chemistry because of OA cause reductions in the recruitment, abundance, and net calcification of CCA. In this thesis, the effects of OA on net calcification, regeneration of wounds, and competition was quantified for two species of CCA common in the back reefs of Mo'orea, French Polynesia; *Porolithon onkodes* and *Lithophyllum insipidum*. Three separate experiments were conducted in four flowing seawater tanks (flumes), each

set to a different target pCO_2 level representative of ambient (~ 400 µatm) or predicted end of the 21 century pCO_2 (~ 700, 1000, and 1300 µatm).

P. onkodes, was found to be the most abundant species of CCA in the back reefs of Mo'orea, followed by *L. flavescens* and *L. insipidum*. The abundance of *P. onkodes* is likely a direct result of its competitive ability. *P. onkodes* is thicker on average than the other common CCA in the back reefs of Mo'orea, and thicker species generally become dominant in areas of intense herbivory, such as coral reefs. In a flume experiment conducted from January to March 2016, net calcification declined 85% in *P. onkodes* at elevated pCO_2 compared to a decline of 42% in *L. insipidum*, indicating that *P. onkodes* may be more negatively affected by OA. The differential responses to OA found here could alter the outcome of competitive interactions between *P. onkodes* and *L. insipidum*, leading to changes in the abundances of these species in CCA communities.

Few studies have quantified the potential for OA to interact with natural disturbances, such as wounding of the thallus by herbivores. A flume experiment conducted from May to July 2016 found that there was a 58% reduction in the rate of vertical regeneration of artificial wounds within *P. onkodes* at elevated pCO_2. This result could have important implications for the response of *P. onkodes* to grazing by excavating herbivores like parrotfish and sea urchins. Inability for CCA to recover from wounding, could increase the susceptibility of CCA to further wounding. In addition, the reductions in vertical regeneration of the wounds could also be indicative of reduced vertical growth rates. CCA with thicker thalli generally outcompete thinner CCA. Reduced vertical growth rates could reduce thallus thickness, and affect the outcome of competitive interactions among CCA.

A flume experiment conducted from June to July 2016 found that there was no effect of elevated pCO_2 on the outcome of competitive interactions between *P. onkodes* and *L. insipidum*. It is likely that this result may have been due to the relatively short duration of this experiment (one month). There was, however, an effect of the identity of the competitor on the proportion of live tissue remaining in focal individuals of *P. onkodes*. The proportion of live tissue remaining in focal individuals of *P. onkodes* was significantly lower in intraspecific pairings than in interspecific pairings or when paired with non-living substrate (controls). This result highlights the importance of including both intraspecific and interspecific interactions in future studies of the effects of OA on competition. Experiments of longer durations may elucidate the potential for elevated pCO_2 to affect competition among CCA. CCA are ecologically important members of coral reefs. Changes in the community composition of CCA on coral reefs, because of altered competitive abilities under elevated pCO_2, could affect the roles that CCA play in building and maintain coral reef ecosystems.

INTRODUCTION

Climate change is leading to drastic changes in species distributions in both terrestrial and marine environments, altering the phenologies of many organisms, and causing cascading effects on ecological networks (Walther et al. 2002, Harley et al. 2006, Walther 2010, Kordas et al. 2011). Climate change is increasing the frequency and severity of coral bleaching events around the world through increasing sea surface temperatures, with variable bleaching among different coral taxa, creating both "winners" and "losers" (Loya et al. 2001, Depczynski et al. 2013, Hughes et al. 2017). Edmunds et al. (2014) found that there have been increases in the absolute and relative abundance of some coral genera, while others have declined over the past few decades. They suggest that there may be changes in the composition in coral reefs at the generic level by the end of the present century because of climate change (Edmunds et al. 2014). However, climate change is just one of the results of increased atmospheric CO_2. The ocean is the largest sink for anthropogenically produced CO_2 (Sabine et al. 2004). As CO_2 is absorbed by the ocean, it is altering oceanic carbonate chemistry. The current rate at which CO_2 is being deposited into the atmosphere has the potential to alter ocean geochemistry to an extent not seen in the past ~300 million years (Hönisch et al. 2012). Carbonate ion concentrations, calcium carbonate saturation states, and pH are decreasing (Hoegh-Guldberg et al. 2007, Doney et al. 2009, Pachauri et al. 2014). Collectively these changes in carbonate chemistry are referred to as ocean acidification (OA, Caldeira & Wickett 2003).

Experimental studies indicate that organisms are responding variably to OA (Ries et al. 2009, Kroeker et al. 2010, Kroeker et al. 2013). In general, the effects tend to be

more negative for calcifying marine organisms, such as corals and calcified algae, than non-calcifying organisms (Ries et al. 2009, Hofmann et al. 2010, Kroeker et al. 2010, Kroeker et al. 2013). Coral reefs are biogenic structures created by calcifying organisms, namely corals and calcified algae. These calcium carbonate structures serve many economic and ecological functions (reviewed in Moberg & Folke 1999). Because of this importance, calcifying organisms on coral reefs have been the focus of much of the OA research to date. Studies have found reductions in the calcification and productivity of many coral species (Anthony et al. 2008, Chan & Connolly 2013, Comeau et al. 2013, 2014). However, there is some evidence that the responses among corals to OA may be variable. Comeau et al. (2014) found that there was a significant difference in the response of corals that calcify fast compared to corals that calcify more slowly. They suggest that corals that calcify more rapidly may be more affected by OA due to increased difficulty in removing H^+ from the sites of calcification, which is important to maintaining high rates of calcification (Comeau et al. 2014). The variable responses of corals to OA could affect coral communities in a similar manner to climate change. Along an *in situ* gradient in pH near natural CO_2 vents on the coral reefs of Papua New Guinea, Fabricius et al. (2011) documented a reduction in the species richness of hard coral adults and juveniles as pH declined from 8.1 to 7.8. There was a significant reduction in the abundance of structurally complex, fast growing corals at reduced pH, which were replaced by slow-growing, mounding massive *Porites* (Fabricius et al. 2011). Fabricius et al. (2011) also found an increase in the cover of fleshy non-calcareous algae and a decrease in calcified red algae at low pH sites. Porzio et al. (2011) found significant shifts in macroalgal communities as pH fell near these natural CO_2 vents, including

decreases in the cover and richness of calcitic calcified algae, while non-calcified species became dominant.

Crustose coralline algae (CCA; Rhodophyta) are important calcifying organisms on coral reefs. CCA are often abundant space holders on coral reefs, where they consolidate and accrete the calcium carbonate framework (Littler & Doty 1975, Adey et al. 1982, Nelson 2009, Dean et al. 2015). CCA are also important promoters of settlement and recruitment of coral larvae (Heyward & Negri 1999, Harrington et al. 2004, Arnold et al. 2010, Price 2010). However, CCA secrete a highly soluble form of calcium carbonate, high-Mg calcite, which may make them more vulnerable to the effects of OA (Morse et al. 2006). Experimental evidence suggests that OA may reduce the recruitment, abundance, productivity, and calcification of tropical CCA (Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Diaz-Pulido et al. 2012, Comeau et al. 2013, 2014). As in corals, there may be some species-specificity among CCA in their responses to OA (Comeau et al. 2014). These species-specific responses to OA, could result in shifts in community composition among tropical CCA. However, despite the potential for OA to affect community structure, few studies have quantified the interaction between OA and ecological processes.

While much of the current research on the effects of OA has focused on the scale of the individual, there has been a recent push to use ecological theory to quantify the effects of OA at larger scales (e.g., community and ecosystem; Gaylord 2008, McCoy & Kamenos 2015, Edmunds et al. 2016). Two of the most important processes shaping benthic marine communities are disturbance and competition. Grime (2006) defined a disturbance as any process that either partially or totally removes biomass. Spatial and

temporal heterogeneity within habitats is maintained in part by the natural disturbance regime, combined biological and physical disturbances, of a community (Sousa 1984). It also has been hypothesized that intermediate levels of disturbance maintain diversity, in part by preventing competitive exclusion (Connell 1978). Human disturbance is altering natural disturbance regimes on coral reefs (Nyström et al. 2000). Disturbance in the form of herbivory plays an important role in shaping the structure of CCA communities. CCA have coevolved with herbivores capable of excavating their calcareous structures, such as sea urchins and parrotfish (Scaridae), and with few other mechanisms to prevent overgrowth, CCA rely on grazing to keep their thalli free of epiphytes (Steneck 1982, 1983).

CCA are relatively resistant to herbivory. Their region of growth, the intercalary meristem, and reproductive structures are protected to some extent by an epithallus (Steneck 1983). Regeneration of deeper wounds can either be achieved through regeneration of the perithallus (photosynthetic tissue) or through the formation of a new hypothallus within the wounds (Steneck 1983). Only one study to date has quantified the interaction between OA and grazing among CCA. Johnson & Carpenter (2012) found that elevated pCO_2 increased the susceptibility of *Porolithon onkodes*, a common CCA throughout the Indo-Pacific, to grazing by the sea urchin *Echinothrix diadema*. Increased susceptibility could be the result of a weakened, or thinner thallus, but this has yet to be demonstrated for this species. However, McCoy & Ragazzola (2014) found that the thalli of modern samples of thicker species of temperate CCA were 2-3 times thinner than historic samples as a result of OA. This reduction in thallus thickness in thick species because of OA could also affect the outcome of competition among CCA.

Competition is an ecological interaction in which individuals of the same or different species harm one another while seeking a common resource, such as space (Birch 1957). Competition is an important process structuring benthic marine communities (Connell 1961, Menge & Sutherland 1976). However, OA has the potential to alter competitive interactions by acting as a resource (e.g., increasing carbon fixation rates) for some taxa, while negatively affecting others (Connell et al. 2013). CCA regularly compete for space through overgrowth interactions. In general, thicker individuals win competitive bouts against thinner individuals, although in the absence of intense grazing competitive reversals can occur, in which thinner species become more dominant (Steneck et al. 1991). Differential responses among CCA to OA conceivably could alter competitive outcome for space. McCoy & Pfister (2014) attribute changes in interaction strength among a temperate guild of CCA off the coast of Washington, USA, to OA. The decreased thallus thickness among thick members of this guild of CCA in response to experimental OA could be a possible mechanism behind the changed outcomes of competitive interactions (McCoy & Ragazzola 2014). If tropical species of CCA behave similarly to temperate species, CCA community structure could change on coral reefs as pH falls, potentially affecting key ecological processes in which they are important, including coral settlement and recruitment.

The goal of this thesis was to quantify the effect of OA on two species of CCA that are common in the back reefs of Mo'orea, French Polynesia, *Porolithon onkodes* and *Lithophyllum insipidum*. In the first chapter, the responses of *P. onkodes* and *L. insipidum* to OA were quantified. Upon discovering differences in the responses of these species to OA, two further experiments were performed to quantify the potential interaction

between OA and the ecological processes of disturbance and competition. In the second chapter, both species were subjected to elevated pCO_2 and simulated herbivory to test the following null hypotheses: (1) there will be no interaction between OA and wounding on the calcification and tissue health of *P. onkodes* and *L. insipidum*, and (2) there will be no effect of OA on the ability of *P. onkodes* and *L. insipidum* to regenerate wounded thalli. In the third chapter, the effect of OA on competition between *P. onkodes* and *L.insipidum* was quantified to test the null hypothesis that OA would not affect competitive outcome for space.

CHAPTER 1

Differential effects of longer-term exposure to elevated pCO_2 on the calcification of two tropical crustose coralline algae

Introduction

Climate change because of increased atmospheric CO_2 is a major threat to global ocean ecosystems. Climate change has already begun to affect many different species in both terrestrial and aquatic environments, altering their phenologies as well as their distributions, leading to cascading effects on ecological networks (Walther et al. 2002, Harley et al. 2006, Walther 2010). On coral reefs, climate change has the potential to drastically alter community structure through differential effects on multiple taxa (Kroeker et al. 2010, Kroeker et al. 2013). The community structure of corals has changed on a few reefs following bleaching events, with shifts toward dominance by more tolerant species (Loya et al. 2001, Depczynski et al. 2013). *In situ* studies of community structure along gradients of pH near natural CO_2 vents in Papua New Guinea have provided some evidence that ocean acidification could also lead to changes in the structure of reef communities (Fabricius et al. 2011, Porzio et al. 2011).

Ocean acidification (OA, Caldeira & Wickett 2003) is the byproduct of increased atmospheric pCO_2, produced by continued human consumption of fossil fuels. Roughly 30 % of human-produced CO_2 has been absorbed by the ocean (Sabine et al. 2004, Pachauri et al. 2014). Increased absorption of anthropogenic CO_2 by the ocean has led to changes in seawater chemistry, including a reduction in pH of 0.1 units, reduced calcium carbonate saturation states, and reduced carbonate ion concentrations (Doney et al. 2009,

Pachauri et al. 2014). These changes in seawater chemistry are expected to negatively affect many calcareous marine organisms at various stages in their life histories, including corals and calcified algae (Ries et al. 2009, Hofmann et al. 2010). Recent meta-analyses have indicated that the responses among marine taxa have been varied, potentially resulting in changes in abundances of the different taxa (Kroeker et al. 2010, Kroeker et al. 2013). Heavily calcified organisms appear to be most affected by OA, while non-calcifying organisms like fleshy algae and seagrasses are unaffected, or positively affected (Kroeker et al. 2010, Kroeker et al. 2013). Studies have found reductions in the net calcification of corals because of experimentally elevated pCO_2, and there may be species-specific responses among corals to projected increases in pCO_2 (Comeau et al. 2013, 2014). Comeau et al. (2014) found that the reduction in the net calcification of corals classified as fast calcifiers (> 1 mg $CaCO_3$ cm^{-2} d^{-1}) was greater in magnitude than the reductions in net calcification found for more slowly calcifying corals (< 1 mg $CaCO_3$ cm^{-2} d^{-1}). Along an *in situ* gradient of increased pH, Fabricius et al. (2011) found a reduction in the richness of hard coral adults and juveniles and an increase in the cover of fleshy macroalgae and seagrasses. They found a reduction in the abundance of structurally complex, fast growing corals, and an increase in the abundance of long-lived, slow growing massive *Porites* at the low pH sites (Fabricius et al. 2011). This finding agrees with the results of Comeau et al. (2014), who found that fast calcifiers may be more affected by OA. Importantly, Fabricius et al. (2011) also found a reduction in cover of crustose coralline algae and other calcified algae at the low pH sites.

Crustose coralline algae (CCA) are an important group of calcifying organisms on coral reefs. They cement coral reef frameworks, consolidating and stabilizing reef

substrate to prevent erosion (Nelson 2009). There also is evidence that some species of CCA can facilitate the settlement and recruitment of coral larvae to the benthos, helping them to navigate early bottlenecks in their life histories (Heyward & Negri 1999, Harrington et al. 2004, Arnold et al. 2010, Price 2010). The high-Mg calcite (> 12% Mg) polymorph of $CaCO_3$ deposited by CCA is more soluble than other polymorphs of calcium carbonate, including the aragonite polymorph deposited by corals (Morse et al. 2006). This means that CCA could be one of the first groups of organisms to respond to increasing seawater pCO_2 and the resulting changes in seawater chemistry. In contrast to corals, CCA are relatively understudied in the context of OA.

Recent experimental and observational field studies have found that OA negatively affects CCA. A manipulative experiment by Kuffner et al. (2008) found reduced recruitment and abundance among CCA because of elevated seawater pCO_2. The calcification rates of several tropical species of coralline algae have been depressed by experimental conditions mimicking projected OA (Anthony et al. 2008, Comeau et al. 2013). Additionally, there appears to be some level of species-specificity among tropical calcified algae in their responses to elevated pCO_2 (Comeau et al. 2013, 2014). Comeau et al. (2014) found that there were significant differences in the effect of elevated pCO_2 on net calcification when comparing non-branched versus branched species, species with cell-wall versus intercellular calcification, and fast (> 20 mg $CaCO_3$ d^{-1} g^{-1}) versus slow (< 20 mg $CaCO_3$ d^{-1} g^{-1}) calcifying species of coralline algae. Doropoulos et al. (2012) found that species of CCA that were most important in inducing the settlement of coral larvae were the most negatively affected by elevated pCO_2 in their experimental study. This could, through reductions in the rate of coral larval settlement, affect the resilience

of coral reefs to future disturbances (Doropoulos et al. 2012). These species-specific responses to OA could also result in alterations in the outcome of competitive interactions between coralline algae for space on the benthos, favoring species that are more robust to predicted changes in seawater chemistry.

Competition for this space is an important determinant of community structure and diversity on reefs (Jackson & Buss 1975, Buss & Jackson 1979, Connell et al. 2004). The effects of OA on competitive interactions among CCA and other benthic organisms on coral reefs has been understudied. However, Diaz-Pulido et al. (2011) demonstrated that mortality in the coral *Acropora intermedia* increased significantly when competing with the macroalga *Lobophora papenfussii* at elevated pCO_2 levels when compared to ambient pCO_2. Therefore, it may be important to quantify the effects of OA on interactions between key species of benthic organisms on coral reefs. The effects of OA on competitive interactions among CCA on coral reefs have yet to be quantified. CCA compete for space through overgrowth interactions, and morphology is important in determining the outcome of these competitive interactions. In the presence of intense herbivory, species with thick thalli will generally win competitive interactions against thinner species (Steneck et al. 1991). Species of CCA that have a thallus thickness averaging more than 500 µm are considered thick species (Steneck 1986). In the absence of herbivory, reversals of the outcomes of competitive interactions can occur to favor thinner, faster growing species that are less defended against herbivory (Steneck et al. 1991). It is possible that OA could result in similar competitive reversals. A recent experiment performed on a group of temperate CCA off the coast of Washington State, USA found that there were reductions in the thallus thickness of thick species of CCA

under elevated pCO_2, while there was little effect on the thallus thickness of thinner species (McCoy & Ragazzola 2014). The species-specific responses in morphology within this guild of CCA are hypothesized to be the mechanism behind *in situ* changes in competitive interaction strengths and increased intransitivity in the competitive network reported for this group of CCA over the past 30 years (McCoy & Pfister 2014). Similar changes among CCA communities on coral reefs could have lasting effects on key ecological processes, including coral recruitment and reef accretion.

Understanding the extent to which tropical CCA differ in their response to OA could be crucial to predicting how their community structure may change because of climate change. The present study quantified the effects of projected increases in pCO_2 on two species of crustose coralline algae commonly found on open substrata (i.e., tops of coral bommies of massive *Porites*) on the back reefs of Moorea, French Polynesia; *Porolithon onkodes* and *Lithophyllum insipidum*. This study tested the null hypothesis that ocean acidification would not influence the net calcification of either of these species.

Methods

Experimental and field studies were performed in Mo'orea, French Polynesia at the University of California, Berkeley, Richard B. Gump South Pacific Research Station. The station is in Cook's Bay on the North Shore. The reefs surrounding the island are monitored extensively as part of the Moorea Coral Reef NSF Long-Term Ecological Research site (MCR LTER), offering an opportunity to track long-term changes in community structure as pH falls because of OA.

CCA abundance and composition

P. onkodes and *L. insipidum* were selected for this study following surveys of CCA composition and abundance on the back reefs surrounding Mo'orea in January 2015. For the composition and abundance surveys, three 10-meter transect lines were placed haphazardly throughout six different back reef sites around Mo'orea adjacent to the six long term research sites (NSF Moorea Coral Reef LTER) to quantify whole-island abundances. On each transect line, a point-intercept quantification of percent cover of CCA was performed using a 0.0625 m^2 quadrat with a grid of sixteen points (0.25 cm spacing) placed at five randomly-determined locations along the transect line. If the randomly determined quadrat location was more than 50% sand or coral, the quadrat was moved to the nearest suitable substrate for CCA (consolidated carbonate substratum). Species composition of the CCA assemblage was recorded at the species level, using morpho-anatomical methods of identification *in situ*, including tissue color, patterning of the thallus (e.g., tessellation present in *L. insipidum*; Adey et al. 1982), and the presence of trichocyte fields (present in *P. onkodes*; Adey et al. 1982).

Experimental design

The manipulative experiment was performed between January 4, 2016 and March 3, 2016 in four flow-through flumes at the Gump Research Station (Figure 1, Comeau et al. 2014). The four flumes were each randomly assigned a target pCO_2 value: 400, 700, 1000, or 1300 µatm. The ambient flume had a target value of 400 µatm pCO_2 to match current conditions. The elevated levels were a range of pCO_2 values that may be expected under the RCP6.0 and RCP8.5 scenarios put forth by the IPCC, which assume that there will be no effort to mitigate emissions of greenhouse gases (Pachauri et al. 2014). Carbon

chemistry varies both spatially and temporally on coral reefs, including diel variation, and this variation is controlled in part by the species composition and abundances of the reef community (Smith 1973, Anthony et al. 2011, Gray et al. 2012, Price et al. 2012). To mimic the diel variation specific to the reefs of Moorea, a nighttime reduction of 0.1 pH units was programmed for each flume (Hofmann et al. 2011). Temperature within the flumes was maintained as close as possible to mean sea surface temperatures from January to March in Mo'orea, ~ 28.35 °C, calculated from mean monthly nighttime sea surface temperature data available for 2003-2014 (Maritorena 2015 Moorea Coral Reef LTER). Temperature values presented here (Table 1) are averages over the entire experimental period including both diel and seasonal variation. Eight, approximately 2.25-cm^2 samples of both *P. onkodes* and *L. insipidum* were placed randomly in each of the four flumes immediately following curing of the marine epoxy used to attach samples to plastic bases (Coral Glue, EcoTech Marine, Allentown, PA).

Sample collection for experiments

Samples of both *P. onkodes* and *L. insipidum* were collected from the back reef around Cook's Bay Pass on the north shore of Moorea using a pneumatic drill (McMaster-Carr) fitted with a 2.4- cm diameter diamond grit hole-saw. Each sample was transported back to the lab in seawater from the collection site, and promptly placed within a flowing seawater table upon arrival at the research station. After collection, samples were cut in half with a diamond grit blade of a band saw. Half of each sample then was rinsed with fresh water and dried at 60 °C for 24 hours, to save them for possible further analysis. The other halves of each sample were scrubbed with a toothbrush to remove epiphytes and glued to randomly numbered plastic bases using

Coral Glue (EcoTech Marine, Allentown, PA). The glued the samples were left in the seawater tables for at least 24 hours to allow complete curing of the adhesive before being distributed randomly among treatments.

Maintenance of seawater chemistry

Carbonate chemistry was measured and controlled using methods similar to Comeau et al. (2015). Pure CO_2 was bubbled into each of the high pCO_2 flume treatments to achieve the desired pCO_2 values, while atmospheric air was bubbled into the ambient pCO_2 flume. pH within each flume was monitored continuously by pH probes within each flume. Bubbling of CO_2 was regulated by a solenoid valve that added pure CO_2 to flumes when the pH measured within the flumes was higher than their set points, which were updated daily on the controller (AquaController, Neptune Systems, USA). A chiller system was used to control temperature in the flumes. Temperature and mV readings were taken daily, around mid-day, using a digital thermometer (Traceable Calibration Control Company) and a handheld pH meter (Thermo Scientific, Orion 3 STAR) fitted with pH probe (Mettler Toledo, DGi115-SC), and used to calculate pH_T. These data were used to update the pH setpoints for the AquaControllers, adjusting for the difference between probe values and pH_T. The handheld pH probe was calibrated as necessary to ensure accuracy using TRIS/HCl buffers (SOP 6a, Dickson et al. 2007). Total alkalinity of each flume was measured once per week using open-cell, potentiometric titrations on an automatic titrator (Mettler Toledo, T50) following SOP 3b of Dickson et al. (2007). TA analyses were done in duplicate (~50 g seawater per replicate sample) for each flume at room temperature, and compared against certified reference material provided by A.G. Dickson. TA was calculated as described in SOP 3b

of Dickson et al. (2007). Salinity also was measured for these weekly water samples with a conductivity meter (Thermo Scientific Orionstar A212, Waltham, MA). Salinity, temperature, pH_T, and TA then were used to calculate carbonate chemistry using the seacarb package for R statistical software (Lavigne et al. 2011).

Photosynthetically active radiation (PAR) was measured in each flume every 30 minutes by an Odyssey Integrating PAR cosine sensor (Dataflow Systems Limited, Christchurch, New Zealand) placed at the center of each flume. Daily integrated PAR (mol quanta m^{-2} d^{-1}) was calculated for the duration of the experiment for each flume. For the first 10 days, samples were exposed to full light, but for the remainder of the experiment samples were shaded by window screening that blocked ~16% of incoming light.

Measured variables

Net calcification was measured for both *P. onkodes* and *L. insipidum* using the buoyant weight technique (Davies 1989). Each sample of *P. onkodes* and *L. insipidum* was buoyant weighed in seawater after the epoxy had cured, before being placed in their respective treatment flumes. They were then buoyant weighed once (due to time constraints) at day 59 of the experiment. The initial and final buoyant weights were converted to dry weights using the density of calcite (2.71) and the density of the seawater. To calculate the density of the seawater, a reference object was weighed in air, and in freshwater and seawater of known temperatures. The density of the reference object was calculated from the weight of the object in freshwater and in air, and the density of seawater was then calculated from the weight of the reference object in seawater and its density. The difference between the final and initial dry weights was

used to calculate net calcification. Net calcification was normalized to planar surface area, using the average surface area of living tissue at the beginning and end of the 59 day experiment. Planar surface area was measured from before (15.9 MP resolution, Nikon COOLPIX AW130) and after (2.5 MP resolution, Canon Powershot S90) planar photographs of each individual using ImageJ (NIH). This method assumes that any tissue mortality occurred in a linear fashion throughout the experiment. Samples for which surface area measurements could not be made confidently (i.e., blurry photograph or tissue was obscured) were not included in the analysis.

There was tissue mortality at the end of the experiment, so an analysis of the proportion of dead tissue at the end of the experiment was performed. The proportion of dead tissue was estimated by subtracting the surface area of living tissue from the total surface area of the sample, and dividing by the total sample area. As in the analysis of net calcification, samples for which surface area measurements could not be made confidently (i.e., blurry photograph or tissue was obscured) were not included in the analysis.

Statistical analyses

Differences in species abundance were analyzed for the three most common species that occurred on the transects. Other species were uncommon (much less than 1 %), and were not included in the statistical analysis. Species abundance was analyzed with a Model I ANOVA after arcsine square root transformation to meet the assumptions of homogeneity of variance and normality using transect as the replicate after averaging the quadrats for each transect. Species was the main factor in the analysis. This ANOVA was followed by a Tukey's Honestly Significant Differences (Tukey's HSD) post-hoc

analysis. These analyses were performed in the statistical software program SYSTAT (SYSTAT Version 13).

Seawater chemistry parameters and daily integrated PAR for the flume experiment were analyzed with a one-way ANOVA using flume as the independent variable with a null hypothesis of no difference between flumes. Post-hoc comparisons of the flumes were performed using Tukey's HSD to determine the relationship among the flumes. If the assumptions of homogeneity of variance and/or normality were not met, variables were transformed to meet them. If transformations to meet these assumptions was not possible, a non-parametric Kruskall-Wallis Test was performed with a Dwass-Steel-Chritchlow-Fligner Test for pairwise comparisons. These analyses were performed using the statistical software SYSTAT (SYSTAT Version 13).

Net calcification data for the flume experiment were analyzed using a generalized linear model (GLM) fit to a Gaussian (normal) distribution, treating individuals within each flume as independent replicates. The data for proportion of dead tissue at the end of the experiment were transformed into binary data and were analyzed using a GLM fit to a binomial distribution, treating individuals within each flume as independent replicates. Proportion dead tissue values that were larger than the median value for the full model were transformed into 1s, and values smaller than the median value were transformed into 0s. For graphical representation, the true values for the proportion of dead tissue for each sample were plotted against pCO_2 (µatm). Reduced model GLMs of net calcification and proportion dead tissue also were performed for each species to determine the effects of pCO_2 on each species individually.

The large volume (~500 L) and the high turnover of seawater within the flumes were used to justify the assumption of independence for these models. The samples also were not in contact with one another during the experiment, and there is no published evidence to indicate any sort of chemical competition among CCA. Therefore, it is unlikely that individuals within the flumes would have been affecting one another, other than directly through competitive interactions, and were assumed to be independent. To statistically test for autocorrelation within the residuals of the models, Durbin-Watson tests of independence were performed for the full model GLMs testing the effect of elevated pCO_2 and species on net calcification and the proportion of dead tissue. The analyses for net calcification and the proportion of dead tissue were performed using the statistical software R (stats package, R Core Team 2015).

Results

Field surveys

Abundance and composition surveys of CCA growing on back reefs surrounding Moorea indicated that *P. onkodes*, *Lithophyllum flavescens*, and *L. insipidum* were the most abundant. There were significant differences in the abundances between species ($p = 0.001$; Table 1.1). *P. onkodes* (10.6 % of total cover) was more abundant than either *L. flavescens* (2.4 % of total cover) or *L. insipidum* (0.56 % of total cover) as indicated by Tukey's HSD ($p < 0.001$ for both, Figure 1.2). *L. flavescens* also was significantly more abundant that *L. insipidum* (Tukey's HSD, $p = 0.001$).

Seawater chemistry

pCO_2 was maintained near the target values throughout the experiment (Table 1.2). The increase in pCO_2 in elevated treatments resulted in expected changes in other aspects of seawater chemistry including a reduction in pH, reduced carbonate ion concentrations, increased bicarbonate ion concentration, increased dissolved inorganic carbon, and decreases in both aragonite and calcite saturation states. Each flume was significantly different from every other flume with respect to these parameters (Tukey's HSD $p < 0.05$). In contrast, there was no difference in alkalinity, salinity, or temperature between flumes ($p > 0.05$).

Samples were exposed to full light for the first 10 days of the experiment, but were covered with window screening after that period because of concerns that high irradiance may be leading to tissue mortality. There was a significant difference in daily integrated PAR between flumes 1 and 2, and 2 and 4 as indicated by Tukey's HSD ($p = 0.01$ and $p = 0.02$ respectively). The daily integrated PAR was higher in flume 2 (18.46 ± 1.01 mol quanta m^{-2} day^{-1}, mean ± SE) than in both flumes 1 and 4 (14.78 ± 0.75 and 14.82 ± 0.76 mol quanta m^{-2} day^{-1} respectively, mean ± SE). However, the mean instantaneous PAR without the window screening was greater than 342 µmol m^{-2} s^{-1} for all treatments, well above the *in situ* and in-lab irradiance at the onset of light saturation (I_k) reported for *P. onkodes* (205 and 124 µmol m^{-2} s^{-1} respectively; Chisholm 2003). Therefore, it is unlikely that the higher irradiance in flume 2 would affect net calcification rates. A summary table of selected seawater parameters as well as daily integrated PAR is presented in Table 1.2.

Net calcification

There was no significant autocorrelation between residuals of the full GLM model testing the effects of pCO_2 and species on net calcification ($p > 0.05$), and the assumptions of homogeneity of variance, normality, and linearity were met without transformation. There was a significant difference in net calcification between *P. onkodes* and *L. insipidum* in the elevated pCO_2 treatments (Table 1.3; $p = 0.003$). There was a significant reduction in net calcification in both *P. onkodes* and *L. insipidum* under elevated pCO_2, indicated by reduced model analyses ($p < 0.001$ and $p < 0.01$ respectively). However, the effect of elevated pCO_2 was more negative for *P. onkodes* (slope = -0.0008) than for *L. insipidum* (slope = -0.0003). Mean net calcification declined 85 % in P. onkodes, from 0.82 ± 0.06 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, n = 8) at 340.95 µatm pCO_2 to 0.12 ± 0.10 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, n = 8) at 1202.26 µatm pCO_2 (Figure 1.3). Mean net calcification declined 42 % in *L. insipidum*, from 0.71 ± 0.08 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, n = 7) at 340.95 µatm pCO_2 to 0.41 ± 0.05 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, n = 8) at 1202.26 µatm pCO_2 (Figure 1.3).

Tissue necrosis

There was no significant autocorrelation between residuals of the full GLM model testing the effects of pCO_2 and species on the proportion of dead tissue ($p > 0.05$). There was a significant difference in the proportion of dead tissue in *P. onkodes* and *L. insipidum* resulting from elevated pCO_2 (Table 1.4; $p < 0.001$). There was an effect of elevated pCO_2 on the proportion of dead tissue in *P. onkodes* (reduced model GLM, $p < 0.001$), in which the mean proportion of dead tissue increased 783 % from 0.06 ± 0.04 (mean ± SE, n = 7) at 340.95 µatm pCO_2 to 0.53 ± 0.12 (mean ± SE, n = 8) at 1202.26

μatm pCO_2 (Figure 1.4). There was no significant difference in the proportion of dead tissue in *L. insipidum* across pCO_2 (Figure 1.4; reduced model GLM, $p = 0.07$).

Discussion

The climate is changing rapidly because of human fossil fuel consumption, which results in cascading effects on ecological networks in many ecosystems, both terrestrial and aquatic (Walther et al. 2002, Harley et al. 2006, Walther 2010). Climate research on coral reefs has begun to focus on determining what species of coral reef organisms might be winners or losers in a changing ocean environment (e.g., Loya et al. 2001). Globally, ocean warming has led to mass coral bleaching events, which have aided in shifts towards less structurally complex communities of corals on some reefs (i.e., reductions in branching morphologies; Loya et al. 2001, Depczynski et al. 2013). Changes in community structure on coral reefs because of human-induced OA have not yet been documented. OA may negatively affect the physiological responses of heavily calcified taxa, like corals and calcified algae, more so than other taxa, like fleshy macroalgae (Kroeker et al. 2010, Kroeker et al. 2013). The variability in the responses of different taxa could lead to changes in their abundance in marine ecosystems, and species-specific responses within these taxa could also influence species interactions and therefore abundances (Kroeker et al. 2013). *In situ* studies of CO_2 vents surrounding the volcanic islands of Papua New Guinea, have indicated that OA could affect coral reef community structure (Fabricius et al. 2011). Fabricius et al. (2011) found that there was an increase in the abundance of slow growing massive *Porites* corals at lower pH sites near CO_2 vents in Papua New Guinea, and a reduction in the abundance of structurally complex corals. In addition, along the gradient of pH at these volcanic seeps, there were increases

in the abundance of non-calcifying algae and decreases in the abundance of calcified algae, like CCA (Fabricius et al. 2011, Porzio et al. 2011).

CCA are important members of coral reef communities, stabilizing reef substrate and in some cases facilitating coral settlement and recruitment (Nelson 2009). Recent manipulative studies have demonstrated that ocean acidification could have a substantially negative effect on CCA, reducing their recruitment, abundance, and net calcification rates (Anthony et al. 2008, Kuffner et al. 2008, Comeau et al. 2013). In addition, Comeau et al. (2014) found that there were significant differences in the response of tropical calcified algae dependent on the type of calcification (cell-wall v. intercellular), morphology (branched v. encrusting), and rate of calcification (fast v. slow). The present study provides further evidence of species-specificity in the response of CCA to OA. Net calcification was significantly depressed by elevated pCO_2 in both *P. onkodes* and *L. insipidum*, but the effect of elevated pCO_2 on net calcification was greater in *P. onkodes*. There was also a significant increase in the proportion of dead tissue in samples of *P. onkodes* because of elevated pCO_2. Conversely, there was no effect of elevated pCO_2 on the proportion of dead tissue in *L. insipidum*. Comeau et al. (2014) found that fast calcifiers of both corals and coralline algae were more negatively affected by elevated pCO_2 than species that calcify more slowly. The results of the present study are consistent with this, as *P. onkodes* had a higher rate of net calcification than *L. insipidum* at ambient pCO_2. In a study of temperate species of CCA, McCoy & Ragazzola (2014) found that there were differences in the response of thick and thin species to elevated pCO_2. CCA are classified as thick when their thalli greater than 500 µm thick (Steneck 1986). They found a reduction in the thickness of the thalli of thick

species, and reductions in interfilament cell-wall thickness in thin species (McCoy & Ragazzola 2014). *P. onkodes* can be many centimeters thick in some cases, and is generally much thicker than *L. insipidum* (Adey et al. 1982, personal observation). Therefore, the results of the present study could also be indicative of a difference in response between thick and thin CCA to OA, though there was not an explicit test of this hypothesis.

The differential responses of coralline algae to OA, demonstrated in this study and others (Comeau et al. 2014), could lead to similar shifts in species abundances in CCA communities as those found in coral communities following bleaching events and along gradients of pH near natural CO_2 vents (e.g., Loya et al. 2001, Fabricius et al. 2011, Depczynski et al. 2013). There is evidence of a change in the competitive interaction strengths and increased intransitivity within a community of temperate CCA off the coast of Washington, USA (McCoy & Pfister 2014). These changes have occurred in the presence of increased OA over the past 30 years (McCoy & Pfister 2014). Similar changes have not yet been quantified for CCA on coral reefs, but there is evidence that OA could affect competition between organisms on coral reefs. For example, Diaz-Pulido et al. (2011) found that elevated pCO_2 led to increased mortality in *Acropora intermedia* when competing with the macroalga *Lobophora papenfussii*. Connell et al. (2013) also found that by acting as a resource for some taxa, such as mat-forming algae, OA may result in shifts in species dominance. They found an increase in the abundance of mat-forming algae along a gradient of increased CO_2 at both temperate and tropical CO_2 vents, which appeared to correspond with decreased cover of calcified taxa (Connell et al. 2013).

On the back reefs of Moorea, and on other reefs throughout the Indo-Pacific, *P. onkodes* is an abundant species of CCA (Littler & Doty 1975, Adey et al. 1982). If OA reduces the competitive ability of *P. onkodes* through reductions in net calcification, it is possible that *L. insipidum* and other CCA could become more competitive with *P. onkodes* for space on the benthos. This could lead to shifts in dominance and changes in community structure. Changes in community structure among CCA could have profound effects on ecological processes like coral settlement and recruitment, particularly if species that are more important facilitators of coral recruitment are more adversely affected by OA (as in Doropoulos et al. 2012). Given the importance of CCA to coral reefs, more effort should be made to quantify the effects that OA may have on competitive interactions among CCA in the coming decades, particularly as this and other studies have demonstrated varied responses among CCA to OA.

CHAPTER 2

Differential effects of ocean acidification and simulated graze wounding on two species of tropical crustose coralline algae

Introduction

Grime (2006) defined disturbance as any event that causes either partial or total loss of biomass of an organism. Natural communities are subjected to a variety of disturbances, both physical and biological, leading to spatial and temporal heterogeneity in community structure among habitats (Sousa 1984). It has been hypothesized that intermediate levels of disturbance maintain species diversity within communities and ecosystems, like coral reefs (Connell 1978). On coral reefs, humans have altered the natural disturbance regimes and introduced new disturbances (Nyström et al. 2000). Press disturbances (e.g., global climate change) could reduce rates of reef recovery following severe disturbances (Nyström et al. 2000). Connell (1997) found that coral reefs exposed to press disturbances were less likely to recover than reefs exposed to pulse disturbances. Anthropogenic press disturbances also may lead to reduced biodiversity by favoring a few generalist species (winners), and causing a decline in other more specialized species (losers; McKinney & Lockwood 1999).

One of the more serious anthropogenic disturbances affecting coral reefs is ocean warming. Ocean warming has accelerated in the past 28 years on coral reefs around the world as a result of climate change, increasing bleaching stresses experienced by corals (Heron et al. 2016). These increases in thermal stresses have increased the scale and frequency of coral bleaching events (Hoegh-Guldberg et al. 2007, Hughes et al. 2017).

An additional effect of increased atmospheric pCO_2 is ocean acidification (OA). The ocean is the largest sink for anthropogenically produced CO_2 (Sabine et al. 2004). OA is caused by the uptake of this atmospheric pCO_2, and results in changes in seawater chemistry (Caldeira & Wickett 2003). The pH of seawater has already decreased by 0.1 units since the beginning of the industrial revolution, and it is expected to drop by an additional ~0.3 units by the end of the current century under the more pessimistic scenarios posed by the Intergovernmental Panel on Climate Change (RCP 8.5; Pachauri et al., 2014). In addition to reductions in seawater pH, OA is also marked by reductions in carbonate ion concentrations and calcium carbonate saturation states (Hoegh-Guldberg et al. 2007, Doney et al. 2009). Changes in seawater chemistry due to OA could negatively affect coral reefs, particularly calcifying organisms such as corals and calcified algae.

Crustose coralline algae (CCA) are important habitat builders found throughout the photic zone of all major coastal ecosystems (Nelson 2009). On coral reefs, CCA consolidate and stabilize reef substrata, and are important to reef accretion (Nelson 2009). There also is evidence that the larvae of some species of coral preferentially settle and metamorphose on different species of CCA (Heyward & Negri 1999, Harrington et al. 2004, Ritson-Williams et al. 2009, Arnold et al. 2010, Price 2010). However, CCA produce calcium carbonate skeletons composed of high-Mg calcite, a more soluble form of calcium carbonate than the aragonite polymorph secreted by corals (Morse et al. 2006). For this reason, CCA are expected to be some of the first organisms to respond to the decreased seawater pH and calcium carbonate saturation states expected under OA (Morse et al. 2006).

Manipulative laboratory experiments have demonstrated reductions in calcification in temperate and tropical CCA at seawater pCO_2 levels expected by the end of the century under the pessimistic RCP 8.5 put forth by the IPCC (~ 1000 µatm; Comeau et al. 2013, 2014, Noisette et al. 2013, Pachauri et al. 2014). A few studies have also demonstrated interactive effects of OA and ocean warming (Anthony et al. 2008, Diaz-Pulido et al. 2012). However, few studies have quantified the interactive effect of OA and natural disturbances, such as herbivory, on CCA. CCA have coevolved with herbivores such as sea urchins and parrotfishes, and rely to some extent on this grazing to prevent overgrowth by epiphytes (Steneck 1982, 1983). Recent experimental evidence suggests that OA may be altering the morphology of some temperate species of CCA, particularly by reducing the thallus and cell-wall thicknesses of certain species (McCoy & Ragazzola 2014). These changes could increase the susceptibility of CCA to grazing and wounding. Johnson & Carpenter (2012) found that there was an increase in the susceptibility of the CCA *Porolithon onkodes* to grazing by the urchin *Echinothrix diadema* when grown in conditions mimicking future OA. Similarly, OA could reduce the ability for these organisms to recover from wounding to their thalli by affecting rates of net calcification. Similarly, Rotjan et al. (2006) found that grazing by parrotfish slowed the recovery of *Montastraea* spp. corals following a coral bleaching event, with potential implications for fitness of these corals.

The present experiment quantified the effects of OA and wounding on the rates of net calcification and wound regeneration in two species of CCA common to the back reefs of Mo'orea, French Polynesia, *Porolithon onkodes* and *Lithophyllum insipidum*. The study tested the following hypotheses in a manipulative experiment: (1) There will

be interactive effects of wounding and elevated pCO_2 on these CCA, and (2) elevated pCO_2 will cause a reduction in wound regeneration.

Methods

Sample collection and preparation

The experimental manipulations were performed at the Richard B. Gump South Pacific Research Station in Mo'orea, French Polynesia from May 24 to July 14, 2016. Two species of CCA were selected for this experiment; *P. onkodes* and *L. insipidum*. Both species are common members of the back-reef communities of Mo'orea (Manning and Carpenter, in prep.), as well as on other reefs throughout the tropical Pacific Ocean (Adey et al. 1982, Keats 1997). Cores of each species were collected using an air-powered drill (McMaster-Carr) with a 3-cm diameter, diamond-tipped hole saw (McMaster-Carr). The cores were collected at a depth of ~ 2 meters on the back reef east of Cook's Pass on the north shore, and transported to the Gump Research Station in ambient seawater. Samples were promptly placed in flowing seawater tables supplied with unfiltered seawater from Cook's Bay.

The cores were mounted to plastic bases using a marine epoxy (Coral Glue, EcoTech Marine, Allentown, PA, USA), and allowed to cure for at least 24 hours in the flowing seawater tables. Prior to placement within experimental treatments, half of the samples for each species were cut to a depth of ~300 µm across their diameters. This was achieved by using a hacksaw blade extending ~300 µm from a handheld grip (as measured using a digital dissecting microscope, Dino-lite Edge AM4815ZTL, Dunwell Tech, Inc., Torrance, CA). This depth was chosen to induce the formation of a new

hypothallus within the wound, which occurs within deeper wounds to CCA thalli, and would be readily identifiable in cross-section (Steneck 1983). Eight wounded and eight non-wounded samples of each species were assigned randomly to each of the four flumes used in the experiment after a two-day acclimation period in the ambient pCO_2 flume.

Experimental design

This experiment was performed in four flow-through seawater tanks, flumes, each set at one of four target pCO_2 levels. The flumes were supplied with filtered seawater pumped from ~ 12-m depth in Cook's Bay, and passed through a sand filter (nominal pore size ~ 100 µm). The seawater within each flume was replaced at a rate of ~ 5 L/min (as in Comeau et al. 2015). There was a flume maintained at 400 µatm pCO_2 to mimic ambient seawater, and three elevated pCO_2 treatments. The three elevated pCO_2 treatments with target pCO2 values of 700, 1000, and 1300 µatm, were representative of predicted increases in seawater pCO_2 by the end of the current century under the RCP6.0 and RCP8.5 scenarios put forth by the IPCC (Pachauri et al. 2014). pH was controlled for a 0.1-unit reduction at night to simulate natural diel variation of pH on the reefs of Mo'orea (Hofmann et al. 2011). Temperature within the flumes was maintained at as close as possible to mean sea surface temperatures from May to July in Mo'orea, ~ 27.15 °C, calculated from mean monthly nighttime sea surface temperature data available for 2003-2014 (Maritorena 2015 Moorea Coral Reef LTER). Temperatures were taken every day at mid-day using a digital thermometer (Accuracy = ± 0.05 ° C, Digital Thermometer, Traceable Calibration Control Company, Webster, TX, USA). The flumes were exposed to natural sunlight, but a piece of window screening was used to further reduce light by ~29% to prevent light oversaturation. Mean instantaneous PAR within the

flumes was higher than the *in situ* and in-lab irradiance at the onset of light saturation (I_k) reported for *P. onkodes* (205 and 124 µmol m^{-2} s^{-1} respectively; Chisholm 2003). The experiment ran for 51 days for *P. onkodes*, but *L. insipidum* was removed after 29 days due to increasing tissue mortality.

Maintenance of seawater chemistry and other physical parameters

Target pCO_2 levels of the elevated treatments were achieved by bubbling pure CO_2 into each elevated flume, and non-CO2-enriched air into the ambient flume. Carbonate chemistry was measured and controlled using methods like those used by (Comeau et al. 2015). pH within each flume was monitored continuously by pH probes within each flume and provided feedback to regulated bubbling of CO2 by a solenoid valve that added pure CO_2 to flumes when the pH measured within the flumes was higher than their set points, which were updated daily on the controller (AquaController, Neptune Systems, USA). Temperature and conductivity readings were taken daily, around mid-day, using a digital thermometer and a handheld pH meter (Thermo Scientific, Orion 3 STAR, Beverly, MA, USA) fitted with a pH probe (Mettler Toledo, DGi115-SC), and used to calculate pH_T. These data were used to update the pH setpoints for the AquaControllers, adjusting for the difference between probe values and pH_T. The handheld pH probe was calibrated using TRIS/HCl buffers (SOP 6a, Dickson et al. 2007) whenever there was large variation in day to day conductivity values (no set interval). Total alkalinity of the water in each flume was measured once per week using open-cell, potentiometric titrations on an automatic titrator (Mettler Toledo, T50) following SOP 3b of Dickson et al. (2007). TA analyses were conducted in duplicate (~50 g seawater per replicate sample) for each flume at room temperature, and compared against certified

reference material provided by A.G. Dickson (Scripps Inst. of Oceanography, San Diego, CA). TA was determined as described in SOP 3b of Dickson et al. (2007). Salinity also was measured for these weekly water samples with a conductivity meter (Thermo Scientific Orionstar A212, Waltham, MA). Salinity, temperature, pH_T, and TA then were used to calculate carbonate chemistry using the seacarb package for R statistical software (Lavigne et al. 2011).

Daily integrated photosynthetically active radiation (PAR; mol quanta m^{-2} d^{-1}) was quantified for each flume using measurements of PAR taken every 30 minutes by an Odyssey Integrating PAR cosine sensor (Dataflow Systems Limited, Christchurch, New Zealand) placed at the center of each flume. However, after June 4th there was a sensor failure which resulted in a sudden reduction in the daily integrated PAR within flumes 3 and 4. The flumes had been running continuously since November 2015, and measurements of PAR had been taken using these sensors throughout that period. During the approximately 6 months prior to June 4th, there was little difference in the mean PAR for each flume (flume 1: 326.99, flume 2: 374.15, flume 3: 342.79, flume 4: 348.56; µmol quanta m^{-2} s^{-1}). Therefore, it is likely that flumes 3 and 4 did not differ from flumes 1 and 2 by the amount measured between June 4 and July 23. Therefore, a correction was made for the PAR data measured in flumes 3 and 4 following June 4th. The correction factor was determined by taking the difference between the pooled mean PAR (µmol quanta m^{-2} s^{-1}) for flumes 1 and 2, and the pooled mean PAR (µmol quanta m^{-2} s^{-1}) for flumes 3 and 4 (using data at 30-minute intervals). The correction factor was then added to the PAR (µmol quanta m^{-2} s^{-1}) values recorded for flumes 3 and 4 at 30 minute

intervals. These corrected values were then used to quantify the daily integrated PAR (mol quanta m^{-2} d^{-1}) for the duration of the experimental period, May 24th to July 14th.

Calcification and tissue mortality

Net calcification was quantified for both *P. onkodes* and *L. insipidum* using the buoyant weight technique (Davies 1989). All samples for both species were buoyant weighed in duplicate prior to being placed within the 400 µatm treatment for acclimation, and again at day 29 of the experiment. The buoyant weights were then converted to dry weights using the density of calcite (2.71 g cm^{-3}) and the density of the seawater. The density of seawater was quantified by weighing a reference object in air, freshwater, and seawater. The weight of the object in air and in freshwater was used to calculate the density of the reference object. The density of the reference object and its weight in the seawater was then used to calculate the density of the seawater. The difference between dry weights (final-initial) then was normalized to the planar surface area of living tissue for each individual sample at day 0, and divided by the total number of days in treatment (29, including a 2-day acclimation). Measurements of the planar surface area of living tissue were made by analyzing photographs taken at the beginning and end of the experiment (~16 MP resolution, Nikon COOLPIX AW130) in the software ImageJ (NIH). The proportion of living tissue remaining was calculated for each individual sample by dividing the total planar area of living tissue at day 29 by the total planar area of living tissue at day 0.

Wound regeneration

After the experiment (29 days for *L. insipidum* and 51 days for *P. onkodes*), all CCA samples were rinsed with freshwater, dried for 24 hours at ~60 °C in a drying oven, and transported back to California State University, Northridge for microscopic analysis of the wounds. Analysis of the wounds was conducted for *P. onkodes*. Analysis of the wounds was not conducted for *L. insipidum* because there were not enough *L. insipidum* samples with living tissue within the wounds at the end of the experiment to allow for this analysis.

To quantify the rate of vertical regeneration within the wounds of the algae, scanning electron microscopy (SEM) was used to image cross-sections of living tissue present within the wounds of the thalli of *P. onkodes* (Figure 2.1). Sample preparation for SEM work included cutting, mounting, and applying a conductive coating of gold to the thalli of *P. onkodes*. Cross sections of the thalli of *P. onkodes* were prepared using wire cutters to cut perpendicular to the wound. These cross sections were then mounted facing up on SEM stubs with an adhesive tab, sprayed with moisture-free air to remove any loose debris, and coated with 5 nm of gold using a sputter coater (Cressington 108auto, Watford, England) and thickness controller (Cressington MTM-20, Watford, England).

Images of the cross sections were obtained using the FEI Quanta 600 SEM at California State University, Northridge. Secondary electron microscopy images of the wounds from each sample of *P. onkodes* with live tissue present (n = 13) were collected under high vacuum. Wounds were imaged at both 150x and 349x magnification. The SEM working conditions during imaging included an accelerating voltage of 20kV, a

beam spot size of 4, a working distance of ~ 10 mm, and filament and emission currents ranging from 1.8-2.0 A and 96-102 μA, respectively.

The wounds were identifiable by their noticeable hypothallial cell layer (Figure 2.1), which forms within deeper wounds in CCA (Steneck 1983). The imaging software ImageJ (NIH) was used to make measurements of the thickness of the regenerated tissue within the images collected with the SEM. The rate of wound regeneration was quantified by measuring the thickness of the perithallus, intercalary meristem, and epithallus (PME layer; Figure 2.1), the region of vertical growth, at three locations within the wound. The thickness of the PME layer was obtained by averaging the thicknesses at the deepest point within the wound, and at the midpoints (approximate) between the outer edges of the wound and the deepest point. The thickness of the PME layer then was divided by the number of days in treatment (51 including the 2-day acclimation) to create a proxy for the rate of vertical regeneration. The proportion of vertical regeneration (thickness of the PME layer) to lateral regeneration (thickness of the hypothallial cell layer) also was measured.

Statistical Analysis

Seawater chemistry parameters and daily integrated PAR for the flume experiment were analyzed with a one-way ANOVA using flume as the independent variable with a null hypothesis of no difference between flumes. Post-hoc comparisons were performed using Tukey's honestly significant differences to determine the relationship among the flumes. If the assumptions of homogeneity of variance and/or normality were not met, variables were transformed to meet them. If transformations to meet these assumptions were not possible, a non-parametric Kruskall-Wallis Test was

performed with a Dwass-Steel-Chritchlow-Fligner Test for pairwise comparisons. These analyses were performed using the statistical software SYSTAT (SYSTAT Version 13).

Net calcification, proportion of live tissue remaining, the rate of vertical regeneration within the wounds (for *P. onkodes*), and the ratio of vertical to lateral regeneration within the wounds (for *P. onkodes*) were analyzed using generalized linear models (GLM) fit to Gaussian (normal) distributions. If the assumptions of normality and homogeneity of variance were not met, transformation was used to meet these assumptions. For net calcification and the proportion of living tissue remaining, the full model included three independent variables, pCO_2 (mean pCO_2 of each flume), wounding treatment (wounded or non-wounded), and species (*P. onkodes* and *L. insipidum*), and their interactions. The three-way interaction was dropped from the model if it was found that it was not significant ($p > 0.25$) to get more powerful estimates for the two-way interactions. After testing the full-model for net calcification, individual GLMs were tested for each species using wounding treatment and pCO_2 as the independent variables. For the rate of vertical regeneration and the ratio of vertical to lateral regeneration within the wounds of *P. onkodes*, pCO_2 was the only independent variable used, as there was no basis for comparison between the two species and only wounded samples were analyzed. All generalized linear model analyses were performed using the statistical software R (stats package, R Core Team 2015).

Independence was assumed for the individual samples within each flume, as the seawater volume (~500 L) and turnover rate within the flumes were relatively high. As there is no evidence of chemical interactions between CCA in the literature, and the samples were not in contact during the experiment, it is likely that the results were not

influenced by interaction between samples. However, to statistically test the assumption of independence of the residuals, a Durbin-Watson test of independence was performed for each model.

Results

Seawater chemistry

pCO_2 was different from target values in each of the flumes throughout the experiment, but nonetheless created a gradient in pCO_2 and pH within the flumes (Table 2.1). The only significant differences in carbonate chemistry found between flumes were in parameters expected to change because of increased pCO_2 (i.e., pH, aragonite and calcite saturation states, carbonate and bicarbonate ion concentrations, etc.), and each flume was significantly different from every other with respect to these parameters (Tukey's HSD, $p < 0.05$). There were no statistically significant differences in total alkalinity, temperature, or salinity between flumes ($p > 0.05$). However, there was a significant difference in the daily integrated PAR between flumes 1 (16.04 ± 0.63 mol quanta m^{-2} d^{-1}, mean ± SE) and 2 (13.98 ± 0.53 mol quanta m^{-2} d^{-1}, mean ± SE; Tukey's HSD, $p = 0.02$). Flume 2 was not significantly different from either flume 3 (14.84 ± 0.28 mol quanta m^{-2} d^{-1}, mean ± SE) or 4 (14.92 ± 0.26 mol quanta m^{-2} d^{-1}, mean ± SE). Despite the difference in daily integrated PAR, the mean instantaneous PAR was > 320 µmol m^{-2} s^{-1} for all treatments were well above the *in situ* and in-lab irradiance at the onset of light saturation (I_k) reported for *P. onkodes* (205 and 124 µmol m^{-2} s^{-1} respectively; Chisholm 2003). It is therefore, unlikely that the higher daily integrated PAR in flume 1 would have affected net calcification through increased photosynthetic ability. A summary of key environmental parameters is given in Table 2.1.

Net calcification

There was no residual autocorrelation for the full model GLM testing of the effects of pCO_2 and wounding on net calcification in *P. onkodes* and *L. insipidum* (Durbin Watson, $p > 0.05$). The summary for the full-model GLM is presented in Table 2.2. The data were square-root transformed to better meet the assumptions of homogeneity of variance and normality, and the three-way interaction was dropped from the model as the p-value was greater than 0.25.

There was a significant difference in the effect of elevated pCO_2 on the net calcification of *P. onkodes* and *L. insipidum* (Figure 2.2; $p = 0.05$). Net calcification was reduced by 41 % in *P. onkodes*, from 0.66 ± 0.05 (n = 15) to 0.38 ± 0.04 (n = 16) mg $CaCO_3$ cm^{-2} d^{-1} (means ± SE at 429.31 and 1179.39 µatm respectively; reduced model, $p < 0.001$), but there was no effect of elevated pCO_2 on net calcification in *L. insipidum*.

There also was a significant difference in the effect of wounding on net calcification in *P. onkodes* and *L. insipidum* (Figure 2.2; $p < 0.001$). There was significant reduction in net calcification of *L. insipidum* from 0.99 ± 0.08 (n = 28) to 0.41 ± 0.05 (n = 27) mg $CaCO_3$ cm^{-2} d^{-1} in wounded samples (mean ± SE; reduced model, $p = 0.018$). There was no effect of wounding on *P. onkodes*.

Tissue mortality

There was no residual autocorrelation for the full model GLMs testing of the effects of pCO_2 and wounding on net calcification in *P. onkodes* and *L. insipidum* (Durbin Watson, $p>0.05$). The summary for the full-model GLM is presented in Table

2.3. The three-way interaction was dropped from the model as the p-value was greater than 0.25.

L. insipidum had significantly less living tissue remaining after 29 days than *P. onkodes*, regardless of pCO_2 or wounding treatment (Figure 2.3; full model $p < 0.001$). *P. onkodes* averaged 87.8 ± 0.03 % (mean ± SE, n = 62) living tissue at the end of the experiment, while *L. insipidum* averaged only 30.7 ± 0.03 % (mean ± SE, n = 55) living tissue. There was a significant reduction in the proportion of living tissue remaining in both species, regardless of wounding, at elevated pCO_2 (Figure 2.3; full model $p < 0.001$). Elevated pCO_2 reduced the proportion of living tissue remaining in *P. onkodes* from 100 ± 0.02 % (429.31 µatm, mean ± SE, n = 15) to 76.1 ± 0.05 % (1179.39 µatm, mean ± SE, n = 16), and from 45.8 ± 0.08 % (429.31 µatm, mean ± SE, n = 15) to 23.2 ± 0.05 % (1179.39 µatm, mean ± SE, n = 11) in *L. insipidum* (Figure 2.3). There were no significant effects of wounding on the proportion of living tissue remaining in either species ($p > 0.05$).

Wound regeneration

There was no significant residual autocorrelation in the GLM testing the effect of pCO_2 on the rate of vertical regeneration in *P. onkodes* (Durbin Watson, $p > 0.05$). Statistical results of the GLM are provided in Table 2.4. The average depth of the simulated wounds was 182 ± 15 µm. The rate of vertical regeneration, estimated by the thickness of the PME layer within the wounds, was reduced from 2.10 ± 0.18 (n = 3) to 0.89 ± 0.36 (n = 3) µm d^{-1} (mean ± SE at 429.31 and 1179.39 µatm respectively, Figure 2.4; $p = 0.010$). There was no significant residual autocorrelation in the GLM testing the effect of pCO_2 on the ratio of vertical to lateral growth within the wounds of *P. onkodes*

(Durbin Watson, $p > 0.05$). Results of the GLM are provided in Table 2.5. The ratio of vertical to lateral growth within the wounds of *P. onkodes* was not significantly affected by elevated pCO_2 ($p = 0.106$), although there is a trend toward more hypothallial tissue than PME in samples at elevated pCO_2 (Figure 2.5).

Discussion

Disturbance plays an important role in determining the structure of natural communities, and plays a role in maintaining diversity (Connell 1978, Sousa 1984). Humans increasingly have been altering the natural disturbance regimes on coral reefs, and coral reefs are now exposed to chronic stresses that are lowering their resilience (Connell 1997, Nyström et al. 2000, Anthony, Maynard, et al. 2011). OA is a press disturbance resulting from the increases in anthropogenically produced CO_2 in the atmosphere (Caldeira & Wickett 2003). OA, and the associated changes in seawater chemistry are expected to negatively affect many calcifying marine organisms, including corals and calcified algae (Anthony et al. 2008, Ries et al. 2009, Büdenbender et al. 2011, Diaz-Pulido et al. 2012, Doropoulos et al. 2012, Comeau et al. 2013, 2014, Noisette et al. 2013). Coral reefs, biogenic structures created by calcifying organisms, have been a major focus of research on the effects of OA. Experimental studies have found reductions in calcification in many coral species exposed to increased pCO_2 levels (Chan & Connolly 2013, Comeau et al. 2013, 2014). However, due to their high-Mg calcite skeletons, which are more soluble than the aragonite polymorph of calcium carbonate used by corals, CCA may respond more rapidly than corals to elevated seawater pCO_2 levels (Morse et al. 2006).

CCA consolidate the reef structure, and can facilitate the settlement and recruitment of coral larvae (Harrington et al. 2004, Nelson 2009, Arnold et al. 2010, Doropoulos et al. 2012). Therefore, understanding how OA affects them is crucial if we are to understand how OA will affect coral reef community structure and function. Natural disturbances, such as herbivory, are important drivers of community structure (Sousa 1984). Despite the importance of natural disturbance in influencing community structure, only one study on the effects of OA on CCA has incorporated the effects of natural disturbance (Johnson & Carpenter 2012). CCA rely on grazing by herbivores to remain free of epiphytes, and grazing can mediate the outcome of interactions for space between CCA species (Steneck 1983, Steneck et al. 1991). The results of the current study indicate that there may be species-specific responses to elevated pCO_2 and simulated wounding in two tropical CCA species common to the back reefs of Mo'orea, French Polynesia. The net calcification of *P. onkodes* was depressed by an increase in pCO_2, but there was no effect of elevated pCO_2 on the net calcification of *L. insipidum*. Past studies have found reductions in the net calcification of *P. onkodes* when exposed to elevated pCO2 (Anthony et al. 2008, Comeau et al. 2013), but there have been no studies quantifying the effects of elevated pCO_2 on *L. insipidum*. The present results indicate that the net calcification of *L. insipidum* may not be affected by elevated pCO_2, at least on the short term, but that elevated pCO_2 could increase tissue mortality. There was no effect of wounding on the net calcification of *P. onkodes* in this study, but there was a significant decrease in the net calcification of *L. insipidum* regardless of pCO_2 level, because of wounding. *P. onkodes* and *L. insipidum* are both relatively thick species (Adey et al. 1982). However, *P.* onkodes, often reaching many centimeters thick, usually is thicker

than *L. insipidum* which can be up to a centimeter thick (Adey et al. 1982, personal observation). *P. onkodes* has a multilayered epithallus one to three cells thick (Adey et al. 1982). In contrast, *L. insipidum* has a single-celled epithallial layer (Adey et al. 1982). The epithallus protects the intercalary meristem, the cell layer that produces reproductive structures (conceptacles) and photosynthetic tissue (perithallial cells), from damage during grazing (Steneck 1982, 1983). Therefore, the thicker epithallus of *P. onkodes* may confer an advantage when dealing with wounding, which could explain why net calcification was not affected by wounding in *P. onkodes*.

In this study, the proportion of live tissue at elevated pCO_2 (1179.39 µatm) for both wounded and non-wounded samples of *P. onkodes* and *L. insipidum* was reduced by 23.9 and 49.3 % from the proportion of live tissue remaining at ambient pCO_2. Two prior studies found that elevated pCO_2 leads to decreases in the percentage of healthy tissue (i.e., not bleached, pale, or colonized by endophytic algae) in *P. onkodes* (Anthony et al. 2008, Diaz-Pulido et al. 2012). There was, however, no effect of wounding on the proportion of live tissue remaining in either species. This is not surprising as CCA are resistant to wounding, particularly due to grazing by herbivores, and CCA abundance often is correlated positively with the abundance of the herbivores that graze on them (Steneck 1986).

This study is the first to quantify the effects of OA on the rate of wound regeneration in CCA. There was an ~ 58 % reduction in the rate of vertical regeneration at elevated pCO_2 within the wounds of *P. onkodes*. This could be the result of the reduction in net calcification at elevated pCO_2. Though the wounds created in this study may not be representative of the areal extent of a parrotfish grazing wounds, the depth of

the wounds (182 ± 15 µm, mean ± SE) could be considered analogous to a shallower parrotfish grazing wounds. These results could therefore indicate that elevated pCO_2 may be detrimental to the recovery of CCA from graze wounds. Furthermore, the reduction in the ability to vertically regenerate tissue within wounds, as shown in this study, could make *P. onkodes* increasingly susceptible to subsequent grazing. Johnson & Carpenter (2012) found that *P. onkodes* was more susceptible to grazing by the urchin *Echinothrix diadema*, which removed 60% more calcium carbonate when pCO_2 was increased from 420 to 830 µatm. Corallines have evolved mechanisms that have helped them cope with excavation by herbivores, such as sea urchins and parrotfish, including the ability to regenerate by forming a new hypothallus within deeper wounds (Steneck 1983), as was the case in the present study. However, a reduced ability to regenerate wounds under OA could affect the relationship between CCA and their grazers.

The reduction in vertical regeneration found here could also represent a reduction in the vertical growth rate of *P. onkodes*. Although this was not tested for in the present experiment, a study of temperate CCA found species-specific reductions in the thallus thickness of thicker species in response to increased seawater pCO_2 (McCoy & Ragazzola 2014). Species-specific reductions in thallus thickness because of OA in these temperate species is hypothesized to have resulted in changes in competitive interaction strength among CCA in this system (McCoy & Pfister 2014). If the reduction in the rate of vertical regeneration within wounds of *P. onkodes* found in this study is representative of a decrease in vertical growth rate at elevated pCO_2, it is possible that this could result in similar shifts in competitive interaction strengths among tropical guilds of CCA, and

possible shifts in CCA community structure. This could have profound effects on the ecological processes in which they play key roles (e.g., coral settlement and recruitment).

Few studies have explored the interaction between natural disturbances and climate change on coral reefs (but see Rotjan et al. 2006, Johnson & Carpenter 2012). The effect of elevated pCO_2 on the rate of wound regeneration in *P. onkodes* in this study is evidence of the potential for interactive effects between natural disturbances like grazing, and climate change. *P. onkodes* is among the most ecologically important CCA on coral reefs throughout the Indo-Pacific, contributing significantly to reef structure (Littler & Doty 1975, Adey et al. 1982). The ability to resist and recover from grazing events is key to this functional importance. Reductions in this ability because of OA could indirectly influence important ecological processes in which CCA play roles.

CHAPTER 3

Effect of ocean acidification on competition between two tropical crustose coralline algae

Manning J.C.

Introduction

Disturbance plays an important role in structuring and maintaining diversity in marine communities (Connell 1978, 1997, Sousa 1979, 1984). The intensity and frequency of disturbances, both biotic and abiotic, make up the disturbance regime experienced by an ecosystem (Sousa 1984). On coral reefs, human disturbance (i.e., nutrient enrichment, overfishing, etc.) is altering natural disturbance regimes, and increasing the chronic stresses experienced by reef organisms (Nyström et al. 2000). Ocean acidification (OA) is a press disturbance caused by increased atmospheric pCO_2 (Caldeira & Wickett 2003). The ocean is the Earth's largest sink for anthropogenically-produced CO_2 (Sabine et al. 2004). Increased oceanic pCO_2 is causing changes in seawater chemistry including reductions in pH, carbonate ion concentration, and calcium carbonate saturation states (Doney et al. 2009). Average seawater pH has already dropped by 0.1 pH units, and more pessimistic scenarios (RCP 8.5) predict that pH may drop a further 0.3 pH units by the end of the 21st century (Pachauri et al. 2014). The current rates of CO_2 increase in the atmosphere may lead to changes in ocean geochemistry, for which there are no analogs in the past ~ 300 million years (Hönisch et al. 2012).

Marine organisms are responding variably to changes in seawater chemistry (Kroeker et al. 2013), and calcified organisms, such as corals and coralline algae, may be affected more negatively than non-calcifying organisms (Hofmann et al. 2010, Kroeker et

al. 2010, Kroeker et al. 2013). Species-specific responses to OA have the potential to alter competitive interactions among marine organisms. Birch (1957) defined competition as interactions between organisms of the same or different species in which the competing groups harm one another in seeking a common resource (i.e., food and space). Competition is an important process structuring many benthic marine communities (Connell 1961, Menge & Sutherland 1976).

Along *in situ* gradients of pH near natural temperate and tropical CO_2 vents, previous studies indicate that reduced pH alters benthic community structure (Hall-Spencer et al. 2008, Fabricius et al. 2011, Porzio et al. 2011, Kroeker, Gambi, et al. 2013). Fabricius et al., (2011) found that the abundance of structurally complex corals and calcified red algae (including crustose coralline algae) was significantly reduced along a gradient of reduced pH (~ 8.1 to 7.7), while fleshy algae and seagrasses increased in abundance. Connell et al., (2013) argued that increased seawater pCO_2 (reduced pH) may act as a resource (e.g., increasing productivity) for some taxa, and result in shifts in ecosystem dominance. However, there is little direct evidence of the effect of OA on competitive interactions between marine organisms (e.g., Diaz-Pulido et al. 2011), despite multiple studies that have indicated differential effects of OA on different taxa (Ries et al. 2009, Hofmann et al. 2010, Kroeker et al. 2013).

Coral reefs are diverse biogenic structures produced by marine calcifying organisms, primarily scleractinian corals and calcified algae. Coral reefs are extremely biodiverse ecosystems, and provide humans with a number of important ecological goods and services (reviewed in Moberg and Folke, 1999). Due to the importance of coral reefs, many studies have focused on the effects of OA on coral reef calcifiers. A number of

studies have demonstrated reduced calcification among corals exposed to experimentally elevated pCO_2 (Chan & Connolly 2013, Comeau et al. 2013, 2014). Additionally, there is some evidence that there could be differential responses to OA among coral species. Comeau et al. (2014) found that corals classified as fast calcifiers (calcifying > 1 mg $CaCO_3$ cm^{-2} d^{-1}) were more negatively affected by experimentally elevated pCO_2 than corals classified as slow calcifiers (calcifying < 1 mg $CaCO_3$ cm^{-2} d^{-1}). However, despite the potential for OA to alter competition through differential effects, there has been little effort to quantify the effects of OA on interspecific competition among corals. Empirical evidence of the performance of six coral species in intra- and interspecific interactions in both ambient and elevated pCO_2, in combination with modeling data, has indicated that there is potential for OA to alter competitive hierarchies within coral communities (Horwitz et al. 2017).

Coralline algae (Phylum Rhodophyta) are important members of many benthic communities globally, including coral reefs (reviewed in Nelson, 2009). On coral reefs, crustose coralline algae (CCA) cement and accrete reef framework, sometimes dominating large portions of the benthos (Littler & Doty 1975, Adey et al. 1982, Nelson 2009, Dean et al. 2015). There also is evidence that the larvae of some species of coral preferentially settle and recruit to CCA (Heyward & Negri 1999, Harrington et al. 2004, Ritson-Williams et al. 2009, Arnold et al. 2010, Price 2010). CCA deposit a high-Mg polymorph of calcium carbonate that is much more soluble than the aragonite polymorph deposited by corals, and therefore are likely to be negatively affected by OA. Indeed, recent experimental evidence has demonstrated reduced recruitment, abundance, and calcification among tropical CCA under elevated pCO_2 (Anthony et al. 2008, Jokiel et al.

2008, Kuffner et al. 2008, Diaz-Pulido et al. 2012, Comeau et al. 2013, 2014). Consistent with experimental evidence, Fabricius et al. (2011) quantified *in situ* reductions in the abundance of CCA at lower pH sites along a natural gradient of pH on the coral reefs of Papua New Guinea. As in corals, there appears to be some level of species-specificity in the responses of CCA to OA (Comeau et al. 2014). However, despite these species-specific responses, there have been no attempts to quantify the effects of OA on interspecific competition between tropical CCA. CCA compete for space on the benthos through overgrowth interactions, in which thicker individuals generally win (Steneck et al. 1991). In a temperate guild of CCA on the coast of Washington State, USA, McCoy & Ragazzola (2014) found that there were significant reductions in thallus thickness in thick species under experimentally elevated pCO_2, but no significant reductions for thinner species. These morphological changes under OA are hypothesized to be the mechanism behind significant changes in competitive interaction strengths (e.g., increased intransitivity) among this guild of CCA over the past 30 years (McCoy & Pfister 2014).

In this study, the effects of elevated pCO_2 on interspecific interactions between two species of CCA common to the back reefs of Mo'orea, French Polynesia, *Porolithon onkodes* and *Lithophyllum insipidum*. *P. onkodes* contributes significantly to the reef structure of many Indo-Pacific reefs, often dominating large areas of reefs (Littler & Doty 1975, Adey et al. 1982). *L. insipidum* also is relatively common on reefs throughout the Pacific (Adey et al. 1982, Keats 1997). Both species are considered thick (> 500 μm; Steneck 1986), although *P. onkodes* typically is thicker (in some cases many cm thick) than *L. insipidum* (up to 1 cm thick, Adey et al. 1982, personal observation). *P. onkodes*

is more abundant on the back reefs of Mo'orea, and as it is generally thicker, it is likely competitively dominant to *L. insipidum* (Manning and Carpenter, in prep). In this study, we tested the null hypothesis that elevated pCO_2 would not affect the outcome of competitive interactions between *P. onkodes* and *L. insipidum*, which has implications for CCA community structure in the back reefs of Mo'orea.

Methods

Collection and preparation

Cores of *P. onkodes* and *L. insipidum* were collected using an air-powered drill (McMaster-Carr) with a 3-cm diameter, diamond-tipped hole saw (McMaster-Carr). The cores were collected at a depth of ~ 2 meters on the back reef east of Cook's Pass on the north shore of Mo'orea, and transported to the Richard B. Gump South Pacific Research Station in ambient seawater. Samples were placed promptly in flowing seawater tables supplied with unfiltered seawater from Cook's Bay. A total of 112 unique individuals (each sample collected from separate thalli) were collected for both *P. onkodes* and *L. insipidum*. Each sample was cut in half with a diamond grit blade on a band saw. One half of each sample was scrubbed with a toothbrush to remove epiphytes and allocated randomly to one of five competitive pairings: *P. onkodes* v. *L. insipidum*, *P. onkodes* v. *P. onkodes* (different individual), *P. onkodes* v. non-living coral skeleton, *L. insipidum* v. *L. insipidum* (different individual), and *L. insipidum* v. non-living coral skeleton (Figure 3.1). The other halves of each of the unique individuals were discarded. For the non-living coral skeleton controls, non-living coral tissue was cored from dead coral heads of massive *Porites*. These non-living cores were then halved and sectioned into pieces corresponding to the thickness of the CCA they were paired with. Samples were paired

with the flat portions of the halves in contact, and held in place on a plastic base using a marine epoxy (Coral Glue, EcoTech Marine, Allentown, PA, USA; Figure 1). The competitive pairings cured for at least 24 hours in flowing seawater tables. Seven of each of the five competitive pairings were then were allocated randomly to each of four experimental pCO2 treatments.

Experimental design

This experiment was performed between June 2 to July 1, 2016 in four flow-through seawater tanks, flumes, each set at one of four target pCO_2 levels. The flumes were supplied with filtered seawater pumped from ~ 12-m depth in Cook's Bay, and passed through a sand filter (nominal pore size ~ 100 µm). The seawater within each flume was replaced at a rate of ~ 5 L/min (as in Comeau et al. 2015). There was a flume maintained at 400 µatm pCO_2 to mimic ambient seawater, and three elevated pCO_2 treatments. The three elevated pCO_2 treatments with target pCO2 values of 700, 1000, and 1300 µatm, were representative of predicted increases in seawater pCO_2 by the end of the current century under the RCP6.0 and RCP8.5 scenarios put forth by the IPCC (Pachauri et al. 2014). pH was controlled for a 0.1-unit reduction at night to simulate natural diel variation of pH on the reefs of Mo'orea (Hofmann et al. 2011). Temperature within the flumes was maintained at as close as possible to mean sea surface temperatures from May to July in Mo'orea, ~ 27.15 °C, calculated from mean monthly nighttime sea surface temperature data available for 2003-2014 (Maritorena 2015 Moorea Coral Reef LTER). Temperatures were taken every day at mid-day using a digital thermometer (accuracy = ± 0.05 ° C, Digital Thermometer, Traceable Calibration Control Company, Webster, TX, USA). The flumes were exposed to natural sunlight, but a piece of window

screening was used to further reduce light by ~29% to prevent light oversaturation. Mean instantaneous PAR within the flumes were higher than the *in situ* and in-lab irradiance at the onset of light saturation (I_k) reported for *P. onkodes* (205 and 124 µmol m^{-2} s^{-1} respectively; Chisholm 2003).

Maintenance of seawater chemistry and other physical parameters

Target pCO_2 levels of the elevated treatments were achieved by bubbling pure CO_2 into each elevated flume, and non-CO_2-enriched air into the ambient flume. Carbonate chemistry was measured and controlled using methods like those used by (Comeau et al. 2015). pH within each flume was monitored continuously by pH probes within each flume and provided feedback to regulated bubbling of CO2 by a solenoid valve that added pure CO_2 to flumes when the pH measured within the flumes was higher than their set points, which were updated daily on the controller (AquaController, Neptune Systems, USA). Temperature and conductivity readings were taken daily, around mid-day, using a digital thermometer and a handheld pH meter (Thermo Scientific, Orion 3 STAR, Beverly, MA, USA) fitted with a pH probe (Mettler Toledo, DGi115-SC), and used to calculate pH_T. These data were used to update the pH setpoints for the AquaControllers, adjusting for the difference between probe values and pH_T. The handheld pH probe was calibrated using TRIS/HCl buffers (SOP 6a, Dickson et al. 2007) whenever there was large variation in day to day conductivity values (no set interval). Total alkalinity of the water in each flume was measured once per week using open-cell, potentiometric titrations on an automatic titrator (Mettler Toledo, T50) following SOP 3b of Dickson et al. (2007). TA analyses were conducted in duplicate (~50 g seawater per replicate sample) for each flume at room temperature, and compared against certified

reference material provided by A.G. Dickson (Scripps Inst. of Oceanography, San Diego, CA). TA was determined as described in SOP 3b of Dickson et al. (2007). Salinity also was measured for these weekly water samples with a conductivity meter (Thermo Scientific Orionstar A212, Waltham, MA). Salinity, temperature, pH_T, and TA then were used to calculate carbonate chemistry using the seacarb package for R statistical software (Lavigne et al. 2011).

Daily integrated photosynthetically active radiation (PAR; mol quanta m^{-2} d^{-1}) was quantified for each flume using measurements of PAR taken every 30 minutes by an Odyssey Integrating PAR cosine sensor (Dataflow Systems Limited, Christchurch, New Zealand) placed at the center of each flume. However, after June 4th there was a sensor failure which resulted in a sudden reduction in the measured daily integrated PAR within flumes 3 and 4. The flumes had been running continuously since November 2015, and measurements of PAR had been taken using these sensors throughout that period. During the approximately 6 months prior to June 4th, there was little difference in the mean PAR for each flume (flume 1: 326.99, flume 2: 374.15, flume 3: 342.79, flume 4: 348.56; µmol quanta m^{-2} s^{-1}). Therefore, it is likely that flumes 3 and 4 did not differ from flumes 1 and 2 by the amount measured between June 4 and July 23. Therefore, a correction was made for the PAR data measured in flumes 3 and 4 following June 4th. The correction factor was determined by taking the difference between the pooled mean PAR (µmol quanta m^{-2} s^{-1}) for flumes 1 and 2, and the pooled mean PAR (µmol quanta m^{-2} s^{-1}) for flumes 3 and 4 (using data at 30-minute intervals). The correction factor was then added to the PAR (µmol quanta m^{-2} s^{-1}) values recorded for flumes 3 and 4 at 30 minute

intervals. These corrected values were then used to quantify the daily integrated PAR (mol quanta m^{-2} d^{-1}) for the duration of the experimental period.

Quantifying the effects of OA on competition

The proportion of live tissue interaction won by the focal individual of the interaction (i.e., focal individual overgrew the non-focal) within each of the five interaction types was measured to quantify the effect of elevated pCO_2 on the outcome of competitive interactions. For the *P. onkodes* versus *L. insipidum* interaction, *P. onkodes* was the focal individual. For the two conspecific interactions (i.e., *P. onkodes* versus *P. onkodes* and *L. insipidum* versus *L. insipidum*), the left individual (as indicated by markings on the plastic bases) was considered the focal individual. For the interactions with *P. onkodes* and *L. insipidum* competing with non-living massive *Porites*, the CCA were the focal individuals. The perimeter of live interaction (i.e., where interacting tissue for each individual was living) for each interaction pair and the perimeter of that total that was won by the focal individual (i.e., thallus of the focal individual overtopped the thallus of the non-focal individual) were measured on the software DinoCapture 2.0 (Dunwell Tech, Inc., Torrance, CA) from photos taken at the end of the experimental period using a digital dissecting microscope (Dino-lite Edge AM4815ZTL, Dunwell Tech, Inc., Torrance, CA). The perimeter of the live interaction won by the focal individual was then divided by the total live interaction perimeter to provide the proportion of live tissue interaction won by the focal individual.

The proportion of living tissue surface area of the focal individual in pairs in which interaction had occurred was quantified from images (~ 15.9 MP resolution, Nikon COOLPIX AW130) analyzed using ImageJ (NIH). This was done to quantify any

potential interactive effect of interaction type and elevated pCO_2 on living tissue within these two species.

Statistical Analysis

Seawater chemistry parameters and daily integrated PAR for the flume experiment were analyzed with a one-way ANOVA using flume as the independent variable with a null hypothesis of no difference between flumes. Post-hoc comparisons were performed using Tukey's honestly significant differences to determine the relationship among the flumes. If the assumptions of homogeneity of variance and/or normality were not met, variables were transformed to meet them. If transformations to meet these assumptions were not possible, a non-parametric Kruskall-Wallis Test was performed with a Dwass-Steel-Chritchlow-Fligner Test for pairwise comparisons. These analyses were performed using the statistical software SYSTAT (SYSTAT Version 13).

To analyze the effect of elevated pCO_2 on the proportion of living tissue interactions won by the focal individual in each of the five interaction types, the proportions were made binary. If the proportion of a given interaction won by the focal individual was > 0.50, it was assigned a value of 1 (a win). If the proportion of a given interaction won by the focal individual was ≤ 0.50, it was assigned a value of 0 (a loss). The data was then analyzed using a generalized linear model (GLM) fit to a binomial distribution.

To analyze the combined effects of elevated pCO_2 and interaction type (e.g., heterospecific, conspecific, and non-living) on the proportion of live tissue remaining in the thallus of the focal individuals, data again was converted to a binary response (0 and

1) prior to analysis. The median of the proportion of living tissue remaining in the thallus of the focal individuals was calculated for all data in which either *P. onkodes* or *L. insipidum* was the focal individual (0.96 and 0.35 respectively). For each data set, proportions greater than the median were given a value of 1, and those below the median were give a value of 0. The new binary response variable was analyzed separately for *P. onkodes* and *L. insipidum* using GLMs fit to binomial distributions to determine the interactive effect of interaction type (heterospecific, conspecific, and non-living for *P. onkodes*; conspecific and non-living for *L. insipidum*) and elevated pCO_2 on the proportion of live tissue remaining at the end of the experiment. When not significant ($p > 0.250$), the interaction terms were dropped from the full model and the results were interpreted from the reduced model.

Independence was assumed for the individual interaction pairs within each flume, as the seawater volume (~500 L) and seawater turnover rate within the flumes were relatively high. As there is no evidence of chemical interactions between CCA in the literature, it is likely that pCO_2 and direct interaction between samples within interacting pairs were the cause of any differences. However, to statistically test for autocorrelation within the residuals, a Durbin-Watson test of independence was performed for each model. All generalized linear model analyses were performed using the statistical software R (stats package, R Core Team 2015).

Results

Seawater chemistry

pCO_2 was different from target values in each of the flumes throughout the experiment, but nonetheless created a gradient in pCO_2 and pH within the flumes (Table 3.1). The only significant differences in carbonate chemistry found between flumes were in parameters expected to change because of increased pCO_2 (i.e., pH, aragonite and calcite saturation states, carbonate and bicarbonate ion concentrations, etc.), and each flume was significantly different from every other with respect to these parameters (Tukey's HSD, $p < 0.05$). There were no statistically significant differences in total alkalinity, temperature, or salinity between flumes ($p > 0.05$). However, there was a significant difference in the daily integrated PAR between flumes 1 (16.04 ± 0.63 mol quanta m^{-2} d^{-1}, mean ± SE) and 2 (13.98 ± 0.53 mol quanta m^{-2} d^{-1}, mean ± SE; Tukey's HSD, $p = 0.02$). Flume 2 was not significantly different from either flume 3 (14.84 ± 0.28 mol quanta m^{-2} d^{-1}, mean ± SE) or 4 (14.92 ± 0.26 mol quanta m^{-2} d^{-1}, mean ± SE). Despite the difference in daily integrated PAR, the mean instantaneous PAR was > 320 µmol m^{-2} s^{-1} for all treatments were well above the *in situ* and in-lab irradiance at the onset of light saturation (I_k) reported for *P. onkodes* (205 and 124 µmol m^{-2} s^{-1} respectively; Chisholm 2003). It is therefore, unlikely that the higher daily integrated PAR in flume 1 would have affected net calcification through increased photosynthetic ability. A summary of key environmental parameters is given in Table 3.1.

Interaction boundary

There was no significant effect of elevated pCO_2 on the outcome of interactions when *P. onkodes* and *L. insipidum* were competing with non-living massive *Porites* ($p > 0.05$). When interaction occurred, living tissue always overgrew the non-living substratum. There were no significant differences in the proportion of interactions in which the focal individuals of *P. onkodes* and *L. insipidum* overgrew their competitors in conspecific interactions ($p > 0.05$). There also was no significant effect of elevated pCO_2 on the proportion of interactions won by *P. onkodes* when competing with *L. insipidum* (Figure 3.2; $p > 0.05$).

Comparisons of the proportion live tissue remaining

There was no significant interaction between pCO_2 and interaction type (heterospecific, conspecific, and non-living) or individual effect of pCO_2 on the proportion of live tissue remaining in focal individuals of *P. onkodes* or *L. insipidum* ($p > 0.250$). Therefore, the interaction terms were dropped from both full models, and reduced models were used to interpret the main effects of pCO_2 and interaction type on the proportion of live tissue remaining (Table 3.2). There was a significant effect of interaction type (i.e., conspecific, heterospecific, and non-living) on the proportion of living tissue remaining in focal individuals of *P. onkodes* (Figure 3.3; $p = 0.03$). The mean proportion of live tissue remaining in focal individuals of *P. onkodes* was 1.01 ± 0.03 ($\pm$ SE, $n = 21$) when competing with *L. insipidum*. When competing with conspecifics and non-living coral tissue the mean proportion of live tissue remaining was lower, 0.79 ± 0.07 and 0.92 ± 0.06 respectively ($\pm$ SE, $n = 22$ and 16 respectively). There was no effect of interaction type on the proportion of live tissue remaining in the thallus

of focal individuals of *L. insipidum* (Figure 3.4, $p > 0.05$), but there was a significant reduction in the proportion of live tissue remaining because of elevated pCO_2 (Figure 3.4, $p = 0.01$). The mean proportion of live tissue remaining within the thalli of focal individuals of *L. insipidum* decreased from 0.60 ± 0.10 (± SE, n = 12) at ambient pCO_2 to 0.18 ± 0.06 (± SE, n = 9) at 1179.39 µatm.

Discussion

Coral reefs are diverse ecosystems, and depend in part on intermediate levels of disturbance to maintain that diversity (Connell 1978). Human disturbance is altering the natural disturbance regimes of coral reefs, and increasing the chronic stresses experienced by many coral reef organisms (Nyström et al. 2000). OA is one of the most serious human disturbances affecting coral reefs today. The associated changes in seawater chemistry may have a negative effect on a wide variety of taxa, but particularly on calcifying organisms like corals and calcifying algae (Kroeker et al. 2010, Kroeker et al. 2013). Additionally , OA may act as a resource for other taxa such as fleshy macroalgae aiding in their ability to outcompete other organisms (Connell et al. 2013). Competition is another important factor shaping benthic marine communities (Connell 1961, Menge & Sutherland 1976), but despite its importance, few studies have attempted to quantify the effect of OA on competition among benthic organisms (but see Diaz-Pulido et al. 2011, Evensen et al. 2015). There is experimental evidence that mortality in the coral *Acropora intermedia* when competing with the macroalga *Lobophora papenfussii* may increase under OA (Diaz-Pulido et al. 2011). Fabricius et al. (2011) found that the abundance of fleshy algae on coral reefs increased along a gradient of decreased pH along natural CO_2 seeps in Papua New Guinea, while the cover of structurally complex corals and calcified

algae declined. Therefore, it may be important to consider the effects OA could have on competition for space on the benthos, and begin to incorporate ecological theory into OA research (Gaylord et al. 2008).

CCA are important members of coral reef communities, consolidating and building the reef framework, and aiding in the settlement and recruitment of coral larvae (reviewed in Nelson 2009). However, as a result of their highly soluble high-Mg calcite skeletons, CCA may be especially vulnerable to changes in ocean chemistry as a result of OA (Morse et al. 2006). Multiple studies of tropical CCA have found that experimental OA has negative effects on calcification, recruitment, and abundance (Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Diaz-Pulido et al. 2012, Comeau et al. 2013, 2014). It is conceivable that OA may affect competitive outcome, through species-specific effects on the physiology of CCA.

Both *P. onkodes* and *L. insipidum* are abundant CCA on the back reefs of Mo'orea, French Polynesia, as well as other locations throughout the Pacific Ocean (Littler & Doty 1975, Adey et al. 1982, Keats 1997, Chapter 1). *P. onkodes* is the dominant space-holder on the back reefs of Mo'orea (Chapter 1). In the present study, there was no significant effect of experimentally-elevated pCO_2 on the strength of the competitive interaction between *P. onkodes* and *L. insipidum*. This may be due to the limited live interactions available for analysis, a result of the general decline in live tissue of *L. insipidum* throughout the experiment. The decline in the proportion of living tissue remaining in *L. insipidum* in this experiment also could explain why there was more living tissue remaining in *P. onkodes* in the heterospecific pairings. Due to tissue mortality (bleaching) in *L. insipidum*, *P. onkodes* may have grown to occupy the space

vacated by *L. insipidum* (with little competition) as it had done in the interactions with non-living massive *Porites*. However, when competing with other healthy individuals of *P. onkodes*, intraspecific competition may have slowed lateral expansion or caused increased tissue mortality.

Competition for space is a key determinant of community structure, and alterations to competitive interactions could lead to changes in community structure and ecosystem function. Changes in competitive interactions that favor a few species could reduce diversity and lead to habitat simplification. Indeed, Fabricius et al. (2011) found that the richness of coral adults and juveniles was reduced along a gradient of decreasing pH in Papua New Guinea, leading to a shift toward an abundance of less structurally complex corals. The results of the present study do not suggest that elevated pCO_2 will affect competitive interactions between *P. onkodes* and *L. insipidum*. However, this study was of short temporal duration, 29 days, and may not be indicative of the effects that elevated pCO_2 might have at larger temporal scales. The time it takes for elevated pCO_2 to sufficiently affect competitive ability through reductions in net calcification rates and decreases in thallus thickness may be beyond the scope of a shorter experiment like this. Nonetheless, this study demonstrated the potential for elevated pCO_2 to open bare substrate by causing the death of thallus tissue in *L. insipidum*, which could allow *P. onkodes* to become increasingly abundant as it grows to occupy the vacated space.

CONCLUSION

Increasing concentrations of atmospheric CO_2 from fossil fuel consumption is negatively affecting marine ecosystems, like coral reefs. Ocean warming has led to an increase in the frequency and spatial scale of coral bleaching events (Hughes et al. 2017). These bleaching events have resulted in altered coral communities in some cases (Loya et al. 2001, Depczynski et al. 2013). However, warming is not the only threat to coral reefs due to increased atmospheric CO_2. The oceans are the largest sink for anthropogenically produced CO_2 (Sabine et al. 2004). The absorption of CO_2 is altering oceanic carbonate chemistry, namely reductions in pH, carbonate ion concentrations, and calcium carbonate saturation states (Hoegh-Guldberg et al. 2007, Doney et al. 2009). Global ocean pH has dropped by 0.1 pH units since the beginning of the industrial revolution, and more pessimistic scenarios put forth by the Intergovernmental Panel for Climate Change (IPCC, Representative Concentration Pathway 8.5) expect pH to decline a further 0.3-0.4 pH units by the end of the 21st century (Pachauri et al. 2014). These changes in seawater chemistry are termed ocean acidification (OA; Caldeira & Wickett 2003). Experimentally, OA has variable effects on marine organisms, but calcifying organisms seem to be most negatively affected (Ries et al. 2009, Hofmann et al. 2010, Kroeker et al. 2010, Kroeker et al. 2013). Among the calcifying marine organisms expected to be negatively affected by OA are crustose coralline algae (CCA).

CCA are globally important members of benthic marine communities within the photic zone (reviewed in Nelson 2009). However, the soluble high-Mg calcite polymorph of calcium carbonate that they deposit, makes them vulnerable to OA (Morse et al. 2006). CCA are important members of coral reef communities, where they are often abundant

space holders (Littler & Doty 1975, Adey et al. 1982, Nelson 2009, Dean et al. 2015). They consolidate and build upon the reef framework, and are involved in the process of coral settlement and recruitment (Littler & Doty 1975, Heyward & Negri 1999, Harrington et al. 2004, Nelson 2009, Arnold et al. 2010, Price 2010). Due to their importance to coral reefs, CCA have become the focus of much of the research on the effects of OA on coral reefs. Numerous experimental studies have found that OA reduces the calcification, productivity, recruitment, and abundance of CCA (Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Diaz-Pulido et al. 2012, Comeau et al. 2013, 2014). Many of these studies have focused on one species of CCA, but recent evidence suggests that there may be species-specificity in the response of CCA to OA (Comeau et al. 2014).

The results of the first chapter of this thesis further support the potential for species-specific responses to OA among CCA. Net calcification in *Porolithon onkodes* was more negatively affected by elevated pCO_2 than the net calcification of *Lithophyllum insipidum*. In addition, the proportion of living tissue within the thalli of *P. onkodes* was more significantly reduced under experimentally elevated pCO_2 than it was in the thalli of *L. insipidum*. These results indicate that *P. onkodes* may respond negatively to future increases in seawater pCO_2, as has been found in prior studies (Anthony et al. 2008, Diaz-Pulido et al. 2012). They also support the findings of Comeau et al. (2014) that fast calcifiers may be more affected by OA than slow calcifiers, as *P. onkodes* calcified more quickly under ambient conditions than *L. insipidum.* The differential responses found within this study could have implications for ecological interactions among these two species of CCA. Both *P. onkodes* and *L. insipidum* are common members of the back

reefs of Mo'orea, French Polynesia. *P. onkodes* is much more abundant than *L. insipidum* on the back reefs of Mo'orea. However, it is possible that this could change because of reduced competitive ability in *P. onkodes* at elevated pCO_2.

CCA compete for space through overgrowth interactions, in which thicker individuals will overgrow thinner individuals (Steneck et al. 1991). Thicker species are typically dominant when there is frequent low intensity grazing, as they are more defended against herbivory than thin species (Steneck et al. 1991). However, more intense grazing can lead to competitive reversals (Steneck et al. 1991). In this way herbivory is an important process determining the structure of CCA communities. Only one study has considered the potential interaction between OA and grazing on CCA. Johnson & Carpenter (2012) found that experimentally elevated pCO_2 levels increased the susceptibility of *P. onkodes* to grazing by the sea urchin *Echinothrix diadema*. There is evidence from temperate species of coralline algae that OA may reduce the thickness of cell walls and reduce cell density (Ragazzola et al. 2012, McCoy & Ragazzola 2014). This could make the thallus weaker, and therefore more susceptible to grazing. There also is evidence that the thallus thickness of temperate CCA may be reduced by OA, making it easier for grazers to wound more deeply (McCoy 2013, McCoy & Ragazzola 2014). No studies in tropical species of CCA have quantified changes in morphology because of elevated pCO_2. In chapter two, there were differences in the responses of *P. onkodes* and *L. insipidum* elevated pCO_2 and wounding. Net calcification in *P. onkodes* was significantly reduced at elevated pCO_2 levels regardless of wounding treatment. In contrast, there was no effect of elevated pCO_2 on the net calcification of *L. insipidum*, but wounding significantly reduced net calcification in *L. insipidum*. The effect of wounding

on *L. insipidum* is likely a consequence of the relative thickness of this species. *P. onkodes* is a very thick CCA, growing multiple centimeters thick in some cases, whereas *L. insipidum* is generally much thinner (≤ 1 cm; personal observation, Adey et al. 1982, Keats 1997). Therefore, injuries to the thalli of *L. insipidum* are likely to be more damaging. There was also a significant reduction in the rate of vertical regeneration within the wounds in the thalli of *P. onkodes* at elevated pCO_2. This could be a potential mechanism explaining the increased susceptibility of *P. onkodes* to urchin grazing at elevated pCO_2 found by Johnson & Carpenter (2012). These reduced rates of vertical regeneration at elevated pCO_2 within the wounds of *P. onkodes*, could also be indicative of a reduction in vertical growth rates.

As thallus thickness is an important factor in determining the outcome of competition between CCA, a reduction in the rate of vertical growth could result in reduced competitive dominance for *P. onkodes*. McCoy & Pfister (2014) found that there have been alterations in the strengths of competitive interactions and increased competitive intransitivity among a guild of temperate CCA on the coast of Washington, USA, presumably because of increasing OA over the past three decades. No such shift in the interactions among CCA has been documented in the tropics, but there is evidence that OA may alter competitive interactions among other benthic coral reef organisms (e.g., Diaz-Pulido et al. 2011, Connell et al. 2013). In the present study (chapter 3), there was insufficient data to reject the null hypothesis that there would be no change in the strength of the interaction between *P. onkodes* and *L. insipidum* because of elevated pCO_2. Despite the differential responses of these two species to conditions mimicking future OA, there was no significant difference in the proportion of times that *P. onkodes*

overgrew *L. insipidum*. However, there was a significant effect of elevated pCO_2 on the proportion of live tissue remaining at the end of this experiment in *L. insipidum*. This may explain why there significantly more live tissue remaining within *P. onkodes* when it was interacting with *L. insipidum* than when it was interacting with other individuals of *P. onkodes.* Intraspecific competition with healthier *P. onkodes* individuals may have slowed the rate of lateral extension, while *P. onkodes* may have been able occupy the space vacated by the dying tissue of *L. insipidum* more readily.

In conclusion, the results of these experiments build upon prior research quantifying the effects of OA on tropical CCA. However, much of the literature has not considered the potential for OA to interact with ecological process that control the structuring of CCA communities. The research presented in this thesis has demonstrated the importance of such research if we are to understand the effect that OA will have on tropical CCA communities. Detrimental changes in the structure of CCA communities could have implications for the resilience of coral reefs to human disturbance.

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APPENDIX A: TABLES

Chapter 1

Table 1.1: Model I ANOVA of the arcsine square root transformed species abundances for the three most common species, *P. onkodes*, *L. insipidum*, and *L. flavescens*.

	SS	d.f.	F-ratio	p-value
Species	0.758	2	64.700	< 0.001
Error	0.299	51		

Table 1.2: Summary of seawater parameters. Values are means ± standard errors for temperature (n = 18), pCO_2 (n = 18), pH (n = 18), TA (n = 18), salinity (n = 18), and daily integrated PAR (n = 60) calculated over the duration of the 59-day experiment.

	Flume 1	Flume 2	Flume 3	Flume 4
Temperature (°C)	28.78 ± 0.11	28.88 ± 0.10	29.00 ± 0.15	29.04 ± 0.08
pCO_2 (µatm)	1202.26 ± 63.50	650.87 ± 43.47	340.95 ± 18.37	883.50 ± 31.70
pH	7.64 ± 0.02	7.88 ± 0.02	8.10 ± 0.02	7.75 ± 0.01
TA (µmol/kg)	2338.10 ± 5.34	2323.21 ± 6.66	2327.91 ± 8.46	2316.48 ± 14.15
Salinity (psu)	35.78 ± 0.03	35.78 ± 0.03	35.78 ± 0.03	35.78 ± 0.03
Daily Integrated PAR (mol quanta m^{-2} day^{-1})	14.78 ± 0.75	18.46 ± 1.01	15.78 ± 0.91	14.82 ± 0.76

Table 1.3: Summary table for the full model GLM (Gaussian) for net calcification.

Interaction	SS	d.f.	F-value	p-value
pCO_2	0.285	1	7.587	0.008
Species	0.238	1	6.332	0.015
pCO_2 x Species	0.362	1	9.622	0.003
Residuals	2.219	59		

Table 1.4: Summary table for the full model GLM (binomial) for tissue necrosis.

Interaction	Likelihood Ratio χ^2	d.f.	p-value
pCO_2	3.215	1	0.073
Species	7.167	1	0.007
pCO_2 x Species	14.230	1	< 0.001

Chapter 2

Table 2.1: Summary of key environmental parameters for each flume. Values are means ± standard errors of the mean. N = 18 for temperature, pCO_2, pH, TA, and salinity. N = 51 for daily integrated PAR.

	Flume 1	Flume 2	Flume 3	Flume 4
Temperature (°C)	27.93 ± 0.22	27.90 ± 0.24	27.88 ± 0.23	27.52 ± 0.18
pCO_2 (µatm)	1179.39 ± 88.85	636.54 ± 27.29	429.31 ± 20.84	827.33 ± 38.51
pH	7.65 ± 0.02	7.88 ± 0.01	8.02 ± 0.02	7.78 ± 0.02
TA (µmol/kg)	2313.16 ± 3.71	2299.18 ± 5.29	2297.34 ± 5.96	2292.69 ± 8.99
Salinity (psu)	35.20 ± 0.09	35.27 ± 0.07	35.23 ± 0.06	35.15 ± 0.05
Daily integrated PAR (mol quanta m^{-2} day^{-1})	16.04 ± 0.63	13.98 ± 0.53	14.84 ± 0.28	14.92 ± 0.26

Table 2.2: Summary table for the full model GLM (Gaussian) for square-root transformed net calcification (mg $CaCO_3$ cm^{-2} d^{-1}) with the three-way interaction dropped ($p = 0.928$).

Interaction	SS	d.f.	F-ratio	p-value
pCO_2	0.002	1	0.075	0.784
Wounding	0.530	1	17.054	< 0.001
Species	0.041	1	1.314	0.254
pCO_2 x Wounding	0.015	1	0.473	0.493
Species x Wounding	1.198	1	38.585	< 0.001
pCO_2 x Species	0.122	1	3.937	0.050
Residuals	3.447	111		

Table 2.3: Summary table for the full model GLM (Gaussian) for proportion of live tissue remaining with the three-way interaction dropped ($p = 0.543$).

Interaction	SS	d.f.	F-ratio	p-value
pCO_2	0.841	1	18.071	< 0.001
Wounding	0.019	1	0.418	0.520
Species	0.867	1	18.630	< 0.001
pCO_2 x Wounding	0.019	1	0.410	0.523
Species x Wounding	0.053	1	1.138	0.289
pCO_2 x Species	0.018	1	0.386	0.536
Residuals	3.447	110		

Table 2.4: Summary table for the full model GLM (Gaussian) for vertical wound regeneration ($\mu m\ d^{-1}$).

Interaction	SS	d.f.	F-ratio	p-value
pCO_2	2.164	1	9.701	0.010
Residuals	2.454	11		

Table 2.5: Summary table for the full model GLM (Gaussian) for the ratio of vertical to lateral regeneration.

Interaction	SS	d.f.	F-ratio	p-value
pCO_2	2.815	1	3.102	0.106
Residuals	9.983	11		

Chapter 3

Table 3.1: Summary of key environmental parameters for each flume. Values are means ± standard errors of the mean. N = 18 for temperature, pCO_2, pH, TA, and salinity. N = 51 for daily integrated PAR.

	Flume 1	Flume 2	Flume 3	Flume 4
Temperature (°C)	27.93 ± 0.22	27.90 ± 0.24	27.88 ± 0.23	27.52 ± 0.18
pCO_2 (µatm)	1179.39 ± 88.85	636.54 ± 27.29	429.31 ± 20.84	827.33 ± 38.51
pH	7.65 ± 0.02	7.88 ± 0.01	8.02 ± 0.02	7.78 ± 0.02
TA (µmol/kg)	2313.16 ± 3.71	2299.18 ± 5.29	2297.34 ± 5.96	2292.69 ± 8.99
Salinity (psu)	35.20 ± 0.09	35.27 ± 0.07	35.23 ± 0.06	35.15 ± 0.05
Daily integrated PAR (mol quanta m^{-2} day^{-1})	16.04 ± 0.63	13.98 ± 0.53	14.84 ± 0.28	14.92 ± 0.26

Table 3.2: Summary table for the reduced model binomial GLMs for the proportion of live tissue surface area remaining within each of the species.

Porolithon onkodes	Likelihood Ratio χ^2	d.f.	p-value
pCO_2	0.791	1	0.374
Interaction Type	6.954	2	0.031
Lithophyllum insipidum			
pCO_2	6.194	1	0.013
Interaction Type	0.012	1	0.912

APPENDIX B: FIGURE CAPTIONS

Chapter 1

Figure 1.1: Four flow-through seawater systems at the Richard B. Gump South Pacific Research Station. Photo by Maureen Ho.

Figure 1.2: Mean ± SE of percent cover of most abundant coralline algal species on the open, hard substrate of the back reefs in Moorea, French Polynesia. n = 54

Figure 1.3: Net calcification rate (mg cm^{-2} day^{-1}) for *P. onkodes* (solid line, circles) and *L. insipidum* (dashed line, crosses) plotted against mean pCO_2. Individual points represent individual samples within each of the four flumes. Ordinary least squares regression lines through the individual points are plotted to demonstrate trends in the data. R^2 values are reported for each line.

Figure 1.4: Proportion of dead tissue for both *P. onkodes* (solid line, circles) and *L. insipidum* (dashed line, crosses) plotted against mean pCO_2. Individual points represent individual samples within each of the four flumes. Ordinary least squares regression lines through the individual points are plotted to demonstrate trends in the data.

Chapter 2

Figure 2.1: A secondary electron SEM image (346 x magnification) of a cross-section through the plane of an artificial wounding for *P. onkodes* from the ambient pCO_2 treatment. The wound in this sample is outlined in white. The arrow is pointing to a single hypothallial cell within the hypothallus that has formed within the wound. The vertical line is indicating the thickness of the region of vertical growth, composed of the perithallus, intercalary meristem, and epithallus (PME layer). A 200-µm scale bar is

presented in the bottom right corner. *Inset*: Representative portion of the thallus of *P. onkodes* (5000 x). P = perithallus, M = intercalary meristem, and E = epithallus. A 10 µm scale bar is presented in the bottom right corner.

Figure 2.2: Net calcification (mg $CaCO_3$ cm^{-2} day^{-1}) plotted against mean pCO_2 for each flume. The points represent individual samples, and the lines are ordinary least-squared regression lines through the non-transformed data. *P. onkodes* is in black and *L. insipidum* is in red. Wounded samples are solid lines and closed circles, and the non-wounded samples are dotted lines and open circles. *P. onkodes* non-wounded $R^2 = 0.23$, *P. onkodes* wounded $R^2 = 0.14$, *L. insipidum* non-wounded $R^2 < 0.01$, *L. insipidum* wounded $R^2 = 0.01$.

Figure 2.3: Proportion of living tissue remaining plotted against average pCO_2 for each flume. The points represent individual samples, and the lines are ordinary least squared regression lines. *P. onkodes* is in black and *L. insipidum* is in red. Wounded samples are solid lines and closed circles, and the non-wounded samples are dotted lines and open circles. *P. onkodes* non-wounded $R^2 = 0.23$, *P. onkodes* wounded $R^2 = 0.10$, *L. insipidum* non-wounded $R^2 = 0.22$, *L. insipidum* wounded $R^2 = 0.08$.

Figure 2.4: Rate of vertical regeneration (µm day^{-1}) within the wounds of *P. onkodes* plotted against average pCO_2 within the flumes. Points represent individuals within each flume and the line is an ordinary least squares regression line.

Figure 2.5: (A) Scanning electron image (346x) of a cross-section across the plane of an artificial wounding for *P. onkodes* from the ambient pCO_2 treatment. A 200 µm scale bar is presented in the bottom right corner. (B) Scanning electron image (346 x) of a cross-

section across the plane of an artificial wounding for *P. onkodes* from the elevated 1300 µatm pCO_2 treatment. A 200 µm scale bar is presented in the bottom right corner. (C) The ratio of vertical to lateral growth within the artificial wounds of *P. onkodes* plotted against average pCO_2 within the flumes. Points represent individuals within each flume and the line is an ordinary least squares regression line.

Chapter 3

Figure 3.1: Representative images of the five interaction types. P = *P. onkodes,* L = *L. insipidum,* and NL = non-living. From left to right: *P. onkodes* vs. *L. insipidum*, *P. onkodes* vs. *P. onkodes*, *P. onkodes* vs. non-living, *L. insipidum* vs. *L. insipidum*, and *L. insipidum* vs. non-living.

Figure 3.2: Proportion of total live interaction won by the focal individual (*P. onkodes*) plotted against mean pCO_2 (µatm) for the *P. onkodes* vs. *L. insipidum* interaction. Individual points represent individual interaction pairs, with a least-squares trendline fitted to illustrate trends.

Figure 3.3: Proportion of live tissue remaining at the end of the experiment for focal individuals of *P. onkodes* within three interaction types, plotted against mean pCO_2 (µatm). Individual points represent individual interaction pairs, with 3 least-squares trendlines fitted to illustrate trends for each of the three interaction types. *P. onkodes* vs. *L. insipidum* interactions are represented by the black closed circles and black trendline. *P. onkodes* vs. *P. onkodes* interactions are represented by the light gray closed circles and light gray trendline. *P. onkodes* vs. non-living massive *Porites* interactions are represented by the red closed circles and red trendline.

Figure 3.4: Proportion of live tissue remaining at the end of the experiment for focal individuals of *L. insipidum* within two interaction types, plotted against mean pCO_2 (µatm). Individual points represent individual interaction pairs, with three fitted trendline to illustrate trends for each of the three interaction types. *L. insipidum* vs. *L. insipidum* interactions are represented by the black closed circles and black trendline. *L. insipidum* vs. non-living massive *Porites* interactions are represented by the red closed circles and red trendline.

APPENDIX C: FIGURES

Chapter 1

Figure 1.1

Figure 1.2

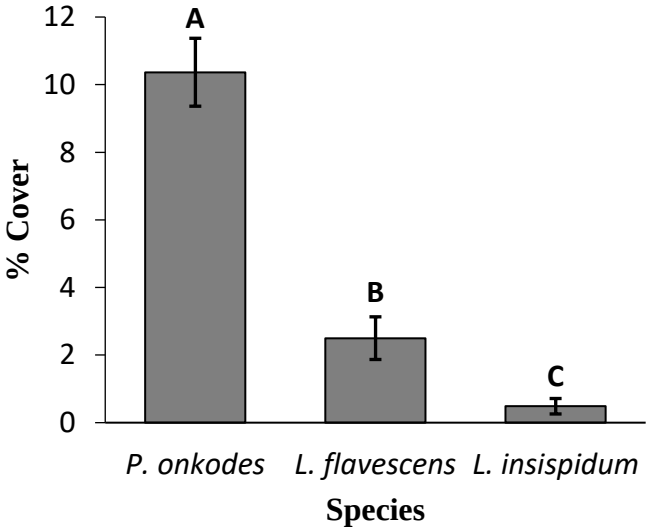


Figure 1.3

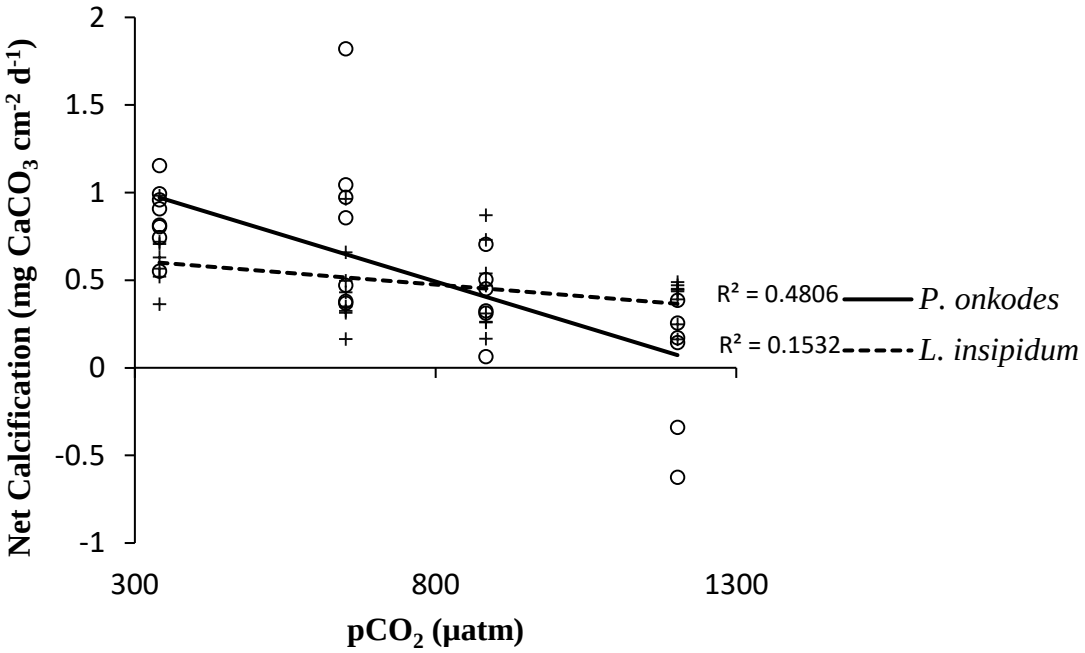
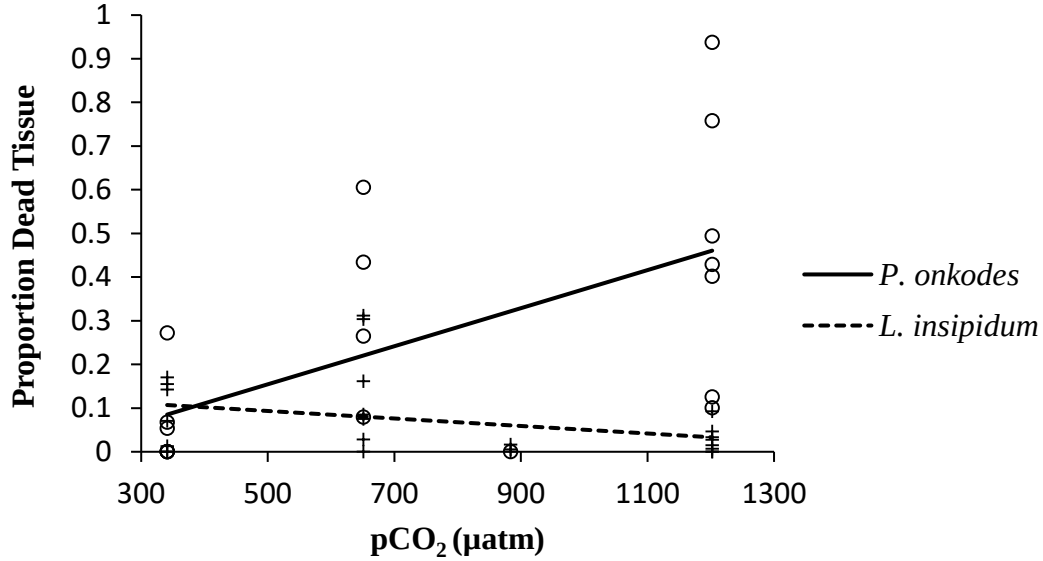
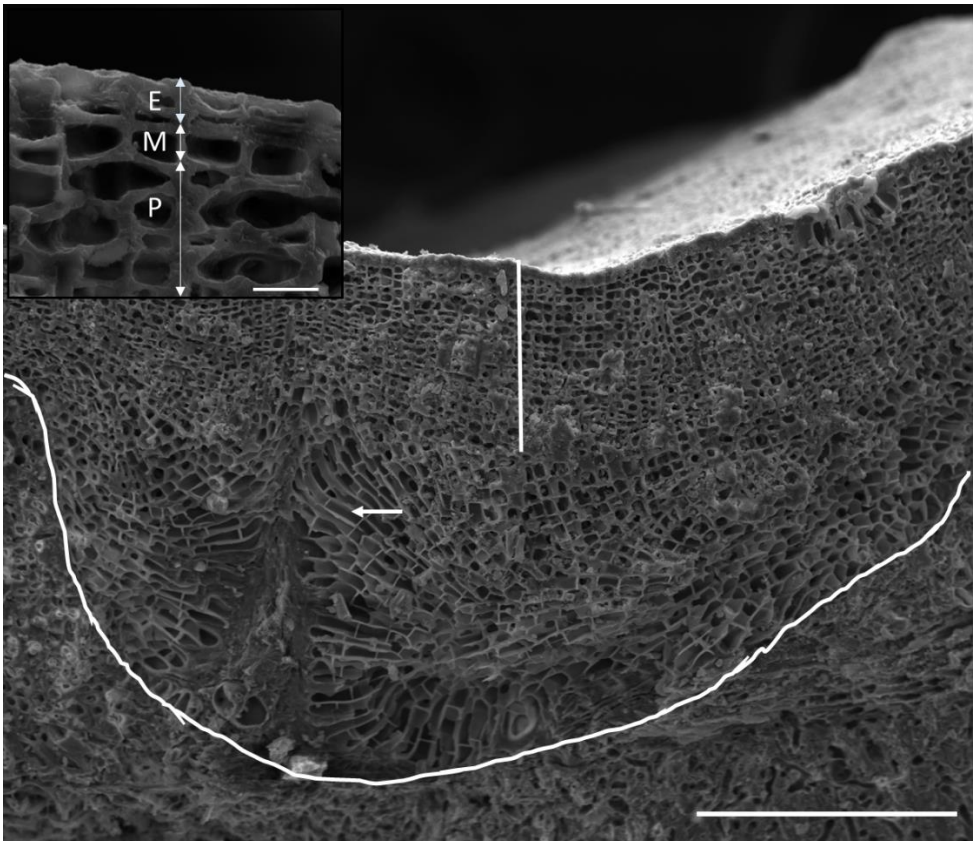


Figure 1.4



Chapter 2

Figure 2.1



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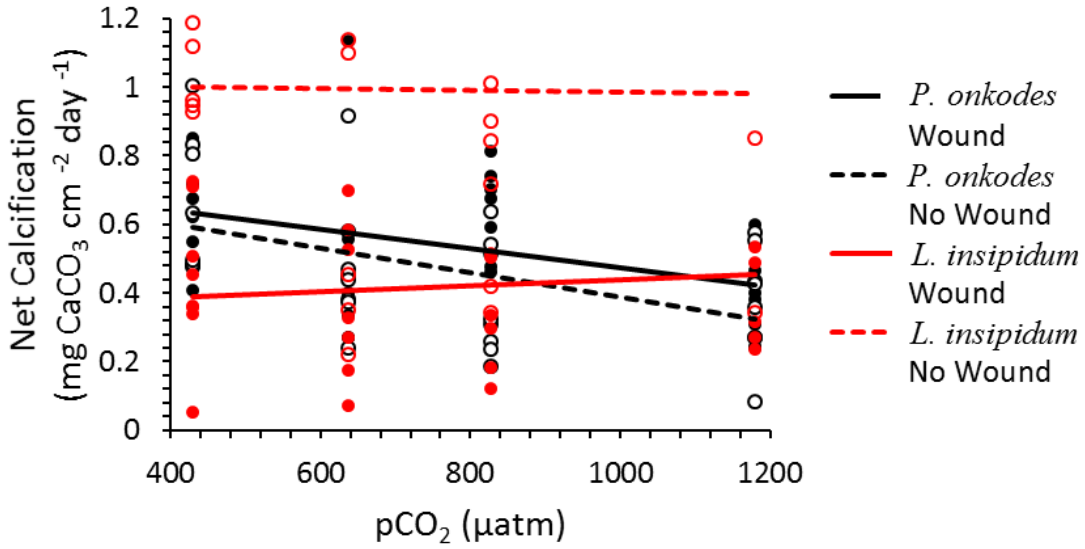


Figure 2.3

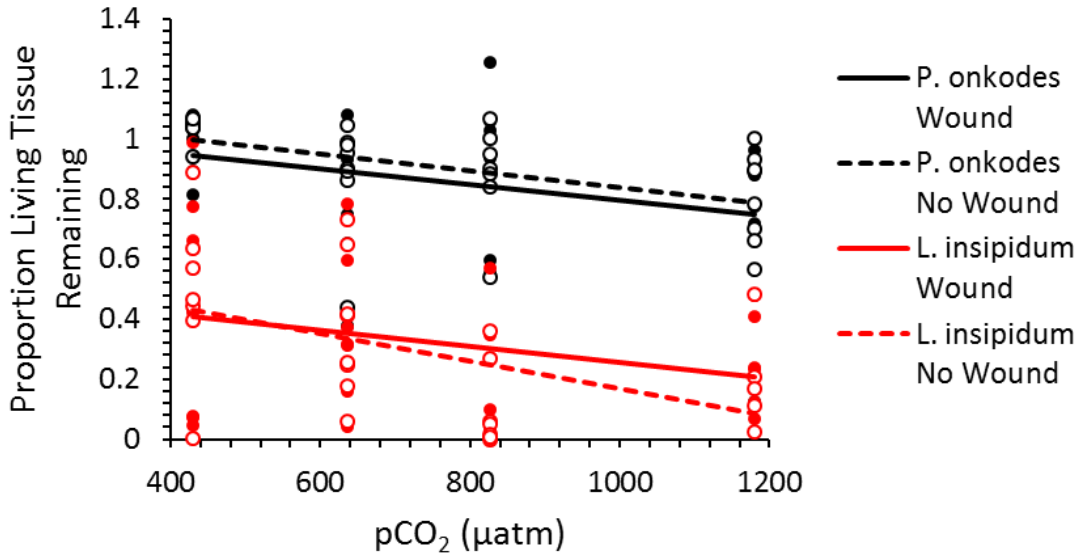


Figure 2.4

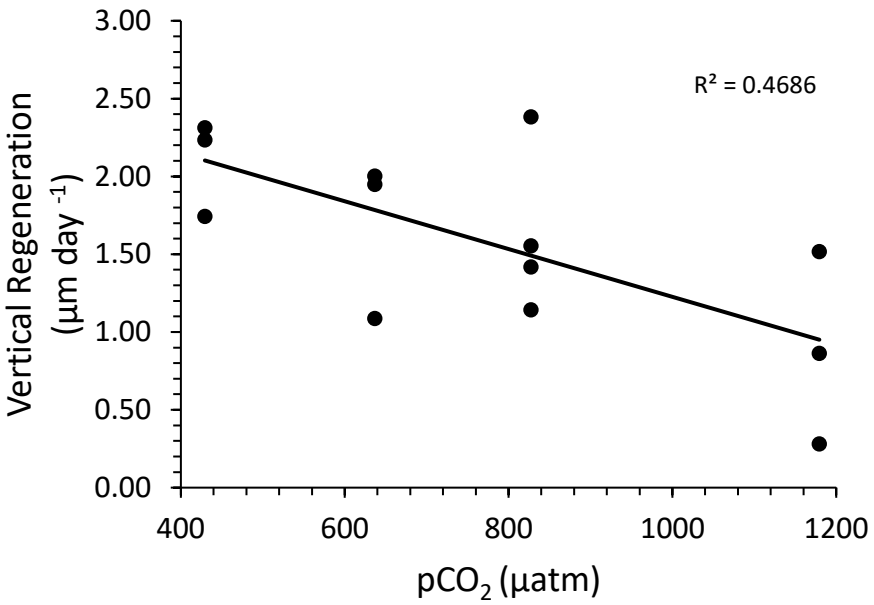
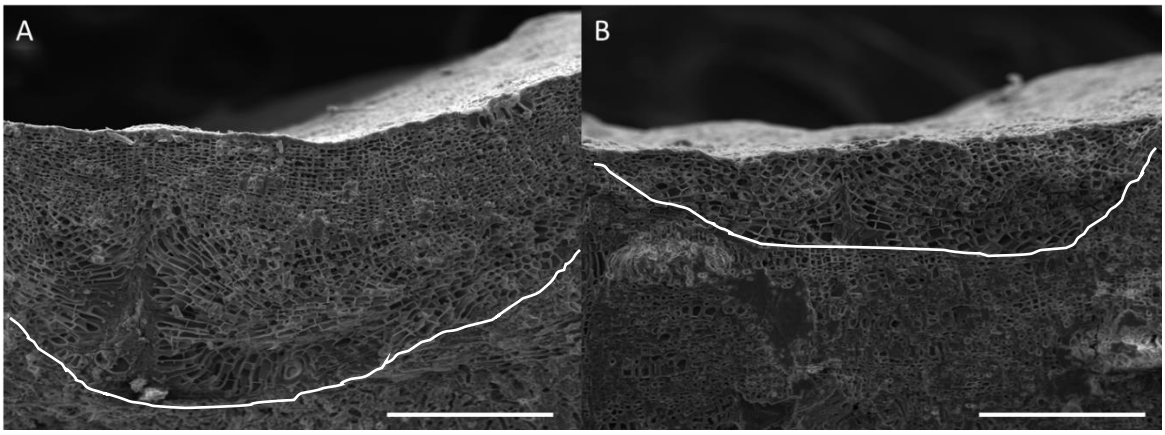
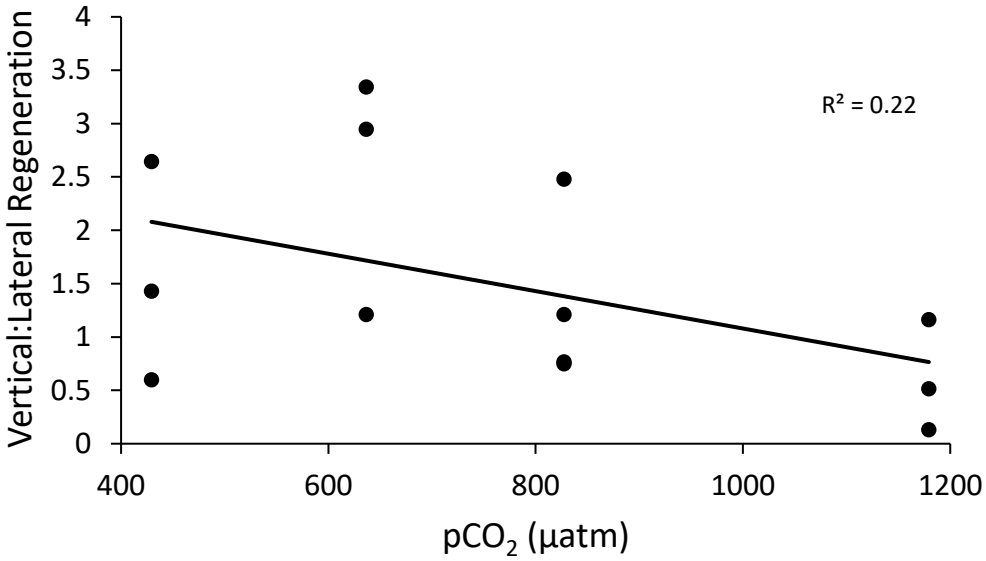

3.00
2.50
2.00
1.50
1.00
0.50
0.00
Vertical Regeneration
(µm day -1)
R² = 0.4686
400
600
800
1000
1200
pCO2 (µatm)

Figure 2.5


A
B

C


Vertical:Lateral Regeneration
4
3.5
3
2.5
2
1.5
1
0.5
0
400
600
800
1000
1200
pCO2 (µatm)
R² = 0.22

Chapter 3

Figure 3.1

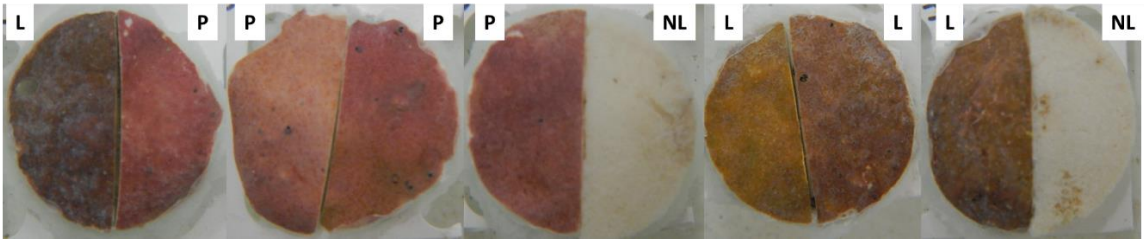


Figure 3.2

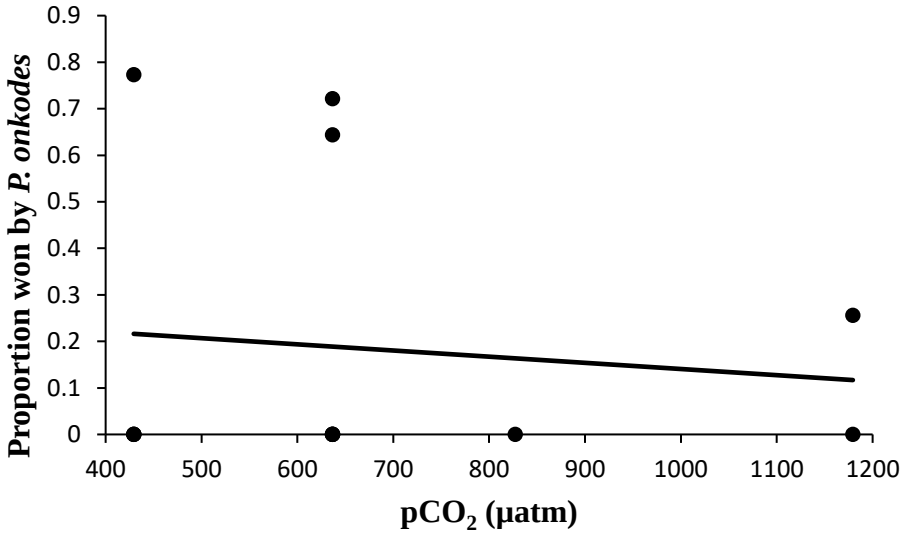


Figure 3.3

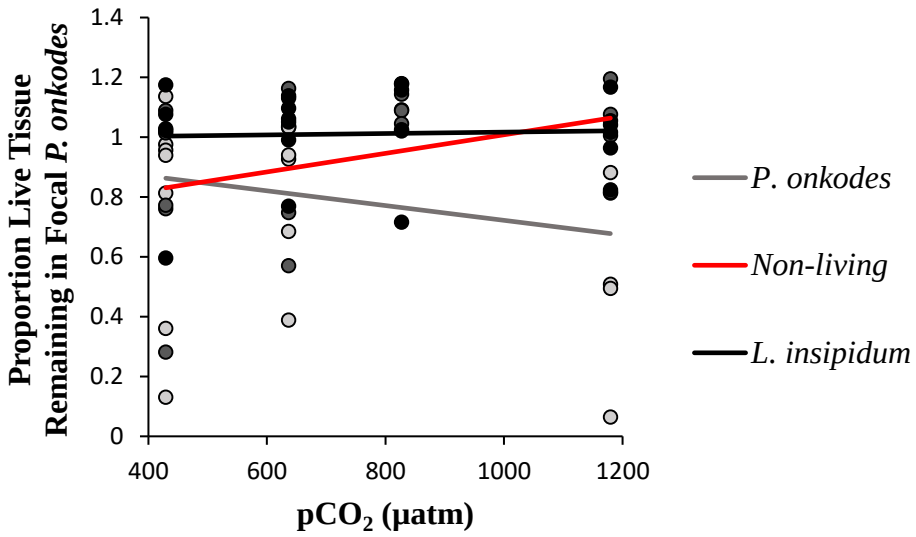


Figure 3.4