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The Combined Effects of Ocean Acidification with Fleshy Macroalgae and Filamentous Turfs on Tropical Crustose Coralline Algae

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

By

Lansing Yun Perng

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The thesis of Lansing Y. Perng is approved by:

Peter J. Edmunds, Ph.D.	Date
Casey P. terHorst, Ph.D.	Date
Robert C. Carpenter, Ph.D., Chair	Date

California State University, Northridge

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ABSTRACT

The Combined Effects of Ocean Acidification with the Presence of Fleshy Macroalgae and Filamentous Turfs on Tropical Crustose Coralline Algae

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Global climate change induces multiple stressors on tropical coral reefs that threaten their persistence. Ocean acidification decreases calcification in most dominant reef builders, such as crustose coralline algae (CCA). Climate change also has the potential to increase the biomass of fleshy macroalgae and filamentous turf in coral reef ecosystems. While fleshy macroalgae and turf may shade, abrade, and have otherwise negative consequences on CCA metabolism, their high rates of photosynthesis may mitigate OA locally through carbon uptake, resulting in a local increase in pH. This thesis explored the effects of OA, combined with the presence of either fleshy macroalgae or algal turfs, on *Lithophyllum kotschyanum*, an abundant species of CCA in Moorea, French Polynesia. In a mesocosm study, three canopy types, clear mimics, dark mimics, and *S. pacificum*, were crossed with two CO_2 levels, ambient (400 μatm) and elevated (1000 μatm). The clear, dark, and *S. pacificum* canopies resulted in stepwise decreases in calcification of *L. kotschyanum*. This response suggests that shading and likely flow moderation decrease CCA calcification. To separate the effects of fleshy macroalgal metabolism from the effects of its physical structure, a

subsequent mesocosm and field experiment were performed. In the mesocosm study, a header tank that provided *S. pacificum*-treated seawater to treatment tanks was used to determine the metabolic effect of *S. pacificum* on *L. kotschyanum*. In the field, *S. pacificum* canopies were attached to 20 × 30 cm grids, upstream from CCA samples. Data from the mesocosm study support a positive effect of carbon uptake by *S. pacificum*, but the metabolic effect did not translate into the field. Because *S. pacificum* was placed in closer proximity to CCA samples in the field than in lab, the difference in *L. kotschyanum* calcification between the mesocosm and field experiment may be due to physical effects of the canopy in the field, such as shading. The combined results of these two studies suggest that upstream macroalgal communities have the potential to mitigate the negative effects of OA to downstream calcifiers, but will not benefit understory calcifiers. Finally, a mesocosm experiment was conducted to address the combined effects of OA and the presence of epiphytic turf algae on host CCA. In a factorial experiment, *L. kotschyanum* samples with and without epiphytic turf algae were placed in flow through tanks where pCO_2 was ambient (400 μatm) or elevated (1000 μatm). Results indicated a significant effect of elevated pCO_2 on CCA calcification and a negative effect of turf presence, despite a higher pH in the presence of turf during light incubations. This indicates that any benefit of higher daytime pH within the DBL of *L. kotschyanum* was outweighed by the negative effects, such as shading, nighttime anoxia and low pH. Overall, these studies indicate that fleshy macroalgae and filamentous turfs can raise seawater pH locally, but any benefit of this effect is outweighed by the negative effects of fleshy macroalgae and turf presence. The only instance during which CCA may incur a net benefit from fleshy macroalgae occurs when calcifiers are situated downstream of a dense

macroalgal community, entirely unaffected by its physical structure. Ultimately, fleshy macroalgae and turf affect CCA negatively, regardless of OA treatment.

Chapter 1

General Introduction

The most biodiverse ecosystems in the marine environment, tropical coral reefs support approximately 25% of all marine species, while covering less than 1% of the benthic space in the ocean (McAllister, 1991; Reaka-Kudla, 2005). These ecosystems have a high level of habitat complexity due to rugose morphologies of foundation species, creating shelter for a vast diversity of organisms (Knowlton et al., 2010). Reefs are also a source of food and medicine and provide an array of vital ecosystem services such as primary production, carbon and nitrogen fixation, and erosion protection (Moberg and Folke, 1999). Coral reefs also facilitate biodiversity and ecosystem functioning in important adjacent ecosystems, such as mangroves and seagrass beds (Guannel et al., 2016). Due to these ecosystem services, along with high demand of tourism and recreation, coral reefs are essential to the global economy, and supply hundreds of thousands of jobs (Dixon et al., 1993; Klein et al., 2004). However, climate change triggered by technological and agricultural development in the past few decades have resulted in the loss of a large portion of coral reefs that have formed over millions of years (Stanley Jr., 1988; Hoegh-Guldberg et al., 2007).

Ocean Acidification

Within recent decades, reefs have been undergoing shifts in calcifier abundance and declines in reef accretion due to global climate change as well as local anthropogenic stressors (Hoegh-Guldberg, 1999; Perry et al., 2013). As a consequence of human activities such as the burning of fossil fuels and deforestation, the concentration of CO_2 in

the atmosphere has risen exponentially since the Industrial Revolution (Solomon et al., 2007; Doney et al., 2009). The ocean has served as the world's largest CO_2 sink, absorbing approximately a third of the CO_2 that has been released (Sabine et al., 2004; Solomon et al., 2007). The resulting increase in seawater pCO_2 initiates a host of carbon chemistry changes that ultimately reduces ocean pH in a process known as ocean acidification (OA). OA occurs as dissolved CO_2 reacts with seawater to form carbonic acid (H_2CO_3), which subsequently dissociates into bicarbonate ions (HCO_3^-) and hydrogen ions (H^+), driving down seawater pH. In order to buffer this pH change, carbonate ions (CO_3^{2-}) in the water react with the excess protons to form more bicarbonate, reducing carbonate concentration in the ocean and lowering carbonate saturation state (Ω) (Hoegh-Guldberg et al., 2007).

Calcifying organisms form coral reef structures by precipitating carbonate ions out of the seawater to build calcium carbonate skeletons, and are therefore sensitive to changes in Ω (Hofmann and Bischof, 2014). As Ω declines, dissolution of carbonate skeletons is favored over precipitation (Orr et al., 2005). Under OA conditions, reef accretion declines as calcifiers lose the ability to precipitate their calcium carbonate skeletons (Johnson and Carpenter, 2012; Comeau et al., 2014). At the current rate of CO_2 emissions on which Representative Concentration Pathway (RCP) 8.5 is based, a three-fold increase in atmospheric carbon dioxide from pre-industrial levels is predicted to occur by the end of the current century. Ocean pH is projected to decrease by up to 0.40 units, with pCO_2 levels reaching ~1000 μatm, compared to pre-industrial levels of ~280 μatm (Moss et al., 2010; IPCC, 2014). Experimental studies suggest that even a doubling of pre-industrial carbon dioxide levels in the atmosphere can be accompanied by a ~40%

decrease in reef net calcification, and some reefs may even experience skeletal dissolution (Kleypas and Langdon, 2006; Silverman et al., 2009; Pandolfi et al., 2011).

Coralline Algae

Coralline algae comprise vital reef building taxa that are ubiquitous in a vast majority of tropical marine habitats (Littler, 1976). Most calcifying algae belong to the Phylum Rhodophyta (Family Corallinaceae) and can have distinct morphologies that are either crustose or branching. Branching taxa can increase reef habitat complexity and provide habitat space for small invertebrates and cryptofauna, while crustose coralline algae (CCA) can increase reef structural stability and resistance to wave erosion (Adey, 1998; Enochs and Manzello, 2012). CCA can cover as much as 40% of the benthic space on a coral reef, providing habitat and food for a variety of reef organisms, contributing ecologically significant levels of primary production and accretion to the reef ecosystem, and facilitating coral settlement (Bosence, 1983; Fabricius and De'ath, 2001; Chisholm, 2003; Price, 2010). Coralline algae are particularly sensitive to OA because their skeletons are formed by precipitating high-magnesium calcite, the most soluble form of $CaCO_3$ in seawater (Hofmann and Bischof, 2014). Because they are slow calcifiers relative to corals, secreting $CaCO_3$ at an approximate rate of 0.03 – 0.37 g cm^{-2} y^{-1} (Chisholm, 2000; Comeau et al., 2013b), coralline algae may be unable to persist under OA-driven dissolution predicted under pessimistic OA projections (Anthony et al., 2008; Diaz-Pulido et al., 2012; IPCC, 2014). As OA slows CCA calcification, crusts may become thinner, leaving the reef structure less resistant to physical disturbance and more vulnerable to collapse (Steneck, 1986).

For coralline algae, the metabolic processes of calcification, photosynthesis, and respiration are thought to be tightly-linked, and the three must be explored together to promote a complete understanding of how OA will affect reef builders. As OA decreases carbonate concentration in seawater and reduces calcification by CCA, it also increases the availability of CO_2 and bicarbonate for use in CCA photosynthesis (Hoegh-Guldberg et al., 2007). In turn, CCA photosynthesis and respiration affect calcification by changing seawater pH in opposing directions at the thallus surface, thus altering Ω at calcification sites (Chisholm, 2003; Comeau et al., 2013a). Additionally, photosynthesis and respiration may display a mutually beneficial relationship, as one process utilizes the products of the other (Kuhl et al., 1996). Currently, studies examining photosynthesis and respiration of calcified algae under OA have demonstrated variable responses, ranging from positive to negative, and some reporting no response (Semesi et al., 2009; Yildiz et al., 2013; Johnson et al., 2014). As climate change continues to impact coral reefs negatively, it is critical to examine physiological responses of important reef builders, such as CCA, and ensuing changes in reef accretion and carbon cycling.

Benthic associations between calcified and non-calcified macroalgae

As climate change lowers seawater pH and raises seawater temperature, the competitive abilities of corals and other reef calcifiers decrease due to poor calcification conditions (Doney et al., 2009; Fisher et al., 2012). Global stressors like OA and warming are accompanied by local stressors such as overfishing and pollution, whose combined effects suppress the growth of reef builders while favoring the proliferation of fast-growing, non-calcifying (fleshy) macroalgae (McCook, 1999; Nyström et al., 2000;

Szmant, 2002; Hughes et al., 2003). Future environmental conditions may promote shifts from coral to macroalgal-dominated states, vastly reducing habitat complexity, biodiversity, and the provision of ecosystem services (McManus and Polsenberg, 2004; Bruno et al., 2009; Cheal et al., 2010; Pratchett et al., 2014).

Coral reef benthic community structure may transform as canopy-forming macroalgae shade and smother newly settled spat and juvenile corals, as well as calcareous algae (Hughes et al., 2006; Vogel et al., 2015; Smith et al., 2016). Reduced light availability has been shown to reduce calcification of CCA, likely due to slower photosynthetic rates affecting pH at the surface of the calcifying individual (Comeau et al., 2014; Vogel et al., 2015). Abrasion by non-calcified algae on corals has also been observed, along with facilitation of microbial activity as a result of DOC release (Mumby and Box, 2007; Haas et al., 2011). Additionally, canopy structure can modulate water flow to understory calcifiers and increase the thickness of the diffusion boundary layer (DBL) (Gardella and Edmunds, 2001). The DBL is a layer of water at the surface of an organism through which metabolites such as HCO_3^- and CO_2 are transferred between the organism and the mainstream seawater by diffusion (Dennison and Barnes, 1988; Lesser et al., 1994). Boundary layer dynamics affect the seawater chemistry experienced by the organism, as well as the rate of metabolite exchange between the organism and its environment (Cornwall et al., 2015). Because slower flow favors thicker DBLs, macroalgal canopies may ultimately reduce the rate of CCA metabolite exchange, slowing calcification, photosynthesis, and respiration (Koch, 1994). However, thicker DBLs may also ameliorate the effects of OA as CCA photosynthesis maintains high pH, relative to mainstream seawater, in the DBL (Cornwall et al., 2014). Under present

carbonate conditions in seawater, flow attenuation by canopy-forming macroalgae may be detrimental to understory CCA due to the reduction of metabolic rates; in future OA conditions, however, thicker DBLs may temper the effect of low pH.

The increase of non-calcified macroalgal abundance on coral reefs is regarded as a sign of habitat degradation, as it reduces reef structural complexity and thus, biodiversity (McManus and Polsenberg, 2004). When unchecked by herbivory, fleshy macroalgae may outcompete reef building corals and calcifying algae for benthic space (Hughes et al., 2007; Houk et al., 2010). Due to high rates of photosynthesis, however, fleshy algae have the potential to promote calcification by acting as a local carbon sink. Under OA conditions, macroalgae may have the capacity to create microhabitat refuges through metabolic moderation of dissolved inorganic carbon (DIC), favoring higher calcification rates than in the acidified mainstream seawater. During photosynthesis, the uptake and dehydration of bicarbonate (HCO_3^-) to usable CO_2 can increase seawater pH locally by reducing the concentration of H^+ ions in the surrounding seawater, countering the effect of OA and increasing the carbonate saturation state (Anthony et al., 2011). A common species of non-calcifying macroalgae found abundantly on Pacific reefs is *Sargassum pacificum*, a fucoid phaeophyte that forms dense aggregations on reef crests and on the tops of coral bommies (Mattio et al., 2008, 2009). Due to its abundance, *S. pacificum* likely interacts with a variety of calcified species, including coralline algae, and represents an ideal species for examining the effects of canopy-forming maroalgae on CCA.

Benthic associations between calcified algae and epiphytic turf algae

Another highly prevalent functional group of fleshy algae found on coral reefs comprises a group of highly productive, filamentous algae that forms cropped, extensive, and fast-growing mats, also known as algal turfs (Connell et al., 2014). This type of algae are opportunistic and are often initial colonizers of newly available benthic space after a disturbance (Kendrick, 1991; Airoldi, 1998). On coral reefs, algal turfs often are found growing on dead coral and live coralline algae and can cover 40 – 50% of the benthos (Airoldi, 2000; Rogers and Miller, 2001; Vroom and Braun, 2010; Smith et al., 2016). Some types of filamentous turf, particularly when grown in the absence of herbivory, impede coral settlement and recruitment through competition for space and associated microbial activity (Birrell et al. 2005; Vermeij et al. 2008). Epiphytic algal turfs have also been shown to impede recruitment and growth of calcifying algae by overgrowing live tissue (Kendrick, 1991; Kuffner et al., 2008). These dense mats of turf algae can shade calcareous algae, reducing photosynthesis and, consequently, calcification (Bulleri, 2006). CCA photosynthesis is thought to facilitate calcification within the same individual by increasing pH at the thallus surface, thus increasing Ω at calcification sites (Chisholm 2003; Comeau et al. 2013), and by supplying needed energy to support ion transport (Borowitzka, 1981). The close association of turf algae, an epiphytic group, with CCA allows their physical structure to reduce water flow at the CCA thallus surface, which thickens the DBL and reduces the rate of metabolite exchange (Koch, 1994; Gardella and Edmunds, 2001; Hurd and Pilditch, 2011; Cornwall et al., 2015). To counter these effects, certain species of CCA have an antifouling mechanism in which they actively slough off their outer layer to remove epiphytes (Keats et al. 1997).

Like fleshy macroalgae, epiphytic turf algae may act as a local carbon sink under OA conditions, exhibiting a mutualistic relationship with the CCA on which they grow. Relative to their percent cover, turf biomass is generally low. However, as an epiphytic group, turf algae exhibit an exceptionally close association with CCA, causing the effects of turf to be extremely localized on the thallus surface of CCA. During photosynthesis, algal turfs have the potential to create higher pH microenvironments within the DBL than the mainstream seawater, thus sheltering CCA from the effects of OA (Short et al., 2015). Therefore, turf algae can potentially facilitate CCA calcification by strengthening the protective barrier provided by the boundary layer and creating a refuge for CCA from acidification. Conversely, algal turfs have also been shown to facilitate microbial activity at the thallus surface of host CCA, which may lead to hypoxia within the DBL of host CCA (Haas et al., 2011). In CCA with more complex morphologies, algal turfs may affect separate regions of the thallus in different ways. Turf often fill in crevices and cracks of their host organisms because these low flow environments facilitate spore settlement and offer effective protection from herbivory (Hay, 1981). As a result, CCA with branching morphologies may exhibit more noticeable responses to turf algae between branches than at branch tips. In this manner, algal turfs have the potential to alter CCA morphology over longer periods of time as differential growth rates occur in different regions of the thallus.

Thesis Objectives

OA conditions are expected to worsen as environmental conditions change, and fleshy macroalgae, as well as filamentous turfs, are expected to become more abundant

on coral reefs. The combined effects of abiotic shifts in seawater chemistry and biotic community changes impose increasingly stressful environmental conditions on coral reefs. Under these conditions, studies of physiological responses of CCA, a key taxon in the most biodiverse marine environment, become indispensable in predicting future coral reef community structure. The objective of this thesis was to elucidate how two growing environmental stressors on coral reefs, ocean acidification and increased algal (fleshy and turf) abundance, may interact to alter the physiology of an important reef builder, coralline algae. A series of complementary experiments were performed between May 2016 and August 2018 in Moorea, French Polynesia. These studies were designed to explore the effects of OA and non-calcified algae on calcification, photosynthesis, and respiration of *Lithophyllum kotschyanum*, an abundant crustose coralline algal taxon in these reef habitats that has been shown to be sensitive to OA (Comeau et al., 2014).

In Chapter 2, I explored the combined effects of OA and the presence of a fleshy macroalgal canopy on crustose coralline algae. Three canopy types, a clear mimic, a dark mimic, and a live *S. pacificum* canopy, were used to differentiate between the physical and biological effects of a macroalgal canopy on understory CCA. These treatments were crossed with two levels of pCO_2, ambient (400 µatm) and elevated (1000 µatm), in a 23-day mesocosm experiment, after which CCA photosynthesis, respiration, and calcification were measured. I predicted that *S. pacificum* metabolism would work to ameliorate the effects of OA on *L. kotschyanum*, while the physical structure would impede CCA metabolism.

In Chapter 3, a laboratory and subsequent field experiment were conducted with the objective of isolating the chemical effect of fleshy macroalgae on downstream

calcifiers without interference from the effects of its physical structure. A short-term mesocosm experiment was performed using a header tank containing *S. pacificum* that drained into treatment tanks of *L. kotschyanum*. Using this method, CCA samples experienced seawater with pH and O_2 levels modified by macroalgal canopy photosynthesis and respiration without any effects of canopy physical structure, such as shading, abrasion, and flow moderation. To determine whether this effect would translate into a natural setting, a 6-week long field experiment was conducted during which *S. pacificum* thalli were placed upstream of *L. kotschyanum* samples on plastic grids attached to coral bommies. The field experiment also examined the effect of natural flow variations on *L. kotschyanum* by placing grids in both a high flow reef crest habitat, as well as a lower flow back reef habitat. I predicted that *S. pacificum*-treated water would affect CCA metabolism in both the laboratory and the field, the field response would be nominal in comparison with the laboratory study, and that the field response would differ based on habitat treatment.

In Chapter 4, the effects of turf algae on *L. kotschyanum* were explored under OA conditions. Two turf treatments, present and absent, were crossed with two pCO_2 treatments, ambient (400 µatm) and elevated (1000 µatm), in a 26-day mesocosm study. Calcification and linear growth were measured at the end of the experiment, and boundary layer pH was quantified. Photosynthesis and respiration were not measured in this study due to the lack of observable responses in the previous two studies. I predicted that epiphytic turf algae would increase CCA calcification and growth between CCA branches, where turf are abundant.

Chapter 2

Effects of ocean acidification and canopy-forming macroalgae on the crustose coralline alga, *Lithophyllum kotschyanum*

Introduction

Coral reefs are critically important and biodiverse marine ecosystems created by the biological activity of benthic calcifiers, primarily corals (Reaka-Kudla and Wilson, 1997; Knowlton et al., 2010). High levels of primary productivity and structural complexity in coral reefs allow them to sustain a disproportionately high species diversity, provide important ecosystem goods and services, and supply billions of dollars to the global economy (Dixon et al., 1993; Moberg and Folke, 1999; Klein et al., 2004). Unfortunately, vital ecosystem services, as well as species endemic to these ecosystems, are under threat due to climate change and other anthropogenic impacts (Roberts et al., 2002; Hughes et al., 2003). A large proportion of CO_2 released by human activities has been absorbed by the ocean, increasing seawater pCO_2 globally and reducing ocean pH in a process known as ocean acidification (OA) (Sabine et al., 2004; Hoegh-Guldberg et al., 2007; Doney et al., 2009). As ocean pH continues to decline globally, coral reefs experience a slow transition from net reef accretion to dissolution, resulting in substantial loss of habitat and biodiversity (Pandolfi et al., 2011; Perry et al., 2013; Fabricius et al., 2014).

OA induces a decline in reef net calcification due to a decrease in carbonate ions that reef builders use to precipitate their skeletons (Johnson and Carpenter, 2012; Comeau et al., 2014). The absorption of atmospheric CO_2 into the ocean initiates a series of chemical reactions that result in increased hydrogen (H^+) and bicarbonate ion (HCO_3^-) concentrations,

ultimately decreasing carbonate ion (CO_3^{2-}) concentration and lowering the carbonate saturation state (Ω) (Hofmann and Bischof, 2014). Calcification rate is directly related to Ω, with low values favoring dissolution of carbonate skeletons and high values favoring precipitation (Hoegh-Guldberg, 1999; Orr et al., 2005). By the end of the current century, ocean pH is projected to decrease by up to 0.40 units in a business-as-usual scenario (Representative Concentration Pathway 8.5), accompanied by Ω levels too low to sustain reef accretion (Raven et al., 2005; Hoegh-Guldberg et al., 2007; IPCC, 2014).

Calcifying algae are a vital and prevalent component of coral reef community structure, sometimes covering more than 20% of the reef surface (Fabricius and De'ath, 2001). Particularly, crustose coralline algae (CCA) increase reef structural stability by binding together dead coral, sand, and debris to create solid substrata and new habitat for small invertebrates and cryptofauna (Adey, 1998; Nelson, 2009; Enochs and Manzello, 2012). These ecosystem engineers provide food and shelter for reef fishes and invertebrates, facilitate coral larval settlement, and enhance reef productivity and accretion (Bosence, 1983; Chisholm, 2003; Price, 2010). Due to their use of the most soluble mineral of $CaCO_3$ in calcification, high-magnesium calcite, coralline algae are particularly sensitive to OA (Hofmann and Bischof, 2014). Under OA-driven dissolution, coralline algal abundance and diversity may decrease, resulting in lowered biodiversity and structural stability of the coral reefs on which they reside.

While CCA calcification directly affects reef accretion as well as structural stability and complexity, other physiological responses such as photosynthesis and respiration are also important measures of reef function and contribute to net community metabolism. Although OA negatively affects calcifiers, it increases the availability of inorganic carbon in seawater

for use in photosynthesis (Hoegh-Guldberg et al., 2007). Within a single CCA individual, photosynthesis and respiration also can affect calcification by moderating seawater pH at the thallus surface, thus altering Ω at the sites of calcification (Chisholm, 2003; Comeau et al., 2013a). Lastly, rates of photosynthesis and respiration may be positively correlated, as one process utilizes the products of the other (Kuhl et al., 1996). To understand how coral reefs will respond to OA, it is necessary to assess the changing physiology of calcifying algae and consequent effects on reef community structure and net accretion.

As ocean pH becomes more acidic and temperatures increase, environmental conditions become less conducive to the growth of calcifying organisms on which coral reef frameworks rely (Doney et al., 2009; Fisher et al., 2012). Additionally, local overfishing suppresses herbivory on reefs, promoting the proliferation of fast-growing, non-calcifying (fleshy) macroalgae (Wooldridge et al., 2005; Hughes et al., 2006). Local nutrient addition due to agricultural runoff and pollution can exacerbate the effects of overfishing by expediting fleshy macroalgal growth while negatively impacting coral growth and reproduction (Koop et al., 2001; Fabricius, 2005; Jessen et al., 2013). Combined, these environmental conditions may facilitate shifts from coral to macroalgal-dominated states, leading to a major loss in marine biodiversity and ecosystem services (McManus and Polsenberg, 2004; Bruno et al., 2009; Cheal et al., 2010; Pratchett et al., 2014). Restructuring of the reef benthos may occur as canopy-forming macroalgae shade and smother newly settled spat and juvenile corals, as well as calcareous algae (Mumby and Box, 2007; Vogel et al., 2015). Beneath these canopies, low light levels may hinder photosynthesis of understory CCA, thus decreasing pH at the CCA thallus surface and reducing calcification rates (Borowitzka, 1981; Flukes et al., 2014).

Additionally, calcifiers growing in the understory of a macroalgal canopy experience reduced water flow, which may interact with OA to alter CCA physiology (Comeau et al., 2014). Prior studies have shown that increased water velocity accelerates metabolic processes such as calcification, photosynthesis, and respiration (Jokiel, 1978; Dennison and Barnes, 1988; Patterson et al., 1991). Despite known effects of water flow on calcifier metabolism, its effect on calcifiers has rarely been studied in combination with OA. Slow flow at the CCA thallus surface thickens the diffusion boundary layer (DBL), a region where the exchange of solutes and gases between an organism and the bulk seawater is dominated by molecular diffusion (Cornwall et al., 2015). Thicker DBLs lead to reduced metabolic rates, but decrease organismal sensitivity to stressors in the mainstream seawater, such as low pH (Koch, 1994; Gardella and Edmunds, 2001). Photosynthesis in CCA allows individuals to modify their boundary layer pH and counteract the negative effects of OA, particularly under slow flow conditions. Under ambient seawater pH, flow moderation due to the physical canopy structure may be detrimental to CCA metabolism; under future OA conditions, however, thicker DBLs may ameliorate the effect of low pH.

While non-calcified macroalgae in coral reefs compete for benthic space with corals and calcifying algae, they can also regulate local pH conditions through daytime photosynthesis and nighttime respiration (Christopher E Cornwall et al., 2013). Within a single macroalgal thallus, photosynthesis generally evolves O_2 at a greater rate than respiration uptakes O_2, resulting in a net pH increase (Burris, 1977; Cheshire et al., 1996; Kleypas et al., 2011). Daytime photosynthesis by a dense macroalgal assemblage elevates seawater pH through the uptake and dehydration of bicarbonate (HCO_3^-) to usable CO_2 (Axelsson and Uusitalo, 1988). This lowers H^+ ion concentration and increases carbonate

saturation state in the surrounding seawater, potentially offsetting the effect of OA on seawater carbon chemistry (Anthony et al., 2011). Therefore, a community of canopy-forming macroalgae has the capacity to create microhabitat refuges from low seawater pH for calcifiers through the moderation of dissolved inorganic carbon (DIC). Under OA conditions, these localized regions of macroalgal photosynthesis may facilitate higher calcification rates of understory CCA than in acidified mainstream seawater. The carbon moderation effect of macroalgal metabolism may be greater under OA conditions, as there is higher availability of carbon for photosynthesis (Zou et al., 2011). Under ambient pH conditions, CCA calcification is not hindered and macroalgal photosynthesis may occur slower than under elevated pH conditions. Therefore, the effect of pH moderation by fleshy macroalgae on CCA calcification may be stronger under OA conditions.

In many Pacific coral reefs, *Sargassum pacificum* is a common fucoid phaeophyte that forms dense canopies at shallow depths of coral reef lagoons, where light availability is high (Mattio et al., 2008, 2009). A variety of calcified species, including coralline algae, occurs in these same reef habitats and likely interacts with the common canopy-forming *S. pacificum*. *Lithophyllum kotschyanum* is an abundant crustose coralline algal taxon that has been observed in close proximity with as well as in the understory beneath *S. pacificum*, and exhibits sensitivity to OA (Comeau et al., 2014) (Fig. 1). To assess the effectiveness of non-calcified algae in mitigating OA effects on calcifiers by acting as a natural carbon sink, the present study separately examined the physical and chemical effects of a *S. pacificum* canopy on understory CCA, *L. kotschyanum*. The goal of the study was to evaluate whether canopy-forming macroalgae have the potential to mitigate the effects of OA on understory CCA. I hypothesized that: 1) canopy shading would reduce net calcification and photosynthesis, and

increase respiration of *L. kotschyanum*, 2) the effect of metabolic moderation of dissolved CO_2 by *S. pacificum* on *L. kotschyanum* calcification would depend on CO_2 treatment, and 3) elevated pCO_2 would affect CCA calcification negatively, but that this effect would be diminished by the presence of *S. pacificum*.

Methods

The effects of ocean acidification combined with the presence of a macroalgal canopy on CCA were quantified in a mesocosm study. The laboratory experiment separately addressed the effects of *S. pacificum* metabolism, physical structure, and shading on metabolic rates of understory CCA. The objective of this experiment was to identify whether canopy metabolism or structure alters the effects of OA by exploring potential interactions between OA and both the physical and biological effects of canopy macroalgae.

Sample collection and preparation

Using a hammer and chisel, 144 individuals of *L. kotschyanum* were collected at depths of ~1-2 m from the fringing reef on the north shore of Moorea, French Polynesia. At the Richard B. Gump South Pacific Research Station, samples were cut to similar sizes (2-4 cm W/L) using a diamond bandsaw (Gryphon C-40, California, USA), cleaned of epibionts, and attached to plastic supports using Coral Glue (EcoTech Marine, Pennsylvania, USA). Following a 7-day acclimation period in a seawater table under ambient light, with a constant inflow of fresh seawater, samples were randomly assigned to the treatments described below (n=12 per tank).

Treatment maintenance

Twelve, 150-L mesocosm tanks (Aqualogic, San Diego, CA) were maintained under two replicated pCO_2 levels (ambient = 400 µatm; elevated = 1000 µatm) crossed with three replicated canopy treatments (*S. pacificum*/dark mimic/clear mimic). Fresh seawater, pumped from Cook's Bay at 12-m depth and sand-filtered (pore size ~550 µm) was used in the

ambient pCO_2 treatment, while the elevated treatment was based on pessimistic end-of-century projections (IPCC, 2014). In the live canopy treatment, freshly-collected *S. pacificum* was used and weighted at the holdfasts. The biomass of *S. pacificum* was chosen to mimic the highest densities (g/m^2) recorded from back reef field sampling. In a dense patch of *S. pacificum*, thalli covering 0.25 m^2 of the benthos were collected and wet weighed in the laboratory. The resulting biomass, ~1000 g, was used in the mesocosm tanks, which are also 0.25 m^2 at the base. To simulate the physical structure and shading effect of *S. pacificum*, dark mimics were constructed from plastic dowel rods (Poly-Dowels, USA) and dark green flagging tape (Tape Brothers, FL, USA). Clear mimics, constructed from plastic dowel rods and strips of clear plastic sheeting (Husky, USA), were used to simulate the canopy structure without the shading effect. Seawater entered the mesocosms at a mean (± SE) rate of 310 ± 1.2 mL min^{-1}, resulting in a full tank turnover of water every 8 hours. Additionally, flow within each tank was created using a pump (Rio 8HF, 2082 L h^{-1}). Each tank received a constant rate of air bubbled in through airstones and was fitted with a plexiglass lid to minimize gas exchange at the water surface. Tank temperatures were maintained at ~27 °C to match austral winter temperatures at the study site (Washburn, 2018). Photosynthetically Active Radiation (PAR, 400-700 nm) was provided on a 12:12 h light:dark photoperiod with 4-h ramp times, using 75W Light Emitting Diode (LED) modules (Sol White LED Module, Aquaillumination, IA, USA) that supplied ~600 μmol quanta m^{-2} s^{-1} individually to each tank. PAR in each tank was measured with a 2π underwater sensor (model LI-192 UWQ 7060) attached to a LI-COR LI-1400 data logger.

Throughout the 23-day experiment, seawater parameters were measured daily and CCA samples and algal mimics were cleaned and rearranged haphazardly every 2-4 days to

minimize positioning effects. *S. pacificum* canopies were refreshed weekly to maintain high rates of photosynthesis. During the experiment, pH fluctuations throughout the day were monitored in the *S. pacificum* tanks from ~8:00 to ~20:00 to determine canopy metabolism effects on ambient seawater pH as well as pH beneath the canopies. Every other day, water samples (10 mL) were drawn from locations above and beneath each *S. pacificum* canopy every ~2 hours with syringes. The pH value of each sample was calculated by measuring temperature (Traceable Digital Thermometer, Fisher Scientific, accuracy: 0.05 °C, resolution: 0.01 °C) and mV (Thermo-Scientific Orion 3-star portable meter with Mettler Toledo InLab Expert Pro probe, calibrated on the total scale with TRIS buffers at a salinity of 35.0 psu). The elevated pCO_2 treatment was maintained by a pH-stat aquacontroller (Neptune Systems, Morgan Hill, CA) that bubbled pure CO_2 into the mesocosm tanks when pH rose above the set point. Daily pH values of all tanks were calculated from temperature and mV as outlined above. Every other day, water samples were collected from each tank to measure salinity (Orion Star A212 Conductivity Benchtop Meter) and total alkalinity (A_T). A_T was measured using open-cell potentiometric titrations (Mettler Toledo T50 titrator with a Mettler Toledo DGi 115-SC probe) on 50-mL aliquots of treatment seawater (Dickson et al., 2007). Accuracy and precision of titrations were determined using titrations of certified reference materials (A. G. Dickson batch 158), which yielded A_T values within 9.3 μmol kg^{-1} of the certified value (SD = 3.03 μmol kg^{-1}; n= 15). Carbonate system parameters were calculated daily for each treatment tank from A_T, pH, temperature, and salinity measurements using the R package seacarb (Lavigne and Gattuso, 2012).

Response variables

Net calcification, photosynthesis, and respiration were measured to determine the effect of metabolically altered seawater carbon chemistry on the physiology of *L. kotschyanum*. All response variables were standardized to surface area (SA), which was quantified using the dye-dipping method (Hoegh-Guldberg, 1988). Samples were dipped in a solution of methylene blue dye (0.1 g 100 mL^{-1} with ~2.5% Triton X-100 detergent) and rinsed in 100 mL of seawater. Using a spectrophotometer (Shimadzu UV-2450), the absorbance of each seawater sample was measured at 620 nm and compared to a standard curve constructed from foil pieces of known surface areas. Sample SA then was predicted from the resulting standard curve ($y = 355.2x - 17.99$, regression of absorbance to known SA). Mean calcification over the entire experiment was measured using the buoyant weighing method (Davies, 1989). Initial and final buoyant weights (Mettler Toledo PB303-S balance, resolution: 0.001 g) of each sample were recorded, and the differences (weight change) were converted to dry weights using the measured density of seawater and a density of pure calcite of 2.71g cm^{-3}.

Photosynthesis and respiration rates were measured by incubating each sample in chambers in the light and dark, respectively. Samples were placed in 250-mL acrylic chambers and incubated with water from the corresponding treatment tanks. To avoid interference with oxygen measurements, canopies were not used during incubations. A constant temperature of 27 °C was maintained by a water jacket surrounding the chamber that received circulating flow from a chilled water bath. Stir bars at the base of each chamber, separated from samples by a plastic mesh, maintained constant water mixing during incubations. Oxygen concentrations were recorded every second by PreSens (Precision

Sensing GmbH, Germany) oxygen optodes and temperature probes attached to Fibox 3 minisensor oxygen meters. The probes were calibrated using a 2-point calibration in water-saturated air (100% air saturation) and water supersaturated with sodium hydrosulfite (0% air saturation). To measure photosynthesis, incubations were performed in saturating light (~200 μmol quanta m^{-2} s^{-1}), estimated with PI curves calculated using a Walz Diving PAM. Incubation light levels were similar to maximum light levels in the treatments, supplying ~600 μmol quanta m^{-2} s^{-1} using two fiber optic halogen lights (Ace 1, Schott North America, Inc.). Respiration rates were measured in dark incubations, during which lights were turned off and chambers were covered with black plastic. Incubations ended when consistent slopes (ΔO_2/time) were achieved, representing stable metabolic rates following a 5-minute acclimation period. Control incubations were performed with only treatment seawater and these O_2 fluxes were subtracted from the rates measured in the algal incubations. Metabolic rates were calculated from linear slopes of oxygen concentrations over time and standardized to surface areas of the samples.

Statistical Analyses

All response variables were analyzed using separate linear mixed-effects models. For pH variation in the *S. pacificum* treatment tanks, water sample location (above/below) and pCO_2 treatment were input into the model as fixed factors, and tank as a random factor. In the *S. pacificum* tanks with ambient pCO_2 levels, 1^{st} to 5^{th} order polynomial curves were fit to pH values, and the best model, chosen based on Akaike information criterion (AIC), was used to describe the diel pH fluctuation observed in the tanks. For net calcification, photosynthesis, and respiration, canopy type and pCO_2 were input into the model as fixed

factors, and tank as a random factor. Models were compared with and without the tank factor, and tank was dropped from the model when AIC values were lower without the tank factor. Assumptions of normality and homoscedasticity were evaluated through graphical analyses of residuals and data were log or square root transformed when necessary. Analyses were performed using R software package lme4 (R Foundation for Statistical Computing).

Results

Mean (± SE) temperature and salinity in the tanks were 26.9 ± 0.02 °C and 36.3 ± 0.01, respectively. Mean (± SE) PAR experienced by samples in the clear mimic, dark mimic, and *S. pacificum* treatment tanks was 145 ± 9.7 µmol quanta m^{-2} s^{-1}, 58 ± 8.5 µmol quanta m^{-2} s^{-1}, and 72 ± 7.2 µmol quanta m^{-2} s^{-1}, respectively (Fig. 2). Although these do not represent saturating light levels for algal photosynthesis, the clear mimic treatments received significantly more light than the dark mimic and *S. pacificum* treatments ($p < 0.001$). In the ambient pCO_2 treatment, mean (± SE) pH was 7.99 ± 0.004, representing mean (± SE) pCO_2 levels of 473.6 ± 6.08 µatm. In the elevated pCO_2 treatment, mean (± SE) pH was 7.72 ± 0.01, representing mean (± SE) pCO_2 levels of 996.6 ± 18.05 µatm. In the *S. pacificum* treatment tanks, there was a significant difference in mean pH between the ambient and elevated pCO_2 treatments ($F_{1,2} = 435.98$, $p = 0.002$), but pH was not significantly different above and beneath the canopies ($F_{1,384} = 1.17$, $p = 0.28$). No interaction between location (above/beneath canopy) and pCO_2 treatment was found ($F_{1,384} = 0.05$, $p = 0.83$). In the ambient pCO_2 treatments, a 4^{th} order polynomial was the best-fit model for daily pH fluctuations both above and below the canopy ($r^2 = 0.85$ and 0.86, respectively) (Fig. 3). Daily pH maxima were ~8.2 and occurred at ~15:00. No diel fluctuation was observed in the elevated pCO_2 treatment tanks due to the method of pH control in maintaining a target pH of 7.7.

Net calcification rates were significantly different in the three canopy treatments ($F_{2,137} = 16.87$, $p < 0.001$). In the dark mimic treatment, net calcification decreased by 21% from the clear mimic treatment, from 0.13 ± 0.007 mg cm^{-2} d^{-1} to 0.10 ± 0.011 mg cm^{-2} d^{-1} (means ± SEs) (Fig. 4). The *S. pacificum* treatment further decreased net calcification by

29% from the dark mimic treatment, and by 44% from the clear mimic treatment, to 0.07 ± 0.005 mg cm^{-2} d^{-1} (mean ± SE) (Fig. 3). No effect of pCO_2 was detected on calcification ($F_{1,137} = 0.01$, $p = 0.93$), but there was a marginally significant interaction between pCO_2 and canopy type ($F_{2,137} = 2.96$, $p = 0.055$). For CCA photosynthesis, there was a significant effect of canopy ($F_{2,138} = 4.78$, $p = 0.01$) and CO_2 ($F_{1,138} = 4.31$, $p = 0.04$), and there was no interaction ($F_{2,138} = 0.25$, $p = 0.78$) (Fig. 5). From the clear to dark mimic treatments, photosynthesis increased by 23%, from 0.100 ± 0.016 μmol O_2 cm^{-2} hr^{-1} to 0.123 ± 0.017 μmol O_2 cm^{-2} hr^{-1} (means ± SEs). At a rate of 0.181 ± 0.030 μmol O_2 cm^{-2} hr^{-1} (mean ± SE) in the *S. pacificum* treatment, photosynthesis further increased by 47% from the dark mimic treatment, and by 81% from the clear treatment. For respiration, there was no effect of canopy ($F_{2,137} = 0.22$, $p = 0.80$) or CO_2 ($F_{1,137} = 1.03$, $p = 0.31$), and there was no interaction ($F_{2,137} = 0.58$, $p = 0.56$) (Fig. 6).

Discussion

The purpose of this study was to test whether canopy-forming macroalgae can mitigate or exacerbate the effect of OA on understory CCA. Using *S. pacificum*, dark algal mimics, and clear mimics, this study demonstrated that canopy macroalgae have overall negative effects on understory CCA calcification. The limited number of tanks available for use in the experiment did not allow for a fourth canopy treatment where no canopy was present. However, canopy structure was standardized across all treatments by using algal mimics, to avoid confounding the results with canopy flow moderation. Net calcification decreased from the clear mimic treatment to the dark mimic treatment, suggesting a negative effect of shading regardless of pCO_2 treatment. Canopy-forming macroalgae are known to shade understory species, reducing gross photosynthesis in CCA samples, and, consequently, calcification (Cornwall et al., 2015). This is supported by the PAR measurement results, which show a ~60% decrease in light from the clear to dark mimic treatment.

Net calcification further decreased from the dark mimic treatment to the *S. pacificum* treatment, indicating a potentially negative biological effect of the live algal canopy. It is also possible that the *S. pacificum* canopy represented a more complex physical structure than the mimics, thus obstructing flow to a higher degree. This may be partially responsible for the decrease in calcification, as lower water flow thickens the DBL and slows the rate of metabolite exchange (Hurd and Pilditch, 2011). Because light levels were not significantly different between the dark mimic and *S. pacificum* treatments, it is unlikely that shading caused the reduction in calcification in the live canopy treatment. Alternative explanations include allelopathy and possible macroalgal facilitation of microbial activity (Tanaka and Asakawa, 1988; Haas et al., 2011). In *S. pacificum*, allelopathy remains unexplored, but other

species within this genus have exhibited differing levels of allelopathy during different studies (Wang et al., 2007; Rasher et al., 2011).

This study demonstrated that *S. pacificum* can raise daytime seawater pH locally, as evidenced by the diel pH fluctuations observed in the ambient pCO_2 tanks with *S. pacificum* canopies. However, this "buffered" seawater did not increase net calcification rates of CCA, likely because the potentially positive effects of canopy metabolism were comingled with the negative effects of shading and flow moderation. Alternatively, in these same tanks, nighttime respiration by *S. pacificum* may have lowered pH substantially, decreasing CCA respiration and calcification rates at night (Anthony et al., 2011). Due to the method of CO_2 control, the *S. pacificum* tanks held at elevated pCO_2 followed a steady pH regime with a constant target pH of 7.7. Although pH variability differed between *S. pacificum* tanks of different pCO_2 treatments, the calcification response was similar. This indicates that short-term temporal pH variability may not induce an observable effect in *L. kotschyanum*, and that the overall mean pH may drive CCA calcification rates.

Contrary to expectations, there was no observed effect of pCO_2 on CCA calcification. This result is inconsistent with prior findings, which showed a decrease in calcification rates in response to elevated pCO_2 treatments (Kuffner et al., 2008; Comeau et al., 2013b). The discrepancy between the results of the present study and prior studies suggests that the presence of a macroalgal canopy may have altered the response of understory CCA to elevated pCO_2. High water flow has the capacity to enhance CCA sensitivity to elevated pCO_2, due to the thinning of DBLs on the CCA thallus surface (Cornwall et al., 2014). In all treatments, water flow experienced by CCA samples were obstructed by a form of canopy structure, either a plastic mimic, or a live *S. pacificum* canopy. These slower flow rates may

have thickened the DBL surrounding *L. kotschyanum* individuals, ultimately lowering their sensitivity to elevated pCO_2 and minimizing its effect on calcification. Therefore, thicker boundary layers may be beneficial in future OA conditions despite slowing metabolic processes (Cornwall et al., 2014).

Photosynthesis was significantly affected by both pCO_2 and canopy treatments. The increase in light-saturated photosynthesis from the clear to dark mimic treatments may be due to shaded samples increasing both the size and number of their photosynthetic units (Falkowski and Owens, 1980; Carpenter, 1985). A further increase from the dark mimic to *S. pacificum* treatment may be explained by decreased DIC availability in the *S. pacificum* treatment causing CCA to increase the efficiency of their carbon concentrating mechanisms (Kaplan et al., 1980). Elevated pCO_2 also resulted in significantly lower photosynthetic rates, which may be due to decreased photochemical efficiency under OA conditions (Briggs and Carpenter, 2019). Respiration rates for *L. kotschyanum* were not affected significantly by canopy type or pCO_2 treatment. The absence of an effect of pCO_2 on CCA respiration is consistent with prior studies (Comeau et al., 2016). It is also important to note that slower CCA calcification due to canopy shading likely occurs by way of reduced photosynthesis in treatment, since these processes interact as they alter carbon chemistry at the CCA thallus surface. Because rates of photosynthesis and respiration were measured without treatment canopies, these responses measure only effects that carried over from treatment to incubation. This suggests that these responses may be plastic enough to change in the short period between treatment and incubation.

This study suggests that the negative effects of canopy-forming macroalgae outweigh their benefits to understory CCA. Despite their ability to alter seawater carbon chemistry by

raising daily pH maxima and lowering daily minima, other effects such as shading, flow moderation, and possible allelopathy ultimately render them a detriment to calcifying competitors. Due to logistical limitations, this study could not isolate the effect of canopy physical structure on understory CCA through a comparison with a control treatment where no canopy was present. To isolate this effect, a subsequent experiment was conducted and is discussed in the following chapter. Aside from the flow moderation, another potential effect of canopy physical structure is abrasion (Grace, 2004). It is also important to note that *L. kotschyanum* have thick skeletons and encrusting algae may be resistant to abrasion by a non-calcified species (Connell, 2003).

Although this study ultimately strengthens the case that increasing macroalgal abundance on coral reefs will contribute to further reef decline, few studies have examined the potential benefits of canopy metabolism without the negative effects of canopy physical structure. Consistent with my hypotheses, this laboratory experiment demonstrated that canopy macroalgae can locally increase seawater pH. While understory CCA may not be able to reap the benefits of heightened pH, CCA and other calcifiers that live downstream of dense communities of macroalgae may be able to benefit from this mitigation of OA effects while avoiding the negative effects of the physical canopy structure. To elucidate the potential role of non-calcifying algae as a mechanism to ameliorate OA effects on downstream calcifiers, future studies investigating the isolated effect of metabolic carbon chemistry control are necessary.

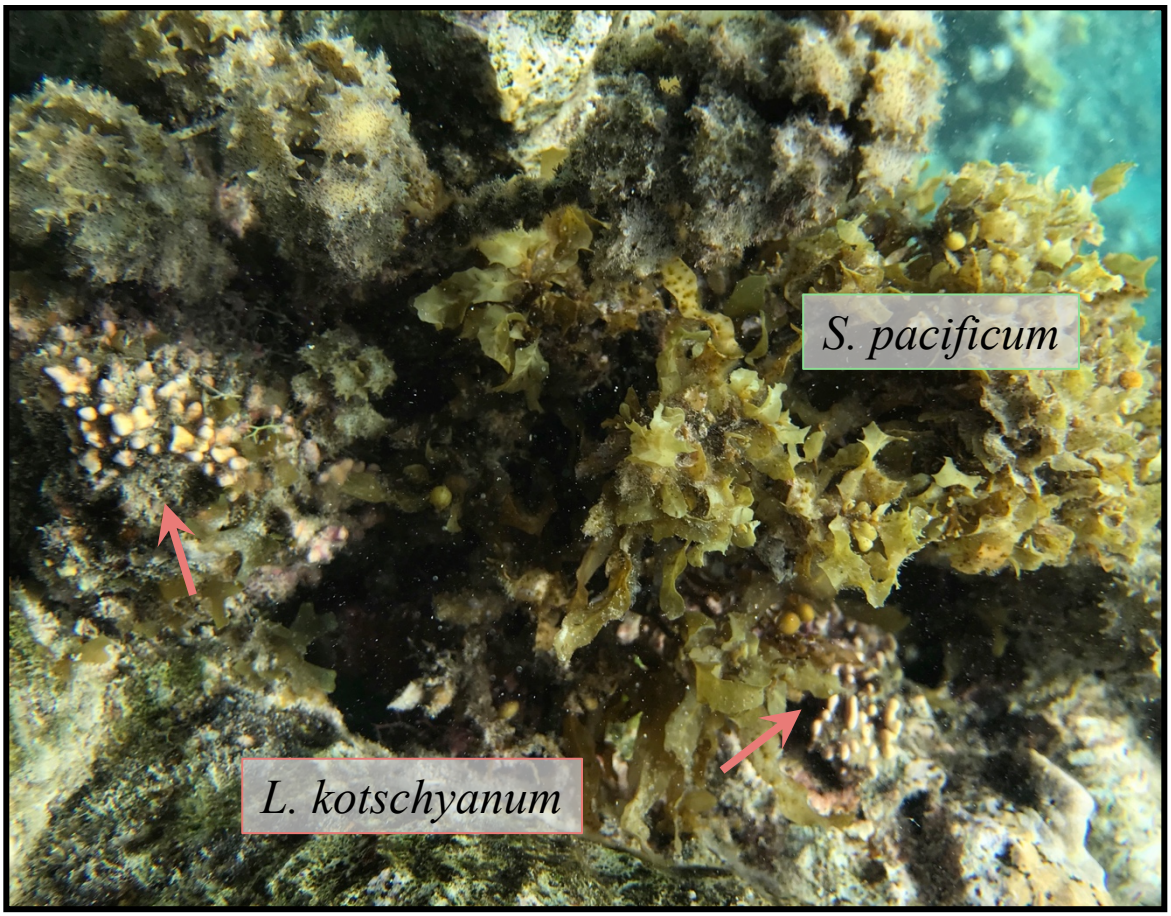

Figure 1. *L. kotschyanum* naturally growing near and beneath *S. pacificum* at the study site.

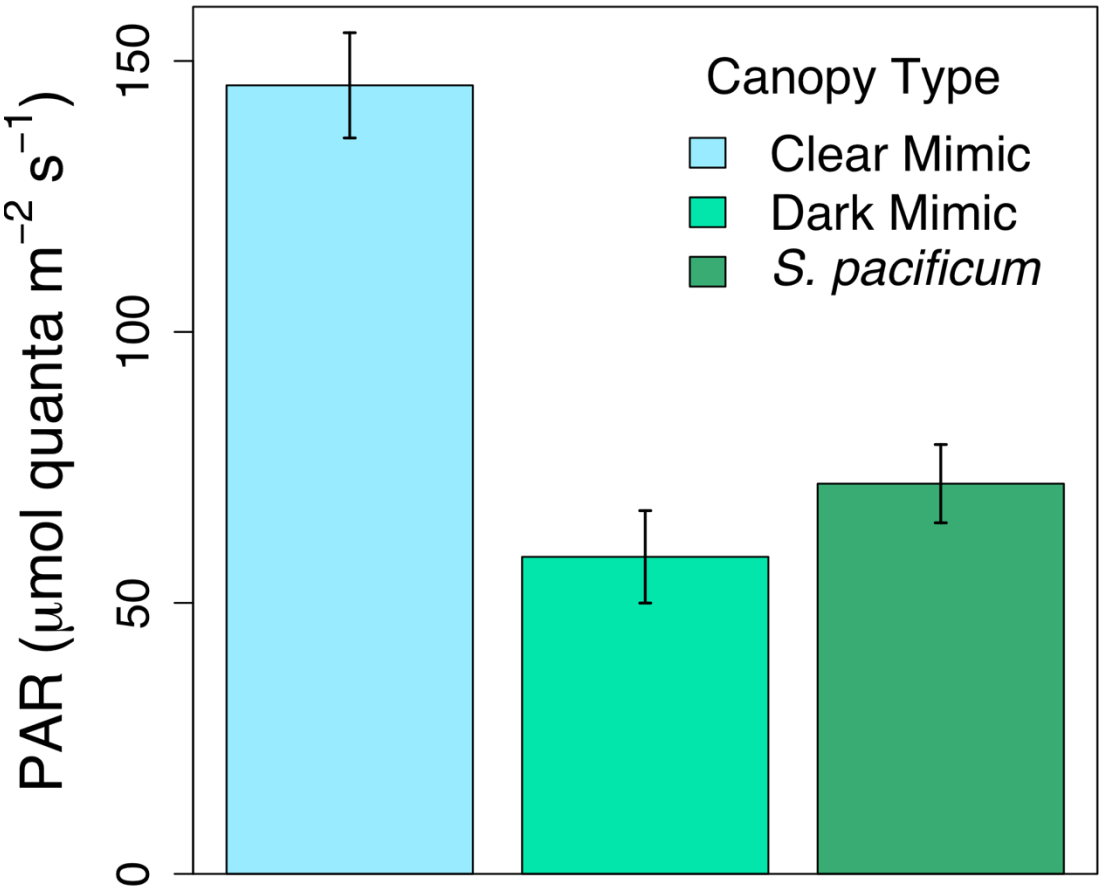

Figure 2. Mean ± SE photosynthetically active radiation (PAR) supplied to samples in the three canopy treatments (n = 4). There is a significant effect of canopy type ($p < 0.001$) A tukey's post-hoc analysis showed that the clear mimic treatment was significantly different from the dark mimic and *S. pacificum* treatments.

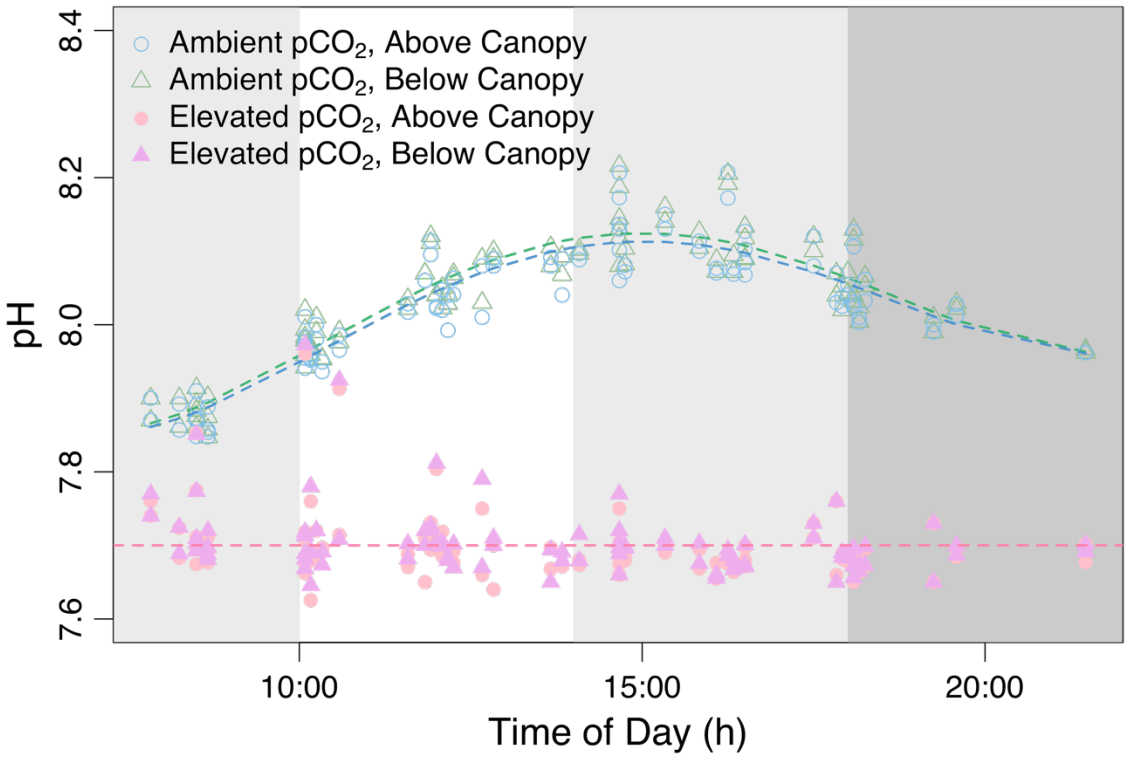

Figure 3. Daily pH fluctuations in the 4 *S. pacificum* treatment tanks (n = 8 days). Dark gray areas are periods during which lights are off in the mesocosms, light gray areas represent ramp periods, and the white area represents the period during which lights are on at full capacity. Dashed curves are 4th order polynomial fit curves. The dashed line represents the target pH (7.7) for elevated pCO_2 treatments.

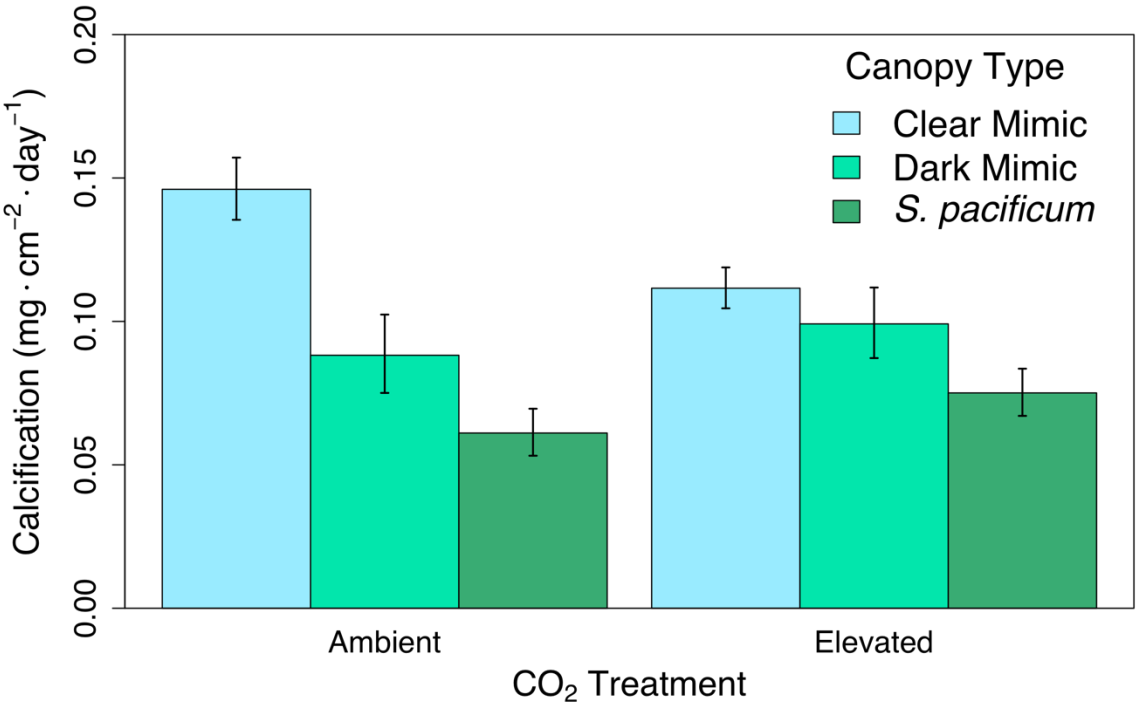

Figure 4. Mean ± SE calcification of *L. kotschyanum* (n = 12). There is a significant effect of canopy type (p = 0.04).

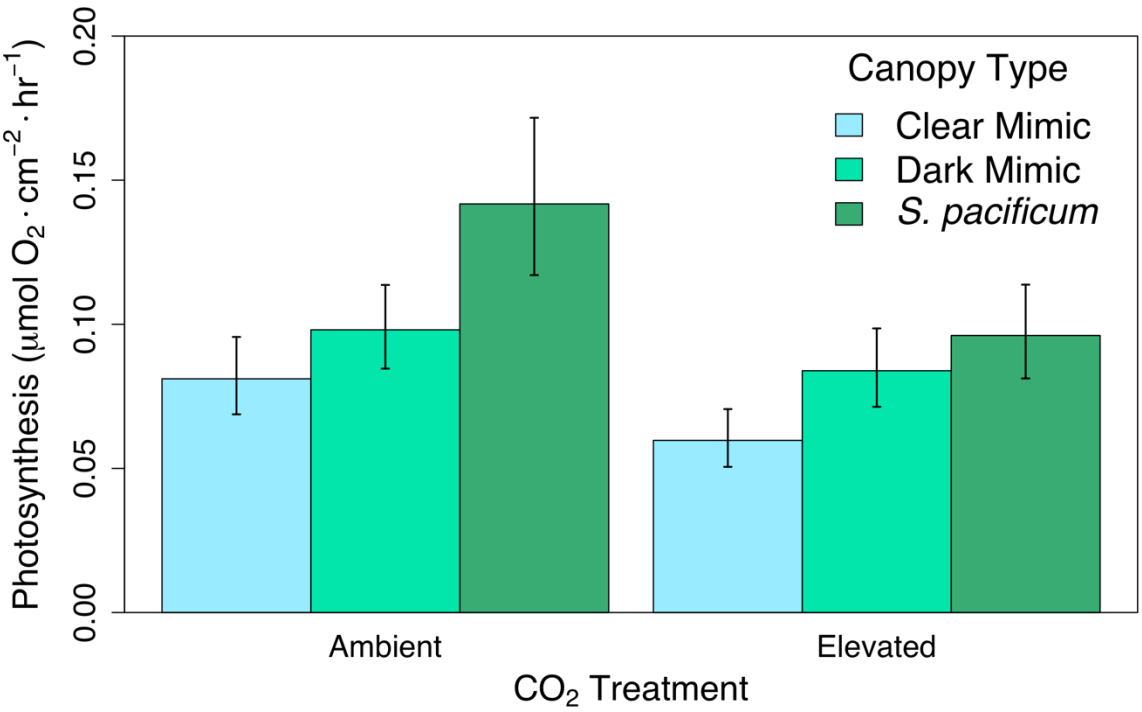

Figure 5. Mean ± SE photosynthesis of *L. kotschyanum* (n = 12). There are significant effects of both canopy type (p = 0.01) and pCO_2 (p = 0.04) on photosynthesis, and no significant interaction.

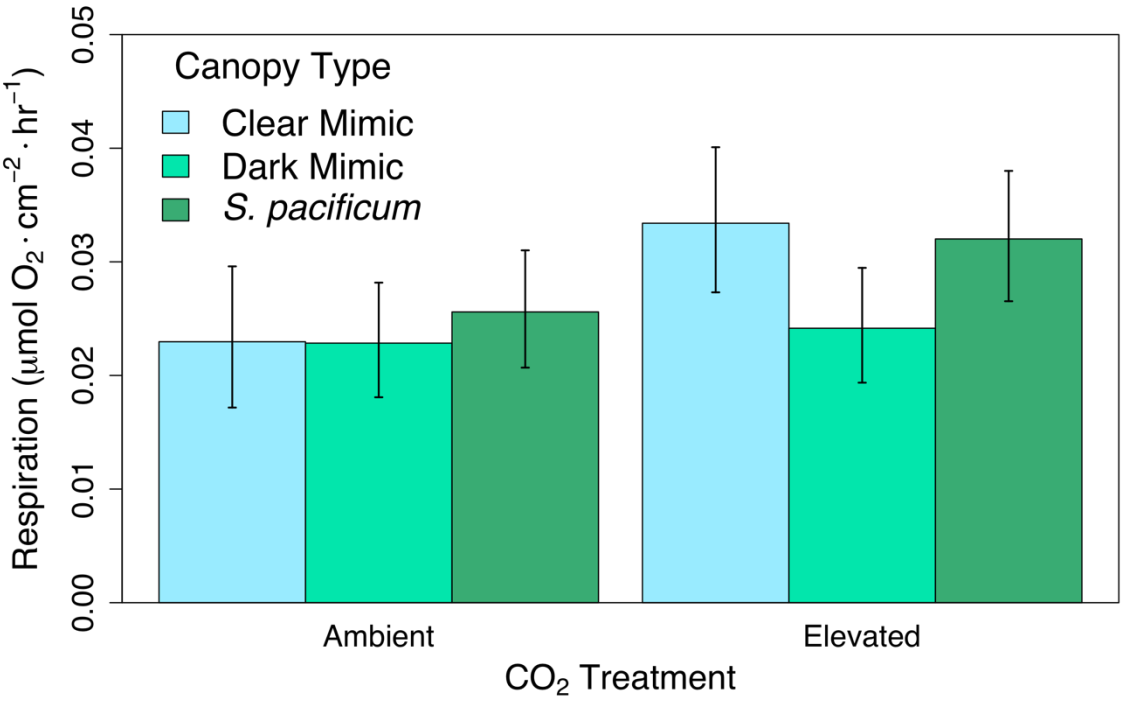

Figure 6. Mean ± SE respiration of *L. kotschyanum* in terms of oxygen uptake (n = 12). There is no significant effect of either treatment, and no significant interaction.

Chapter 3

Effects of photosynthetic CO_2 drawdown and shading by *Sargassum pacificum* on *Lithophyllum kotschyanum*

Introduction

Among the most productive and biodiverse ecosystems in the world, coral reefs are ecologically and economically important ecosystems that are threatened by climate change and other anthropogenic impacts (Dixon et al., 1993; Moberg and Folke, 1999; Hughes et al., 2003). Reef builders create complex biogenic structures in coral reefs, allowing these ecosystems to support the highest level of biodiversity in the ocean (Knowlton et al., 2010). Within recent decades, climate change has become a global threat to these ecosystems, inducing declines in coral abundance and reef accretion, which may even transition into dissolution as atmospheric CO_2 continues to be absorbed by the ocean (Pandolfi et al., 2011; Perry et al., 2013). Human activities in recent centuries, such as the burning of fossil fuels, have increased the concentration of atmospheric CO_2, a third of which has been absorbed by the ocean (Sabine et al., 2004; Solomon et al., 2007). This increased seawater pCO_2 leads to an increased concentration of hydrogen (H^+) and bicarbonate ions (HCO_3^-), ultimately reducing ocean pH in a process known as ocean acidification (OA) (Hoegh-Guldberg et al., 2007; Doney et al., 2009).

Under OA conditions, the ability of calcifiers to precipitate their calcium carbonate skeletons declines (Johnson and Carpenter, 2012; Comeau et al., 2014). The addition of CO_2 to seawater initiates a series of chemical reactions that results in

decreased carbonate ion (CO_3^{2-}) concentration and a lowered carbonate saturation state (Ω) (Hofmann and Bischof, 2014). Because calcifiers build their skeletons by precipitating carbonate ions out of the seawater, Ω is a direct measure of reef calcification (Orr et al., 2005; Pandolfi et al., 2011). Net carbonate accretion is reported to occur only on coral reefs with Ω levels that exceed 3.3 (Kleypas et al., 1999). In response to a doubling of pre-industrial levels of carbon dioxide in the atmosphere to 560 ppm, coral reefs may transition into net dissolution globally (Kleypas and Langdon, 2006; Silverman et al., 2009). According to a business-as-usual projection (Representative Concentration Pathway 8.5), a three-fold increase in atmospheric carbon dioxide from pre-industrial levels is predicted to occur by the end of the current century, driving down ocean pH by up to 0.40 units (IPCC, 2014).

Corals, the primary foundation species found on coral reefs, have been the focus of the majority of OA studies, but calcifying algae remain understudied (Carpenter et al., 2008; Albright et al., 2010; Comeau et al., 2015). As they create new regions of biogenic reef and cement the dead coral skeletons together, calcifying algae, especially crustose coralline taxa, stabilize the reef framework and increase reef resistance to wave erosion (Adey, 1998). These critical reef builders provide a substratum for coral settlement, are a source of food and shelter for reef fishes and invertebrates, and contribute to reef productivity and accretion (Bosence, 1983; Chisholm, 2003; Price, 2010). Coralline algae are particularly sensitive to OA because they precipitate high-magnesium calcite, the most soluble form of $CaCO_3$ in seawater (Hofmann and Bischof, 2014). Additionally, they are slow calcifiers, secreting $CaCO_3$ at an approximate rate of 0.03 – 0.37 g cm^{-2} y^{-1} (Chisholm 2000; Comeau et al. 2013). Due to their sensitivity to OA and their slow

calcification rates, coralline algal taxa may be among the first reef calcifiers lost as OA effects permeate through tropical coral reefs. As ocean pH declines, crustose coralline algae (CCA) may form thinner crusts, which are less resistant to grazing and wave action, leaving the reef structure more vulnerable to collapse (Steneck, 1986).

As OA reduces the growth and calcification of corals and CCA, fast-growing, non-calcifying (fleshy) macroalgae may become more common on the reef benthos at shallow depths. OA increases the availability of CO_2 and bicarbonate in seawater, potentially facilitating photosynthesis in both fleshy and coralline algae (Hoegh-Guldberg et al., 2007; Zou et al., 2011). Persistent stressors such as warming, nutrient enrichment, and overfishing contribute to a positive feedback cycle that favors macroalgal abundance on coral reefs (Nyström et al., 2000; Szmant, 2002; Hughes et al., 2003; Wooldridge et al., 2005). Documented effects of macroalgal-dominated conditions include reduced reef structural complexity as a result of coral loss, leading to a decline in biodiversity and important ecosystem services (Chong-Seng et al., 2012; Pratchett et al., 2014). Additionally, canopy-forming macroalgae can shade and smother newly settled spat and juvenile corals, as well as calcareous algae (Hughes et al., 2006; Vogel et al., 2015; Smith et al., 2016).

The canopy physical structure also modulates water flow to understory calcifiers, resulting in a thicker diffusion boundary layer (DBL) (Cornwall et al., 2015). Thicker DBLs may provide a protective barrier from OA-induced low pH in the bulk seawater, but reduce the rate of metabolite exchange, thus affecting calcification, photosynthesis, and respiration (Koch, 1994; Gardella and Edmunds, 2001). Therefore, flow moderation by canopy macroalgae may be detrimental to associated CCA in current seawater pH

conditions, but beneficial in future OA conditions. Habitat-specific flow regimes on reefs may also affect spatial distribution and abundance of calcifiers, due to differential metabolic rates under different flow velocities (Gourlay and Colleter, 2005; Monismith, 2007). Within a single coral reef, high-flow regions may be associated with faster growth, and, therefore, higher abundance of calcifiers. CCA occurring in close proximity to macroalgal canopies may be particularly affected by canopy flow obstruction in these high-flow environments, compared to low-flow regions of the reef.

Studies that examine the role of non-calcified macroalgae in coral reefs focus on their competition for benthic space with corals and calcifying algae, but few have examined the potential benefits of fleshy algae as a local carbon sink. Contingent on the density at which they occur, macroalgal canopies have variable capacities to modify their immediate chemical environment. As the climate changes, the ocean is expected to become warmer and more acidic, favoring the propagation of fleshy macroalgae over reef-building corals (Hoegh-Guldberg et al., 2007; Ledlie et al., 2007). In these conditions, metabolic moderation of dissolved inorganic carbon (DIC) by fleshy algae could provide microhabitat refuges from OA for closely associated calcifiers, such as CCA. Through the uptake and dehydration of bicarbonate (HCO_3^-) to CO_2 in photosynthesis, a dense assemblage of fleshy algae potentially might offset the effect of OA on seawater carbon chemistry (Axelsson and Uusitalo, 1988). By reducing the concentration of H^+ ions in the surrounding seawater and increasing pH, fleshy macroalgae can raise the carbonate saturation state, thereby influencing calcification rates of adjacent CCA (Anthony et al., 2013). These changes to seawater carbon concentration may also alter the effect of OA on CCA photosynthesis and respiration (Zou et al., 2011;

Cornwall et al., 2012). In turn, photosynthesis and respiration affect calcification by changing seawater pH in opposing directions at the thallus surface, thus altering Ω at calcification sites (Chisholm, 2003; Comeau et al., 2013a). Photosynthesis and respiration may also display a positive relationship with one another, as one process utilizes the products of the other (Kuhl et al., 1996).

Previous studies of seawater CO_2 moderation by fleshy macroalgae were conducted in the absence of calcifiers and were conducted in exceptionally controlled laboratory conditions (Anthony et al., 2011). To assess the capacity of non-calcifying algae as a means to mitigate the negative effects of OA on calcifiers, the present study examined both the physical and chemical effects of a canopy-forming fleshy alga on a crustose coralline alga. A mesocosm experiment was performed in the laboratory, followed by a field experiment to determine whether results differed between controlled and natural environments. Potential differences between habitat types within the reef were also examined, as macroalgae is more abundant near the reef crest than further down in the back reef. Additionally, water flow may be different between these habitats because wind-driven swells break near the reef crest with high energy and slow down as they flow mostly unidirectionally through dense stands of macroalgae before reaching the lower flow lagoon (Hench et al., 2008). *Sargassum pacificum* is a common fucoid phaeophyte on many Pacific coral reefs and forms dense aggregations on reef crests and on the tops of coral bommies (Mattio et al., 2008, 2009). Because of its high abundance, it is likely that *S. pacificum* interacts with a variety of calcified species, including coralline algae. An abundant crustose coralline algal taxon in these same reef habitats is *Lithophyllum kotschyanum*, a species in which calcification is known to be sensitive to

OA (Comeau et al., 2014). I hypothesized that *S. pacificum* would increase seawater pH through uptake of DIC, thus increasing calcification of associated *L. kotschyanum*, while decreasing photosynthesis and respiration. In the field, I hypothesized this effect would be present, but weaker, due to more turbulent flow under field conditions than in the laboratory. I also expected pH moderation by *S. pacificum* to be less pronounced near the reef crest, where water flow is expected to be higher than further back in the lagoon.

Methods

The effects of seawater carbonate chemistry alteration and the physical structure of canopy-forming macroalgae on CCA physiology were quantified in a mesocosm study and a subsequent field study. The laboratory experiment addressed the isolated effect of *S. pacificum* metabolism on seawater chemistry and the net calcification of CCA, while the field experiment evaluated the combined effects of DIC moderation and the physical canopy structure on CCA in a natural setting. Together, these experiments assessed the capacity of canopy-forming macroalgae to mitigate the effects of OA on CCA in both controlled and natural environments.

Experiment 1—Indirect effects of a macroalgal canopy

Sample collection and preparation

Using a hammer and chisel, 48 individuals of *L. kotschyanum* were collected from the fringing reef at ~1-2 m depth on the north shore of Moorea, French Polynesia. Using a diamond bandsaw (Gryphon C-40, CA, USA), samples were cut to similar sizes (2-3 cm W/L) at the Richard B. Gump South Pacific Research Station. *L. kotschyanum* individuals then were cleaned of epibionts and attached to plastic supports using Coral Glue (EcoTech Marine, PA, USA). To minimize environmental variation in samples, were acclimated for 4 days in an outdoor seawater table with constant input of fresh seawater pumped up from Cook's Bay. Samples then were randomly assigned to the treatments described below (n=12 per tank).

Treatment maintenance

Four, 150-L mesocosm tanks (Aqualogic, San Diego, CA) were maintained under two replicated seawater regimes: 1) ambient and 2) *S. pacificum* treated. The ambient seawater treatment consisted of fresh seawater, pumped from Cook's Bay at 12-m depth and sand-filtered (pore size ~550 μm). In the *S. pacificum* treatment, filtered seawater was fed through a header tank containing *S. pacificum* and gravity-fed into the replicated treatment tanks. The biomass of *S. pacificum* was chosen to mimic the highest densities (g/m^2) recorded from back reef field sampling. In a 0.25 m^2 patch of high *S. pacificum* percent cover, thalli were collected and wet weighed in the laboratory. The resulting biomass, ~1000 g, was used in the mesocosm tanks, which are also 0.25 m^2 at the base. Seawater entered the mesocosms at a mean (± SE) rate of 315 ± 2.6 mL min^{-1}, resulting in a full tank turnover of water every 8 hours. Additionally, flow within each tank was created using a pump (Rio 8HF, 2082 L h^{-1}). Each tank received a constant rate of air bubbled in through airstones and was fitted with a plexiglass lid to minimize gas exchange at the water surface. Tank temperatures were maintained at ~27 °C to match austral winter temperatures observed at the study site (Washburn, 2018). Photosynthetically Active Radiation (PAR, 400-700 nm) was provided on a 12:12 h light:dark photoperiod with 4-h ramp times, using 75W Light Emitting Diode (LED) modules (Sol White LED Module, Aquaillumination, IA, USA) that supplied ~600 μmol quanta m^{-2} s^{-1} individually to each tank. PAR in each tank was measured with a 2π underwater sensor (model LI-192) attached to a LI-COR LI-1400 data logger.

Throughout the 8-day experiment, seawater parameters were measured daily and CCA samples were cleaned and rearranged haphazardly every 3 days to minimize

positioning effects. The elevated pCO_2 treatment was maintained by a pH-stat aquacontroller (Neptune Systems, Morgan Hill, CA) that bubbled pure CO_2 into the mesocosm tanks when pH rose above the set point. Daily pH values were calculated by measuring temperature (Traceable Digital Thermometer, Fisher Scientific, accuracy: 0.05 °C, resolution: 0.01 °C) and mV (Thermo-Scientific Orion 3-star portable meter with Mettler Toledo InLab Expert Pro probe, calibrated on the total scale with TRIS buffers at a salinity of 35.0 psu). Every other day, water samples were collected from each tank to measure salinity (Orion Star A212 Conductivity Benchtop Meter) and total alkalinity (A_T). A_T was measured using open-cell potentiometric titrations (Mettler Toledo T50 titrator with a Mettler Toledo DGi 115-SC probe) on 50-mL aliquots of treatment seawater (Dickson et al., 2007). Accuracy and precision of titrations were determined using titrations of certified reference materials (A. G. Dickson batch 158), which yielded A_T values within 4.2 μmol kg^{-1} of the certified value (SD = 1.27 μmol kg^{-1}; n= 9). Carbonate system parameters were calculated daily for each treatment tank from A_T, pH, temperature, and salinity measurements using the R package seacarb (Lavigne and Gattuso, 2012).

Response variables

Net calcification, photosynthesis, and respiration were measured to determine the effect of metabolically-altered seawater carbon chemistry on the physiology of *L. kotschyanum*. All response variables were standardized to surface area (SA), which was quantified using the dye-dipping method (Hoegh-Guldberg, 1988). Samples were dipped in a solution of methylene blue dye (0.1 g 100 mL^{-1} with ~2.5% Triton X-100 detergent)

and rinsed in 100 mL of seawater. Using a spectrophotometer (Shimadzu UV-2450), the absorbance of each seawater sample was measured at 620 nm and compared to a standard curve constructed from foil pieces of known surface areas. Sample SA then was predicted from the resulting standard curve (y = 257.84x + 11.292, regression of absorbance to known SA). Mean calcification over the entire experiment was measured using the buoyant weighing method (Davies, 1989). Initial and final buoyant weights (Mettler Toledo PB303-S balance, resolution: 0.001 g) of each sample were recorded, and the differences (weight change) were converted to dry weights using the measured density of seawater and a density of pure calcite of 2.71g cm^{-3}.

Photosynthesis and respiration rates were measured by incubating each sample in chambers in the light and dark, respectively. Samples were placed in 250-mL acrylic chambers and incubated with water from the corresponding treatment tanks. A constant temperature of 27 °C was maintained by a water jacket surrounding the chamber that received circulating flow from a chilled water bath. Stir bars at the base of each chamber, separated from samples by a plastic mesh, maintained constant water mixing during incubations. Oxygen concentrations were recorded every second by PreSens (Precision Sensing GmbH, Germany) oxygen optodes and temperature probes attached to Fibox 3 minisensor oxygen meters. The probes were calibrated using a 2-point calibration in water-saturated air (100% air saturation) and water supersaturated with sodium hydrosulfite (0% air saturation). To measure photosynthesis, incubations were performed in saturating light (~200 μmol quanta m^{-2} s^{-1}), estimated using PI curves calculated with a Walz Diving PAM. Similar to maximum light levels in the treatments, PAR was supplied during incubations at ~600 μmol quanta m^{-2} s^{-1} by two fiber optic halogen lights (Ace 1,

Schott North America, Inc.). Respiration rates were measured in dark incubations, during which lights were turned off and chambers were covered with black plastic. Incubations ended when consistent slopes (ΔO_2/time) were achieved, representing stable metabolic rates following a 5-minute acclimation period. Control incubations were performed with only treatment seawater and these O_2 fluxes were subtracted from the rates measured in the algal incubations. Metabolic rates were calculated from linear slopes of oxygen concentrations over time and standardized to surface areas of the samples.

Statistical Analyses

Data were analyzed using a linear mixed-effects model with seawater treatment as a fixed factor and tank as the nested factor. Models with and without the tank factor were compared, and the tank effect was dropped from the final model when Akaike's information criterion (AIC) values were lower without the tank factor. Assumptions of normality and homoscedasticity were evaluated through graphical analyses of residuals and data were log or square root transformed when necessary to meet assumptions. Analyses were performed using R software package lme4 (R Foundation for Statistical Computing).

Experiment 2: Direct Effects of a macroalgal canopy

Collection, Setup and Treatment Maintenance, Response Measurement

To test the hypothesis that fleshy macroalgae can alter CCA physiology under natural conditions, a field experiment was conducted using *S. pacificum* canopies. Following the methods outlined in the prior experiment, 144 individuals of *L.*

kotschyanum were collected, prepared, and acclimated for two weeks in a flowing seawater table. Samples were assigned randomly to one of four treatment groups placed at reef locations on the north shore of Moorea (Fig. 1). Two back reef habitats (upstream/downstream) were crossed with two canopy treatments (*S. pacificum* canopy/no canopy). The upstream habitat was ~30 m behind the reef crest, while downstream habitat was ~450 m behind the reef crest (Fig. 1). The *S. pacificum* treatment was created by attaching live *S. pacificum* thalli (~130 g) to the upstream edge of a 20×30 (W×H) cm plastic grid (BOEN Green Plastic Garden Fence). This density represented ~50% algal cover on the benthos and is similar to present-day macroalgal densities found near the reef crest on the north shore of Moorea (Fig. 3). For the "no canopy" treatment, only the plastic grid was used (Figs. 2a, 2b). For both treatments, three *L. kotschyanum* samples (2-3 cm diameter) were attached to the downstream edge of each grid (n=12 grids per treatment). This experimental setup was designed to compare responses of *L. kotschyanum* to two seawater treatments: water with potentially modified carbonate chemistry due to upstream macroalgal photosynthesis, and ambient seawater. *S. pacificum* individuals of moderate height (24-28 cm) were used to avoid shading of samples and canopies were refreshed weekly to maintain high rates of photosynthesis.

Temperature and light (PAR) were measured using the same methods as described in Experiment 1, while relative water flow rates between the four treatments (two habitat types and two canopy treatments) were estimated using clod card dissolution (Doty, 1971; Brown and Carpenter, 2015). To measure *in situ* pH, water samples were taken from three haphazardly chosen grids per treatment group using 150-mL bottles. Following the method outlined in Experiment 1, pH was calculated from temperature

(Traceable Digital Thermometer, Fisher Scientific, accuracy: 0.05 °C, resolution: 0.01 °C) and mV (Thermo-Scientific Orion 3-star portable meter with Mettler Toledo InLab Expert Pro probe, calibrated on the total scale with TRIS buffers at a salinity of 35.0). Samples were taken four times during the 6-week experiment from the base of each plastic grid, at which the CCA samples were placed. Clod card dissolution was used as a proxy for relative flow differences between the reef crest and back reef habitat types. Following the method outlined in Doty 1971, Plaster of Paris (DAP) was used to mold similarly-sized (4.7 × 2.7 × 2.2 cm) shapes in ice cube trays. Replicate clod cards (n = 2) were attached to grids adjacent to the CCA samples, downstream of canopies, to determine relative flow rates in the four different treatment groups. Clod cards were deployed during replicate trials (trial 1: n = 3; trial 2: n =4 grids/treatment group), assigned randomly to treatment grids, and remained in the field for 48 ± 1 h (mean ± SE). Final weights were measured after drying for a minimum of 7 days in the laboratory. To correct for dissolution in still water, controls were placed in containers of still treatment water while each batch was deployed. Control dissolution was subtracted from field dissolution to obtain dissolution values resulting from water flow. These values then were normalized to the initial weight to obtain percent dissolution values.

Net calcification of *L. kotschyanum* was measured using changes in buoyant weight over the experiment using the same methods as described for the mesocosm experiment. Measurements were standardized to surface area following the dye method outlined above and SA was predicted from the resulting standard curve (y = 271.85x + 2.26, regression of absorbance to known SA). ().

Statistical Analyses

Linear mixed-effects model was used to analyze all response variables. For calcification, habitat and canopy treatment were fixed factors, and grid was added as a random factor. Models with and without grid as a factor were compared, and grid was retained based on Akaike's information criterion (AIC). Unequal variances were found among grids and mean values for each grid were used in the final model. For clod card dissolution, canopy and habitat were input as fixed factors, and trial as a random factor. Models with and without trial as a factor were compared and trial was dropped from the model based on AIC. For seawater pH, canopy and habitat were fixed factors and day of sampling was a random factor. After model comparison using AIC values, day was retained in the final model. Assumptions of normality and homoscedasticity were evaluated through graphical analyses of residuals and data were log transformed when necessary to meet assumptions. Analyses were performed using R software package lme4 (R Foundation for Statistical Computing).

Results

Experiment 1 - Mesocosm Experiment

Mean (± SE) temperature and salinity in the tanks were 27.5 ± 0.06 °C and 35.3 ± 0.07, respectively. Mean (± SE) PAR was supplied at 589 ± 21.9 μmol quanta m^{-2} s^{-1}. In the *S. pacificum* treated seawater, the live canopy induced natural pH fluctuations, with the lowest levels observed at night, and highest during the day. In the ambient seawater treatment, pH ranged from 7.93 to 8.12, representing pCO_2 levels of approximately 350 – 550 μatm. In the treatment where water was exposed to *S. pacificum*, pH ranged from 7.85 to 8.24, representing pCO_2 levels of approximately 230 – 700 μatm. Net calcification rates were significantly higher in the *S. pacificum* seawater treatment ($F_{1,45}$ = 10.38, p = 0.002). The *S. pacificum* treated seawater increased calcification by approximately 40% from 0.148 ± 0.011 mg cm^{-2} d^{-1} to 0.207 ± 0.015 mg cm^{-2} d^{-1} (means ± SE) (Fig. 4a). No effect of seawater treatment was detected on photosynthesis ($F_{1,46}$ = 0.01, p = 0.91) or respiration ($F_{1,2}$ = 0.12, p = 0.76) (Figs 4b, c).

Experiment 2 - Field experiment

Seawater temperatures in the field ranged from 23.3 °C to 28.7 °C, and light levels from 830 to 850 μmol quanta m^{-2} s^{-1}. Analysis of clod card dissolution indicated that flow was significantly higher in the upstream location than downstream ($F_{1,51}$ = 24.01, p < 0.001). For trial 1, mean (± SE) dissolution occurred at 0.43 ± 0.05 % hr^{-1} in the back reef, and at 0.65 ± 0.08 % hr^{-1} in the reef crest, a 51% increase (Fig. 5). For trial 2, mean (± SE) dissolution occurred at 0.43 ± 0.04 % hr^{-1} in the back reef, and at 0.70 ± 0.04 % hr^{-1} in the reef crest, a 63% increase (Fig. 5). Canopy treatment had no effect on

clod card dissolution ($F_{1,51} = 0.07$, $p = 0.80$), and the interaction (canopy × habitat) was not significant ($F_{1,51} = 0.65$, $p = 0.42$). Significant differences in pH were found between the two habitat types, averaging 8.16 ± 0.006 (mean ± SE) upstream, and 8.18 ± 0.005 downstream ($F_{1,38} = 100.06$, $p < 0.001$). Canopy treatment had no effect on pH ($F_{1,38} = 0.90$, $p = 0.22$), and no interaction between canopy and habitat was found ($F_{1,38} = 0.08$, $p = 0.78$) (Fig. 6). No effect of habitat ($F_{1,44} = 2.14$, $p = 0.15$) or canopy ($F_{1,44} = 2.46$, $p = 0.12$) on net calcification was detected, and there was no significant interaction ($F_{1,44} = 0.87$, $p = 0.36$) (Fig. 7).

Discussion

The purpose of this study was to test the hypothesis that non-calcifying macroalgae could potentially mitigate the effects of ocean acidification on a species of CCA. The laboratory study demonstrated that *S. pacificum* can raise seawater pH locally, and that this chemically altered seawater can increase net calcification rates of CCA. The *S. pacificum* treated seawater resulted in larger daily pH fluctuations, evidenced by higher daily maxima pH and lower daily minima. Overall, daytime photosynthesis of *S. pacificum* raised pH more than nighttime respiration lowered pH. This suggests that *L. kotschyanum* may be resistant to short-term temporal pH variability, and that CCA calcification responds to the overall mean pH. This is one of the first studies to provide evidence that macroalgae on coral reefs may indirectly promote increased calcification in CCA. As the effects of OA on coral reefs become more pronounced, the capacity of photosynthetic organisms to locally buffer seawater from increases in CO_2 concentration becomes more critical (Comeau et al., 2015). Due to their ability to alter seawater carbon chemistry, macroalgae may play a more beneficial role in future reefs (Ledlie et al., 2007; Houk et al., 2010). However, this beneficial effect can be variable. This indirect effect of macroalgal canopies, when coupled with direct effects of their physical structure in the field experiment, exhibited contrasting results when juxtaposed with the results of the laboratory experiment.

The beneficial effect of *S. pacificum* canopies did not translate from the laboratory into the field, where canopy algae could impose both direct and indirect effects, in the face of interactions with other biotic and abiotic variables. The laboratory study focused solely on the metabolic CO_2 moderation ability of *S. pacificum*, while the field study also took into account the effects of the physical structure of the canopy, and it was conducted under

natural water flow conditions. In close proximity to *L. kotschyanum* samples, *S. pacificum* may have shaded, abraded, or chemically impaired samples through allelopathy (Reed and Foster, 1984; Tanaka and Asakawa, 1988; Grace, 2004). However, *L. kotschyanum* have thick skeletons and encrusting algae often are robust to effects of abrasion by a non-calcified species (Connell, 2003). Additionally, allelopathy in *S. pacificum* has not been studied, but studies conducted on allelopathy in other species within this genus have yielded inconclusive results (Wang et al., 2007; Rasher et al., 2011). Shading of understory species by canopy-forming macroalgae is widely-documented and may have occurred in the field experiment (Cornwall et al., 2015). It may have reduced photosynthesis in CCA samples, negatively affecting calcification, and counteracting any potential benefit of seawater carbonate chemistry moderation. *S. pacificum* density also was higher in the laboratory experiment than the field experiment, and water residence time longer. No significant effect of canopy on pH was observed in the field, suggesting that, under these field conditions, *S. pacificum*, could not alter seawater carbon chemistry to the same degree as in the mesocosm experiment. Lastly, *S. pacificum* canopies had no effect on flow rate, suggesting that the distance at which CCA samples were placed from the canopies was sufficient to avoid flow obstruction due to the physical canopy structure. Since the clod cards were placed immediately next to the samples, they are representative of the relative flow regimes experienced by the *L. kotschyanum* samples.

Habitat type also had no effect on CCA net calcification, despite the significantly different flow and pH conditions. Contrary to expectations, pH was higher in the downstream location than the upstream location, and while statistically different, it is unlikely to be meaningful biologically. However, both values are elevated above the oceanic pH levels of

8.03 (Turley et al., 2006), suggesting that present-day densities of fleshy macroalgae in this lagoon habitat may already be altering seawater carbon chemistry in coral reefs, potentially affecting back reef calcification. Although fleshy macroalgal biomass was much higher upstream, near the reef crest, low water residence times due to higher flow may force photosynthetically altered seawater to leave the upstream habitat rapidly. Water then slows down as it passes through dense stands of macroalgae near the reef crest (Hench et al., 2008). As water slowly passes through the downstream lagoon, macroalgal metabolism may result in higher pH values during the day, and lower pH values at night (Anthony et al., 2011).Empirical studies generally utilize a 0.2 - 0.5 unit reduction in pH as an ecologically significant change in seawater chemistry (Semesi et al., 2009; Johnson et al., 2014; Comeau et al., 2015). Despite a statistically significant difference in pH between the two habitats, the pH differential of ~0.02 pH is minor, and most likely not ecologically significant. In the upstream habitat, clod card dissolution was significantly higher, indicating higher water flow conditions. This is consistent with my prediction, which was based on the observation that wave-driven flows rapidly move over the shallowest point of the reef, the reef crest, then slows down as it flows across the back reef (Monismith, 2007). Because faster flow results in faster exchange of metabolites across the algal surface, faster calcification in the upstream samples might be favored by this flow regime (Reidenbach et al., 2006; Carpenter and Williams, 2007). However, the consequent thinning of the DBL may result in higher sensitivity of CCA calcification to OA, suggesting that high flow conditions near the reef crest may be detrimental to calcifiers in future ocean conditions (Cornwall et al., 2014).

In the laboratory experiment, photosynthesis and respiration in CCA were unaffected by *S. pacificum* photosynthesis. Because no effect was observed on these response variables,

they were omitted from the field experiment. These results indicate that calcification in CCA is more sensitive to pH changes than are photosynthesis and respiration (Hurd et al., 2009; Kamenos et al., 2013). However, in cases where macroalgal density is comparable to the density used in the laboratory experiment (1000 g/0.25 m^2), nighttime respiration and daytime photosynthesis may deplete O_2 and DIC enough to limit CCA respiration and photosynthesis, respectively. Various types of algae have the photosynthetic capacity to raise the pH of an isolated volume (700 mL) of seawater above 9.0 (Axelsson and Uusitalo, 1988). Because the highest pH observed during the study was 8.24, the DIC in the treatment seawater may still have been high enough that photosynthesis was not carbon-limited. However, this would depend on whether *L. kotschyanum* are CO_2 or HCO_3^- users and would require further exploration. Lastly, while the 8-day experimental duration was sufficient to result in observable differences in calcification, it may have been too short to alter photosynthetic and respiration mechanisms in *L. kotschyanum*.

The set of experiments conducted in the present study evaluated the indirect metabolic effects and the direct physical effects of a canopy forming macroalga on a tropical crustose coralline alga. My results indicate that the moderation of seawater carbon chemistry by fleshy macroalgae has the potential to mitigate the negative effects of OA on calcifiers. However, this benefit is qualified by 3 caveats: 1) algal biomass must be high, and water residence time long enough for photosynthetic moderation of seawater pH to occur, 2) calcifiers cannot be in close proximity to canopy macroalgae, due to the negative effects of shading, and possibly abrasion and allelopathy, and 3) calcifiers must live downstream of macroalgae. In a coral reef like that of Moorea, these criteria may be met in the future. In present day conditions, algal biomass is already higher near the reef crest than further

downstream. Under these conditions, a significant, albeit slight, difference in pH between the upstream and downstream habitats was detectable. As global stressors continue to favor growth of fast-growing fleshy macroalgae, their biomass per unit area is expected to increase on tropical coral reefs (McCook et al., 2001; Mumby et al., 2005). As a consequence of increased fleshy algal biomass, combined with a more acidic ocean pH, the pH differential between the upstream and downstream habitats may increase under future conditions. Additionally, CCA and corals are found commonly in back reef lagoons of barrier reefs such as the Moorea Coral Reef, a downstream environment that receives continuous water input from the macroalgae-dense habitat on the reef crest. A dense upstream macroalgal community may have the capacity to take up a limited quantity of DIC before the seawater reaches downstream calcifiers, ultimately raising seawater pH downstream. Overall, the present study suggests the potential for a beneficial relationship between fleshy macroalgae and calcifiers under projected future conditions. Therefore, future studies exploring this upstream photosynthetic buffering effect, conducted on a larger spatial scale, may provide critical information in predicting reef community structure and function in response to OA.

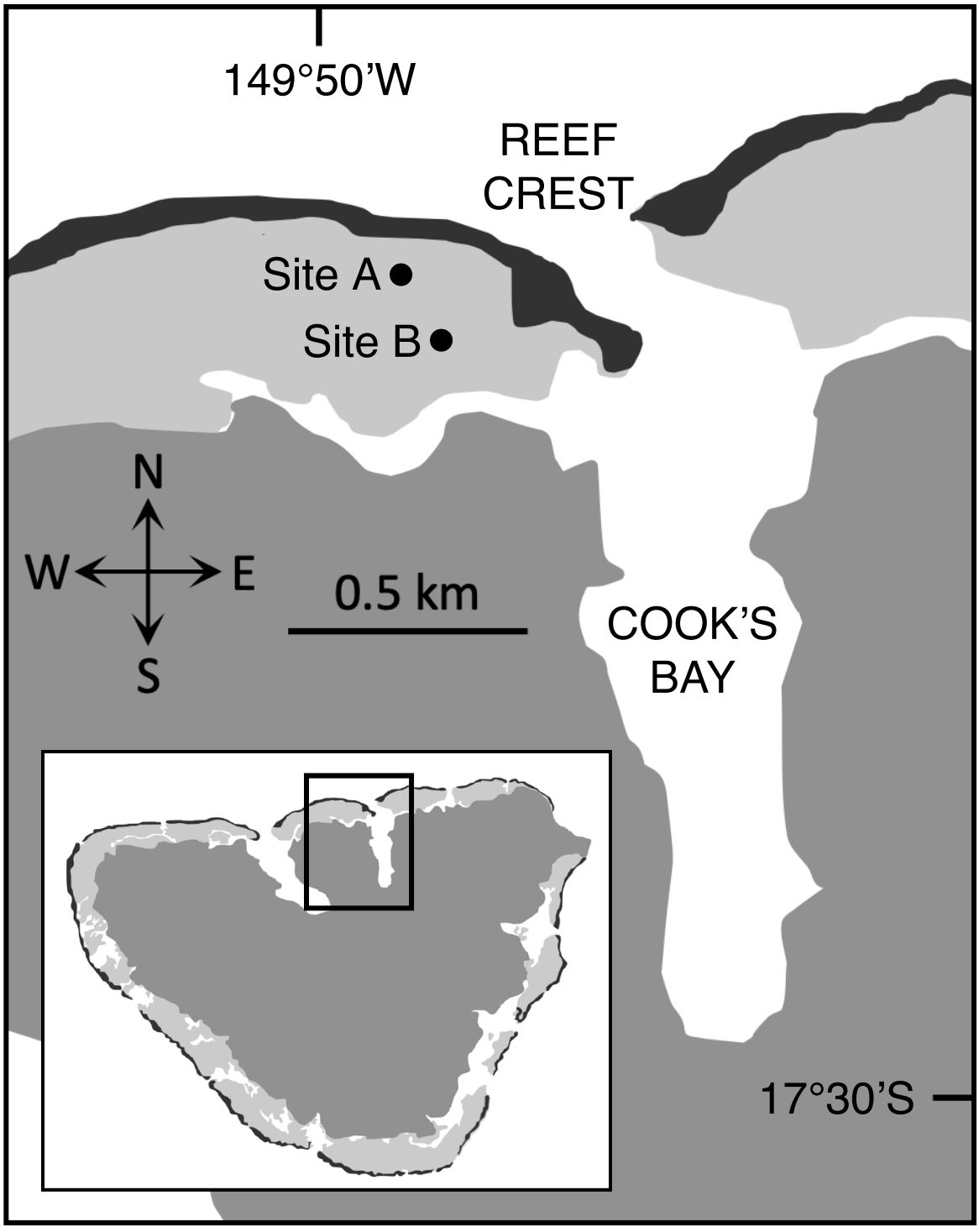

Figure 1. Map of Moorea, French Polynesia indicating the approximate locations of the upstream and downstream sites.

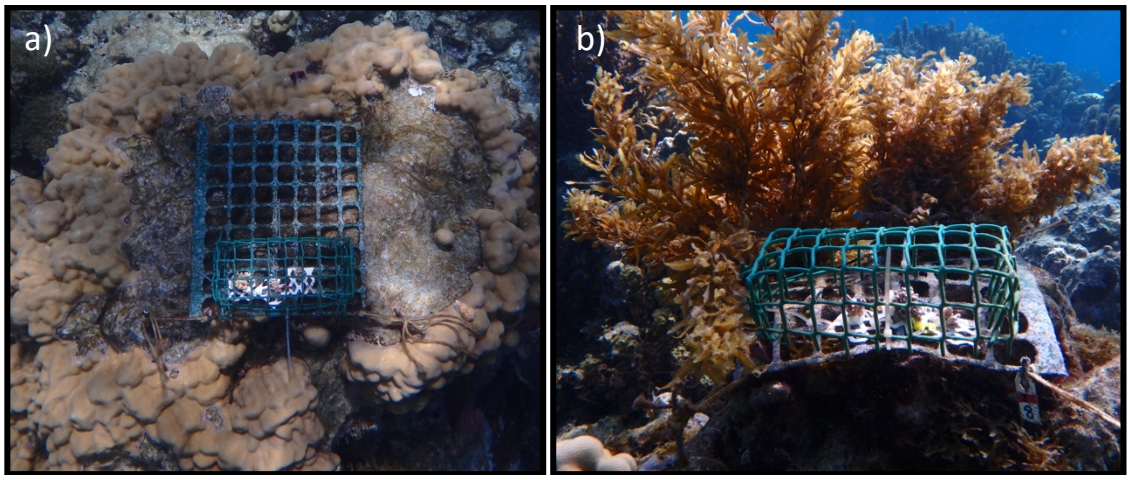

Figure 2. Field setup of control **(a)** and *S. pacificum* **(b)** treatment grids.

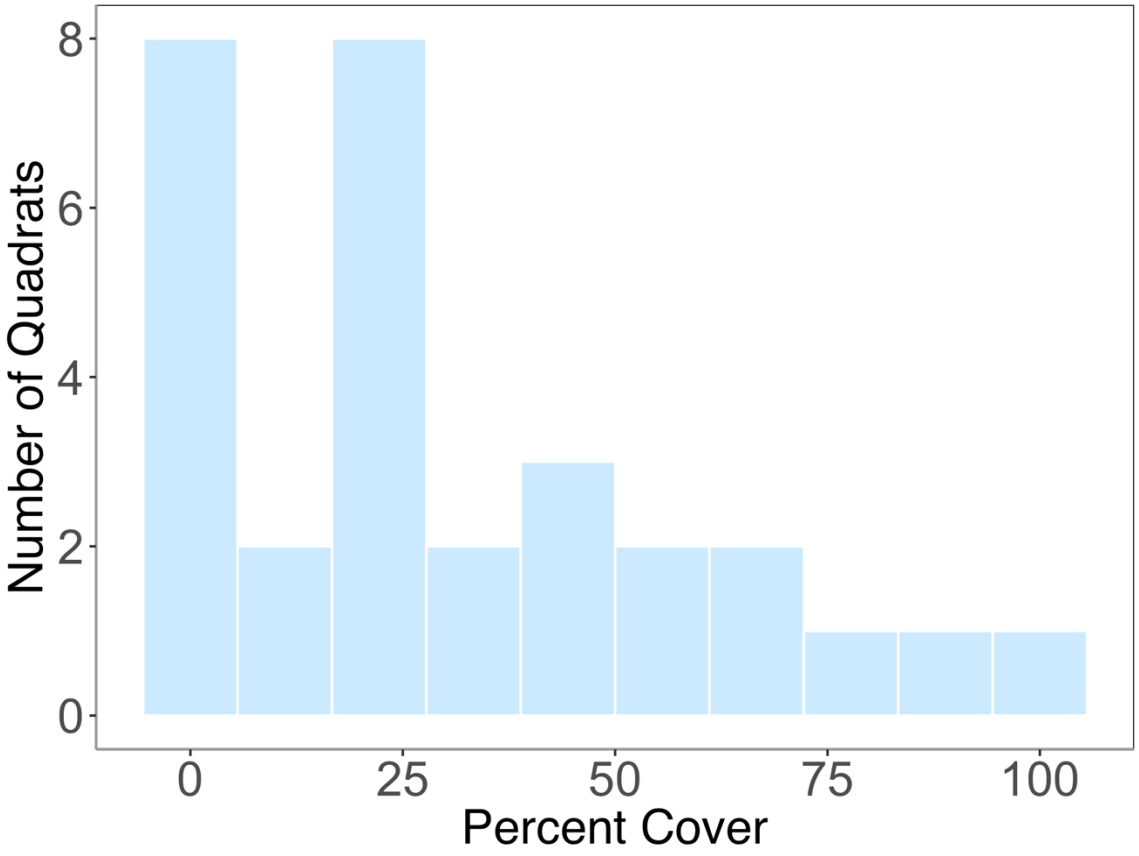

Figure 3. Histogram of *Sargassum pacificum* percent cover at the field site (n = 30).

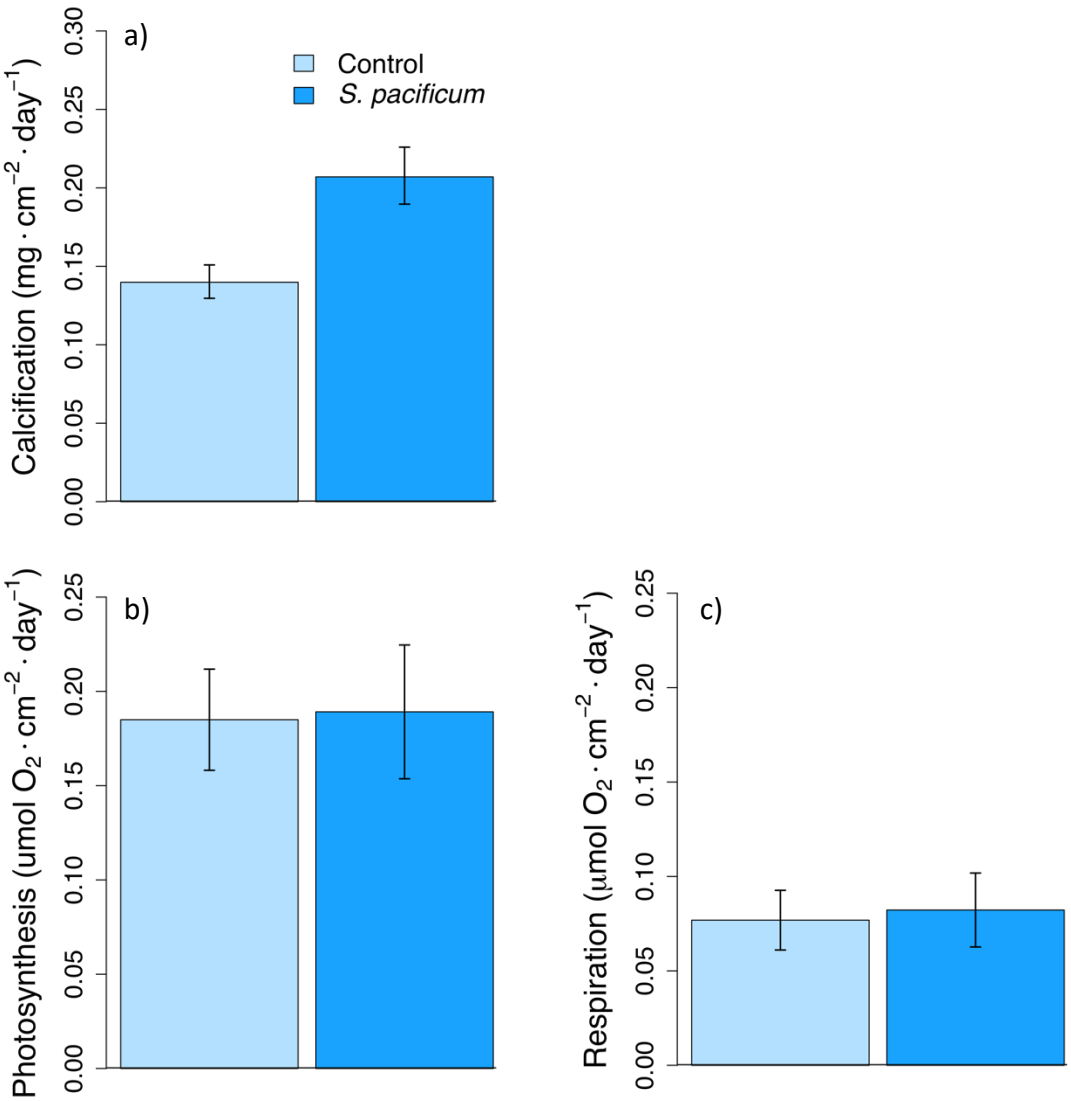

Figure 4. Mean calcification **(a)** , photosynthesis **(b)**, and respiration (O_2 uptake) **(c)** rates for the mesocosm experiment. Graphs show means ± SE (n = 12). There is a significant effect of *S. pacificum* treatment on calcification (p = 0.002).

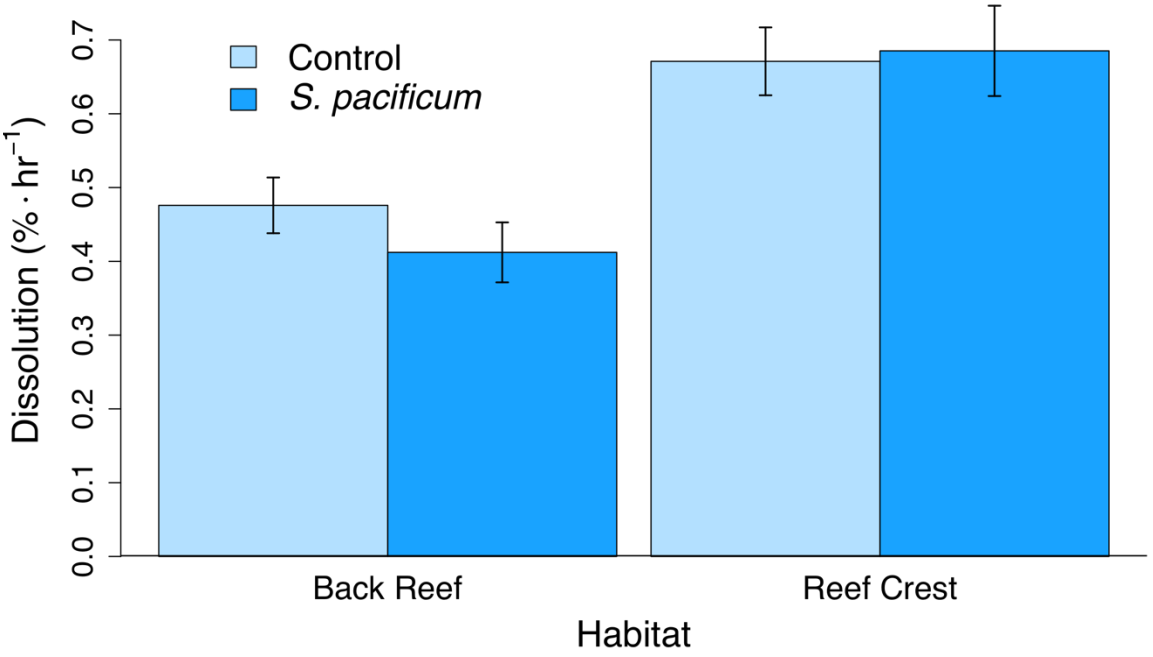

Figure 5. Mean ± SE dissolution for two batches of clod cards in the field experiment (trial 1: n = 3; trial 2: n = 4 grids). There is a significant effect of habitat type on dissolution ($p < 0.001$).

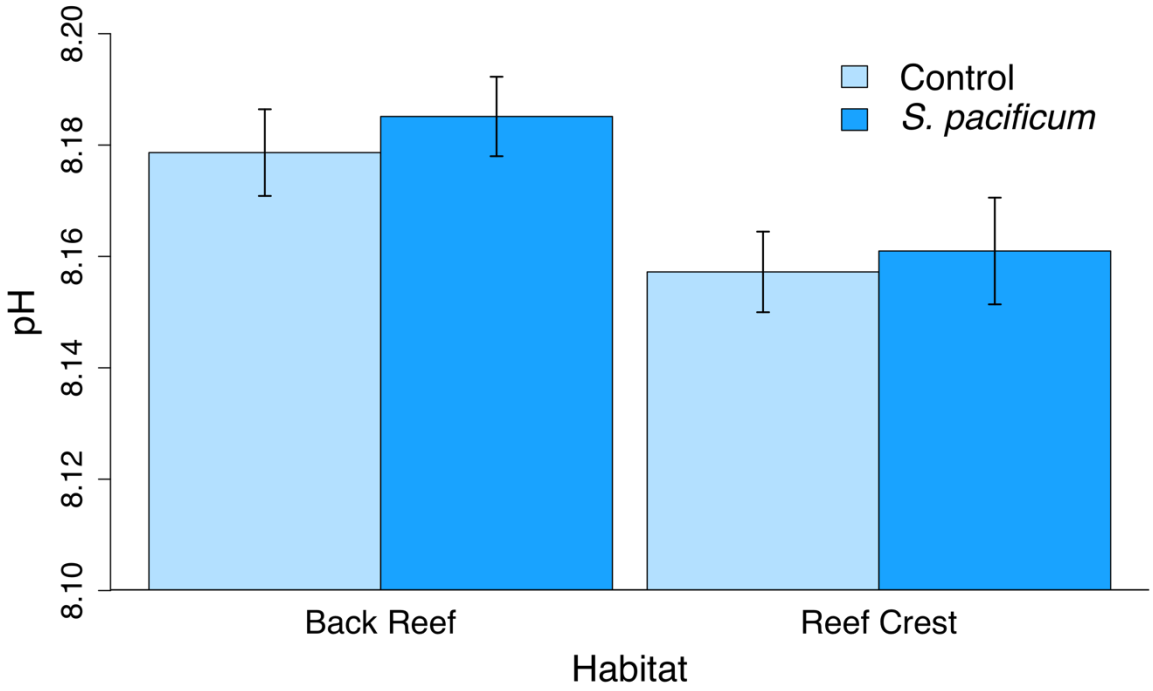

Figure 6. Mean ± SE pH for each treatment group in the field experiment after 4 trials (n = 3). There is a significant effect of habitat type on pH ($p < 0.001$).

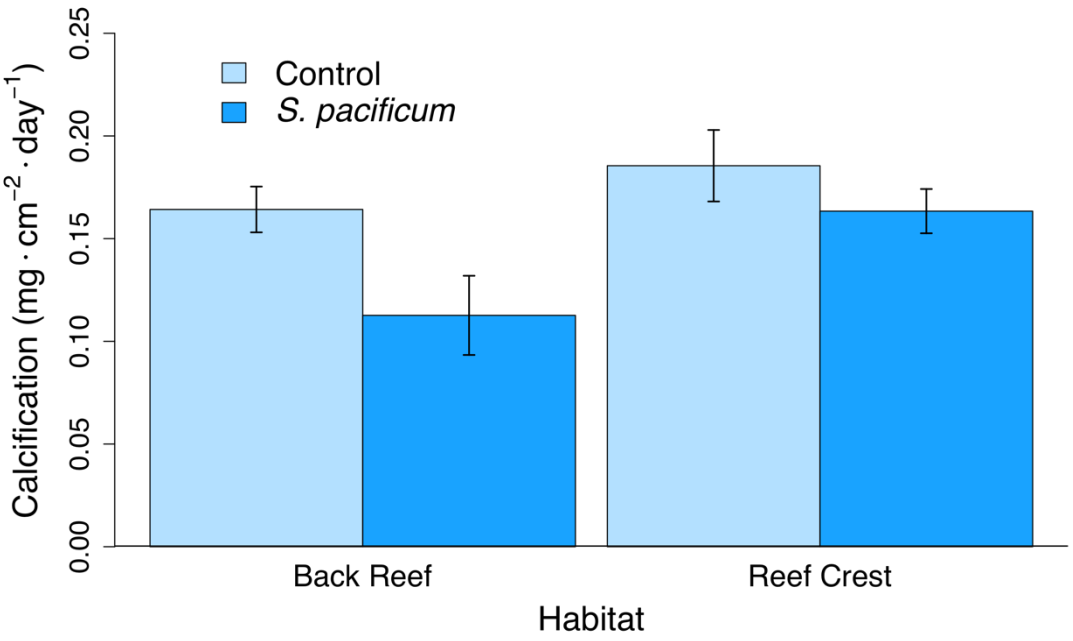

Figure 7. Mean ± SE calcification of *L. kotschyanum* in the field experiment (n = 12 grids/treatment group; n = 3 samples/grid). There is no significant effect of either treatment, and no significant interaction.

Chapter 4

Effects of ocean acidification and algal turfs on calcification and morphology of *Lithophyllum kotschyanum*

Introduction

Economically and ecologically important marine ecosystems, coral reefs boost the global economy through tourism and recreation, and provide vital ecosystem services (McAllister, 1991; Dixon et al., 1993; Moberg and Folke, 1999). These ecosystems support the highest level of biodiversity found in the ocean, due to reef builders that create complex biogenic structures in coral reefs (Knowlton et al., 2010). Within recent decades, declines in coral abundance and reef accretion have been attributed to climate change and other anthropogenic processes (Perry et al., 2013). The ocean has served as a major carbon sink, absorbing approximately a third of atmospheric CO_2, which has been steadily increasing since the Industrial Revolution (Sabine et al., 2004; Solomon et al., 2007). The resulting increase in seawater pCO_2 leads to ocean acidification (OA), a process by which ocean pH declines as the concentration of hydrogen (H^+) and bicarbonate ions (HCO_3^-) increases (Hoegh-Guldberg et al., 2007; Doney et al., 2009).

Under OA conditions predicted by Representative Concentration Pathway (RCP) 8.5, a three-fold increase in atmospheric CO_2 from pre-industrial levels by the end of the century will occur if CO_2 emissions are not mitigated (IPCC, 2014). However, experimental studies have shown that even a doubling of pre-industrial atmospheric CO2 can lead to a 40% decrease in calcification and may transition coral reefs into net dissolution globally (Silverman et al., 2009; Kleypas et al., 2011). In order to precipitate

their calcium carbonate skeletons, calcifiers depend on a high carbonate (CO_3^{2-}) saturation state (Ω), which is lowered through OA as excess hydrogen ions react with carbonate ions to form more bicarbonate (Hoegh-Guldberg, 1999; Hofmann and Bischof, 2014). In present day reefs, a Ω of 3.3 is required to sustain net reef accretion (Kleypas et al., 1999), and projections suggest that Ω may potentially fall below this level as early as 2050 (Hoegh-Guldberg et al., 2007).

There is an extensive literature outlining the effect of OA on corals, the primary foundation species found on coral reefs, but studies on calcifying algae are less numerous (Carpenter et al., 2008; Albright et al., 2010; Comeau et al., 2015). Calcifying algae can cover up to 20% of the reef benthos, creating new regions of biogenic reef and binding together reef fragments to increase the structural stability of the reef framework (Adey, 1998; Fabricius and De'ath, 2001). Ecologically important in these habitats, crustose coralline algae (CCA) facilitate coral settlement, serve as food and shelter for a variety of reef organisms, and contribute significantly to reef productivity and accretion (Bosence, 1983; Chisholm, 2003; Price, 2010). Unfortunately, coralline algal skeletons are particularly sensitive to OA because the $CaCO_3$ mineral they precipitate during calcification, high-magnesium calcite, is the most soluble form in seawater (Hofmann and Bischof, 2014). Due to their sensitivity to OA and slow calcification rates relative to corals, CCA may be among the earliest reef calcifiers to transition into dissolution in changing seawater carbonate conditions (Chisholm, 2000; Comeau et al., 2013b).

While OA slows growth in calcifying algae, non-calcifying (fleshy) algae may increase in abundance due to higher availability of CO_2 for photosynthesis and (Kroeker et al., 2012). A highly prevalent group of fleshy algae found on coral reefs forms fast-

growing and extensive mats, or algal turfs (Connell et al., 2014). This functional group of algae comprises a group of highly productive, filamentous algae and they are among the first to colonize the benthos after a disturbance (Kendrick, 1991; Airoldi, 1998). Algal turfs commonly cover 30 – 50% of the benthos on healthy reefs and often are found on dead coral and live coralline algae (Airoldi, 2000; Rogers and Miller, 2001; Vroom and Braun, 2010; Smith et al., 2016). These filamentous algae are known to impede coral settlement and recruitment through competition for space and facilitation of microbial activity through release of dissolved organic carbon (DOC) as a photosynthetic product (Wild et al., 2010; Haas et al., 2011).

Overgrowth by filamentous turfs has also been shown to impede recruitment and growth of calcifying algae (Kendrick, 1991; Kuffner et al., 2008). These algal turfs can overgrow and shade calcareous algae, reducing photosynthesis and, consequently, calcification (Bulleri, 2006). Within a CCA individual, photosynthesis is thought to facilitate calcification by increasing pH at the thallus surface, thus increasing Ω at calcification sites (Chisholm 2003; Comeau et al. 2013), and by supplying needed energy to support ion transport (Borowitzka, 1981). Epiphytic turf may also reduce water flow in localized regions of the thallus surface of host CCA, resulting in a thicker diffusive boundary layer (DBL) (Hurd and Pilditch, 2011; Cornwall et al., 2015). Thicker DBLs reduce the rate of metabolite exchange, thus slowing physiological processes such as calcification (Koch, 1994; Gardella and Edmunds, 2001). To counter these effects, certain species of CCA have an antifouling mechanism in which they actively slough off their outer layer to remove epiphytes (Keats et al. 1997).

Alternatively, in elevated pCO_2 conditions, epiphytic turf algae may act as a local carbon sink and promote sustained calcification of host CCA under OA stress. Through high rates of photosynthesis, algal turfs have the potential to create higher pH microenvironments within the DBL of CCA than the mainstream seawater (Short et al., 2015). The elevated boundary layer pH of host CCA leads to higher carbonate saturation at the sites of calcification, thus shielding CCA from the effects of OA (Anthony et al., 2013). The biomass of turf algae is generally low relative to their percent cover due to their tendency toward horizontal instead of vertical propagation (Connell et al., 2014). However, a close association between epiphytic turfs and their host CCA suggests that this OA mitigation effect by turf algae would be extremely localized to within a few mm of the thallus surface of CCA. Therefore, turf algae can potentially facilitate CCA calcification by strengthening the chemical refuge from acidification provided by the boundary layer.

As oceanic pH continues to decline, it becomes increasingly important to examine the physiological responses of important reef builders such as calcifying algae, and to assess how such changes may alter reef accretion and carbon cycling. Past studies have focused on the negative effects of turf overgrowth on calcifier metabolism, and few have studies have been conducted on CCA, relative to corals (McCook et al., 2001; Bulleri, 2006; Arnold et al., 2010; Vermeij et al., 2010). In Moorea, French Polynesia, a layer of dense algal turf commonly is seen growing on an abundant species of crustose coralline alga (CCA), *Lithophyllum kotschyanum*, which has demonstrated sensitivity to OA in experimental conditions (Comeau et al., 2014). *L. kotschyanum* has a moderately complex morphology with smooth branches, and turf algae grow abundantly within the

interstitial spaces between branches. Because of this, CCA morphology may be affected as growth between branches is impeded by turf overgrowth. Alternatively, under high pCO_2 conditions, turf algae between branches may shield CCA from OA stress, thus increasing growth between branches, relative to branch tips, conforming *L. kotschyanum* to a thick, encrusting morphology. The main objective of the present study was to assess the capacity of epiphytic turf algae to act as a localized carbon sink and a means to mitigate OA effects at the CCA thallus surface. I hypothesized that 1) epiphytic turfs would increase mean seawater pH encountered by host *L. kotschyanum* through DIC uptake, thus increasing CCA calcification and 2) the effect would be stronger under the elevated pCO_2 treatment and restricted to regions of turf growth between branches.

Methods

The effects of turf algae and OA on CCA physiology were quantified in a mesocosm study. The goal of this experiment was to assess the capacity of turf algae to mitigate the effects of OA within the DBL of CCA. Under ambient and elevated pCO_2 conditions, the laboratory experiment addressed the effect of algal turfs on seawater carbon chemistry at the thallus surface of *L. kotschyanum*, as well as CCA net calcification and linear extension.

Sample collection and preparation

Individuals (n = 80) of the common *L. kotschyanum* were collected from the fringing reef on the north shore of Moorea, French Polynesia at depths of ~1-2 m. Samples were separated from the reef substratum using a hammer and chisel and transported to the Richard B. Gump South Pacific Research Station. A diamond bandsaw (Gryphon C-40, CA, USA) was used to create samples of similar sizes (2-3 cm W/L) samples, which then were attached to plastic supports using Coral Glue (EcoTech Marine, PA, USA). To anesthetize and remove epifaunal invertebrates while leaving epiphytic turf algae undisturbed, *L. kotschyanum* samples were immersed in an $MgCl_2$ seawater bath (50 g L^{-1}) for 1 hour (Arafa et al., 2007; Suquet et al., 2009). Calcein, a visible fluorochrome marker shown to be non-toxic to CCA (Lewis and Diaz-Pulido, 2017), was then used to mark a baseline for measurement of linear extension. Following an 8-day acclimation period in a flowing seawater table, samples were immersed in a calcein bath (50 mg L^{-1}) overnight for 12 hours, a staining time that was sufficient to yield usable baseline in prior studies (Linard et al., 2011; Cornwall et al., 2017). To

enhance solubility and buffer the acidic calcein, (Sigma-Aldrich CAS 148504-34-1), the solution was prepared with enough sodium hydroxide (NaOH) to maintain the solution at an ambient seawater pH of ~8.1. The final pH of the solution was calculated by measuring temperature and mV using the method described below. Temperature and PAR during staining matched the treatment conditions in the mesocosm tanks during the study. An airstone bubbled air into the calcein bath and maintained water movement throughout the immersion period. Following staining, individuals were rinsed in a fresh seawater bath for 1 hour to remove any residual stain and assigned randomly to treatments (n = 10 per tank).

Treatment maintenance

Eight, 150-L mesocosm tanks (Aqualogic, San Diego, CA) were maintained under two replicated turf treatments (present/absent) crossed with two replicated pCO_2 levels (ambient = 400 μatm; elevated = 1000 μatm). In the treatment where turf was absent, samples were cleaned of epiphytes. In the turf present treatment, samples were brushed gently to avoid shading of CCA samples by turf algae, removing turf from branch tips while leaving turf in interstices undisturbed (Fig. 1). CCA samples were cleaned and rearranged haphazardly every 3-4 days to minimize positioning effects. The ambient pCO_2 treatment consisted of fresh seawater, pumped from Cook's Bay at 12-m depth and sand-filtered (pore size ~550 μm), while the elevated treatment was based on a business-as-usual scenario, Representative Concentration Pathway (RCP) 8.5 (IPCC, 2014). Seawater entered the mesocosms at a mean (± SE) rate of 315 ± 2.6 mL min^{-1}, resulting in approximately three complete turnovers of water in each tank daily. A water

pump (Rio 8HF, 2082 L h^{-1}) produced constant flow in each tank and airstones were used to supply air at a constant rate. To minimize gas exchange at the water surface and stabilize pCO_2 in treatments, each tank was fitted with a plexiglass lid. The mean austral winter temperature at the study site, ~27 °C, was the target temperature for all treatments (Washburn, 2018). Diel light cycles in each tank followed a 12:12 h light:dark photoperiod with 4-h ramp times, supplying Photosynthetically Active Radiation (PAR, 400-700 nm) using 75W Light Emitting Diode (LED) modules (Sol White LED Module, Aquaillumination, IA, USA). Maximum light levels were set at ~600 µmol quanta m^{-2} s^{-1}, similar to ambient light levels at the study site, and measured throughout the experiment with a 2π underwater sensor (model LI-192 UWQ 7060) attached to a LI-COR LI-1400 data logger (Johnson et al., 2014).

Throughout the 26-day experiment, the elevated pCO_2 treatment was maintained by a pH-stat aquacontroller (Neptune Systems, Morgan Hill, CA) that bubbled pure CO_2 into the mesocosm tanks when pH rose above the set point. Carbonate system parameters were calculated daily from temperature, pH, and total alkalinity (A_T) measurements. Temperature (Traceable Digital Thermometer, Fisher Scientific, accuracy: 0.05 °C, resolution: 0.01 °C) and mV (Thermo-Scientific Orion 3-star portable meter with Mettler Toledo InLab Expert Pro probe, calibrated on the total scale with TRIS buffers at a salinity of 35.0 psu) measurements were used to calculate pH. Every other day, water samples were collected from each tank to measure salinity (Orion Star A212 Conductivity Benchtop Meter) and total alkalinity (A_T). A_T was measured using open-cell potentiometric titrations (Mettler Toledo T50 titrator with a Mettler Toledo DGi 115-SC probe) on 50-mL aliquots of treatment seawater (Dickson et al., 2007). Accuracy and

precision of titrations were determined using titrations of certified reference materials (A. G. Dickson batch 158), which yielded A_T values within 9.9 μmol kg^{-1} of the certified value (SD = 5.96 μmol kg^{-1}; n= 9). Carbonate system parameters were calculated daily for each treatment tank from A_T, pH, temperature, and salinity measurements using the R package seacarb (Lavigne and Gattuso, 2012).

Response variables

Net calcification and linear extension were measured to determine the effect of turf algae on the growth of *L. kotschyanum*, and whether the effect differed between branch tips and interstices. Mean calcification over the entire experiment was measured using the buoyant weighing method (Davies, 1989). Initial and final buoyant weights (Mettler Toledo PB303-S balance, resolution: 0.001 g) of each sample were recorded, and the differences (weight change) were converted to dry weights using the measured density of seawater and a density of pure calcite of 2.71g cm^{-3}. Net calcification was standardized to surface area (SA), which was quantified using the dye-dipping method (Hoegh-Guldberg, 1988). Samples were dipped in a solution of methylene blue dye (0.1 g 100 mL^{-1} with ~2.5% Triton X-100 detergent) and rinsed in 100 mL of seawater. Using a spectrophotometer (Shimadzu UV-2450), the absorbance of each seawater sample was measured at 620 nm and compared to a standard curve constructed from foil pieces of known surface areas. Sample SA then was predicted from the resulting standard curve ($y = 271.85x + 2.26$, regression of absorbance to known SA). Linear extension was measured for a random subset of 40 samples (n = 5 per tank) using the calcein marker as the starting point of growth in treatment. Samples were reinforced with clear, non-brittle

glue (Scotch High Performance Repair Glue) and cross-sectioned with a bandsaw (Powermatic, Model 148, TN, USA). Each cross-section was subsequently sanded with 180, 300, and 2000 grit sandpaper, respectively, to achieve smooth surfaces for viewing under a microscope. Fluorescence microscopy (Olympus IX71, Model U-LH100HG Fluorescence Light Source) with a FITC filter block was used to detect the excitation and emission peak wavelengths of calcein at 494 nm and 517 nm, respectively. Linear extension then was quantified using ImageJ by measuring the distance from the outside edge of the calcein marker to the growing edge of the sample (Fig. 2). Three measurements per sample were used to obtain average vertical growth at the branch tips. Calcein markers were not visible in any of the interstices between branches, likely due to minimal calcification during calcein immersion. Therefore, comparisons were made between branch tip calcification and total net calcification measurements.

Boundary layer pH was measured at the branch tips and in the interstices between branches to determine the localized effect of turf algae on seawater chemistry within these interstices. A random subset of 40 samples (n = 5 per tank) were placed in 250-mL acrylic chambers and incubated with water from the corresponding treatment tanks. A water jacket that received circulating flow from a chilled water bath maintained incubation temperature at 27 °C. Constant water mixing throughout incubations was generated by stir bars at the base of each chamber, separated from samples by a plastic mesh. Samples were incubated in saturating light (~200 μmol quanta m^{-2} s^{-1}), which was estimated with PI curves calculated using a Walz Diving PAM. Two fiber optic halogen lights (Ace 1, Schott North America, Inc.) delivered ~600 μmol quanta m^{-2} s^{-1} to samples in incubation, similar to maximum light levels in the treatments. Temperature (Traceable

Digital Thermometer, Fisher Scientific, accuracy: 0.05 °C, resolution: 0.01 °C) and mV (Thermo-Scientific Orion 3-star portable meter with Mettler Toledo InLab Ultra Micro-ISM probe, calibrated on the total scale with TRIS buffers at a salinity of 35.0 psu) measurements between branches and at branch tips were used to calculate boundary layer pH in regions with and without turf algae, respectively. Measurements of ΔpH were divided by incubation duration (mean ± SE = 19.9 ± 0.56 min).

Statistical Analyses

Calcification and linear extension were analyzed using linear mixed-effects models with pCO_2 and turf presence/absence as fixed factors, and tank as a random factor. Models with and without the tank factor were compared, and the tank effect was dropped from the final model when Akaike's information criterion (AIC) values were lower without tank. Assumptions of normality and homoscedasticity were evaluated through graphical analyses of residuals and data were square root transformed when necessary to meet assumptions. Analyses were performed using R software package lme4 (R Foundation for Statistical Computing). For analysis of standardized measurements of ΔpH over time, location (branch tip or between branches) was added as a third fixed factor. A generalized linear mixed-effects model using package nlme was used to allow for unequal variances among locations, which data transformations were unable to resolve.

Results

Mean (± SE) temperature and salinity in the tanks were 27.05 ± 0.04 °C and 35.56 ± 0.05, respectively. Mean (± SE) PAR was supplied at 543.2 ± 22.3 μmol quanta m^{-2} s^{-1}. In the ambient pCO_2 treatment, mean (± SE) pH was 8.08 ± 0.008, representing mean (± SE) pCO_2 levels of 378.9 ± 12.18 μatm. In the elevated pCO_2 treatment, mean (± SE) pH was 7.70 ± 0.009, representing mean (± SE) pCO_2 levels of 1054.47 ± 33.75 μatm. Net calcification rates were significantly higher in the treatment where turf was absent ($F_{1,76}$ = 67.33, $p < 0.001$). Presence of turf decreased calcification by approximately 56%, from 0.163 ± 0.008 mg cm^{-2} d^{-1} to 0.071 ± 0.008 mg cm^{-2} d^{-1} (means ± SEs) (Fig. 3). From the ambient pCO_2 treatment, elevated pCO_2 decreased calcification by 18%, from 0.128 ± 0.011 mg cm^{-2} d^{-1} to 0.106 ± 0.010 mg cm^{-2} d^{-1} (means ± SEs) ($F_{1,76}$ = 4.11, $p = 0.046$) (Fig. 3). There was no significant interaction between turf treatment and CO_2 ($F_{1,76}$ = 0.17, $p = 0.68$). For linear extension rates at branch tips, no significant effect of turf treatment or pCO_2 was found, and there was no significant interaction ($F_{1,36} \leq 0.59$, $p \geq 0.45$) (Fig. 4). A significant interaction between turf treatment (present/absent) and location (branch tips/between branches) on seawater pH was found ($F_{1,68}$ = 47.15, $p < 0.001$). The presence of turf resulted in an approximate threefold increase in the rate of pH change between branches, from 0.13 ± 0.03 pH units h^{-1} to 1.33 ± 0.02 pH units h^{-1} (means ± SE) (Fig. 5). There was no significant effect of CO_2 on seawater pH ($F_{1,4}$ = 3.36, $p = 0.14$), and there was no significant interaction between CO_2 and turf treatment ($F_{1,4}$ = 0.21, $p = 0.67$) or between CO_2 and location on seawater pH ($F_{1,68}$ = 0.66, $p = 0.42$). Lastly, the three-way interaction was not significant ($F_{1,68}$ = 0.71, $p = 0.40$).

Discussion

On the fringing and back reefs of Moorea, French Polynesia, *L. kotschyanum* rarely is found without a layer of epiphytic turf algae on its thallus surface. Therefore, to fully understand the response of these important ecosystem engineers to the OA, it is necessary to consider the ecological interactions between CCA and the epiphytes to which they provide a substratum for growth. In a controlled laboratory experiment, the presence of turf algae decreased CCA calcification, despite significantly increasing pH within the DBL between *L. kotschyanum* branches during light incubations. Although the low-lying (several mm to a few cm) structure of algal turfs results in a low biomass per unit area relative to other non-calcifying algal taxa, their high photosynthetic rates and close association with CCA allow them to influence CCA success. Incubations lasting ~20 minutes were sufficient to measure pH increases between branches of over 1 pH unit. These results suggest that *L. kotschyanum* may experience large diel fluctuations in pH within their DBLs due to their close association with epiphytic turf algae.

High daytime photosynthesis by epiphytic turfs suggests that nighttime respiration may also substantially lower pH, likely surpassing end-of-century decreases in oceanic pH predicted by RCP 8.5 (IPCC, 2014). Additionally, this may lead to sustained periods of hypoxia or anoxia among CCA branches at night (Wangpraseurt et al., 2012). Although the effects of nighttime anoxia on the physiology of CCA are not well-studied, it is likely detrimental due to lack of O_2 for respiration and low pH for nighttime calcification (Short et al., 2015). Experimental studies have shown that hypoxia or anoxia can lead to reduced metabolic efficiency and even mortality in corals (Simpson et al., 1993; Murphy and Richmond, 2016), and may also be stressful to CCA. These diel

amplifications of pH range experienced by CCA may be greater in *L. kotschyanum* than in less complex, knobby forms, such as *Hydrolithon* spp., or in flat, adherent forms, such as *Porolithon* spp. Because of this, *L. kotschyanum* may experience particularly high pCO_2 levels in future conditions, as OA is augmented by diel pH fluctuations at the thallus surface. Consistent with my hypothesis, elevated pCO_2 significantly decreased *L. kotschyanum* calcification (Fig. 3) and lends further support to the negative effect of OA on CCA calcification shown in prior studies (Hofmann and Bischof, 2014; Fabricius et al., 2015).

Elevated pCO_2 had no significant effect on the rate at which turf photosynthesized during incubations, suggesting that algal turfs are not-carbon limited in present day conditions. Turf algal abundance has exhibited variable responses to elevated pCO_2 conditions, including positive, negative, and species-specific responses (Connell and Russell, 2010; Porzio et al., 2011, 2013). Overall, the 56% reduction in calcification of CCA attributed to the presence of epiphytic turf indicates that any benefit of higher daytime pH within the DBL of *L. kotschyanum* was outweighed by the negative effects, such as shading or nighttime anoxia and low pH (Bulleri, 2006; Wangpraseurt et al., 2012). Turf algae can also promote microbial proliferation through the photosynthetic release of DOC for consumption by microbes (Wild et al., 2010). In turn, microbial respiration can deplete oxygen and exacerbate nighttime hypoxia due turf respiration (Haas et al., 2011).

Another factor affecting the response of CCA to the presence of epiphytic turf algae may be the species of turf growing on the CCA thallus. Algal turfs are commonly studied as multispecies assemblages and, to my knowledge, studies have not been

conducted on individual turfing species (Connell et al., 2014). As a result, species-specific physiological characteristics of turf-forming species are not well described. As photosynthetic physiology is highly variable among larger marine macrophytes (Brylinsky, 1977; Raven and Hurd, 2012), physiological differences among turf species may affect overall photosynthetic rate and production within a single assemblage. Thus, multispecies turf assemblages of comparable biomass may exhibit high variation in the presence and magnitude of microbial activity.

Additionally, different densities of algal turfs have been shown to have contrasting impacts on CCA physiology. A previous study showed that moderate levels of turf overgrowth (~ 43% cover) could benefit CCA calcification under high pCO_2, but higher turf densities influenced CCA negatively, due to reduced recruitment and growth of CCA (Short et al., 2015). Densities of epiphytic algae used in treatment described here were not experimentally manipulated and are therefore similar to natural densities found in the field at the time of sample collection. This suggests that present levels of turf overgrowth on CCA may already be high enough to pose a threat to CCA persistence. However, experimental samples were collected from the fringing reef, where water flow is low and a layer of epiphytic turf is found on much of the benthos (Carpenter, 2018). *L. kotschyanum* occurring in habitats with lower cover of algal turfs may be less impacted by their overgrowth.

Data from linear extension measurements indicate that net growth at CCA branch tips did not differ between pCO_2 or turf treatments. However, overall calcification in CCA individuals was lower in the presence of epiphytic turf, indicating that lowered calcification between branches may be responsible for this decrease. This is consistent

with the hypothesis that the effects of turf are restricted to the interstitial spaces between branches, but differs in that turf appears to inhibit CCA calcification despite raising daytime pH. These results suggest that the negative effects of turf on CCA calcification stem from its physical structure, not from toxic chemical effects on the seawater, such as allelopathy (Littler and Littler, 1997). If the latter were true, it is probable that the branch tips would also exhibit a reduction in growth in the presence of turf (Maida et al., 1995; Mulderij et al., 2006).

Due to their ability to affect CCA calcification at localized regions of the thallus surface, turf algae have the potential to modify CCA morphology over longer time scales. In high densities, turf algae may facilitate the formation of longer, thinner branches of host CCA, such as *L. kotschyanum*, as calcification between branches is lowered. This morphology may support a higher density of turf algae and higher diversity of invertebrates, as there will be more interstitial space between branches (Stachowicz and Hay, 1996; Steller et al., 2003; Gabara et al., 2018). In low densities, epiphytic turfs may instead facilitate the development of thicker crusts, as growth between branches increases due to a localized rise in pH (Short et al., 2014). Thick crusts can increase reef structural integrity and resistance to wave erosion, as well as host a higher diversity of cryptic infauna (Bosence, 1983; Chenelot et al., 2011).

Overall, the results of this 26-day mesocosm study demonstrated a negative effect of turf algae on CCA net calcification between branches. This confirms that current densities of epiphytic turf on CCA in the fringing reef of Moorea (~ 20 – 40% cover) may be high enough to reduce net calcification on localized regions of the thallus surface. Thus, the current densities at which epiphytic turf occur on *L. kotschyanum* may

ultimately alter their branching morphology. In ecosystems like Moorea, nutrient-poor water conditions are less conducive to turf algal growth (Gorgula and Connell, 2004). Because the natural densities of turf on CCA observed in this study were sufficient to reduce calcification of host CCA by 56%, nutrient-rich ecosystems may further increase the ability of turf to overgrow its calcifying benthic competitors (Vermeij et al., 2010). Although the effect would be smaller, sparser aggregations of turf-forming algae may still produce higher pH microclimates on regions of the CCA thallus, allowing continued calcification under future OA conditions (Short et al., 2015). Consistent with many previous studies, a significant effect of OA on CCA was demonstrated in this study, indicating that *L. kotschyanum* is sensitive to elevated pCO_2 levels (Comeau et al. 2013; Comeau et al. 2014).

Currently, it remains unclear whether the combined effects of OA and turf will be additive, synergistic, or antagonistic under changing climate conditions. The present study, in conjunction with prior studies (Airoldi, 2000; Diaz-pulido and McCook, 2002; Connell and Russell, 2010), suggests that OA and warming will enhance the rate of turf overgrowth, resulting in overall negative effects on CCA success in future coral reefs. This study demonstrates the causal relationship between turf overgrowth and reduced CCA calcification. However, similar studies performed *in situ* would be required to confirm whether this effect persists in a natural environmental setting. Lastly, this study raises questions regarding the effect of nighttime anoxia on CCA, and studies elucidating CCA tolerance to this phenomenon will inform a better understanding of CCA vulnerability to projected OA conditions. Untangling the effects of a changing climate and ecological interactions, which are in turn affected by a dynamic environment, is

crucial to understanding ongoing changes in coral reef trophic dynamics as well as in predicting future benthic community structure.

Figure 1. *Lithophyllum kotschyanum* individuals with natural densities of turf growing in interstitial spaces between branches.

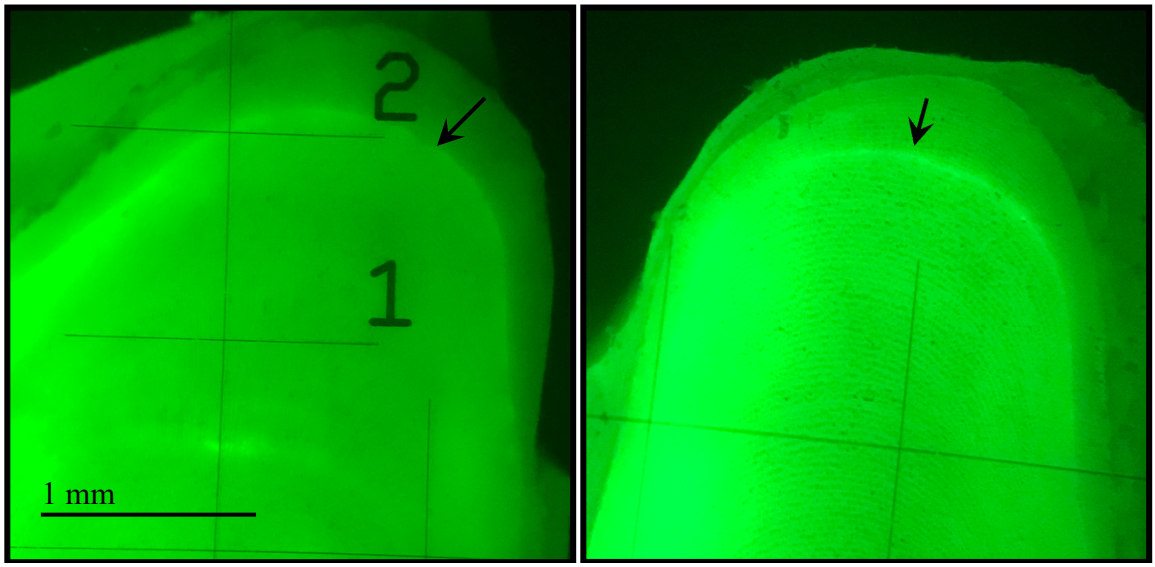

Figure 2. Fluorescent images of calcein markers from two individuals viewed under a FITC filter block.

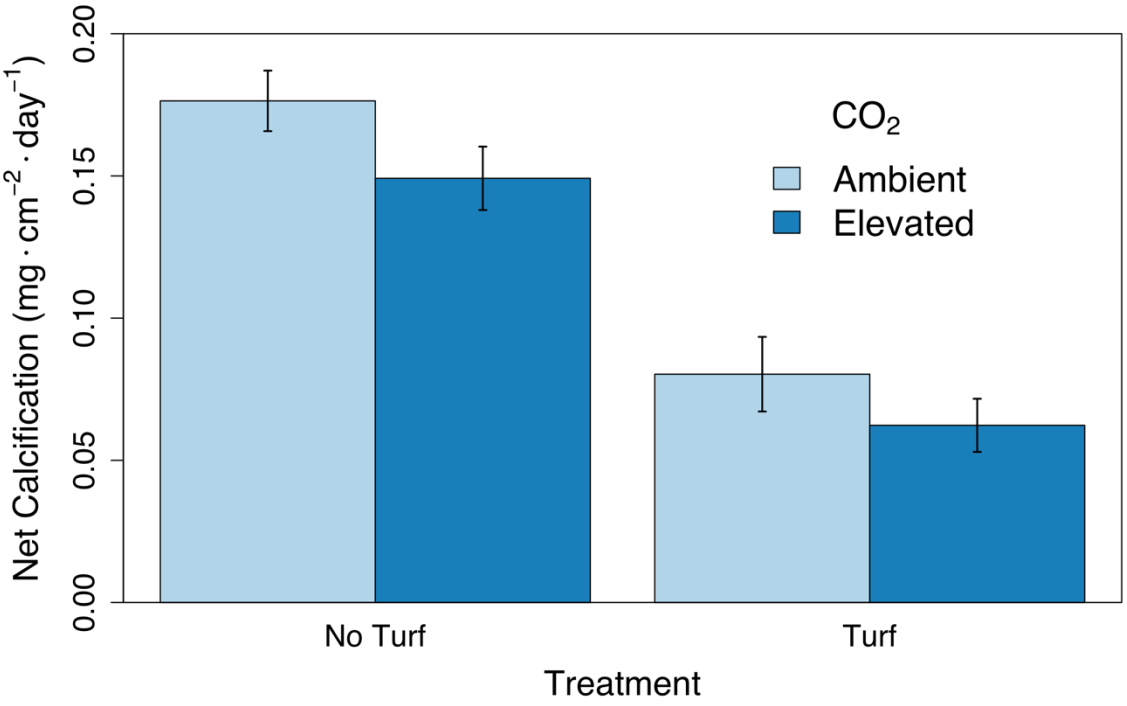

Figure 3. Mean ± SE net calcification rates of *L. kotschyanum* (n = 10). There is a significant effect of turf treatment ($p < 0.001$) and pCO_2 ($p = 0.046$) on calcification.

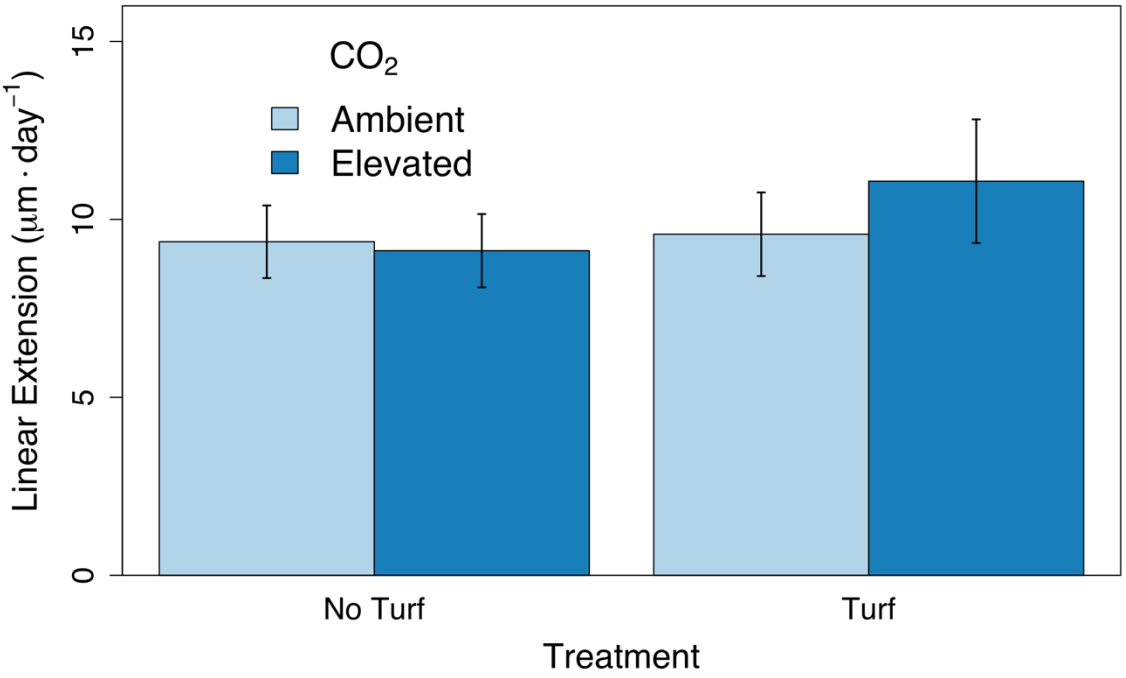

Figure 4. Mean ± SE linear extension rates at branch tips (n = 5). There is no significant effect of either treatment and no significant interaction.

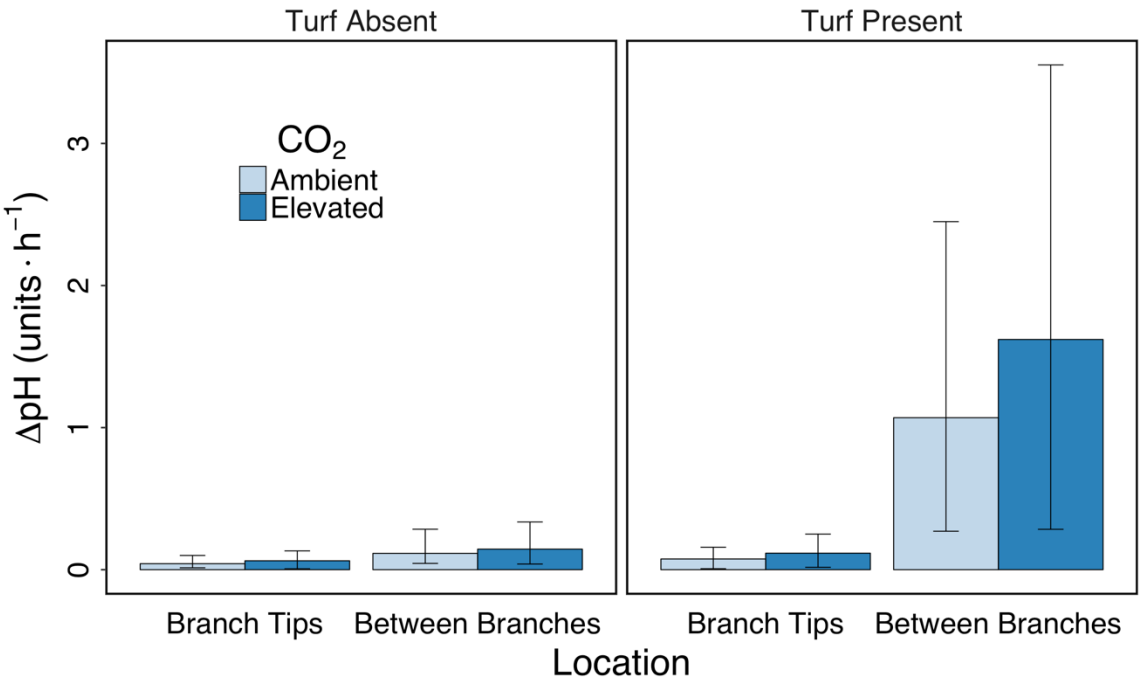

Figure 5. Mean ± SE rate of surface area normalized pH change at branch tips and between branches (n = 5). There is a significant interaction between location on thallus and turf treatment ($p < 0.001$).

Chapter 5

Conclusions

Through gradual reef accretion (7-70 mm/year) (Buddemeier and Smith, 1988), corals and other calcifying species have formed structurally complex biogenic reefs that provide a variety of important ecosystem services and are economically important (Stanley Jr., 1988; Dixon et al., 1993; Moberg and Folke, 1999; Knowlton et al., 2010). Due to a variety of global and local anthropogenic impacts, the future success of coral reefs remains uncertain (Pandolfi et al., 2011). Ocean acidification (OA) is a global stressor that reduces habitat complexity and slows reef accretion by lowering the seawater concentration of carbonate (CO_3^{2-}), an ion needed for calcification by reef builders (Hoegh-Guldberg et al., 2007). Local stressors such as overfishing and pollution, as well as global stressors such as OA and warming, drive a positive feedback loop that favors the rapid growth of non-calcifying macroalgae on reefs while suppressing the growth of reef builders (McCook, 1999; Pandolfi et al., 2011). Historically, stony corals, the primary reef building taxonomic group, have been the focus of coral reef research (Doney et al., 2009; Edmunds et al., 2014; Comeau et al., 2015). However, crustose coralline algae (CCA) are also recognized as a vital reef building group (Bosence, 1983; Adey, 1998) whose physiology responds to environmental stressors.

CCA are critical in stabilizing the reef framework as they bind together coral rubble and contribute to reef calcification. Furthermore, they provide habitat and food for a variety of reef organisms, enhance primary production and reef accretion, and facilitate coral settlement (Bosence, 1983; Chisholm, 2003; Price, 2010). The changing physiology of CCA, a key taxon in the most biodiverse marine environment, must be examined as

the changing climate induces increasingly stressful environmental conditions on coral reefs. Through a pH reduction of ~0.3 – 0.4 units, OA decreases calcification rates in CCA, while increasing the concentration of dissolved inorganic carbon (DIC), which is used in photosynthesis by fleshy algae (Hughes et al., 2006; Ledlie et al., 2007; Johnson and Carpenter, 2012). On coral reefs, fleshy algae compete with CCA for benthic space (Vermeij et al., 2011; Hofmann et al., 2012), but may also create microhabitat refuges from OA through carbon dioxide drawdown during photosynthesis (Anthony et al., 2013; Vogel et al., 2015). This thesis elucidated the effects of two ongoing stressors on coral reefs, ocean acidification and increased fleshy algal abundance, on a common species of CCA in the shallow reef lagoons of Moorea, French Polynesia.

In Chapter 2, I sought to tease apart the canopy effects of shading and canopy metabolism under OA conditions on a common CCA species, *Lithophyllum kotschyanum*, under standardized flow conditions. Consistent with prior studies, the experiment confirmed that canopy macroalgae reduces light to understory CCA to a level low enough to impair calcification, likely by reducing CCA photosynthesis (Chisholm, 2003; Cornwall et al., 2015). *L. kotschyanum* calcification was further reduced between the dark mimic and the *S. pacificum* treatment, possibly representing a biological effect of the canopy. *L. kotschyanum* photosynthesis was also affected by canopy type and CO_2, potentially indicating plasticity in photosynthetic mechanisms (Falkowski and Owens, 1980; Kaplan et al., 1980; Carpenter, 1985; Briggs and Carpenter, 2019). The density of *S. pacificum* used was sufficient to alter pH within the tanks, resulting in a conspicuous diel fluctuation in seawater pH. Daytime photosynthesis raised seawater pH, and nighttime respiration presumably follows the same diel pattern, lowering pH. Because

algal photosynthesis typically evolves oxygen at a higher rate than algal respiration uptakes oxygen (Burris, 1977), increases in daytime pH were likely larger than nighttime decreases (Christopher E Cornwall et al., 2013). If this is true, calcification should exhibit a positive response to the higher overall mean seawater pH. To confirm this inference, a study focused on the biological effect of an *S. pacificum* canopy on CCA was performed in Chapter 3. However, since calcification was lowest in the *S. pacificum* treatment, it is possible that the negative effects of a fleshy macroalgal canopy, potentially attributed to a reduction in flow (Christopher E. Cornwall et al., 2013) or increased microbial density beneath the canopy (Haas et al., 2011), outweigh the benefits. Additionally, the macroalgal canopy may have been more morphologically complex than the plastic mimics, resulting in slower water flow at the CCA thallus surface. This would thicken the diffusion boundary layer (DBL) of CCA and, consequently, slow the rate of exchange of metabolites between CCA individuals and the bulk seawater (Reidenbach et al., 2006; Carpenter and Williams, 2007).

In Chapter 3, I conducted laboratory and field experiments with the objective of isolating the biological effects of canopy-forming macroalgae from the effects of its physical structure. During the mesocosm manipulation, *S. pacificum* (~1000 g) raised the pH of 150 L of seawater, and *L. kotschyanum* net calcification increased as a result. The laboratory experiment also confirmed that *S. pacificum* raises daytime pH more than it lowers nighttime pH. This study represents some of the first evidence that fleshy macroalgae may promote calcification. However, the field study provided no evidence that the canopy created by *S. pacificum* can modify the dissolved inorganic carbon or pH of the seawater beneath, thus potentially explaining why there was no effect on CCA

calcification. The disconnect between the laboratory and field experiment may be explained by the relatively lower density of *S. pacificum* (~ 50 % cover) used in the field experiment. It may not have been sufficient to alter the pH of seawater in the back reef and reef crest, as water residence times were low relative to the mesocosm tanks. In present-day conditions, *S. pacificum* densities used in the mesocosms (100% cover) do occur in regions of the reef crest, and fleshy macroalgal density will likely increase as oceanic pH drops (Porzio et al., 2011). As these densities become more common, downstream calcifiers may experience seawater pH that has been altered by upstream macroalgae and may exhibit the same response in calcification rate observed in the mesocosm experiment. There was also no observable effect of habitat (reef crest/back reef) on CCA calcification. The significant, but minute difference in pH between the reef crest and back reef habitats likely was not biologically meaningful. However, this difference may increase as macroalgal abundance increases in the reef crest and water flows through this high flow environment and settles temporarily in the back reef (Hench et al., 2008). Although no difference in calcification was detected between samples placed in the reef crest and back reef, there was a trend toward higher calcification at the reef crest. Perhaps the higher flow rates at the reef crest may favor faster calcification due to a thinning of the DBL around *L. kotschyanum* individuals. However, this effect may become detrimental in future OA conditions, as thinner DBLs increase sensitivity to stressors in the bulk seawater, such as OA (Cornwall et al., 2014).

In Chapter 4, a manipulative mesocosm experiment was used to identify potential interactions between the presence of turf algae and elevated pCO_2 on the calcification response of *L. kotschyanum*. While turf algal photosynthesis significantly raised pH

within the DBL of *L. kotschyanum*, CCA calcification decreased. It is possible that high levels of nighttime respiration of algal turfs led to anoxia at the surface of the CCA thallus, thus having detrimental consequences on CCA calcification (Wangpraseurt et al., 2012). CCA calcification also decreased in response to elevated pCO_2, which is consistent with previous studies (Hofmann and Bischof, 2014; Fabricius et al., 2015). CCA morphology is also an important factor in the role that turf plays in influencing calcification. Morphologies with higher structural complexity generally provide more habitat for algal turfs, and lead to larger pH fluctuations between branches of the CCA thallus. Therefore, more complex morphologies may exhibit stronger responses to the presence of algal turfs. Additionally, measurements of linear extension suggest that slower calcification rates between branches, as opposed to branch tips, is responsible for the decrease in calcification in the presence of turf. This may alter *L. kotschyanum* morphology over time, resulting in longer and thinner branches and altering available habitat space for turf and associated invertebrates, as well as the resistance of *L. kotschyanum* individuals to wave action.

Several conclusions may be drawn from this synthesis of manipulative experiments defining the relationship between CCA and fleshy algae under changing environmental conditions. This thesis indicates that CCA are highly sensitive to associations with fleshy while sensitivity to elevated pCO_2 can be dependent on abiotic factors, such as water flow. In Chapters 2, 3, and 4, CCA parameters varied in response to the presence of fleshy and turf algae and CCA calcification decreased due to elevated pCO_2 in Chapter 4. However, in Chapter 2, canopy-forming macroalgae appear to decrease the sensitivity of understory CCA to OA due to a thickening of the DBL by flow

attenuation. Combined, the experiments outlined in Chapters 2, 3, and 4 also indicate that both fleshy macroalgae and algal turfs can locally alter seawater carbon chemistry through the algal metabolic processes of photosynthesis and respiration. In Chapter 3, this effect, when isolated from physical structure effects of shading and possible abrasion/allelopathy, can enhance CCA calcification. However, in both laboratory experiments and in the field, fleshy macroalgae were shown to impede calcification of CCA. This leads to the conclusion that downstream calcifiers can only benefit from high levels of photosynthesis by dense communities of fleshy macroalgae under conditions where the macroalgal physical structure does not interfere. While similar to *S. pacificum* with respect to modulation of local carbonate chemistry, turf algae differ in their opportunistic ability to colonize the CCA thallus and chemically alter microscopic volumes of seawater between CCA branches, disproportionately affecting CCA calcification. The results of Chapter 4 indicate that, although turf density (biomass/area) is much lower than that of canopy macroalgae, the extremely localized effect still can inhibit CCA calcification. Additionally, *S. pacificum* raises daytime pH to a greater degree than it lowers nighttime pH, resulting in overall better pH conditions for calcification than when fleshy algae are absent. In contrast, algal turfs may induce chemically stressful seawater conditions on the CCA thallus as nighttime respiration creates potentially anoxic conditions.

It is also important to note that boundary layer dynamics over and within CCA thalli may alter CCA physiology and is a vital subject for study. The results of Chapter 2 suggest that flow moderation by the morphologically complex *S. pacificum* thickens the DBL in *L. kotschyanum*, consequently decreasing its sensitivity to OA. In Chapter 3,

distinct flow environments between the reef crest and back reef indicate that boundary layer dynamics may play a vital role in determining differential CCA metabolism in different regions of the same coral reef. As dissimilar flow rates lead to differing DBL thicknesses, CCA growing in different reef habitats may also exhibit varying sensitivities to future OA conditions. The results of Chapter 4 show distinctly, on a finer scale, that carbon chemistry within the DBL of a CCA thallus can differ vastly from that of ambient seawater. Due to the presence of turf algae within this boundary layer, pH can fluctuate greatly within the span of a single day and may have significant physiological consequences on CCA. These dynamics must be better understood to predict the response of an important reef builder to rapidly changing environmental conditions. Photosynthesis was affected by canopy type and pCO_2 in Chapter 2, but was unaffected by *S. pacificum* treated seawater in Chapter 3, indicating that photosynthesis is more sensitive to canopy physical structure than to its biology. Lastly, CCA respiration was not affected by canopy or elevated pCO_2 in Chapters 2 and 3, which suggests that respiration is less sensitive to environmental changes than are CCA calcification or photosynthesis.

The experiments discussed in this thesis show that two of the most influential contemporary stressors on coral reefs, OA and increasing algal abundance, can influence an important reef builder, CCA, in complex ways. Their ability to facilitate coral recruitment and strengthen the reef structure renders them significant contributors to long-term reef resilience (Bosence, 1983; Morse and Morse, 1984). As macroalgal abundance increases under stressors of high fishing incidence, rising sea surface temperatures, nutrient enrichment, and increasing pCO_2 (Jouffray et al. 2015; McManus & Polsenberg 2004; Spalding & Brown 2015), it becomes more crucial to understand

how CCA will behave under a regime of dense fleshy algal biomass and changing ocean chemistry. Studies involving experiments such as those described in this thesis are necessary to disentangle the effects of climate change and a dynamic environment on CCA physiology, allowing for predictions of future coral reef community structure and to understand the importance of mitigating anthropogenic stressors to these valuable ecosystems.

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