

CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

THE ROLE OF *SARGASSUM PACIFICUM* IN OCEAN ACIDIFICATION  
EFFECTS ON CORALS

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

By

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## ABSTRACT

The role of *Sargassum pacificum* in ocean acidification effects on corals

By

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Master of Science in Biology

Ocean acidification (OA), the decrease in the seawater pH and carbonate ion concentration due to the uptake of anthropogenic carbon dioxide (CO<sub>2</sub>) with no change in total alkalinity, threatens coral reefs worldwide as studies predict the decline of calcifiers and increasing growth of non-calcified macroalgae. Future recovery of coral reefs may depend on the ability of corals to survive in association with macroalgae. However, few studies have incorporated multiple species into OA experiments. This research sought to explore the understudied combined impact of macroalgae and elevated pCO<sub>2</sub> on corals. Macroalgae can indirectly (e.g., shading, allelochemicals) and directly (e.g., abrasion, overgrowth) harm corals. Alternatively, algae could facilitate coral calcification by reducing pH through photosynthesis. The reef crest surrounding Moorea, French Polynesia, is covered by the fleshy, macroalgal species *Sargassum pacificum*. Results from photoquadrat surveys showed that scleractinian corals and other biogenic calcifiers covered 15% of the sub-canopy habitat. The same microenvironment experiences on average, increases in pH by  $0.031 \pm 0.012$  pH<sub>T</sub> and decreases in irradiance by ~98% compared to surrounding, above-canopy conditions. On a larger scale, diel fluctuations in pH (up to 0.197 pH<sub>T</sub>) were recorded behind the *S.*

*pacificum*-dominated reef crest. To help predict the future of coral reef dynamics accurately, the present research examined how non-calcifying macroalgae could mitigate, by metabolically reducing CO<sub>2</sub> levels, or exacerbate, by shading, the effects of OA on associated scleractinian corals.

In Chapter 2, the role of macroalgae as a chemical refuge from OA was addressed through a combined laboratory and field experiment testing the effect of the presence/absence of *Sargassum pacificum* on juvenile *Porites rus* exposed to ambient and elevated pCO<sub>2</sub>. In the field, corals with *S. pacificum* exhibited less bleaching but did not significantly alter their net calcification rates. *P. rus* in the laboratory experiment displayed ~23% higher net calcification rates in elevated pCO<sub>2</sub> and ~16% reduced calcification in the macroalgal treatments. *P. rus* was resistant to OA but sensitive to *S. pacificum*. These results suggest the potential ability of *S. pacificum* to outcompete corals for a shared carbon resource (bicarbonate) or to induce other harmful chemical changes on the coral.

The hypothesis that *Sargassum pacificum* could positively (chemical refuge) or negatively (shading) alter the effects of OA on the growth and photosynthesis of the coral species *Acropora pulchra* was tested in Chapter 3 with a combined field and laboratory experiment. A fluctuating pCO<sub>2</sub> treatment was used in both experiments to replicate the natural pH fluctuations created by *S. pacificum* on the reef crest. Similar to *Porites rus*, *A. pulchra* did not respond significantly to the manipulated CO<sub>2</sub> treatments. In the mesocosm experiment, light played a substantial role in controlling coral growth; shaded corals exhibited significantly reduced net calcification, linear extension and photosynthesis rates,

and increased chlorophyll *a*. In addition, shading slightly exacerbated the impact of fluctuating and elevated pCO<sub>2</sub> on coral linear extension and photosynthesis rates. In the field, corals displayed somewhat higher net calcification rates in fluctuating pCO<sub>2</sub> over stable pCO<sub>2</sub>. Thus, the presence of a macroalgal-induced chemical refuge from OA is only apparent qualitatively for *A. pulchra* while light appeared to be the main driving factor affecting coral growth and metabolism. Across both field experiments, corals with *Sargassum pacificum* consistently exhibited reduced calcification rates compared to algal mimic treatments. This suggests that *S. pacificum* may have other harmful chemical effects on corals. The present study provides novel approaches to creating more ecologically relevant OA experiments and motivates OA research to examine the complex relationships between marine organisms and the abiotic environment of a coral reef.

## Chapter 1

### General Introduction

Tropical coral reefs, one of the most biologically diverse and productive systems on earth, are undeniably economically valuable ecosystems creating \$29.8 billion a year in revenue by protecting coastlines from storms, sustaining fisheries, and supporting the livelihood of communities worldwide (Cesar et al. 2003). However, anthropogenic activities from overfishing, climate change, coastal development and pollution are altering crucial abiotic and biotic factors that maintain the existence, recovery and resilience of tropical coral reefs. Climate change particularly endangers reefs as warming sea surface temperatures and elevated carbon dioxide ( $p\text{CO}_2$ ) concentrations will exacerbate massive bleaching events of corals (Hoegh-Guldberg 1999) and limit calcification of key reef builders (Hoegh-Guldberg et al. 2007). In the future, the multitude of stressors is predicted to cause more frequent and permanent phase shifts from coral-dominated to macroalgal-dominated reefs (Diaz-Pulido et al. 2007, Hoegh-Guldberg et al. 2007, Diaz-Pulido et al. 2009, Cheal et al. 2010, Anthony et al. 2011b). The recovery of a coral reef from a macroalgal phase shift will depend heavily on healthy herbivorous fish populations (Tanner 1995, Jompa and McCook 2002, Hughes et al. 2007, Cheal et al. 2010, Adam et al. 2011) and the recruitment and survival of corals (Carpenter and Edmunds 2006, Diaz-Pulido et al. 2009). Understanding the role of macroalgae on coral reefs in future acidified conditions, will be crucial to accurately predict future coral reef dynamics.

## *Ocean Acidification*

In 2008, human activities, mainly burning fossil fuels, producing cement, and deforestation, emitted ~10 billion tons of carbon dioxide (Guinotte and Fabry 2008, Doney et al. 2009). Currently, CO<sub>2</sub> emissions are accelerating at an unprecedented rate and the oceans are absorbing ~25-30% of the atmospheric CO<sub>2</sub> through air-sea gas exchange (Sabine and Feely 2004, Hoegh-Guldberg et al. 2007, Doney et al. 2009). Ocean acidification (OA) is the process whereby the absorption of excess CO<sub>2</sub> into the ocean results in a change in seawater carbonate chemistry and an overall decrease in the pH while total alkalinity (A<sub>T</sub>) remains the same. Since the industrial period, the pH of the ocean has dropped by 0.1 units (Caldeira and Wickett 2003, Orr et al. 2005, Hoegh-Guldberg et al. 2007) and is predicted to drop by another 0.3-0.4 by the end of the current century. As shown in Equation 1, the reduction in pH corresponds with a 100-150% increase in hydrogen ion (H<sup>+</sup>) concentration, a 50% decrease in carbonate ion (CO<sub>3</sub><sup>2-</sup>) concentration, and a 9% increase in bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) (Caldeira and Wickett 2003, Orr et al. 2005, Guinotte and Fabry 2008, Feely et al. 2009, Hurd et al. 2009, Ries et al. 2009b).



The reduction of seawater carbonate ion concentration simultaneously occurs with a reduction in the saturation state ( $\Omega$ ) of the calcium carbonate minerals: calcite, aragonite and high-magnesium calcite.

The persistence of coral reefs depends on the precipitation of calcium carbonate (CaCO<sub>3</sub>) by important ecosystem engineers such as scleractinian corals

and crustose coralline algae (CCA). Calcifiers use calcium ions ( $\text{Ca}^{2+}$ ) and carbonate ions ( $\text{CO}_3^{2-}$ ) to build their shells or skeletons (Equation 2).



Many calcifying marine organisms (e.g., sea urchins, CCA, mollusks, scleractinian corals, coccolithophores) exhibit depressed calcification rates in seawater with pH reduced by  $\sim \leq 0.5$  from ambient conditions (Kroeker et al. 2010). The majority of OA studies has focused on physiological responses of individual species to elevated  $\text{pCO}_2$ , with up to 50% reduction in net calcification rates by hard corals and coralline algae (Orr et al. 2005, Guinotte and Fabry 2008, Kleypas and Yates 2009). With the current ongoing reduction of seawater saturation states, overall rates of reef accretion are decreasing and rates of dissolution are rising (Kleypas and Yates 2009). Ocean acidification poses a critical threat to coral reef communities, as the low pH seawater may make building calcium carbonate structures increasingly difficult for calcifying organisms.

A meta-analysis from 2010 called attention to the prevailing net negative effects of OA on most marine flora and fauna (Kroeker et al. 2010). However, the study also pointed out the large heterogeneity in organismal responses to OA conditions depending on several factors such as species, ability to calcify, type of carbonate mineral used by the organism, and presence of carbon-concentrating mechanisms (CCM) (Kroeker et al. 2010). The studies incorporated into the meta-analysis focused on the differential susceptibility of individual species to elevated  $\text{pCO}_2$ . A more recent meta-analysis, equated the rise in heterogeneous responses

reported in Kroeker et al. (2013) to the surge in mixed-species experiments. For example, the loss of some invertebrates under elevated CO<sub>2</sub> in the low intertidal community in Plymouth, UK, lessened competition and predation, allowing other species, vulnerable to elevated pCO<sub>2</sub> in single-species incubations, to thrive in multi-species assemblages exposed to acidified conditions (Hale et al. 2011). Some organisms may be able to physiologically tolerate or adapt to more acidic conditions (Lohbeck et al. 2012), giving them a competitive advantage over other species and indirectly altering future community composition. Another study in 2014 found sediment dissolution to be a main contributor to the overall depression in reef community calcification by 59% under elevated pCO<sub>2</sub> (1300 µatm) (Comeau et al. 2014b). More importantly, multi-species experiments are revealing an overall loss of diversity and community complexity under OA scenarios (Fabricius et al. 2011, Hale et al. 2011, Porzio et al. 2011). To more accurately predict the future “winners” and “losers” on coral reefs, manipulative field and laboratory experiments should incorporate the direct and indirect (through species interactions) effects of ocean acidification.

### *Reef Metabolism*

By including species interactions into OA research, one should consider the impact of these benthic communities on seawater carbonate chemistry. In shallow coastal habitats, ocean acidification does not occur uniformly as the composition of the marine, benthic community, water flow, nutrient concentrations, and seawater residence time can lead to significant diel

fluctuations in seawater carbonate chemistry (Diaz-Pulido et al. 2007, Anthony et al. 2011a). Coral reefs in particular experience inorganic carbon fluctuations due to the abundance of benthic organisms and their associated metabolic processes. Reef metabolism comprises two main processes: organic production of carbon through photosynthesis and respiration (net ecosystem production) and inorganic production of carbon through dissolution and calcification (net ecosystem calcification) (Bates et al. 2009). Respiration and calcification release  $H^+$  and  $CO_2$ , lowering seawater pH, while dissolution and photosynthesis reduce  $H^+$  and  $CO_2$ , increasing the pH (Gattuso et al. 1999b, Bates et al. 2009, Anthony et al. 2011a, Kleypas et al. 2011). Since ocean acidification is altering the composition of carbon species in seawater, reef metabolism also will be impacted, which will in turn modify the effect of OA on seawater carbonate chemistry. Thus, to fully understand the effects of elevated  $pCO_2$  on marine communities, studies need to elucidate the complex relationships and feedbacks between ocean acidification and reef metabolism.

Besides scleractinian corals, fleshy and calcified algae greatly influence reef metabolism (McConnaughey et al. 2000, Semesi et al. 2009a, Hurd et al. 2011a, Connell et al. 2013, Jokiel et al. 2014a). As coral reefs experience more frequent disturbances and anthropogenic stressors, it will be important to understand the impacts of macroalgae, as they become more dominant benthic organisms. Across marine systems, dissolved inorganic carbon availability can determine macroalgal photosynthesis. Thus, elevated  $CO_2$  may up-regulate photosynthesis, carbon storage and overall growth of fleshy, upright macroalgae

depending on the presence of a CCM (Gao and McKinley 1994). CCM's allow algae to use  $\text{HCO}_3^-$ , the most abundant species of dissolved inorganic carbon (DIC) in seawater, which then is dehydrated extracellularly or intracellularly into  $\text{CO}_2$  via the enzyme carbonic anhydrase (Hurd 2000, Israel and Hophy 2002, HongYan et al. 2008, Hurd et al. 2009). In high pH environments, CCM's allow algae to photosynthesize; in low pH environments, the activity of CCM's may be down-regulated as  $\text{CO}_2$  is more readily available (Cornwall et al. 2012). An increase in macroalgal photosynthesis at elevated  $\text{pCO}_2$  also will depend on the light environment in addition to water flow (Carpenter et al. 1991, Hurd 2000, Diaz-Pulido et al. 2007). Porzio et al. (2011) described a shift in macroalgal communities along a pH gradient near  $\text{CO}_2$  vents in Italy. Calcareous algae were replaced by four fleshy macroalgae species, including a *Sargassum* species, at the lowest pH of 6.7 (Porzio et al. 2011). Thus, *Sargassum* spp. may have utilized the excess carbon dioxide (Cornwall et al. 2012) to outcompete other species. However, most studies have not considered the ability of benthic macroalgae to alter seawater  $\text{pCO}_2$ .

On a shallow coral reef such as the back reef of Moorea, French Polynesia, the benthic community can alter the seawater chemistry through reef metabolism. On the north shore of Moorea, seawater water flows from the fore reef over the reef crest, across the back reef, and back to the open ocean through a boat channel and reef pass (Hench et al. 2008, Leichter et al. 2012). The reef crest has been and is currently covered by *Sargassum pacificum* in addition to patches of *Turbinaria ornata* (Stiger and Payri 1999, Spitler 2009) that may be altering

the seawater carbonate chemistry and influencing the entire back reef. Gattuso et al. (1997) determined that the fringing reef, also dominated by macroalgae (85%) in 1997, at Tiahura (Moorea) was a CO<sub>2</sub> sink. Between the two algal-dominated areas, patches of scleractinian corals, usually in the form of mounding coral bommies covers 21% of the back reef (Adjeroud 1997, Holbrook et al. 2002, Adjeroud et al. 2009, Comeau et al. 2014c). Thus, the variability in cover of macroalgae and corals makes the coral reef surrounding Moorea a suitable location to study the effects of reef metabolism on seawater carbon speciation.

### *Coral–Algal Interactions*

Algae represent one of the main primary producers and dominant benthic organisms on coral reefs, providing habitat and food for numerous marine organisms. However, algae can readily compete with scleractinian corals, a key reef-builder, for light and space (Carpenter 1990). Some algal species overgrow corals during times of degradation from external stressors, such as overfishing, resulting in a regime shift to algal dominance (Diaz-Pulido et al. 2007, Hoegh-Guldberg et al. 2007, Hughes et al. 2007, Diaz-Pulido et al. 2009, Cheal et al. 2010, Anthony et al. 2011b). Coral–algal interactions represent a key ecological process shaping the composition of coral reef communities. Corals and algae compete through six mechanisms: 1) overgrowth, 2) shading, 3) abrasion, 4) allelopathy, 5) epithelial sloughing, and 6) recruitment barrier (McCook 2001). Macroalgae can impair coral growth, survival and calcification by altering flow, increasing sedimentation (Stamski and Field 2006, Jokiel et al. 2014b), releasing

secondary metabolites (Jompa and McCook 2003a, Titlyanov et al. 2007, Rasher and Hay 2010, Rasher et al. 2011), reducing irradiance (Box and Mumby 2007), or physically abrading the coral (River and Edmunds 2001). The presence of some algae may even facilitate coral predation by corallivores (Wolf and Nugues 2013). However, the outcome of coral-algal competition also will depend on the algal functional group and morphological characteristics of the alga (mats vs. canopies) (Jompa and McCook 2003b, Hauri et al. 2010) in addition to abiotic factors such as water flow (Brown and Carpenter 2014).

Not all coral–algal interactions negatively impact coral species. Titlyanov et al (2007) recorded no effect of shading by *Dictyota dichotoma* on the growth of *Porites lutea*. McCook (2001) observed net positive growth of massive *Porites lobata* when competing for space with turf algae; this result was further supported by Jompa and McCook (2003a). In addition, *Sargassum* species, which produce large, leathery canopies, have been known to shade and thus, protect corals in the Great Barrier Reef from bleaching (Jompa and McCook 1998). Similarly, some macroalgal species can even protect juvenile corals from herbivorous fish such as parrotfish (Venera-Ponton et al. 2011). An earlier study by Tanner (1995) recorded negative growth of *Pocillopora damicornis* when algae was removed. Understanding the complexity and variety of coral–algal interactions and outcomes will be crucial in predicting the community composition of coral reefs during and after phase shifts.

Ocean acidification is another crucial abiotic factor driving coral reef dynamics and surprisingly few studies have examined the effects of OA on coral-

macroalgal relationships. Overall, fleshy algae grow on average 22% more rapidly in seawater with a 0.5 pH reduction compared to normal conditions (Kroeker et al. 2013). In the future, macroalgae could more readily outcompete corals for space if they become more abundant and grow more quickly. A previous study reported a shift in the competitive balance in favor of one macroalgal species, *Lobophora papenfusii*, over the coral, *Acropora intermedia*, at high CO<sub>2</sub> concentrations (Diaz-Pulido et al. 2011). Similarly, a study in 2010 suggested that a reduced pH under the canopy and mats of several algal species in the field potentially suppressed calcification of associated corals (Hauri et al. 2010). The majority of OA research demonstrates overall suppression of coral growth and metabolism by algae exposed to elevated pCO<sub>2</sub>. However, macroalgae along with scleractinian corals will not only have species-specific responses to elevated CO<sub>2</sub> but may metabolically modify the seawater pH flowing over the reef.

In fact, the presence of a *Chondria* spp., a fleshy macroalga, incubated with *Acropora* and *Montipora* spp., promoted coral calcification (McConnaughey et al. 2000). Other calcifying organisms such as crustose coralline algae (CCA) and *Halimeda* spp., elicit higher rates of calcification in the presence of seagrass beds in Chwaka Bay, Zanzibar (Semese et al. 2009b). In both instances, the macroalga and seagrass were actively photosynthesizing and increasing the pH in the habitat surrounding the calcifier (McConnaughey et al. 2000, Semese et al. 2009a). Kleypas et al. (2011) and Anthony et al. (2011) suggest the potential for macroalgal-dominated areas upstream to ameliorate the impacts of OA on downstream habitats comprised of calcifying corals by drawing down CO<sub>2</sub> and

elevating the saturation state. In a coral reef ecosystem, modified DIC concentrations associated with macroalgal canopies may mitigate the effects of ocean acidification on seawater carbonate chemistry and the associated corals.

### *Research Objectives*

To fully understand which organisms will be the “winners” (increase growth) and “losers” (decrease growth) in an increasingly acidic seawater environment, OA experiments need to focus on ecological interactions between multiple species (Diaz-Pulido et al. 2011, Hale et al. 2011) and how these species may in turn alter the carbonate chemistry (Connell et al. 2013, Jokiel et al. 2014a). The main objective of the present research was to explore how non-calcified macroalgae (*Sargassum pacificum*) could modify the impact of elevated pCO<sub>2</sub> on associated scleractinian corals. In Chapter 2, I characterize the benthic community and chemical environment (pH) in the understory microhabitat of small patches of *Sargassum pacificum*. These preliminary data were expanded on in Chapter 3 to include information about the chemical and light environment below and adjacent to large areas of *S. pacificum*. This knowledge was used to support the main objective and following hypotheses.

First, the hypothesis that *Sargassum pacificum* could alter the calcification of juvenile *Porites rus* by reducing pCO<sub>2</sub> through photosynthesis *in situ* and under elevated CO<sub>2</sub> conditions was tested with a combined laboratory and field experiment presented in Chapter 2. The field experiment also tested whether or not *S. pacificum* affects the frequency of coral bleaching. In Chapter 3, I expanded

the hypothesis to include a coral species more sensitive to elevated pCO<sub>2</sub> (*Acropora pulchra*) and a potential negative impact of *S. pacificum*, shading. I tested the hypothesis that *S. pacificum* could intensify, through shading, or lessen, through photosynthesis, the deleterious effects of OA on the calcification of *Acropora pulchra* fragments. It is becoming increasingly important to understand how other abundant reef organisms (i.e., fleshy algae) may interact with ocean acidification and alter key abiotic factors (i.e., light and pH) influencing coral population dynamics. In addition, the present study helped to clarify if certain macroalgal species can be used as a means to lower carbon dioxide levels (Gao and McKinley 1994).

## Chapter 2

### **The effect of *Sargassum pacificum* on juvenile *Porites rus* corals in natural and elevated CO<sub>2</sub> conditions**

#### **Introduction**

Coral reefs are among the most diverse and socioeconomically important ecosystems, providing invaluable services to humans globally through storm protection, a source of local livelihood, and economic revenue (Costanza et al. 1997). Yet, natural disturbances, from storms to outbreaks of corallivorous sea stars, and anthropogenic impacts, from coastal development to overfishing, drive mass coral bleaching events, coral disease outbreaks, and shifts to macroalgal-dominated reefs. Indo-Pacific coral reefs, representing three fourths of the coral reefs worldwide, consisted of 22.1% live coral cover in 2003, a ~52% decrease since the 1980s (Bruno and Selig 2007). Similarly, coral cover has declined precipitously throughout the entire Caribbean from the late 1970s until the early 2000s due to overfishing and mass mortality of echinoids (Gardner et al. 2003). The profound loss of critical, high quality coral habitat and the associated loss of reef invertebrates, fish and algae (Hodgson 1999, Hughes et al. 2003, Bruno and Selig 2007, Knowlton and Jackson 2008), equates to a tremendous shift in community dynamics (Pandolfi et al. 2003) and compromises ecosystem services provided by coral reefs (Worm et al. 2006). Coral reefs worldwide now face the added stressors of climate change, specifically decreasing ocean pH and increasing sea surface temperatures, which can exacerbate existing natural and anthropogenic disturbances.

Humans have been releasing carbon dioxide (CO<sub>2</sub>) into the atmosphere at increasingly rapid rates since the industrial revolution. With medium CO<sub>2</sub> emissions (Representative Concentration Pathway 6.0), the Intergovernmental Panel on Climate Change (IPCC) predicts a rise in atmospheric CO<sub>2</sub> to 850 μatm by 2100 (van Vuuren et al. 2011). The absorption of excess atmospheric CO<sub>2</sub> (around 25%) into the ocean and the corresponding changes in seawater carbonate chemistry, with no change in total alkalinity (A<sub>T</sub>), is a process known as ocean acidification (Caldeira and Wickett 2003, Sabine and Feely 2004). Ocean acidification (OA) elicits the formation of carbonic acid (H<sub>2</sub>CO<sub>3</sub>) which readily dissociates and decreases the carbonate ion (CO<sub>3</sub><sup>2-</sup>) concentration, and increases the bicarbonate (HCO<sub>3</sub><sup>-</sup>) and hydrogen (H<sup>+</sup>) ion concentrations (Feely et al. 2004). The equations below represent the change in seawater carbonate chemistry (Equation 1) and the precipitation and dissolution of calcium carbonate (Equation 2). A reduction in available carbonate ions (Equation 1) and a simultaneous decrease in carbonate saturation state (Ω), can suppress the process of building calcium carbonate skeletons (Equation 2) for critical coral reef ecosystem engineers (Kroeker et al. 2010).



In the last decade, studies on the effects of ocean acidification have increased in number and diversity of marine systems examined, revealing heterogeneous growth responses of calcifying (Ries et al. 2009a) and non-calcifying marine organisms. The majority of studies show a negative effect of

OA on scleractinian coral calcification and growth (average reduction by 22-39%) as revealed in the meta-analysis of 228 OA studies (Kroeker et al. 2013). However, some corals and calcifying macroalgae (Price et al. 2011, Johnson et al. 2012) do not respond uniformly to OA conditions (Comeau et al. 2013c). For example, various perforate coral species survive in natural locations where pH is 7.8, the predicted increase by the end of the century (Fabricius et al. 2011). Similarly, some fleshy macroalgae exhibit increased tissue growth under acidified conditions (Kuffner et al. 2008, Hurd et al. 2009) while other species have reduced tissue growth (Israel and Hophy 2002). Thus, ocean acidification differentially affects individual species; some will be “winners” and some considered “losers”. To create management and conservation strategies that ensure the persistence of coral dominated reefs in the future, research needs to clarify the responses of biogenic calcifiers and non-calcifiers to OA.

As ocean acidification increases coral dissolution (van Woesik et al. 2013a) and anthropogenic stressors intensify, coral reefs will experience more frequent and prolonged shifts to a macroalgal-dominated state (Diaz-Pulido et al. 2007, Hoegh-Guldberg et al. 2007, Diaz-Pulido et al. 2009, Cheal et al. 2010, Anthony et al. 2011b). OA can exacerbate macroalgal dominance by stimulating carbon fixation for photosynthesis (Harley et al. 2012, Koch et al. 2013) and thus algal growth (Kuffner et al. 2008, Hurd et al. 2009). With the presence of a carbon-concentrating mechanism (CCM), some algal species can gain a competitive advantage over corals in elevated pH seawater (Connell et al. 2013) as the CCM allows for the use of  $\text{HCO}_3^-$  and  $\text{CO}_2$  (Hurd 2000, Israel and Hophy

2002, HongYan et al. 2008, Hurd et al. 2009). The macroalgal tolerance of OA and the potential ability to flourish in acidic seawater conditions could drive shifts in the dominant community members. Porzio et al. (2011) described a shift in macroalgal communities along a pH gradient near CO<sub>2</sub> vents in the Gulf of Naples (Italy), with fleshy macroalgae (*Sargassum* spp.) dominating areas of the lowest pH (6.7) and calcareous algae dominating areas 60-90 m away from the vents (pH 8.1). On coral reefs, some fleshy macroalgal species may benefit from ocean acidification and become dominant reef community members in the future.

Algae, being a main primary producer providing habitat and food for numerous marine species, can readily outcompete scleractinian corals indirectly or directly for light and space (Carpenter 1990). Six main mechanisms of coral-algal competition have been described as: 1) overgrowth, 2) shading, 3) abrasion, 4) allelopathy, 5) epithelial sloughing, and 6) recruitment barrier (McCook 2001). Macroalgal canopies, depending on the morphology and density of the algae, can decrease coral growth and metabolism indirectly (Hauri et al. 2010) by reducing flow (Escartin and Aubrey 1995), decreasing light (Box and Mumby 2007), intensifying sediment accumulation (Stamski and Field 2006, Rasher et al. 2012, Gowan et al. 2014), increasing dissolved organic carbon and microbial activity (Barott et al. 2011, Wangpraseurt et al. 2012), and exuding harmful metabolites into the water column or directly onto the corals (Rasher et al. 2011, Andras et al. 2012, Bonaldo and Hay 2014, Rasher and Hay 2014). Generally, macroalgae have deleterious effects on coral survival and growth. However, some macroalgae, such as *Sargassum* spp. on the Great Barrier Reef, positively impacted corals by

preventing bleaching (Jompa and McCook 1998). Surprisingly, little OA research incorporates coral-algal interactions. One study reported a shift in the competitive balance in favor of the macroalgal species *Lobophora papenfusii* when in direct contact with *Acropora intermedia* at high CO<sub>2</sub> concentrations (Diaz-Pulido et al. 2011). However, the role and dominance of macroalgae over corals in the future is complicated by their potential to alter carbonate conditions under current and elevated CO<sub>2</sub> conditions.

The majority of research on coral—algal interactions have examined the direct effects of algae on corals, however, with continuing research, the indirect relationships between corals and algae are proving to be increasingly complex. Macroalgae, by using excess CO<sub>2</sub> as a resource to increase photosynthetic rates (Longphuir et al. 2013), can elevate seawater pH (Anthony et al. 2011a, Connell et al. 2013) and could potentially facilitate coral calcification (McConnaughey et al. 2000). Thus, macroalgae indirectly could increase coral growth by creating a refuge from more acidic conditions. In one instance, coral calcification increased when downstream of macroalgal-dominated habitats (Anthony et al. 2011a, Kleypas et al. 2011). Similarly, crustose coralline algae surviving in seagrass and *Ulva* beds, displayed increased calcification rates (Semese et al. 2009b) during the day due to elevated mid-day pH (~9) from seagrass photosynthesis (Semese et al. 2009a, Semese et al. 2009b, Unsworth et al. 2012). Understanding the indirect, potentially facilitative relationship macroalgae may have on coral calcification at a microhabitat scale is becoming increasingly important as the persistence of coral reefs is threatened by elevated CO<sub>2</sub> levels and algal-dominance.

The objective of this research was to determine if non-calcified macroalgae could provide a refuge for juvenile corals in which they increase net calcification, by metabolically lowering the pH under natural and elevated CO<sub>2</sub> levels. In addition, as *Sargassum* spp. prevented coral bleaching in previous work (Jompa and McCook 1998), the research also sought to examine the effect of on coral bleaching in the field. On the island of Moorea, French Polynesia, *Sargassum pacificum*, an introduced brown alga, blankets the entire reef crest surrounding the island (personal observation) and is found commonly on the tops of coral colonies (bommies) along the back reef (Stiger and Payri 1999, Stewart 2008, Spitler 2009). Recent work has shown little-to-no effect of other *Sargassum* spp., either through allelochemicals or shading and abrasion, on coral photosynthetic efficiency, bleaching or mortality (Rasher and Hay 2010, Rasher et al. 2011, Rasher and Hay 2014). Due to its lack of harmful chemicals, its current abundance surrounding Moorea, and potential resilience to and ability to thrive in more acidified conditions (Porzio et al. 2011, Longphuir et al. 2013), *S. pacificum* was used in this study. Juvenile corals, defined as <4 cm in diameter (Bak and Engel 1979), were employed in this study because of their importance in the recovery of a coral reef from a macroalgal-dominated phase shift (Carpenter and Edmunds 2006, Diaz-Pulido et al. 2009). *Porites rus*, a perforate coral, was chosen as the study organism due to its abundance in the back reef and previously recorded reduction in calcification rates under elevated CO<sub>2</sub> levels (Edmunds et al. 2012). Through a combined laboratory and field experiment, the hypothesis that the net calcification of juvenile *P. rus* would be affected by the presence of

macroalgae (*S. pacificum*) *in situ* and under elevated CO<sub>2</sub> conditions was tested. Lastly, the present field experiment tested the hypothesis that macroalgal shading would affect the frequency of *P. rus* bleaching.

## **Methods**

### *Site and Field Surveys*

This study was completed at the University of California Berkeley, Richard B. Gump South Pacific Research Station located on the island of Moorea (Fig. 1). Moorea (17° 30' S, 149° 50' W), a 132-km<sup>2</sup> island surrounded by a lagoon and coral reef, is part of the Society Island chain in French Polynesia. The north shore of Moorea, with abundant macroalgae, presented a suitable location to explore the potential existence of a macroalgal physiological refuge for coral growth and survival through a combined laboratory and field experiment.

To determine if corals naturally inhabited the substratum under the canopy of *Sargassum pacificum*, photoquadrat surveys were completed. In the surveys, all corals in addition to other calcifying organisms were photographed using a camera (Canon PowershotD10) mounted on a metal photoquadrat (12 cm<sup>2</sup>) at haphazardly chosen *Sargassum* patches along the back reef and reef crest ( $n=25$ , Fig.1). Due to lack of sufficient water depth at the top of the reef crest, surveys were only completed on the landward edge of the reef crest. *Sargassum* thalli were either removed or pushed aside to take a clear picture of the substratum. If calcifiers were present, the genus and species (when possible) of the calcifying organism were identified and recorded. The area (mm<sup>2</sup>) of each calcifier was

measured with ImageJ (1.47v) and used to calculate percent cover of the three main calcifiers.

To estimate the thallus densities of *S. pacificum* in the laboratory and field experiments, naturally occurring thallus densities first was established. Over four days in early June, *S. pacificum* thalli were counted in 0.25-m<sup>2</sup> quadrats placed every 10-15 meters along the edge of the reef crest of the north shore ( $n=35$ , Fig.1).

### *Field Experiment*

To test the hypothesis that *Porites rus* net calcification and frequency of bleaching is altered in the presence of *Sargassum pacificum* under natural CO<sub>2</sub> conditions, a field experiment controlling herbivores and manipulating the presence of *Sargassum pacificum* was conducted. First, preliminary water sampling was conducted underneath *S. pacificum* patches to characterize the chemical environment. The amount of *S. pacificum* used in the preliminary water sampling informed the amount of algae necessary for the field experiment.

### Preliminary Water Sampling

*Sargassum* thalli were collected from the reef crest, cleaned of epiphytes with a toothbrush, wet weighed and zip tied to 0.25 m<sup>2</sup> coated, metal grids at two different densities (20 thalli and 30 thalli) based on the average natural density of *S. pacificum* thalli recorded on the reef crest (~20 thalli/0.25 m<sup>2</sup>) and a greater density. The two grids were nailed to the tops of two separate, dead coral

bommies on the back reef in June 2012 (Fig. 1). An integrated water sampler was attached to a bommie nearby each grid location (Fig. 2). The algae were replaced four times over four days, giving a total of five trials for the lower density ( $n=5$ ) and seven trials for the higher density ( $n=7$ ). The water samplers simultaneously collected water from inside (2 cm above the substrate) and outside the *S. pacificum* canopies for one and two-hour intervals into 1-L bags (Nalgene) pre-rinsed with fresh seawater at each location (Fig. 2). Each water sample was transferred within an hour to the lab to record temperature, salinity, pH ( $\text{pH}_T$ ) and total alkalinity ( $A_T$ ) via spectrophotometric techniques with m-cresol purple dye (SOP 6b, Dickson et al. 2007) and with an open cell potentiometric titrator (T50, Mettler-Toledo; SOP 3b, Dickson et al. 2007). The pH probe (DG I115-SC, Mettler) on the titrator was calibrated every other day with Tris buffer (Batch 10) from the Andrew Dickson Lab at Scripps Institution of Oceanography. The  $A_T$  and  $\text{pCO}_2$  was calculated with the R package Seacarb (Lavigne and Gattuso 2012). Certified reference material (CRM, Batch 105) from the Dickson lab was processed before running water samples to evaluate the accuracy of  $A_T$  values (Table 1). Average  $A_T$  of the CRM samples ( $2235 \pm 2.4 \mu\text{mol kg}^{-1} \pm \text{SE}$ ) was kept within  $4 \mu\text{mol kg}^{-1}$  of the certified  $A_T$  value of  $2235 \mu\text{mol kg}^{-1}$ . The effects of algal wet weight, holdfast density and trial duration on the seawater carbonate parameters were analyzed with a three-way ANOVA. The three factors were not significant ( $P>0.05$ ). The trials were pooled and a paired t-test used to analyze the pH inside and outside the canopy.

### Coral Collection

Plating *Porites rus* juveniles were collected via hammer and chisel in June 2012 from the back reef of the north shore of Moorea at ~2-m depth and brought back to the Gump Research Station in bags (Ziploc) filled with fresh seawater. The corals were identified as juveniles based on traits such as the presence of healthy, living coral tissue and a generally circular shape. These characteristics prevented misidentifying coral fission products as juveniles. The samples were placed in a water table (240 L) with rapid flow ( $\sim 10 \text{ L min}^{-1}$ ) of fresh seawater from Cook's Bay. Each coral was attached to a plastic base using marine epoxy (Z-spar Splash Zone Compound <sup>TM</sup>, A788). Epoxy covered all exposed dead skeletons to minimize changes in weight due to skeletal dissolution. Corals ( $n=33$ ) remained in the seawater table for three days before being deployed.

### Field Setup

To test the effects of *Sargassum pacificum* on *Porites rus* net calcification and bleaching, a field experiment was deployed on the back reef of the north shore of Moorea where the preliminary seawater chemistry was measured (Fig. 1). Three caged treatments were blocked on individual coral bommies ( $n=11$ ): 1) *S. pacificum* + *P. rus*, 2) *P. rus*, and 3) *P. rus* + algal mimics (Fig. 3B). Cages were necessary to keep herbivores and corallivores from consuming the algae and the *Porites rus*. The bommies were chosen based on sufficient space for three cages (avg. surface area=  $7.09 \text{ m}^2 \pm 0.97 \text{ SE}$ ), 25% or less live coral cover and similar depths ( $1.41 \text{ m} \pm 0.06 \text{ SE}$ ) to ensure the corals experienced the same

abiotic conditions. The cylindrical cages (2-cm<sup>2</sup> galvanized, welded mesh fence) were 0.41 m high with a 1.25 m circumference and square (0.41 m<sup>2</sup>) bases and tops.

Algal mimics were used to account for the effects of *Sargassum pacificum* on water flow and light. The algal mimics were plastic aquarium plants (Tetra, WaterWonders, Red Ludwigia Plant, 30.5 cm) interspersed with brown plastic tubing, and were attached to the caging material with brown pipe cleaners. Since the preliminary field water sampling exhibited an increase in pH with 20-30 thalli, which also represented the natural density of *S. pacificum*, ~30 thalli were deployed in each algal treatment. The thalli were collected haphazardly from the reef crest, cleaned of all epiphytes, wet weighed and placed in tanks with running seawater before being deployed in each cage. The field experiment ran for 23 days and the cages were cleaned every 3-4 days to minimize the growth of turf algae and other epiphytes.

Irradiance and relative flow differences were measured to test whether the algal mimics created a similar flow and light environment to *Sargassum pacificum* and to determine if the cage had an effect on water flow and light. Between 1100-1300 hours, the PAR light sensor (1-mm active diameter) on a Diving PAM (Heinz Walz), calibrated to a 2 $\pi$  light sensor (Li-Cor, LI-192), was used to measure irradiance inside and outside the cage, inside the algal mimics, and inside the *S. pacificum* patch on top of each bommie. To determine relative flow differences between the algal mimics versus *S. pacificum* and inside versus outside the cages, calcium sulfate dissolution rates were estimated using plaster

clod cards (Doty 1971, Jokiel and Morrissey 1993, Thompson and Glenn 1994). Three batches of clod cards, made of two parts plaster (Plaster of Paris, Dap©) to one part water poured into ice cube trays (4.7 x 2.7 x 3 cm), were allowed to set overnight. The plaster cubes were dried in an oven at 60 °C for 24 hours and sanded to an average  $27.48 \pm 0.05$  g ( $\pm$  SE,  $n=59$ ). The plaster cubes were glued (aquarium silicone) to plastic cards and dried for 24 hrs. On each bommie, a clod card was zip-tied for 22 hours inside the control cage ( $n=11$ ), outside the control cage ( $n=11$ ), inside the algal mimic ( $n=11$ ) and inside the *S. pacificum* ( $n=11$ ). To account for any dissolution in calm water and calculate the control factor (CF), control clod cards from each batch were placed in a bucket with fresh seawater from Cook's Bay at ambient temperature ( $n=15$ ). After ~22 hours, all clod cards were retrieved, dried for one week and re-weighed to determine dissolution rates and calculate relative flow differences with the following equation (W=weight, T=time, i=initial, f=final, c=control clod cards, e=clod cards deployed in the experiment (Brown 2012):

$$\text{(Equation 1) Control Factor} = (W_{ic} - W_{fc}) / W_{ic}$$

$$\text{(Equation 2) Dissolution Rate} = ((W_{ie} - W_{fe}) - (CF \times W_{ie})) / T$$

The irradiance and dissolution rates were analyzed with a blocked ANOVA with bommie as the random blocked factor and treatments as a fixed effect.

### Net Calcification and Bleaching

Net calcification and percent bleaching of the corals were measured at the end of the 23-day experiment. The *Porites rus* juveniles were buoyant weighed

before and after the experiment (Davies 1989). The buoyant weights were converted to dry weights using the aragonite density of  $2.93 \text{ g cm}^{-3}$ . Surface area was measured with the aluminum foil technique (Marsh 1970) and used to normalize the net calcification rates. After deploying the corals, immediate changes in the tissue color were observed. Thus, a  $6.5 \text{ cm}^2$  photoquadrat (Canon Powershot D10) with a scale bar (0-6.5 cm) on one side and 5 color standards made of electrical tape (red, yellow, white, green, and blue) were taken between 1100 and 1300 hours every three days to document any bleaching (Fig. 3A). At the end of the experiment, corals were assigned to two categories based on pigmentation: partial to fully bleached (distinct areas of white coral tissue or the entire coral was white), unbleached (no white tissue).

### *Mesocosm Experiment*

To test the effect of elevated  $\text{pCO}_2$  in addition to macroalgae on coral calcification, a fully factorial mesocosm experiment crossing two  $\text{CO}_2$  treatments (ambient:  $400 \text{ } \mu\text{atm}$ , and high:  $800 \text{ } \mu\text{atm}$ ) with the presence/absence of *Sargassum pacificum* was conducted. To establish the amount of algae required to increase seawater pH due to photosynthesis in the laboratory experiment, a pH drift experiment was performed in closed tanks with the presence of *S. pacificum*.

### Preliminary Water Sampling

Algal thalli, averaging  $\sim 800 \text{ g}$  wet weight, were collected from the field and allowed to acclimate for 30 minutes in 150-L tanks (Aqua Logic, San Diego,

CA) with 75 W LED lights (Sol White LED Module, Aquillum) and 125 L of fresh, filtered seawater from Cook's Bay. For each three-hour trial ( $n=4$ ), new algae were collected, cleaned of epiphytes and wet weighed. Two 50-ml water samples were collected before each trial and at the end of each hour to measure changes in carbonate chemistry. Water samples were analyzed using m-cresol purple dye with a spectrophotometer (SOP 6b, Dickson et al. 2007) and with an open cell potentiometric titrator (T50, Mettler-Toledo) (SOP 3b; Dickson et al. 2007) where the pH probe (DG I115-SC, Mettler) was calibrated with Tris buffer (Batch 10). To ensure the titrations were accurate, certified reference material (CRM, Batch 105) from the Dickson lab were processed before running water samples (Table 1). To calculate the carbonate parameters ( $A_T$ ,  $pCO_2$ ,  $[HCO_3^-]$ ,  $[CO_3^{2-}]$ , DIC and  $\Omega_{arag}$ ), the salinity, temperature, pH and  $A_T$  values were input into the R package Seacarb (Lavigne and Gattuso 2012). No control incubations were performed because previous analysis of seawater chemistry without algae after ~4 hours showed no significant change in pH (personal observation) or <1% change in DIC in previous work (Anthony et al. 2011a).

### Coral Collection

Juvenile *Porites rus* ( $n=96$ ) were collected via hammer and chisel along the back reef of the north shore of Moorea at ~2-m depth. Each coral was brought back to the lab and epoxied (Z-spar Splash Zone Compound™, A788) to plastic cards in flow-through water tables. The corals acclimated for ten days in a separate, custom made acclimation tank (Aqualogic, San Diego, 1000 L) with

filtered seawater from Cook's Bay and four 75 W LED lights (Sol White LED Module, Aquaillumination) set to a 12:12 hour photoperiod cycle. The light intensity increased for four hours from 0 to 100%, stayed at 100% for four hours and decreased to 0% light for the last four hours. The light cycle mimicked the light levels found naturally occurring over the period of a day on the back reef (2-m depth) of Moorea (Carpenter 2014a). The corals were placed on a circular table that rotated twice each day, ensuring all of the corals experienced the same light and flow levels. The light and temperature ( $\sim 28$  °C) of the acclimation tank simulated the ambient natural conditions commonly recorded on the back reef (2-4 m depth) in June (Putnam and Edmunds 2011, Carpenter 2014a, Leichter 2014), in addition to the mesocosm conditions.

### Tank Setup

A flow-through seawater system pumped fresh seawater from 12-m depth in Cook's Bay through a sand filter with a 25-100  $\mu\text{m}$  mesh (Triton II) before entering 150-L tanks (Aqua Logic, San Diego, CA) at  $\sim 200$   $\text{ml min}^{-1}$ . The temperature was maintained at  $\sim 28$  °C and the water flow in each tank was controlled with submersible water pumps (Rio 8HF, 2,082  $\text{L h}^{-1}$ ). Each tank had a 75W LED light (Sol White LED Module, Aquaillumination) set to the same levels and photoperiod cycle as described above. Irradiance in each tank was measured before and after the experiment with a  $2\pi$  light sensor (Li-Cor, LI-192). Tanks were assigned randomly to  $\text{CO}_2$  treatments, with four tanks at ambient ( $\sim 400$   $\mu\text{atm}$ ) and four tanks at elevated  $\text{pCO}_2$  (800  $\mu\text{atm}$ ). The high  $\text{CO}_2$  treatment

was based on the IPCC scenario projected for 2100 with medium CO<sub>2</sub> emissions (RCP6, van Vuuren et al., 2011). A solenoid (Model A352, Qubit Systems, Ontario, Canada) controlled the amount of pure CO<sub>2</sub> and ambient air needed to create the elevated CO<sub>2</sub> treatment. CO<sub>2</sub>-enriched air was monitored on a LabPro program (Venier Software and Technology) and bubbled into the tanks via pumps (Gast pump DOA-P704-AA). Ambient CO<sub>2</sub> treatments received ambient air bubbled into the tanks from similar Gast pumps. Two ~ 50-ml water samples were collected in glass bottles every two days to analyze the carbonate chemistry (see description for pH drift).

Two tanks from each CO<sub>2</sub> treatment were chosen randomly as the “algae present” treatment. To create the algal treatment, *Sargassum pacificum* was collected from the reef crest, cleaned of epiphytes with toothbrushes, wet weighed and zip-tied to triangular grids (2.5 cm<sup>2</sup> galvanized and PVC-coated, welded mesh fence). The algal treatments ( $n=4$ ) contained two *S. pacificum* grids in opposite corners of the tank to avoid abrading or shading the corals. Each tank had ~60 algal thalli averaging  $801.08 \pm 0.97$  g wet weight. The *S. pacificum* densities and weights were based on the amount of algae used in the pH drift experiment (~60 thalli, ~800 g wet weight) which mimicked natural densities and weights (see results). The tanks were cleaned every 2-3 days with scrub pads to remove turf algal growth. The treatments were maintained for 21 days.

Each tank ( $n=8$ ) contained 12 juvenile *Porites rus* placed on top of an acrylic stand centered in the tank (i.e., not shaded by the algae). The corals ( $n=96$ ) were moved every two days to minimize position effects in the tanks. The

juvenile corals were buoyant weighed at the beginning of the experiment and after 21 days (Davies 1989). The aragonite density of  $2.93 \text{ g cm}^{-3}$  was used to convert the buoyant weights into dry weight. The calcification rate was normalized to tissue surface area estimated by the aluminum foil technique (Marsh 1970).

### *Statistical Analysis*

For the field experiment, coral net calcification rates were analyzed using a blocked ANOVA with coral bommies as the blocking factor and treatment as a fixed effect. Two measurements of net calcification rate were removed from the analyses because they were judged as measurement errors; one was unrealistically high ( $4.8 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) for the length of the experiment and the other was unrealistically low ( $-1.8 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) compared to previously recorded *P. rus* growth rates (Comeau et al. 2013a, Comeau et al. 2013b, Comeau et al. 2013c). The coral bleaching data were analyzed with a log-linear model because the percent of bleached and unbleached corals fit a binomial distribution. For both the blocked ANOVA and log-linear models, the assumptions of normality and equality of variance were evaluated with the Shapiro-Wilk test statistic and examination of the residuals.

To evaluate whether tank treatments in the mesocosm experiment were maintained with precision and accuracy, the physical conditions were analyzed with a partly nested 2-way ANOVA. The  $\text{pCO}_2$  level (ambient/high) and algal treatment (presence/absence) were fixed effects, and tank was a random effect nested within the treatments. The tank did not significantly affect the physical

conditions ( $P \geq 0.25$ ) and therefore the data were pooled within treatments (Quinn and Keough 2002). Net calcification rates were analyzed with the same ANOVA model (Table 2). Once again, the random, nested tank factor was not significant ( $F_{4,87}=242$ ,  $P=0.91$ ), thus the tank factor was dropped and replicates from the tanks were pooled, doubling the replicate size ( $n=24$ ). All data met the assumptions of normality and equality of variance with the Shapiro-Wilk and Levene's test statistics. The data were normally distributed. The program SYSTAT 12 was used for all statistical analyses.

## Results

### *Field Surveys*

From the photoquadrat surveys, the average percent cover of calcifying organisms (mean  $\pm$  SE,  $n=25$ ) living below *Sargassum pacificum* canopies was  $5.76 \pm 1.75\%$  for scleractinian corals,  $1.01 \pm 0.51\%$  for *Halimeda* spp., and  $8.48 \pm 2.54\%$  for crustose coralline algae. The maximum area of each calcifier surviving in association with *S. pacificum* was calculated: scleractinians and hydrocorals ( $43.3 \text{ mm}^2$ ), crustose coralline algae ( $94.0 \text{ mm}^2$ ) and *Halimeda* spp. ( $17.6 \text{ mm}^2$ ). The coral cover represented five genera (listed in descending percent cover): *Porites* spp., *Millepora*, *Psammocora*, *Montipora*, and *Pocillopora*. Species were not determined as the small sizes of the individuals made identification difficult. The other  $\sim 85\%$  of the substratum consisted of macroalgae, sand, and bare substratum. *Sargassum pacificum* was found at a mean density of  $19.51 \pm 1.47$  thalli/ $0.25 \text{ m}^2$  ( $n=35$ ) and weighed (wet weight)  $801.15 \pm 77.95 \text{ g}/0.25 \text{ m}^2$  ( $n=4$ ).

### *Field Experiment*

The trials conducted with the water sampler were pooled as the three-way ANOVA revealed no significant ( $P > 0.05$ ) effects of trial duration, algal density or algal wet weight. Paired t-tests revealed a significant difference in pH inside and outside the *Sargassum pacificum* canopy ( $t = 2.467$ ,  $df = 10$ ,  $P = 0.033$ ). On average ( $\pm$  SE), the pH inside the canopy during the day was  $0.031 \pm 0.012$  ( $n = 11$ ) higher than outside the canopy (Table 2).

The cage significantly affected irradiance levels ( $F_{1,10} = 27.3$ ,  $P \leq 0.001$ ). The cage reduced light by  $\sim 166 \mu\text{mol quanta m}^2 \text{ s}^{-1}$ ; however, all treatments were caged and thus, experienced the same light reduction (Table 3). The cages did not significantly affect clod card dissolution rates ( $F_{1,10} = 0.023$ ,  $P = 0.882$ ). Clod card dissolution and light levels were not significantly different between the algal mimics and *Sargassum pacificum* patches (Table 3). Thus, the algal mimics successfully imitated the light and flow environment in *S. pacificum* canopies.

Overall, the treatments did not affect *Porites rus* net calcification significantly ( $F_{2,18} = 1.34$ ,  $P = 0.286$ , Table 4A) or the percent of colonies categorized as bleached ( $\chi^2 = 44.99$ ,  $P = 0.441$ , Table 4B). While there was no statistically significant difference, the average net calcification rate of *Porites rus* ( $\pm$  SE) in the *Sargassum pacificum* treatment ( $0.45 \pm 0.11 \text{ mg cm}^{-2} \text{ day}^{-1}$ ,  $n = 11$ ) was 35-39% lower than the control corals ( $0.74 \pm 0.48 \text{ mg cm}^{-2} \text{ day}^{-1}$ ,  $n = 9$ ) and corals in the algal mimic treatment ( $0.69 \pm 0.13 \text{ mg cm}^{-2} \text{ day}^{-1}$ ,  $n = 11$ ). Less than half ( $\sim 46\%$ ) of the algal-treatment corals and a little more than half ( $\sim 56\%$ ) of the corals + algal mimic were bleached, whereas  $\sim 73\%$  of the control corals were

bleached by the end of the experiment. Biologically, the *S. pacificum* negatively affected the calcification and growth of *Porites rus* (Fig. 6).

### *Mesocosm Experiment*

An average of  $802.18 \pm 0.57$  g wet weight (mean  $\pm$  SE) of *Sargassum pacificum* was used in the pH drift trials ( $n=4$ ). Due to *S. pacificum* photosynthesis, over the course of three hours, the pH increased from  $\sim 8.04$  to  $\sim 8.19$ , for an average change in  $\text{pH}_T$  of  $0.15 \pm 0.01 \text{ pH}_T \text{ hr}^{-1}$  ( $n=4$ ) (Table 5 and Fig. 4A). While the average pH and carbonate concentration ( $200.44 \pm 45.58 \mu\text{mol kg}^{-1} \text{ hr}^{-1}$ ) increased, there was a corresponding decrease in  $\text{pCO}_2$  of  $48.63 \pm 3.12 \mu\text{atm hr}^{-1}$  (mean  $\pm$  SE),  $\text{HCO}_3^-$  by  $53.23 \pm 14.80 \mu\text{mol kg}^{-1} \text{ hr}^{-1}$  and DIC by  $34.46 \pm 10.83 \mu\text{mol kg}^{-1} \text{ hr}^{-1}$  (Fig. 4).

The four tanks with elevated  $\text{pCO}_2$  treatments maintained mean  $\text{pCO}_2$  values of  $811 \pm 16 \mu\text{atm}$  (Tank 1),  $833 \pm 13$  (Tank 6),  $827 \pm 72 \mu\text{atm}$  (Tank 8) and  $758 \pm 36 \mu\text{atm}$  (Tank 11). The ambient  $\text{pCO}_2$  treatments ranged between  $440 \pm 87 \mu\text{atm}$  (Tank 3),  $524 \pm 73 \mu\text{atm}$  (Tank 4),  $415 \pm 54 \mu\text{atm}$  (Tank 2), and  $414 \pm 92 \mu\text{atm}$  (Tank 9) (Table 6). All tank temperatures were kept within  $0.3 \text{ }^\circ\text{C}$  of  $28.3 \text{ }^\circ\text{C}$ ; however, the temperatures of the  $\text{pCO}_2$  treatments were significantly different ( $F_{1,64}=8.78$ ,  $P=0.004$ ). The differences were minor ( $0.2 \text{ }^\circ\text{C}$ , Table 6) and likely were not biologically important as the sample corals were collected from an area on the back reef (2-4 m depth) of Moorea that regularly experiences fluctuations (0.9-4.7%) in temperature (Putnam and Edmunds 2011, Leichter 2014). The carbonate parameters between  $\text{pCO}_2$  treatments were significantly different (Table

7). A slight increase in bicarbonate concentrations ( $F_{1,64}=4.98$ ,  $P=0.029$ ) and a corresponding increase in total alkalinity by  $29.75 \mu\text{mol kg}^{-1}$  ( $F_{1,64}=0.06$ ,  $P<0.001$ ) occurred in the algal treatments (Table 6 & 7). The increase in  $A_T$  of the algal treatments most likely had little-to-no effect on the corals, as the corals were collected from an area on the north shore of Moorea that naturally experiences diel fluctuations in  $A_T$  by  $\sim 40 \mu\text{mol kg}^{-1}$  (Alldredge and Carlson 2013).

The presence of macroalgae neither exacerbated nor ameliorated the effect of elevated carbon dioxide levels on coral calcification. Instead, *P. rus* net calcification rates responded in opposite directions to the presence of macroalgae and elevated  $p\text{CO}_2$  (Fig. 7). Coral net calcification rates significantly decreased by  $\sim 16\%$  in the algal treatments ( $F_{1,91}=3.92$ ,  $P=0.05$ ) and significantly increased by  $\sim 23\%$  in elevated  $p\text{CO}_2$  conditions ( $F_{1,91}=5.91$ ,  $P=0.02$ ) (Table 8). Although not statistically significant, the corals in the elevated  $p\text{CO}_2$  + algae treatments had on average  $\sim 19\%$  higher net calcification rates ( $1.73 \pm 0.16 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) than the corals in the ambient  $p\text{CO}_2$  + algae treatments ( $1.45 \pm 0.16 \text{ mg cm}^{-2} \text{ day}^{-1}$ ). Similarly, *Porites rus* in the elevated  $p\text{CO}_2$  – algae treatment exhibited higher net calcification rates ( $2.11 \pm 0.14 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ day}^{-1}$ ) than the corals in the ambient  $p\text{CO}_2$  – algae treatment ( $1.66 \pm 0.14 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ day}^{-1}$ ).

## Discussion

The main objective of this research was to test for an indirect effect of macroalgae, mediated by algal metabolism, on coral calcification under ambient and lowered pH conditions. There was no evidence of macroalgae positively

affecting corals by creating a chemical refuge (increasing seawater pH through photosynthesis) for corals from ocean acidification, primarily because *Porites rus* was not sensitive to reduced seawater pH. Some scleractinians, for example massive *Porites* spp., appear to be resilient to naturally occurring low pH (~7.8) environments (Fabricius et al. 2011). *Porites rus* also is resistant to high pCO<sub>2</sub>, even under high light (~1000 μmol photons m<sup>-2</sup> s<sup>-1</sup>) and food (zooplankton) availability (Comeau et al. 2013a). Similar to Comeau et al. a negative effect of elevated pCO<sub>2</sub> on *P. rus* calcification was not recorded during the 23-day incubation. Instead, a significant increase in coral calcification (~23%) at elevated pCO<sub>2</sub> was recorded (Fig. 7). *Porites rus* may have used the elevated HCO<sub>3</sub><sup>-</sup> availability associated with the ~800 μatm treatment for calcification and daytime photosynthesis, as evidenced by an increase in net calcification shown in other studies (Comeau et al. 2013b). Thus, the juvenile corals in the present study most likely were able to mitigate the effects of OA by using bicarbonate as an alternate resource for calcification. Since *P. rus* appeared to benefit from elevated bicarbonate concentrations, the presence of a macroalgal physiological refuge from more acidic conditions was unlikely for this coral species.

Although *Sargassum pacificum* significantly increased the seawater pH in the pH drift experiment and during field trials with the integrated water samplers, algal presence had no effect on coral calcification or bleaching in the field experiment (Fig. 6), and decreased calcification rates of *Porites rus* at both the ambient and elevated CO<sub>2</sub> conditions in the mesocosm experiment (Fig. 7). It is possible that the macroalgae and corals were competing for the same source of

DIC, because both *P. rus* calcification and *S. pacificum* photosynthesis use bicarbonate ions. Future research should repeat long-term pH drift trials to determine the pH compensation point and thus, the presence of a carbon concentrating mechanism (CCM) in *S. pacificum* (Hepburn et al. 2011, Cornwall et al. 2012). A CCM would increase the carbon available at the site of carbon fixation, either by actively acquiring dissolved CO<sub>2</sub> or dehydrating HCO<sub>3</sub><sup>-</sup> (91% of DIC) extracellularly or intracellularly into dissolved CO<sub>2</sub> with the enzyme carbonic anhydrase (Hurd 2000, Israel and Hophy 2002, HongYan et al. 2008, Hurd et al. 2009). Some algal species will be superior competitors in future OA conditions, depending on the alga's carbon capturing strategies and the presence of a CCM (Connell et al. 2013). Although less common, other algae can actively consume carbon dioxide directly from seawater, without CCMs (Raven et al. 2005). Furthermore, current research indicates that specific species of algae under OA conditions can shift from using CCMs to a less costly process using dissolved CO<sub>2</sub> for the Rubisco substrate, instead of converting bicarbonate to CO<sub>2</sub> (Connell et al. 2013). With a CCM, *S. pacificum* photosynthesis presumably could have reduced CO<sub>2</sub> and bicarbonate concentrations in the surrounding seawater, which, in turn, could have decreased the bicarbonate available for *P. rus* calcification. Competition for a common carbon resource (bicarbonate) may have elicited a negative response in coral growth rates to macroalgae in both the field and laboratory experiment.

Not only were the two study organisms potentially competing for the same resource, but the density of algae used in the experiments only resulted in a slight

increase in pH. With larger macroalgal patches in the natural reef environment, the impact of macroalgae on ocean acidification could be much more influential, as shown by the model simulations in Kleypas et al. (2011) on the Tiahura coral reef flat community of Moorea. Similarly, seagrass beds can increase daily pH to such an extent that green and red algae calcify 1.6 to 5.8 times more (by mass) (Semese et al. 2009b), revealing the potential presence of an important coastal refuge from OA conditions (Manzello et al. 2012). In the Semese et al. (2009b) study, the calcifying organism (*Hydrolithon* spp.) was in the presence of immense seagrass beds covering 50 km<sup>2</sup>. Thus, the amount and density of *S. pacificum* (~800 g wet weight in 0.29 m<sup>2</sup>) used in the present experiments may not have increased the pH sufficiently to positively influence calcification on a small spatial scale.

The ability of macroalgae and other marine organisms to mitigate OA conditions may depend greatly on diffusive boundary layers (DBL) and the influence of water flow on DBLs. The DBL comprises an area surrounding the organism ( $\mu\text{m}$  to cm) where the mass transfer of dissolved compounds occurs through molecular diffusion. Metabolic processes, such as calcification/dissolution and photosynthesis/respiration, modify the chemical environment of the DBL (Hurd et al. 2011b, Cornwall et al. 2013a). With decreasing water flow, the DBL increases in thickness creating a larger divide between the chemical environment surrounding the organism and that of the immediate seawater. Under low flow, the DBL around photosynthesizing organisms can act as a buffer against ocean acidification conditions (Cornwall et

al. 2014). Changes in pH have been recorded up to 68 mm from the surface of an articulate coralline alga (Cornwall et al. 2013b). Larger DBLs can persist below macroalgal canopies where water flow is modulated (Hurd 2000). It is established that macroalgae can alter seawater carbonate chemistry through metabolic processes. However, the greatest change in seawater chemistry most likely will occur in the DBL surrounding the macroalga (Delille et al. 2009, Anthony et al. 2011a, Cornwall et al. 2013a). Corals and other calcifiers would have to be within the macroalgal DBL to experience a macroalgal refuge from OA.

Water flow influences the thickness of DBLs (Hurd 2000), thus, potentially controlling the persistence and effectiveness of a macroalgal refuge for corals from ocean acidification. In the present study, the field treatments were placed in an area with high seawater turnover rates (Hench et al. 2008) and on the tops of bommies, where water velocities are elevated compared to the area behind or downstream of a bommie (Hench and Rosman 2013). The high water flow may have disrupted the development and persistence of a DBL surrounding *Sargassum pacificum*, compromising the chance for corals to experience potential positive changes in seawater chemistry. Similarly, pumps maintained continuous water circulation in the mesocosms, diminishing the thickness of the macroalgal DBL. If the corals were placed within the canopy for the mesocosm experiment and in a lower flow location for the field experiment, they might experience a microenvironment with a larger increase in pH. In both cases where macroalgae and seagrass created a physiological refuge for biogenic calcifiers, the water flow and residence time of water at the study sites were extremely low (Semese et al.

2009b, Cornwall et al. 2013a, Cornwall et al. 2013b, Cornwall et al. 2014). An intriguing line of future research should examine the direct and indirect effects of varying macroalgal densities and spatial variability (areas with longer water residence times) on the carbonate chemistry of DBLs and the associated impact on coral calcification.

Another factor unexplored here, is that the positive influence of elevated pH on corals surviving in close proximity to macroalgae would have to outweigh other harmful chemically mediated coral-algal interactions. In both the laboratory and mesocosm experiments, reduced coral calcification rates were recorded in the presence of *Sargassum pacificum*. Another mechanism to explain the decline in coral growth is through the macroalgal release of dissolved organic carbon (DOC) from living (Haas et al. 2011) or detrital macroalgae (Khailov and Burlakova 1969) and the associated increase in growth, abundance and diversity of microbial communities colonizing corals (Kline et al. 2006). Depending on water flow (Brown and Carpenter 2013), the respiration of the microbiota can establish harmful hypoxic areas at the zone of interaction with corals (Barott et al. 2011, Barott and Rohwer 2012, Wangpraseurt et al. 2012). If *S. pacificum* is similar to other *Sargassum* species with high microbial diversity (Thurber et al. 2012), high DOC exudation rates (Brylinsky 1977), and the ability to directly (Thurber et al. 2012) or indirectly (Morrow et al. 2013) transfer harmful microbes to corals, the juvenile *Porites rus* from these experiment may have experienced microbially-mediated mortality. *In situ*, the corals experienced high flow rates but were deployed in direct contact with *S. pacificum*, potentially accelerating the transfer

of microbial vectors. In the mesocosm experiment, both the senescence of *S. pacificum* and low residence time could have exacerbated microbial growth, reducing coral calcification. With the looming threat of increasing herbivorous fish overexploitation (Hughes et al. 2007) and ocean acidification leading to macroalgal dominated reefs, more experiments are required to document *S. pacificum* DOC release rates and its affiliated microbiota, as it is an already abundant alga on coral reefs throughout Oceania, south-east Asia, south America and central America (Guiry 2014).

While the present results show no existence of a DIC-based macroalgal refuge for corals under the aforementioned conditions, another type of physiological refuge may be apparent. Corals in the field experiment showed reduced bleaching in the presence of both the *Sargassum pacificum* and the algal mimic (Fig. 6). This reduction in bleaching could be caused by beneficial shading from high light (reduction in irradiance), an effect shown in other *Sargassum* spp. (Jompa and McCook 1998); other algal canopies have also been shown to prevent bleaching and enhance growth in sub-story corallines (Figueiredo et al. 2000). However, the short-term duration of the experiment and stress of transplantation into high light may have exacerbated the frequency of mortality of control corals. Although the presence of a macroalgal-mediated physiological refuge for juvenile corals at the present experimental scale is not apparent, the results show a potential for corals associated with macroalgae to have a higher chance of survival, albeit with reduced growth rates. This result is important, because the recovery of a coral reef from a macroalgal-dominated state will depend heavily on

the survival of juvenile corals (Carpenter and Edmunds 2006, Diaz-Pulido et al. 2009).

Ocean acidification research, along with the present study, continues to reveal that marine organisms exhibit species-specific responses to elevated CO<sub>2</sub> levels and reduced pH (Kroeker et al. 2013). With rapidly increasing atmospheric CO<sub>2</sub> and the threat of macroalgal-dominated reefs in the future, researchers need to further explore how indirect, chemically mediated coral-algal interactions may change under OA scenarios. The results of this study corroborate previous research on the resistance of *Porites rus* to ocean acidification conditions and the ability for macroalgae to increase the pH in the local environment. However, a macroalgal-mediated physiological refuge is not apparent. Instead, *Sargassum pacificum* hindered juvenile *P. rus* growth either by outcompeting the coral for a shared carbon resource or increasing microbial growth. The negative effects of *S. pacificum* combined with *P. rus*'s ability to utilize bicarbonate outweighed any positive indirect impacts of algal photosynthesis on seawater pH. A macroalgal physiological refuge from OA will occur most likely within high densities of algae in isolated habitats, where low water flow enhances the presence of DBLs. However, the refuge will only be apparent if it can outweigh negative effects of macroalgae on corals, such as shading, abrasion, and harmful microbial growth. To understand potential future consequences for corals reefs threatened by ocean acidification and other anthropogenic perturbations, researchers need to continue to parse out the indirect and direct influence of macroalgae on coral growth and metabolism.

## Tables

**Table 1.** The average total alkalinity ( $A_T$ ) for CRM samples were recorded for all seawater analyses and kept within 3 to 4  $\mu\text{mol kg}^{-1}$  of the certified value of 2235  $\mu\text{mol kg}^{-1}$  (Batch 105).

Experiment (sample size)	Average $A_T$ ( $\mu\text{mol kg}^{-1} \pm \text{SE}$ )
Preliminary field sampling ( $n=5$ )	$2235 \pm 2.4$
pH drift ( $n=2$ )	$2236 \pm 0.0$
Mesocosm ( $n=10$ )	$2238 \pm 0.9$

**Table 2.** The effects of *Sargassum pacificum*, at two different densities, varying wet weights and trial durations, on *in situ* seawater pH. NA refers to the first two test trials where the wet weight was not recorded.

Date (2012)	Wet weight (g)	Density (thalli/0.25 m <sup>2</sup> )	Trial (hrs.)	Inside pH <sub>T</sub> (i)	Outside pH <sub>T</sub> (o)	Δ pH <sub>T</sub> (i-o)
6/11	NA	20	1	7.890	7.880	0.010
	NA	30	1	7.884	7.844	0.041
6/12	826	20	2	7.694	7.671	0.023
	1217.8	30	2	7.901	7.761	0.141
		30	1	7.937	7.866	0.071
6/13	755.8	20	2	7.985	7.973	0.012
		20	1	7.965	7.946	0.019
	862.4	30	2	7.993	7.973	0.020
		30	1	7.981	7.991	-0.009
6/20	1601.2	30	2	7.990	7.985	0.005
		30	1	7.997	7.985	0.012

**Table 3.** The results of blocked ANOVAs testing the effect of the cage and the mimic versus the *Sargassum pacificum* on the average light levels (A) and relative flow (B) differences. The relative flow was determined by clod card dissolution rates (B).

A						
Treatment (Trt)	Light ( $\mu\text{mol quanta m}^2 \text{sec}^{-1} \pm \text{SE}$ )	Effect	df	MS	F	P
Inside cage	688.52 $\pm$ 62.75	Trt	1	152,167	27.28	< <b>0.001</b>
Outside cage	854.85 $\pm$ 70.89	Block	10	93,015	16.67	< <b>0.001</b>
		Error	10	5,578		
*Inside Algae	228.39 $\pm$ 62.83	Trt	1	0.384	3.28	0.100
*Inside Mimic	246.64 $\pm$ 42.20	Block	10	0.928	7.94	<b>0.001</b>
		Error	10	0.117		
B						
Treatment (Trt)	Dissolution rate ( $\text{g hr}^{-1} \pm \text{SE}$ )	Effect	df	MS	F	P
Inside cage	0.53 $\pm$ 0.03	Trt	1	0.000	0.023	0.882
Outside cage	0.53 $\pm$ 0.03	Block	10	0.017	17.91	< <b>0.001</b>
		Error	10	0.001		
Inside Algae	0.60 $\pm$ 0.03	Trt	1	0.001	0.24	0.634
Inside Mimic	0.61 $\pm$ 0.02	Block	10	0.009	2.87	0.056
		Error	10	0.003		

\* Data transformed to  $\log(x+1)$  to meet normality.

**Table 4.** A) Results of ANOVA testing differences between treatments (algal mimic and presence/absence of *Sargassum pacificum*) and among blocks (bommie) in area-normalized net calcification rates from the field experiment. (B) Results from a log-linear model ( $\ln(\text{MLE})$ = natural log of maximum likelihood estimation) with bleaching as the binomial, categorical response to the three field treatments.

A

Dependent Variable	Effect	df	MS	F	P
Area-normalized net calcification rate (mg cm <sup>-2</sup> day <sup>-1</sup> )	Treatment	2	0.162	1.34	0.286
	Block	10	0.208	1.73	0.149
	Error	18	0.120		

B

Dependent Variable	Effects	df	$\ln(\text{MLE})$	$\chi^2$	P
Bleaching (%)	Treatment	2	-11.013	43.29	0.961
	Bleaching	1	-10.643	44.03	0.365
	Bleaching x Treatment	2	-11.493	44.99	0.441

**Table 5.** The results of pH drift trials ( $n=4$ ), each lasting three hours. The initial and final values for pH: total pH scale,  $p\text{CO}_2$ : partial pressure of  $\text{CO}_2$ ,  $\text{HCO}_3^-$ : bicarbonate concentration,  $\text{CO}_3^{2-}$ : carbonate concentration and DIC: dissolved inorganic carbon concentration, were used to calculate the rate of change ( $\Delta \text{hr}^{-1}$ ).

Parameter (unit)	Initial (mean $\pm$ SE)	Final (mean $\pm$ SE)	$\Delta \text{hr}^{-1}$ (mean $\pm$ SE)
pH ( $\text{pH}_T$ )	$8.04 \pm 0.00$	$8.19 \pm 0.01$	$0.05 \pm 0.01$
$p\text{CO}_2$ ( $\mu\text{atm}$ )	$403.63 \pm 4.43$	$257.75 \pm 8.22$	$-48.63 \pm 3.12$
$\text{HCO}_3^-$ ( $\mu\text{mol kg}^{-1}$ )	$1754.47 \pm 6.04$	$1594.78 \pm 18.10$	$-53.23 \pm 14.80$
$\text{CO}_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	$2262.12 \pm 27.19$	$2863.44 \pm 56.41$	$200.44 \pm 45.58$
DIC ( $\mu\text{mol kg}^{-1}$ )	$1991.54 \pm 3.82$	$1888.15 \pm 12.74$	$-34.46 \pm 10.83$

**Table 6.** Summary of temperature and carbonate conditions in the eight mesocosms. Two tanks were designated per treatment and tank parameters averaged over the course of the experiment. The intended high CO<sub>2</sub> level was ~800  $\mu$ atm treatment and intended ambient CO<sub>2</sub> level was ~400  $\mu$ atm. The algal treatments were designated as presence or absence of *Sargassum pacificum* in the tank. pCO<sub>2</sub>: partial pressure of CO<sub>2</sub>, A<sub>T</sub>: total alkalinity, pH: total pH scale, HCO<sub>3</sub><sup>-</sup>: bicarbonate concentration, CO<sub>3</sub><sup>2-</sup>: carbonate concentration,  $\Omega_{\text{arg}}$ : calcium carbonate saturation state for aragonite

Treatment (CO <sub>2</sub> -Algae)	Tank	Temp (°C ± SE)	pCO <sub>2</sub> ( $\mu$ atm ± SE)	A <sub>T</sub> ( $\mu$ mol kg <sup>-1</sup> ± SE)	pH (pH <sub>t</sub> ± SE)	HCO <sub>3</sub> <sup>-</sup> ( $\mu$ mol kg <sup>-1</sup> ± SE)	CO <sub>3</sub> <sup>2-</sup> ( $\mu$ mol kg <sup>-1</sup> ± SE)	$\Omega_{\text{arg}}$ (± SE)
High-Present	1	28.2 ± 0.1	811 ± 16	2355 ± 4	7.81 ± 0.01	1959.11 ± 38	161.59 ± 14	2.59 ± 0.08
	6	28.6 ± 0.1	833 ± 13	2351 ± 5	7.79 ± 0.01	1966.54 ± 30	157.11 ± 11	2.52 ± 0.07
Amb-Present	3	28.3 ± 0.1	440 ± 87	2351 ± 3	8.02 ± 0.04	1779.09 ± 28	232.79 ± 11	3.73 ± 0.18
	4	28.3 ± 0.1	524 ± 73	2353 ± 2	7.96 ± 0.03	1833.31 ± 34	211.74 ± 13	3.39 ± 0.15
High-Absent	8	28.4 ± 0.1	827 ± 72	2321 ± 3	7.79 ± 0.03	1947.89 ± 24	151.81 ± 9	2.43 ± 0.15
	11	28.6 ± 0.1	758 ± 36	2325 ± 2	7.82 ± 0.03	1922.85 ± 26	163.81 ± 10	2.63 ± 0.17
Amb-Absent	2	28.2 ± 0.0	415 ± 54	2330 ± 3	8.03 ± 0.04	1752.69 ± 15	234.92 ± 5	3.76 ± 0.21
	9	28.2 ± 0.0	414 ± 92	2315 ± 4	8.03 ± 0.04	1742.18 ± 11	231.72 ± 4	3.71 ± 0.23

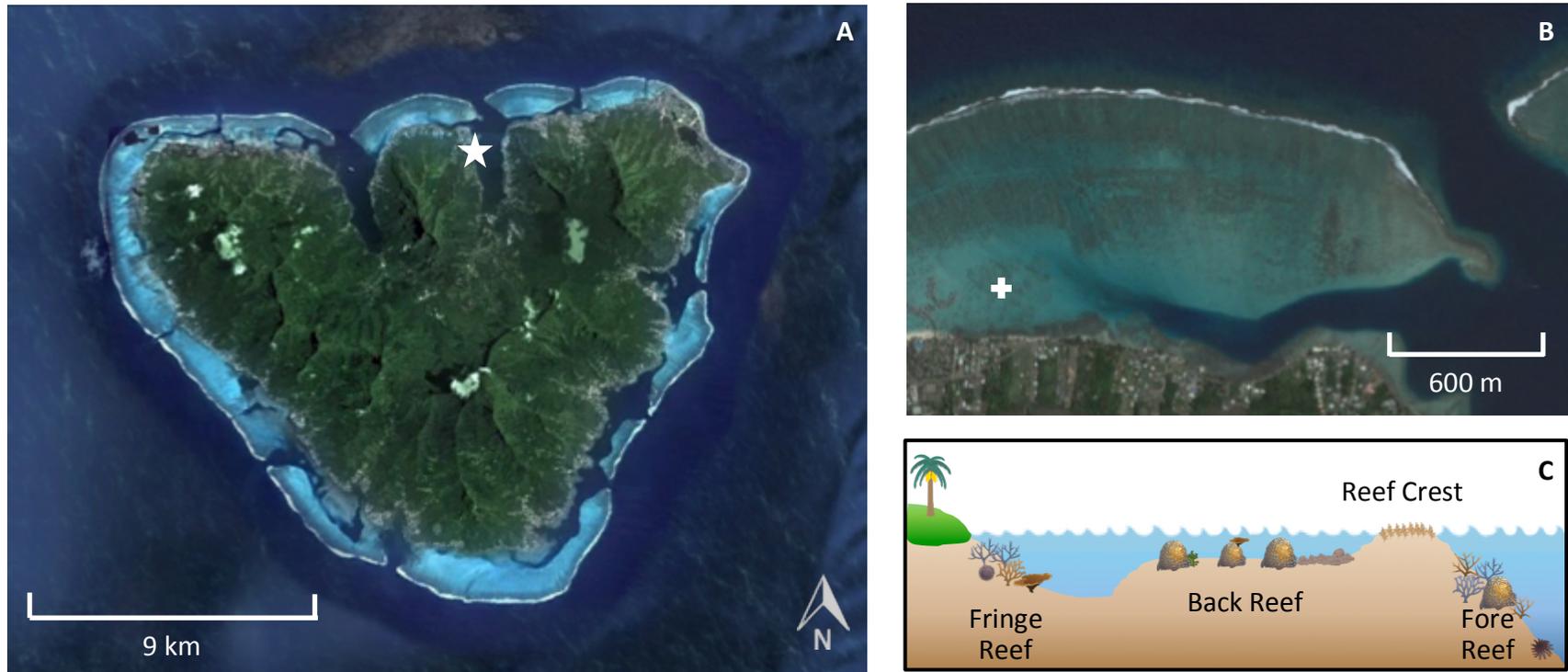
**Table 7.** Results of partly nested two-way ANOVAs analyzing the effects of pCO<sub>2</sub> level and the algal treatments (Algae=absence/presence of *Sargassum pacificum*), on various abiotic tank parameters. Only the main effects of the two treatments are presented below as the interactions (pCO<sub>2</sub> x Algae) and tank effects (Tank(pCO<sub>2</sub> x Algae)) were not significant for all of the tank parameters.

Parameter	Source	SS	df	MS	F	P
Temperature	pCO <sub>2</sub>	0.760	1	0.760	8.78	<b>0.004</b>
	Algae	0.204	1	0.204	2.36	0.130
	Error	4.676	64	0.087		
pH	pCO <sub>2</sub>	0.724	1	0.72	83.83	<b>&lt;0.001</b>
	Algae	0.008	1	0.01	0.84	0.354
	Error	0.553	64	0.01		
A <sub>T</sub>	pCO <sub>2</sub>	6.8	1	6.8	0.06	0.806
	Algae	15129.0	1	15129.0	136.49	<b>&lt;0.001</b>
	Error	7094.1	64	110.8		
pCO <sub>2</sub>	pCO <sub>2</sub>	2186648	1	2186648	63.04	<b>&lt;0.001</b>
	Algae	39929	1	39929	1.15	0.287
	Error	2220067	64	34688		
HCO <sub>3</sub> <sup>-</sup>	pCO <sub>2</sub>	502812	1	502812	79.54	<b>&lt;0.001</b>
	Algae	31483	1	31483	4.98	<b>0.029</b>
	Error	404554	64	6321		
CO <sub>3</sub> <sup>-2</sup>	pCO <sub>2</sub>	81,157	1	81,157	90.64	<b>&lt;0.001</b>
	Algae	383	1	383	0.43	0.515
	Error	57,303	64	895		
Ω <sub>arg</sub>	pCO <sub>2</sub>	20.59	1	20.6	89.65	<b>&lt;0.001</b>
	Algae	0.10	1	0.1	0.44	0.509
	Error	14.70	64	0.2		

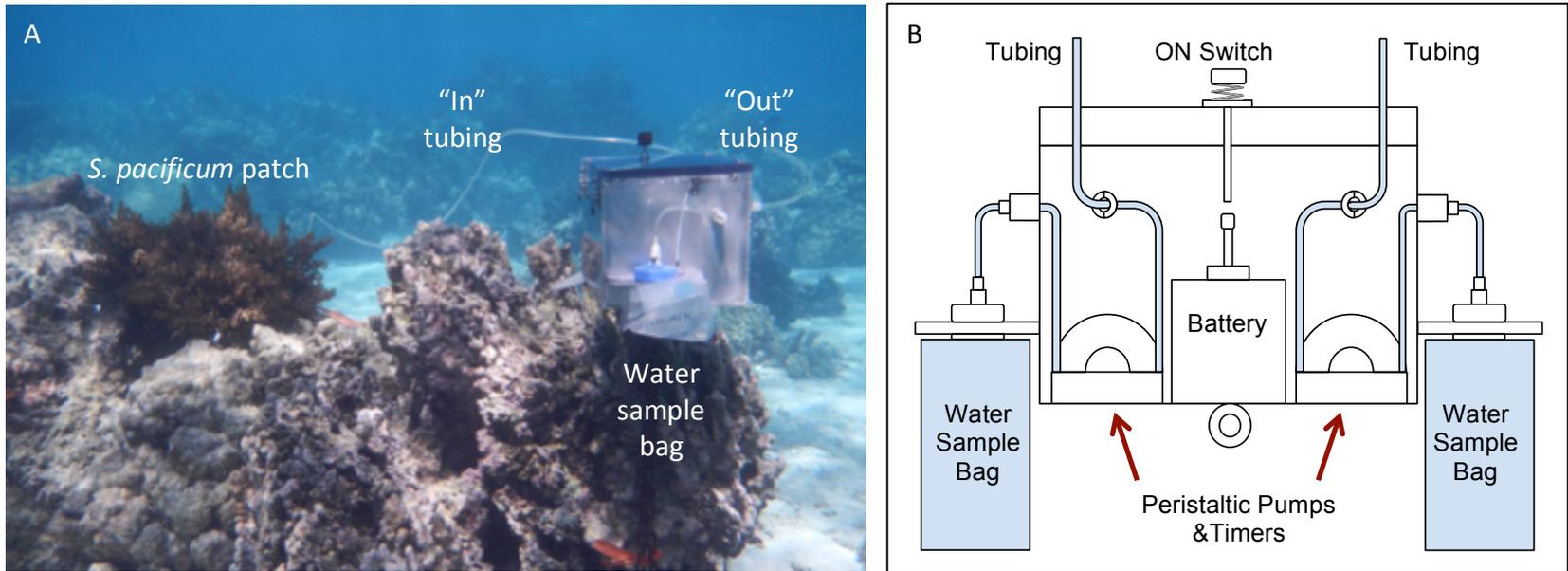
**Table 8.** Results of a partly nested two-way ANOVA on net calcification rates of *Porites rus* from the mesocosm experiment with tank as a nested, random factor.

Dependent Variable	Effect	SS	df	MS	F	P
Area-normalized net calcification rate (mg CaCO <sub>3</sub> cm <sup>-2</sup> day <sup>-1</sup> )	pCO <sub>2</sub>	3.15	1	3.15	23.68	<b>0.01</b>
	Algae	2.07	1	2.07	15.59	<b>0.02</b>
	Algae x pCO <sub>2</sub>	0.14	1	0.14	1.06	0.36
	Tank (Algae x pCO <sub>2</sub> )	0.53	4	0.13	0.24	0.91
	Error	47.83	87	0.55		

## Figures



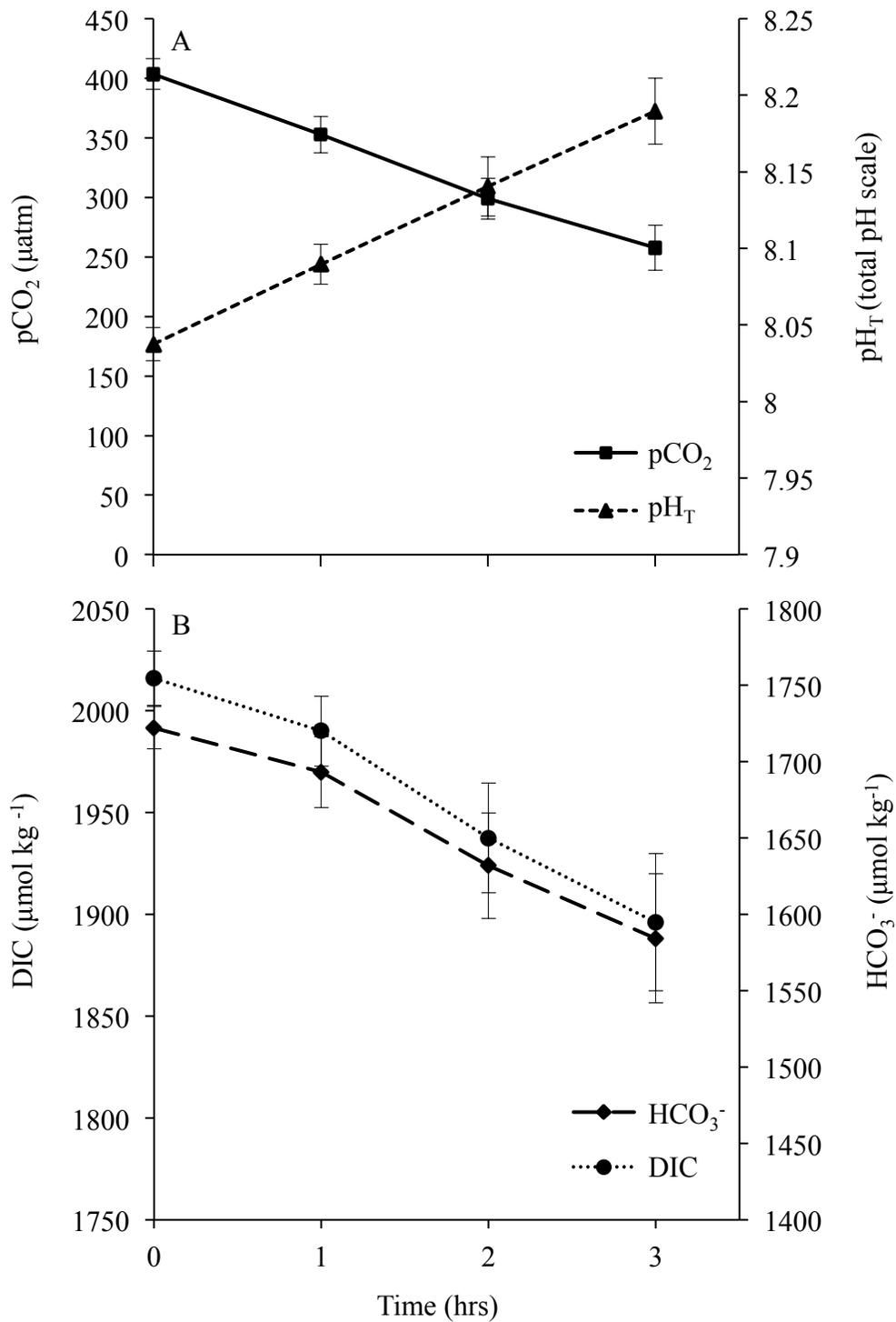
**Figure 1.** The island of Moorea, part of the French Polynesian Society Island chain, with the Richard B. Gump South Pacific Research Station (★) located in Cook's Bay on the north shore (A) and location of field experiment (+) west of Cook's Bay (B). Profile diagram of the reef surrounding Moorea (C).



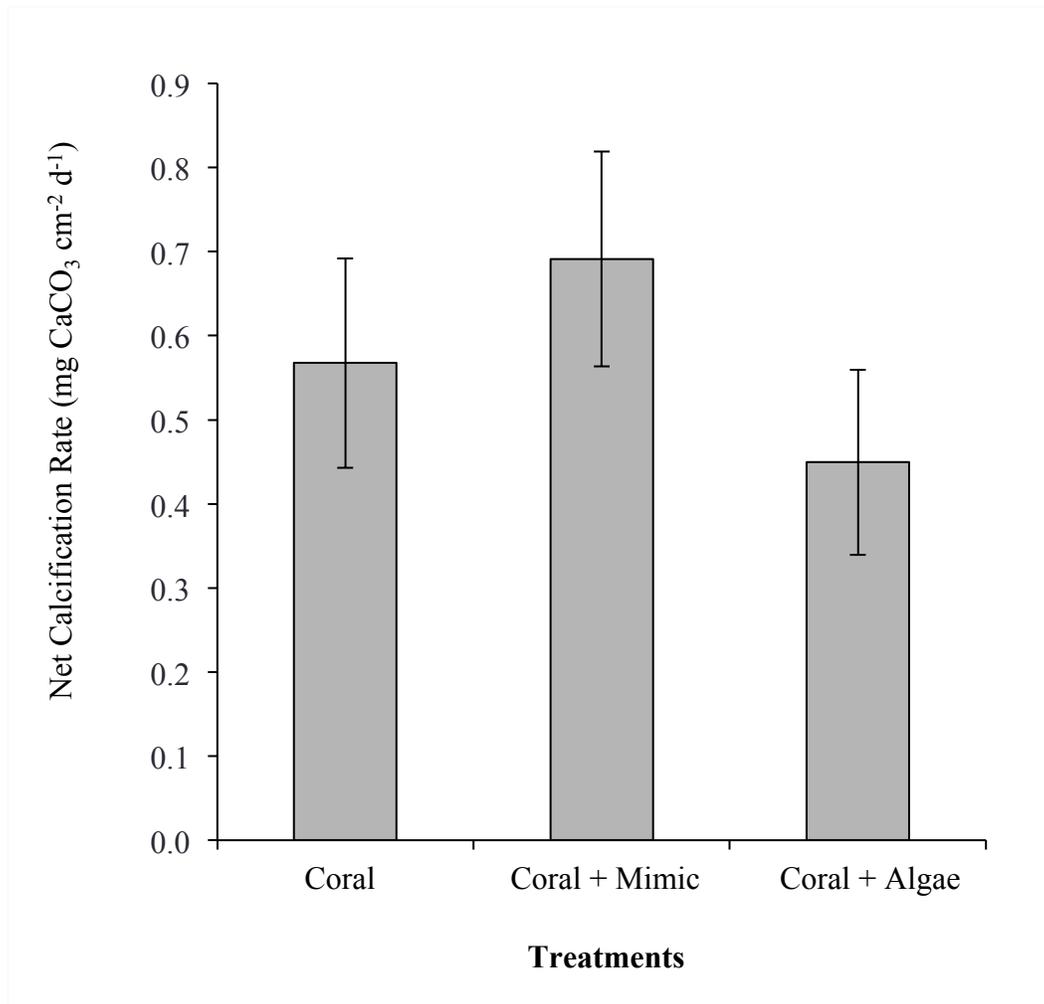
**Figure 2.** The integrated water sampler, chained to the bottom of a dead coral bommie, simultaneously collected water from inside the *Sargassum pacificum* patch and outside the patch (A). The water sampler consisted of a metal welded box with a clear, acrylic lid with two peristaltic pumps (Welco), a battery, two timers (12V Timer Switch) and aquarium tubing connecting the two water sample bags (1 L, Nalgene) to the pumps (B). The ON switch could be activated from the outside of the box.



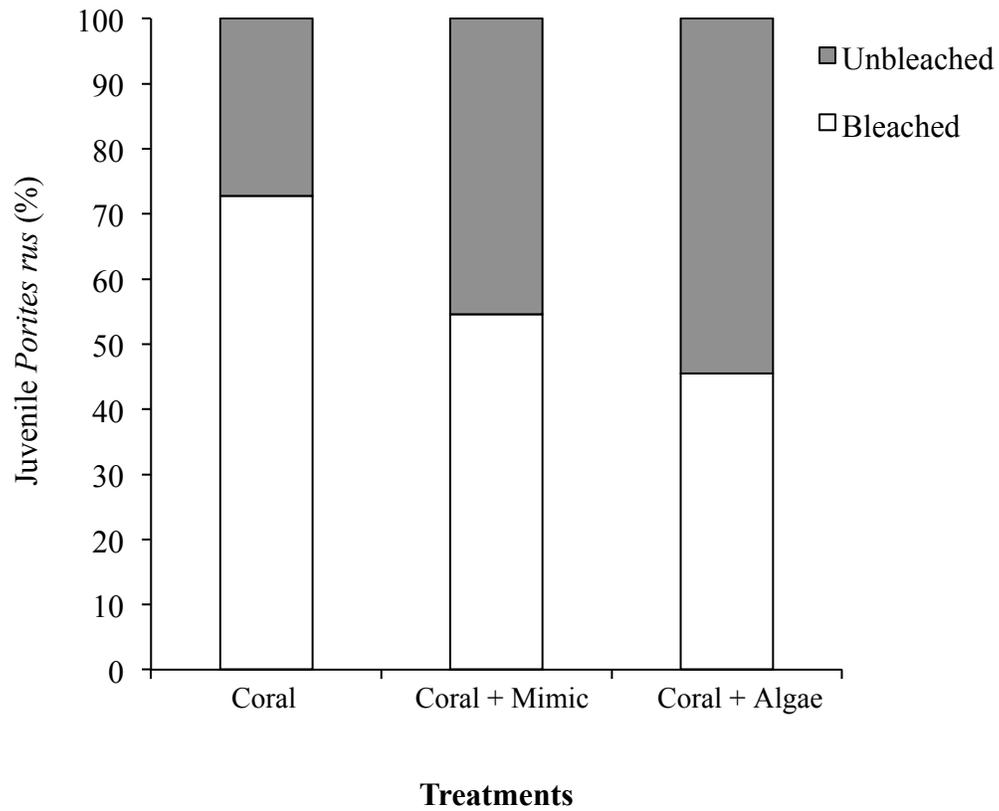
**Figure 3.** Photoquadrat of a juvenile *Porites rus* adhered to gutter guard with marine epoxy and zip-tied to the bottom of the cage (A). A bommie (block) with the caged treatments: 1=*Porites rus* + *Sargassum*, 2=*Porites rus* + algal mimic, 3=isolated *Porites rus* (control) (B). The treatments were nailed in random order on top of each dead coral bommie ( $n=11$ ).



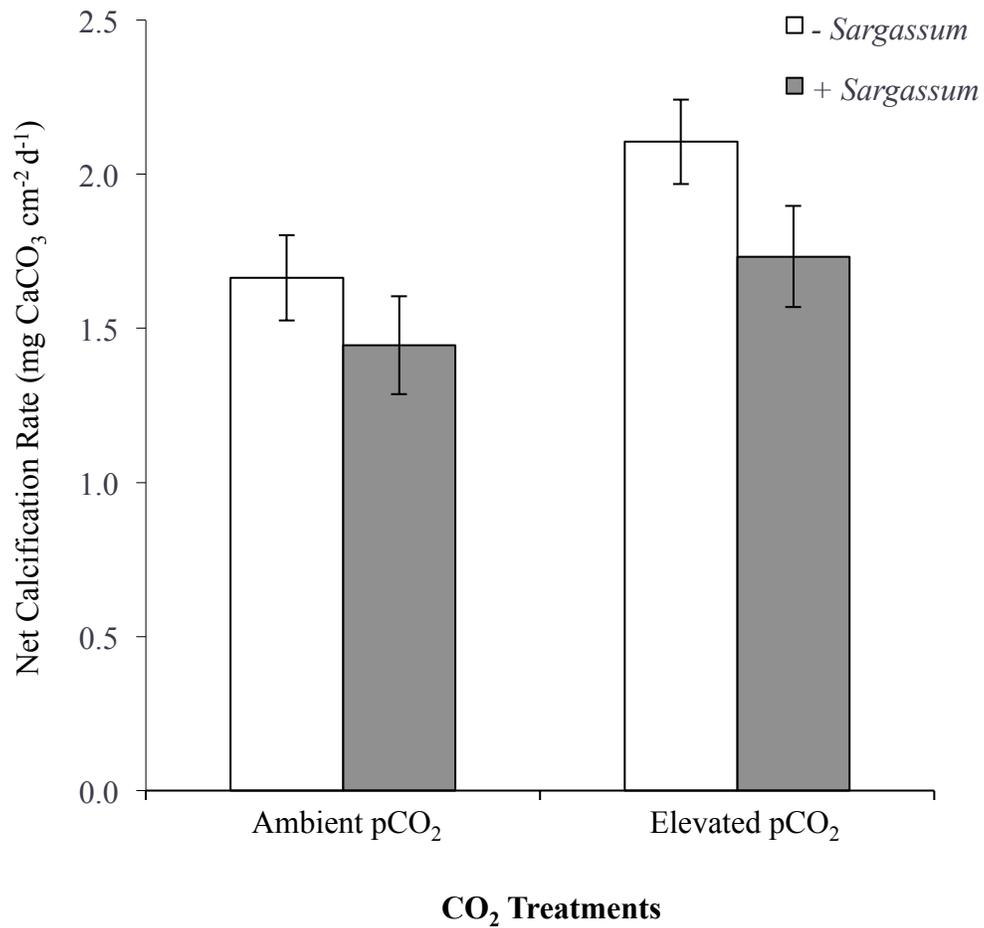
**Figure 4.** A pH drift experiment showing the effects of *Sargassum pacificum* on (A) pCO<sub>2</sub>, pH<sub>T</sub> and (B) HCO<sub>3</sub><sup>-</sup> and DIC over four hours ( $n=4$ ).



**Figure 5.** Average net calcification rates of juvenile *Porites rus* normalized to surface area ( $\pm$  SE,  $n=11$ ) in three caged treatments: isolated (control), + algal mimic, and + *Sargassum pacificum*.



**Figure 6.** At the end of the 23 day field experiment with three treatments ( $n=11$ ), the percent of juvenile *Porites rus* in each treatment were categorized as unbleached (grey bars) if no whiteness was recorded or bleached (white bars) if the coral had any white spots.



**Figure 7.** Area-normalized net calcification rates of juvenile *Porites rus* (mean  $\pm$  SE,  $n=24$ ) in ambient ( $\sim 400$   $\mu\text{atm}$ ) and elevated CO<sub>2</sub> ( $\sim 800$   $\mu\text{atm}$ ) conditions (white bars). While net calcification rates increased with increasing pCO<sub>2</sub>, the rates decreased in the presence of *Sargassum pacificum* (grey bars).

## Chapter 3

### The ability of *Sargassum pacificum* to modify the effects of ocean acidification on *Acropora pulchra*

#### Introduction

Humans are rapidly altering the biosphere by inundating the carbon cycle with excess carbon dioxide (CO<sub>2</sub>) through the burning of fossil fuels, deforestation and other anthropogenic activities. Around 30% of the excess CO<sub>2</sub> is absorbed into the oceans, leading to a process known as ocean acidification (Sabine and Feely 2004). Oceans act as carbon sinks (Gattuso et al. 1997) where the carbon dioxide either can be used in metabolic processes or dissolved in seawater to form inorganic carbon (carbonate, bicarbonate and aqueous CO<sub>2</sub>) and hydrogen ions (H<sup>+</sup>). With a rise in atmospheric CO<sub>2</sub> concentration from ~280 μatm (Feely et al. 2009) to ~400 μatm since the industrial revolution, the oceans have dropped by 0.1 pH units and become less saturated with carbonate ions (Caldeira and Wickett 2003, Orr et al. 2005, Hoegh-Guldberg et al. 2007). As humans continue to accelerate the release of excess carbon dioxide, the oceans are predicted to experience a reduction in pH by 0.3-0.4 by the end of the century (Doney et al. 2009). An ocean undersaturated with carbonate minerals threatens the persistence of key calcifying organisms such as scleractinian corals (Côté et al. 2005, Fabricius et al. 2011, Kroeker et al. 2013). Consequently, ocean acidification threatens tropical coral reefs, one of the most diverse and socioeconomically valuable ecosystems.

With a decreasing carbonate saturation state ( $\Omega$ ) across the oceans, the ability of biogenic calcifiers, such as corals, to build massive structures may

become increasingly compromised (Hoegh-Guldberg et al. 2007). Once  $\Omega$  is undersaturated, the mineral components of an organism's shell or skeletons may even begin to dissolve (van Woesik et al. 2013b). Globally, coral reefs face an uncertain future, as decreasing seawater pH will stress the key reef builders supporting the diverse benthic community. Previous research has documented reduced coral calcification rates when organisms are placed in elevated  $\text{CO}_2$  conditions (reduction in seawater pH by  $\leq 0.5$  units) for short, pulse treatments. Under these conditions, most calcifiers exhibit reduced calcification rates by up to 50%; however, species respond heterogeneously to OA treatments (Kroeker et al. 2013). Some marine calcifiers, such as massive *Porites* (Fabricius et al. 2011), and some macroalgae, such as *Halimeda* spp., (Price et al. 2011) are resilient to high  $\text{pCO}_2$  (750  $\mu\text{atm}$  and  $\sim 950$   $\mu\text{atm}$  respectively). Not only do marine organisms have species-specific responses to OA, but also they can potentially alter the effect of OA by metabolically changing the carbonate chemistry of the surrounding seawater. Ocean acidification research has rarely involved longer-term ( $>1$  month) treatments or taken into account the natural spatial and temporal variation in carbonate chemistry of nearshore marine ecosystems (Hofmann et al. 2011).

Ocean acidification will not occur uniformly across shallow coastal ecosystems such as coral reefs, oyster beds and kelp forests, where benthic organisms metabolically modify the seawater carbonate chemistry (Anthony et al. 2013). Instead, the flux of DIC and thus, effects of ocean acidification, will depend heavily on benthic community composition and other environmental

factors that impact the metabolic activity of the community. Reef metabolism is the organic production of carbon through photosynthesis and respiration (net ecosystem production), and the inorganic production of carbon through dissolution and calcification (net ecosystem calcification) (Bates et al. 2009). Respiration and calcification release  $H^+$  and  $CO_2$ , lowering pH, while dissolution and photosynthesis consume  $H^+$  and  $CO_2$ , increasing pH (Gattuso et al. 1999b, Bates et al. 2009, Anthony et al. 2011a, Kleypas et al. 2011). Thus, benthic organisms in coral reefs create diel fluctuations in dissolved inorganic carbon (DIC) concentrations, due to differences in daytime vs. nighttime metabolic activity (Gattuso et al. 1993). For example, a coral reef dominated by fleshy macroalgae may experience larger carbon fluctuations due to high levels of photosynthetic activity during the day and respiration at night (Suzuki et al., 1995; Gattuso et al., 1999). The presence and magnitude of these carbon fluxes also will depend on abiotic conditions such as water flow, nutrient levels, temperature and water residence time (Diaz-Pulido et al. 2007, Anthony et al. 2011a). However, feedbacks between OA, benthic community composition and reef metabolism could influence  $CO_2$  concentrations, though these relationships remain largely unexplored.

Turf algae, macroalgae, and crustose coralline algae naturally make up a significant portion (~45-80% of reefs in Northwestern Hawaiian Islands) of many coral reef communities (Vroom et al. 2006, Vroom and Braun 2010). However, the abundance of non-calcified algae can increase rapidly and become detrimental to other reef inhabitants when herbivore populations decline, nutrient

concentrations increase (Pandolfi et al. 2003, Hughes et al. 2010) or alternatively, when excess CO<sub>2</sub> from anthropogenic activities stimulates photosynthesis (Longphuir et al. 2013) or growth rates of algae (Kuffner et al. 2008, Hurd et al. 2009, Johnson et al. 2014). As human activities, including overfishing, coastal development (driving nutrient pollution), and carbon emissions, continue to increase, macroalgae are predicted to increase in abundance leading to prolonged shifts to macroalgal-dominated states in coral reefs (Diaz-Pulido et al. 2007, Hoegh-Guldberg et al. 2007, Diaz-Pulido et al. 2009, Cheal et al. 2010, Anthony et al. 2011b). Numerous studies have examined the direct and indirect negative effects macroalgae have on coral survival, growth and metabolism (McCook 2001). More recently, research has begun to examine potential interactions between OA and macroalgae. In one case, mortality of *Acropora intermedia* increased when grown in direct contact with the encrusting, fleshy alga, *Lobophora papenfusii*, at high pCO<sub>2</sub> (Diaz-Pulido et al. 2011). The ability of macroalgae to modify the effects of OA may depend partially on the characteristics of the alga, such as the morphology (Jompa and McCook 2003b, Hauri et al. 2010, Bender et al. 2012) and presence of allelochemicals (Rasher et al. 2011, Andras et al. 2012, Bonaldo and Hay 2014, Rasher and Hay 2014). Larger, fleshy macroalgae can overgrow and shade corals in the macroalgal understory (Box and Mumby 2007). However, macroalgal shading alone has mixed effects on coral health ranging from reducing coral metabolism (Hauri et al. 2010), to preventing bleaching (Jompa and McCook 1998), to eliciting no physiological response by the coral *Porites lutea* (Titlyanov et al. 2007). Due to

the heterogeneous nature of coral-algal interactions, it is unknown whether certain macroalgal species would harm or facilitate corals in elevated CO<sub>2</sub> conditions.

With increasing CO<sub>2</sub> levels, macroalgae could impact coral growth and metabolism positively, by reducing pCO<sub>2</sub> (thereby increasing pH) through photosynthesis. Macroalgal species (e.g. *Chondria* spp.) elevate seawater pH and stimulate the calcification of *Acropora* spp. by 60% and *Montipora* spp. by 130% (McConnaughey et al. 2000). Similarly, increased coral calcification rates were recorded downstream of macroalgae in flumes and from model simulations (Anthony et al. 2011a, Kleypas et al. 2011). Previous work shows how macroalgae can induce physical and chemical changes in the microenvironment below the macroalgal canopy and surrounding benthic invertebrates (Duggins et al. 1990, Hauri et al. 2010). In small patches (~20-30 thalli/0.25 m<sup>2</sup>) on the north shore of Moorea, *Sargassum pacificum* increases seawater pH by  $0.031 \pm 0.012$  pH<sub>T</sub> (mean  $\pm$  SE) below the canopy (Chapter 2). In much larger patches, *S. pacificum* could potentially increase the seawater pH to a greater degree (>0.031 pH<sub>T</sub>) and mitigate the negative impacts of ocean acidification on scleractinian corals. In addition, *S. pacificum* represents a genus that survives in low pH (6.7) seawater near volcanic CO<sub>2</sub> in Italy (Porzio et al. 2011), can prevent coral bleaching through shading on inshore reefs of the Great Barrier Reef (Jompa and McCook 1998), and lacks allelochemicals harmful to corals (Rasher and Hay 2010, Rasher et al. 2011, Rasher and Hay 2014). Despite the variety of mechanisms by which *S. pacificum* may affect corals, the net effect of this common alga on corals under OA conditions remains unknown.

The objective of this study was to experimentally test two alternative mechanisms by which fleshy macroalgae could modify the response of corals to OA:

- 1) Reducing coral growth and metabolism by shading the coral
- 2) Increasing coral growth and metabolism by actively reducing pCO<sub>2</sub> through photosynthesis, which creates a chemical refuge from OA

The present research incorporated ecologically relevant interactions between macroalgae and corals in an OA experiment to determine if a fleshy macroalga would either exacerbate, through shading, or mitigate, through a reduction in pCO<sub>2</sub> by photosynthesis, the effects of ocean acidification on a scleractinian coral.

*Acropora pulchra*, a branching perforate coral, was used in this study because it has previously exhibited sensitivity to elevated pCO<sub>2</sub> (Comeau et al. 2013c). In addition, *Acropora* spp. provide complex structure and critical habitat for sustaining reef fish populations (Chabanet et al. 1997, Holbrook et al. 2002). *Sargassum pacificum*, a large, fleshy alga found throughout the tropical Pacific and dominating the reef crest surrounding Moorea, French Polynesia, was used in the present study (Stiger and Payri 1999, Stewart 2008, Spitler 2009). It does not harbor harmful allelochemicals (Rasher and Hay 2010, Rasher et al. 2011, Rasher and Hay 2014) and is predicted to become abundant with decreasing seawater pH (Porzio et al. 2011). The fore reef in Moorea had low cover (~4% at LTER 1) of fleshy, non-calcified macroalgae in January 2012 (Carpenter 2014b), thus the fore reef and back reef (immediately downstream of the algal-covered reef crest) represent suitable locations to study, *in situ*, the effect of macroalgal metabolism

and shading on *A. pulchra*. To test the effect of macroalgae on corals under elevated CO<sub>2</sub>, mesocosms were used to recreate the light and chemical (pH) environment of the microhabitat below *S. pacificum* canopies. The combined field and mesocosm experiments tested the hypothesis that light and pCO<sub>2</sub> would affect *A. pulchra* growth and photosynthesis

## **Methods**

### *Site*

The study was conducted on the north shore of Moorea (17° 30' S, 149° 50' W), in the Society Island chain of French Polynesia. The laboratory experiment was completed at the University of California, Berkeley, Richard B. Gump South Pacific Research Station and the field experiment was conducted along the reef crest and fore reef of the north shore reef (Fig. 1). On the north shore of Moorea, the fore reef water flows south, over the macroalgal-dominated reef crest and the coral-dominated back reef (Adjeroud 1997, Holbrook et al. 2002, Adjeroud et al. 2009, Comeau et al. 2014c), and then east to west along the lagoon and back to the open ocean through the boat channel (Fig 1.) (Hench et al. 2008, Leichter et al. 2012).

In 2012, the Moorea Coral Reef Long Term Ecological Research Site deployed 5 SeaFETs, ocean pH sensors, at 5 sites from the fore reef to the fringing reef at Site 1 (Fig. 1). The SeaFETs recorded seawater pH every 30 minutes (Fig. 2). The fore reef SeaFET was mounted ~4-5 m below the surface on a mooring line attached at 10 m depth. The SeaFET south of the reef crest was attached to a PVC plate bolted to the reef (1-2 m depth). Before deployment, both

SeaFETs were equilibrated in the same seawater for 2 weeks to ensure the internal and external electrode corresponded. Replicate seawater samples were collected from the SeaFET locations during the deployment and retrieval of each SeaFET (personal communication with Keith Seydel). The pH of the seawater samples, analyzed spectrophotometrically (SOP 6b, Dickson et al. 2007) and with an open cell potentiometric titrator (T50, Mettler-Toledo; SOP 3b, Dickson et al. 2007), was used to calibrate the instruments and account for any drift in the pH measurements. The seawater pH measurements from the two sites (reef crest and fore reef) informed the location of the field experiment and the pCO<sub>2</sub> treatments for the laboratory experiment, in addition, to providing information on the effects of benthic community composition on seawater carbonate chemistry.

To determine the light environment created by *Sargassum pacificum* and thus, inform the light levels needed for the mesocosm and field experiment, irradiance was measured in the field. Using the 2 $\pi$  PAR light sensor (1-mm active diameter) on a Diving PAM (Heinz Walz), calibrated to a 2 $\pi$  light sensor (Li-Cor, LI-192) connected to a light meter (Li-Cor, LI-1400), the light levels were measured inside and outside *S. pacificum* canopies along the reef crest of the North shore. On June 7 and June 9, 3-6 light measurements were recorded at the base of the holdfast, middle (~5-10 cm above substrate) of the canopy and directly above the canopy at 20 different patches of *S. pacificum* on the reef crest. All light measurements ( $n=365$ ) were recorded between the hours of 1100 and 1300 to determine maximum light levels.

### *Mesocosm experiment*

To test the combined effects of ocean acidification and presence of macroalgae, specifically fluctuating pCO<sub>2</sub> and shading, on coral growth and photosynthesis, a fully factorial mesocosm experiment crossing three CO<sub>2</sub> treatments (ambient: 400 μatm, fluctuating: 250-400-800 μatm, high: 800 μatm) with two light levels was conducted. The two light levels were established from the field surveys to mimic the environment below and above the *Sargassum pacificum* canopy. Four response variables were measured on *Acropora pulchra* nubbins: two to describe the growth of the coral (net calcification and linear extension) and two to quantify changes in metabolism (net photosynthetic rate and chlorophyll *a* concentration) that contribute to the coral growth patterns.

*Acropora pulchra* nubbins were collected haphazardly with gardening shears and clipped 4 cm from the tip of the colony. Corals were collected from a fringing reef site on the north shore of Moorea. The samples were placed into Ziploc bags with fresh seawater and, within an hour, transferred into a flow through-seawater table with seawater pumped from Cook's Bay. Each coral nubbin was epoxied (Z-spar Splash Zone Compound<sup>TM</sup>, A788) to pieces of rectangular, plastic gutter guard with the epoxy covering exposed skeleton. The nubbins remained in the water table for 24 hours; afterward, they were moved to a 1000-L acclimation tank (Aqualogic, San Diego) filled with sand-filtered (~100 μm mesh) seawater pumped from 12-m depth in Cook's Bay. Temperature and light levels (Table 1) mimicked the conditions in the mesocosm treatments (~27 °C and ~400 μmol photons m<sup>-2</sup> s<sup>-1</sup>) and fell within the range of levels found

naturally on the back reef (Putnam and Edmunds 2011, Carpenter 2014a). A twelve-hour light:dark cycle was created by four 75 W LED lights (Sol White LED Module; Aquaillumination) mounted above the acclimation tanks. The light intensity increased for four hours from 0 to 100%, stayed at 100% for four hours and decreased to 0% light for the last four hours, representing the natural light cycle on the reef. To avoid any position effects, the corals were placed on a table that rotated every 12 hours.

### Tank Setup

The same water for the acclimation tank was pumped into ten 150-L tanks (AquaLogic, San Diego, CA) at  $9.24 \pm 0.17 \text{ L hr}^{-1}$  ( $n=26 \text{ tank}^{-1}$ ). The temperature was maintained at  $\sim 28 \text{ }^\circ\text{C}$  and the flow of each tank was controlled with submersible water pumps (Rio 8HF,  $2,082 \text{ L h}^{-1}$ ). Each tank had a 75W LED light (Sol White LED Module, Aquaillumination) set to the same 12:12 hr. light: dark cycle for the acclimation tank. In addition, two stands (one shaded and one unshaded) made of clear acrylic (11 x 20.7 x 9 cm) were placed in each tank. To create the shaded treatment, a neutral density filter (Lee 210 ND6, 2 stop Gel Filter Sheet) was clipped to the top of one of the acrylic stands to block out  $\sim 76.5\%$  of the light, mimicking the reduction of light by *S. pacificum* canopies recorded in the field. A stand without a light filter was placed in each tank to ensure all corals experienced the same flow environment created by the stand. Irradiance under each stand was measured before, during and after the experiment with the  $2\pi$  PAR light sensor (1-mm active diameter) connected to a Diving PAM

(Heinz Walz) and calibrated to a  $2\pi$  light sensor (Li-Cor, LI-192). Water temperature was measured every day with a handheld digital thermometer (Fisher Scientific, model 15-077-8,  $\pm 0.1$  °C). Tanks were assigned randomly to the CO<sub>2</sub> treatments with four tanks at ambient ( $\sim 400$   $\mu\text{atm}$ ), four tanks at elevated ( $\sim 800$   $\mu\text{atm}$ ) and 2 tanks at low ( $\sim 250$   $\mu\text{atm}$ ). The high CO<sub>2</sub> treatment was based on the IPCC scenario projected for 2100 if human carbon emissions remain the same (RCP6, van Vuuren et al., 2011).

Twelve *Acropora pulchra* nubbins were placed on PVC-coated metal fencing (2.5-cm<sup>2</sup> galvanized, welded mesh fence) below the shaded and clear canopies. Nubbins were moved within the grid every two days to minimize position effects in the tank. Corals in the fluctuating pCO<sub>2</sub> treatment remained in the ambient pCO<sub>2</sub> tanks from 0800-1000 hours, were moved to the low pCO<sub>2</sub> tanks from 1000-1600 hours, transferred back to the ambient pCO<sub>2</sub> tanks from 1600-1800 hours, and remained in the elevated pCO<sub>2</sub> tanks from 1800-0800 hours (Fig. 3). To account for handling effects, corals in the stable ambient and elevated CO<sub>2</sub> treatments were also moved in and out of their corresponding tanks for the same amount of time and at the same times of day. All tanks were scrubbed every two days to remove turf algal growth.

### Carbonate Chemistry

To create the elevated CO<sub>2</sub> treatments, a solenoid (Model A352, Qubit Systems, Ontario, Canada) controlled the amount of pure CO<sub>2</sub> and ambient air required for a mixture of known pCO<sub>2</sub>. An Infrared Gas Analyzer (Model S151,

Qubit Systems) analyzed the  $p\text{CO}_2$  of the gas mixture. The  $\text{CO}_2$ -enriched air was monitored on a LabPro program (Venier Software and Technology) and bubbled into the tanks via pumps (Gast pump DOA-P704-AA). Ambient  $\text{CO}_2$  treatments received ambient air bubbled into the tanks from similar Gast pumps. For the low  $\text{CO}_2$  treatments, a column of desiccant (Regular Drierite, W.A. Hammond Drierite Co.) followed by a column of soda lime (SODASORB®, W.R. Grace & Co., Conn.) were attached to an aquarium air pump which dried and scrubbed the air of  $\text{CO}_2$  before entering the tank. The soda lime and desiccant were changed every two days. To measure the carbonate parameters, two ~ 50-ml seawater samples were collected in glass bottles every two days and acclimated to laboratory conditions at 25 °C. For each sample, the salinity was measured with an YSI 3100 conductivity meter before being analyzed with spectrophotometric techniques using m-cresol purple dye (SOP 6b, Dickson et al. 2007) and with an open cell potentiometric titrator (T50, Mettler-Toledo; SOP 3b, Dickson et al. 2007). The R package Seacarb (Lavigne and Gattuso 2012) was used to calculate the total alkalinity ( $A_T$ ), pH,  $p\text{CO}_2$ ,  $[\text{HCO}_3^-]$ ,  $[\text{CO}_3^{2-}]$ , DIC and  $\Omega_{\text{arg}}$ . To ensure accurate carbonate measurements, the pH probe (DG I115-SC, Mettler-Toledo) was calibrated every two days with Tris buffer (Batch 13) from the Dickson Lab at Scripps Institution of Oceanography. Certified reference materials (CRM, Batch 122 and 105) were analyzed before each batch of water samples. The average  $A_T$  ( $\mu\text{mol kg}^{-1} \pm \text{SE}$ ) of the CRM samples (Batch 122:  $2231 \pm 0.8$ ,  $n=17$ ; Batch 105:  $2237 \pm 0.8$ ,  $n=27$ ) was kept within 2  $\mu\text{mol kg}^{-1}$  of the expected CRM values (Batch 122: ~2233, Batch 105: ~2235). All CRM, Tris buffer and hydrochloric

acid (~0.1N HCl and 0.6 M NaCl) were acquired from the Andrew Dickson Lab at Scripps Institution of Oceanography. The treatments were maintained for 38 days, after which the response variables were measured.

### Net Calcification and Linear Extension

To determine the effects of shading and CO<sub>2</sub> (both fluctuating and elevated) on coral linear extension and net calcification rates, normalized to surface area, were quantified. Calipers were used to measure the vertical growth (mm) of the coral before and after the experiment to quantify linear extension. The coral nubbins were buoyant weighed before and after the experiment (Davies 1989) to determine net calcification rates. The aragonite density of 2.93 g cm<sup>-3</sup> was used to convert the buoyant weight into dry weight. The calcification rate was normalized to tissue surface area estimated by the wax dipping technique, shown to be accurate for branching corals such as *Acropora* spp. (Stimson and Kinzie 1991, Naumann et al. 2009). The coral tissue was removed with thin jets of filtered (45 µm) seawater from a Water Pik (Johannes and Wiebe 1970) and set aside for chlorophyll *a* measurements (see below). The coral skeletons were rinsed in freshwater and set to dry for 48 hours, after which each coral was weighed. The nubbins were dipped in 65 °C paraffin wax, melted in a 500-ml beaker inside of a water bath, for two seconds and shaken five times to remove excess wax (Veal et al. 2010). After the wax had dried for 24 hours the samples were reweighed.

## Chlorophyll Extraction and Net Photosynthesis

To quantify net photosynthetic rates, a subset of coral nubbins ( $n=4$ ) from each light treatment (shaded and unshaded) in each tank was incubated in a sealed, 250-mL chamber connected to a water bath. The water bath circulated water around the chamber to maintain a constant temperature of  $\sim 28$  °C. The seawater was acquired from the corresponding tank and remained in constant motion with a magnetic stir bar and plate (Multistirrer MC 303, Scinic Co., Tokyo). Two 75W LED light (Sol White LED Module, Aquaillumination) were suspended above the two chambers and set to the average high light level the corals experienced in the tanks (shaded:  $\sim 29$   $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , unshaded:  $\sim 359$   $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Light was measured in the water of the chamber with the  $2\pi$  light sensor (1-mm active diameter) attached to a Diving PAM (Heinz Walz, Germany). Coral specimens were acclimated in the chamber for ten minutes before a fiber optic oxygen probe (Fibox 3, 2-mm diameter, PreSens) and temperature probe measured the change in oxygen (% air saturation), compensated to temperature over time. Seawater was exchanged before each trial. The % air saturation was converted to  $\mu\text{mol O}_2 \text{ L}^{-1}$  by using the values from the Unisense gas tables (N. Ramsing and J. Gundersen at Unisense, <http://www.unisense.com/Default.aspx?ID=1109>). The slope of oxygen evolution for each trial was multiplied by the volume of the water in the chamber (- the volume of the coral) and normalized to surface area.

To calculate chlorophyll *a* concentrations (Jeffrey and Humphrey 1975), the water-picked tissue from the nubbins was placed into 250-mL cups on

magnetic plates with stir bars to keep the solution homogenized. A dismembrator (Model 500, 15-338-550, with 3.2-mm diameter probe, model 15-338-67, Fisher Scientific) set to 10% power, pulsed the slurry for four 10-second bursts to break up the tissue into a more homogenized solution. A volume of 15 mL of the solution was pipetted into a falcon tube and centrifuged at 1400 rpm for 10 minutes. The supernatant was discarded and 3 mL of 90% acetone was added to the falcon tube. Each sample was wrapped in foil, shaken and placed in a freezer at 4 °C for 24 hrs. The samples, along with 90% acetone blanks ( $n=7$ ), were processed in a spectrophotometer and the absorbances at 663 nm and 630 nm were used to calculate chlorophyll *a* concentrations from the equation (Jeffrey and Humphrey 1975):

$$\text{Chlorophyll } a \text{ (}\mu\text{g cm}^{-2}\text{)} = 11.43 E_{663} - 0.64 E_{630}$$

Chlorophyll *a* concentrations were normalized to the surface area of the nubbin.

### *Field Experiment*

To test the hypothesis that pCO<sub>2</sub> and the presence of *Sargassum pacificum* would affect coral growth, a field experiment was deployed in July 2013.

*Acropora pulchra* nubbins, ~4 cm high, were collected from a fringing reef on the north shore of Moorea. Within two hours of collection, the corals were brought back to the lab, trimmed to similar lengths (mean: ~3.8 cm, range: 2.2-5.0 cm), and epoxied (Z-spar Splash Zone Compound™, A788) to rectangular pieces of plastic. The nubbins remained in an outdoor seawater table (2.4 x 1 x 0.1 m) with natural light and a high turnover (~10 L min<sup>-1</sup>) of fresh seawater pumped from 12-

m depth in Cook's Bay. The corals recovered for 5-7 days in water tables before being deployed.

### Field Setup

The field experiment consisted of a blocked design crossing stable and fluctuating pCO<sub>2</sub> (fore reef vs. reef crest) with the presence/absence of *Sargassum pacificum* and an algal mimic to account for shading effects. Half of the treatments were placed at the location of the SeaFETs deployed on the reef crest and fore reef by the Moorea Coral Reef Long Term Ecological Research Site (Site 1, Fig 1.). Across from Cook's Bay, the other half of the experiment was deployed at Site 2 (Fig. 1). Four platforms were installed at each site, two on the reef crest and two on the fore reef. The fore reef platforms ( $n=4$ ) consisted of 0.6 x 0.6 m, 18-mm thick, treated plywood board bases anchored to a chain weighing 136 kg, in 7.6 m of water, floating ~3.5 m below the surface (Fig 3). Each base had a plastic mesh grid (0.6 m x 0.6 m) nailed on top with floats tied to the underside to keep the bases parallel to the sea floor.

On each grid, there were three treatments: + *Sargassum pacificum*, + mimic and a control. Ten algal mimics, each consisting of five 23 cm sections of camouflage-pattern polypropylene rope, were tied in two rows to the far left side of each platform. The density of 10 mimics/0.125 m<sup>2</sup> imitated the natural *Sargassum* holdfast densities found at the reef crest (Chapter 2). Each mimic was zip-tied at 7 cm above the board to prevent any abrasion on the 4-5 cm coral nubbins. The top 16 cm of the ropes were frayed to mimic the *S. pacificum*

canopy. The algal treatments for the fore reef were created by collecting *S. pacificum* from the reef crest, cleaning thalli of epiphytes, wet weighing the algae ( $446.15 \pm 6.41$  g per treatment) and zip-tying it to the center of the mesh grid in the same alignment as the mimics. PVC-coated caging material ( $2.5\text{-cm}^2$  galvanized) was cut into 7 cm wide strips and placed between the algae and the coral nubbins to prevent macroalgal abrasion of the corals but ensure the algae shaded the coral. On the reef crest this setup was replicated, however no platforms were necessary and instead the mesh grids with the 3 treatments were nailed into the substratum within cleared areas. Since *S. pacificum* is found naturally on the reef crest, a third of the corals (with minimal caging to prevent abrasion) were nailed directly below the *S. pacificum* canopy (Fig. 3).

Light levels were measured below all treatments at Site 1 on the reef crest ( $n=113$ ) and at Site 2 ( $n=36$ ) on the fore reef with a  $2\pi$  light sensor (1-mm active diameter) attached to a Diving PAM (Heinz Walz, Germany), calibrated to a  $2\pi$  light sensor (Li-Cor, LI-192). Average ( $\pm$  SE) light levels under the mimics on the reef crest ( $76.51 \pm 12.40 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ ,  $n=18$ ) and the fore reef ( $58.42 \pm 11.11 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ ,  $n=12$ ) were within  $\sim 5\text{-}15 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$  of the irradiance under the natural *S. pacificum* canopy on the reef crest ( $63.37 \pm 6.55 \mu\text{mol quanta m}^{-2} \text{sec}^{-1} \pm \text{SE}$ ,  $n=24$ ). Light measurements were not replicated on the reef crest at Site 2 or fore reef at Site 1 as the exact same arrangement of mimics was used for each grid, meaning the light levels would be the same for each mimic treatment.

## Net Calcification and Linear Extension

To calculate net calcification rates, corals were buoyant weighed before and after the 15-21 day experiment (Davies 1989). The change in buoyant weight was converted to dry weight with the aragonite density of  $2.93 \text{ g cm}^{-3}$  and then normalized to surface area. Surface area for each nubbin was calculated with the wax dipping technique as described above (Stimson and Kinzie 1991, Naumann et al. 2009). The vertical height (mm) of each coral nubbin also was measured with calipers before and after the experiment to quantify linear extension rates.

## *Statistical Analyses*

To determine the effectiveness of the light treatments in the mesocosm experiment, irradiance was analyzed with a partly nested 2-way ANOVA with each mesocosm as a random effect nested within the treatments: pCO<sub>2</sub> level (ambient/low/high) and light (shaded/unshaded). The other physical parameters (temperature, salinity, flow and all carbonate values) of the mesocosms were analyzed with a partly nested 1-way ANOVA where mesocosm was a random effect nested within the pCO<sub>2</sub> treatments.

Net calcification rates and chlorophyll *a* concentrations from the mesocosm experiment were  $\log(x+1)$  transformed to meet assumptions of normality. Linear extension and net photosynthetic rates met the assumptions of normality and equality of variance with the Shapiro-Wilk and Levene's test statistic, respectively. Each variable was analyzed separately in a mixed effects models with pCO<sub>2</sub> (ambient, fluctuating and elevated) and light (shaded and

unshaded) as the fixed effects and mesocosm as a random effect nested within each treatment. If the P value for the mesocosm effect was  $\geq 0.25$  (Quinn and Keough 2002) tank was dropped from the analysis and replicates from the duplicate tanks were pooled. A 2-way ANOVA tested for the main effects.

For the field experiment, net calcification rates and linear extension rates met assumptions of normality and were analyzed with a mixed effects ANOVA model. As all control corals were lost to corallivory on the reef crest at Site 2 and several corals in the control and + algal mimic treatments were dislodged or damaged by the platforms colliding on the fore reef at Site 2, data collected from Site 2 was not included in the analysis. The fixed effects were treatment (+ *Sargassum pacificum*, + mimic and control) and CO<sub>2</sub> environment (reef crest-fluctuating CO<sub>2</sub> and fore reef-stable CO<sub>2</sub>). To account for non-independence among within-platform replicates, the platform was included as a nested factor within the CO<sub>2</sub> factor in the analysis. If the nested platform effect or any higher order interaction had a  $P \geq 0.25$  (Quinn and Keough 2002), the effect was dropped and the analysis was recalculated with individual corals as replicates. The program SYSTAT 12 was used for all statistical analyses.

## Results

On the north shore of Moorea, the largest fluctuation in pH over a day ( $0.197 \Delta \text{pH}_T$ ) was recorded directly behind the macroalgal-covered reef crest (Fig. 2). Over ten days, the average change in pH ( $\pm \text{SE}$ ) was  $0.145 \pm 0.008 \text{ pH}_T$  behind the reef crest and  $0.019 \pm 0.002 \text{ pH}_T$  on the fore reef. Compared to the reef

crest, pH of the seawater on the fore reef remained relatively stable (Fig. 2). Light levels also were recorded within the *Sargassum pacificum* canopies close to the SeaFET location (Table 1). The irradiance inside the middle of the canopy (~5-10 cm from substrate) was  $14.1 \pm 1.1 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ , a ~97% reduction from the light levels outside of the canopy (Table 1). As waves moved the *S. pacificum* thalli, pulses of light penetrated the canopy leading to a wide range of irradiance (0-71  $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ ) throughout the canopy (Table 1). A median irradiance of ~30  $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$  was chosen for the shaded treatments in the mesocosm experiment.

#### *Mesocosm Experiment*

The physical parameters of each tank were analyzed to ensure the treatments were maintained throughout the experiment. The tank factor did not significantly affect light levels ( $F_{7,400}=0.63$ ,  $P=0.735$ ) and was dropped from the analysis (Table 2). The acrylic canopies, shaded and unshaded in each tank, did significantly affect the light levels ( $F_{1,138}=442.2$ ,  $P>0.001$ ). The average light level of the shaded treatment (~22  $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ ) was ~92% reduced from the unshaded treatment (~284  $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ ), mimicking the shading caused by *Sargassum pacificum in situ* (Table 1). The average water flow ( $9.24 \pm 0.17 \text{ L hr}^{-1}$ ) into the tanks and average salinity levels ( $35.93 \pm 0.02 \text{ psu}$ ) remained stable across all of the tanks (Table 3 and 4). However, temperatures were statistically different between tanks ( $F_{7,377}=13.83$ ,  $P<0.001$ ) (Table 4). The range (max-min) in average temperatures was only 0.81 °C in the tanks (Table 3). The

differences were most likely biologically insignificant, as daily seawater temperatures on the north shore of Moorea naturally fluctuated by  $0.92 \pm 0.05$  °C (mean  $\pm$  SE,  $n=15$ ) in June 2013 (Leichter 2014) and have been shown to fluctuate by up to 4.7 °C (Putnam and Edmunds 2011).

Ten tanks were maintained at varying pCO<sub>2</sub> with four ambient tanks averaging  $\sim 407$   $\mu\text{atm}$ , two low tanks remained at  $\sim 270$   $\mu\text{atm}$  and four elevated tanks averaged  $\sim 835$   $\mu\text{atm}$  (Table 3). As expected pCO<sub>2</sub>, pH, [HCO<sub>3</sub><sup>-</sup>], [CO<sub>3</sub><sup>2-</sup>] and  $\Omega_{\text{arg}}$ , varied based on the CO<sub>2</sub> treatment and were not significantly impacted by the nested tank factor (Table 4). However, total alkalinity was statistically different among the tanks ( $F_{7,378}=18.84$ ,  $P<0.001$ ) (Table 4). The range in total alkalinity between the the tank with the lowest A<sub>T</sub> ( $\sim 2307$   $\mu\text{mol kg}^{-1}$ ) and the tank with the highest A<sub>T</sub> ( $2339$   $\mu\text{mol kg}^{-1}$ ) was  $\sim 31.4$  high  $\mu\text{mol kg}^{-1}$ . Previous water samples collected by the LTER showed seawater total alkalinity fluctuating by  $\sim 36$   $\mu\text{mol kg}^{-1}$  across the reef in a single day (Alldredge and Carlson 2013).

No *Acropora pulchra* nubbins bleached or died by the end of the 38-day experiment. Net calcification rates consistently decreased in the shaded treatments and increased in the unshaded treatments by an average  $0.89$   $\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$  throughout all three CO<sub>2</sub> levels (Fig. 5A). The corals in the ambient, unshaded treatment calcified (mean  $\pm$  SE) fastest ( $2.13 \pm 0.19$   $\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=24$ ) while the nubbins in the fluctuating, shaded treatment calcified slowest ( $0.98 \pm 0.12$   $\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=24$ ). Thus, light was the driving factor for calcification ( $F_{1,135}=60.50$ ,  $P<0.01$ ). However, there was some tank-to-tank variation in the magnitude of the effect of the light treatment, as indicated by a significant light x

tank (pCO<sub>2</sub>) interaction ( $F_{3, 129} = 4.41$ ,  $P = 0.01$ ), most likely due to a malfunctioning LED light which reduced light levels in one of the fluctuating treatment tanks (Table 5). When explored graphically for each tank, the net calcification rates qualitatively followed the same trend as shown in Fig. 5A but differed in magnitude. In regard to linear extension rates, the corals grew fastest in the fluctuating pCO<sub>2</sub>, unshaded treatment ( $0.10 \pm 0.02 \text{ mm d}^{-1}$ ,  $n = 24$ ) and slowest in the high pCO<sub>2</sub>, shaded treatment ( $0.03 \pm 0.01 \text{ mm d}^{-1}$ ,  $n = 24$ ). *Acropora pulchra* nubbins exhibited overall increased vertical growth in unshaded treatments and decreased vertical growth under elevated CO<sub>2</sub> levels (Fig. 5B); however, there were no significant effects of shading or pCO<sub>2</sub> on *A. pulchra* linear extension rates (Table 5).

A significant interaction between pCO<sub>2</sub> and the light treatments was recorded for net photosynthetic rates from a subset of corals ( $F_{2, 42} = 11.45$ ,  $P < 0.001$ ) (Table 6). The coral nubbins displayed elevated photosynthetic rates under fluctuating and high pCO<sub>2</sub> in the unshaded treatments and the opposite pattern in the shaded treatments (Fig. 6A). The net photosynthetic rates dropped by an average ~65% or ~204-174  $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$  from the ambient CO<sub>2</sub> treatment ( $66 \pm 39 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ ) to the fluctuating ( $-138 \pm 20 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ ) and elevated ( $-108 \pm 29 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ ) CO<sub>2</sub> treatments in the reduced light environment. The greatest mean ( $\pm$  SE) photosynthetic rates were recorded in the high light, elevated CO<sub>2</sub> treatment ( $1016 \pm 74 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ ,  $n = 8$ ) followed by the high light, fluctuating CO<sub>2</sub> treatment ( $773 \pm 98 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ ,  $n = 8$ ). The corals in the shaded, elevated CO<sub>2</sub> treatment displayed the highest

chlorophyll *a* concentrations ( $8.2 \pm 0.9 \mu\text{g cm}^{-2}$ ,  $n=22$ ) while the corals in the unshaded, ambient CO<sub>2</sub> treatments exhibited the lowest chlorophyll *a* concentrations ( $5.4 \pm 1.8 \mu\text{g cm}^{-2}$ ,  $n=22$ ). Overall, corals in the shaded treatments had on average ~29% higher chlorophyll *a* concentrations than the corals in the unshaded treatments (Fig. 6B). Thus, chlorophyll *a* concentrations were only affected by irradiance ( $F_{1,135}=12.30$ ,  $P<0.001$ ) (Table 6).

### *Field Experiment*

The *Sargassum pacificum* treatment was not maintained on the fore reef and the algae disappeared by the end of the experiment, compromising the effectiveness of the + algae treatment and increasing light levels (Table 7). With data from both sites pooled, the highest calcification rates were among the corals in the stable CO<sub>2</sub> (fore reef), + algae treatment ( $3.08 \pm 0.28 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=34$ ). The next highest calcification rates ( $3.03 \pm 0.20 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=24$ ) were recorded among the control corals in the fluctuating CO<sub>2</sub> treatment, representing a ~6% rise above control corals in the stable carbonate environment. Similarly, the *Acropora pulchra* nubbins in the fluctuating CO<sub>2</sub> conditions increased in net calcification by 24% in the mimic treatment (Fig. 8A). Discounting the + algae treatment, the pooled averages of the net calcification rates ( $1.51 \pm 0.09 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=59$ ) and linear extension rates ( $0.03 \pm 0.01 \text{ mm d}^{-1}$ ,  $n=59$ ) from the corals in the + mimic treatments were much lower than the growth rates of the control corals. Among the + mimic treatments, the corals in the stable CO<sub>2</sub> had ~89% reduced vertical growth compared to the corals

in the fluctuating CO<sub>2</sub> environment (Fig. 7A). Control corals in the stable CO<sub>2</sub> treatment exhibited elevated vertical growth by ~0.03 mm d<sup>-1</sup> compared to the control corals in the fluctuating CO<sub>2</sub> treatment (0.07 ± 0.02 mm d<sup>-1</sup>, n=24).

With the loss of control corals at Site 2, data from Site 1 was presented separately (Fig. 7B and 8B) and analyzed (Table 8). Control corals from both CO<sub>2</sub> conditions exhibited, on average, the highest linear extension rates (0.10 mm day<sup>-1</sup>, n=36). Among the treatments at the stable CO<sub>2</sub> environment (fore reef), the corals + mimics had the slowest linear extension rate (-0.03 ± 0.02 mm day<sup>-1</sup>, n=11) and the control corals had fastest rate of linear extension (0.12 ± 0.02 mm day<sup>-1</sup>, n=12). Across the treatments in fluctuating pCO<sub>2</sub>, the nubbins steadily increased their average vertical growth from the + algae treatment to the + mimics treatment to the control corals (Fig. 7B). The interaction between Treatment and Platform (CO<sub>2</sub>) was not significant (F<sub>4,87</sub>=1.70, P=0.158) but it was included in the ANOVA (Table 8) since the P-value was below 0.25 (Quinn and Keough 2002). The algal treatments significantly affected *Acropora pulchra* linear extension rates (F<sub>2,87</sub>=6.35, P=0.048).

Similar to the linear extension rates recorded in the fluctuating CO<sub>2</sub> treatment, the *Acropora pulchra* steadily increased their net calcification rates from the + algae treatment to the + mimic treatment to the control (Fig. 8B). The coral nubbins in the + mimic and coral only treatments at the fluctuating CO<sub>2</sub> locations, exhibited, on average, ~7% higher net calcification rates than the corresponding corals in the stable CO<sub>2</sub> treatments. As seen in the pooled data from both sites, the corals + algae in the stable CO<sub>2</sub> location displayed much

higher average ( $\pm$  SE) net calcification rates ( $2.41 \pm 0.43 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=10$ ) than the corals in the algal treatments at the fluctuating  $\text{CO}_2$  location ( $0.94 \pm 0.13 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ ,  $n=20$ ), most likely due to the inability to replenish the algae on the fore reef platforms. The two-way interaction between algal treatment and the platform effect, nested within  $\text{CO}_2$ , ( $F_{4,86}=0.11$ ,  $P=0.979$ ) was dropped from the mixed model analyzing net calcification rates (Table 8). The platform ( $\text{CO}_2$ ) effect was included in the analysis ( $F_{2,90}=3.10$ ,  $P=0.050$ ) as the P value was not  $>0.25$  (Quinn and Keough 2002). Even though the platform affected the impact of the  $\text{CO}_2$  environment on the net calcification rates, qualitatively, the coral nubbins displayed the same pattern of net calcification on both platforms at Site 1.

## Discussion

The present study examined the role of macroalgae as either an additional inhibitor or facilitator of *Acropora pulchra* growth and metabolism under ocean acidification. The research sought to address whether or not macroalgae could modify the effects of elevated  $\text{pCO}_2$  on corals. Although *Sargassum pacificum* locally can decrease  $\text{pCO}_2$  through photosynthesis and potentially create large diel fluctuations in pH (Fig. 2), the macroalgae did not provide corals a refuge from ocean acidification in either the mesocosm or field experiment. Instead, the present results demonstrated the potential resilience and/or acclimation of *Acropora pulchra* to OA conditions and the importance of macroalgal shading in affecting coral growth and metabolism. Light primarily influenced *Acropora*

*pulchra* calcification, supporting extensive research on light-enhanced calcification (Gattuso et al. 1999a) in other *Acropora* (Chalker and Taylor 1978, Enochs et al. 2014). The *Acropora* nubbins, if pooled among the CO<sub>2</sub> treatments and tanks, exhibited a 45% decrease in net calcification and 39% decrease in linear extension in the shaded treatments compared to the unshaded treatments. A similar study observed a 59% reduction in *A. pulchra* calcification in low light ( $95.8 \pm 2.1 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) compared to high light levels of  $411.4 \pm 8.6 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (both  $\pm$  SE) (Comeau et al. 2014a). This research along with studies by Comeau et al. (2014a) and Enochs et al. (2014) support light-enhanced calcification in *Acropora* spp. and other scleractinian corals (Schutter et al. 2008). Although extensively studied, the mechanisms behind light-enhanced calcification remain unclear (Allemand et al. 2011).

Unexpectedly, CO<sub>2</sub> level did not significantly affect net calcification rates. Similar to results reported by Comeau et al. (2014), *Acropora pulchra* nubbins did not respond to pCO<sub>2</sub> in a statistically significant manner. However, the pooled calcification rates from the fluctuating and elevated CO<sub>2</sub> treatments were, on average, 15% lower than coral calcification rates from the ambient pCO<sub>2</sub> tanks. Although most *Acropora* spp. are thought to be sensitive to OA (Comeau et al. 2013c), longer exposures to elevated pCO<sub>2</sub> in the present study and other studies may have allowed *Acropora pulchra* to acclimate (Form and Riebesell 2012, McCulloch et al. 2012) to new carbonate conditions, unlike shorter, perturbation experiments (Comeau et al. 2013c). Additionally, recent research suggests that biogenic calcifiers can mitigate the effects of decreased carbonate saturation states

by using bicarbonate, as well as carbonate, for calcification (Comeau et al. 2013b) or by buffering the pH internally at the site of calcification (McCulloch et al. 2012). Either of these processes may have allowed the corals to adjust to the fluctuating pCO<sub>2</sub> treatment, reducing the effectiveness of a macroalgal chemical refuge in the mesocosm experiment.

Contrary to the net calcification data, pCO<sub>2</sub> condition did significantly alter the vertical growth of the coral fragments. Corals in the fluctuating pCO<sub>2</sub> treatment had the highest linear extension rates without a corresponding increase in net calcification rates. These results suggest that skeletons of corals inhabiting areas with large pH fluctuations, e.g., an area of macroalgal dominance, may continue to extend linearly, but this skeleton may be less dense or smaller in diameter and thus, more fragile. Little research has explored the effects of ocean acidification on coral skeletal density (Enochs et al. 2014), which will become increasingly important to understand if corals are more fragile and susceptible to breakage in increasingly acidic seawater in the future. While *Acropora pulchra* may be more fragile under fluctuating CO<sub>2</sub> conditions and is shown in the present study to be more resilient to changes in pH than previously recorded, light played a central role in modulating the impact of OA on coral growth.

Shading appeared to exacerbate the effects of OA on coral vertical growth and did not alter the effects of OA on coral calcification. Although not statistically significant, reduced light amplified the depression in calcification at fluctuating and elevated pCO<sub>2</sub> by 14% compared to the corals in ambient pCO<sub>2</sub>. These results coincide with other findings that low light can increase the negative effects of OA

on *Acropora pulchra* calcification (Comeau et al. 2014a). Similarly, the vertical growth of the shaded corals was reduced by 54% in elevated pCO<sub>2</sub> compared to the corals in the fluctuating and ambient CO<sub>2</sub> conditions. *Acropora horrida* exhibited an equivalent pattern of reduced growth under low light ( $100 \pm 11 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) and elevated pCO<sub>2</sub> at  $\sim 735 \mu\text{atm}$  (Suggett et al. 2013). Lower light conditions likely increase the vulnerability of corals to OA. However, some corals species that are resistant to changes in pCO<sub>2</sub> (e.g., *Porites* spp.) did not modify their growth or metabolism based on irradiance (Marubini et al. 2001, Comeau et al. 2013a). Similar to the present study, an increase in light by  $\sim 195 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  actually lessened the impacts of elevated pCO<sub>2</sub> on the calcification of larval *Pocillopora* spp. (Dufault et al. 2013). Thus, the role of irradiance in either intensifying, having no effect, or even ameliorating effects of OA depends on the life stage of the coral (Dufault et al. 2013), coral species (Comeau et al. 2013a) and the level of irradiance (Suggett et al. 2013), as discussed by Comeau et al. (2014) and Enochs et al. (2014).

Consistent with Dufault et al. (2013), high irradiance may offset the effects of OA on hermatypic corals. Increases in irradiance stimulate the photosynthetic rates of *Symbiodinium*, endosymbiotic zooxanthellae that reside in the coral tissue and provide energy for coral growth (Gattuso et al. 1999a). Among the unshaded treatments, *Acropora pulchra* calcification declined by an average  $\sim 10\%$  in the fluctuating and elevated CO<sub>2</sub> conditions. However, the effect of OA may have been more severe if not for the significant increase in net photosynthetic rates by 25-43% in these treatments. Corals can acclimate to

different light conditions by altering their zooxanthellae density (Drew 1972, Titlyanov et al. 2001) and/or photosynthetic pigments (Falkowski and Dubinsky 1981, Porter et al. 1984, Titlyanov et al. 2001, Stambler 2011). In the shaded treatments, the nubbins acclimated by increasing chlorophyll *a* concentrations by ~28%, reinforcing results from Titlyanov et al. (2001). It is well known that light enhances zooxanthellae production and zooxanthellae photosynthesis is coupled with coral calcification (Gattuso et al. 1999a). However, research has only begun to investigate the impacts of changing carbonate chemistry on the coral calcification-zooxanthellae photosynthesis relationship. In the present case, excess bicarbonate, in addition to higher light, may have additionally enhanced the *Symbiodinium* photosynthetic rates (Crawley et al. 2010), increasing the internal pH at the site of calcification (McCulloch et al. 2012). Similar to corals (Comeau et al. 2013b), *Symbiodinium* also may use bicarbonate as a resource for photosynthesis. While the present laboratory study did not examine coral physiology, the high light and excess bicarbonate may have physiologically enhanced *Acropora pulchra* resilience to ocean acidification.

On the north shore of Moorea, the field experiment yielded limited results due to multiple natural and experimental problems that prevented an analysis of all of the data and created a significant effect of the study platform. The data from the reef crest at Site 2 was compromised when all of the control corals and most of those in the algal mimic treatments were lost to corallivory. Corallivory in this location was unexpected because large patches of *Sargassum* or other macroalgae usually deter reef fish (Hoey and Bellwood 2011, Venera-Ponton et al. 2011) and

no corallivory was witnessed on a test assay at Site 1 before the experiment. Nevertheless, the present study suggests that dense stands of macroalgae may not deter corallivores. The distance at which a macroalgal refuge occurs is unclear since the eaten corals were adjacent to (not inside of) dense areas of macroalgae covering the reef crest. Differences in the abundance, species, and behavior of corallivores between sites may have also accounted for the loss of corals in only one location. Lastly, the *Sargassum pacificum* treatment on the fore reef became a pulse treatment since most of the fronds disappeared by the end of the experiment due to either herbivory (Quinn and Keough 2002, Hoey and Bellwood 2009, Vergés et al. 2012) or wave-induced fragmentation. With the *Sargassum* gone, the corals were not shaded (Table 7) and thus had significantly higher net calcification and vertical growth rates than the nubbins in the algal mimic treatment.

Although no statistically significant results could be gleaned from the field experiment, the *Acropora pulchra* nubbins in the field experiment did exhibit slightly higher net calcification rates (6-24%) in the fluctuating pCO<sub>2</sub> environment of the reef crest than at the stable pCO<sub>2</sub> environment of the fore reef. Whether this slight rise was due to the increase in available bicarbonate for calcification (Comeau et al. 2013b) in the fluctuating pCO<sub>2</sub> or due to higher flow (Comeau et al. 2014c) at the reef crest, is unclear and needs to be explored further. However, these results provide some evidence for the ability of larger patches of dense *S. pacificum* to chemically facilitate sub-canopy or downstream coral calcification. Interestingly, the linear extension rates followed the opposite

trend (lower rates in fluctuating CO<sub>2</sub> conditions), potentially due to the waves knocking the platforms together on the fore reef and causing skeletal breakage on some of the nubbins. Other factors, such as irradiance (Gattuso et al. 1999a) or water flow (Jokiel 1978, Nakamura and Yamasaki 2006, Todd 2008) may have affected coral vertical growth at the two CO<sub>2</sub> locations differently, leading to the observed increase in vertical growth among the corals on the fore reef. The flow environment in particular may have significantly altered the growth of the corals on the reef crest as they were less than 0.5 m below the surface of the water, whereas the corals on the fore reef were ~3 m below the surface. Previous research shows turbulent water (i.e., the water of the reef crest) can induce shorter, more stunted growth morphologies of scleractinian corals (Kaandorp 1999, Nakamura and Yamasaki 2006, Veron 2011). For field experiments on OA, thoroughly characterizing the abiotic and biotic environment of various coral reef habitats is crucial to creating and maintaining successful treatments.

Parallel to the laboratory experiment, the effect of shading was prevalent in the field experiment. As expected, the *Acropora pulchra* nubbins shaded by the algal mimic had reduced vertical growth and calcification compared to unshaded corals (Fig. 7A and 8A). Light has frequently been shown to impair coral growth (Marubini et al. 2001) by depressing coral photosynthesis (Anthony and Hoegh-Guldberg 2003). However, among the pooled and separate data (Site 1), the algal mimic and live algae elicited different results. In the fluctuating CO<sub>2</sub> environment at Sites 1 and 2, the corals surrounded by *Sargassum pacificum* consistently displayed, on average, a ~41% reduction in net calcification compared to the

corals surrounded by algal mimics (Fig. 7A and 7B). The vertical growth of the same corals, but only at Site 1, followed the same pattern, with ~82% slower linear extension rates than the corals in the algal mimics. The coral fragments in the algal mimic treatment on the fore reef had the slowest linear extension rates, potentially due to wave-induced platform contact. Overall, lower calcification and extension rates may be evidence for other harmful chemically mediated changes in the sub-canopy of *Sargassum pacificum*.

Seawater pH will continue to decrease with increased carbon deposition into the world's ocean (Feely et al. 2009), and macroalgal abundance is expected to increase with reductions in consumer pressure (due to fishing) and increases in nutrient availability (fueled by coastal development) (Diaz-Pulido et al. 2007, Hoegh-Guldberg et al. 2007, Hughes et al. 2007, Diaz-Pulido et al. 2009, Cheal et al. 2010, Anthony et al. 2011b). Thus, to accurately predict the future of coral reefs, more experiments are needed to understand the mechanisms behind coral calcification and physiological responses to algae and OA. In particular, the disparity between the indirect and direct ways macroalgae can modulate the effects of elevated pCO<sub>2</sub> on biogenic calcifiers must be clarified. The present research in the context of the literature stresses the importance of *in situ* OA experiments, because organisms in multi-species assemblages may exhibit very different responses from those in solitary OA incubations. Additionally, the absence of ocean acidification research integrating naturally occurring fluctuations in carbonate chemistry into experiments is concerning. The ocean, especially in shallow coastal ecosystems where the majority of OA research

focuses, experiences natural variability in seawater pH (Hofmann et al. 2011) partially due to the metabolism of benthic primary producers (Anthony et al. 2013). The present study emphasizes the need for longer term research that 1) addresses the effect of OA on diel pH fluctuations and the marine organisms that survive and create these fluctuations (Comeau et al. 2014a), and 2) clarifies whether or not organisms can acclimate to fluctuating, versus stable, elevated pCO<sub>2</sub>.

## Tables

**Table 1.** The average ( $\pm$  SE) and range of irradiance measured inside (bottom and middle) and above the canopy of *Sargassum pacificum* found on the reef crest.

Canopy Location	Average Light ( $\mu\text{mol quanta m}^{-2} \text{sec}^{-1} \pm \text{SE}$ )	Light Range (min-max $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ )
Bottom	$4.4 \pm 0.5$ ( $n=149$ )	0 - 30
Middle	$14.1 \pm 1.1$ ( $n=144$ )	0 - 71
Above	$512.8 \pm 30.8$ ( $n=72$ )	121 - 1050

**Table 2.** A) Average light measurements ( $\mu\text{mol quanta m}^{-2} \text{sec}^{-1} \pm \text{SE}$ ) under each shaded and unshaded canopy in the mesocosms. Light levels were analyzed with a partly nested 2-way ANOVA with tank as the random, nested factor. The tank factor was not significant ( $P>0.25$ ) and dropped. B) ANOVA table with the results of the reduced 2-way ANOVA testing the main fixed effects of pCO<sub>2</sub> (low, ambient and high) and light (shaded and unshaded) on irradiance.

A

Treatment	CO <sub>2</sub> Level	Tank	Shaded	Unshaded
Stable	Ambient	1	26 ± 3	263 ± 20
		2	25 ± 4	330 ± 27
	High	7	19 ± 3	253 ± 33
		12	15 ± 1	294 ± 24
Fluctuating	Ambient	9	15 ± 1	297 ± 56
		10	19 ± 1	283 ± 64
	Low	5	24 ± 2	317 ± 23
		6	23 ± 2	323 ± 27
	High	8	29 ± 2	284 ± 48
		11	15 ± 1	192 ± 25

B

Source	SS	df	MS	F	P
pCO <sub>2</sub>	56,789	2	28,394	1.9	0.156
Light	6,715,908	1	6,715,908	442.2	<b>&lt;0.001</b>
Light x pCO <sub>2</sub>	61,585	2	30,792	2.0	0.133
Error	6,288,275	138	15,189		

**Table 3.** Physical parameters (mean  $\pm$  SE) in tanks with for stable (A) and fluctuating (B) CO<sub>2</sub> treatments in the mesocosm experiment. pCO<sub>2</sub>: partial pressure of carbon dioxide, A<sub>T</sub>: total alkalinity, pH: pH on total scale, HCO<sub>3</sub><sup>-</sup>: bicarbonate concentration, CO<sub>3</sub><sup>2-</sup>: carbonate concentration,  $\Omega_{\text{arg}}$ : aragonite saturation state.

A									
CO <sub>2</sub> Level	Tank	Temperature (°C)	Salinity (psu)	pCO <sub>2</sub> (μatm)	A <sub>T</sub> (μmol kg <sup>-1</sup> )	pH (pHt)	HCO <sub>3</sub> <sup>-</sup> (μmol kg <sup>-1</sup> )	CO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> )	$\Omega_{\text{arg}}$
Ambient	1	27.09 $\pm$ 0.08	36 $\pm$ 0.1	424 $\pm$ 19	2307 $\pm$ 4	8.03 $\pm$ 0.01	1756.80 $\pm$ 12	223.37 $\pm$ 4	3.55 $\pm$ 0.07
	2	27.40 $\pm$ 0.05	36 $\pm$ 0.1	411 $\pm$ 9	2311 $\pm$ 3	8.03 $\pm$ 0.01	1751.68 $\pm$ 7	226.99 $\pm$ 2	3.61 $\pm$ 0.04
High	7	27.80 $\pm$ 0.08	36 $\pm$ 0.1	849 $\pm$ 15	2320 $\pm$ 3	7.77 $\pm$ 0.01	1973.58 $\pm$ 5	141.29 $\pm$ 2	2.25 $\pm$ 0.03
	12	27.19 $\pm$ 0.05	36 $\pm$ 0.0	812 $\pm$ 24	2318 $\pm$ 2	7.79 $\pm$ 0.01	1963.02 $\pm$ 7	144.81 $\pm$ 3	2.30 $\pm$ 0.05
B									
CO <sub>2</sub> Level	Tank	Temperature (°C)	Salinity (psu)	pCO <sub>2</sub> (μatm)	A <sub>T</sub> (μmol kg <sup>-1</sup> )	pH (pHt)	HCO <sub>3</sub> <sup>-</sup> (μmol kg <sup>-1</sup> )	CO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> )	$\Omega_{\text{arg}}$
Ambient	9	27.12 $\pm$ 0.05	35.9 $\pm$ 0.0	390 $\pm$ 5	2337 $\pm$ 1	8.05 $\pm$ 0.00	1758.37 $\pm$ 5	235.50 $\pm$ 2	3.75 $\pm$ 0.03
	10	27.00 $\pm$ 0.04	35.9 $\pm$ 0.0	401 $\pm$ 7	2320 $\pm$ 2	8.04 $\pm$ 0.01	1758.64 $\pm$ 8	228.27 $\pm$ 2	3.63 $\pm$ 0.04
Low	5	27.37 $\pm$ 0.20	35.9 $\pm$ 0.1	278 $\pm$ 9	2339 $\pm$ 1	8.17 $\pm$ 0.01	1627.94 $\pm$ 10	288.62 $\pm$ 4	4.59 $\pm$ 0.06
	6	27.00 $\pm$ 0.07	35.9 $\pm$ 0.1	262 $\pm$ 9	2335 $\pm$ 1	8.19 $\pm$ 0.01	1604.69 $\pm$ 13	296.27 $\pm$ 5	4.71 $\pm$ 0.08
High	8	27.60 $\pm$ 0.03	35.9 $\pm$ 0.0	857 $\pm$ 20	2323 $\pm$ 2	7.77 $\pm$ 0.01	1977.96 $\pm$ 7	140.69 $\pm$ 3	2.24 $\pm$ 0.04
	11	27.41 $\pm$ 0.05	35.9 $\pm$ 0.1	821 $\pm$ 19	2335 $\pm$ 1	7.79 $\pm$ 0.01	1979.18 $\pm$ 7	145.35 $\pm$ 3	2.31 $\pm$ 0.04

**Table 4.** The results of nested ANOVAs testing the effect of tank and pCO<sub>2</sub> on the abiotic conditions of each mesocosm. \* Denotes each physical parameter that had a significant tank effect.

Parameter	Source	SS	df	MS	F	P
Temperature*	pCO <sub>2</sub>	11.12	2	5.559	2.73	0.133
	Tank(pCO <sub>2</sub> )	14.41	7	2.058	13.83	<b>&lt;0.001</b>
	Error	56.13	377	0.149		
Salinity	pCO <sub>2</sub>	0.30	2	0.152	1.22	0.352
	Tank(pCO <sub>2</sub> )	0.87	7	0.124	1.34	0.229
	Error	34.98	378	0.093		
Flow	pCO <sub>2</sub>	3.74	2	1.867	0.98	0.418
	Tank(pCO <sub>2</sub> )	12.84	7	1.835	0.25	0.972
	Error	1,804.51	246	7.335		
pH	pCO <sub>2</sub>	10.12	2	5.060	912.14	<b>&lt;0.001</b>
	Tank(pCO <sub>2</sub> )	0.04	7	0.006	1.63	0.124
	Error	1.28	378	0.003		
A <sub>T</sub> *	pCO <sub>2</sub>	16,534	2	8,267	2.12	0.192
	Tank(pCO <sub>2</sub> )	27,317	7	3,902	18.84	<b>&lt;0.001</b>
	Error	78,320	378	207		
pCO <sub>2</sub>	pCO <sub>2</sub>	22,144,154	2	11,072,077	890.67	<b>&lt;0.001</b>
	Tank(pCO <sub>2</sub> )	86,892	7	12,413	1.38	0.211
	Error	3,389,789	378	8,967		
HCO <sub>3</sub> <sup>-</sup>	pCO <sub>2</sub>	7,622,553	2	3,811,276	1,458.29	<b>&lt;0.001</b>
	Tank(pCO <sub>2</sub> )	18,301	7	2,614	0.93	0.483
	Error	1,063,107	378	2,812		
CO <sub>3</sub> <sup>-2</sup>	pCO <sub>2</sub>	1,296,496	2	648,248	951.48	<b>&lt;0.001</b>
	Tank(pCO <sub>2</sub> )	4,759	7	679	1.69	0.111
	Error	152,485	378	403		
Ω <sub>arg</sub>	pCO <sub>2</sub>	326.86	2	163.43	963.74	<b>&lt;0.001</b>
	Tank(pCO <sub>2</sub> )	1.19	7	0.17	1.68	0.113
	Error	38.10	378	0.10		

**Table 5.** The results of a mixed effects model analyzing linear extension rates and log+1 transformed area-normalized net calcification rates from the mesocosm experiment. The model includes the tank as the random, nested factor, the interactions and the main fixed effects of pCO<sub>2</sub> (ambient, fluctuating and high) and light (shaded and unshaded).

Dependent Variable	Effect	SS	df	MS	F	P
Linear extension rate (mm d <sup>-1</sup> )	pCO <sub>2</sub>	0.028	2	0.014	1.15	0.43
	Light	0.033	1	0.033	3.05	0.18
	Light x pCO <sub>2</sub>	0.005	2	0.003	0.24	0.80
	Tank(pCO <sub>2</sub> )	0.036	3	0.012	1.13	0.46
	Light x Tank(pCO <sub>2</sub> )	0.032	3	0.011	2.51	0.06
	Error	0.564	132	0.004		
Area-normalized net calcification rate (mg CaCO <sub>3</sub> cm <sup>-2</sup> d <sup>-1</sup> )	pCO <sub>2</sub>	0.354	2	0.177	1.09	0.44
	Light	4.850	1	4.850	15.12	<b>0.03</b>
	Light x pCO <sub>2</sub>	0.069	2	0.035	0.11	0.90
	Tank(pCO <sub>2</sub> )	0.486	3	0.162	0.51	0.71
	Light x Tank(pCO <sub>2</sub> )	0.962	3	0.321	4.41	<b>≤0.01</b>
	Error	9.381	129	0.073		

**Table 6.** Results of mixed effects models analyzing coral net photosynthetic rates and log+1 transformed chlorophyll *a* concentrations from the mesocosm experiment. The full models include the tank as the random, nested factor and the reduced models analyzed the main fixed effects of pCO<sub>2</sub> (ambient, fluctuating and high) and light (shaded and unshaded).

Dependent Variable	Model	Effect	SS	df	MS	F	P
Area-normalized net photosynthetic rate ( $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ hr}^{-1}$ )	Full	pCO <sub>2</sub>	262,244	2	131,122	10.38	0.123
		Light	8,365,630	1	8,365,630	1,280.64	0.033
		Light x pCO <sub>2</sub>	547,883	2	273,941	47.13	0.212
		Tank(pCO <sub>2</sub> )	44,419	3	14,806	1.60	0.407
		Light x Tank(pCO <sub>2</sub> )	18,517	2	9,258	0.25	0.779
		Error	1,362,560	37	36,825		
	Reduced	pCO <sub>2</sub>	193,453	2	96,726	2.85	0.069
		Light	8,630,466	1	8,630,466	254.28	<b>&lt;0.001</b>
		Light x pCO <sub>2</sub>	777,370	2	388,685	11.45	<b>&lt;0.001</b>
		Error	1,425,498	42	33,940		
Chlorophyll <i>a</i> ( $\mu\text{g cm}^{-2}$ )	Full	pCO <sub>2</sub>	0.654	2	0.327	0.37	0.720
		Light	6.864	1	6.864	7.17	0.075
		Light x pCO <sub>2</sub>	0.071	2	0.035	0.04	0.964
		Tank(pCO <sub>2</sub> )	2.676	3	0.892	0.93	0.523
		Light x Tank(pCO <sub>2</sub> )	2.873	3	0.958	1.70	0.171
		Error	72.863	129	0.565		
	Reduced	pCO <sub>2</sub>	0.583	2	0.292	0.502	0.607
		Light	7.154	1	7.154	12.304	<b>0.001</b>
		Light x pCO <sub>2</sub>	0.061	2	0.030	0.052	0.949
		Error	78.491	135	0.581		

**Table 7.** The average ( $\pm$  SE) irradiance and relative flow rates (clod card dissolution rate) in the three algal treatments and at each CO<sub>2</sub> level (location) for the field experiment. ND means no data, as the data could not be collected.

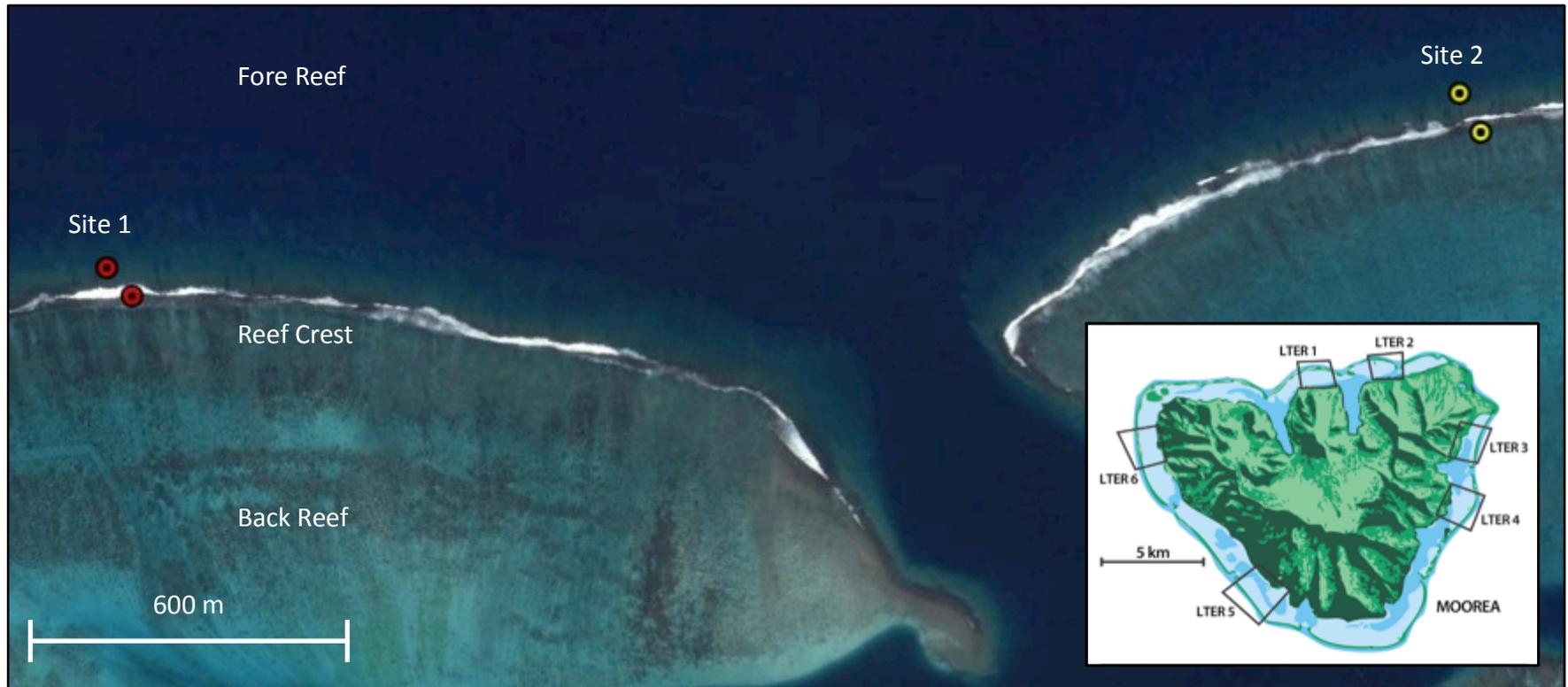
CO <sub>2</sub> Level (Location)	Algal Treatment	Light ( $\mu\text{mol quanta m}^2 \text{ sec}^{-1}$ )	Dissolution rate (g hr <sup>-1</sup> )
Fluctuating (RC)	+ Algae	63.37 $\pm$ 6.55	0.12 $\pm$ 0.03
	+ Mimic	76.51 $\pm$ 12.40	0.12 $\pm$ 0.03
	Control	939.35 $\pm$ 38.92	0.19 $\pm$ 0.01
Stable (FR)*	+ Algae	549 $\pm$ 55.78	ND
	+ Mimic	58.42 $\pm$ 11.11	ND
	Control	685.58 $\pm$ 38.25	ND

\* Data was collected after the deployment

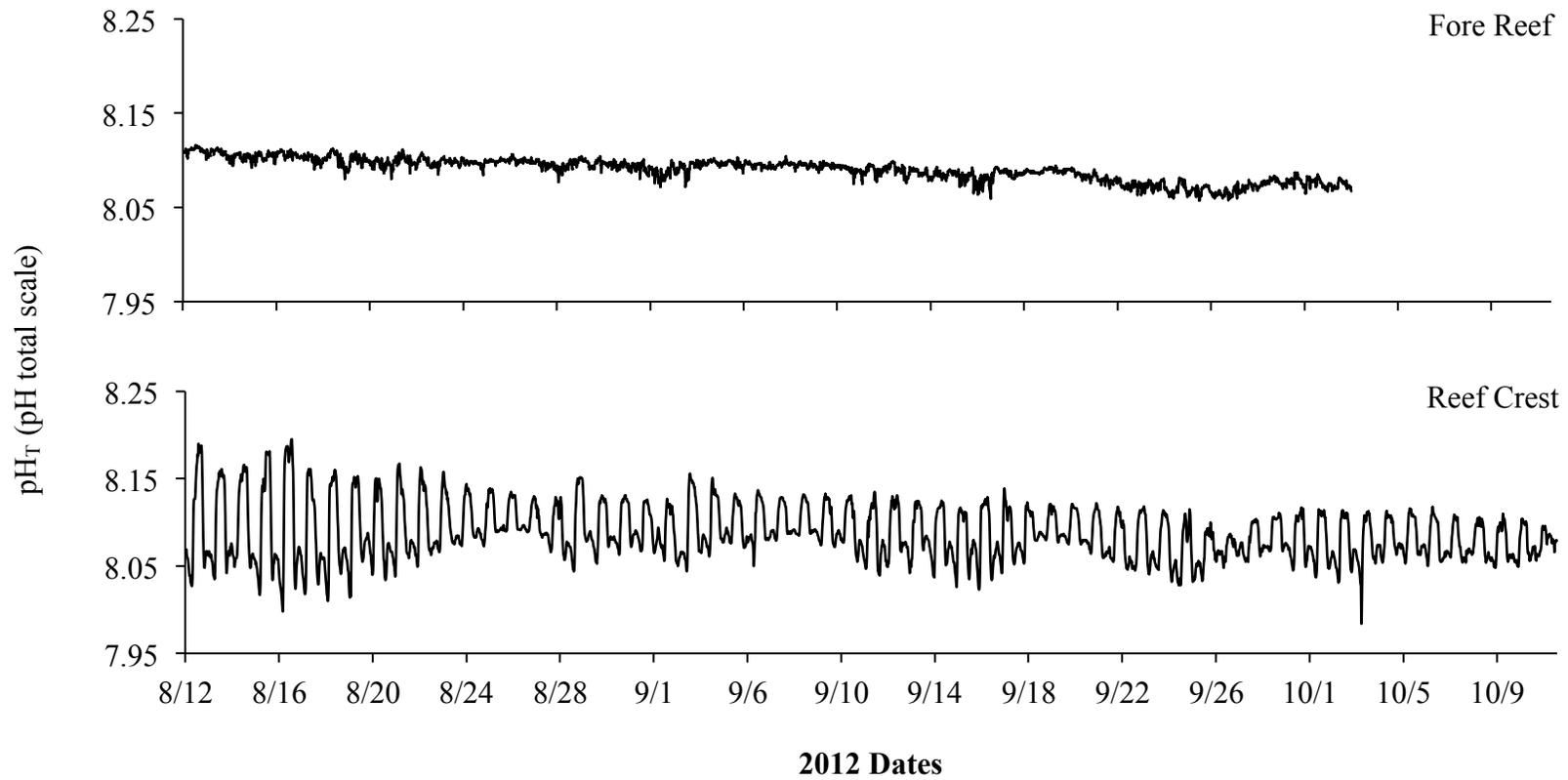
**Table 8.** Results of ANOVA on average net calcification rates and average linear extension rates for *Acropora pulchra* nubbins from Site 1. A mixed effects model tested for the effect of CO<sub>2</sub> level (fluctuating and stable), three treatments (+ algae, + mimic and control) and a random platform effect on each response variable. The Treatment x Platform (CO<sub>2</sub>) interaction for area-normalized net calcification rate was dropped (F<sub>4,86</sub>=0.12, P=0.979) since P>0.25.

Dependent Variable	Effect	SS	df	MS	F	P
Area-normalized net calcification rate (mg CaCO <sub>3</sub> cm <sup>-2</sup> d <sup>-1</sup> )	CO <sub>2</sub>	0.346	1	0.346	1.64	0.320
	Treatment	3.543	2	1.772	24.13	<b>&lt;0.001</b>
	Treatment x CO <sub>2</sub>	1.778	2	0.889	12.12	<b>&lt;0.001</b>
	Platform (CO <sub>2</sub> )	0.456	2	0.228	3.10	<b>0.050</b>
	Error	6.609	90	0.073		
Linear extension rate (mm hr <sup>-1</sup> )	CO <sub>2</sub>	0.003	1	0.003	0.37	0.598
	Treatment	0.109	2	0.054	6.35	<b>0.048</b>
	Treatment x CO <sub>2</sub>	0.081	2	0.040	4.70	0.078
	Platform (CO <sub>2</sub> )	0.016	2	0.008	0.90	0.474
	Treatment x Platform (CO <sub>2</sub> )	0.036	4	0.009	1.70	0.158
	Error	0.460	87			

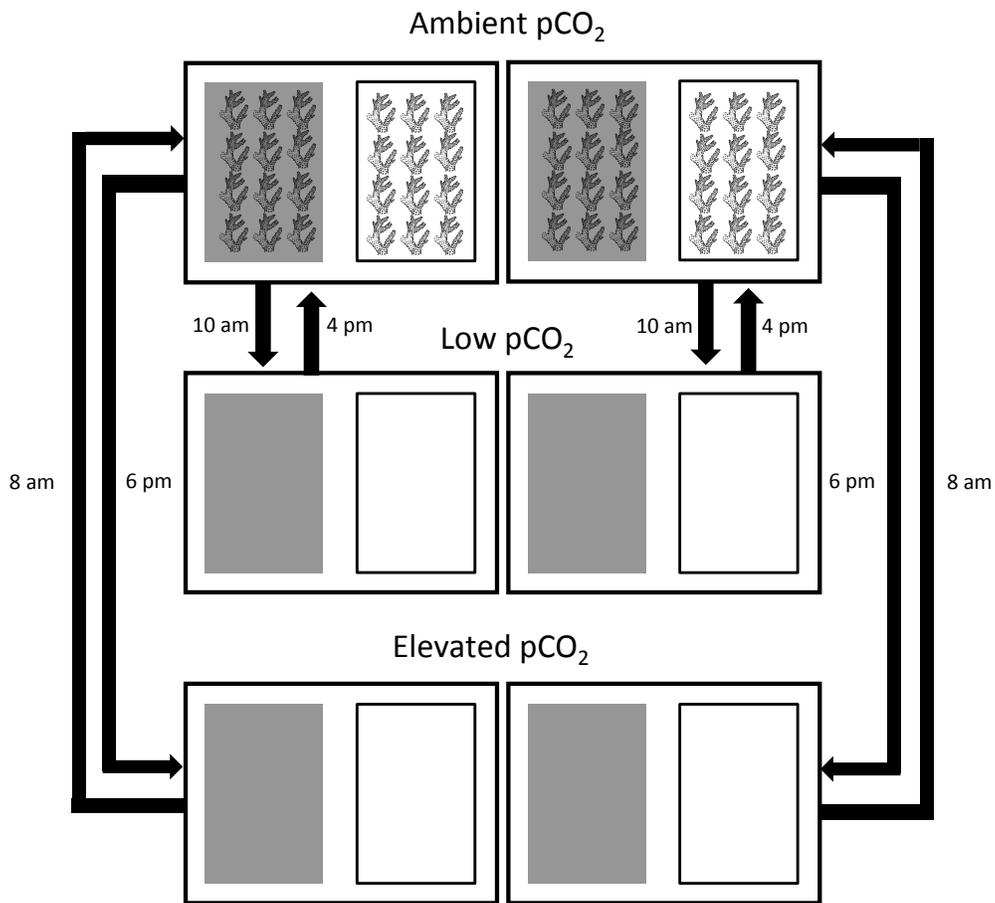
## Figures



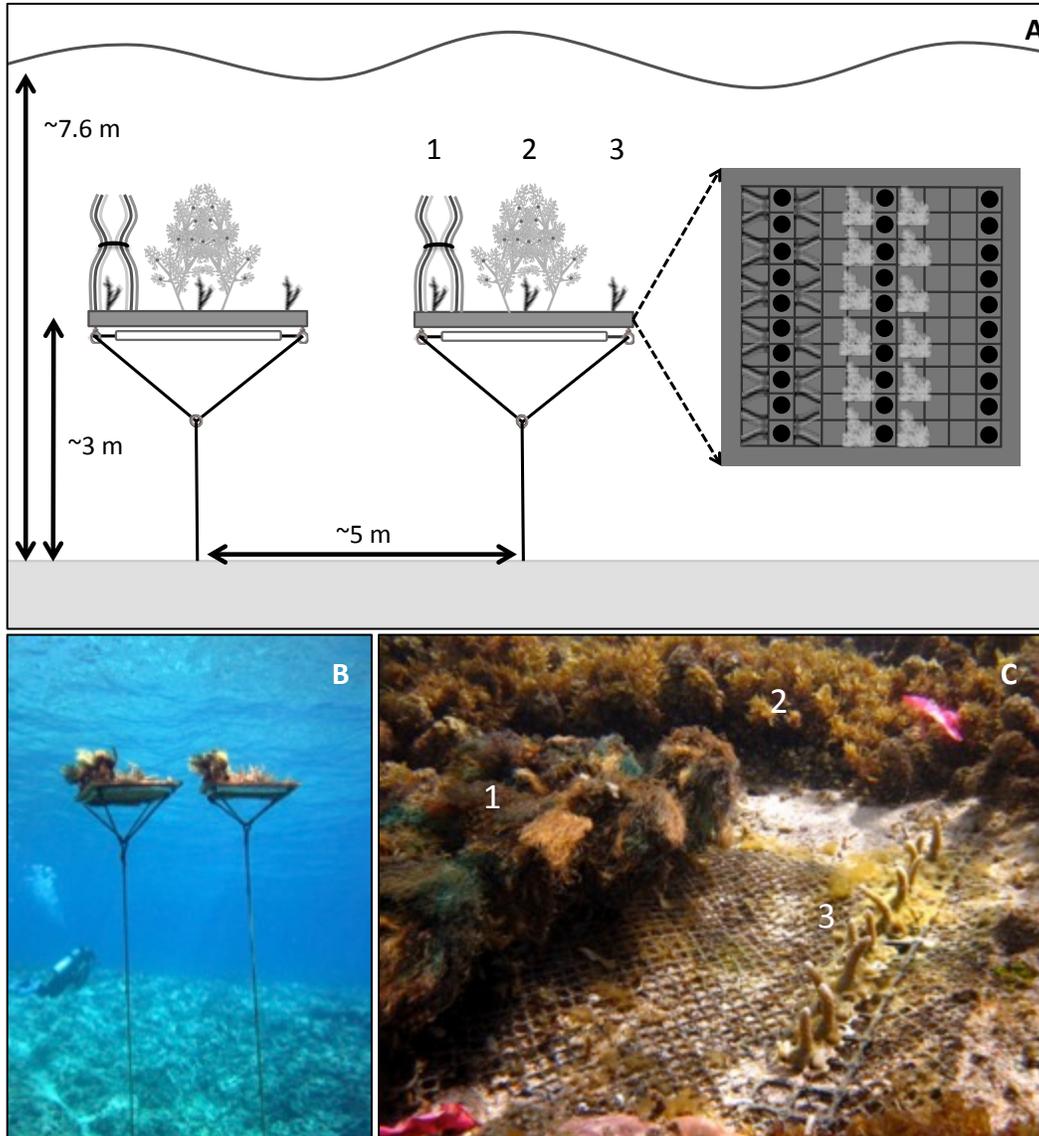
**Figure 1.** The location of the field experiment was between LTER 1 and LTER 2 (inlaid picture from Leichter et al. 2013) on the north shore of Moorea, French Polynesia. The experiment was deployed at two sites (Site 1 in red and Site 2 in yellow). Four platforms with all three treatments were placed at each site, two on the fore reef and two on the reef crest.



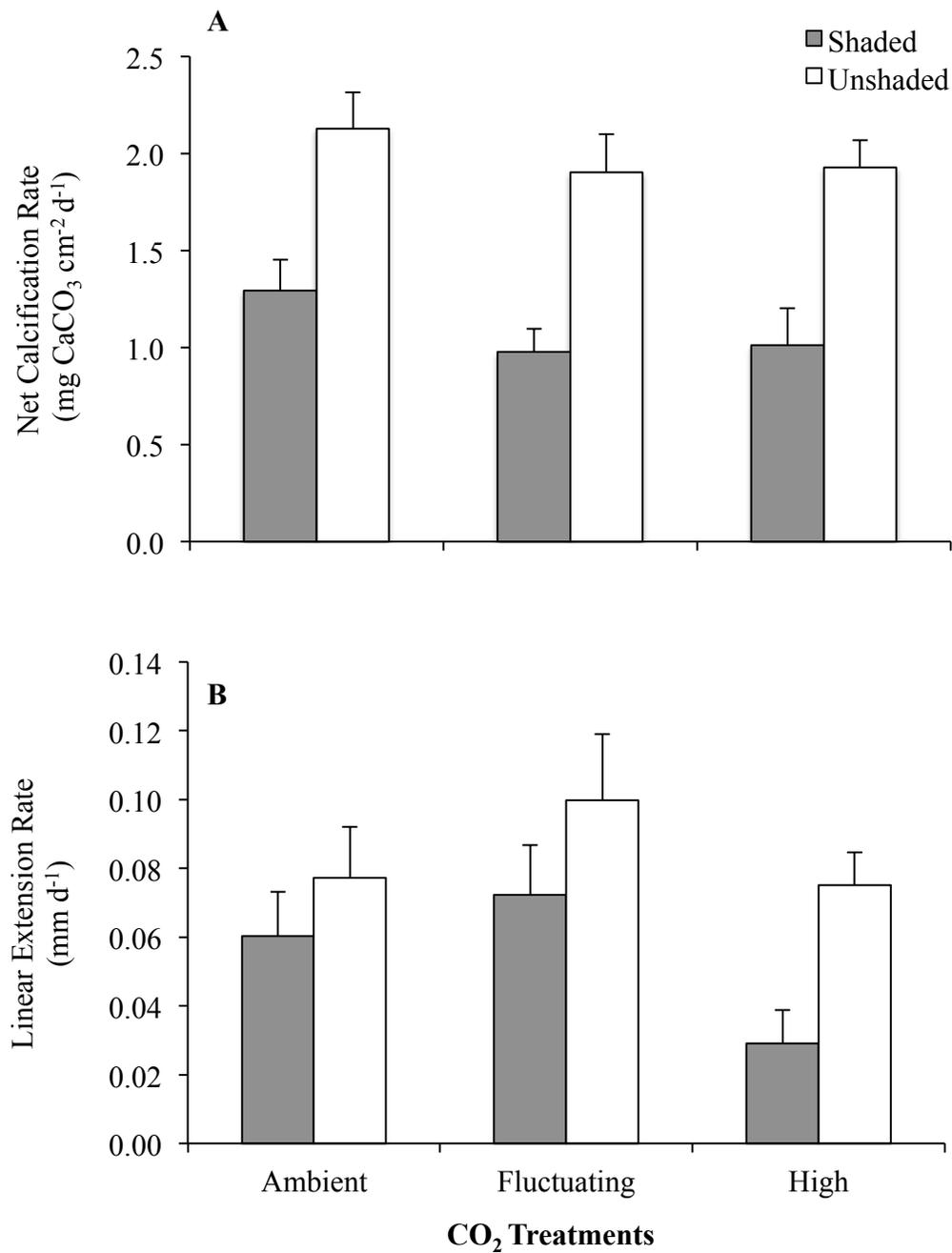
**Figure 2.** pH data collected from two SeaFETS on the fore reef and reef crest at LTER 1 (Fig. 1) from August to October of 2012. The seawater on the reef crest exhibited large diel fluctuations in pH compared to the seawater on the fore reef.



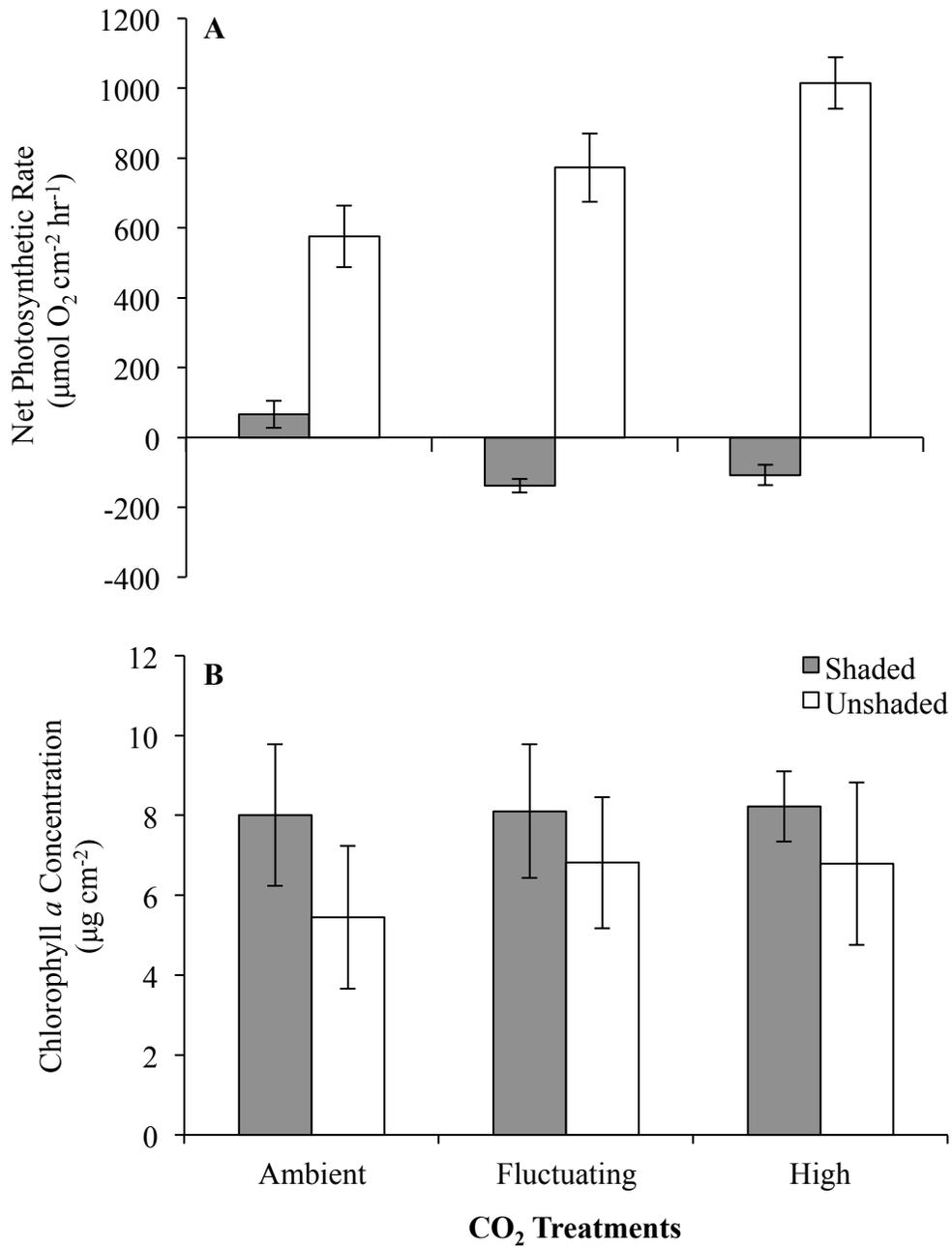
**Figure 3.** In the mesocosm experiment each tank contained a shaded (grey) and unshaded (white) acrylic canopy. One of the CO<sub>2</sub> conditions was a fluctuating treatment consisting of an entirely separate set of tanks with two tanks at each CO<sub>2</sub> level (low, ambient, elevated). To mimic the diel fluctuations in pH recorded by the SeaFET at the reef crest (Fig. 2), the corals were placed in the ambient tanks from 8-10 am, transferred to the low tanks from 10-4 pm, transitioned back into the ambient tanks from 4-6 pm and lastly, moved to the elevated tanks from 6 pm-8 am.



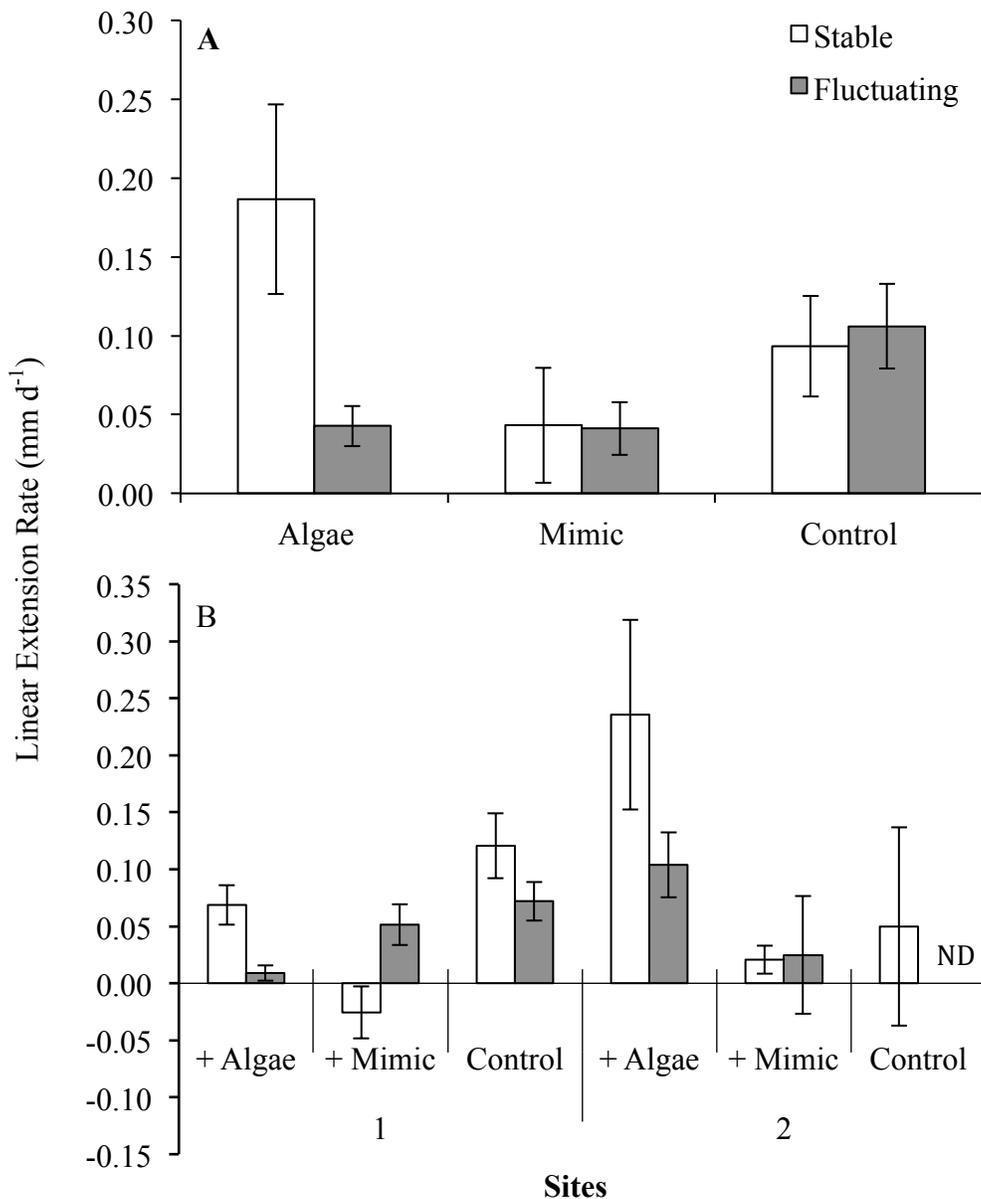
**Figure 4.** At each site, the field experiment consisted of two platforms anchored to the fore reef (A, B) and two grids nailed to the reef crest (C). On each platform and grid, corals were placed in three treatments: 1) + algal mimic, 2) + *Sargassum pacificum*, and 3) isolated coral (control).



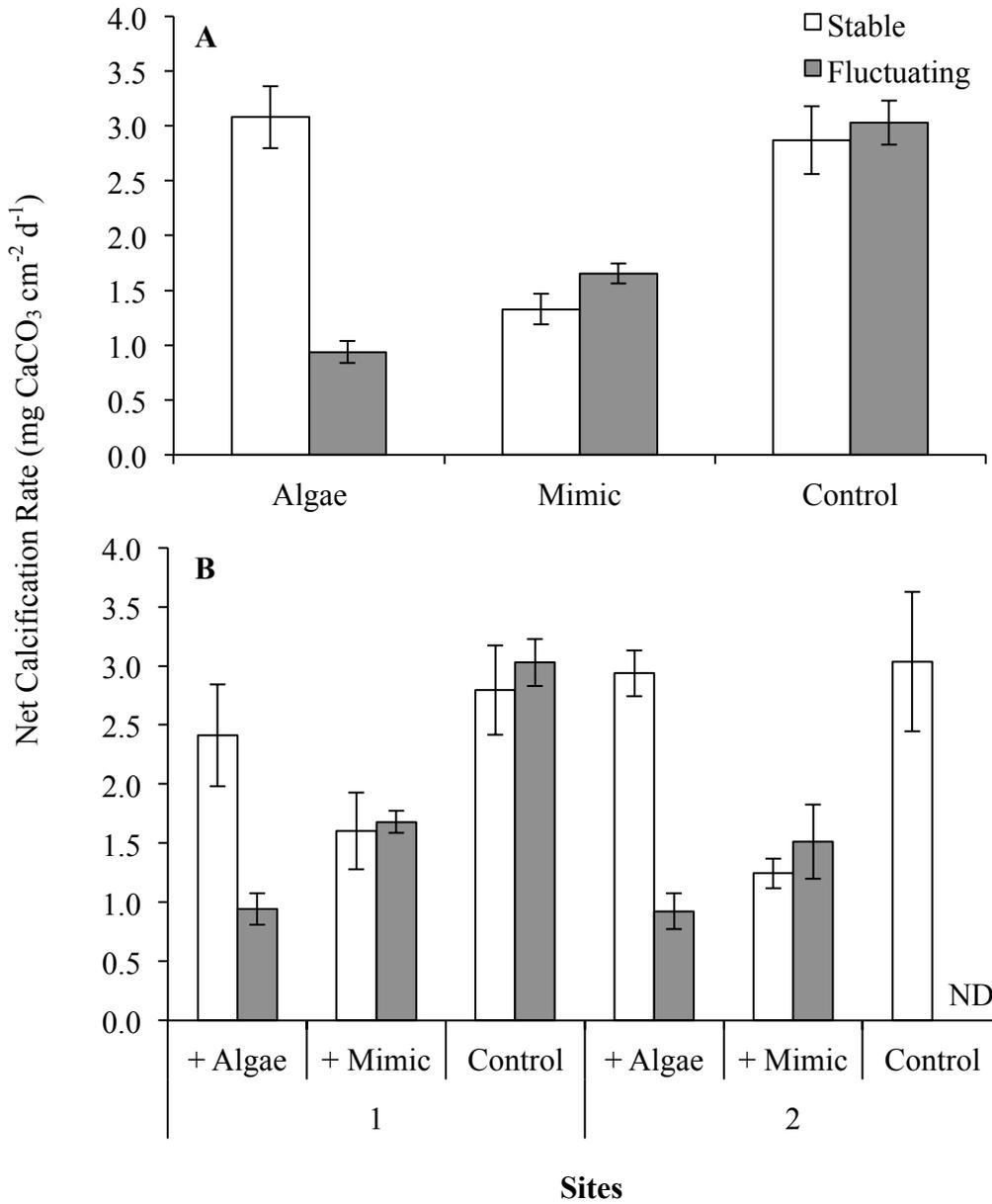
**Figure 5.** (A) The average area-normalized net calcification rates (mean  $\pm$  SE,  $n=24$ ) and (B) the average rates of linear extension (mean  $\pm$  SE,  $n=24$ ) of the *Acropora pulchra* nubbins in the shaded (grey bars) and unshaded (white bars) treatments at ambient, fluctuating and elevated CO<sub>2</sub> conditions.



**Figure 6.** The average (A) net photosynthetic rates (mean  $\pm$  SE,  $n=8$ ) and (B) chlorophyll *a* concentrations (mean  $\pm$  SE,  $n=22-24$ ) of a subset of *Acropora pulchra* nubbins from the mesocosm experiment crossing three CO<sub>2</sub> treatments and two light levels in each tank.



**Figure 7.** The average rates of *Acropora pulchra* linear extension (mean ± SE) from the field experiment are pooled by site (A) and separated by site (B). No data (ND) was collected from the control, fluctuating treatment at Site 2. The experiment consisted of three treatments: + *Sargassum pacificum* (Algae), + algal mimic and the control, deployed in fluctuating CO<sub>2</sub> treatments at the reef crest (grey bars) and stable CO<sub>2</sub> conditions on the fore reef (white bars).



**Figure 8.** The average area-normalized net calcification rates (mean  $\pm$  SE) of *Acropora pulchra* nubbins pooled by site (A) and separated by site (B). No data (ND) was collected from the control, fluctuating treatment at Site 2. The field experiment consisted of three treatments: + algae, + algal mimic and the control, deployed in fluctuating CO<sub>2</sub> treatments at the reef crest (grey bars) and stable CO<sub>2</sub> conditions on the fore reef (white bars).

## Chapter 4

### Summary

Abiotic and biotic factors control and drive the dynamics and set the composition of all ecological communities. Tropical coral reefs only cover less than 0.1% of the ocean's surface but are a vital part of the biosphere (Spalding and Grenfell 1997). Coral reefs sustain immense amounts of biodiversity and provide invaluable resources (Cesar et al. 2003) and ecosystem services (Costanza et al. 1997). Abiotic factors, such as high light, warm temperatures and low nutrient levels, limit the distribution of tropical coral reefs between 30 °N and 30 °S. Biotic factors, such as herbivory by fishes and sea urchins, influence the reef community composition (Hughes et al. 2007, Rasher et al. 2012). For decades, humans have been directly and indirectly altering the abiotic and biotic factors controlling the persistence of coral-dominated reefs. Overfishing alone, a prevalent activity worldwide, is severely decreasing the diversity and functioning of reefs (Jackson et al. 2001). Climate change, created by the release of excess greenhouse gases (e.g., carbon dioxide) into the atmosphere by anthropogenic activities, is profoundly modifying crucial marine abiotic factors, such as seawater temperature and pH. Oceans are becoming more acidic in a process known as ocean acidification (OA). Ocean acidification threatens the persistence of coral reefs as corals depend on a certain pH and carbonate saturation state to maintain reef accretion.

Since the 1990's, the amount of ocean acidification research has expanded rapidly. With more research, studies are showing species-specific responses to

ocean acidification, even among scleractinian corals and calcifying algae (Comeau et al. 2013c, Edmunds et al. 2013). Some hard corals are proving to be resistant to changes in carbonate chemistry and thus, deemed future “winners”. As seen in Chapter 2, *Porites rus* seemed to be resilient to elevated pCO<sub>2</sub> and may have used bicarbonate for calcification (Comeau et al. 2013b). Similarly, *Acropora pulchra* in Chapter 3 did not exhibit statistically different calcification rates in elevated pCO<sub>2</sub> than those recorded from the corals in the ambient and fluctuating pCO<sub>2</sub>. *Porites rus* may already have the physiological means to tolerate OA whereas *Acropora pulchra* may be able to acclimate (Fabricius et al. 2011) or eventually even adapt (Lohbeck et al. 2012) to more acidified conditions. This would require longer term, cross-generational experiments. Previous research has consisted mostly of short-term OA perturbations with variability in organismal responses (Kroeker et al. 2013) potentially due to the organisms ability or inability to acclimate quickly to shifts in the abiotic environment (Guinotte and Fabry 2008). The magnitude of the effects of elevated pCO<sub>2</sub> on coral reefs needs to be reevaluated with longer-term incubations that allow organisms to adapt or acclimate.

Incorporating biotic interactions between species further complicates predictions of organismal responses to OA. In Moorea, French Polynesia, calcifiers were found commonly surviving below macroalgal communities such as the *Sargassum pacificum* covering the reef crest (Chapter 2). Macroalgae can affect corals through numerous indirect and direct pathways, ranging from abrasion (River and Edmunds 2001) to shading (Box and Mumby 2007) to

altering the microbial community associated with a coral (Barott et al. 2011, Wangpraseurt et al. 2012). The present research sought to explore whether or not *S. pacificum* could facilitate coral growth (McConnaughey et al. 2000) under OA conditions by metabolically increasing the pH (Anthony et al. 2011a, Connell et al. 2013) in the sub-canopy habitat surrounding the associated coral. On a small spatial scale, *Sargassum pacificum* can raise seawater pH, if only slightly (Chapter 2), and at a larger scale, create significant diel fluctuations in pH (Chapter 3). However, both the *Acropora pulchra* and *Porites rus* samples exhibited depressed calcification rates in the presence of *S. pacificum*. On the other hand, all of the corals from the field experiment in Chapter 3 displayed slight increases in net calcification in the fluctuating pCO<sub>2</sub> environment compared to the stable CO<sub>2</sub> environment. Although there was no way to incorporate a stable elevated CO<sub>2</sub> level in the field, these results suggest corals may be more equipped to survive in future (i.e., elevated, fluctuating pH) carbonate conditions than previously realized. Overall, the present research shows only minimal evidence for a macroalgal chemical refuge from ocean acidification and the significant need for more experiments utilizing ecologically relevant pH conditions (Hofmann et al. 2011).

Instead, these results more strongly suggest that *Sargassum pacificum* has a competitive advantage over sub-canopy juvenile corals by inducing other chemical changes in the microhabitat. The constraints of this research prevented an investigation of the direct effects of OA on algal physiology and potential mechanism of competition. This research focused on seawater pH changes in sub-

canopy habitats due to the importance of balanced seawater carbonate chemistry for coral calcification and the impending threat of ocean acidification. However, other factors that could have influenced coral growth and metabolism (e.g., dissolved oxygen, the presence of allelochemicals, dissolved organic carbon, soluble reactive phosphorous) were not taken into account (Hauri et al. 2010). To date, there is no evidence of toxic allelochemicals in other *Sargassum* spp. (Rasher and Hay 2010, Rasher et al. 2011, Rasher and Hay 2014). Thus, *Sargassum pacificum* and corals may have been competing for bicarbonate, an alternate resource for coral calcification and primary resource for macroalgal (with a carbon concentrating mechanism) photosynthesis. The presence of a CCM would increase algal photosynthesis and growth under elevated pCO<sub>2</sub> (Longphuir et al. 2013). More research on *S. pacificum* physiology, e.g. the presence/absence of allelopathy and a CCM, will further clarify the outcome and mechanisms of this specific coral-algal interaction.

While *Sargassum pacificum* negatively affected both coral species, shading did not seem to magnify the effects of ocean acidification on *Acropora pulchra* calcification. However, macroalgal shading did slightly exacerbate the effects of OA on net photosynthetic and linear extension rates of *A. pulchra* nubbins (Chapter 3). Under low light and increasing pCO<sub>2</sub>, net photosynthesis dropped precipitously. Shading alone can potentially drive coral growth and make corals more susceptible to ocean acidification (Dufault et al. 2013, Comeau et al. 2014a). Scleractinian corals in low light environments may be the first “losers” in more acidic seawater. This is an important result for managers and

conservationists to consider when creating plans and actions to help protect coral reefs from further degradation. On the contrary, higher light environments may ameliorate the negative effects of OA by increasing coral photosynthetic rates (Chapter 3) while increasing the risk of bleaching (Chapter 2). Examining in detail the ability of light to modify the overall impact of OA on calcifiers is imperative to understanding how OA will impact whole coral reef communities (Edmunds et al. 2013). Similarly, the importance of water flow in altering the transfer of bicarbonate and carbonate to marine calcifiers is proving to be another important abiotic factor that can modify the effects of OA (Comeau et al. 2014c). As seen in Chapter 2, the water flow in the mesocosm and on the back reef in the field experiment may have disrupted the diffusive boundary layer and canopy boundary layer, the areas most likely to have a higher pH due to algal photosynthesis, below the *Sargassum pacificum* canopy. Unfortunately, the high flow conditions may have prevented *Porites rus* from ever experiencing positive changes in pH. Incorporating ecologically relevant levels of water flow and light may significantly alter the outcome of OA studies.

In conclusion, this research sought to understand the indirect and direct effects of macroalgae on corals exposed to manipulated pH conditions in the lab and to natural pH levels *in situ*. Unexpectedly, both coral species proved to be resilient, or were able to acclimate, to elevated pCO<sub>2</sub>, motivating the need for more research using long-term incubations to address the physiological capability of calcifiers to adapt to OA (Lohbeck et al. 2012). Research addressing the mechanisms behind coral calcification will help inform longer-term, cross-

generational studies. Overall, the results suggest that *Sargassum pacificum*, at small and large spatial scales, has a net negative impact on associated corals that outweigh any mitigation of the effects of OA. However, the magnitude of the macroalgal impact may depend on the flow environment, proximity to the coral and other, potentially harmful, chemical alterations. On the contrary, the present data also illustrate the potential for macroalgal-dominated areas to remediate the effects of lowered pH; however, OA in turn may readily facilitate the growth and competitive ability of macroalgae over corals in the future. The complex relationships and feedback loops between the abiotic environment and coral-algal communities should be a priority for future OA studies. Lastly, the majority of OA experiments expose corals to stable, elevated pH. In reality, corals in shallow coastal habitats experience and contribute to diel fluctuations in pH (Hofmann et al. 2011). The water sampling in Chapter 2 and SeaFET data from Chapter 3 provided a novel approach for motivating an ecologically relevant OA experiment in Chapter 3. Thus, continuous, long-term monitoring of coral reef metabolism is crucial for informing future OA studies. The present study is a small step toward fully understanding the impacts of ocean acidification on coral reef communities under conditions of macroalgal domination.

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