THE COMBINED EFFECTS OF OCEAN ACIDIFICATION WITH WATER FLOW
AND TEMPERATURE ON TROPICAL NON-CALCAREOUS MACROALGAE

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

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Dedication

This is dedicated in loving memory of my grandfather, who always told me to persevere through the obstacles because it will always be worth it if I’m working towards my passion. And for teaching me the greatest lessons in life—how to bake, and the importance of patience, humility, and generosity.
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Abstract

The Combined Effects of Ocean Acidification With Water Flow and Temperature on Tropical Non-Calcareous Macroalgae

By

Maureen Ho

Master of Science in Biology

The vulnerability of coral reefs has substantially increased in the past few decades due to accelerating human-driven global change. The effects of ocean acidification (OA) and global warming individually and interactively have resulted in varying degrees of responses in benthic reef organisms. For non-calcareous macroalgae, the physiological and ecological responses to physical environmental changes can alter their relative abundances, which are often used as an indicator of the overall coral reef status. To better understand how fleshy macroalgae will respond to various physical parameters, three separate experiments were conducted from June 2014 to July 2015 in Moorea, French Polynesia.

An important physical driver in transferring nutrients and dissolved gases to benthic reef organisms is water motion. In 2014, I tested the hypothesis that increased water motion and elevated pCO₂ would benefit Amansia rhodantha (a CO₂ user) more than Dictyota bartayresiana and Lobophora variegata (HCO₃⁻ users). The highest and lowest growth rates were at the intermediate and highest flow speed, respectively, for all three species. A. rhodantha exhibited the greatest reduction in biomass at reduced flow under ambient pCO₂, indicating high sensitivity to mass transfer and carbon limitation. In
2015, the interactive effects of temperature and OA were tested in a two-part study on the metabolic (i.e. photosynthesis and respiration) and growth responses of *D. bartayresiana* and *A. rhodantha*. The first study in January 2015 showed that net photosynthesis in both species was affected by high $pCO_2$ but not temperature, and the combination of temperature and OA affected respiration rates. In the second study in July 2015, metabolic rates were affected by temperature but not $pCO_2$. Net photosynthesis and respiration of *A. rhodantha* were highest under OA conditions at 27.5 °C, but were reduced at 30 °C. There was no effect on metabolic rates of *D. bartayresiana* across all temperature treatments. The relative growth rates for *D. bartayresiana* were higher than *A. rhodantha* in the first study, while both species exhibited varying responses to treatments in the second study. Lastly, from May to June 2015, massive *Porites* spp. was paired with *D. bartayresiana* in competitive interactions at low and high flow speeds under ambient and elevated $pCO_2$ levels. I tested the hypothesis that increased water flow would increase algal growth rates, enhancing the competitive ability of the alga against the coral. For corals, I predicted that OA and reduced water flow would negatively affect the corals, thus increasing susceptibility to algal overgrowth. Net calcification and the photosynthetic efficiency of corals were used as a proxy for fitness and health status, respectively, however neither was affected by water flow or OA. On the contrary, growth rates of *D. bartayresiana* were significantly reduced under low flow. The negative effects of reduced water motion on macroalgae may potentially compromise the ability of the alga to compete. The variation in water motion can affect resource acquisition and when combined with OA, can have significant implications on species interactions. These results indicate the importance of water motion in influencing macroalgal growth and
provide insights to the varying responses in fleshy macroalgae to global change. Furthermore, their physiological responses may be attributed to their different carbon uptake strategies, as *A. rhodantha* was more sensitive to reduced flow and temperature than *D. bartayresiana*. 
Chapter 1

General Introduction

Among the highly diverse groups of benthic organisms occupying coral reefs, macroalgae are considered one of the more diverse groups (Diaz-Pulido and McCook 2008). Macroalgae exist in a wide range of morphologically different functional groups, consisting of filamentous algae, non-calcareous fleshy or encrusting algae, and calcareous crustose or non-crustose algae (Diaz-Pulido et al. 2007). Collectively, macroalgae contribute to the high biological diversity found in tropical reef habitats, and serve important ecological roles including providing structural habitat (Graham and Nash 2013), contributing to benthic primary production (Adey 1998), nitrogen fixation (Doney et al. 2009), and for some species, facilitating coral settlement (Negri et al. 2001). They also can replace coral populations, shifting benthic community composition to algal domination (Diaz-Pulido et al. 2007). Global environmental changes are creating more suitable habitats for some algal species than others (i.e. non-calcifying vs. calcifying algae), contributing to increasing algal abundances. In turn, tropical coral reefs can become less economically valuable, decreasing tourism services due to reduced structural complexity and diversity. Immense attention has been focused on understanding the ecological and functional changes in coral reefs directly and indirectly affected by rising anthropogenic impacts (Jokiel 2015; Pendleton et al. 2016).
Global threats

Coral reefs have been subjected to a number of environmental stressors, notably ocean acidification (OA) and climate change (Pendleton et al. 2016). The absorption of increasing anthropogenic CO₂ emissions into the ocean is affecting a variety of marine organisms (Kroeker et al. 2010). Increasing seawater CO₂ concentrations are altering the relative proportions of dissolved inorganic carbon (DIC) species utilized by reef organisms for various physiological processes and lowering seawater pH with no change in total alkalinity, a process termed ocean acidification (Doney et al. 2009). Currently, DIC in seawater is composed of 92% bicarbonate (HCO₃⁻), 1% CO₂, and the remaining 7% as carbonate (CO₃²⁻) (Zou et al. 2011). As seawater pH decreases, the relative abundances of DIC species will shift, increasing concentrations of HCO₃⁻ and CO₂, the two sources of inorganic carbon (Ci) macroalgae utilize for photosynthesis and growth (Fernández et al. 2014). Concurrently, the calcium carbonate saturation state is decreasing with decreasing pH, affecting calcifying marine organisms utilizing the different forms of calcium carbonate for their skeletons (Kleypas et al. 2006). Because coral reefs are composed of complex assemblages of benthic reef organisms with different physiological adaptations for biological processes, there appears to be a broad range of responses to OA that are species-specific. As a result, scaling individual responses to predict population- and ecosystem-level responses has proven difficult.

The number of studies examining the possible effects of OA has substantially increased within the last couple of decades (Riebesell and Gattuso 2015). There has been extensive research examining how calcifying organisms will be affected, as they are responsible for the structural complexity of coral reefs. The reduction in saturation state
(Ω) of calcium carbonate (CaCO₃) has affected calcifiers differentially, because Ω differs with each carbonate form (i.e. high-magnesium calcite, aragonite, and/or calcite) that calcifying organisms use (Veron 2011; Comeau et al. 2013). Generally, among marine organisms, studies have shown calcifying organisms to be more sensitive to OA conditions, but the magnitude of OA effects on organisms is still unknown. A meta-analysis by Kroeker et al. (2010) revealed that several biological responses of calcifying and non-calcifying organisms varied in the degree of sensitivity to OA among taxa. The meta-analysis indicated a general negative effect on calcification, survival, and reproduction for calcifiers, with the most sensitive response being calcification. Increasing number of studies exploring the implications for OA on calcifying organisms, particularly the survival, growth, and recruitment of corals, because a decrease in carbonate ions correlates with a reduction in Ω_{aragonite} (Veron 2011). As a result, this can reduce the ability of corals to calcify, while increasing susceptibility to other environmental stressors, potentially shifting reef community structure to favor less structurally complex species (Andersson et al. 2009; Hofmann et al. 2010; Edmunds 2011; Fabricius et al. 2011).

Simultaneously, studies are focusing on how non-calcifying macroalgae will respond to OA and whether a shift in algal abundance is the cause or consequence of coral mortality (McCook et al. 2001; McManus and Polsenberg 2004; Smith et al. 2006; Diaz-Pulido et al. 2007; Koch et al. 2013). Contrary to calcifying organisms, non-calcifying macroalgae have exhibited either negligible (Israel and Hophey 2002; Fernández et al. 2015; Rautenberger et al. 2015) or positive responses (Kübler et al. 1991; Connell and Russell 2009; Diaz-Pulido et al. 2011) to higher ρCO₂. However,
similar to calcifiers, the level of sensitivity can vary between species, potentially due to their different carbon assimilation (Kroeker et al. 2010; Cornwall et al. 2012; Fernández et al. 2015). Since macroalgae can only utilize CO₂ and/or HCO₃⁻ for photosynthesis and growth, their ability to take up the different Ci sources depends on how much CO₂ is present (Hurd et al. 2009). With the small concentration of CO₂ available and the slow diffusion rate of CO₂ in seawater, many macroalgae have developed carbon-concentrating mechanisms (CCMs) to convert HCO₃⁻ to CO₂. The ability to assimilate HCO₃⁻ suggests an advantage for macroalgae possessing CCMs under current CO₂ concentrations (Giordano et al. 2005; Zou et al. 2011). However, under OA conditions, obligate CO₂ users may respond positively to the predicted ~190% increase in CO₂ concentrations projected by 2100 (Roleda et al. 2012; IPCC 2013; Fernández et al. 2015). Thus, to better predict and understand how macroalgal abundances on tropical reefs will be affected by global change, it is essential to elucidate the underlying mechanisms influencing the physiological functions in non-calcifying algae.

Together with OA, emissions of greenhouse gases are contributing to rising sea surface temperatures (SST), with an expected increase of 0.6-2.0 °C in SST by the end of the century (IPCC 2013). Algal performance typically is associated with temperature because it can affect rates of photosynthesis (Padilla-Gamiño and Carpenter 2007a). Temperature is critical in regulating metabolic processes and can be disrupted easily if changes in temperatures are drastic (Kordas et al. 2011). The thermal limits among algal species depend on their habitat, and for tropical macroalgae, the optimum temperature for photosynthesis is 27-34 °C (Koch et al. 2013). Despite varying thermal tolerances in organisms, once the temperature exceeds the optimum temperature, it can result in
detrimental effects to their physiology. However, if temperature is maintained within their thermal tolerance window, an increase in temperature can enhance metabolism and growth (Harley et al. 2012; Kram et al. 2015). When combined with OA, increases in SST are predicted to affect physiological responses positively in macroalgae (Kram et al. 2015). Still, the interactive effects of warming and OA on fleshy macroalgae are less known and can vary with their different carbon uptake strategies. Facultative HCO$_3^-$ users currently are carbon-saturated and are predicted to have little benefit with increasing $p$CO$_2$, while obligate CO$_2$ users (i.e. lacking CCMs) are predicted to have enhanced photosynthetic and growth rates with increasing $p$CO$_2$ (Zou 2005). The increase in CO$_2$ concentrations potentially can reduce carbon-limitation in obligate CO$_2$ users, and alter macroalgal population dynamics on tropical coral reefs. Studies also have shown varying or neutral physiological responses to combinations of OA and increasing temperature (Kroeker et al. 2013; Harvey et al. 2013; Kram et al. 2015). Regardless, non-calcareous macroalgae are predicted to exhibit a positive response to global climate change (Harley et al. 2012). Hence, the interactive effects of elevated CO$_2$ and seawater warming warrants further study, as it suggests a potential for synergistic positive effects on metabolic processes among tropical macroalgae that may be species-specific.

*Effects of water flow on macroalgae*

In addition to multiple stressors affecting macroalgal abundances, the physical environment plays a key role in facilitating resource acquisition by transferring nutrients and dissolved gases (Hurd 2015). Notably, water motion is an essential parameter influencing a number of physiological and biological processes in macroalgae (Hurd
2000). Water flow can vary greatly in a reef-lagoon system as seen in Moorea, French Polynesia because it is often driven by wave-driven flows (Monismith 2007). In Moorea, as a wave approaches the reef, it breaks near the reef crest and into a lagoon, creating much stronger mean velocities at the reef crest and back reef regions of the lagoon (Hench et al. 2008). As a result, the high variation in water motion can differentially affect the morphological and physiological adaptations among corals and algae. With the predicted increase in atmospheric CO₂ concentrations in the next century, incorporating the effects of water motion into laboratory studies can provide insight into how interactive effects can influence algal physiology and how that may differ based on their DIC use.

The rate and direction of water flow can affect a variety of factors from organism to reef scale. It can affect the rate of mass transfer of nutrients and gases, metabolism, larval dispersal, and the overall structure of reef communities (Lowe and Falter 2015). Increasing water motion can increase photosynthesis and growth in benthic reef organisms, while reduced flow speeds reduce gas and carbon exchange across the surface of the organism (Wheeler 1980; Atkinson and Bilger 1992; Stewart and Carpenter 2003). At low flow speeds, thicker boundary layers can form around algal thalli, limiting mass transfer of nutrients (Hurd 2000). In turn, it can alter physiological performance, negatively affecting benthic organisms. However, a thicker boundary layer due to reduced flow also has been shown to act as a buffer against the negative effects of OA on coralline algae (Cornwall et al. 2014). Thus, the hydrodynamics of coral reefs have important implications for sessile reef organisms occupying either high flow or low flow habitats.
The variation in water motion can affect the degree of carbon limitation of metabolism (Enríquez and Rodríguez-Román 2006) and the location of corals and algae on coral reefs also can be affected by flow conditions. For example, macroalgae that solely take up CO$_2$ are currently carbon-limited due to the low concentration of CO$_2$ available (Giordano et al. 2005). Most obligate CO$_2$ users are commonly distributed in light-limited microhabitats (e.g. crevices or deeper depths) and are represented primarily by some red algal species (Maberly 1980; Diaz-Pulido et al. in press). As a result, obligate CO$_2$ users may be at a disadvantage under current CO$_2$ conditions. However, this may shift under OA conditions to the benefit of algae lacking CCMs, potentially alleviating carbon limitation and competitive disadvantages with facultative HCO$_3^-$ users. Conversely, algae with CCMs will benefit less under OA conditions, but can increase their uptake in CO$_2$ diffusion by downregulating their CCM activity, thus allocating energy to other biological processes instead of photosynthesis (Raven and Hurd 2012).

The variability in carbon uptake strategies among species of macroalgae can greatly influence their biological and physiological responses to water motion, and when combined with global environmental changes, might influence the relative abundances and composition of macroalgal communities.

*Interaction of water motion and ocean acidification on coral-algal competition*

Macroalgal abundance commonly has been used to represent the overall status of coral reefs. For example, rich coral and algal assemblages indicate high habitat heterogeneity and structural complexity (Fabricius et al. 2011). While lower cover of coralline algae and corals, and higher cover of turf or fleshy algae can indicate a degraded
reef (Birrell et al. 2008; Diaz-Pulido et al. in press). When processes such as OA threaten reefs, shifts in benthic community structure can occur with algal abundances increasing, further inhibiting coral growth and decreasing coral cover (Diaz-Pulido and McCook 2008; Fabricius et al. 2011).

On coral reefs, the two dominant benthic groups frequently interacting are scleractinian corals and algae (Lang and Chornesky 1990), and the most common resource corals and algae compete for is space (Carpenter 1990). The availability of space can be strongly limited by benthic algae due to its rapid ability to colonize bare substratum or dead corals (Birrell et al. 2008; Diaz-Pulido et al. 2007). In addition, during reef degradation, competition between corals and macroalgae typically result in negative effects on certain corals by the algae (McCook et al. 2001). As reefs face both natural and increasing anthropogenic disturbances, the ability for fleshy macroalgae to potentially outgrow corals may further limit coral survival. There is rising concern that corals are becoming increasingly vulnerable to human activities, altering or disrupting physiological processes (Kroeker et al. 2010). For example, Comeau et al. (2014) reported a general reduction in calcification of various corals differing in functional groups to OA and a difference in those rates of calcification. The study indicates some species to be more sensitive under OA conditions than others. On the contrary, in some cases, OA has been shown to enhance macroalgal growth while reducing calcification rates in corals (e.g. Rasher and Hay 2010; Diaz-Pulido et al. 2011; Kram et al. 2015). As a result, increasing sensitivity to human impacts in corals can provide an advantage for macroalgae to grow or outcompete corals for space.
Competitive interactions play an important role in modulating reef community structure, and alterations to these interactions due to environmental changes can affect ecological processes (Barott and Rohwer 2012). Thus, it has become critical to examine the physical and chemical drivers and mechanisms influencing coral-algal interactions at the organismal level to better understand and predict changes at the population and community-level. One of the most common mechanisms by which algae compete with corals is through direct overgrowth (McCook et al. 2001). This can hinder the ability of corals to grow and survive, leading to tissue necrosis and/or mortality (Diaz-Pulido et al. 2009; Jompa and McCook 2003). Furthermore, it can increase the susceptibility of corals to competition, as macroalgae are predicted to have enhanced metabolic and growth rates from increasing atmospheric CO₂ concentrations. In addition, coral reef communities are composed of densely packed sessile benthic organisms that depend on water motion to facilitate nutrient acquisition and competition (Koehl 1986). Increases in water motion can further enhance macroalgal growth rates on coral reefs, potentially exacerbating the direct competitive effect on corals.

To date, there are a limited (but growing) number of studies examining the effects of OA on coral-algal competitive interactions (e.g. Diaz-Pulido et al. 2011; Kroeker et al. 2012). The predicted negative effects of OA on calcifying organisms (e.g. corals) and positive effects on fleshy macroalgae potentially can shift competitive interactions to favor macroalgae. However, despite water flow being an important physical parameter on coral reefs, there are no studies examining the interactive effects of water flow and OA on the competitive interactions between corals and algae. Additionally, higher water flow can further promote macroalgal growth, increasing the ability of macroalgae to overgrow
corals, and their ability to colonize and compete for space. While reduced water flow typically is associated with mass transfer limitations (Koehl 1984), the reduction in flow also creates a thicker boundary layer around the organism. For some calcifying organisms, this can ameliorate the negative effects of OA (Cornwall et al. 2014). Conversely, increase water flow can enhance the photosynthetic efficiency (a parameter used to assess coral health) in corals, potentially improving their ability to recover from an environmental stressor (Finelli et al. 2006). The rate of flow varies with the location of where the organisms are on coral reefs. For instance, slow-flow habitats can potentially reduce the vulnerability in calcifying organisms by producing thicker boundary layers, while high-flow habitats can increase metabolic processes. Hence, water flow can play an important role in modulating the physiology of reef organisms, especially for species that are more sensitive to OA. Considering the effects of water motion under OA on corals and algae might provide further insight into how corals and algae will respond to OA. Water flow has the potential to mitigate or exacerbate negative effects of OA on calcifying organisms and under competition, can influence the competitive abilities between corals and fleshy macroalgae

**Thesis objectives**

With growing interest in how coral reefs will respond to global environmental changes, increasing studies are incorporating multiple abiotic and biotic factors to provide a more ecological approach to understanding how individual and population dynamics will be affected. As anthropogenic impacts continue to increase, ocean acidification has become the focus for how reef organisms will respond to increasing
atmospheric CO₂ concentrations. Additionally, it is of interest how OA can influence the biological and physiological responses of organisms when combined with other physical factors. The goal of this thesis was to investigate how non-calcareous macroalgae differing in their DIC use respond to the combined effects of ocean acidification with a physical parameter (i.e. water flow and temperature). Different responses (i.e. photosynthesis, respiration, and growth rates) were measured to evaluate how algal physiology was affected. Additionally, the competitive ability between a dominant coral and alga was examined to better understand the underlying mechanisms involved in competition and how that may affect coral-reef dynamics.

Three separate experiments were conducted in Moorea, French Polynesia, from June 2014 to July 2015 in order to explore the interactive effects of different abiotic factors. In Chapter 2, relative growth rates of three dominant non-calcifying macroalgae, Lobophora variegata, Dictyota bartayresiana, and Amansia rhodantha were measured. They were chosen based on their different carbon uptake strategies and exposed to ambient and elevated pCO₂ under three different flow regimes.

In Chapter 3, I examined how elevated temperature and pCO₂ influence the metabolic (i.e. photosynthesis and respiration) and growth responses of two macroalgae species differing in their DIC use. Two experiments were conducted in mesocosms in Moorea, in which Amansia rhodantha and Dictyota bartayresiana were subjected to 400 µatm (ambient pCO₂) and 1000 µatm (elevated pCO₂) at either two or three different temperatures in January and July 2015, respectively. Under OA conditions, I predicted A. rhodantha (an obligate CO₂ user) to have enhanced photosynthesis, respiration, and growth rates than D. bartayresiana (a HCO₃⁻ user). Additionally, increasing temperature
can enhance metabolic rates, so I predicted temperature to synergistically interact with OA, positively increasing physiological responses in both species.

Finally, in Chapter 4, I investigated how the combined effects of water motion and OA influence the competitive interaction between massive *Porites* spp. and *D. bartayresiana*. I hypothesized that increased water motion would increase algal growth rates, enhancing the competitive ability of the alga over the coral. In addition, under competition, I hypothesized that the coral would be more susceptible to OA, resulting in reduced calcification rates. I staged interactions between a dominant coral massive *Porites* spp. with a dominant alga, *Dictyota bartayresiana* to test the competitive ability for space, and measured growth rates and photosynthetic efficiency of the corals. Lastly, I predicted OA and water flow would alter the coral-algal competitive interaction to favor the alga. To investigate the direct effect of the alga on the coral, algal overgrowth was used as a proxy for the underlying competitive mechanism to measure the direct effect of algae on corals.
Chapter 2

Differential growth responses to water flow and lowered pH in tropical marine macroalgae

Introduction

The increase in dissolution of atmospheric CO$_2$ into the ocean leading to a reduction in seawater pH, termed ocean acidification (OA), is expected to have varying effects on coral reefs by altering community and population dynamics of reef organisms. OA effects have been extensively studied on calcifying organisms as they are considered the framework of coral reefs, and are currently threatened due to elevated CO$_2$ concentrations lowering carbonate saturation states, reducing their ability to calcify (Doney et al. 2009; Anthony et al. 2011a). An increased number of studies also have focused on how non-calcifying algae are affected by OA and the potential shifts in reef communities from coral-dominated to algal-dominated reefs (Lirman 2001; McCook et al. 2001; McManus and Polsenberg 2004; Diaz-Pulido et al. 2011). Under OA, the partitioning of dissolved inorganic carbon (DIC) is altered, increasing HCO$_3^-$ while decreasing CO$_3^{2-}$ concentrations (Andersson and Mackenzie 2011; Veron et al. 2011). This can cause various responses in calcifying and non-calcifying macroalgae to shift depending on their carbon uptake strategy, thereby potentially affecting their relative abundances (Koch et al. 2013).

A number of studies have shown organisms varying in their biological and physiological responses to ocean acidification (Guinotte and Fabry, 2008; Anthony et al. 2008; Kroeker et al. 2010; Price et al. 2011; Koch et al. 2013; Johnson et al. 2014). The
changes in productivity, calcification, and dissolution rates of benthic reef organisms affect the carbonate chemistry in seawater based on their DIC use (Leclercq et al. 2000; Kleypas et al. 2006; Anthony et al. 2011b). Contrary to calcifying organisms, many fleshy macroalgal species seem to be resilient (Israel and Hophey 2002) or in some cases, have benefitted under OA conditions (Connell and Russell 2010; Diaz-Pulido et al. 2011; Porzio et al. 2011; Johnson et al. 2012). However, sensitivity to OA varies across taxa of fleshy macroalgae (Kroeker et al., 2010; Cornwall et al., 2012). Algae depend on the supply of inorganic carbon for growth and production, and increases in CO₂ concentrations can enhance metabolic processes such as photosynthesis (Roleda et al. 2012). All macroalgae acquire CO₂ and/or HCO₃⁻ for photosynthesis and growth, either via diffusion (for CO₂) or active uptake from carbon-concentrating mechanisms (CCMs; for HCO₃⁻) (Beardall et al. 1998; Giordano et al. 2005; Raven and Hurd 2012; Koch et al. 2013). The capability to assimilate inorganic carbon (Ci) depends on the availability of CO₂, and in most cases CO₂ is insufficient for maximum photosynthesis (Giordano et al. 2005; Hurd et al. 2009). However, for facultative HCO₃⁻ users, the ability to convert HCO₃⁻ to CO₂ can be done in two ways: 1) using extracellular carbonic anhydrase (CA), or 2) CCMs actively take up HCO₃⁻ with CA acting intracellularly (Hurd et al. 2009). This evolutionary development has provided macroalgae with CCMs a more energetically costly but beneficial advantage, eliminating the risk of being carbon limited under present pCO₂ in most environments (Giordano et al. 2005). For obligate CO₂ users restricted to diffusive CO₂ uptake, current concentrations of CO₂ in seawater are relatively lower than concentrations of HCO₃⁻ and, therefore, may have slower photosynthetic rates due to carbon limitation (Kübler et al. 1999; Zou 2005).
Consequently, OA can have various (positive) effects on photosynthesis, growth, and carbon acquisition for both CO\textsubscript{2} and HCO\textsubscript{3}^- users. For obligate CO\textsubscript{2} users lacking CCMs, increase atmospheric CO\textsubscript{2} dissolution in seawater can alleviate carbon limitation and potential disadvantages in their competitive ability with other noncalcareous algae. For HCO\textsubscript{3}^- users, OA may not enhance photosynthetic rates as expected for CO\textsubscript{2} users (Zou 2005; Hurd et al. 2009), but they may downregulate their CCM activity, thus reducing energetic investment for CO\textsubscript{2} active uptake (Raven and Hurd 2012). These responses are species-specific (Kram et al. 2015), and the majority of these studies have focused on OA effects in isolation (e.g. Cornwall et al. 2012; Connell et al. 2014; Fernández et al. 2015; Diaz-Pulido et al. in press). This is partly because predicting effects of increased CO\textsubscript{2} are difficult because coral reefs are simultaneously subjected to an array of abiotic and biotic factors that are rarely independent of each other. Studies manipulating one abiotic factor allows can focus on changes in physiological and ecological performance of benthic reef organisms on both the species and population level. However, in an environment where reefs are continuously exposed to changing conditions such as increase CO\textsubscript{2}, incorporating various environmental factors provide better insight in understanding physiological changes in benthic organisms.

In reef ecosystems, many organisms depend on water motion for the transfer of dissolved gases and nutrients across the boundary layer (Wheeler 1980). The physical environment plays a critical role influencing how benthic reef organisms settle and acquire resources, because many are sessile, which favors competition for resources like space or light when limited by mobility (Carpenter 1990). For marine macroalgae, water movement is a key factor influencing a number of biological processes: morphology
production and mass transfer (Adey and Steneck 1985; Carpenter et al. 1991), photosynthetic and growth rates (Koch et al. 2013), some facilitating larval/spore dispersal (Lowe and Falter 2015), light attenuation (Carpenter 1985), and carbon acquisition (Hurd 2000; Raven et al. 2005). The rate and mode of water motion (e.g., fast vs. slow; oscillatory vs. unidirectional) also contributes greatly to the variable effects of water motion on algal metabolism (Denny 1988; Carpenter et al. 1991). An increase in water motion is beneficial for resource acquisition, ultimately increasing photosynthesis and growth, while a decrease in water motion (i.e., slow flow) can increase mass transfer limitation of gases and nutrients, potentially leading to negative effects on their metabolic rates (Atkinson and Bilger 1992; Hurd 2000). These physiological fluctuations occur when the diffusion boundary layer (DBL) is altered due to hydrodynamic forces. DBLs represent concentration gradients that form through the diffusion of gas or molecules from the bulk fluid to the thallus surface where they are taken up (Wheeler 1980; Tansik et al. 2015). Therefore, depending on slow- or fast-flow environments, the DBL thickness varies (inversely with water velocity), resulting in an increase or decrease of mass transfer and uptake of DIC, ultimately affecting photosynthesis and growth (Carpenter and Williams 2007; Raven and Hurd 2012; Cornwall et al. 2014).

Under natural conditions, benthic algae can experience both unidirectional and oscillatory flow, and while there is increasing effort to understand how the physical environment affects algal physiology, mimicking field conditions has been proven difficult (Williams and Carpenter 1998; Carpenter et al. 1991; Comeau et al. 2014). The objective of this study was to examine the combined effects of unidirectional flow speeds
and lowered pH on growth rates of three dominant non-calcifying macroalgal species, *Lobophora variegata* (Phaeophyta), *Dictyota bartayresiana* (Phaeophyta), and *Amansia rhodantha* (Rhodophyta) found in Moorea, French Polynesia. Based on the review by Koch et al. (2013) on photosynthetic pathways and carbon acquisition of marine macroalgae, *L. variegata* is a HCO$_3^-$ user. To determine the inorganic carbon assimilation of the other two species, a pH drift experiment (Maberly 1990) was conducted prior to treatment on *D. bartayresiana* and *A. rhodantha*. This technique measures the change in pH when carbon is assimilated through photosynthesis, and when there is a rapid increase in pH (>9), the alga is typically utilizing HCO$_3^-$ (Axelsson and Uusitalo 1988; Maberly 1990). *D. bartayresiana* raised the seawater pH >9 indicating the ability to use bicarbonate while photosynthesis of *A. rhodantha* was carbon saturated at a pH of 8.51 (R. Carpenter unpublished data). I tested the hypothesis that the combined effects of water motion and lowered pH will positively affect macroalgal growth rates. As flow increases, mass transfer of gases increases, thus increasing growth rates. I predicted that: (H1) algal growth rates would increase with increasing flow speed, (H2) *A. rhodantha* (CO$_2$ user) would benefit under elevated pCO$_2$ by having higher growth rates than *D. bartayresiana* and *L. variegata*, (H3) the growth rates of HCO$_3^-$ users would not vary between current and elevated pCO$_2$ conditions, and (H4) when OA is combined with increased flow, *A. rhodantha* would result in higher growth rates than the other two species. Relative growth rates were measured as a proxy of fitness to assess how the interaction of OA and flow can affect algal growth.
Methods

Study site and collection

This study was conducted in Moorea, French Polynesia in June-July 2014. Three dominant non-calcifying species, *Lobophora variegata*, *Dictyota bartayresiana*, and *Amansia rhodantha* of similar size were collected from the back reef of the north shore at a depth of ~1-2 m. *L. variegata* exists in three morphologies, a ruffled form, a decumbent form, and an encrusting morph (Coen and Tanner 1989). The decumbent and encrusting forms were commonly seen in Moorea and therefore, the decumbent morphology was used, as it was most similar to the other two fleshy macroalgal species. These species were chosen based on preliminary field surveys on the overall abundance of macroalgae on the back reef of the north shore in 2014 and from the Moorea Coral Reef Long Term Ecological Research (MCR-LTER) dataset (knb-lter-mcr.8) on benthic algal cover for 2014.

Algal samples were collected weekly for each flow treatment, 2 days prior to each flow/pCO$_2$ experiment, and brought to the Richard B. Gump South Pacific Research station in Cook’s Bay, Moorea. Algae were transferred to a flow through seawater table, cleaned of epiphytes, then placed into nylon monofilament mesh bags with 1.3-cm openings (Fig. 1B and 1C; $n=40$ per species). Algae were placed in mesh bags to retain algal biomass and minimize fragmentation. Mesh bags were made by cutting nylon monofilament mesh into 21 cm x 10 cm sheets, folded in half and the ends were tied in opposite directions with monofilament line to inflate the mesh bags and allow space for each alga to grow. Each bag was attached to a 5-cm$^2$ PVC plate (0.6-cm thick) acting as a weight. Light (photosynthetically active radiation, PAR) measurements were taken inside
and outside the mesh bags using the Walz pulse amplitude modulated fluorometer (PAM) 2π cosine light sensor calibrated to a LI-COR 2π quantum sensor (LI 192-sa), giving PAR experienced by the algae. Algal individuals were kept in a flow-through sea water table for 2 days during preparation before being transferred to their respective treatments.

Experimental conditions

The experiment was conducted in four outdoor flumes, each with a working section of 0.3 x 0.3 x 5.0 m, together containing 700L of seawater (Fig. 1A). Seawater was pumped from Cook’s Bay at 12-m depth and filtered through a sand filter (nominal filter size ~100 µm) then directly into the upstream end of each flume at 5L min⁻¹. The seawater was dispensed into a rectangular section that transitioned to a circular section with flow straighteners (20-cm long, 3-cm diameter stacked PVC pipes) positioned at each end of the flume. Seawater was re-circulated back to a 12.5-cm return section using a W. Lim WAVE II 375W pump. Weekly light levels were measured for overall light conditions using a LiCor 4π quantum sensor (LI-193) and a LI-1400 meter, and the outdoor flumes experienced natural light conditions (PAR ~900 µmol photons m⁻² s⁻¹). Light measurements were taken 15 cm above the flume bottom at the upstream, middle, and downstream end of each flume, and averaged to account for variability in light exposure along the flume.

Three different flow speeds were used: 8.5 cm s⁻¹ representing an average flow speed in the back reef at 2-3 m depth (Hench et al. 2008), 5 cm s⁻¹, and < 1 cm s⁻¹ treatment. Each flow speed ran for 7 days, with two flumes at ambient pCO₂ and the other two at high pCO₂ treatment. At < 1 cm s⁻¹ flow, incoming fresh seawater naturally
created slight water movement, but to prevent flow, the main pumps were turned off during the treatment. Flow rates were measured on day 1 and 4 of the treatments for consistent velocities throughout the experiment using a Nortek Vectrino Acoustic Doppler Velocimeter, positioned 15 cm above the flume bottom. After 7 days, the flumes were drained and rinsed with freshwater before the next flow speed treatment began with newly collected algal individuals.

**Carbonate chemistry**

\( p\text{CO}_2 \) was regulated using a pH-stat aquacontroller (Neptune systems) that bubbled in either pure CO\(_2\) or CO\(_2\)-free air. Two flumes were regulated at \(~400\ \mu\text{atm}\) (ambient \( p\text{CO}_2 \) level) and the other two flumes at \(~1000\ \mu\text{atm}\) (high \( p\text{CO}_2 \)), the expected atmospheric \( p\text{CO}_2 \) level projected in 2100 under a “business as usual” scenario from the IPCC (2013) Representative Concentration Pathways (RCP) 8.5 (Van Vuuren et al. 2011).

Flumes were maintained at a mean temperature of \( 27.1 \pm 0.5 \, ^\circ\text{C} \), mimicking the back reef temperature conditions (\(~27.6 \, ^\circ\text{C}\) during May-July 2014 (MCR-LTER Dataset ID: knb-lter-mcr.1035). Temperature and pH were recorded daily using a hand-held pH meter (Orion 3-stars) fitted with a Mettler DG 115-SC pH probe, which was calibrated every other day against certified TRIS buffer (A. Dickson, San Diego, USA). pH also was measured weekly using m-cresol dye (Dickson et al. 2007), providing results that were within \( \pm 0.03 \) units of those obtained with the portable pH meter. Total alkalinity (\( A_T \)) was measured every 2 days using an automatic titrator (T50, Mettler-Toledo) using 50-mL seawater samples collected the day of sampling following the standard procedure of Dickson et al. (2007). Titrations of certified reference materials (CRM) provided by
A.G. Dickson (Batch 130) were used to determine the accuracy and precision of the titrated samples, yielding $A_T$ values within 9-$\mu$mol kg$^{-1}$ of the certified value. The R package seacarb (Lavigne and Gattuso 2013) was used to calculate the carbonate system parameters with salinity, temperature, $A_T$ and pH$_T$.

**Growth Measurements**

To determine growth rates, initial and final wet (spun dry) weight was measured for each individual. Algae in their mesh bags were spun in a salad spinner for 10 seconds and immediately weighed ($\pm 0.001g$). The relative growth rate is a common measure of change in weight over time by using the initial and final weights. To calculate the relative growth rate (RGR), the following equation adapted from Yong et al. (2013) was used:

$$
\text{RGR} (\% \, \text{day}^{-1}) = \log \left( \frac{W_f}{W_i} \right) \times 100 \, \frac{t}{t}
$$

Growth is expressed as percent change per day, where $W_f$ and $W_i$ are the final and initial wet weights, respectively, and $t$ is the total time in days the algae were subjected to a treatment.

**Statistical Analyses**

Physical conditions in the flumes were analyzed using a two-way partially-nested analysis of variance (ANOVA), with $p$CO$_2$ and flow as fixed effects and flume as a random factor nested in the interaction of $p$CO$_2$ and flow. All effects were tested using $\alpha=0.05$, however to increase statistical power, the flume was dropped from the statistical model when not significant under a more conservative criterion of $\alpha=0.25$ (Quinn and Keough, 2002). Growth rates of the algal species were analyzed using a three-way
partially-nested ANOVA with two fixed, between-plot factors (pCO₂ and flow), the random plot as replicate flumes nested within the interaction of pCO₂ and flow treatment, and algal species as a fixed, within-plot factor. Relative growth rates of each species were used as the dependent variable to test for treatment effects after 7 days. Tukey’s post hoc tests were used to analyze differences between species and treatments (flow and pCO₂). All analyses were performed using RStudio software (R Foundation for Statistical Computing), and assumptions of normality and equality of variance were evaluated through graphical analyses of residuals.

Results

Mean PAR in the four flumes during the three 1-week incubation was 843 ± 19 μmol photons m⁻² s⁻¹ and 1040 ± 66 μmol photons m⁻² s⁻¹ in the ambient treatments and 872 ± 14 μmol photons m⁻² s⁻¹ and 808 ± 14 μmol photons m⁻² s⁻¹ in the elevated pCO₂ treatments (±SE, n=18). Weekly light levels did not differ among flumes (F₃,₈=1.495, p=0.288). Light inside and outside the mesh bags was comparable (~190 μmol photons m⁻² s⁻¹), indicating no shading effects. Total alkalinity (Aₜ) did not vary significantly between pCO₂ (F₁₆=1.235, p=0.309) or flow (F₁₆=1.987, p=0.218) treatments, or within flumes among pCO₂ and flow treatments (F₆₂₅=1.391, p=0.230). Aₜ averaged 2316 ± 3, 2320 ± 3, and 2317 ± 2 μmol kg⁻¹ at 0.1, 5, and 8.5 cm s⁻¹ flow speed (±SE, n=8), respectively, for ambient pCO₂, and 2309 ± 1, 2319 ± 4, and 2319± 2 μmol kg⁻¹ at 0.1, 5, and 8.5 cm s⁻¹ flow speed (±SE, n=8; Table 1) for elevated pCO₂. Temperature was consistent across all flumes, and did not differ between pCO₂ (F₁₆=0.045, p=0.839) or flow (F₂₆=2.100, p=0.204).
Mean $p\text{CO}_2$ for the ambient treatments were maintained at 397 ± 8, 373 ± 14, and 404 ± 9 μatm at 0.1, 5, and 8.5 cm $s^{-1}$, respectively (±SE, $n=14$). For the elevated $p\text{CO}_2$ treatments, values were maintained at 990 ± 47, 998 ± 45, and 1065 ± 35 μatm at 0.1, 5, and 8.5 cm $s^{-1}$, respectively (±SE, $n=14$). $p\text{CO}_2$ differed between treatments ($F_{1,6}=865.06$, $p<0.001$) but not between flow treatments ($F_{2,6}=2.674$, $p=0.148$) or among flumes within the interacting treatments ($F_{6,72}=0.682$, $p=0.664$). Similarly, pH differed between ambient and elevated $p\text{CO}_2$ treatments ($F_{1,6}=1411.03$, $p<0.001$), but not between flow treatments ($F_{2,6}=3.571$, $p=0.095$), or between flumes within treatments ($F_{6,72}=0.644$, $p=0.695$).

Algal growth rates were significantly different between $p\text{CO}_2$ ($F_{1,342}=4.384$, $p=0.037$) and flow treatments ($F_{2,342}=161.998$, $p<0.001$), but the interaction between the two was not significant ($p=0.183$). There were differences in growth among species ($F_{2,342}=87.473$, $p<0.001$) and planned comparisons revealed $L. \text{variegata}$ having overall higher growth rates than both $D. \text{bartayresiana}$ and $A. \text{rhodantha}$, but no difference in growth rates between $D. \text{bartayresiana}$ and $A. \text{rhodantha}$. RGR also varied by species within each flow treatment ($F_{4,342}=29.12$, $p<0.001$; Fig. 2). Growth of $L. \text{variegata}$ and $A. \text{rhodantha}$ were affected by all three flow treatments, having the greatest increase at 5 cm $s^{-1}$. Under ambient and elevated $p\text{CO}_2$, growth of $L. \text{variegata}$ increased 3.27 ± 0.26 % day$^{-1}$ and 2.64 ± 0.35 % day$^{-1}$, respectively (n=20) and for $A. \text{rhodantha}$, increased by 1.36 ± 0.31 % day$^{-1}$ and 1.26 ± 0.16 % day$^{-1}$, respectively (n=20). However, under no flow, $A. \text{rhodantha}$ had negative growth of -1.66 ± 0.20 % day$^{-1}$ (n=20) at ambient conditions, which was the greatest reduction among the three species (Fig. 2C). $D. \text{bartayresiana}$ only differed in RGR between the mid flow and no flow treatment, having
a 0.62 ± 0.30 % day\(^{-1}\) (n=20) increase in biomass at 5 cm s\(^{-1}\) at ambient pCO\(_2\), while under no water motion, it demonstrated a negative growth rate under both CO\(_2\) treatments, with a greater reduction at elevated pCO\(_2\) (-0.80 ± 0.15 % day\(^{-1}\), n=20, Fig. 2A). However, there was neither a species-within-CO\(_2\) treatment (F\(_{2,342} = 2.219, p=0.11\)) effect, nor a species within interacting flow and pCO\(_2\) effect (F\(_{4,342} = 0.540, p=0.707\)).

**Discussion**

This study examined the interactive effects of CO\(_2\) enriched seawater with flow on three dominant non-calcifying macroalgal species commonly found in the back reefs of Moorea. Macroalgae were subjected to three flow speeds within the range they experience in the back reef community, and relative growth rates were used as a proxy for fitness. The results of this study were not consistent with the hypothesis that algal growth rates are significantly affected by both pCO\(_2\) and flow, with the greatest increase in growth under the highest flow speed. While there was no significant difference among species within pCO\(_2\) treatments or within the interaction of pCO\(_2\) and flow treatments, relative growth rates differed among species within flow treatments. Furthermore, growth rates were affected independently by pCO\(_2\) and flow. However, this effect seemed to be driven mainly by the increase in RGR of *L. variegata*. *L. variegata* had a positive RGR at 0.1 cm s\(^{-1}\) and double and five-fold the growth rate of *A. rhodantha* and *D. bartayresiana* at 5 cm s\(^{-1}\), respectively. These differential responses between algal species indicate the importance of examining the effects of physical and chemical parameters on multiple taxa of benthic reef organisms.

Under current atmospheric CO\(_2\), the most abundant form of DIC is HCO\(_3^-\) (92%) and the least is CO\(_2\) (1%) (Royal Society 2005). With the current large HCO\(_3^-\) pool
available, algae with CCMs have become efficient at CO₂ fixation compared to obligate CO₂ users (Fernández et al. 2015). However, the relative proportions of DIC in seawater will shift under OA, with an expected increase of 194% in dissolved CO₂ and a 14% increase in HCO₃⁻ (Royal Society 2005). This increase in dissolved CO₂ has been hypothesized to benefit obligate CO₂ users under future acidic seawater conditions, potentially enhancing metabolic processes and growth in non-calcareous macroalgae (Cornwall et al. 2012), while bicarbonate users are expected to have minimal response. There was an overall CO₂ effect on macroalgal growth, but it did not differ among species within the CO₂ treatments, suggesting that OA does not enhance growth rates of these algal species regardless of their DIC use.

A significant difference was found among species within flow treatments in the present study. *D. bartayresiana* and *A. rhodantha* exhibited similar responses to all three flow speeds. Both species experienced the strongest effect of flow at intermediate flow speeds (5 cm s⁻¹), negative growth rates under no flow (0.1 cm s⁻¹), and minimal growth under the highest flow speed (8.5 cm s⁻¹) in both CO₂ conditions. *L. variegata* also had similar trends at 5 and 8.5 cm s⁻¹, but had positive growth rates at 0.1 cm s⁻¹ compared to the other two species. This might be explained by differences in boundary layer dynamics and morphology between the species. Diffusion boundary layers (DBL) are related inversely to flow speed, so under slower flow speeds, a thicker DBL should be present, increasing mass transfer limitation and leading to a reduction in macroalgal primary productivity (Hurd 2000). Additionally, surface area of the alga can be a determining factor for diffusion of gases (Stewart and Carpenter 2003). Algae tend to have more flexible morphologies in high-flow habitats to decrease vulnerability to dislodgement by
having lower surface area/volume ratios (SA/V) (Koehl and Alberte 1988). Conversely, algae in low-flow habitats have higher SA/V ratios to maximize nutrient uptake despite increasing area and forces exerted on the algae (Stewart and Carpenter 2003). While low flow speeds generate thicker boundary layers that can limit delivery of materials, higher SA/V such as for the decumbent blades of *L. variegata* may explain why there was a positive growth when subjected to no water flow and, an overall higher RGR across all three flow treatments.

Contrary to my hypothesis that increasing flow speed will increase growth rates, all three species exhibited minimal growth under the highest flow treatment. A potential threshold may exist for algae to retain the flux of CO$_2$, O$_2$, and nutrients for growth under high flow, although it is unclear whether this has been tested. However, there are two possible explanations for low growth rates at this flow speed. First, both *D. bartayresiana* and *A. rhodantha* are fleshy algae with undulated blades and flexible thalli. Under higher flow speed, the shear stress and drag forces cause the thallus to collapse and result in self-shading (Hurd 2000), reducing photosynthetic activity and nutrient acquisition (Hay 1981). However, this is only applicable to the two species with fleshy morphology. The negative growth at 8.5 cm s$^{-1}$ for *D. bartayresiana* may be an artifact of self-shading and fragmentation and at 0.1 cm s$^{-1}$ an insufficient uptake of nutrients, likewise with *A. rhodantha*. While *L. variegata* displayed similar results to the other species under the highest flow speed, self-shading likely was not responsible since it is morphologically different. The decumbent form of *L. variegata* has convoluted blades and lobed margins (Enríquez and Rodríguez-Román 2006) and when grown, form flat, semi-circular blades (Coen and Tanner 1989). Instead, it may be affected by having
external carbonic anhydrase (CA). Some bicarbonate users have CA acting externally while others that actively take up HCO$_3^-$, have CA acting internally (Hurd et al. 2009). CA is an enzyme that many CCM-macroalgae possess to help catalyze the conversion of bicarbonate to CO$_2$ (Raven et al. 2014). However, high flow speeds can remove the external CA surface due to shear stress, compromising its efficiency to convert HCO$_3^-$ and CO$_2$. Enríquez and Rodríguez-Román (2006) tested the decrease in photochemical efficiency of L. variegata under higher flows and after adding a CA inhibitor, resulted in no change in photosynthetic efficiency, indicating the loss of the external CA.

Second, although increased flow speeds can enhance growth rates (Carpenter et al. 1991), it can also have negative effects on the thalli or decrease the ability to uptake and retain CO$_2$ and/or HCO$_3^-$. CO$_2$ is an important substrate for photosynthesis and under high flow speeds, the thickness of DBL decreases. But in turn, could also increase the potential efflux of CO$_2$ out of the cell, as the alga is unable to retain the ions (OH$^-$) rapidly diffusing across the cell membrane (Hurd 2000). Collectively, potential effects of self-shading or thalli clumping, external CA dislodgement, and changes in the flux of substrates (CO$_2$, O$_2$, and nutrients) under higher flow speeds can result in adverse effects on metabolism and growth that outweigh any benefits.

Interestingly, L. variegata showed markedly higher RGR at 5 cm s$^{-1}$ compared to the two fleshy species. Enríquez and Rodríguez-Román’s (2006) demonstrated similar results, revealing a photosynthetic optima for L. variegata at an intermediate flow speed and photosynthesis declining with increasing seawater velocity. It is possible that as irradiance increases, the use of CCMs and carbon fixation are enhanced, thus increasing photosynthesis and growth for algae with CCMs that typically are found in lower light
environments (Hepburn et al. 2011). *L. variegata* is typically seen in crevices or lower surfaces of coral structures and the transition to the outdoor flumes where light is not a limiting factor may have been beneficial for algal growth.

Yet the two HCO$_3^-$ users exhibited different patterns of RGR, despite being from the same family, Dictyotaceae, both of which have CCMs. *D. bartayresiana* resulted in lower growth rates than *L. variegata* across all three flow speeds. One explanation for the poor growth response of *D. bartayresiana* to flow is that it is quite susceptible to fragmentation (M. Ho pers. obs.). Although algae were placed in mesh bags with openings large enough to not restrict growth, measurements of the growth rates of *D. bartayresiana* still were compromised due to fragmentation, with smaller pieces escaping the openings in the mesh bags. Renken et al. (2010) also reported a high rate of fragmentation of *Dictyota pulchella* due to its susceptibility to dislodgement due to wave exposure. Between the two fleshy species, *D. bartayresiana*, a bicarbonate user generally exposed to more dynamic environmental conditions, had overall lower growth rates across treatments than *A. rhodantha*. Therefore, it is likely that the RGR reported here is not a good representation of growth rates for *D. bartayresiana*.

Despite the two HCO$_3^-$ users exhibiting contrasting growth rates between treatments, *L. variegata* still displayed an overall higher RGR than *A. rhodantha*, a CO$_2$ user. Under OA conditions, it is possible that algae with CCMs can down-regulate their CCM activity (Giordano et. al. 2005) and instead allocate more energy toward growth (Zou and Gao 2009; Raven et al. 2011). Additionally, *L. variegata* has a larger surface area that potentially could result in increased light capture, offering an advantage to having flat, decumbent blades than flexible, undulated blades regardless of flow.
velocities (Koehl and Alberte 1988). Still, it is expected that bicarbonate users would show little response to OA conditions compared with CO$_2$ users (Zou 2005). Yet, *A. rhodantha* had negligible differences between treatments while *L. variegata* had reduced growth rates notably under elevated CO$_2$. Additionally, when combined with restricted water motion, *A. rhodantha* exhibited the strongest negative effect on growth rates for the three species. This might be due to being carbon and mass-transfer limited, resulting in overall lower growth rates than for *L. variegata*. Variable water motion can affect the accumulation of OH$^-$ and O$_2$ at the thallus surface, both important by-products for photosynthesis (Hurd 2000). Under slow flow, OH$^-$ can accumulate rather than O$_2$, lowering photosynthetic rates and reducing growth (Gonen et al. 1995). The responses of *A. rhodantha* contradicts the prediction that CO$_2$ users are expected to benefit with increasing CO$_2$ concentrations, and have higher growth rates under future conditions compared to HCO$_3^-$ users. Similarly, Cornwall et al. (2012) examined photosynthetic responses of five macroalgal species based on their carbon use under OA conditions and detected no differences in photosynthetic rates for any of the species. In addition, obligate CO$_2$ users did not benefit under OA conditions as predicted in their study. Although they did not measure the same response variables, differences in photosynthetic rate would be predicted to affect algal growth rates. However, other studies (e.g. Kübler et al. 1999) demonstrated that increasing CO$_2$ concentrations increased growth rates for obligate CO$_2$ species. This argues for additional and longer-term experiments to test the effects of OA with various light and flow regimes to better understand how macroalgal responses are influenced based on their carbon acquisition.
As an increasing number of studies are focusing on understanding the underlying mechanisms involved in organismal responses to global threats, it is important to examine multiple environmental and physical factors simultaneously to better understand the variability in algal responses. For coral reefs, many benthic organisms are exposed to a dynamic flow environment that may result in species-specific responses due to different physiological adaptations to flow conditions (Cornwall et al. 2014). Differing responses of macroalgae to water flow and OA might alter competitive outcomes between species, potentially changing community composition. This present study demonstrates the variability in responses of macroalgae to be species specific when various environmental factors are involved. There was a significant effect of flow and of $p$CO$_2$ on macroalgal growth rates but not an interactive effect. Additionally, growth rates differed among species within flow treatments. Contrary to the a priori hypothesis, the obligate CO$_2$ user, *A. rhodantha* did not have higher RGR compared to the facultative HCO$_3^-$ users, *L. variegata* and *D. bartayresiana*.

The increase in CO$_2$ concentrations is predicted to benefit macroalgae and enhance their ability to photosynthesize and grow (Hepburn et al. 2011; Cornwall et al. 2012; Koch et al. 2013; Kroeker et al. 2013; Kram et al. 2015). This might have broader implications on community structure if OA promotes algal growth, increasing its ability to compete for space with other benthic organisms, in particular corals. The different carbon uptake strategies can affect how they will respond to different environmental conditions, making it difficult to predict how OA will affect physiological responses in macroalgae. While studies suggest that OA may enhance metabolic processes, studies on the underlying mechanisms influencing algal physiology and the activity of CCMs are
lacking (Raven et al. 2011; Johnson et al. 2014). If macroalgal responses are affected by their ability to utilize different DIC species, it might determine future competitive interactions among benthic organisms and drive the trajectories of the community structure of future coral reefs.
Table 1. Mean carbonate chemistry for each flow speed treatment (0.1 cm s\(^{-1}\), 5 cm s\(^{-1}\), 8.5 cm s\(^{-1}\)) at ambient and high \(pCO_2\) treatments. The partial pressure of CO\(_2\) \((pCO_2)\) was calculated from salinity (PSU), pH\(_T\) (total scale), total alkalinity \((A_T)\), and temperature. SE of temperature and salinity were < 0.2. Values are mean ± SE (n=14 except \(A_T\)).

<table>
<thead>
<tr>
<th>Flow Speeds</th>
<th>Treatment</th>
<th>T (°C)</th>
<th>Sal</th>
<th>pH(_T)</th>
<th>pCO(_2) (µatm)</th>
<th>(A_T) (µmol • kg(^{-1}))</th>
<th>HCO(_3) (µmol • kg(^{-1}))</th>
<th>CO(_2) (µmol • kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 cm • s(^{-1})</td>
<td>Ambient CO(_2)</td>
<td>27.2</td>
<td>35.4</td>
<td>8.05 ±0.01</td>
<td>397.44 ±7</td>
<td>2316.17 ±3(8)</td>
<td>1751.19 ±7</td>
<td>10.65 ±0.2</td>
</tr>
<tr>
<td></td>
<td>High CO(_2)</td>
<td>27.3</td>
<td>34.9</td>
<td>7.71 ±0.02</td>
<td>989.84 ±47</td>
<td>2309.03 ±1(8)</td>
<td>2006.99 ±11</td>
<td>26.47 ±1.2</td>
</tr>
<tr>
<td>5 cm • s(^{-1})</td>
<td>Ambient CO(_2)</td>
<td>27.3</td>
<td>36.0</td>
<td>8.07 ±0.01</td>
<td>372.91 ±14</td>
<td>2320.14 ±3(8)</td>
<td>1718.27 ±14</td>
<td>9.93 ±0.4</td>
</tr>
<tr>
<td></td>
<td>High CO(_2)</td>
<td>27.2</td>
<td>35.9</td>
<td>7.71 ±0.02</td>
<td>998.30 ±45</td>
<td>2319.22 ±4(8)</td>
<td>2012.21 ±11</td>
<td>26.62 ±1</td>
</tr>
<tr>
<td>8.5 cm • s(^{-1})</td>
<td>Ambient CO(_2)</td>
<td>27.0</td>
<td>35.8</td>
<td>8.04 ±0.01</td>
<td>403.53 ±10</td>
<td>2316.50 ±2(8)</td>
<td>1753.28 ±9</td>
<td>10.82 ±0.3</td>
</tr>
<tr>
<td></td>
<td>High CO(_2)</td>
<td>26.9</td>
<td>35.8</td>
<td>7.68 ±0.01</td>
<td>1065.21±35</td>
<td>2318.91 ±2(8)</td>
<td>2033.00 ±8</td>
<td>28.64 ±1</td>
</tr>
</tbody>
</table>
Figure 1. (A) Photograph of four outdoor flumes with a working section of 0.3 x 0.3 x 5.0 m for each flume. (B) Closer view of macroalgal species randomly allocated along an individual flume subjected to various $pCO_2$ and flow speed conditions. (C) Photographic images of *D. bartayresiana* (left) and *A. rhodantha* (right) placed in nylon monofilament mesh bags with $\frac{1}{2}''$ square openings attached to a 2 in.$^2$ light grey PVC plate acting as a weight.
Figure 2. Relative growth rates (RGR) of *Dictyota bartayresiana*, *Lobophora variegata*, and *Amansia rhodantha* subjected to three flow speeds (0.1 cm s$^{-1}$, 5 cm s$^{-1}$, and 8.5 cm s$^{-1}$) and two $p$CO$_2$ levels. White bars represent RGR measured in ambient $p$CO$_2$ conditions (400 µatm) and black bars represent RGR measured in elevated $p$CO$_2$ conditions (~1,000 µatm). Bars represent mean ± standard error (n=20).
Chapter 3

Effects of elevated pCO₂ and temperature on metabolic and growth responses for
two species of tropical non-calcifying macroalgae

Introduction

Algae utilize inorganic carbon as their sole carbon source for growth and photosynthesis. Under current ambient seawater, the relative partitioning of dissolved inorganic carbon (DIC or C_T) is 92% of inorganic carbon occurs as HCO₃⁻, 1% as CO₂, and the remainder as CO₃²⁻ (Zou et al. 2011). The increase in atmospheric CO₂ due to anthropogenic activities will alter the proportions of DIC while reducing seawater pH, a process known as ocean acidification (OA). A recent meta-analysis revealed OA to have adverse effects on many benthic reef organisms (Kroeker et al. 2010), particularly calcifying organisms that depend on CO₃²⁻ for their skeletal structures since less CO₃²⁻ is available for calcification under OA conditions (Hofmann and Bischof 2014). Additional studies have shown that the level of sensitivity varies across taxa (Harvey et al. 2013; Kamenos et al. 2013; Comeau et al. 2014), with much of the focus on (crustose) coralline algae, as this taxonomic group serves important ecosystem functions such as inducing coral larval settlement (Doropoulos and Diaz-Pulido 2013) and providing habitat structure (Diaz-Pulido et al. 2012). Moreover, many major contributors to the carbon budgets of coral reefs (Martin et al. 2013). However, many organisms also rely on non-calcifying macroalgae for food and habitat (Raven and Hurd 2012) and their ability to assimilate different inorganic carbon (Ci) species (i.e. HCO₃⁻ or CO₂) depends on how abundant DIC is. As atmospheric [CO₂] continues to increase, how macroalgae respond
to the changes in DIC partitioning will have important implications and effects on the composition of reef ecosystems.

CO₂ is an important component of the carbonate buffering system and while all marine macroalgae can acquire CO₂ diffusively, it can be limiting under present CO₂ conditions (Hurd et al. 2009). Many species cannot achieve maximum photosynthesis using CO₂ alone (Kübler et al. 1999; Giordano et al. 2005) and have developed carbon-concentrating mechanisms (CCMs) for active uptake of HCO₃⁻ (Beardall et al. 1998). The interconversion of CO₂ and HCO₃⁻ involves the enzyme carbonic anhydrase (CA) located either intracellularly or extracellularly (Maberly 1990). Since the rate of diffusion of CO₂ in water is relatively slow, the ability to utilize HCO₃⁻ and/or CO₂ aids RUBISCO as a more efficient method to capture CO₂ (Raven and Hurd 2012). Although transporting HCO₃⁻ across cellular membranes requires energy, the relatively large HCO₃⁻ pool available enhances carbon acquisition for HCO₃⁻ users, benefitting their photosynthetic activity (Koch et al. 2013). The facultative ability to use HCO₃⁻ or CO₂ is thought to provide an advantage over obligate CO₂ users by eliminating the risk of carbon limitation under low CO₂ concentrations (Raven et al. 2011). As a result, there has been growing interest in elucidating the physiological responses to OA and understanding the mechanisms by which algae are influenced based on their mode of photosynthetic carbon acquisition.

The predicted increase in atmospheric CO₂ from current concentrations of 400 µatm to near or above 1,000 µatm by 2100 will shift the Ci speciation in seawater, increasing CO₂ availability by 194% and HCO₃⁻ by only 14% (The Royal Society 2005). Since many macroalgal species utilizing HCO₃⁻ are nearly saturated with respect to
photosynthesis under current DIC concentrations in seawater, the increase in atmospheric
CO$_2$ is expected to have little benefit photosynthetically (Kübler and Dudgeon 2015).
However, bicarbonate users may downregulate their energy intensive CCM activity, and
reduce energy expenditures on active carbon uptake, perhaps directing their energy
instead towards growth or other purposes (Hurd et al. 2009). However, those exclusively
using CO$_2$ as a substrate for photosynthesis (i.e. lacking CCMs) are predicted to have
enhanced growth and/or photosynthetic rates under OA conditions (Zou 2005; Hepburn
et al. 2011). This can potentially alleviate carbon limitation and increase or decrease
competition among non-calcareous algae. Simultaneously, the continuous release of
atmospheric CO$_2$ is contributing to global warming, leading to increased sea surface
temperatures (SST). By the end of the century, SSTs are expected to increase by 0.6-2.0
°C (IPCC 2013). Together, global warming and ocean acidification are considered the
two greatest global threats marine ecosystems currently face. Both can have deleterious
effects on organismal growth, survival, and relative abundances, which can ultimately
affect community structure and marine biodiversity.

Many studies have examined the effects of temperature on macroalgae,
documenting the range over which macroalgae can persist depending on their geographic
distribution (Padilla-Gamiño and Carpenter 2007b; Keith et al. 2014). Thermal limits can
vary among species, and their tolerance can be dependent on their habitat. Species in
habitats where temperatures vary seasonally may be more tolerant to longer-term
temperature fluctuations than those experiencing a more consistent temperature year
round. The optimum temperature for photosynthesis ranges from 21-32 ºC for temperate
macroalgae and 27-34 ºC for tropical macroalgae (Koch et al. 2013). Having a narrower
thermal window may result in higher sensitivity to warming, as many tropical species are currently growing in the upper temperatures of their ranges. Photosynthesis relies on enzymatic activity and the transport of carbon substrate, both of which can be affected strongly by temperature. Increasing temperature can enhance metabolic rates of most organisms within their thermal tolerance window (Davison 1991), however, beyond their thermal tolerance limit can result in detrimental effects to their physiology. Due to differences in physiological performance and adaptation among species, the changes in photosynthesis and respiration rates in macroalgae can significantly alter carbon metabolism (Zou et al. 2011).

Currently, elevated CO$_2$ concentrations in seawater are negatively impacting a number of calcifying species arising from the dissolution of carbonate skeletal structures (Kroeker et al. 2010). Conversely, studies have examined bleaching as a result of warmer temperatures, negatively affecting growth and survival of calcifiers (Anthony et al. 2011b; Diaz-Pulido et al. 2012; Kram et al. 2015). Thus, the increase in SST is expected to exacerbate the negative effects of OA. The opposite effect has been seen for non-calcifying species and this could be due to having different carbon uptake strategies (Kübler et al. 1999; Hurd 2000; Cornwall et al. 2012; Kram et al. 2015). OA has resulted in positive or neutral physiological responses for non-calcifying macroalgae (e.g. Israel and Hophey 2002; Connell and Russell 2010; Johnson et al. 2012) and increased SST is predicted to interact positively with OA, enhancing metabolic and growth responses. Harvey et al. (2013) conducted a meta-analysis examining the direct and interactive effects of OA and warming on various response variables of both calcifying and non-calcifying marine organisms. But for corals and non-calcifying macroalgae, the effects of
OA and warming in isolation exhibited negative and positive effects on corals vs. macroalgae, respectively. When the two stressors were combined, OA and warming synergistically reduced calcification, reproduction, and survival of calcifiers, and increased photosynthesis but had no effect on growth. For non-calcifiers, the responses varied but growth generally was promoted by the interactive effect. The inconsistent responses demonstrate the lack of knowledge about the underlying mechanisms resulting in the varying responses among algae and the difficulty to make predictions of their sensitivity to environmental fluctuations difficult. Hence, understanding differences in physiological responses calls for incorporating multiple stressors for a more ecologically relevant approach.

The focus of this study was to examine two tropical, non-calcifying macroalgal species *Amansia rhodantha* and *Dictyota bartayresiana* differing in their carbon uptake strategy, and their metabolic (i.e. photosynthesis and respiration) and growth responses to direct and combined effects of elevated $pCO_2$ and temperature. *A. rhodantha* and *D. bartayresiana* are two morphologically similar macroalgal species common on the back reefs of Moorea, French Polynesia. Their carbon uptake strategy was determined using the pH drift method (Axelsson and Uusitalo 1988), which indicated that *D. bartayresiana* is a HCO$_3^-$ user, and *A. rhodantha* as a CO$_2$ user (R. Carpenter unpublished data). Based on their mechanism of carbon acquisition, I hypothesized *A. rhodantha* (CO$_2$ user) would have enhanced metabolic and growth rates under OA conditions while *D. bartayresiana* (HCO$_3^-$ user) would not be affected by $pCO_2$ treatments. Additionally, increased temperature within their tolerance window is expected to enhance metabolic rates. Therefore, I predicted both species to have higher metabolic rates under elevated
temperature and when combined with elevated $p\text{CO}_2$, these combined factors would increase the photosynthesis, respiration, and growth responses of $A.\ rhodantha$ compared to $D.\ bartayresiana$.

**Methods**

To test the hypothesis that the combined effects of elevated $p\text{CO}_2$ and temperature affects the metabolic and growth responses of both non-calcifying macroalgal species, two similar experiments were conducted in laboratory mesocosms in Moorea for 7 and 10 days in January and July 2015, respectively. The first experiment examined two temperature levels that mimicked the low (25 °C) and high (30 °C) levels exhibited in the seasonal range in the back reef of Moorea (MCR-LTER Dataset ID: knb-lter-mcr.1035). This range was used to determine if metabolic and growth responses were affected by a 5 °C difference within the annual temperature range. The second experiment in July 2015 incorporated the ambient temperature (27.5 °C) that algae were currently experiencing. Both experiments were crossed with the same ambient ($\sim$400 µatm) and elevated ($\sim$1000 µatm) $p\text{CO}_2$.

**Study organisms and collection**

Experiments were conducted at the Richard B. Gump South Pacific Research Station in Cook’s Bay, Moorea. Similar sized ($\sim$4.5 g) samples of $D.\ bartayresiana$ and $A.\ rhodantha$ were collected in the back reef of the north shore at 1-2 m depth. Samples were collected 1-2 days prior to treatment, cleaned of epiphytes, and placed individually into nylon monofilament mesh bags with 1.3-cm openings. Due to the propensity for
fragmentation for *D. bartayresiana*, mesh bags were used to retain algal biomass and both species were placed in mesh bags to standardize treatment effect. Bags containing the algal samples were attached to 2.5-cm round, 3.8-cm high PVC bases with zip-ties that positioned the algae upright provided exposure to water movement and light. Light (photosynthetically active radiation, PAR) measurements were made inside and outside the mesh bags in the mesocosms using the Walz (Model 210, Effeltrich, Germany) pulse amplitude modulated fluorometer (PAM) $2\pi$ cosine light sensor calibrated to a LI-COR (LI 192-sa) sensor. Algal samples were kept in a flow-through seawater table for 2 days during preparation before being transferred to their respective treatments.

*Experimental design*

In experiment 1, eight 150-L mesocosms were divided into four elevated $pCO_2$ treatment tanks and four ambient $pCO_2$ treatment tanks (Fig. 1). Due to logistical limitations, an additional 150-L tank was used as a header tank where the pH was controlled to create the elevated CO$_2$ treatments. While this experimental setup creates pseudoreplication when measuring individual responses from algae within each tank, the continuous replacement of the large volume of seawater and re-allocation of algal individuals every 2 d within each tank minimizes the potential effects of a lack of independence (Comeau et al. 2016a). The header tank and four ambient tanks were supplied with flowing ambient seawater pumped from Cook’s Bay at 12-m depth and filtered through a sand filter (nominal filter size ~100 µm). To create the elevated $pCO_2$ treatment, pure CO$_2$ was bubbled into the header tank, creating a target elevated $pCO_2$ of ~1,000 µatm. The CO$_2$-enriched seawater then was dispensed into four, randomly
assigned elevated CO$_2$ treatment tanks. Five individuals of each species were allocated randomly to one of eight 150-L mesocosms (n=40 per species). Two target levels of temperature, 25 °C and 30 °C were crossed with two pCO$_2$ treatments, ~400 µatm and ~1000 µatm. This created four combinations of treatments with two tanks per treatment: low temperature-ambient pCO$_2$ (LT-ACO$_2$), low temperature-elevated pCO$_2$ (LT-HCO$_2$), high temperature-ambient pCO$_2$ (HT-ACO$_2$), and high temperature-elevated pCO$_2$ (HT-HCO$_2$). The header tank was maintained at the ambient temperature of 27.5 °C and each treatment tank was independently heated and chilled to the respective temperatures.

The second experiment incorporated the ambient seawater temperature in July to examine if algal species are experiencing the thermal optima for metabolic responses. Experiment 2 consisted of 12, 150-L mesocosms, six elevated and six ambient pCO$_2$ treatment tanks filled with fresh filtered seawater pumped from Cook’s Bay. Each elevated CO$_2$ tank was individually controlled for pH and had pure CO$_2$ bubbled in, targeting the same elevated pCO$_2$ of ~1,000 µatm. An additional temperature level was added, consisting of three target levels: 25 °C (low), 27.5 °C (ambient), and 30 °C (high), each crossed with the same two pCO$_2$ treatments as the first experiment. This created six treatment combinations with two tanks per treatment: low temperature-ambient pCO$_2$ (LT-ACO$_2$), low temperature-elevated pCO$_2$ (LT-HCO$_2$), ambient temperature-ambient pCO$_2$ (AT-ACO$_2$), ambient temperature-elevated pCO$_2$ (AT-HCO$_2$), high temperature-ambient pCO$_2$ (HT-ACO$_2$), and high temperature-elevated pCO$_2$ (HT-HCO$_2$). Additionally, the sample size was increased to ten individuals of each species randomly distributed to one of twelve 150-L tanks (n=120 per species).
In both experiments, each tank had a 75-W LED module (Sol White LED Module, Aqua-Illumination) on a 12 hour light : 12 dark cycle to mimic the natural light cycle, with the light intensity gradually ramping up from 0-100% starting at 06:00 h over 4 h, staying at the maximum intensity at 10:00 h for 4 h, then gradually ramping down for the last 4 h. Mid-way through each experiment, irradiance was measured below the seawater surface and a few cm above the algae using the PAM 2π cosine light sensor. Fresh seawater was replaced continuously at ~210 mL min\textsuperscript{−1} and was re-circulated within the tanks with Rio 8HF Hyper flow water pumps. Tanks were cleaned daily and algal individuals were moved within the tanks randomly every 2 days to eliminate position effects.

Carbonate chemistry and maintenance

The targeted ambient $p$CO\textsubscript{2} (400 µatm) and elevated $p$CO\textsubscript{2} (1000 µatm) levels were maintained in tanks using a pH-stat aquacontroller (Neptune systems) that bubbled in either pure CO\textsubscript{2} or CO\textsubscript{2}-free air. $p$CO\textsubscript{2} conditions were representative of current conditions and the projected condition at the end of the century under a “business as usual” scenario from the IPCC (2013) Representative Concentration Pathways (RCP) 8.5 (van Vuuren et al. 2011).

Temperature was measured daily at 08:00, 14:00, and 17:30 local time using a certified digital thermometer (Fisher Scientific, 15-07708, ± 0.05 °C). In addition, daily pH (total scale) measurements were recorded using a hand-held pH meter (Orion 3-stars) fitted with a Mettler DG 115-SC pH probe, which was calibrated every third day against certified TRIS buffer (A. Dickson, San Diego, USA). Weekly pH measured using a
spectrophotometer and m-cresol dye (Dickson et al. 2007), providing similar results that were within ± 0.006 units of those obtained with the portable pH meter. Seawater samples were collected from the tanks at ~08:00 every third day to analyze seawater chemistry for salinity, \( pCO_2 \), and total alkalinity (\( A_T \)). Measurements of \( A_T \) were made using 50-mL samples of seawater in an automatic titrator (T50, Mettler-Toledo) following the standard procedure of Dickson et al. (2007). Titrations of certified reference materials (CRM) provided by A.G. Dickson (Batch 130) were used to analyze the accuracy and precision of the titrated samples, yielding \( A_T \) values within 8 and 2 µmol kg\(^{-1}\) of the certified value for the first and second experiment, respectively. The R package seacarb (Lavigne and Gattuso 2013) was used to calculate the carbonate system parameters with salinity, temperature, \( A_T \) and pH\( _T \).

Response variables

**Growth.** The fresh weight (FW) algal biomass was used to estimate growth measurements. This was determined by the wet (spun dry) weight before and after incubation. Each individual in a mesh bag was spun in a salad spinner for 10 s and immediately weighed (± 0.001g). To determine daily growth rates, relative growth rate (RGR) expressed as percent per day was calculated using the following equation adapted from Yong et al. (2013):

\[
\text{RGR (\% day}^{-1}\) = \log \left( \frac{W_f}{W_i} \right) \times \frac{100}{t}
\]

where \( W_f \) is the final and \( W_i \) is the initial wet weight of the algae, and \( t \) the time in days the algae were subjected to treatments.
**Photosynthesis and respiration.** Measurements were taken within 2-3 days of the end of treatment incubations. All algal samples were weighed for wet biomass and a subsample of algal individuals (n=2 for experiment 1 and n=3 for experiment 2) were selected randomly from each tank (i.e. treatment replicate) to measure photosynthesis and respiration as the change in dissolved oxygen over time. Two 250-mL acrylic chambers were used concurrently, each fitted with a temperature probe and a PreSens dipping oxygen sensitive optode connected to a Fibox 3 minisensor oxygen meter (Precision Sensing GmbH, Germany), simultaneously measuring oxygen concentration and temperature in the chamber. At the start of incubations, the oxygen probe was calibrated with temperature compensation, using a 2-point calibration in 100% air-saturated water and water with zero oxygen (supersaturated sodium dithionite, Na$_2$S$_2$O$_4$). The acrylic chamber was surrounded by a water jacket connected to a circulating water bath, allowing control for the appropriate temperature (25 °C, 27.5 °C, 30 °C). At the base of the chamber was a stir bar to create water motion, and a circular transparent mesh screen separating the stir bar from the incubated alga to prevent contact with the alga. Two fiber optic halogen lights were used to provide light for photosynthesis, providing PAR levels similar to what the algae experienced in the mesocosms (~200 μmol quanta m$^{-2}$ s$^{-1}$; measured with a Walz PAM 2π cosine light sensor).

The photosynthetic rate was estimated by measuring O$_2$ evolution over time forming a steady slope (usually within 25 minutes). Subsequent to the photosynthesis incubations, individuals were allowed to acclimate for 5 minutes in chambers covered with an opaque shroud creating a dark treatment, and respiration measured for O$_2$ consumption over time. Each algal individual was incubated in seawater from its
corresponding treatment ($pCO_2$ and temperature) in the chamber. At the end of each incubation (i.e. photosynthesis and respiration), the incubated seawater was replaced with new seawater from the appropriate treatment. Afterwards, algal individuals were dried at 80 ºC (Fisher Scientific Isotemp Oven) for 24 h and metabolic rates were normalized to dry mass as $\mu$mol O$_2$ g$^{-1}$ hr$^{-1}$.

Statistical analyses

Tank parameters were compared among tanks with a two-way analysis of variance (ANOVA), with temperature and $pCO_2$ as fixed treatment effects and tank representing a random factor nested within the interaction of treatments. The tank effect was dropped from the statistical analyses when not significant with $p > 0.25$ (Quinn and Keough, 2002) to increase statistical power. Normalized rates of photosynthesis and respiration and the relative growth rates of algae were analyzed using a three-way partially nested ANOVA with temperature (25 ºC, 27.5 ºC, 30 ºC) and $pCO_2$ (ambient or elevated) as the fixed, between-plot effects, replicate tanks as a random nested factor, and algal species as a fixed, within-plot factor. Data were log-transformed when necessary to meet ANOVA requirements for normal distribution. Analyses indicating significant main effects were followed by Tukey’s post hoc tests to analyze differences between species and treatments (temperature and $pCO_2$). In Experiment 2, extensive fragmentation of $D$. bartayresiana occurred during the treatment. This precluded the analysis with a nested experiment in the design. Therefore, in order to interpret the results of growth rates, a post hoc analysis was conducted in which tanks were pooled and a normal three-way ANOVA was applied with $pCO_2$, temperature, and species as fixed factors. All analyses
were performed using RStudio software (R Foundation for Statistical Computing), and assumptions of normality and equality of variance were evaluated through graphical analyses of residuals.

Results

Experiment 1

The conditions within tanks were regulated at a mean irradiance of 202 ± 13 μmol quanta m⁻² s⁻¹. Mean PAR inside and outside the mesh bags were ~190 μmol quanta m⁻² s⁻¹, indicating that there were no shading effects by placing algae inside the bags. The mean values of the pCO₂ treatments differed slightly from the targeted values. For the low and high temperature treatments, the elevated pCO₂ level was maintained at 870 ± 28 and 965 ± 28 μatm (± SE, n=16), respectively. The mean pCO₂ levels for the ambient treatments were maintained at 325 ± 4 μatm for the low temperature and 391 ± 8 μatm for the high temperature treatments (± SE, n=16). Mean pH and pCO₂ levels differed between pCO₂ treatments (F₁,₄ ≥ 2307.53, p < 0.001) and temperature treatments (F₁,₄ ≥ 48.38, p < 0.002) but not between tanks within treatments (F₄,₅₆ ≤ 0.317, p ≥ 0.865). Temperature treatments were maintained at a mean of 25.52 ± 0.05 °C and 25.46 ± 0.06 °C (± SE, n=16) under ambient and elevated pCO₂ levels, respectively, for the low temperature treatment and the targeted high temperature treatments were maintained at 30 ± 0.07 °C and 29.13 ± 0.13 °C (± SE, n=16) under ambient and elevated pCO₂ levels, respectively (Table 1). Temperatures differed between treatments (F₁,₄ = 2361.69, p < 0.001) but not between tanks within the interacting treatments (F₄,₅₆ = 1.05, p > 0.39). Total alkalinity (A_T) did not vary between either treatments (F₁,₄ ≤ 0.148, p ≥ 0.720) but
did differ between tanks within the interacting treatments ($F_{4,56} = 3.607, p = 0.011$). However, the variation between paired tanks was small (means differing $<4 \mu$mol kg$^{-1}$).

Under ambient CO$_2$ conditions, $A_T$ averaged $2297.4 \pm 2 \mu$mol kg$^{-1}$ at $25 \degree C$ treatment and $2299.3 \pm 2 \mu$mol kg$^{-1}$ at $30 \degree C$. Under elevated CO$_2$ conditions, $A_T$ averaged $2300 \pm 1 \mu$mol kg$^{-1}$ for the $25 \degree C$ treatment and $2298 \pm 1 \mu$mol kg$^{-1}$ for the $30 \degree C$ treatment ($\pm SE, n=8$).

The nested tank factor for both metabolic responses was removed ($p > 0.25$) from subsequent analyses and tanks were pooled across treatments. All response variables for Experiment 1 were log-transformed to meet assumptions of normality. In general, photosynthetic rates increased under elevated $p$CO$_2$ conditions for both species. The rates of net photosynthesis normalized to biomass dry weight were affected significantly by $p$CO$_2$ treatment ($F_{1,24} = 10.75, p = 0.003$), with $D. bartayresiana$ having higher rates of net photosynthesis at $25 \degree C$ ($\sim 94 \mu$mol O$_2$ g$^{-1}$ hr$^{-1}$) than $A. rhodantha$ ($\sim 76 \mu$mol O$_2$ g$^{-1}$ hr$^{-1}$). There was neither a difference between species, a significant effect of temperature, nor significant for any of the interacting factors ($F_{1,24} \leq 1.590, p \geq 0.22$). For rates of respiration of $A. rhodantha$ and $D. bartayresiana$, there was a significant difference between species ($F_{1,24} = 19.074, p = 0.0002$) and species within each temperature treatments ($F_{1,24} = 5.83, p = 0.023$). In both temperature treatments, $D. bartayresiana$ had higher rate of respiration than $A. rhodantha$ (Fig. 2). Although there was no main effect of $p$CO$_2$ ($F_{1,24} = 0.080, p = 0.780$) or temperature ($F_{1,24} = 0.226, p = 0.639$), there was a significant interactive effect between temperature and $p$CO$_2$ ($F_{1,24} = 6.605, p = 0.017$).

The growth rates of $A. rhodantha$ and $D. bartayresiana$ were not affected significantly by temperature ($F_{1,72} = 0.271, p = 0.604$) or $p$CO$_2$ ($F_{1,72} = 0.025, p = 0.874$).
However, growth rates did differ by species ($F_{1.72} = 8.01, p = 0.005$). Similar to photosynthetic rates, *D. bartayresiana* had overall higher growth rates, specifically under the HT-HCO$_2$ treatment ($2.93 \pm 0.57 \%$ day$^{-1}$). Although there were no differences between treatments or the interacting treatments, planned comparisons revealed marginal differences between the two species under high $p$CO$_2$ treatment ($p = 0.066$). The growth rates of *D. bartayresiana* increased in comparison to the growth rates of *A. rhodantha* under elevated $p$CO$_2$ (Fig. 4).

**Experiment 2**

The $p$CO$_2$ conditions at 25 °C (low), 27.5 °C (mid), and 30 °C (high), respectively, were maintained at two levels with the following averages ($\pm$ SE, n=16): 366 ± 12, 397 ± 16, and 423 ± 12 µatm under ambient conditions and 975 ± 12, 1005 ± 10, and 1032 ± 16 µatm under elevated conditions (Table 2). Mean pH and $p$CO$_2$ differed between $p$CO$_2$ ($F_{1.6} \geq 3439.51, p < 0.001$) and temperature ($F_{2.6} \geq 9.995, p \leq 0.012$) treatments, but not between tanks within the interaction ($F_{6,84} \leq 0.963, p \geq 0.455$). $A_T$ did not vary between tanks ($F_{6,84} < 0.0001, p = 0.986$) or between either $p$CO$_2$ ($F_{1.6} < 0.0001, p = 0.618$) or temperature ($F_{2.6} = 1.927, p = 0.226$) treatments. Mean $A_T$ for 25 °C, 27.5 °C, and 30 °C, respectively, were 2313 ± 14, 2302 ± 16, and 2304 ± 15 µmol kg$^{-1}$ under ambient $p$CO$_2$ conditions and 2312 ± 14, 2306 ± 16, and 2305 ± 16 µmol kg$^{-1}$ under elevated $p$CO$_2$ conditions ($\pm$ SE, n=8). Temperatures did not differ between tanks within treatments ($F_{6,84} = 1.309, p = 0.262$) but did differ between temperature treatments ($F_{2.6} = 578.08, p \leq 0.0001$). Mean temperature parameters for the two $p$CO$_2$ levels (ambient and elevated, respectively) were 25.18 ± 0.04 and 25.10 ± 0.15 °C for the low temperature, 27.31 ±
0.06 and 26.91 ± 0.07 °C for the mid temperature, and 29.76 ± 0.05 and 29.84 ± 0.17 °C for the high temperature (± SE, n=16).

Both photosynthetic and respiration rates were log-transformed and tanks were pooled across treatments when there was no nested tank effect. In LT-HCO₂, one individual of *D. bartayresiana* was dropped from net photosynthesis analysis due to measurement error, resulting in n=5 when pooled. Net photosynthesis differed between temperature treatments ($F_{2,59} = 3.714$, $p = 0.03$) and by species between temperature treatments ($F_{2,59} = 3.36$, $p = 0.041$). This was driven mainly by *A. rhodantha* having higher rates at 27.5 °C than 25 °C ($p = 0.024$), increasing from the 25 °C to 27.5 °C treatment by 84% at 400 µatm and by 195% under the elevated CO₂ treatment (Fig. 3). Similarly, respiration rates of both species differed between temperature treatments ($F_{2,59} = 4.785$, $p = 0.0119$), with respiration rates of *A. rhodantha* notably higher at 27.5 °C under elevated $p$CO₂ than *D. bartayresiana*. However, between the 27.5 °C and 30 °C temperature treatments, there was a 42% and 53.5% reduction in respiration rates of *A. rhodantha* under ambient $p$CO₂ and elevated $p$CO₂, respectively (Fig. 3).

*D. bartayresiana* fragmented extensively during the treatment incubations, resulting in a significant tank effect. Algal individuals were treated as the statistical replicate instead of tanks, in order to facilitate the interpretation of the results. Therefore, statistical analysis revealed algal growth rates to differ by temperature treatments ($F_{2,228} = 3.524$, $p = 0.031$) and among species ($F_{1,228} = 55.429$, $p < 0.001$). Within treatments, RGR differed among species within temperature ($F_{2,228} = 12.008$, $p < 0.002$), within $p$CO₂ ($F_{1,228} = 15.425$, $p < 0.001$), and within the interacting treatments ($F_{2,228} = 15.777$, $p < 0.001$).
Discussion

Increasing atmospheric CO$_2$ is contributing simultaneously to lowered seawater pH and increasing sea surface temperatures, bringing significant attention to evaluating the interactive effects of these multiple stressors on benthic reef organisms. The present study examined the effects of temperature with ocean acidification on metabolic and growth responses of two common non-calcareous macroalgal species. One experiment conducted in January 2015 and one in July 2015 subjected algae to two and three (respectively) different temperatures within the range experienced in the back reefs and these were crossed with two pCO$_2$ levels. The first experiment resulted in overall higher photosynthetic rates under elevated pCO$_2$ conditions and differing respiration rates between *D. bartayresiana* and *A. rhodantha*. *D. bartayresiana* exhibited higher respiration rates within temperature treatments and between temperature and pCO$_2$ treatments. Additionally, both species had reduced rates under the HT-HCO$_2$ treatment. RGR for *D. bartayresiana* were generally higher than *A. rhodantha*. The results in the second experiment have to be treated with caution due to high fragmentation in *D. bartayresiana* but analyses revealed algal growth rates unaffected by pCO$_2$ but affected by temperature and all interacting treatments. Metabolic rates were insensitive to pCO$_2$ treatments and the interacting effect of pCO$_2$ and temperature. However, between temperature treatments, *A. rhodantha* exhibited varying responses but not *D. bartayresiana*. Temperature affected photosynthetic rates of *A. rhodantha*, resulting in higher net photosynthesis under the 27.5 ºC treatment. Within temperature treatments, rates of respiration differed between the two species, with increased respiration rates for *A. rhodantha* at 27.5 ºC under elevated pCO$_2$ and a decrease under elevated temperature.
Variations in temperature directly affect photosynthesis and respiration responses of organisms and these results suggest that temperature is likely to have a stronger impact on metabolic responses than OA conditions.

Typically as temperature increases, photosynthesis also increases until it reaches a maximum rate at an optimum temperature (Davison 1991). Beyond the optimum range, photosynthetic rates rapidly decline. When algal species were exposed to only two temperatures (i.e. 25 or 30°C), net photosynthesis was unaffected by temperature but enhanced by elevated $p$CO$_2$. When algae were exposed to 25, 27.5 or 30 °C, the $p$CO$_2$ effect disappeared, and net photosynthesis was affected by temperature. The differential responses to temperature variations may be explained by the temperature conditions to which algae are acclimatized. The mean temperature of water over the back reefs of Moorea is ~28.5 °C for January and ~26.3 °C for July. Algae experiencing the ambient temperature of 28.5 °C in January were insensitive to temperature treatments when exposed to the lower and higher temperatures within their thermal range, but had increased photosynthetic rates under elevated $p$CO$_2$. If algal species currently are acclimatized to higher temperatures, then individuals may have a greater tolerance to increases in temperature. However, in July when seawater temperatures in Moorea are ~2 °C lower, algae acclimatized to a lower ambient temperature were either unaffected or negatively affected by warming with effect of CO$_2$. Similar findings of either positive or no responses of photosynthesis to OA and/or warming have been reported in several macroalgae (e.g. Connell and Russell 2010; Kroeker et al. 2013; Harvey et al. 2013). Net photosynthesis of $A$. rhodantha were enhanced by temperature, specifically between 25 and 27.5 °C, then reduced at 30 °C. Interestingly, under the 30 °C treatment, net
photosynthesis of *A. rhodantha* decreased to a comparable photosynthetic rate that was exhibited in Experiment 1. This suggests the potential photosynthetic optimum for *A. rhodantha* to be under the current local temperature the alga is experiencing. However, as seen in other studies, these responses are species-specific and require further studies on HCO$_3^-$ and CO$_2$ users to understand fully how different carbon assimilation can affect photosynthetic responses to OA and/or warming.

Macroalgal photosynthesis is affected by physiological adaptations to various environmental factors and these differences in photosynthetic rates could depend on their ability to utilize CO$_2$ and/or HCO$_3^-$. CO$_2$ users are expected to have higher metabolic rates under elevated CO$_2$ conditions compared to their bicarbonate using counterparts. In Experiment 2, photosynthetic rates for *A. rhodantha* increased under elevated $p$CO$_2$ at ambient and elevated temperatures. However, elevated temperature negatively affected photosynthesis of *A. rhodantha* under ambient $p$CO$_2$, but photosynthesis increased under elevated $p$CO$_2$, suggesting that OA may potentially mitigate the negative effects of increase temperature on *A. rhodantha*. Furthermore, increases in atmospheric CO$_2$ could help alleviate carbon limitation for obligate CO$_2$ users, allowing them to become better competitors for resources under OA conditions, if higher photosynthesis results in increased growth rates. Conversely, HCO$_3^-$ users are predicted to have little response to OA, yet in Experiment 1, photosynthetic rates of both species were enhanced by elevated $p$CO$_2$. Interestingly, *D. bartayresiana* (HCO$_3^-$ user) exhibited higher net photosynthesis than *A. rhodantha*, particularly at 25 ºC under OA conditions. One potential explanation for this is that although bicarbonate concentrations are not expected to increase as much as [CO$_2$] under OA conditions, CCM activity typically is reduced under lower
temperatures (Beardall et al. 1998; Raven 1991). Decreasing the necessity for active transport may benefit *D. bartayresiana* (bicarbonate user) as this species can use CO₂ facultatively.

Along with photosynthesis, respiration also can increase with increasing temperature. Eventually, photosynthesis declines while respiration continues to increase until reaching an optimum temperature, where respiration also declines (Smith and Smith 2009). Contrary to net photosynthesis, both species had decreased respiration rates from ambient to elevated pCO₂ conditions under elevated temperature. While I predicted increase temperature would increase metabolic rates (e.g. respiration) particularly for *A. rhodantha*, I also expected elevated CO₂ to benefit the CO₂ user. In Experiment 1, *D. bartayresiana* exhibited overall higher respiration rates than *A. rhodantha* within temperature treatments, particularly at 25 ºC. In addition, an interactive pCO₂ x temperature effect was present. At 25 ºC, rates of respiration increased by 9% for *D. bartayresiana* and by 30% for *A. rhodantha* under elevated pCO₂ but when temperatures were elevated, rates declined. However, under ambient CO₂ conditions, rates of respiration for *A. rhodantha* were positively affected, increasing by 53% between temperature treatments despite being acclimated to a higher ambient temperature. This suggests temperature has a greater effect on respiration rates than elevated CO₂.

Similar to net photosynthesis in Experiment 2, respiration rates were insensitive to pCO₂ treatments when algae were exposed to three different temperatures. Elevated temperature negatively affected *A. rhodantha*, but the effect was mitigated when combined with elevated pCO₂. Comparable to photosynthetic rates, *A. rhodantha* exhibited higher rates of respiration at the 27.5 ºC treatment. This increase in metabolic
response to temperature was not seen in January, suggesting that species responses are influenced by different thermal acclimatization. *D. bartayresiana* had minimal differences between CO$_2$ treatments and the lack of responses to OA from algae possessing CCMs is expected, as they are currently carbon-saturated, but studies have shown varying responses of respiration rates to elevated $p$CO$_2$. Comeau et al. (2016b) saw a positive response to CO$_2$ in a calcifying macroalga while Zou et al. (2011) found no effects of CO$_2$ on respiration of non-calcifying macroalgae. However, it is unclear why *D. bartayresiana* was unaffected by the temperature treatments. Perhaps *D. bartayresiana* has a wider thermal range resulting in less sensitivity to temperature or the potential to downregulate CCM activity thereby allowing energy to be allocated to other metabolic processes. Despite metabolic processes being temperature dependent, few studies have examined the effects of OA and warming on respiration in non-calcifying macroalgae.

Photosynthetic responses of macroalgae are mediated (in part) by temperature and those responses are typically used to make inferences about their growth rates. However, this can be difficult because photosynthetic optima tend to occur a few degrees higher than growth optima (Davison 1991). While photosynthesis commonly is linked to changes in algal growth rates, other metabolic processes (e.g. dark respiration) regulate growth as well (Davison 1991). Therefore, differences in growth rates are not always coupled directly to changes in net photosynthesis.

The results from both experiments are not consistent with my hypothesis that the combined effects of temperature and ocean acidification would increase growth rates, with a greater positive effect on *A. rhodantha*. In Experiment 1, algal growth rates
differed among species. *D. bartayresiana* generally exhibited a higher RGR than *A. rhodantha*. When exposed to a higher temperature, the result was a slight reduction in RGR for *D. bartayresiana* under ambient *p*CO₂ but an increase in RGR under elevated *p*CO₂. Similar findings by Kram et al. (2015) found growth rates of a temperate fleshy macroalga negatively affected by elevated temperature, but when combined with elevated CO₂, growth rates increased, suggesting CO₂ can alleviate the negative effects of increased temperature. Conversely, growth rates of *A. rhodantha* were slightly enhanced under ambient CO₂ and decreased when exposed simultaneously to elevated CO₂ and temperature. Further limitations to linking temperature effects on photosynthesis and growth occurred in the second experiment due to high fragmentation in *D. bartayresiana*. Although *A. rhodantha* is less susceptible to fragmentation, there was also a negative response to elevated CO₂ at 25 °C. At 27.5 °C, growth rates of *A. rhodantha* increased under elevated CO₂, similar to the responses of net photosynthesis. However, at 30 °C, net photosynthesis was negatively affected yet growth rates increased with increasing temperature. Therefore, interpretation of growth rates should be made with caution as the loss in biomass due to fragmentation limits the understanding of growth responses to OA and warming.

Overall, the adverse responses in the present study from both experiments to OA and warming continue to illustrate the importance of incorporating interactive abiotic factors to better understand the threats to marine ecosystems. Increasing dissolution of atmospheric CO₂ into the oceans has already been seen to have negative impacts on the health of a number of calcifying organisms responsible for the framework of coral reefs (Anthony et al. 2008; Diaz-Pulido et al. 2012; Comeau et al. 2016c). Along with OA,
rising sea surface temperatures also can alter physiological responses of reef organisms. Increases in temperature can increase metabolic rates, and is predicted to act synergistically when simultaneously exposed to OA conditions, positively affecting non-calcareous macroalgae (Olischlager and Wiencke 2013). This would alleviate carbon limitation on obligate CO\textsubscript{2} users by providing more carbon substrate for photosynthesis, potentially providing a competitive advantage with other non-calcareous macroalgae. When algae were acclimatized to higher temperatures in January and then were exposed to two different temperatures, OA had a greater effect on metabolic rates. In July when ambient temperatures were \textasciitilde2 °C lower, elevated temperatures negatively affected net photosynthesis and rates of respiration of \textit{A. rhodantha} while \textit{D. bartayresiana} was insensitive to the separate and combined treatments. This supports the hypothesis that HCO\textsubscript{3}\textsuperscript{-} users are predicted to have little benefit under climate change conditions. The lack of responses in \textit{D. bartayresiana} to temperature suggests that it may have a wider thermal tolerance window than \textit{A. rhodantha}, a CO\textsubscript{2} user. This may change the predictions of physiological responses for HCO\textsubscript{3}\textsuperscript{-} users currently carbon saturated. In addition, the growth rates for this study did not relate to net photosynthesis and only differed among species, which other studies have shown growth rates unaffected by warming and CO\textsubscript{2} (Liu and Zou 2015). Examining short-term temperature effects to changes in metabolic responses has proven useful to better understand physiological adaptations (Davison 1991; Kuebler et al. 1991; Padilla-Gamiño and Carpenter 2007b). However, short-term photosynthetic responses do not always correspond to the thermal tolerance for growth. In order to improve our understanding of how future climate change will affect non-calcifying algae, additional studies need to incorporate multiple stressors affecting
various organismal processes and whether there is a general response for algae that differ in their mode of carbon utilization. The interactive effects of these two global threats will provide better insight on the predictive capability of how sensitive or resilient organisms are in order to scale up individual and population responses to ecosystem level.
Table 1. Tank parameters for Experiment 1 in January 2016 over a 7-day treatment. Each temperature treatment was crossed with either ambient $p$CO$_2$ (ACO2; 400 µatm) or elevated $p$CO$_2$ (HCO2; target-1,000 µatm). Values are mean ±SE (n=16; except $A_T$). Salinity (PSU), pH$_T$ (total scale), total alkalinity ($A_T$), and temperature were used to calculate the partial pressure of $p$CO$_2$. SE of salinity and pH$_T$ were <0.07 and <0.02, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T (°C)</th>
<th>Sal</th>
<th>pH$_T$</th>
<th>$p$CO$_2$ (µatm)</th>
<th>$A_T$ (µmol kg$^{-1}$)</th>
<th>HCO$_3$ (µmol kg$^{-1}$)</th>
<th>CO$_2$ (µmol kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C (LT)</td>
<td>ACO2</td>
<td>25.52 ± 0.05</td>
<td>35.44</td>
<td>8.11</td>
<td>324.86 ± 4</td>
<td>2297.42 ± 2(8)</td>
<td>1690.86 ± 5</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>25.46 ± 0.06</td>
<td>35.34</td>
<td>7.76</td>
<td>870.32 ± 28</td>
<td>2299.99 ± 1(8)</td>
<td>1987.17 ± 7</td>
</tr>
<tr>
<td>30 °C (HT)</td>
<td>ACO2</td>
<td>30.0 ± 0.07</td>
<td>35.49</td>
<td>8.04</td>
<td>391.37 ± 8</td>
<td>2299.30 ± 2(8)</td>
<td>1691.34 ± 8</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>29.13 ± 0.13</td>
<td>35.49</td>
<td>7.72</td>
<td>965.99 ± 28</td>
<td>2298.03 ± 1(8)</td>
<td>1974.13 ± 6</td>
</tr>
</tbody>
</table>
Table 2. Tank parameters for Experiment 2 in July 2016 over a 10-day treatment consisting of three temperature treatments, 25, 27.5 and 30 ºC, under ambient (ACO2; 400 µatm) and elevated (HCO2; target-1,000 µatm) pCO₂ conditions. Values are mean ±SE (n=16; except A_T). Salinity (PSU), pH_T (total scale), total alkalinity (A_T), and temperature were used to calculate pCO₂. SE of salinity and pH_T were <0.04 and <0.01, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T (ºC)</th>
<th>Sal</th>
<th>pH_T</th>
<th>pCO₂ (µatm)</th>
<th>A_T (µmol kg⁻¹)</th>
<th>HCO₃ (µmol kg⁻¹)</th>
<th>CO₂ (µmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ºC (LT)</td>
<td>ACO2</td>
<td>25.18 ± 0.04</td>
<td>35.88</td>
<td>8.08</td>
<td>366.36 ± 12</td>
<td>2312.71 ± 14(8)</td>
<td>1749.64 ± 10</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>25.10 ± 0.15</td>
<td>35.88</td>
<td>7.71</td>
<td>974.83 ± 12</td>
<td>2311.91 ± 14(8)</td>
<td>2035.60 ± 7</td>
</tr>
<tr>
<td>27.5 ºC (AT)</td>
<td>ACO2</td>
<td>27.31 ± 0.06</td>
<td>35.88</td>
<td>8.05</td>
<td>396.79 ± 16</td>
<td>2301.75 ± 16(8)</td>
<td>1737.26 ± 15</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>26.91 ± 0.07</td>
<td>35.88</td>
<td>7.70</td>
<td>1004.78 ± 10</td>
<td>2305.79 ± 16(8)</td>
<td>2021.65 ± 28</td>
</tr>
<tr>
<td>30 ºC (HT)</td>
<td>ACO2</td>
<td>29.76 ± 0.05</td>
<td>35.88</td>
<td>8.02</td>
<td>422.52 ± 12</td>
<td>2304.85 ± 15(8)</td>
<td>1729.65 ± 48</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>29.84 ± 0.17</td>
<td>35.88</td>
<td>7.69</td>
<td>1032.27 ± 16</td>
<td>2305.39 ± 15(8)</td>
<td>1997.88 ± 29</td>
</tr>
</tbody>
</table>
Figure 1. Schematic of the experimental design of Experiment 1 consisting of two temperature levels, 25 °C (LT) and 30 °C (HT), crossed with two $p$CO$_2$ levels, 400 µatm (ACO2) and 1,000 µatm (HCO2). Four combinations of treatments: LT-ACO2, LT-HCO2, HT-ACO2, and HT-HCO2 created two replicates per treatment containing 5 individuals of each algal species.
Figure 2. Metabolic responses of *Dictyota bartayresiana* and *Amansia rhodantha* to ocean acidification and warming conditions for Experiment 1 in January. Bars are mean rates (±SE) of net photosynthesis and respiration (*n*=4 for each bar) on both algal species after 7 days of incubation. Solid bars represent ambient $p$CO$_2$ (ACO2) and striped bars represent elevated $p$CO$_2$ (HCO2) treatments. The different shades represent different temperature treatments. Net photosynthesis significantly differed between $p$CO$_2$ treatments. Lower case letters denotes differences among species and by temperature treatments on rates of respiration retrieved from *post hoc* multiple comparisons analysis.
Figure 3. Mean rates (± SE; n=6) of photosynthetic and respiration rates of *Dictyota bartayresiana* (n=5 for net photosynthesis of LT-HCO₂) and *Amansia rhodantha* in July (Experiment 2). Algal species were subjected to ambient (solid bars) and elevated (striped bars) pCO₂ levels at various temperatures for 10 days. Different shades represent different temperature treatments. For each response variable, treatments sharing the same small case letters are not significantly different.
Figure 4. Mean relative growth rates of *Dictyota bartayresiana* and *Amansia rhodantha* subjected to different temperatures (reflected by different shades) crossed with two $pCO_2$ levels. (A) Algal species in the first experiment were subjected to two temperatures, 25 and 30 °C, under ambient (solid bars) and elevated (striped bars) $pCO_2$ conditions for 7 days ($n=10$ for each bar). (B) Algal species in the second experiment were subjected to three temperatures, 25, 27.5 and 30 °C in ambient and elevated $pCO_2$ levels for 10 days ($n=20$ for each bar). Data represent mean ± SE.
Chapter 4

The effects of water flow and ocean acidification on interactions between juvenile Porites spp. and the alga Dictyota bartayresiana

Introduction

One of the most important ecological processes determining structures of reef communities is the competition for space among benthic organisms (Tanner 1995). Specifically, corals and macroalgae often are the dominant benthic groups commonly interacting on coral reefs (Lang and Chornesky 1990). Their relative abundances and competitive interactions are generally indicators to the status of coral reefs (McCook et al. 2001). For example, reef degradation commonly has been associated with a decrease in coral cover and increases in macroalgal abundance (Diaz-Pulido et al. 2007; Birrell et al. 2008; Barott and Rohwer 2012). Local impacts such as overfishing (Pandolfi et al. 2003; Rasher and Hay 2010) and nutrient availability (Burkepile et al. 2013) combined with coral bleaching, coral disease, and cyclones have contributed to reef degradation (Hughes et al. 2003; Diaz-Pulido et al. 2009), promoting rapid colonization by macroalgae either by actively overgrowing live corals or occupying dead coral substrata (Diaz-Pulido et al. 2007). The increase in macroalgal abundance can increase competition between corals and algae, further reducing coral settlement and potentially lead to macroalgal-dominated states.

Although overall algal biomass is predicted to increase in coral reef communities, macroalgae serve important ecological and functional roles on coral reefs by contributing to primary production, nitrogen fixation, and providing habitat structure (Diaz-Pulido et al. 2007; Raven and Hurd 2012). However, the concern arises as further anthropogenic
stressors are increasing favorable conditions for macroalgae and creating less favorable conditions for corals. This can alter species composition, changing the overall complex assemblages of coral reefs composed of various calcifying and non-calcifying organisms to a more simplified community structure dominated by fleshy and turf algae (Swierts and Vermeij 2016). Increases in macroalgal cover already have been observed on a number of reefs due to several causes, including storm damage and coral predator and disease outbreaks (McManus et al. 2000). The concern of increasing algal abundance is the potential shift to reefs becoming dominated by algae. For example, phase shifts have been documented on an Australian reef where a red macroalga dominated a region due to a shipwreck spill (Hatcher 1984). It was enhanced further from the low abundance of herbivorous fishes and led to reduced coral cover. More notably, shifts from coral to algal dominance have been reported in the Caribbean due to (but not limited to) hurricanes and reduced herbivory (Hughes 1994; Bruno et al. 2009). The potential shift in community composition can have profound consequences on the economic and ecological value of coral reefs (Hughes et al. 2003). With the ongoing global degradation of reefs due to human impacts, more studies need to address the ecological and physical drivers that may alter competitive interactions between coral and algae.

Together with the number of local impacts coral reefs currently face, global threats such as ocean acidification (OA) are being evaluated as a potential driver in changes to community structures of coral reefs (Connell et al. 2014). The increase in dissolution of CO₂ absorption potentially can alter the competitive balance between corals and algae by reducing growth rates of calcifying organisms (i.e. corals) and perhaps increasing the growth and abundance of algae. The increase in coral mortality
provides substratum for macroalgae to colonize, inhibiting coral recruitment and resulting in an altered state for reefs that often proves difficult to reverse back to coral-domination (Mumby 2009). However, the full extent of how OA affects macroalgae is still unknown. Generally, OA is predicted to benefit macroalgal productivity (Beardall et al. 1998) because the relative abundances of dissolved HCO$_3^-$ and CO$_2$ (Hurd et al. 2009) continue to increase with increasing atmospheric CO$_2$, which are two important carbon sources non-calcareous macroalgae utilize for photosynthesis and growth. For corals, the reduction in carbonate saturation state associated with OA has generally resulted in negative effects on growth rates (i.e. calcification) (Kroeker et al. 2010). This increases their susceptibility to being outcompeted by macroalgae in the benthos. However, studies have shown varying responses by macroalgae to OA (Israel and Hophy 2002; Diaz-Pulido et al. 2007; Kram et al. 2015), making predictions in how OA will drive coral-algal interactions difficult.

Coral-algal competitive interactions can be mediated or exacerbated by a number of abiotic and biotic factors. In coral reefs where space is limited, the physical environment plays a critical role in the facilitation of competition. Specifically, water motion is a key driver in transferring dissolved gases and nutrients (Wheeler 1980), influencing various biological and physiological processes. Additionally, the rate and mode of water motion can affect metabolic rates (Carpenter et al. 1991; Denny 1988); increases in water motion promotes resource acquisition and productivity while decreases in water motion can result in mass transfer limitations of nutrients and gases (Atkinson and Bilger 1992; Hurd 2000). Differences in flow speed affects the thickness of the diffusion boundary layer (DBLs; inversely related to flow speed) that forms on the
surface of benthic organisms, either increasing or decreasing nutrient supply. A decrease in water motion can result in negative effects on growth rates for macroalgae (Hurd 2009). However, Cornwall et al. (2014) found that a thicker DBL due to reduced water motion can ameliorate the negative effects of OA on calcifying organisms (e.g. coralline algae) by acting as a buffer. Therefore, this may decrease the vulnerability of calcifying organisms to competition when combined with OA conditions. Conversely, higher flow speeds combined with the predicted benefit of elevated $p\text{CO}_2$ on macroalgae could potentially exacerbate competitive interactions for corals by further promoting algal growth.

The ability of macroalgae to colonize and/or outcompete corals can be complemented by a variety of environmental factors that can make predictions in competitive outcomes difficult. Additionally, the outcome of competitive interactions can result from a variety of mechanisms that directly or indirectly affect competition (McCook et al. 2001). One such mechanism by which corals and algae compete is the direct effect of overgrowth of coral by macroalgae. This has been documented in a number of studies that have described tissue necrosis for the coral and/or mortality (e.g. Tanner 1995; Jompa and McCook 2003; Nugues and Bak 2006; Box and Mumby 2007; Diaz-Pulido et al. 2009; Diaz-Puldio 2011) and in some cases, together with disturbances have resulted in community shifts from coral- to algal-dominated states (Hughes 1994; McClanahan and Muthiga 1998; Birrell et al. 2008; Cheal et al. 2010). While predicting competitive outcomes in such a dynamic ecosystem is challenging, examining the underlying mechanisms involved in competition can provide a better understanding of the performance of organisms in a changing environment. The frequency and intensity of
competitive interactions can increase due to combined effects of environmental factors (e.g., OA and water flow). When corals are exposed to a disturbance or stressor that affects their physiology negatively, they become more susceptible to being outcompeted. OA has the capacity to reduce the growth and survival rates of corals while increasing algal proliferation. Once established, negative feedbacks can result from the increase in algal abundance and inhibition of coral recruitment, and when combined with reduced herbivory can further suppress coral cover (Rasher et al. 2011).

With increasing concentrations of atmospheric CO$_2$ posing a threat to calcifying organisms, OA potentially might shift the competitive interaction in favor of algae. Additionally, water motion can exacerbate the direct competitive effect on calcifiers (i.e. corals) by promoting algal growth and result in physical contact between algae and corals as flexible algal thalli are moved by oscillatory water motion. The present study investigated the effect of water motion on coral-algal competitive interactions exposed to OA conditions by testing the hypothesis that the combined effects of OA and water flow will alter the competitive interactions between massive *Porites* spp. and *Dictyota bartayresiana*, to favor the alga. These species were chosen based on surveys of coral-algal interaction on the north shore, *D. bartayresiana* is an abundant alga commonly interacting with massive *Porites* spp. in the back reef habitats of Moorea, French Polynesia (M. Ho unpub. data). Massive *Porites* spp. is a dominant coral genus in the Pacific (Done and Potts 1992; Edmunds 2014) and has been shown to be relatively resilient to OA conditions (Fabricius et al. 2011; Edmunds 2012). However, their susceptibility to OA when interacting with a competitor is unknown and therefore, it was chosen as the focal coral genus. The genus *Dictyota* is common on many coral reefs,
particularly in the Caribbean where it frequently interacts with corals (McCook et al. 2001). The high abundance of this species is also documented in the Moorea Coral Reef Long-Term Ecological Research data set (knb-iter-mcr.8). In addition, *D. bartayresiana* utilizes bicarbonate as the main carbon source for photosynthesis and growth and currently is carbon-saturated, thus the expected increase in CO$_2$ is predicted to have little effect on HCO$_3^-$ users since bicarbonate is the dominant form of DIC in seawater (Zou 2005). However, this species is still predicted to benefit under OA conditions compared to calcifiers. Based on this notion, the following hypotheses were tested: (i) elevated pCO$_2$ will positively affect *D. bartayresiana*, (ii) a low flow speed will negatively affect growth rates of *D. bartayresiana* and massive *Porites* spp., (iii) in a competitive setting for space, OA will alter the coral-algal interaction in favor of the alga, and (iv) when OA is combined with increased water motion, it will increase algal growth rates, enhancing the ability of the alga to overgrow the coral, thus exacerbating competitive effects on the coral.

**Methods**

*Study site and collection*

This research was carried out between May and June 2015 at the Richard B. Gump South Pacific Research Station in Cook’s Bay, Moorea, French Polynesia. Large mounding corals commonly on the back reefs of Moorea are composed of two species (*Porites lobata* and *Porites lutea*) that are visually indistinguishable (Edmunds 2009), and thereby referred to as massive *Porites* spp. Study organisms consisted of a dominant macroalgal species, *Dictyota bartayresiana*, and a dominant coral, juveniles (≤ 4-cm
diameter) of massive *Porites* spp., collected from 2-3-m depth on the back reef of the north shore.

*D. bartayresiana* of similar sizes (~5.5-6 g) were hand collected from bare substratum 2 days prior to treatment and attached to plastic supports using a zip-tie. Juvenile corals were collected from the back reef substratum using a hammer and chisel 5 days prior to treatment and attached to plastic supports using EcoTech Coral Glue, then placed in a flow through seawater table for a day to recover. Afterwards, corals were transferred to two outdoor flumes with fresh sand-filtered seawater pumped from Cook’s Bay at 12-m depth, to acclimate for four days prior to treatment. Both flumes were set to a flow speed of ~10 cm s\(^{-1}\) (mean flow speed of the back reef) at ~27 °C (mean ambient temperature of the back reef from May-July 2015) under sunlight. Both organisms were collected in the field where they were not in contact with other corals or algae to avoid any previously established interactions. Due to minimal handling and high sensitivity to fragmentation of the algae, algae were not acclimated and were directly placed in a flow-through sea water table for preparation.

*Competitive interactions*

To stage the competitive interactions, organisms on plastic supports were placed together with a zip-tie with organisms ~1 cm apart. To retain and reduce algal fragmentation, rectangular cages were built for each pairing by wrapping nylon monofilament mesh sheets with 0.65 x 0.65 cm openings along the edges of a 1 cm thick, 7 x 12 cm white PVC base, with a height of 10 cm. Each pairing then was attached onto the PVC base with nylon monofilament line. Tops of the cages were cut on three of the
four sides and formed a lid to allow for easy opening and closing. After pairings were placed into the mesh containers, the lid was tied shut with monofilament line (Fig. 1b and 1c). Flow and light levels were measured inside and outside the mesh containers to ensure that the mesh she did not disrupt flow treatments or create shading effects. Attachment to PVC bases allowed for better stability and for moving the pairings every three days to reduce positional effects within the flumes. The competitive interactions consisted of three pairings: (i) algae paired with corals ~1 cm apart (A + C) (Fig. 2a), (ii) algae paired with a coral mimic (bare carbonate rock collected from the back reef; A + CM) (Fig. 2b), and (iii) isolated live juvenile corals oriented on one side of the PVC base (controls) (Fig. 2c). These pairings mimicked the experimental design of Nugues and Bak (2006) examining the change in algal growth on dead and live corals in the Caribbean. While other studies have incorporated algal mimics to discern chemical effects or abrasion on the coral, coral damage typically does not resulted from algal mimics (Rasher et al. 2011; Diaz-Pulido 2011). Therefore, due to space limitation and to increase the power of individual statistical comparisons of interest, algal mimics were not used. Twenty-eight replicates of each pairing were created, with seven replicates of each pairing randomly allocated to one of four outdoor flumes subjected to different flow and CO₂ treatments (see below).

**Experimental conditions**

Four outdoor flumes were used, each with a working section of 0.3 x 0.3 x 5.0 m receiving continuous fresh filtered seawater (filtered through a sand filter with nominal filter size ~100 µm) pumped from Cook’s Bay at 12-m depth. Seawater was dispensed at
5 L min⁻¹, directly into the rectangular section at the upstream end of each flume, then transitioned to a section stacked with flow straighteners composed of 20-cm long, 3-cm diameter stacked PVC pipes, and re-circulated using a W. Lim WAVE II 375W pump back through a 12.5-cm diameter return section. The outdoor flumes experienced natural sunlight (PAR ~900 µmol photons m⁻² s⁻¹) and weekly light levels were measured to obtain overall light conditions (using a 4π quantum sensor LI-193 and a LiCor LI-1400 meter). To account for variability in light exposure along the flume, light measurements were taken 15 cm above the flume bottom at the upstream, middle, and downstream end of each flume and averaged. To obtain PAR experienced by algae inside the mesh cages, light levels were measured inside and outside the mesh cages of competitive pairings using the Walz pulse amplitude modulated fluorometer (PAM) 2π cosine light sensor calibrated to a LI-COR (LI 192SA) sensor.

This study was conducted in two trials where competitive interactions were subjected to treatments for two weeks in each trial, and then using newly collected organisms, pairings were established and incubated in a second trial for two weeks. The experimental design for this experiment consisted of two flow treatments crossed with two pCO₂ treatments, creating four different treatments for each trial (Fig. 2a). Flow treatments were established at 4 cm s⁻¹ (low flow) and 10 cm s⁻¹ (high flow mimicking the average flow conditions experienced in the back reef) under either 400 µatm (ambient pCO₂) or the targeted 1000 µatm (expected in 2100 under a pessimistic scenario; IPCC 2013). Flow rates were measured using a Nortek Vectrino Acoustic Doppler Velocimeter (ADV) positioned 15 cm above the flume bottom. Measurements were recorded on the
initial day of treatment and every 7 days thereafter to ensure consistent velocities throughout the incubation.

**Carbonate chemistry**

CO₂ levels were regulated using a digital controller attached to pH electrodes (Aquacontroller, Neptune Systems, USA) and bubbled in either pure CO₂ or CO₂-free air into the flumes. Mean temperature in the flumes was maintained at 26.6 ± 0.4 °C, close to the temperature conditions (~27.6 °C) experienced in the back reef during June-July (MCR-LTER Dataset ID: knb-lter-mcr.1035). Daily temperature and pH on the total scale (pHₜ) were recorded at 08:00 local time using a hand-held pH meter (Orion 3-stars) fitted with a Mettler DG 115-SC pH probe calibrated every 2 d against certified TRIS-HCl buffers (A. Dickson, San Diego, USA). Weekly pHₜ also was measured spectrophotometrically using m-cresol dye (Dickson et al. 2007), yielding results within ≤ 0.02 units of those obtained with the portable pH meter. Seawater samples (50 mL) were collected every 2 days and used to measure total alkalinity (ₐₜ) using an automatic titrator (T50, Mettler-Toledo). Titrations of certified reference materials (A.G. Dickson batch 130) provided ₐₜ values within 5 µmol kg⁻¹ of the certified value. The R package seacarb (Lavigne and Gattuso 2013) was used to calculate carbonate chemistry parameters using ₐₜ, temperature, pH, and salinity.

**Response variables**

Relative growth rates. Growth measurements were recorded using the wet (spun dry) weight of each algal individual before and after incubation. To reduce fragmentation,
each individual was placed in the same mesh bag before being spun in a salad spinner for 10 seconds, then immediately weighed (± 0.001g). Relative growth rates (RGR) were calculated using the following equation (Yong et al. 2013),

\[
\text{RGR} \left( \frac{\% \text{ day}^{-1}}{\text{day}^{-1}} \right) = \log \left( \frac{W_f}{W_i} \right) \times \frac{100}{t}
\]

where \(W_f\) and \(W_i\) are the final and initial wet weights, respectively, and \(t\) is the total time in days the algae were subjected to a treatment. Growth rates are expressed as percent change per day.

**Net Calcification rates.** The growth rates in corals were measured using the buoyant weight (± 1 mg) technique (Spencer-Davies 1989) recorded before and after 14 days of treatment. The change in buoyant weight during the incubation was converted to dry weight using the density of aragonite (2.93 g cm\(^{-3}\)). Net calcification rates were normalized to surface area using the aluminum foil technique (Marsh 1970) and reported as mg cm\(^{-2}\) day\(^{-1}\).

**PAM fluorometry.** The diving-pulse-amplitude-modulated (PAM) underwater fluorometer typically is used to measure the health and physiological status of an organism. It can provide a quantifiable measure of the photosynthetic response to bleaching or environmental stressors by measuring the photosynthetic efficiency (effective quantum yield; \(Y\)) within light-adapted organisms (Beer et al. 1998). The values of quantum yield are unitless and can range from 0 to 1, but typically healthy corals range from 0.5 to 0.8 and damaged/bleached corals range from 0.0 (= coral mortality) to 0.2 (Smith et al. 2006; Rasher and Hay 2010). Yield measurements were
made at the end of treatment trials between 1100- 1300 h local time at two locations on each coral, 90 ° on the interacting side of the coral with an alga and on the side opposite from the interaction (i.e. non-interacting side), and were compared between experimental and control competitive treatments.

**Statistical Analyses**

The physical conditions in the flumes were tested between trials using a three-way ANOVA, with Trial, flow, and $pCO_2$ treatments as fixed treatment effects. To test for differences in response variables between trials, relative growth rates and calcification rates were analyzed with a four-way split-plot ANOVA. Temperature, $pCO_2$, and Trial served as the main fixed factors, competitive pairing as a fixed within plot factor, and relative growth rates and calcification rates as dependent variables. Trial was dropped from the analyses when not significant ($p \geq 0.25$; Quinn and Keough 2002) and analyzed using a two-way ANOVA for flume parameters and, a three-way ANOVA for algal growth and calcification rates, treating individual competitive pairings as statistical replicates. Prior to analysis, statistical assumptions of the ANOVA were tested through graphical analyses of residuals.

PAM (effective quantum yield) measurements were analyzed using Wilcoxon pair-wise tests to assess differences between the opposite sides on the coral controls and the interacting and non-interacting sides of each coral when paired with an algal individual.
**Results**

There was no significant Trial effect ($p \geq 0.30$) for pH, $p\text{CO}_2$, and temperature and therefore, Trial was dropped from subsequent analyses of flume conditions. Mean elevated $p\text{CO}_2$ was successfully maintained at $1066 \pm 32$ and $1002 \pm 25$ µatm and mean ambient $p\text{CO}_2$ was $392 \pm 5$ and $379 \pm 6$ µatm ($\pm$ SE; $n=28$) for the 4 and 10 cm s$^{-1}$ flow treatments, respectively. $p\text{CO}_2$ and pH were significantly different between CO$_2$ treatments ($F_{1,108} \geq 987.8$, $p < 0.0001$) but not between flow or flow x CO$_2$ treatment ($F_{1,108} \leq 3.565$, $p \geq 0.0617$). Temperature also differed between CO$_2$ treatment ($F_{1,108} = 1.608$, $p = 0.0171$) but differences between treatments were trivial (means differing $<0.1$ °C). Total alkalinity ($A_T$) resulted in a Trial effect ($p < 0.0001$) but the variation between the two trials was small (means differing $< 3$ µmol kg$^{-1}$) and was dropped from the analysis. $A_T$ at 4 cm s$^{-1}$ was $2327 \pm 2$ and $2328 \pm 2$ µmol kg$^{-1}$, and at 10 cm s$^{-1}$ was $2324 \pm 2$ and $2325 \pm 2$ µmol kg$^{-1}$ ($\pm$ SE; $n=16$) under ambient and elevated $p\text{CO}_2$, respectively (Table 1). Light measurements made inside and outside the mesh containers resulted in no Trial effect ($p = 0.228$), with mean light levels of $\sim 187$ µmol quanta m$^2$ s$^{-1}$ inside the cages and $\sim 209$ µmol quanta m$^2$ s$^{-1}$ outside the cages, indicating no shading effect from cages.

Relative growth rates of *D. bartayresiana* did not differ between Trial ($p > 0.954$) and a three-way ANOVA was conducted, resulting in a significant $p\text{CO}_2$ ($F_{1,104} = 4.680$ and $p = 0.033$) and flow ($F_{1,104} = 24.194$, $p < 0.0001$) effect, and a significant interactive effect between the two ($F_{1,104} = 7.66$, $p = 0.007$). Between flow treatments, algal growth rates in their respective competitive interactions (i.e. algae with coral mimic; A+CM or algae with coral; A+C) increased under ambient $p\text{CO}_2$ conditions. *Post-hoc* analysis revealed RGR in ambient treatments were significantly higher than in elevated treatments...
at 10 cm s$^{-1}$. Growth rates of *D. bartayresiana* in different competitive pairings resulted in significant interactive effect of $p$CO$_2$ and flow treatments ($F_{1,104} = 6.110, p = 0.0151$). When *D. bartayresiana* was paired with live juvenile corals under ambient $p$CO$_2$, the RGR positively increased ten-fold between 4 and 10 cm s$^{-1}$ but resulted in no differences in growth rates under elevated $p$CO$_2$ (Fig. 3). Additionally, the high flow treatment decreased algal growth rates in the coral-algal competitive pairings by 58%, from 1.37 ± 0.22 % day$^{-1}$ in the ambient treatment to 0.57 ± 0.14 % day$^{-1}$ in the elevated treatment (±SE; $n=14$). RGR of algae in A+CM pairings had reduced growth rates between $p$CO$_2$ treatments but increased growth rates between flow treatments.

Net coral calcification rates had a significant Trial effect (<0.001), however, when treatments were plotted, it differed only by magnitude and not response direction. Therefore, Trial was dropped from subsequent analyses and a three-way ANOVA was performed. Net calcification rates differed between coral controls and when corals were paired with algae ($F_{1,104} = 8.362, p = 0.0047$), with coral calcification generally higher when paired with algae rather than alone. Coral calcification had a significant $p$CO$_2$ effect ($F_{1,104} = 4.20, p = 0.0429$) but this was driven mainly by an increase in calcification of coral controls at 10 cm s$^{-1}$ under elevated $p$CO$_2$ (Fig.4). There were negligible differences in calcification rates between flow treatments or any of the interaction treatments ($F_{1,104} \leq 2.459, p \leq 0.120$).

Photosynthetic efficiency measured using PAM fluorometry revealed no differences between the combination treatments (ambient $p$CO$_2$, low flow and high flow; elevated $p$CO$_2$, low flow and high flow) on the opposite or interacting sides for both coral controls and corals paired with algae ($W \leq 61, n_1 = n_2 = 14, p \geq 0.093$) suggesting no
direct negative effect of algae on coral photosynthetic physiology over the 2-week treatment period (Fig. 5).

**Discussion**

This study addressed two main questions, (1) with *a priori* knowledge that massive *Porites* spp. can be relatively resilient to OA, does it become more or less susceptible to OA when in the presence of a competitor? And (2) is algal growth enhanced under elevated $p$CO$_2$, shifting the competitive interaction to favor the alga when combined with increased water motion? The results of this experiment demonstrated that between two frequently interacting coral-algal species, the effects of water motion strongly enhanced algal growth rates compared to the effects of elevated $p$CO$_2$, while corals were less affected by water motion and OA regardless of the presence/absence of a competitor. The higher water flow increased growth rates of *D. bartayresiana* in both coral-algal competitive pairings and when algae were paired with coral mimics. Elevated $p$CO$_2$ reduced algal growth rates except under low flow when algae were paired with live corals. For corals, OA had a minor effect on coral calcification rates with growth rates slightly increasing under elevated $p$CO$_2$ treatments but no effect of water flow.

Furthermore, measurements of photosynthetic efficiency showed no differences between the interacting side with algae or on the opposite side (i.e. non-interacting side), suggesting no strong direct effect by the algae on the corals.

As coral reefs are facing increasing anthropogenic pressures contributing to reef degradation, the frequency and intensity of coral-algal competition can potentially increase due to calcifiers (e.g. corals) becoming more vulnerable. The capacity of corals
to survive after a disturbance or an acute environmental change will depend in part on the
direct or indirect effects algae have on corals (Smith et al. 2006). Similarly, the capacity
of algae to rapidly colonize dead coral or overgrow live corals will depend in part on the
direct or indirect effects of environmental changes (González-Rivero et al. 2016).
However, the impacts of combined abiotic factors such as ocean acidification with water
flow on coral-algal competitive interactions are less known. The results of this study
revealed a strong interactive effect between water flow and $pCO_2$ on algal growth rates.
Regardless of the type of competitive pairing, a decrease in water motion resulted in a
reduction in algal growth rates. Under flow speeds naturally experienced in the back reef
(10 cm s$^{-1}$), growth rates under the ambient $pCO_2$ treatment (~400 µatm) in both
competitive treatments were much higher compared to the elevated $pCO_2$ treatment
(~1000 µatm). This supports the assumption that algae like *D. bartayresiana* currently are
carbon-saturated and are able to utilize HCO$_3^-$ to achieve higher growth rates under
current CO$_2$ conditions because of the relatively large HCO$_3^-$ pool available (Koch et al.
2013). A similar response was seen by Diaz-Pulido et al. (2011) examining the effects of
OA on coral-algal competition, specifically on *Lobophora papenfussii* (a HCO$_3^-$ user)
competing with *Acropora intermedia* across a gradient of $pCO_2$ levels. In that study, the
growth of algal blades without a live coral were enhanced when $pCO_2$ were < 560 ppm
but declined at the highest $pCO_2$ level (1140 ppm), further supporting the predicted minor
response to OA in algae that use bicarbonate (Zou 2005). In addition, the results
presented here support the hypothesis that lower flow speeds negatively affect algal
growth rates. Interestingly, under higher flow, growth rates of *D. bartayresiana* were
greatest when paired with corals and decreased with increasing $pCO_2$. Conversely, at low
flow, algal growth rates were lowest when paired with corals under ambient $p$CO$_2$
conditions, indicating potential mass transfer limitation, while in the elevated treatment,
growth rates between flow speeds did not differ. These results suggest that water motion
has a stronger effect on algal growth rates than OA.

Although facultative HCO$_3^-$ users are expected to have little response to OA (Zou
et al. 2011), algae still are expected to benefit in comparison to corals, as corals generally
are negatively affected by OA. I predicted enhanced growth rates of *D. bartayresiana*
from the combined effects of increased water motion and OA would exacerbate their
negative competitive effect on corals. The increase in water motion did enhance algal
growth rates, but neither increased $p$CO$_2$ nor decreased water flow affected the juvenile
corals negatively. Massive *Porites* spp. has been shown to be resilient to OA conditions
(Edmunds et al. 2012). However, I predicted juvenile corals to become more susceptible
to OA under competition due to increase algal growth. In turn, this would enhance the
ability of algae to shade and overgrow corals, leading to increased vulnerability to
competition. While the present results did not explicitly demonstrate direct damage to
coral health, there were several of the coral-algal pairings, particularly under the low flow,
elevated CO$_2$ treatment where corals were paling or had discoloration (pers. observations
and photographs), but no distinct overgrowth, tissue necrosis, or coral mortality.
Therefore, potentially extending to a longer-term experiment may result in negative
effects of reduced flow on coral growth and health.

Many studies have examined competition with algae directly in contact with the
corals (de Ruyter van Steveninck 1988; McCook 2001; Jompa and McCook 2003;
Nugues et al. 2004; Titlyanov et al. 2006; Rasher and Hay 2010; Diaz-Pulido et al. 2011)
and observed direct negative effects on coral growth. In this study, competitive pairings were placed ~1 cm away from each other to test for competition for space via overgrowth. Algal growth rates were enhanced but it did not exacerbate effects on the coral. However, it is likely that under conditions of longer contact, overgrowth could occur. In some cases, algal individuals attached onto the coral or coral mimic (pers. obs.), suggesting the potential to grow on the coral. To assess the health status of the coral, photosynthetic efficiency of the coral was used as a proxy. All measurements of quantum yield fell within, what is considered, the healthy range (Rasher and Hay 2010), suggesting that there was no competitive effect on the photosynthetic function of corals within these treatments. But when examining values among the types of competitive pairings, corals paired with algae typically had a slightly higher quantum yield on the interacting side than the non-interacting side, regardless of $pCO_2$ or flow treatment. This is somewhat expected as the interacting side of the coral is subjected to shading by the alga which would result in higher quantum yields (Ralph et al. 2005). This can be indicative of the potential for direct overgrowth if treatment persisted because as algal biomass increases, it can lead to both shading and abrasion on the coral (Lirman 2001). Shading can reduce the photosynthetic rates of zooxanthellae in corals (quantum yield and photosynthetic rates are related inversely), eventually reducing coral health and resulting in coral overgrowth by the algae.

I also predicted lower flow to affect coral calcification rates negatively and when combined with OA, would exacerbate these negative effects. To the contrary, there was a general increase in net calcification of corals to elevated $pCO_2$. A $pCO_2$ effect was present but seemed mainly driven by the coral controls under high flow at the ambient
$pCO_2$ treatment. Although the thickness in diffusion boundary layers (DBLs) was not measured, corals unaffected by a reduction in flow speed could be related to having a thicker DBL. Cornwall et al. (2014) exposed a coralline macroalga to two pH treatments and two velocities, finding that low flow speeds produces thicker DBLs that can act as a surrounding buffer, ameliorating the negative effects of OA. Additionally, the carbonate chemistry at the surface of an organism can vary with flow conditions and at slower flows can be favorable to higher net calcification, thus resulting in no differences in growth rates under OA conditions (Cornwall et al. 2014). This may explain why massive Porites spp. still was unaffected by elevated $pCO_2$ when flow speeds were reduced. Overall, there were no differences in calcification rates among or between treatments.

These results are congruent with other studies concluding massive Porites spp. as a more resilient species to OA (Fabricius et al. 2011; Edmunds et al. 2012; Wall and Edmunds 2013). If consistent, this species can become the more dominant coral species on coral reefs under future, more acidic conditions. Field investigations on coral reefs at volcanic CO$_2$ seeps by Fabricius et al. (2011) showed how lowered pH reduced coral diversity, structural reef complexity, and shifted competitive interactions to favor more slow-growing and structurally simple corals such as massive Porites. However, in some regions juvenile massive Porites decreased in population density with increasing $pCO_2$. If juvenile corals are more susceptible to OA, it could increase their vulnerability to competition by other organisms such as macroalgae. So while massive Porites spp. may be resilient to OA, particularly during short exposures, the implications for future reef communities may be severe. The relative abundance of corals may shift from a mix of
sensitive and resilient taxa to those that are resilient, providing a less structurally complex habitat for other reef organisms.

A variety of competitive mechanisms can be involved between coral-algal interactions but elucidating whether it is a direct or indirect effect is critical to understanding the potential effects of increased algal biomass on coral survival. In addition, the algal functional group may also influence the success of macroalgal colonization as some species may fare better than others under global climate change. The capability of macroalgae to overgrow corals can determine the level of competitive success and help predict how competitive outcomes can influence coral-algal distributions (Airoldi 2000). This is critical for coral reefs facing a number of local and global threats, but predictions have proven difficult as the physical environment also plays an important role in modulating these dynamic interactions. Flow conditions can differ significantly from open-ocean to waves breaking over the reef crest into the back reef and through the lagoon (Hench et al. 2008). Back reef habitats are a suitable study habitat because: (1) the direction of flow is mostly unidirectional from waves breaking at the reef crest, and (2) the high variability in water motion (Hench et al. 2013), affecting seawater chemistry and individual and community metabolism (Anthony et al. 2011a; Kleypas et al. 2011). On the north shore of Moorea, water exchange is highly variable due to swells, with periods of high flow and periods of slower flow (Hench et al. 2008). Additionally, the spatial distribution of coral bommies have upstream and downstream effects, modifying flow patterns and with the potential to influence biological processes (Hench et al. 2013). This combined with the variation in carbonate chemistry across the reef can modulate changes in overall coral-algal abundances. Organisms can influence
changes in seawater chemistry and differences in their relative abundance can affect the availability of carbon sources (Kleypas et al. 2011). This also will vary in different flow environments, as water motion is an essential physical driver affecting responses in organisms. Thus, the differential responses to OA and water flow will have important implications for understanding species interactions and potential changes in community structure.

The present study examined the direct competitive effect of algae on coral by subjecting competitive pairings to two $pCO_2$ levels and two flow speeds. This study tested whether the juveniles of a resilient coral species becomes susceptible to OA when competing for a common resource (i.e. space) with a dominant macroalga. The combined effects of OA and water motion did not exacerbate the competitive effect, but *D. bartayresiana* exhibited increased growth rates at a higher flow speed under current $pCO_2$ conditions when paired with corals while causing negligible damage to the coral. Not only can water motion heavily influence organism’s responses, but increasing anthropogenic impacts can lead to a reduction in structurally complex reefs and create an environment that favors an increase in algal biomass. Enhanced growth of *D. bartayresiana* under current CO$_2$ conditions suggests a more favorable environment than under OA conditions. RGR were reduced under OA conditions and were severely affected at low flow, suggesting that the competitive ability of this species is compromised under reduced flow, which reduces nutrient and gas availability. Therefore, it is equally as critical to incorporate fast-growing or more sensitive coral species to study the potential detrimental effects of algae on more susceptible species. Additionally, more studies are needed to evaluate other facultative HCO$_3^-$ users with obligate CO$_2$ users to
examine whether some species are a stronger competitor than others under current versus predicted future CO₂ conditions, and whether there is a generalization to these responses. Species interactions may be altered depending on the flow environments and how organisms modify seawater chemistry, thereby affecting organism-level responses to OA. If OA affects corals negatively and reduces the complex reef framework, then changes in community structure may modify flow patterns, further affecting species interactions. Evaluating the direct and indirect effects of competitive mechanisms is critical to improving predictions of competitive outcomes between foundational coral species and macroalgae.
Table 1. Flume parameters of carbonate chemistry pooled across trials. Two flow speeds, 4 cm s$^{-1}$ and 10 cm s$^{-1}$, were crossed with either ambient $p$CO$_2$ (400 µatm; ACO2) or elevated $p$CO$_2$ (1000 µatm; HCO2). Salinity (PSU), pH$_T$ (total scale), total alkalinity ($A_T$), and temperature were used to calculate partial pressure of CO$_2$ ($p$CO$_2$). Values are mean ± SE ($n=28$; except $A_T$). SE of salinity and temperature was < 0.05 and < 0.1, respectively.

<table>
<thead>
<tr>
<th>Flow speed</th>
<th>Treatment</th>
<th>Sal</th>
<th>T (°C)</th>
<th>pH$_T$</th>
<th>$p$CO$_2$ (µatm)</th>
<th>$A_T$ (µmol kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 cm s$^{-1}$</td>
<td>ACO2</td>
<td>35.4</td>
<td>26.7</td>
<td>8.05 ± 0.01</td>
<td>392 ± 5</td>
<td>2327 ± 2 (16)</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>35.2</td>
<td>26.5</td>
<td>7.69 ± 0.01</td>
<td>1066 ± 32</td>
<td>2328 ± 2 (16)</td>
</tr>
<tr>
<td>10 cm s$^{-1}$</td>
<td>ACO2</td>
<td>35.4</td>
<td>26.8</td>
<td>8.06 ± 0.01</td>
<td>379 ± 6</td>
<td>2324 ± 2 (16)</td>
</tr>
<tr>
<td></td>
<td>HCO2</td>
<td>35.4</td>
<td>26.6</td>
<td>7.71 ± 0.01</td>
<td>1002 ± 25</td>
<td>2325 ± 2 (16)</td>
</tr>
</tbody>
</table>
**Figure 1.** Diagram showing the three competitive treatments on plastic support bases consisting of (a) a coral paired with an alga ~1 cm apart, (b) coral mimic (bare carbonate rock) paired with an alga ~1 cm apart, and (c) an isolated coral control treatment. Each support base was 1-cm thick, 7 x 12 cm wide and surrounded by a nylon monofilament mesh cage with 0.65 x 0.65 cm opening.
Figure 2. (a) Experimental setup showing one of the four outdoor flumes at 4 cm s\(^{-1}\) or 10 cm s\(^{-1}\) flow speed crossed with two PCO levels, 400 or 1000 μatm, containing the three competitive pairings. (b) and (c) show a direct overview and side view of a staged competitive pairing between a juvenile massive *Porites* spp. and an individual *Dictyota bartayresiana* ~ 1 cm apart, attached onto a plastic support base with a surrounding nylon monofilament mesh cage.
Figure 3. Relative growth rates (% day$^{-1}$) of *Dictyota bartayresiana* paired with either coral mimics (bare carbonate rock) or live juvenile massive *Porites* spp. under the low flow (4 cm s$^{-1}$) or high flow (10 cm s$^{-1}$) treatment at ambient pCO$_2$ (ACO2) or elevated pCO$_2$ (HCO2). Bars represent means ± SE (n=14) pooled across trials.
Figure 4. Coral net calcification responses (mg CaCO$_3$ cm$^2$ d$^{-1}$) to the ambient and elevated $p$CO$_2$ treatments at low (4 cm s$^{-1}$) and at high (10 cm s$^{-1}$) flow treatments. Juvenile massive Porites spp. were either paired with an individual alga or was isolated as the control. Bars represent mean ± SE ($n$=14) pooled across trials.
Figure 5. The photochemical efficiency represented as effective quantum yield ($Y$; mean ± SE) measured on the interacting and opposite side of each coral at A) 400 $\mu$atm (ACO$_2$) treatment and B) 1000 $\mu$atm (HCO$_2$) treatment ($n=14$) under the two flow treatments, pooled across trials.
Chapter 5

Concluding remarks

An increasing body of literature is focusing on how organisms will respond to a changing environment heavily influenced by accelerating human activities. Attention has shifted to coral reefs subjected to ongoing degradation due to pollution and overfishing, and further compounded by global warming and ocean acidification (Pandolfi et al. 2011). Coral reefs are biodiversity hotspots well known for their roles in providing ecologic and economic value (Pandolfi et al. 2011) and associated with a diverse community assemblage by having structurally complex frameworks (Graham and Nash 2013). However, the substantial increase in human activity has established coral reefs as one of the most vulnerable marine ecosystems to global change. The oceanic absorption of anthropogenic CO$_2$ is altering seawater chemistry and shifting the relative proportion of dissolved inorganic carbon (DIC) (Hurd et al. 2009). Simultaneously, global warming is contributing to coral bleaching and sea level rising (Koch et al. 2013). The physiological and ecological responses of benthic reef organisms are expected to change in response to these global threats, however, the magnitude of OA and temperature effects, both separately and interactively, are still unknown.

The goal of this research was to investigate the physiological responses in non-calcareous macroalgae differing in their carbon uptake strategies to increased $p$CO$_2$ and temperature conditions. The source of DIC may allow some species to benefit metabolically more than others, potentially making those species better competitors under OA and climate change conditions. Additionally, water motion can have important
implications on how a physical driver can affect macroalgal responses, and whether it can mediate the competitive outcomes between algae and corals.

In Chapter 2, I investigated the interactive effects of ocean acidification and water flow on *Lobophora variegata* (HCO$_3^-$ user), *Dictyota bartayresiana* (HCO$_3^-$ user), and *Amansia rhodantha* (CO$_2$ user). Relative growth rates were affected independently by $p$CO$_2$ and water flow. All three species exhibited the highest growth rates at 5 cm $s^{-1}$ (the intermediate flow speed), minimal growth at 8.5 cm $s^{-1}$ (the highest flow speed), and varying responses at 0.1 cm $s^{-1}$ (lowest flow speed). *A. rhodantha* did not exhibit higher growth rates under OA conditions, contrasting with the predicted 195% increase in CO$_2$ concentration would physiologically benefit obligate CO$_2$ users more than facultative HCO$_3^-$ users. However, under the no flow treatment, *A. rhodantha* exhibited the greatest reduction in growth rate under the ambient treatment ($-1.66 \pm 0.20 \% \text{ day}^{-1}$). These results indicate that water motion has a stronger effect on macroalgal growth rates than OA conditions. When OA was combined with water motion, it resulted in negligible effects. This study did not exhibit a general response in algae based on their DIC source but did demonstrate that responses to OA are species-specific. As reported by a number of studies (e.g. Israel and Hophy 2002; Kram et al. 2015), non-calcareous macroalgae can exhibit neutral or positive responses to elevated $p$CO$_2$, and this can be further complicated by their carbon acquisition strategy.

In Chapter 3, I report the results from two experiments examining the effects of elevated $p$CO and temperature on *D. bartayresiana* and *A. rhodantha*. I examined how the physiological responses differed between the two species differing in their DIC use. The first study in January 2015 resulted in overall higher photosynthetic rates under
elevated pCO₂ conditions for both species and *D. bartayresiana* exhibiting higher respiration rates within temperature and between temperature and pCO₂ treatment. When elevated temperature was combined with elevated pCO₂, photosynthesis increased while respiration decreased for both species. Interestingly, the second study in July 2015 yielded different responses. Metabolic rates were unaffected by pCO₂ treatments and the interaction of pCO₂ and temperature, but a temperature effect was present. *A. rhodantha* exhibited higher net photosynthesis at 27.5 °C as well increased respiration rates, but both decreased at 30 °C. The ambient local temperature is ~2 °C lower in July, suggesting that algae acclimatized to a lower temperature are more affected by increased temperature (Padilla-Gamiño and Carpenter 2007b). However, *D. bartayresiana* was insensitive to temperature treatments, indicating that this species potentially has a wider thermal tolerance window than *A. rhodantha*. This can provide an advantage to certain species, particularly if they also have CCMs, as they would be less sensitive to temperature variations but still facultatively use HCO₃⁻ under OA conditions. Although growth rates were measured, the varying responses and loss of biomass in some cases made it difficult to infer growth responses to photosynthetic rates.

Finally, in Chapter 4, staged interactions of *D. bartayresiana* and juvenile massive *Porites* spp. were used to investigate if the combined effects of water flow and OA exacerbated the competitive ability of the alga on the coral, resulting in overgrowth. Photosynthetic efficiency reported no differences between coral controls or corals interacting with algae, suggesting no direct competitive effect by the alga. Additionally, net calcification rates were not affected by different treatments. Corals were not more susceptible to competition under reduced flow and elevated pCO₂, further supporting the
resilience of massive *Porites* spp. (Edmunds et al. 2012). There were no differences in algal growth rates under OA conditions; however, reduced flow speeds reduced growth rates under ambient treatments, particularly when algae were paired with corals. This suggests that variation in the flow environment can compromise the ability for algae to compete. Furthermore, back reef habitats can experience both variable flow and pCO2 conditions, which might mediate species interactions.

Many studies have focused on investigating the effects of global environmental change on calcifying organisms (i.e. scleractinian corals) as they construct the framework of reef ecosystems (Edmunds et al. 2016). A number of reefs have experienced a steady decline in coral abundance (e.g. McManus et al. 2000; Gardner et al. 2003; Pandolfi et al. 2003; Bruno et al. 2009; Cheal et al. 2010) eliciting growing concern of possible shifts in relative abundances of coral and algae. Reef degradation is typically caused by some disturbance (e.g. storm damage or pollution) that can substantially reduce coral cover, and the recovery or replenishment of corals (i.e. larval recruitment, settlement, and post-settlement; Birrell et al. 2008) can be further hindered by rapid colonization of macroalgae (Diaz-Pulido et al. 2007). This has increased studies on the underlying mechanisms affecting physiological and ecological responses in macroalgae to environmental changes. Gaining a better understanding of organismal responses to disturbances and environmental stressors, can help predict potential population and community responses. However, the challenge to making such predictions is well-established as coral reefs are experiencing a combination of physical and chemical stressors. Water motion is a critical physical parameter facilitating resource acquisition and mediating competition (Hurd 2000). Variation in water motion can increase or
decrease mass transfer of nutrients and gases, differentially affecting benthic reef organisms. Additionally, OA and warming can act independently or synergistically to benefit macroalgae while negatively affecting corals, potentially affecting the frequency and outcomes of interactions between corals and algae. However, responses by macroalgae will not be uniform due to the wide range of functional groups of macroalgae (e.g. non-calcareous vs. calcareous) and their different carbon uptake strategies (Cornwall et al. 2012). OA will alter DIC speciation and the ability of algae to assimilate different carbon sources may influence their responses, potentially making some macroalgal species more successful than others (Koch et al. 2013). Similarly, some coral taxa are more resilient than others, and those more sensitive to global threats can result in changes in population dynamics, potentially shifting community composition to favor the less structurally complex species. Implications of physical and chemical parameters such as OA, warming, and water flow on macroalgal communities can be difficult to predict. However, understanding the physiological responses of individual species will be critical in identifying how post-disturbance or increasing anthropogenic activities will affect benthic populations. In particular, whether increases in algal abundances are short term or long term, which can either shift species interactions or inhibit reef recovery, resulting in different impacts on reef ecosystems.


Fernández, P.A., Hurd, C.L., and Roleda, M.Y. 2014. Bicarbonate uptake via an anion exchange is the main mechanism of inorganic carbon acquisition by the giant kelp


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