UNIVERSITY OF CALIFORNIA, SAN DIEGO

Ecophysiology of coral reef primary producers: Response to natural and anthropogenic environmental change

A dissertation submitted in partial satisfaction of the requirements for the degree
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in

Marine Biology

by

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The Dissertation of Maggie Dorothy Johnson is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

To my loving and supportive parents, Su and Richard, my inspiring and determined brother and sister, Jamie and Jenilyn, and the three Johnson boys that always keep me smiling, Patrick, Jakob and Cameron. I have never met kinder, quirkier or more caring people. Despite the odds and in spite of the downs, you keep going. Thank you for never giving up and for always believing in me.

EPIGRAPH

"Unless someone like you cares a whole awful lot,
Nothing is going to get better.
It's not."

Dr. Seuss
The Lorax

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ABSTRACT OF THE DISSERTATION

Ecophysiology of coral reef primary producers:
Response to natural and anthropogenic environmental change

By

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Natural heterogeneity and global change are key environmental drivers of ecosystem structure and function in both terrestrial and marine ecosystems. At the foundation of all food webs are the primary producers, which require macronutrients and photosynthetic substrate in order to fix inorganic carbon into organic sugars and fuel energy transfer into food webs. This dissertation is an examination of the ecophysiology, or the interaction of organismal physiology with the environment, of key benthic primary producers on coral reefs. Reef-building corals and algae are the most abundant primary producers on coral reefs, and I use coarse functional groupings

categorized as reef-building corals, fleshy macroalgae, calcareous macroalgae, crustose coralline algae (CCA) and turf algae assemblages. I assessed the influence of, 1) a natural gradient in inorganic nutrient availability, and, 2) simulated global change on the ecophysiology of corals and algae by functional group. The Southern Line Islands are an archipelago of islands that span the equatorial upwelling region and demonstrate predictable heterogeneity in inorganic nutrient availability. The dominant species of corals and algae demonstrated higher pigment concentrations and photosynthetic efficiency across the archipelago as a function of increasing inorganic nutrient concentrations. This suggests that natural fluxes of inorganic nutrients have an important positive influence on primary producers. I then conducted laboratory experiments on Palmyra Atoll and in Moorea, French Polynesia to test the effects of ocean acidification (OA) and warming on different functional groups of algae. Across a suite of species, OA increased the growth of fleshy macroalgae and turf algae assemblages, but decreased growth and calcification of calcareous macroalgae and CCA. Ocean acidification had a stronger effect than warming on the biomass of turf algae assemblages. Positive effects of OA on turf algae metabolism were increased by warming. These findings suggest that fleshy and calcifying algae respond differently to global change stressors. Ocean acidification has the potential to increase growth and productivity of fleshy algae, while concurrently decreasing growth and calcification of calcifying algae. Anthropogenic activities are increasingly altering the natural environment, and the results of this dissertation improve our ability to predict the response of corals and algae to increasing exposure to nutrients, OA and warming in the near-future ocean.

CHAPTER 1

Introduction: Ecophysiology of benthic primary producers

Maggie D. Johnson

INTRODUCTION

Primary Production

Primary production is the fundamental biological process that supports the diversity of life and unifies terrestrial and aquatic systems (Lieth and Whittaker 1975; Lindeman 1942). In marine ecosystems, primary production is mainly driven by photosynthesis, and provides half of total annual global carbon production (Field et al. 1998). Photosynthetic biota (autotrophs) harvest energy from the sun to form organic sugars, and net primary productivity is the proportion of that fixed carbon that then becomes available to higher trophic levels (Field et al. 1998; Lieth and Whittaker 1975) (see definitions in Figure 1). These photoautotrophs provide 99% of the organic matter used in marine food webs (Lieth and Whittaker 1975; Raven et al. 2005). Given the vital role of primary production, understanding the biotic and abiotic factors that influence processes and patterns of primary production can shed light on key mechanisms that shape ecosystem structure and function.

Abiotic factors that influence marine primary production include light availability, temperature, availability of inorganic nutrients and access to chemical substrates for photosynthesis (Field et al. 1998). Of these processes, there is a close relationship between primary production and availability of inorganic nutrients and carbon (Elser et al. 2007). Depletion of inorganic macronutrients, such as nitrogen and phosphorous, decreases rates of primary production, while nutrient enrichment enhances primary production (Cullen et al. 1992). Availability of inorganic carbon can similarly influence primary productivity, although the magnitude of this effect is

dependent on carbon acquisition strategies of primary producers (Raven 1970). Processes that influence the availability of nutrients and carbon to autotrophs are therefore key to influencing the rates and total potential for primary production in an ecosystem (Falkowski et al. 1998; Geider et al. 2001). In marine systems, variability in nutrients and anthropogenic global change (e.g. global warming, ocean acidification) (Behrenfeld et al. 2006a; Falkowski et al. 1998; Raven et al. 2005) have the potential to influence primary production with cascading impacts on energy availability to marine food webs.

The importance of primary production extends beyond provisioning of energy for food webs within a single ecosystem. Primary production in the ocean is important for sustaining biodiversity, as well the persistence of global fisheries (Pauly and

Ecophysiology: the interaction between organismal physiology and the physical environment.

Primary production (or *organic production*): the synthesis of new organic material from inorganic molecules.

Net primary production: the surplus of fixed carbon from photosynthesis, after carbon has been consumed by the primary producer through cellular respiration, that provides energy to higher trophic levels.

Gross primary production: the total amount of carbon fixed by photosynthesis, without accounting for carbon used during respiration. Gross production is calculated by adding carbon consumed from respiration (measured in the dark) to net primary production (measured in the light).

Respiration: the amount of carbon consumed through cellular respiration. Respiration uses carbon fixed through photosynthesis to generate ATP.

Areal specific production: the amount of carbon fixed per unit of surface area.

Figure 1.1. Definitions of organic and inorganic production. Frequently used terms for organic and inorganic production are defined here.

Christensen 1995; Ware and Thomson 2005). Global fisheries in turn provide economic revenue (Kent 1997). Fishers supply the main source of protein to billions of people worldwide (Hughes et al. 2005), which is particularly important in developing nations (Kent 1997). Abiotic factors that influence patterns in marine primary production, such as nutrient availability and global change, thus have global implications for the sustainability of fisheries and the human populations they support (Brown et al. 2010; Kent 1997; Pauly 2000).

Environmental Heterogeneity

The natural environment is highly variable, and abiotic environmental heterogeneity has long been recognized as a key factor that influences community structure in terrestrial (Macarthur 1965; Ricklefs 1977; Stein et al. 2014) and marine ecosystems (Barry and Dayton 1991; Menge and Sutherland 1976). In the ocean, environmental heterogeneity manifests as spatial and temporal patterns in resource availability (e.g. inorganic nutrients) (Hayward et al. 1983), and physical forcing (e.g. disturbance events and hydrodynamics) (Barry and Dayton 1991). Marine primary producers are affected particularly by the availability of inorganic nutrients and carbon because of the pivotal role these compounds play in the biochemical pathways of photosynthesis (Cullen et al. 1992; Falkowski et al. 1998). Understanding the physical factors that influence inorganic carbon and nutrient availability in marine systems is essential for elucidating the mechanisms that drive ecosystem capacity for primary production.

Hydrodynamics play an important role in determining both spatial and temporal environmental heterogeneity in marine ecosystems (Barry and Dayton 1991; Hayward et al. 1983). Upwelling, tidal bores and internal tides are examples of widespread hydrodynamic processes that contribute to environmental heterogeneity by delivering subthermocline water to the ocean surface. Subthermocline water is cool and enriched in inorganic nutrients, mainly nitrogen and phosphorous, and through periodic or persistent upwelling events it replaces the warmer and nutrient depleted surface waters (Mann and Lazier 2005). Long-standing currents create regions of persistent upwelling (Cromwell 1953), such as the equatorial upwelling region (Wyrtki 1981), while episodic events like internal tides and tidal bores provide pulses of subthermocline water (Leichter et al. 1996). Spatial and temporal environmental heterogeneity driven by upwelling has direct implications for ecosystem primary production because it provides primary producers with otherwise limiting inorganic nutrients (Cullen et al. 1992) and increases biological productivity (Elser et al. 2007; Falkowski et al. 1998).

Anthropogenic Global Change

Humans began significantly altering the global biogeochemical cycle of carbon with the onset of the Industrial Revolution in the mid 1700's. Since then, atmospheric carbon dioxide (CO_2) has increased due to fossil fuel consumption, land development, and deforestation (Houghton 1995). Over the last 800,000 years, atmospheric CO_2 concentrations remained around 172-300 µatm (Luthi et al. 2008), and in 2013 the concentration of CO_2 in the atmosphere surpassed 400 µatm. It is equally alarming

that the rate of CO_2 emissions continues to increase. In the 1990's atmospheric CO_2 increased by ~ 1.0% yr^{-1}, but by the 2000's that rate increased to ~3.4% yr^{-1} (Le Quere et al. 2009). In business-as-usual emissions scenarios, atmospheric CO_2 is projected to surpass 800 µatm before the end of the century (IPCC 2013). The increase in atmospheric CO_2 has long-lasting implications for the global climate and for ocean chemistry.

Ocean Acidification

On an annual time-scale, the ocean is in equilibrium with respect to atmospheric gases. According to Henry's law, the amount of a gas dissolved in a solution is directly proportional to the partial pressure of the gas above the solution. After CO_2 is emitted, 45% remains in the atmosphere, 29% is absorbed by the terrestrial biosphere, and 26% is absorbed by the ocean (Le Quere et al. 2009). The increase in CO_2 in ocean surface waters causes a shift in the equilibrium of inorganic carbon speciation in seawater, a process referred to as ocean acidification (OA). OA manifests as a decrease in pH and carbonate ions (CO_3^{-2}), an increase in the partial pressure of CO_2 (pCO_2) and bicarbonate ions (HCO_3^-), and no change in total alkalinity (A_T) (Figure 2) (Orr et al. 2005). Mean ocean pH has already decreased by 0.1 units since the Industrial Revolution, and is expected to decrease further by 0.3-0.5 units before the end of the century in business-as-usual carbon emission scenarios (IPCC 2013; Mora et al. 2013). The decrease in CO_3^{-2} affects the solubility of calcium carbonate ($CaCO_3$) in seawater, an important mineral secreted by biogenic calcifiers as stony shells or skeletons. OA decreases the saturation state of $CaCO_3$ (Ω) **(**Figure 2**)**,

and biogenic calcification becomes more difficult at lower Ω (Kleypas et al. 1999a), while dissolution of $CaCO_3$ becomes thermodynamically favorable at $\Omega < 1$ (Milliman and Droxler 1996). Ocean acidification has the potential to alter biological processes that use inorganic carbon by changing the proportion of inorganic carbon species in seawater.

A large effort has been put forth to understand the effects of OA on a vast number of marine organisms and ecosystems (Doney et al. 2009; Kroeker et al.

Carbon dioxide (CO_2^*) is dissolved in seawater as aqueous CO_{2aq}. CO_2^* is the sum of CO_{2aq} and carbonic acid (H_2CO_3).

Carbonate chemistry refers to the marine inorganic carbon species and associated properties in seawater. The dissolved carbon species are related by the following equations:
$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad K_1^*$
$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad K_2^*$

pH is a measure of ocean acidity.
$pH = -\log[H^+]$

Total alkalinity (A_T) is the sum of major anions in seawater minus the sum of major cations.
$A_T \approx [HCO_3^-] + 2[CO_3^{2-}] + [B(OH_4)^-] + [OH^-] - [H^+]$ + minor acid base pairs

Calcification is the biological precipitation of calcium carbonate ($CaCO_3$).
$Ca^{2+} + 2HCO_3^- = CaCO_3 + H_2O + CO_2$

Calcium carbonate ($CaCO_3$) is a biologically secreted limestone in the mineral forms of aragonite, calcite or high magnesium (high-Mg) calcite.
$CaCO_3 = Ca^{2+} + CO_3^{2-} \quad K^*_{SP}$

Calcium carbonate saturation state (Ω) is a thermodynamic property indicating saturation or undersaturation of seawater with respect to $CaCO_3$ mineral forms.
$\Omega = [Ca^{2+}]\,[CO_3^{2-}] / K^*_{SP}$

Ocean acidification (OA) is the reduction in ocean pH and Ω caused by increased uptake of anthropogenic CO_2 from the atmosphere.

Figure 1.2. The essentials of seawater carbonate chemistry. The chemical reactions and definitions for processes associated with ocean acidification are defined here.

2013; Kroeker et al. 2010). The majority of these studies have focused on commercially valuable bivalves and reef-building corals, specifically the effects on biotic calcification (Figure 2). From these studies, OA and the concurrent decrease in Ω have been directly correlated with decreased rates of biogenic calcification and increased rates of skeletal dissolution in scleractinian corals (Gattuso et al. 1998; Langdon et al. 2000). A meta-analysis combining the results of numerous experiments across taxa found overall negative effects of OA on calcification, but also on survival, growth and reproduction across diverse marine biota (Kroeker et al. 2010). Non-calcifying, or fleshy, marine species also respond to OA, but the direction and magnitude of response is often species-specific (Kroeker et al. 2010). However, fewer studies have explored the effects of OA on non-calcifying taxa or on benthic calcifiers other than corals and bivalves. Thus, there remains a gap in our understanding of OA effects on an important group of primary producers, the fleshy and calcifying algae.

Global Warming

Increasing anthropogenic CO_2 emissions are simultaneously causing warming of the global climate. Since the Industrial Revolution, global temperatures have been rising by 0.2 °C per decade, yielding a projected increase of ~2-3 °C over the next century, with a corresponding trend for warming of ocean surface waters (IPCC 2013). Ocean warming has a suite of biological and ecological consequences that ultimately start at the smallest scale by affecting organismal physiology. Increasing temperatures influence marine organisms because temperature plays a fundamental role in biological processes by affecting the rate of biochemical reactions (Somero and

Hochachka 1971). Temperature typically increases metabolic rates to a certain maximum threshold, after which rates precipitously decline. Metabolic rates determine the life history strategies of marine organisms, larval dispersal and population growth and ecosystem structure and function (O'Connor et al. 2007). While most marine organisms have the ability to adapt or acclimatize to an optimum temperature, the rapid rate of warming will outpace the ability of organisms to adapt to warmer temperatures and likely lead to mortality (O'Connor et al. 2007). Effects of warming may manifest as shifts in the distribution and abundance of species and species extinctions, with ultimate consequences for community structure and function (Behrenfeld et al. 2006a; Polovina et al. 2008).

Response to Environmental Heterogeneity and Global Change

Inorganic Nutrients

Biologically available forms of macronutrients are essential for primary producers because they provide the raw materials for synthesizing, maintaining and utilizing photosynthetic machinery. Nutrient availability can thus directly influence photosynthetic rates and primary production, especially in areas where inorganic nutrients are naturally low. Nitrogen and phosphorous, in particular, have predictable and positive effects on biological productivity, and are often the limiting factors for photosynthesis (Cullen et al. 1992; Falkowski et al. 1998). For example, nitrate or phosphate limitation can decrease productivity and growth in phytoplankton (Caperon and Meyer 1972; Thayer 1974), microalgae (Cullen and Horrigan 1981; Falkowski et al. 1993; Muscatine et al. 1989) and macroalgae (Deboer 1977; Delgado and Lapointe

1994; Fong et al. 2003; Gagne et al. 1982; Lapointe 1987; Rosenberg and Ramus 1982; Topinka and Robbins 1976). Although oceanographic upwelling events and the ensuing increase in surface inorganic nutrients are linked to increased surface-ocean productivity by phytoplankton (Barber and Chavez 1991; Bunt 1973; Field et al. 1998), less is known about how natural fluxes in inorganic nutrients influences photosynthesis and primary production of benthic taxa. Although phytoplankton provide the largest contribution to total ocean primary production, benthic primary producers are also important, particularly macroalgae in coastal environments (Duarte and Cebrian 1996; Field et al. 1998).

Global Change

Primary producers are also subject to changes in the availability of inorganic carbon due to anthropogenic activities. Ocean acidification has known effects on rates of biological calcification, but the effects of OA on calcifying primary producers are more complex. Another biological process that is potentially sensitive to OA is photosynthesis, because changes in seawater carbon speciation associated with OA are thought to directly influence the availability of photosynthetic substrates. Carbon dioxide is the primary substrate for the photosynthetic enzyme RubisCo and the increased availability of dissolved CO_2 in the near-future ocean is expected to enhance primary production (Holbrook et al. 1988; Koch et al. 2013). The response of primary producers to OA may depend on the presence and activity of carbon concentrating mechanisms (CCMs) and the extent of carbon-limitation under present-day conditions (Giordano et al. 2005; Raven et al. 2011). Many marine primary producers evolved

CCMs to increase the concentration of CO_2 at the site of photosynthetic fixation in order to compensate for lower concentrations of dissolved CO_2 relative to other carbon species in seawater (e.g. bicarbonate, HCO_3^-) (Beardall et al. 1998; Giordano et al. 2005). Species that are carbon-limited under present day conditions, such as those that do not possess carbon concentrating mechanisms (CCMs), are expected to respond positively to OA. Species that possess CCMs may still respond positively to OA, if the additional CO_2 makes more energy available by decreasing production of energetically costly CCMs. Differences in photosynthetic pathways and relative carbon-limitation may thus influence the effect of OA on marine primary producers. Because CCMs are species-specific, it is important to experimentally test the effects of OA on both calcification and primary production across many different species of primary producers.

Ocean warming has additional impacts on marine primary producers. Total global annual primary production has decreased by at least 6% over the last three decades, and is likely related to acidification and ocean warming (Gregg et al. 2003). These large-scale changes are fundamentally driven by the effects of these abiotic factors on small-scale physiological processes. For example, enzyme reaction rate, diffusion and membrane transport all respond to changes in temperature (Somero and Hochachka 1971). Moderate increases in temperature can increase photosynthetic rates, and thus gross primary production (Figure 2). But temperature has stronger effects on organismal respiration than it does on photosynthesis (Lopez-Urrutia et al. 2006; Somero and Hochachka 1971). Higher rates of respiration could thus counteract the positive effects of temperature on gross production by yielding no change or a net

decrease in primary production. Changes in small and large-scale processes of primary production due to global change have profound implications for the biosphere and biogeochemical cycling of the planet (Falkowski et al. 2000)

The Coral Reef Ecosystem

Coral reefs are one of the most productive and diverse ecosystems on the planet (Connell 1978), supporting one quarter to one third of all marine species (Plaisance et al. 2011), yet covering only 0.1-0.5% of the ocean floor (Smith 1978; Spalding and Grenfell 1997). In addition to providing structural complexity and the carbonate framework that supports a wealth of biodiversity (Paulay 1997), coral reefs are economically and culturally valuable to people. They provide a variety of tangible goods ranging from seafood and raw materials to social and cultural services such as recreation, ecotourism, and aesthetic beauty (Moberg and Folke 1999). Coral reefs provide important ecosystem services including physical protection of shorelines, storing and cycling of essential nutrients, and primary and secondary production (Moberg and Folke 1999; Worm et al. 2006).

However, coral reefs exist within a relatively narrow range of temperature, light, and Ω regimes and are sensitive to changes in the environment (Kleypas et al. 1999b). Modern coral reefs are one of the most vulnerable ecosystems to global change, because they are dominated by calcifying primary producers (e.g. scleractinian corals and calcified algae), that are sensitive to inorganic nutrient availability, decreasing Ω and temperature (Hoegh-Guldberg et al. 2007; Langdon et al. 2000). These factors, combined, make coral reef ecosystems an ideal case study for

exploring how natural heterogeneity in nutrient availability and global change influence growth and photosynthesis in ecologically relevant primary producers.

Coral Reef Primary Producers

Coral reefs are substantial contributors to global primary production (Pauly and Christensen 1995). Corals typically exist in oligotrophic tropical waters, and have high rates of primary production despite lower rates of primary production in the adjacent surface waters (Odum and Odum 1955). Coral reef primary production is accomplished by a suite of benthic primary producers, including corals and their symbiotic zooxanthellae and a variety of macroscopic algae (Odum and Odum 1955; Wanders 1976). Together, these primary producers provide the energetic foundation for coral reef ecosystems, support an incredible diversity of organisms and facilitate key ecosystem services (Moberg and Folke 1999; Odum and Odum 1955).

A functional-group approach can be useful for understanding broader impacts of environmental heterogeneity and global change on coral reef primary producers (Littler and Littler 1980; Steneck and Dethier 1994). Primary producers can be loosely categorized into coarse functional groups based on their anatomy and morphology, which closely relates to their ecological function (Steneck and Dethier 1994), and their contribution to organic and inorganic production in the ecosystem. Coral reef primary producers are thus broken into the following coarse groups throughout this dissertation: corals, fleshy macroalgae, calcareous macroalgae (referred to as upright calcifying algae in Chapter 2), crustose coralline algae and turf algae.

Reef-building Corals

Corals are the foundation species of the coral reef ecosystem that build the carbonate matrix and habitat through biotic calcification of $CaCO_3$ (in the mineral form of aragonite). In addition to building and maintain the three dimensional habitat, corals are also important as a source of primary production (Odum and Odum 1955; Sargent and Austin 1949). Primary production in corals is achieved by their symbiotic zooxanthellae (*Symbiodinium* sp.), which provide organic sugars through photosynthesis and in turn receive excreted nutrients and protection from the coral host (Goreau et al. 1971; Muscatine and Porter 1977). Corals are sensitive to a variety of physical factors, but particularly to the availability of light and inorganic nutrients, as well as increasing temperatures and OA (Hoegh-Guldberg et al. 2007). The role of natural nutrient heterogeneity in inorganic nutrients in structuring modern coral reefs can be difficult to discern because coral reefs across the globe are increasingly exposed to anthropogenic nutrient pollution (Hughes et al. 2007). Thus we have a poor understanding of how natural fluxes of nutrients influence the structure and function of coral reef ecosystems.

Fleshy Macroalgae

Fleshy macroalgae are non-calcifying, benthic algae that are important to reef primary production and provide an important food source for higher trophic levels (Gattuso et al. 1997; Wanders 1976). Although fleshy macroalgae typically have lower areal specific productivity (Figure 1), they remain an important contributor to reef production because of their larger size and high biomass. In areas of the reef where

fleshy algae are abundant, such as the algal ridge or reef crest, rates of algal production exceed those of both corals and algal turfs (Hatcher 1988). Despite the importance of fleshy macroalgae to reef primary production, the majority of published studies on fleshy macroalgae have been in the context of either competition with corals or overfishing of herbivores (e.g. overgrowth of corals by fleshy macroalgae, phase shifts towards fleshy macroalgae dominated systems, or the role of herbivores in controlling fleshy macroalgae) (Hughes 1994; McCook 1999; McCook et al. 2001; Smith et al. 2010; Smith et al. 2006). Thus, there is a relative paucity of literature that has investigated effects of global change and environmental heterogeneity on fleshy macroalgae populations.

Calcareous Macroalgae

Calcareous macroalgae are erect algae that secrete $CaCO_3$ in the mineral form of aragonite (Borowitzka and Larkum 1976). Calcification occurs intercellularly, and segments are connected via non-calcified joints (Drew 1986). The coenocytic green algae in the genus *Halimeda*, order Caulerpales, are the most abundant and cosmopolitan jointed calcareous algae on coral reefs, where they are a major source of organic and inorganic production (Figure 2) (Drew 1986; Hillis-Colinvaux 1983; Rees et al. 2007). *Halimeda* has fast growth rates, and the standing stock on a reef can turn over in as little as one month (Smith et al. 2004; Wefer 1980). After reproduction, the living tissue senesces and the discrete carbonate segments fall to the sediment. This contribution to unconsolidated reef sediments may be quantitatively more important than carbonate production by corals and CCA combined (Neumann and Land 1975;

Stoddart 1969), and transport of *Halimeda* debris from the reef to depths greater than 100 m may be the largest source of carbon export on some coral reefs (Hillis-Collinvaux 1986). *Halimeda* can contribute up to 40% of barrier reef sediment (Macintyre et al. 1987), and is thought to account for 8% of total global carbonate production (Hillis 1997). *Halimeda* provides an important source of food to a variety of fishes that feed either indirectly on their epibionts, or directly on the calcareous segments (Hamilton et al. 2014; Mantyka and Bellwood 2007). Given the fast growth rates and high turnover, and high production rates, *Halimeda* is an important producer organic and inorganic carbon on coral reefs.

Crustose Coralline Algae

Crustose coralline algae (CCA) are red algae that are distributed globally throughout the photic zone of coastal marine waters (Dethier and Steneck 2001; Steneck 1986). CCA are abundant on coral reefs, particularly in in shallow and high wave energy environments where they build algal ridges (Adey et al. 1982; Littler 1973), and in deep reef habitats greater than 200 m in depth (Steneck 1986). On some reefs, crustose corallines account for 90-100% of shallow reef benthic cover (Tribollet and Payri 2001). The crustose coralline thallus consists of a prostrate crust that grows over hard substrata, like dead corals, coral rubble and pavement. Calcification occurs intracellularly (as opposed to intercellularly as in *Halimeda*), where $CaCO_3$ in the form of high magnesium (High-Mg) calcite is deposited directly within the organic matrix of the cell wall (Borowitzka 1981; Steneck 1986). CCA serve a variety of important ecological functions on reefs by contributing to primary productivity and

carbonate production (Chisholm 2003; Goreau 1963), producing settlement cues for coral larvae (Harrington et al. 2004; Ritson-Williams et al. 2009), and maintaining structural integrity of the framework by acting as reef cement (Camoin and Montaggioni 1994).

Due to their prostrate growth form and limited surface area exposed to light and nutrients, crustose corallines are typically thought to have relatively low rates of primary production and growth (Dethier and Steneck 2001; Goreau 1963). Field measurements of coralline metabolism, however, contradict this early hypothesis as Chisholm (2003) found that rates of coralline production are higher than initially thought. Given that cover of corallines on shallow coral reefs can reach 100%, they are undoubtedly a key contributor to organic and inorganic production on coral reefs worldwide. CCA are notoriously difficult to identify to the genus and species level, particularly in the field, and are grossly understudied with respect to both primary production and effects of global change. Given their roles as reef-builders and facilitators of coral settlement, and their potential sensitivity to OA, it is important to understand how natural variability in inorganic nutrients and the progression of global change influence CCA primary production and calcification.

Turf Algae Assemblages

Turf algae are a dense multispecies assemblage of algal filaments, crusts and unicellular algae that are typically less than 1 cm in height (Carpenter 1986; Steneck and Dethier 1994). Turf assemblages are the most ubiquitous and abundant growth form of algae on coral reefs (Hay 1981), and are characterized by rapid growth and

primary production that enable them to take advantage of available nutrients, open substrata, and relief from herbivore pressure (Carpenter 1986). Due to a combination of high abundance and rates of turnover from continual grazing by herbivores, turfs have the highest rates of primary productivity on coral reefs (Adey and Steneck 1985; Hatcher 1988; Marsh 1976; Odum and Odum 1955). Despite these high rates, turf assemblages are likely carbon-limited under present-day conditions due to resource limitation within the diffusive boundary layer (Larkum et al. 2003). Given their high potential for primary production, global change is likely to increase turf assemblage primary production by providing warmer temperatures and additional CO_2 that can fuel turf productivity. Turf assemblages have important ecological roles on coral reefs as competitors with reef-building corals and algae and as inhibitors of juvenile and adult corals (Birrell et al. 2005; Vermeij et al. 2010). Although increased rates of primary production could mean more energy available to coral reef food webs, higher abundances of turfs could mean more frequent competition with corals and could ultimately lead to a shift in community structure by enhancing the competitive ability of fleshy species over reef-building calcifiers.

Assessing Primary Production

In order to assess effects of global change and heterogeneity in inorganic nutrients on coral reef primary production, I conducted a series of field observations and experimental manipulations with the goal of quantifying primary producer ecophysiology. Ecophysiology refers the physiological performance of an organism with respect to the physical environment (Figure 1). To quantify ecophysiology, with a

specific focus on primary production, I used the following methods: 1) sealed incubations to measure metabolic rates (net production, gross production, respiration) as a proxy for primary production, 2) changes in biomass as a long-term estimate of net primary production (carbon fixed into biomass), and 3) measurements of photosynthetic machinery, including fluorometric and pigment analyses. I measured metabolic rates by quantifying oxygen evolution in the light, as a measure of net photosynthesis, and oxygen consumption in the dark, as a measure of respiration. Because oxygen produced is proportional to the amount of carbon fixed during photosynthesis, I used measures of net and gross oxygen production as a proxy for net and gross primary production (Figure 1). I assessed biomass as growth in response to changing environmental conditions, and use this as a cumulative representation of primary production under the assumption that growth is a net result of carbon fixed into biomass. Finally, by directly measuring photosynthetic machinery I quantify the physiological potential of primary producers for photosynthesis, and thus primary production. Each of these approaches provides insight into the ecophysiology of primary producers in response to natural and anthropogenic changes in the environment.

Structure of the Dissertation

In addition to this introduction, this dissertation includes three research chapters and a brief conclusion that summarizes the major findings. As each chapter is intended as a stand-alone publication, there may be some redundancy in the introductory material and the methods.

Chapter 1: *Photosynthetic potential of corals and algae increase across a gradient of inorganic nutrient availability in the central Pacific*

In Chapter 1, I address the impacts of heterogeneity in inorganic nutrient availability on the photosynthetic potential of the primary producers on coral reefs in the central Pacific. The Southern Line Islands (SLI) are a remote archipelago of islands that span a natural gradient of exposure to equatorial upwelling in inorganic nutrient availability. Equatorial upwelling creates an area of persistently high concentrations of inorganic nitrogen and phosphorous, relative to the surrounding tropical gyres, and corresponds to an increase in surface ocean primary production (Chai et al. 1996; Chavez and Barber 1987). Although we know that increasing availability of inorganic nutrients associated with upwelling increases surface-ocean productivity (Geider et al. 2001; Koblents-Mishke 1965), we know less about effects of this natural flux of inorganic nutrients on benthic primary producers. I quantified the ecophysiology of the five dominant corals and algae across the SLI using a series of metabolic incubations to measure net primary production, gross primary production and respiration (and calcification in one species). Further, I extracted photosynthetic pigments and used a pulse-amplitude modulation (PAM) fluorometery to quantify maximum quantum yield and the status of photosynthetic machinery.

I found that photosynthetic potential of all taxa increased as a function of exposure to inorganic nutrients. Maximum quantum yield and photosynthetic pigments showed the strongest responses across all taxa, with lowest values at islands that have lower inorganic nutrient concentrations. Metabolic rates were more variable, but generally showed an increase in net and gross primary production at islands with

higher nutrient concentrations. These data indicate that the environmental heterogeneity in inorganic nutrient availability is an important abiotic driver of benthic primary producer ecophysiology. Higher photosynthetic potential could mean higher ecosystem capacity for primary production, which has important implications for the transfer of energy throughout marine food webs.

Chapter 2: *Contrasting effects of ocean acidification on tropical fleshy and calcareous algae*

In Chapter 2, I experimentally test the effects of OA on a suite of coral reef algae representing three functional groups: fleshy macroalgae, calcareous macroalgae and crustose coralline algae. Few studies have explored the response of coral reef algae to OA, and even less is known about the response of fleshy macroalgae to OA (Koch et al. 2013; Kroeker et al. 2010). The increase in dissolved CO_2 associated with OA has the potential to fuel photosynthesis in these primary producers by providing additional substrate for photosynthesis. However, the simultaneous decrease in pH (and Ω) has negative implications for biotic calcification (Langdon et al. 2000). I conducted a series of experiments with 11 species of coral reef algae common on the reefs of Palmyra Atoll. I exposed algae to simulated OA for 2-3 weeks and quantified the effects on two metrics of primary production: growth/calcification and photophysiology.

I compiled the results of these 11 experiments in a meta-analysis and found differential effects of OA on fleshy and calcareous algal functional groups. Fleshy algae tended to grow more under OA, while calcifying algae (calcareous macroalgae

and CCA) grew less or dissolved. However, there were no effects of OA on algal photophysiology. These results indicate that OA may facilitate the growth of fleshy algae, while inhibiting the growth and calcification of calcifying species. If enhanced growth under OA provides fleshy algae with a competitive advantage over reef-building corals and CCA, reefs of the future could shift from calcifier to fleshy dominated systems (Fabricius et al. 2011) and ultimately compromise coral reef ecosystem functions and services (Moberg and Folke 1999).

Chapter 3: *Complex and interactive effects of ocean acidification and warming on epilithic and endolithic coral reef turf assemblages*

In Chapter 3, I explore the effects of multiple stressors on coral reef turf algae assemblages, the fourth functional group of coral reef algae mentioned above. Turf algae are the most ubiquitous primary producers on coral reefs (Wanders 1976), yet little is known about how they respond to global warming and OA. Given that I found OA increased fleshy algal growth in Chapter 2, I expected that OA and warming would enhance growth of turf assemblages because they are dominated by fleshy algal filaments. Multiple stressor experiments are important for improving our power to predict the response of coral reefs to global change, because warming and acidification will occur simultaneously. Further, warming and OA often have synergistic effects on biological responses (Kroeker et al. 2013). I conducted a controlled laboratory experiment in Moorea, French Polynesia where I exposed turf algae assemblages to high CO_2 and 6 levels of temperature for 3 weeks. I quantified the effect of global change stressors on two metric of primary production: growth by change in biomass

and photosynthetic production. I assessed the growth response of two components of the turf assemblage, the epilithic community, comprised of algal filaments on the surface, and the endolithic community, comprised of boring microalgae within the carbonate matrix.

I found that both the epilithic and endolithic component of turf algae assemblages demonstrated positive growth responses to OA, but not to warming. However, there were also interactive effects of OA and warming on metabolic rates of the whole turf assemblage. With this experiment, I complete my functional group analysis of the response of coral reef algae to OA, and found the results to be consistent with the hypothesis that OA enhances fleshy algal growth. Further, OA exacerbated the effects of warming on primary production, and the effect of OA was temperature-dependent. These findings indicate that multiple stressor experiments that span a full range of environmental variables are important for contextualizing biological responses to OA. Further, the increase in biomass of epilithic turf algae and the endolithic community has important implications for reefs of the future. While turf algae are important for primary production on coral reefs, they also have a suite of negative ecological interactions. Increased rates of epilithic growth could increase the frequency of coral and turf competition, from which turf usually emerges as the competitive dominant. Endoliths play an important role in the coral reef ecosystems as bioeroders, and the increase in endolithic biomass under OA could lead to higher rates of bioerosion. The response of turf algae assemblages to global change was positive, although driven more by OA than warming, which may further fuel the shift of coral

reef ecosystems towards dominance by fleshy species in the near future high-CO_2 ocean.

The results of this dissertation provide new information on the response of coral reef primary producers to natural heterogeneity in inorganic nutrients, and the effects of global change on functional groups of coral reef algae. Environmental factors have a critical role in shaping ecosystem structure and function, and here I show that inorganic nutrients fuel benthic primary producers, OA facilitates fleshy algae but inhibits calcifying algae and that warming has complex interactive effects on the ecophysiology of turf assemblages. This dissertation improves our understanding of how heterogeneity and global change may influence patterns of benthic primary production, and thus ecosystem structure and function, in the near future.

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CHAPTER 2

Photosynthetic potential of corals and algae increase across a natural gradient in inorganic nutrient availability in the central Pacific

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ABSTRACT

Oceanographic upwelling delivers inorganic nutrients to nearshore communities that are important for resident organisms, particularly in nutrient depleted systems. Although coral reefs are often considered oligotrophic systems, some experience persistent or episodic upwelling. However, relatively little is known about the effects of natural nutrient subsidies on coral reef primary producers and ecosystem productivity. To test the hypothesis that coral and algal primary production is increased by exposure to inorganic nutrient availability, we conducted environmental and physiological measurements of the most abundant primary producers across the Southern Line Islands (SLI) in the Republic of Kiribati. The SLI span a natural oceanographic gradient of increasing exposure to equatorial upwelling. The two northernmost islands had cooler water temperatures and higher inorganic nutrient concentrations compared to the three islands in the southern region of the chain. Pigment concentrations and photosynthetic efficiency, cumulatively referred to as photosynthetic potential, of the two dominant genera of coral (*Montipora* and *Pocillopora*) and algae (*Porolithon*, *Avrainvillea* or *Halimeda*) at each of the SLI tracked patterns of nutrient availability. Maximum quantum yield and photosynthetic pigments of all taxa, as well as calcification of *Halimeda*, increased as a function of nutrient availability. The most striking physiological pattern was an increase in coralline algal phycobilin pigments. As marine primary production is driven by photosynthesis, increases in photosynthetic potential of primary producers in regions of persistent upwelling may indicate a higher capacity for ecosystem primary production.

INTRODUCTION

Nutrient availability is known to drive organismal physiology and influence community structure, function and productivity across ecosystems (Elser et al. 2007; Oksanen et al. 1981). In marine ecosystems, oceanographic processes can provide persistent or episodic pulses of nutrients that can fuel primary productivity through the delivery of otherwise limiting inorganic nutrients (Behrenfeld et al. 2006; Bunt 1973; Geider et al. 2001). Persistent upwelling, caused by long-standing currents (Cromwell 1953), and periodic upwelling, caused by episodic processes such as internal tidal bores (Leichter et al. 1996), deliver subthermocline water to the ocean surface. These deep, cooler waters are enriched in nitrate and phosphate and replace the warmer, nutrient-depleted surface waters (Mann and Lazier 2005). The delivery of potentially limiting inorganic nutrients can fuel primary production and thus ecosystem community structure by increasing the amount of fixed carbon available to higher trophic levels (Field et al. 1998). Marine primary production is driven mainly by photosynthesis and is influenced by a variety of abiotic factors including light, temperature and availability of inorganic nutrients. However, nutrient availability may be the most important physical factor affecting autotrophs in systems that are neither light-limited nor highly variable with respect to temperature, such as in the tropics.

Biologically available forms of macronutrients, including nitrogen and phosphorous, are essential for autotrophs because they provide the raw materials for synthesizing, maintaining and utilizing photosynthetic machinery. Nutrient availability can thus directly influence photosynthetic rates and primary productivity, especially in areas where inorganic nutrients are naturally low. Nitrogen and phosphorous, in

particular, have predictable and positive effects on biological productivity, and are often the limiting factors for photosynthesis (Cullen et al. 1992; Falkowski et al. 1998). For example, nitrate and/or phosphate limitation can decrease productivity and growth in phytoplankton (Caperon and Meyer 1972; Thayer 1974), microalgae (Cullen and Horrigan 1981; Falkowski et al. 1993; Muscatine et al. 1989) and macroalgae (Deboer 1977; Delgado and Lapointe 1994; Fong et al. 2003; Gagne et al. 1982; Lapointe 1987; Rosenberg and Ramus 1982; Topinka and Robbins 1976). Although oceanographic upwelling events are linked to increased surface-ocean productivity (Barber and Chavez 1991; Bunt 1973; Field et al. 1998), less is known about how these natural nutrient subsidies influence photosynthesis and primary production of benthic taxa.

Equatorial upwelling is caused by divergence of Ekman transport to the equator (Cromwell 1953) and leads to persistently higher concentrations of inorganic nutrients in surface waters of the equatorial Pacific (Barber and Chavez 1986; Chai et al. 1996), spanning ~8°N to 8°S (Wyrtki 1981). The nutrient subsidies from upwelling support high levels of primary production in equatorial Pacific surface waters relative to the oligotrophic tropical gyres (Barber et al. 1996; Chavez and Barber 1987; Chavez et al. 1996; Pennington et al. 2006), and corresponds to increased surface-ocean productivity (observed via satellite-derived chlorophyll concentrations) closer to the equator (Koblents-Mishke 1965). Persistent exposure to upwelled nutrients is an essential determinant of community structure in temperate systems (Dayton 1985; Parnell et al. 2010), but their impact is less understood in tropical systems.

Coral reefs exist in warm, often oligotrophic tropical waters, yet they sustain paradoxically high rates of production (Darwin 1874; Muscatine and Porter 1977; Odum and Odum 1955). While some reefs exist in these classically nutrient-depleted conditions, natural nutrient subsidies delivered by upwelling processes likely facilitate the persistence of high rates of coral reef primary production (Gove et al. 2016). Indeed, upwelling on coral reefs has been documented across ocean basins, ranging from the Florida Keys (Leichter et al. 1996) and the Colombian Caribbean (Diaz-Pulido and Garzon-Ferreira 2002) to the Seychelles (Novozhilov et al. 1992) and the Great Barrier Reef (Andrews and Gentien 1982). Despite the prevalence of upwelling processes on coral reefs, we have a limited understanding of how these natural nutrient subsidies influence resident reef organisms and ecosystem processes (Beach et al. 2003; Diaz-Pulido and Garzon-Ferreira 2002; Leichter and Genovese 2006; Smith et al. 2004).

Coral reefs are important contributors to global primary production (Crossland et al. 1991; Duarte and Cebrian 1996). The key benthic autotrophs contributing to reef primary production include reef-building corals, turf algae, fleshy macroalgae and calcareous algae (Hatcher 1988). Nutrient enrichment experiments in the field and laboratory consistently demonstrate a positive effect of nitrogen and/or phosphorous availability on growth, calcification, productivity and photosynthetic potential of corals and macroalgae, particularly under nutrient limiting conditions (Klumpp et al. 1990; Lapointe 1985; Lapointe 1997). Inorganic nutrient availability is tightly coupled with photosynthetic processes in algae, where increased availability leads to increases in photosynthetic pigment content, photosynthetic efficiency and photosynthetic

capacity. Quantifying parameters of photosynthesis and calcification in corals and algae across a gradient of upwelling can thus provide insight into the effect of persistent exposure to inorganic nutrients on benthic autotroph ecophysiology.

The role of natural nutrient subsidies in structuring modern coral reefs can be difficult to discern because coral reefs across the globe are increasingly exposed to anthropogenic nutrient pollution (Hughes et al. 2007). Extreme nutrient enrichment associated with human populations can negatively impact coral reefs by decreasing growth rates of corals and fueling rapid growth of macroalgae (Szmant 2002). Coupled with overfishing, anthropogenic nutrient pollution can cause reefs to shift to dominance by fleshy taxa rather than reef-building corals and algae (Hughes 1994; Hughes et al. 2007; Jackson et al. 2001). However, in systems that are devoid of local, human sources of nutrient pollution and overfishing, natural nutrient inputs likely have a positive impact on maintaining a balance between fleshy and calcifying taxa. The aim of this study was to explore the role of equatorial upwelling on coral reef autotrophs in the remote central Pacific, across a series of islands with no history of long-term human habitation.

To better understand the effects of nutrient subsidies on benthic autotrophs we explored the relationship between exposure to equatorial upwelling and ecophysiology of the five dominant autotrophic taxa on reefs of the Southern Line Islands (SLI) in the remote central Pacific (Fig. 1). The SLI are a series of uninhabited islands and atolls that span a natural gradient of surface ocean productivity and inorganic nutrient availability driven by distance from the equatorial upwelling region (Cromwell 1953; Pennington et al. 2006; Wyrtki 1981). We tested the hypothesis that the ecophysiology

of the two dominant genera of corals and algae would vary across the SLI in relation to exposure to equatorial upwelling. We predicted that metabolic rates (production and respiration), maximum quantum yield and photosynthetic pigment concentrations would increase with increasing exposure to equatorial upwelling (decreasing distance to the equator). Photosynthesis by benthic autotrophs drives primary production on coral reefs, and autotrophic responses to equatorial upwelling can therefore inform patterns in benthic primary production. By understanding patterns in ecophysiology of key benthic taxa across the SLI, we can provide insight into the relative importance of oceanographic conditions, such as upwelling, on the physiology of benthic autotrophs.

METHODS

Site Description

This study was conducted in 2013 on an expedition to the SLI aboard the *M/Y Hanse Explorer*. The five uninhabited islands of the SLI are under the jurisdiction of the Republic of Kiribati in the central Pacific, and span ~900 km from Malden Island at the northern end of the island chain to Flint Island at the southern end (Table 1, Fig 1D). The three southernmost islands (Flint, Vostok, Millennium) generally resemble oligotrophic waters characteristic of many coral reef systems, but the two northernmost islands (Malden, Starbuck) exist within purportedly nutrient rich waters of the equatorial upwelling region (Kelly et al. 2014). The coral reefs of the SLI represent “intact” reef ecosystems, characterized by high abundance and biomass of herbivores (Edwards et al. 2014) and top predators and high benthic cover of reef-building corals and coralline algae (Smith et al. 2016). The SLI provide an opportunity

to study the influence of large-scale oceanographic processes on benthic community structure and function without the potentially confounding factors associated with local human populations (e.g. nutrient pollution, runoff).

Surface Ocean Primary Productivity

To estimate surface ocean primary productivity across the SLI, we used the eight-day 0.0417° (4 km) spatial resolution product of chlorophyll *a* (mg mg^{-3}) derived from the Moderate Resolution Imaging Spectroradiometer (MODIS). Data were obtained for September-December 2013 to provide a four month window centered on the time period of the research cruise. The oceanographic conditions during this period are most relevant to the ecophysiology of the organisms studied and provide a reliable estimate of the seasonal conditions across the SLI. Due to data-quality concerns, nearshore pixels were masked and the most appropriate pixels for a nearshore estimate were selected following Gove et al. (2013) and (2016).

Study Design

All physiological studies were conducted aboard the *M/Y Hanse Explorer* at each island for three to four consecutive days, beginning at Flint Island and followed by Vostok Island, Starbuck Island, Malden Island and Millennium Atoll. Two species of coral and algae were collected on scuba at a depth of 10 m from each island (Table 1). In general, we sampled the most abundant species of coral and algae from each island in the archipelago, but due to biogeographic differences we were not always able to use the same species across all islands. Replicates of each species were

collected from different individuals (n = 4 per species) in unshaded habitats. Replicates that were 5-8 cm in length or diameter were collected for the branching coral, *Pocillopora meandrina*, the plating coral, *Montipora aequituberculata* and the crustose coralline alga (CCA), *Porolithon* sp with a hammer and chisel at each of the five islands. Voucher samples of CCA were collected, rinsed in freshwater and identified to genus with basic microscopy. Replicates of the dominant species of *Halimeda* (~8 cm in length) were collected from each island, when present (Flint (*H. opuntia*), Starbuck (*H. micronesica*), and Millennium (*H. taenicola*)), with the holdfast left intact to minimize damage to the photosynthetic portion of the thallus. The three species of *Halimeda* are morphologically similar and are not psammophytic (soft-sediment dwelling), meaning they attach to hard substrata by rhizoids or a small holdfast (Littler et al. 1988). We classified these algal taxa at the genus level, because *Halimeda* physiology varies only slighty by species (Jensen et al. 1985) and different species respond similarly to increasing nutrient concentrations (Beach et al. 2003; Littler et al. 1988). The fleshy green alga *Avrainvillea amadelpha* was collected at Malden and Vostok, with care taken to preserve the holdfast and minimize stress to the thallus. The most abundant species of algae varied by island, and complete absence from some islands precluded the use of the same genera and species across all SLI.

Metabolic Incubations

Production and respiration rates were determined by measuring oxygen exchange in saturating irradiance and total darkness, respectively, with sealed metabolic incubation chambers. Five incubations of one species were run

simultaneously, with each chamber containing one specimen or seawater control and a magnetic stir bar. Chambers were placed in a water bath (142 L cooler), with temperature maintained by a recirculating chiller set to ambient temperature (27-28.5 °C, site dependent). The cooler containing the water bath sat over a magnetized motor rack, and water flow was maintained inside each incubation chamber with magnetic stir bars. Incubation chambers were 2 L translucent plastic food storage containers with a gasket-sealed lid. A hole was drilled in each lid to hold a luminescent/optical dissolved oxygen (DO) probe (Hach IntelliCal LDO101) that was connected to a portable meter (Hach HQ40d) and accurate to ±0.1 mg L^{-1}. The body of the probe was wrapped with parafilm to ensure an air-tight seal with the incubation chamber lid.

Two 7-color LED light fixtures (Aqua Illumination, Hydra) were suspended directly above the incubation chambers. Light levels inside incubation chambers were measured with a submerged 4π quantum sensor (LI-193) attached to a LiCor LI-1400 meter. A preliminary set of incubations were conducted to construct photosynthesis versus irradiance curves (PI curves) and determine saturating irradiance for metabolic incubations (~700 µmol photon m^2 s^{-1}).

Oxygen production and consumption were measured during two separate incubations of the same individual. The first incubation was conducted at saturating irradiance, and the second incubation was conducted in total darkness. A blank control (with no specimen) was run alongside each set of light and dark incubations for each species. Dissolved oxygen was measured every minute during 45-90 minute incubations. Samples were kept in the dark for subsequent dark adapted measurements of photophysiology (see below).

Rates of oxygen production in the light and consumption in the dark were calculated as the linear slope of oxygen concentration against the duration of the incubation. The blank for each set of incubations was subtracted from sample production and respiration rates to account for background changes in oxygen. Oxygen production in the light represents net photosynthetic production (P_N) while net oxygen consumed in the dark represents respiration (R). To estimate total oxygen produced during incubations (gross photosynthetic production (P_G)), light respiration and dark respiration were assumed to be approximately equivalent, and R was added to P_N. Rates were normalized to surface area and are expressed as mg O_2 cm^{-2} hr^{-1}.

Surface area of coralline fragments was measured by the foil-wrapping technique following Marsh (1970). Coral surface area was determined by wax dipping following Stimson & Kinzie (1991). Coral tissue was removed from the carbonate skeleton with an airbrush and seawater (with a subsample for pigment analyses) and the skeleton was dried at 60°C to a constant weight. Coral skeletons were weighed, dipped in paraffin wax (at 62°C) and reweighed. Wax weight was converted to surface area based on a calibration with 15 wax-dipped wooden objects with known surface areas. The green algae genera, *Halimeda* and *Avrainvillea*, were thawed, photographed and subsampled for pigment analyses. Digital photographs were analyzed for planar surface area using imaging software, ImageJ, and these values were multiplied by two to yield total surface area.

Maximum Quantum Yield

Photophysiology was assessed further with a red Pulse Amplitude Modulation Fluorometer (Diving PAM, Walz). Samples were dark adapted for up to 2 hours, and maximum quantum yield (F_v/F_m) was measured at three haphazardly selected points on each alga and coral. Dark adapted quantum yield measurements provide an estimate of the maximal yield of photosystem II (PSII) (Kooten and Snel 1990), and can be used as a proxy for photosynthetic efficiency as it provides a convenient and instantaneous estimate of the performance of the photosynthetic machinery in both algae and corals (Hader and Figueroa 1997; Kolber and Falkowski 1993; Kooten and Snel 1990). We calibrated the fluorometer to produce F_0 measurements within 300-500 units for each genus, and minimized gain to avoid amplifying noise (Fitt et al. 2001). Further, we used the same settings for measuring light intensity, saturation intensity, saturation pulse width, gain and damping for each species at different islands. While there can be variability in maximum quantum yield within an individual, we addressed this by collecting three measurements from approximately the same region (i.e. ~2 cm from the tip) for each sample and calculated an average maximum quantum yield per individual. Due to logistical difficulties, no measurements were taken at Flint.

Pigment Concentrations

Samples were frozen at -20°C immediately after physiological assessments and were transported back to Scripps Institution of Oceanography (SIO) for and pigment analyses. The coralline alga *Porolithon* sp., was analyzed for chlorphyll *a* and

carotenoid pigments, as well as water-soluble phycobilin pigments. A 1 cm^2 punch was collected from the calcified thallus and ground to a fine powder in a cold mortar and pestle over ice and in the dark. Water soluble pigments were extracted first in 5 ml of 0.01 M phosphate buffer and centrifuged for 20 min at 7,000 x g and 4°C (Beer and Eshel 1985). Phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) concentrations were determined from the supernatant using the wavelengths and coefficients of Beer & Eshel (1985) for PE and PC, and Kursar and Alberte (1983) for APC. Chlorophyll *a* and total carotenoids were extracted by homogenizing the remaining pellet in 1 ml of *N,N*-dimethylformamide (DMF) following Moran & Porath (1980). Pigments were extracted for 24 hours in darkness at 4°C. The sample was then centrifuged for 5 min at 7,000 x g. The supernatant was analyzed with a diode array spectrophotometer (Agilent, UV-vis 8453) according to the wavelengths and coefficients of Wellburn (1994) for a spectrophotometer with 1 nm resolution. Pigment concentrations were normalized to surface area of the coralline punch and solvent volume.

For green algae (*Halimeda, Avrainvillea*), chlorophyll *a* and carotenoids were extracted from three intact subsamples of individual thalli in 1 mL of DMF. Pigment concentrations were determined spectrophotometrically according to wavelengths and equations of Wellburn (1994), normalized to the subsample wet weight and multiplied by the volume of DMF used in the extraction (1 mL). The three replicate subsamples of each individual were averaged to yield average chlorophyll *a* and total carotenoid concentrations per individual.

Corals fragments were subsampled for chlorophyll *a* and carotenoid pigments. Coral tissue was isolated in 6 mL of 0.7 µm filtered seawater using an airbrush. The resulting blastate was brought to a final total volume of 10 ml and homogenized for 30 seconds with an electric handheld homogenizer. Two replicate aliquots of 1.5 ml were removed from the homogenized blastate per coral and centrifuged for 2 min at 4,000 x g. The supernatant with the animal tissue fraction was discarded and the remaining zooxanthellae pellet was resuspended and vortexed with 1.5 ml of DMF. Pigments were extracted for 24 hours in darkness at 4°C. Pigment aliquots were then centrifuged again for 4 min at 4,000 x g to pellet all suspended particles and pigment concentrations were determined on the resulting supernatant according to the equations of Wellburn (1994). Chlorophyll *a* and total carotenoids were normalized to coral surface area and the two replicate aliquots were averaged to provide an average pigment concentrations per coral surface area.

Environmental Parameters

Water samples were collected for nutrient analyses at each island over three separate sampling intervals. During a similar cruise to the SLI in 2009, water samples were collected in triplicate from ~ 1 m above the benthos at 1-3 sites per island (referred to as 2009 *in situ*). Seawater was filtered through 1.2 µm GF/C filters (Whatman) into 50 mL falcon tubes and frozen upright at -20 °C. Samples were transported to the University of Hawaii (UH) Hilo EPSCoR analytical laboratory and analyzed for nitrate + nitrite ($NO_3^- + NO_2^-$) and phosphate (PO_4^{-3}). Triplicate samples were averaged across sites to yield nutrient concentrations by island.

During the 2013 cruise, 9-12 water samples were collected from ~1 m above the benthos at the site of algal and coral collections (referred to as 2013 *in situ*). Seawater was filtered through 1.2 µm GF/C filters into 20 mL plastic scintillation vials with foam lined caps; vials and lids were rinsed with sample water three times prior to collecting sample water. Samples were frozen -20 °C and similarly analyzed for NO_3^- + NO_2^- and PO_4^{-3} at the UH Hilo analytical laboratory. Water samples were averaged per island (n = 9-12).

Additionally, three water samples were collected from the bulk seawater used during metabolic incubations at each island (referred to as incubation nutrients). Samples were processed as above and analyzed at the University of California, Santa Barbara Marine Science Institute Analytical Lab for NO_3^- + NO_2^- and PO_4^{-3} concentrations (precision ± 5%), and water samples were averaged per island (n = 3)

We deployed 6-8 autonomous sensors (Manta 2, Eureka Environmental Engineering) at 10 m depth for 3-4 days at each island. Sensors recorded temperature every 5 minutes. Daily average temperature was calculated per sensor, and then averaged across sensors to produce an overall site average.

Net Calcification and Net Photosynthetic Production

Water samples also were collected from *Halimeda* incubations per island (at each island where *Halimeda* was present) for analysis of total carbon (C_T) and total alkalinity (A_T) according to standard protocols (Dickson et al. 2007). One bulk seawater sample was collected for initial carbonate chemistry values prior to the start of *Halimeda* incubations. Immediately after the light incubations, seawater was

collected from the control incubation and a subset of 3 *Halimeda* incubations at each island. Seawater was siphoned directly from incubation chambers into 200 ml Kimax brand screw-top bottle with a cone lid and teflon-taped threads. Samples were immediately poisoned with 120 µl of saturated mercuric chloride ($HgCl_2$). Water samples were analyzed for A_T and C_T at SIO. A_T was determined by potentiometric titrations with an open-cell titrator (Metrohm Dosimat Model 665) following standard operating procedure (SOP) 3b (Dickson et al. 2007). C_T was analyzed with an Automated Infra Red Inorganic Carbon Analyzer (AIRICA, Marianda) system equipped with a Li-Cor 7000. Performance and accuracy of these measurements was checked regularly against certified reference material provided by A. Dickson at SIO and daily precision for each parameter ranged from 2-3 (µmol kg^{-1}).

Halimeda sp. calcification rates were calculated based on the alkalinity anomaly technique (Smith and Key 1975). This approach assumes that formation of one mole of $CaCO_3$ decreases seawater A_T by two moles and C_T by one mole. Net calcification (NC) was calculated according to the equation:

$$NC = - ½ \Delta A_T \rho / \Delta t$$

where ρ is the density of seawater at incubation temperature and salinity, ΔA_T is the final A_T minus initial A_T (µmol kg^{-1}) and Δt is the duration of the incubation. Net photosynthetic production (NPP) was determined from initial and final measurements of C_T, accounting for changes in C_T due to calcification, according to the equation:

$$NPP = - \Delta C_T \rho / \Delta t – NC$$

where ΔC is the final C_T minus initial C_T (µmol kg^{-1}). NC and NPP were normalized to sample surface area and are expressed as mol hr^{-1} cm^{-2}.

Statistical Analyses

Mean environmental parameters, including satellite derived chlorophyll *a* and SST were analyzed across islands with a one-way ANOVA with island as a fixed factor. Inorganic nutrients were analyzed separately for each nutrient sampling interval, 2009 *in situ*, 2013 *in situ* and incubation nutrients. Where significant differences were detected, a Tukey's post-hoc comparison of means identified islands that were significantly different from each other.

Response variables were tested for normality (Shapiro-Wilks) and equality of variances (Bartlett's test). Each response variable was tested separately by species using a one-way ANOVA with island as a fixed factor, followed by Tukey's post-hoc comparisons. Statistical analyses were conducted in JMP v.10.

RESULTS

Environmental Parameters

The environmental conditions of the northernmost islands (Starbuck, Malden) were characteristic by cooler, nutrient enriched waters typical of an upwelling influenced system. There was a striking pattern of significantly higher satellite-derived surface chlorophyll concentrations with decreasing latitude ($F_{4,12} = 29.41$, $P < 0.0001$). From September to December 2013, Malden was within a region of high surface chlorophyll concentration, while Flint was within a region of low surface chlorophyll concentration, and the remaining three islands spanned a gradient increasing from south to north (Fig 2). Malden island surface waters averaged 10% higher chlorophyll

concentrations than Starbuck, while Starbuck, Millennium and Vostok averaged 15-20% more surface chlorophyll than Flint (Figure 3A).

Water samples collected for nutrient analyses *in situ* in 2009 and 2013, and from bulk water prior to incubations showed the same pattern of increasing ambient concentrations of $NO_3^- + NO_2^-$ and PO_4^{-3} from south to north across the SLI (Fig 3C,D). Malden and Starbuck were significantly enriched in $NO_3^- + NO_2^-$ compared to Flint, Vostok and Millennium, regardless of whether the water sampled was from ~1 m above the benthos in 2009 ($F_{4,18} = 24.47$, $P < 0.0001$) and 2013 ($F_{4,47} = 568.08$, $P < 0.0001$) or from nearshore surface water used in metabolic incubations ($F_{4,10} = 119.83$, $P < 0.0001$). *In situ* $NO_3^- + NO_2^-$ concentrations were an order of magnitude higher at Malden than at Flint in 2013, with averages (± SE) of 4.63 ± 0.13 versus 0.46 ± 0.1 µmol, respectively (see other values in Table 3). Additionally, inorganic nitrogen was 4 times higher at Malden than either Vostok or Millennium, 0.93 ± 0.05 and 0.95 ± 0.08 µmol, respectively, and PO_4^{-3} concentrations were 1.5-2.5 times higher at the northern islands (2013 *in situ*: 0.42-0.44 µmol) than at the three southern islands (2013 *in situ*: 0.13-0.16 µmol) (Table 3). PO_4^{-3} concentrations were also highest at Malden and Starbuck from *in situ* samples in 2009 ($F_{4,18} = 10.85$, $P < 0.0001$), 2013 ($F_{4,47} = 136.28$, $P < 0.0001$) and from incubation water samples ($F_{4,10} = 15.35$, $P = 0.0003$).

Short-term temperature data further support the presence of this oceanographic gradient, where the average daily temperature ranged from 28.5 ± 0.04 °C at Flint in the south to 27.3 ± 0.04 °C at Malden in the north (Fig 3B, Table 3).

Metabolic Incubations

There were genera-specific significant differences in metabolic rates across the SLI. The most striking differences were observed for the coralline red alga *Porolithon*, where both net oxygen production ($F_{3,12}$ = 10.50, P = 0.001) and gross oxygen production ($F_{3,12}$ = 13.97, P = 0.0003) were highest at Malden, the northernmost island in the SLI (Table 2), and generally decreased with latittude. Net production of *Porolithon* was 40% higher at Malden than Millennium, and 4-7 times higher than at Vostok and Starbuck. Gross oxygen production ranged from 60-200% higher at Malden than at all other islands. *Porolithon* respiration rates were highest at both Malden and Vostok ($F_{3,11}$ = 6.76, P = 0.008).

Metabolic rates of the coral *Pocillopora* varied significantly across the SLI, with highest metabolic rates at Starbuck, the second most northern island (Table 2). *Pocillopora* net production ($F_{4,15}$ = 7.07, P = 0.002), gross production ($F_{4,15}$ = 13.16, P <0.0001) and respiration ($F_{4,15}$ = 14.68, P <0.0001) were 60-250% higher at Starbuck than all other islands.

Metabolic rates of the green calcifying alga *Halimeda* varied significantly across the three islands where it was found (Flint, Millennium and Starbuck) (Table 2). Gross production ($F_{2,8}$ = 7.00, P = 0.018) and respiration ($F_{2,8}$ = 15.25, P = 0.002) were higher at both Millennium and Starbuck than at Flint. However, net oxygen production did not differ across the three islands ($F_{2,8}$ = 2.98, P = 0.108).

Metabolic rates of the coral *Montipora* and the green fleshy macroalga *Avrainvillea* did not vary across islands (Table 2). There were no differences in *Montipora* net oxygen production ($F_{4,14}$ = 0.105, P = 0.979), gross oxygen production

($F_{4,14}$ = 0.134, P = 0.967) or respiration ($F_{4,14}$ = 1.35, P = 0.301) by island. Rates of net oxygen production ($F_{1,6}$ = 1.89, P = 0.218), gross oxygen production ($F_{1,6}$ = 0.69, P = 0.437) and respiration ($F_{1,6}$ = 3.39, P = 0.115) by *Avrainvillea* did not differ between Vostok and Malden.

Maximum Quantum Yield

Maximum quantum yield, an instantaneous measure of the performance of photosystem II, increased across the SLI from south to north for all taxa. *Porolithon* maximum quantum yield differed significantly across islands ($F_{3,12}$ = 8.66, P = <0.001), and was 40-65 % higher at Malden, the northernmost island, than at Vostok and Starbuck, respectively (Fig 4A). Similarly, maximum quantum yield of *Pocillopora* was highest at Malden ($F_{3,12}$ = 17.98, P < 0.0001) (Fig 4B). Maximum quantum yield of *Montipora* varied significantly across the SLI ($F_{3,12}$ = 99.37, P < 0.0001), with highest values at Starbuck then Malden (Fig 4C). *Montipora* maximum quantum yield was 30% higher at Starbuck and 20% higher at Malden than both Vostok and Millennium at the southern end of the SLI. Maximum quantum yield of the fleshy green alga *Avrainvillea* was 10% higher at Malden than at Vostok ($F_{1,6}$ = 7.64, P = 0.033) (Fig 4D). Maximum quantum yield of *Halimeda* showed a similar, but not significant, trend with increasing values from the southern to the northern islands ($F_{1,6}$ = 1.89, P = 0.219) (Fig 4E).

Chlorophyll a and Carotenoid Concentrations

Chlorophyll *a* and total carotenoids, the primary photosynthetic pigments in coral reef benthic autotrophs, generally increased in concentration across all taxa from south to north along the SLI, although there were no significant differences for the green algae *Avrainvillea* and *Halimeda.* Although chlorophyll *a* concentration of *Porolithon* did not differ across islands ($F_{4,12} = 2.22$, $P = 0.128$), total carotenoid concentration was ~200% higher at Malden than Millennium, and 30-80% higher than Flint, Vostok and Starbuck ($F_{4,12} = 3.71$, $P = 0.035$) (Fig 5A). Chlorophyll *a* and carotenoid pigments of zooxanthellae symbionts in *Pocillopora* showed a similar trend for highest concentrations at Malden ($F_{4,12} = 12.37$, $P = 0.0003$; $F_{4,12} = 10.25$, $P = 0.0008$) (Fig 5B). Chlorophyll *a* concentration was 75-125% higher at Malden and Millennium than at Flint, Vostok and Starbuck, while carotenoids were 75-115% higher at Millennium than Starbuck and Flint, respectively. Chlorophyll *a* and carotenoids of *Montipora* zooxanthellae showed the most striking trend in pigment concentrations, with significantly higher concentrations at the two northernmost islands, Starbuck and Malden ($F_{4,12} = 18.72$, $P < 0.0001$; $F_{4,11} = 41.70$, $P < 0.0001$) (Fig 5C). Chlorophyll *a* concentrations were 150-260% higher at Malden and Starbuck, and carotenoids 100-260% higher, than at Flint, Vostok and Millennium. There were no significant differences in chlorophyll *a* or total carotenoid concentrations in the green algae, *Avrainvillea* ($F_{1,5} = 0.360$, $P = 0.575$; $F_{1,5} = 2.88$, $P = 0.151$) (Fig 5D) and *Halimeda* ($F_{2,7} = 3.14$, $P = 0.106$; $F_{2,7} = 1.70$, $P = 0.25$) (Fig 5E) across the SLI.

Phycobilin Pigment Concentrations

Phycobilin pigment concentrations, including phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC), of the coralline red alga *Porolithon* varied consistently across the SLI with concentrations increasing from south to north ($F_{4,13} = 8.28$, $P = 0.002$; $F_{4,13} = 15.64$, $P < 0.0001$; $F_{4,13} = 12.98$, $P = 0.0009$) (Fig 6). Relative to all other islands, PC and PE concentrations were 3-5 times higher at Malden, and APC concentrations were 2.5-3.5 times higher at Starbuck and Malden, respectively.

Net Calcification and Net Photosynthetic Production

Net calcification (NC) and net photosynthetic production (NPP) of *Halimeda*, as measured by changes in total alkalinity and total carbon, varied across the SLI where *Halimeda* was present ($F_{2,6} = 22.76$, $P = 0.002$; $F_{2,6} = 21.73$, $P = 0.002$). Both NC and NPP were 1.5-2 times higher at Starbuck than at Flint and Millennium, respectively (Table 2).

DISCUSSION

The SLI provide an opportunity to explore the influence of naturally elevated nutrient availability on coral reef organisms. The photosynthetic potential of the dominant benthic autotrophs generally increased across a latitudinal gradient as a function of increasing inorganic nutrient concentrations. The southern islands, Flint, Vostok and Millennium, were characterized by warm, oligotrophic conditions while the northern islands nearest to the equator, Starbuck and Malden, were indicative of

cooler, nutrient enriched waters of an upwelling-influenced system (Fig 3). The gradient in exposure to equatorial upwelling, and thus nutrient availability, was supported by increased concentrations of satellite-derived chlorophyll *a* estimates in surface ocean waters from south to north across the SLI (Fig 2). The ecophysiology of the dominant benthic autotrophs on the coral reefs of these islands, assessed by maximum quantum yield, photosynthetic pigments and net calcification, followed a similar pattern. Environmental and ecophysiological data together provide evidence that patterns of increased surface ocean productivity associated with equatorial upwelling corresponded with increased photosynthetic potential of the dominant benthic autotrophs in the SLI. Therefore, natural fluxes of nutrients can provide an important source of limiting inorganic nutrients to coral reefs (Andrews and Gentien 1982; Leichter et al. 1996), and directly influence rates of photosynthesis, with implications for primary production and general food web productivity.

Nutrient and temperature data across the SLI in this study confirm the existence of a gradient of increasing exposure to equatorial upwelling and are consistent with decades of research documenting persistently higher concentrations of inorganic nutrients, cooler temperatures and higher surface ocean production in the equatorial upwelling region (Barber and Ryther 1969; Barber and Chavez 1986; Chai et al. 1996; Cromwell 1953; Wyrtki 1981). Discrete water samples for nutrient analyses on water used in metabolic incubations (near-shore surface water) and from ~1 m above the benthos (10-15 m depth) across 2 years all showed that the northernmost islands, Malden and Starbuck, had consistently significantly higher ambient concentrations of nitrate and nitrite ($NO_3^- + NO_2^-$) and phosphate (PO_4^{-3}) (Fig

3C,D), the major inorganic nutrients brought to the surface by upwelling (Mann and Lazier 2005). Further, given that this trend was consistent across sampling cruises in April 2009 and October 2013 (Fig 3C,D, Table 3), it suggests these patterns are characteristic of the nutrient environment of these islands. Short-term temperature data further support the presence of this oceanographic gradient. Cooler water temperatures are consistent with the delivery of subthermocline water to reefs by oceanographic processes such as upwelling or internal tidal bores (Coles 1997; Leichter et al. 2003; Leichter et al. 1996; Wolanski and Delesalle 1995). Although nutrient and temperature data are from discrete or short-duration time points, our data were collected at two different times of year on two independent cruises and the long-term persistence of this gradient in both equatorial upwelling and nutrient availability are further supported by the trend in surface chlorophyll concentrations across the SLI (Fig 2)

Given the potential for nutrient availability to influence photosynthesis, we expected the photosynthetic potential of benthic autotrophs to increase across the SLI with increasing exposure to equatorial upwelling and ambient inorganic nutrient concentrations. While extreme nitrogen enrichment through human impacts can have deleterious effects on the coral/algal symbiosis (Szmant 2002), the highest concentrations of nitrate and phosphate found here were 5-fold lower than the minimum concentrations (20-200 μmol) shown to negatively affect coral physiology (Szmant 2002). We also expected coral photosynthetic potential to increase across the SLI as a result of either increased photosynthetic performance by their algal symbionts or increased chlorophyll density (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989). The most striking patterns we found were in the response of maximum

quantum yield and photosynthetic pigmentation of the dominant primary producers to the gradient in upwelling, with more variable effects on gross metabolic rates.

Our findings are consistent with the expected physiological impacts of increased nutrient availability on photosynthetic potential for all five of the taxa studied in the SLI. Maximum quantum yield increased across the SLI upwelling gradient for the dominant coral and algal genera in the SLI, with some variability in the magnitude of increase (Fig 4). Across all taxa, maximum quantum yield was highest at Starbuck and Malden, the northernmost islands which experience consistent and prolonged exposure to naturally high levels of inorganic nitrogen (Barber and Chavez 1986). From Flint, in the south, to Malden in the north, *Porolithon* maximum quantum yield increased by up to 65%, while it increased 20-30% for both coral genera. Maximum quantum yield in the green algae, *Avrainvillea*, was highest at Malden, but to a lesser magnitude (10%), while *Halimeda* showed only a slight trend for higher maximum quantum yield at the northernmost island, Starbuck.

Fluorescence measurements of PSII, such as maximum quantum yield, provide valuable insight into the photosynthetic potential of photoautotrophs and their response to varying environmental conditions (Berges et al. 1996; Kolber and Falkowski 1993), particularly the response of algal PSII efficiency to nitrogen availability (Berges et al. 1996). PSII is the primary reaction center for photosynthesis, or the first step in channeling energized electrons through the light-dependent reactions of photosynthesis, and is comprised of pigment-protein complexes (Barber and Anderson 2002). Chlorophyll *a* is at the core of the PSII complex, and electron transfer across pigment complexes is facilitated by a suite of reaction center proteins

(Barber and Anderson 2002). Nitrogen and phosphorous are the principal macronutrients for building and maintaining properly functioning pigment-protein complexes (Barber and Anderson 2002; Berges et al. 1996). Nutrient limitation inhibits synthesis of core proteins and pigments in PSII and PSI reaction centers (Falkowski et al. 1989; Kolber et al. 1988; Rhiel et al. 1986) and causes degradation of PSII pigment-protein complexes (Kana et al. 1992). Combined, nutrient limitation can lead to an overall loss of active PSII reaction centers by up to 65-85%, and a decrease in the efficiency of energy capture and conversion on the more southern islands (Berges et al. 1996). Thus long-term exposure to ample nitrogen availability likely increases the number, function and efficiency of photosystem reaction centers.

Maximum quantum yield measurements are most informative when coupled with additional estimates of photosynthetic machinery function, such as photosynthetic pigment content and metabolic rates. Normalized pigment content increased markedly from south to north across the SLI for all genera but the green algae, *Avrainvillea* and *Halimeda* (Fig 5). For both coral genera, surface area normalized concentrations of zooxanthellae chlorophyll *a* were highest at the northernmost islands, Starbuck and Malden, and a similar pattern was found for carotenoids in *Montipora*. *Porolithon* pigments generally followed the same pattern, but there was higher variability in pigment estimates, likely because it is difficult to isolate photosynthetic pigments from the calcified thallus of coralline algae. There were no differences in pigment concentrations for the green alga *Avrainvillea*, but a trend for higher chlorophyll *a* concentrations in *Halimeda* that was not significant. Pigment concentrations are flexible in primary producers (Rosenberg and Ramus

1982), and highly responsive to prevailing environmental conditions, especially through photoacclimation to light and ambient nutrient concentrations (Falkowski and Laroche 1991; Gantt 1990). Excess inorganic nutrient availability increases chlorophyll and carotenoid pigment concentrations across a suite of photosynthetic taxa and environmental conditions (Falkowski et al. 1993; Herzig and Falkowski 1989; Kolber et al. 1988; Lapointe and Ryther 1979; Morgan et al. 1980; Paerl et al. 2008). Nitrogen is essential for chlorophyll synthesis and maintenance (Cullen et al. 1992), and increased availability enhances the size and/or the number of active photosystems in algae (Falkowski and Laroche 1991). Similar to photosystems mentioned above, chlorophyll pigment-protein complexes break down when nutrients, particularly nitrogen, become limiting (Geider et al. 1993; Herzig and Falkowski 1989). Thus, increased nutrient availability can facilitate pigment-protein synthesis and increase pigment concentrations. Higher concentrations of photosynthetic pigments enhances the efficiency of energy capture and transfer and the overall capacity for photosynthesis and carbon fixation (Chapman et al. 1978).

Red algae possess additional accessory pigments, or phycobilin complexes, that give them their reddish-brown pigmentation, further broaden the spectrum of light available for photosynthesis and provide luxury nitrogen storage (Beale 1993; Gantt 1990; Kromkamp 1987). Phycobilin pigment concentrations showed a clear pattern of increasing concentrations from south to north across the SLI in the coralline red alga *Porolithon*, with highest concentrations of phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) at Malden (Fig 6), where we measured the highest nutrient concentrations. The magnitude of increase in phycobilin pigments concentrations

across the SLI was dramatic, with 2.5-5 times higher pigment concentrations at Malden than at Flint, mirroring the pattern in nutrient concentrations. Phycobilin pigments are particularly responsive to nutrient conditions (Kana et al. 1992; Kursar and Alberte 1983), in part because nitrogen is actively stored within the phycobilin pigment complexes. Nitrogen storage for luxury consumption by algae is an ecological adaptation to nutrient limitation (Gerloff and Krombhol.Ph 1966) and provides a source of inorganic nitrogen that can be later metabolized to fuel growth when nutrients are limiting (Chapman and Craigie 1977; Vona et al. 1992). Further, a lack of inorganic nutrient availability causes degradation of phycobilin pigment complexes (Allen and Hutchison 1980; Allen and Smith 1969; Kana et al. 1992) and represses phycobilin protein synthesis (Lau et al. 1977). It stands to reason, then, that the strongest detected effect of equatorial upwelling on photosynthetic potential was in the red algae phycobilin pigments that closely tracked ambient nitrogen availability. Primary and accessory photosynthetic pigment content may be ecologically important because higher concentrations increase photosynthetic capacity and photochemical efficiency (Geider et al. 1993; Herzig and Falkowski 1989).

In addition to measurements of photosynthetic machinery, direct measures of metabolic rates, including photosynthesis and respiration, are important for assessing ecophysiological status. Patterns in gross metabolism, including net photosynthetic production, gross photosynthetic production and respiration, were variable across the SLI, although three of the fiver genera studied, *Porolithon*, *Pocillopora* and *Halimeda*, showed significant increases in photosynthetic oxygen production and respiration at the northernmost islands. Oxygen production and respiration were up to 7 times higher

for the coralline alga, *Porolithon*, at Malden, while *Pocillopora* metabolic rates were 2.5 times higher at Starbuck than islands in the south (Table 2). *Halimeda* gross production and respiration were highest at both Millennium and Starbuck, although net oxygen production did not differ. Higher rates of gross production thus were driven by the differences in respiration across the islands. Conversely, *Montipora* and *Avrainvillea* had generally consistent metabolic rates across the SLI suggesting species-specific nutrient demans. Metabolic tended to match the patterns of increasing photosynthetic potential for the algae *Porolithon* and *Halimeda*, and the coral *Pocillopora*, but there was high variability associated with metabolic measurements. High variability may be due to difficulty in detecting signal versus noise in incubations, or perhaps compounding error associated with the several steps involved in normalizing metabolic rates to volume and biomass of each individual (e.g. volume and surface-area measurements) (Edmunds and Gates 2002). Despite the variability associated with these measurements, all genera trended towards increasing metabolic rates with increasing exposure to equatorial upwelling. Differences among islands or treatments may be more readily detected in future experiments by optimizing incubation experiments, reducing error in volume and surface-area measurements and by increasing replication.

Across the three SLI with the green calcified alga *Halimeda*, the increase in photosynthetic potential and metabolic rates (gross oxygen production and respiration) paralleled an increase in both net calcification (NC) and net photosynthetic carbon production (NPP), as measured through changes in total alkalinity (A_T) and total carbon (C_T), respectively. Both NC and NPP calcification rates were 1.5-2 times

higher at Starbuck, in the north, than Millennium and Flint in the south. Inorganic nutrient availability may influence algal calcification rates indirectly through direct effects on photosynthetic rates, because calcification and photosynthesis are tightly coupled in *Halimeda* (Borowitzka 1982; Jensen et al. 1985; Stark et al. 1969). Photosynthesis increases local pH in the intercellular spaces among the utricles (Borowitzka and Larkum 1977) and creates an internal environment that thermodynamically favors calcium carbonate precipitation (Borowitzka 1982; Borowitzka and Larkum 1976; De Beer and Larkum 2001). Here we show that both photosynthetic performance and calcification of *Halimeda* are enhanced by increasing exposure to equatorial upwelling and inorganic nutrients. Although elevated phosphate concentrations have been shown to impair calcification rates of *Halimeda* in controlled experiments (Demes et al. 2009), the concentrations required to elicit negative calcification responses surpassed the maximum phosphate values in the SLI by 40-fold. Our findings concur with previous studies that have documented increased rates of *Halimeda* photosynthesis (Littler et al. 1988; Teichberg et al. 2013), productivity (Delgado and Lapointe 1994; Wolanski et al. 1988) and growth (Vroom et al. 2003) in response to either natural nutrient subsidies or nutrient enrichment from *in situ* experimental manipulations (Smith et al. 2004). The coherence of our results to those of previous studies conducted on much smaller spatial scales or in controlled laboratory conditions suggests that influence of nutrients on coral reef primary production is a globally relevant process that is important to consider in future studies of coral reef ecosystem dynamics.

We used the SLI as a natural laboratory to explore the effects of a gradient in equatorial upwelling and nutrient availability on the ecophysiology of coral reef benthic autotrophs. We documented an increase in photosynthetic potential for the two dominant coral genera, Pocillopora and Montipora, and the three dominant algal genera, Porolithon, Halimeda and Avrainvillea, with increasing exposure to equatorial upwelling. Maximum quantum yield, photosynthetic pigment content and calcification rates were highest for all genera at islands nearest to the equatorial upwelling region, either Malden or Starbuck, relative to the more distant islands in the south, Flint, Vostok and Millennium. Physiological patterns of benthic taxa paralleled increased surface-ocean productivity, as determined from satellite-derived chlorophyll, and increasing exposure to environmental conditions that are indicative of oceanographic upwelling processes (Chai et al. 1996; Leichter et al. 1996; Wyrtki 1981). We have an advanced understanding of the relationship between upwelling and surface-ocean productivity, and our findings show that provide important information on how the physiology of the corresponding benthic primary producers is are similarly impacted by these nutrient subsidies, resulting in increasing metabolic rates with increasing exposure to equatorial upwelling.

Upwelling, internal tides and tidal bores are important oceanographic processes that influence the inorganic nutrient landscape on coral reefs worldwide (Andrews and Gentien 1982; Diaz-Pulido and Garzon-Ferreira 2002; Leichter et al. 1996; Quinn and Johnson 1996). Coral reefs are classically portrayed as highly oligotrophic and nutrient depleted systems that support paradoxically high rates of primary production (Muscatine and Porter 1977; Odum and Odum 1955), with the

exception of coastal reef systems that are impacted by anthropogenic nutrient pollution and effluent (Dubinsky and Stambler 1996). Yet, various processes of upwelling provide a critical and natural subsidy of limiting nutrients that may facilitate high rates of primary production on coral reefs (Gove et al. 2016). The ecophysiology and environmental data we present here demonstrate the surface-ocean productivity may be indicative of the relative potential for primary production of the underlying benthos. However, further research should explore the relationship between nutrient subsidies, organismal ecophysiology and ecosystem primary production, as such upwelling-influenced systems may have higher potential for net ecosystem production and trophic transfers. With the continual degradation of many coral reefs due to human activities, it is becoming increasingly difficult to understand the influence of natural nutrient subsidies on coral reefs without the confounding effects of anthropogenic nutrient pollution. We can thus use remote and uninhabited islands to understand how physical drivers shape the structure and function of coral reefs.

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FIGURES

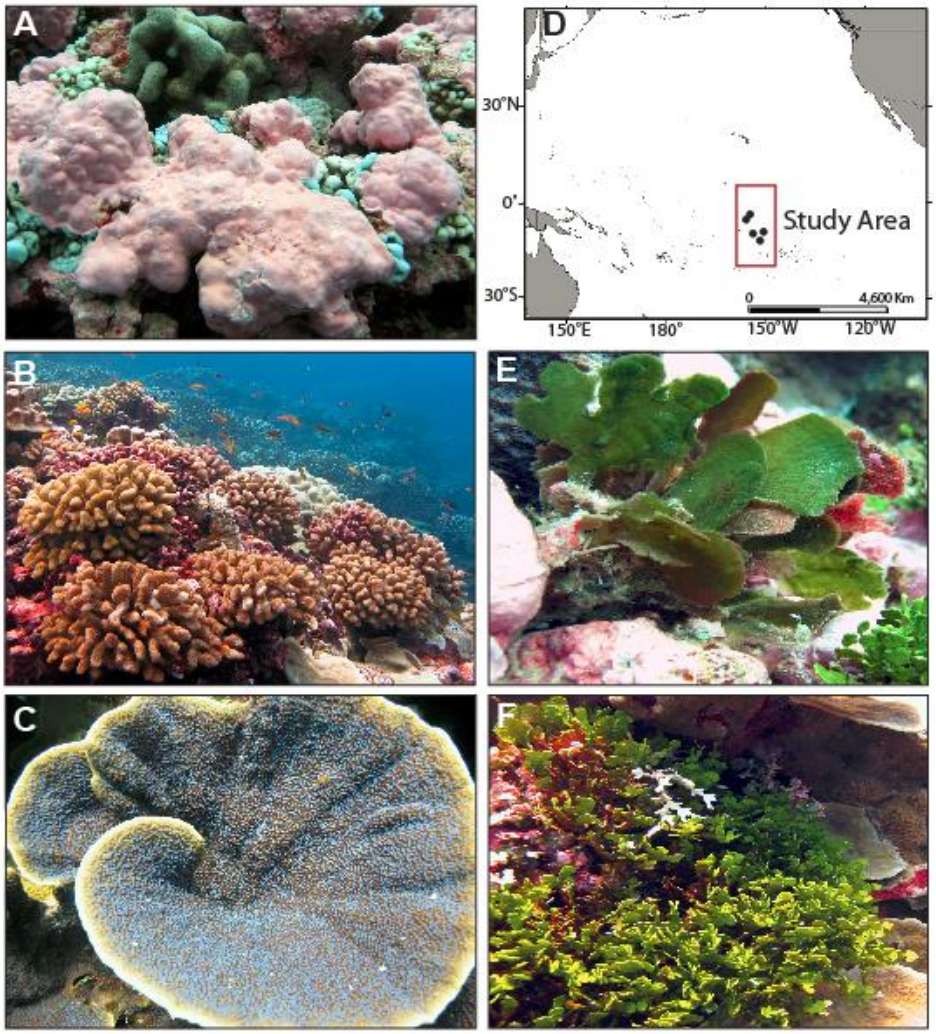

Figure 2.1. The most abundant genera of corals and algae across the Southern Line Islands used in studies of ecophysiology. Ecophysiology of the two most abundant genera of corals and algae was studied across the Southern Line Islands (D) in the Republic of Kiribati, central Pacific. The crustose coralline alga, *Porolithon* (A) and the corals *Pocillopora* (B) and *Montipora* (C) were collected from all five islands, while the fleshy green alga, *Avrainvillea* (E) was found only at Vostok and Malden, and the calcareous green alga, *Halimeda* (F), was found at Flint, Millennium and Starbuck.

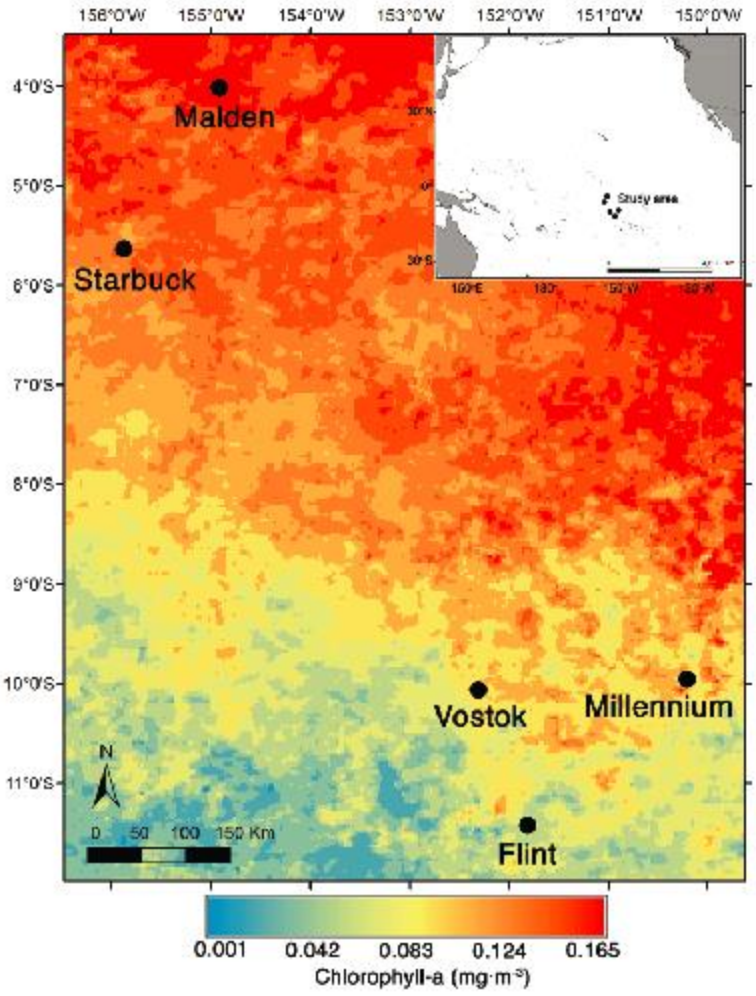

Figure 2.2. Surface ocean productivity across the Southern Line Islands. Average surface ocean productivity increased incrementally from south to north across the Southern Line Islands over the four months encompassing the research expedition. Oceanic primary production is estimated from the eight-day 0.0417° (4 km) spatial resolution product of chlorophyll *a* (mg mg^{-3}) derived from the Moderate Resolution Imaging Spectroradiometer (MODIS). The northernmost islands, Starbuck and Malden, fall within the equatorial upwelling region of higher surface ocean productivity, while Vostok, Millennium and Flint fall within more oligotrophic conditions of the open ocean.

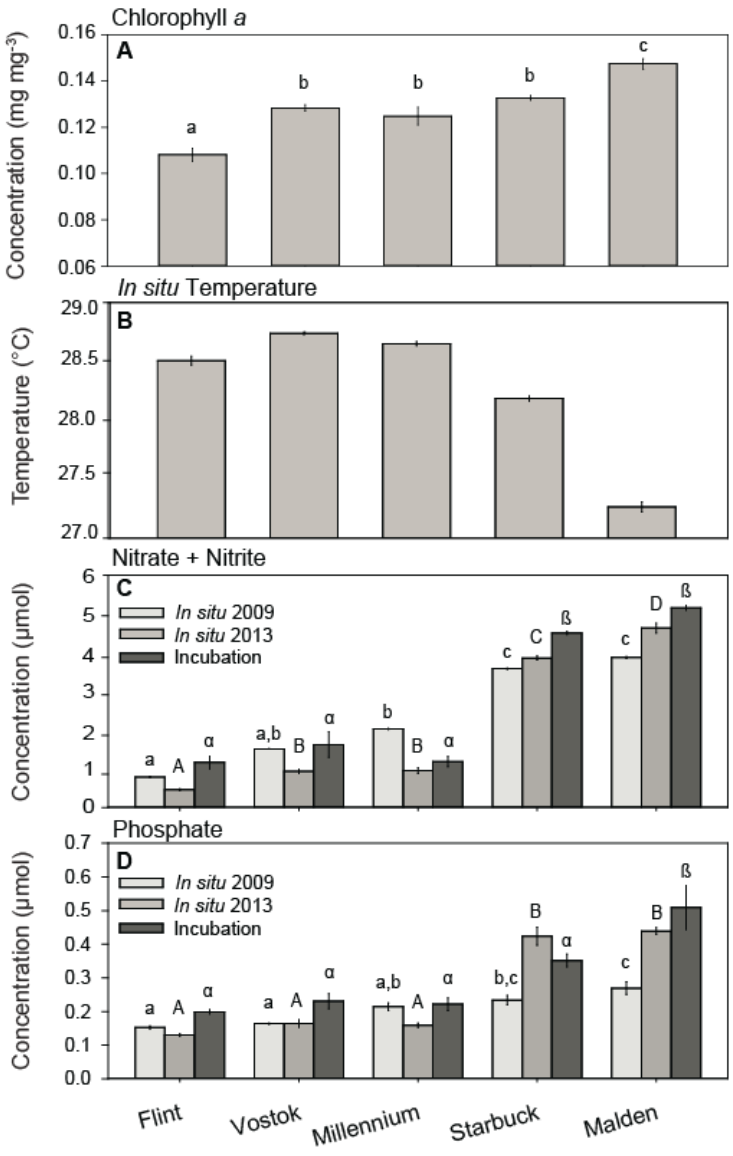

Figure 2.3. Environmental parameters across the Southern Line Islands. Mean (± SE) chlorophyll *a* was calculated as island averages (A). Daily *in situ* temperature was calculated as averaged daily averages of 6-8 sensors per island (B). Nitrate and nitrite (C) and phosphate (D) concentrations were measured on discrete water samples collected *in situ* in 2009 (n = 3-6) and 2013 (n = 9-12), and from nearshore surface water used in metabolic incubations (n = 3). Significant differences between islands were determined by Tukey's post-hoc comparisons, different letters are significantly different ($p < 0.05$).

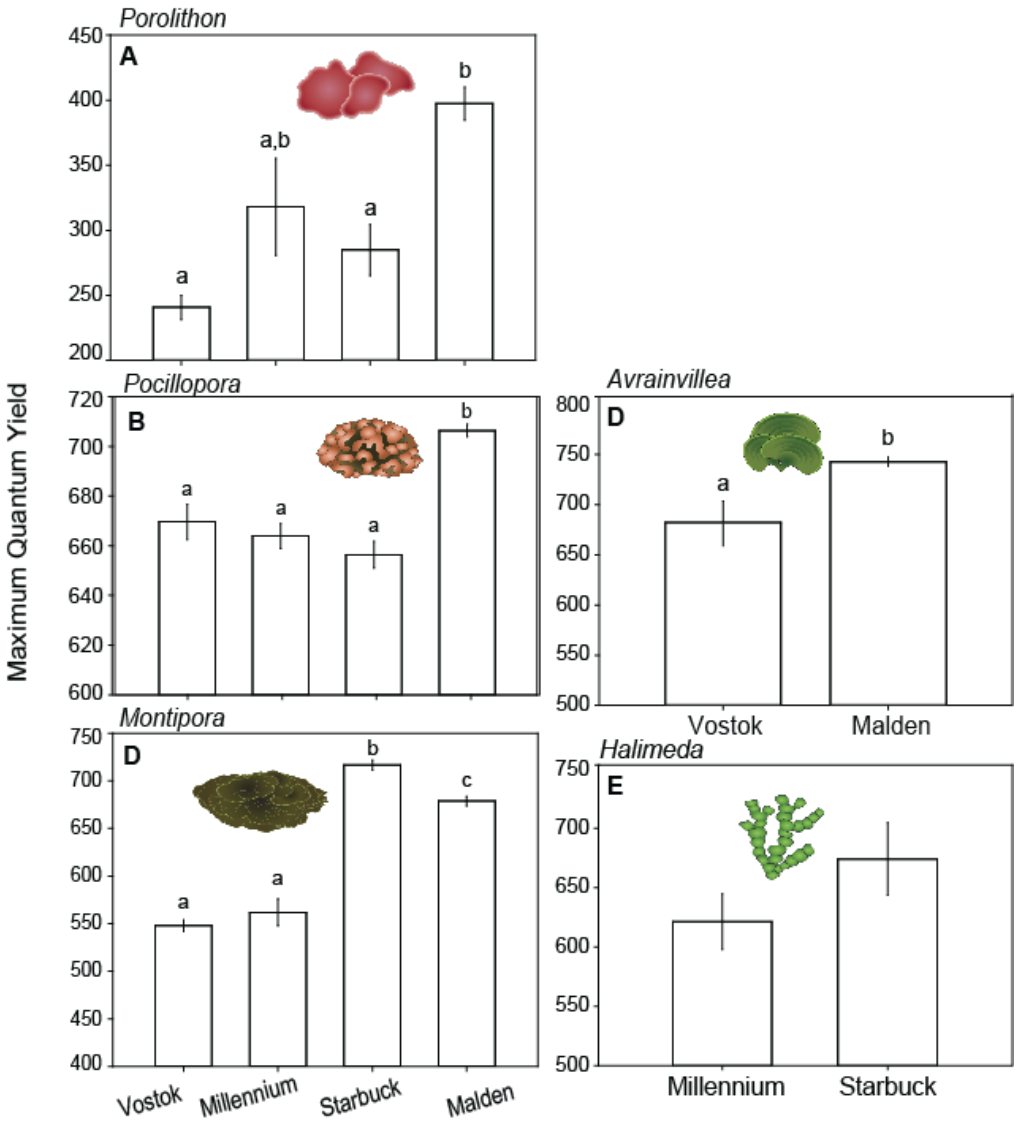

Figure 2.4. Maximum quantum yield of benthic primary producers. Mean (± SE) maximum quantum yield of key benthic taxa across the Southern Line Islands. Taxa include the crustose coralline alga *Porolithon* (A), the corals *Pocillopora* and *Montipora* (B,C), the fleshy green alga *Avrainvillea* (D) and the calcareous green alga *Halimeda* (E). Significant differences in maximum quantum yield across islands were determined per genus with Tukey's post-hoc comparisons, and islands with different letters are significantly different ($p < 0.05$).

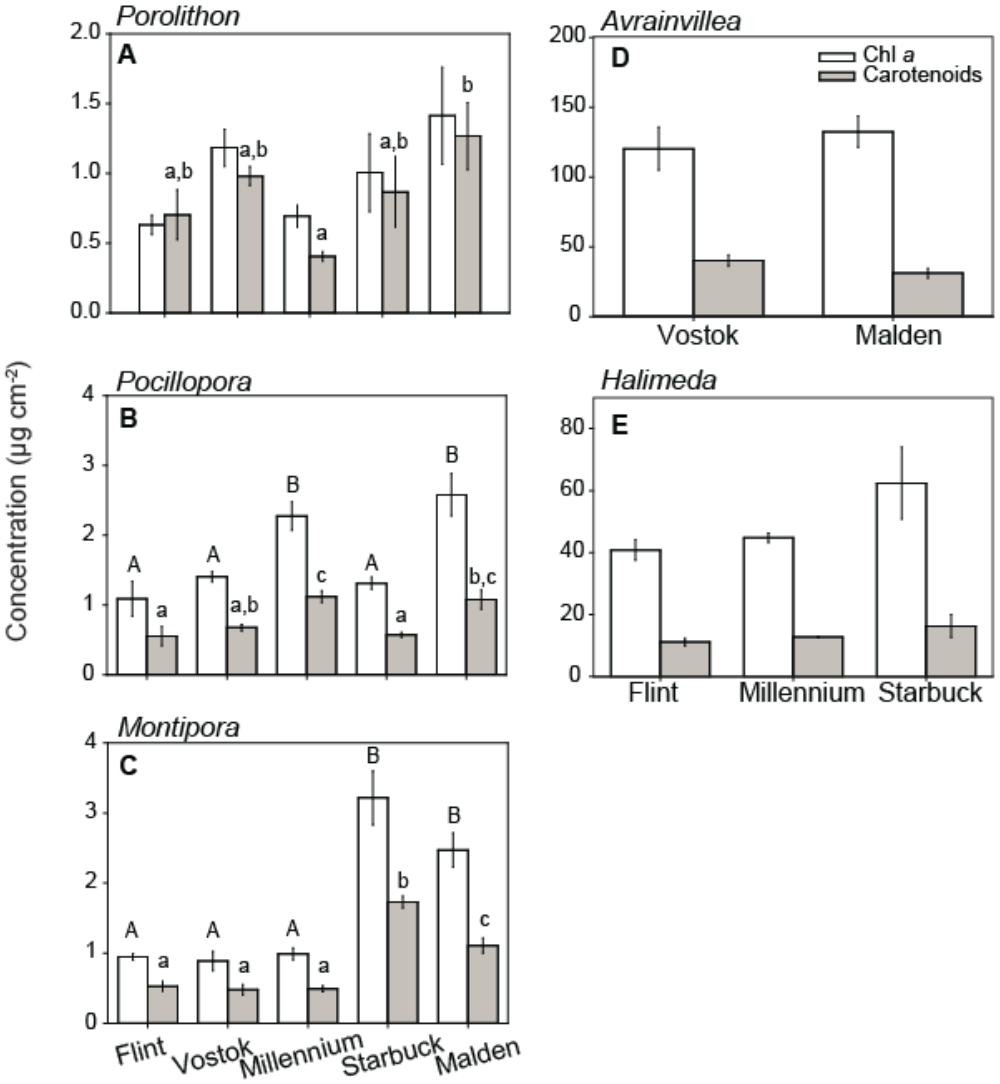

Figure 2.5. **Photosynthetic pigment concentrations.** Mean (± SE) photosynthetic pigment concentrations normalized to surface area for the five abundant taxa across the Southern Line Islands. Chlorophyll *a* (open bars) and total carotenoid (gray bars) concentrations were measured on subsamples (n = 2) of individual replicates (n = 4) per island. Significant differences in pigment concentrations across islands were determined per genus and pigment with Tukey's post-hoc comparisons, and islands with different letters are significantly different ($p < 0.05$).

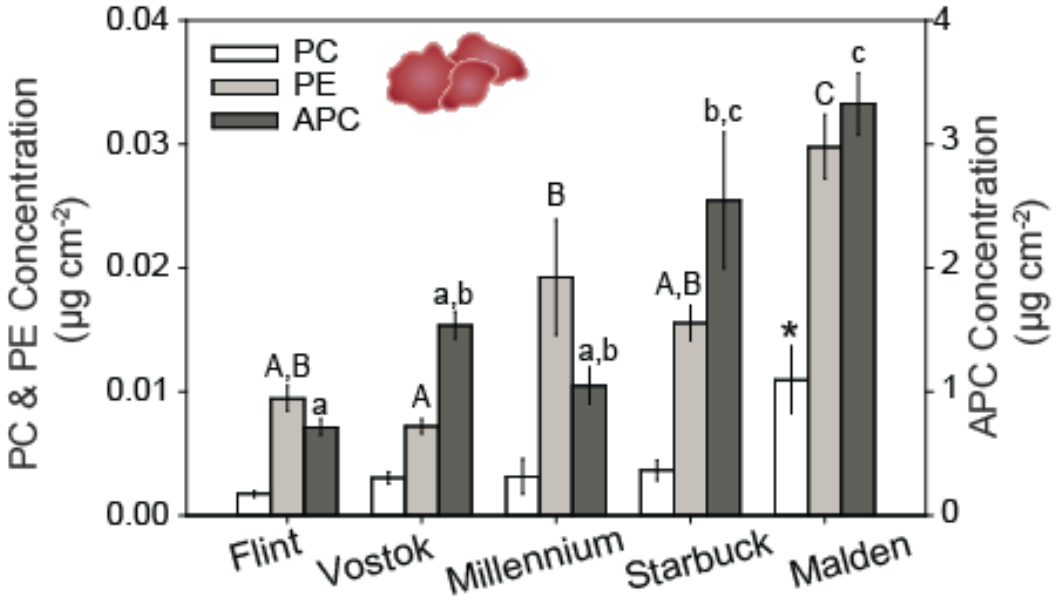

Figure 2.6. **Phycobilin pigment concentrations of the coralline alga *Porolithon*.** Mean (± SE) phycobilin pigment concentrations normalized to surface area for the crustose coralline alga *Porolithon* across the Southern Line Islands, listed in order from south to north. Phycocyanin (PC, open bars), phycoerythrin (PE, light gray) and allophycocyanin (APC, dark gray) concentrations were measured on subsamples (n = 2) of individual replicates (n = 4) per island. Significant differences in pigment concentrations across islands were determined for each pigment with Tukey's post-hoc comparisons, and islands with different letters are significantly different ($p < 0.05$).

TABLES

Table 2.1. The Southern Line Islands in order from south to north. Islands closest to the equator were characterized by higher ambient nutrient concentrations due to equatorial upwelling (in bold). The most abundant genera of corals and algae were collected from one site at each island for physiological measurements.

Island	Lagoon Area (km^2)	Coordinates	Coral	Algae
Flint	0.01	11°26'S 151°48'W	*Pocillopora* *Montipora*	*Halimeda* *Porolithon*
Vostok	-	10°06'S 152°25'W	*Pocillopora* *Montipora*	*Avrainvillea* *Porolithon*
Millennium	6.3	9°57'S 150°13'W	*Pocillopora* *Montipora*	*Halimeda* *Porolithon*
Starbuck	4	5°37'S 155°56'W	*Pocillopora* *Montipora*	*Halimeda* *Porolithon*
Malden	13	4°01'S 154°59'W	*Pocillopora* *Montipora*	*Avrainvillea* *Porolithon*

Table 2.2. Mean metabolic and calcification rates of the dominant taxa across the Southern Line Islands, listed from south to north.

Island	Genus	Net Production (mg cm^{-2} hr^{-1})	Gross Production (mg cm^{-2} hr^{-1})	Respiration (mg cm^{-2} hr^{-1})	Calcification (mol cm^{-2} hr^{-1})
Flint	*Pocillopora*	0.021 ± 0.002	0.037 ± 0.002	0.015 ± 0.001	
	Montipora	0.022 ± 0.006	0.040 ± 0.009	0.018 ± 0.003	
	Halimeda	0.012 ± 0.002	0.013 ± 0.002	0.002 ± 0.0003	0.65 ± 0.16
Vostok	*Pocillopora*	0.012 ± 0.005	0.023 ± 0.004	0.011 ± 0.001	
	Montipora	0.026 ± 0.002	0.049 ± 0.004	0.022 ± 0.002	
	Porolithon	0.006 ± 0.004	0.030 ± 0.005	0.024 ± 0.001	
	Avrainvillea	0.005 ± 0.0002	0.011 ± 0.001	0.007 ± 0.001	
Millennium	*Pocillopora*	0.010 ± 0.003	0.019 ± 0.004	0.009 ± 0.002	
	Montipora	0.028 ± 0.009	0.043 ± 0.014	0.015 ± 0.004	
	Porolithon	0.020 ± 0.005	0.031 ± 0.002	0.014 ± 0.003	
	Halimeda	0.017 ± 0.0009	0.023 ± 0.002	0.006 ± 0.0004	0.53 ± 0.07
Starbuck	*Pocillopora*	0.034 ± 0.005	0.059 ± 0.007	0.025 ± 0.002	
	Montipora	0.027 ± 0.008	0.041 ± 0.010	0.013 ± 0.002	
	Porolithon	0.0004 ± 0.001	0.016 ± 0.001	0.016 ± 0.002	
	Halimeda	0.014 ± 0.001	0.022 ± 0.002	0.009 ± 0.001	1.61 ± 0.12
Malden	*Pocillopora*	0.020 ± 0.001	0.034 ± 0.001	0.015 ± 0.001	
	Montipora	0.025 ± 0.005	0.043 ± 0.008	0.019 ± 0.004	
	Porolithon	0.029 ± 0.005	0.049 ± 0.005	0.021 ± 0.001	
	Avrainvillea	0.006 ± 0.001	0.011 ± 0.001	0.005 ± 0.001	

Table 2.3. Island-scale mean environmental parameters across the Southern Line Islands, from south to north. Chlorophyll concentrations were derived from satellite data of the SLI from September-December 2013. *In situ t*emperature was measured with autonomous sensors for 3-4 days per island. *In situ* water samples were collected for nutrient analyses (NO_3^- + NO_2^-, PO_4) from one site per island in 2013 (n = 9-12), and in triplicate from 3-6 sites per island during a research cruise in 2009. Islands closest to the equatorial upwelling region are in bold.

Island	Year	Temp (°C)	Chlorophyll (mg m^{-3})	NO_3^- + NO_2^- (µmol)	PO_4 (µmol)
Flint	2013	28.5 ± 0.04	0.108 ± 0.003	0.46 ± 0.01	0.13 ± 0.003
	2009			0.78 ± 0.02	0.15 ± 0.003
Vostok	2013	28.7 ± 0.01	0.128 ± 0.001	0.93 ± 0.05	0.16 ± 0.01
	2009			1.51 ± 0.35	0.16 ± 0.003
Millennium	2013	28.7 ± 0.02	0.125 ± 0.004	0.95 ± 0.08	0.16 ± 0.01
	2009			2.03 ± 0.06	0.21 ± 0.01
Starbuck	2013	28.2 ± 0.02	0.133 ± 0.001	3.86 ± 0.05	0.42 ± 0.03
	2009			3.59 ± 0.38	0.23 ± 0.01
Malden	2013	27.3 ± 0.04	0.147 ± 0.003	4.63 ± 0.13	0.44 ± 0.01
	2009			3.87 ± 0.32	0.27 ± 0.02

CHAPTER 3

Contrasting effects of ocean acidification on tropical fleshy and calcareous algae

Maggie D. Johnson, Nichole N. Price, Jennifer E. Smith

ABSTRACT

Despite the heightened awareness of ocean acidification (OA) effects on marine organisms, few studies empirically juxtapose biological responses to CO_2 manipulations across functionally distinct primary producers, particularly benthic algae. Algal responses to OA may vary because increasing CO_2 has the potential to fertilize photosynthesis but impair biomineralization. Using a series of repeated experiments on Palmyra Atoll, simulated OA effects were tested across a suite of ecologically important coral reef algae, including five fleshy and six calcareous species. Growth, calcification and photophysiology were measured for each species independently and metrics were combined from each experiment using a meta-analysis to examine overall trends across functional groups categorized as fleshy, upright calcareous, and crustose coralline algae (CCA). The magnitude of the effect of OA on algal growth response varied by species, but the direction was consistent within functional groups. Exposure to OA conditions generally enhanced growth in fleshy macroalgae, reduced net calcification in upright calcareous algae, and caused net dissolution in CCA. There was no consistent effect of OA on algal photophysiology. Our study provides experimental evidence to support the hypothesis that OA will reduce the ability of calcareous algae to biomineralize. Thus, our results suggest that projected OA conditions may favor non-calcifying algae and influence the relative dominance of fleshy macroalgae on reefs, perpetuating or exacerbating existing shifts in reef community structure.

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Contrasting effects of ocean acidification on tropical fleshy and calcareous algae

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ABSTRACT

Despite the heightened awareness of ocean acidification (OA) effects on marine organisms, few studies empirically juxtapose biological responses to CO_2 manipulations across functionally distinct primary producers, particularly benthic algae. Algal responses to OA may vary because increasing CO_2 has the potential to fertilize photosynthesis but impair biomineralization. Using a series of repeated experiments on Palmyra Atoll, simulated OA effects were tested across a suite of ecologically important coral reef algae, including five fleshy and six calcareous species. Growth, calcification and photophysiology were measured for each species independently and metrics were combined from each experiment using a meta-analysis to examine overall trends across functional groups categorized as fleshy, upright calcareous, and crustose coralline algae (CCA). The magnitude of the effect of OA on algal growth response varied by species, but the direction was consistent within functional groups. Exposure to OA conditions generally enhanced growth in fleshy macroalgae, reduced net calcification in upright calcareous algae, and caused net dissolution in CCA. Additionally, three of the five fleshy seaweeds tested became reproductive upon exposure to OA conditions. There was no consistent effect of OA on algal photophysiology. Our study provides experimental evidence to support the hypothesis that OA will reduce the ability of calcareous algae to biomineralize. Further, we show that CO_2 enrichment either will stimulate population or somatic growth in some species of fleshy macroalgae. Thus, our results suggest that projected OA conditions may favor non-calcifying algae and influence the relative dominance of fleshy macroalgae on reefs, perpetuating or exacerbating existing shifts in reef community structure.

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INTRODUCTION

Changes in ocean chemistry associated with anthropogenic carbon dioxide (pCO_2) emissions, a process known as ocean acidification (OA) (*Kleypas et al., 1999*; *Orr et al., 2005*), have raised widespread concern for the survival and persistence of marine biota (*Kleypas et al., 1999*; *Hoegh-Guldberg et al., 2007*). Identifying the groups of organisms that will be susceptible to rapid OA versus those that may be resistant has prompted numerous studies (*Ries, Cohen & McCorkle, 2009*; *Kroeker et al., 2010*; *Kroeker et al., 2013*). To date, research has focused on understanding how reductions in the saturation state (Ω) of calcium carbonate ($CaCO_3$) and seawater pH associated with OA will impact the growth

and physiology of commercially important calcifying organisms or entire ecosystems, such as coral reefs, that build carbonate platforms (*Kleypas et al., 1999*; *Andersson & Gledhill, 2013*). However, examination of a wider taxonomic representation, including those that calcify and those that do not, within and across ecosystems is critical to developing ecological predictions of community-level responses to OA.

The changes in the carbonate system have important implications for marine calcifiers, namely that OA may inhibit the ability of these species to grow, develop, reproduce and sustain themselves within a community, although plasticity in organismal responses indicates that some species may have wider tolerance limits (*Doney et al., 2009*; *Kroeker et al., 2010*; *Kroeker et al., 2013*; *Johnson, Moriarty & Carpenter, 2014*). Mounting evidence from coral reefs suggests that decreasing carbonate saturation (Ω) has negative effects on calcification (*Langdon & Atkinson, 2005*; *Doney et al., 2009*; *Andersson & Gledhill, 2013*), reproductive success (*Albright, 2011*), and competitive strength (*Diaz-Pulido et al., 2011*) of scleractinian corals. However, less attention has been given to the response of tropical marine primary producers to rising oceanic CO_2, particularly fleshy and calcareous benthic macroalgae which are also among the most dominant constituents of the coral reefs benthos.

The future trajectory of coral reefs may be influenced by concurrent effects of OA on both fleshy and calcified algae (reviewed in *Koch et al., 2013*), which serve key functional roles in reef systems in addition to competing with corals for space and resources. Calcareous algae contribute to framework development and some are active reef builders that account for up to 90% of living benthic cover on reefs (*Tribollet & Payri, 2001*). Crustose coralline algae (CCA) serve important ecological functions on reefs by contributing to primary production and carbonate production (*Chisholm, 2003*), producing settlement cues for coral larvae (*Harrington et al., 2004*; *Price, 2010*), and maintaining structural integrity of the framework by acting as reef cement (*Camoin & Montaggioni, 1994*). Calcareous green algae, such as *Halimeda* spp., are a major source of primary production and $CaCO_3$ (*Rees et al., 2007*) due to their fast growth and turnover rates (*Smith et al., 2004*), and are a preferred food source for many coral reef fishes (*Mantyka & Bellwood, 2007*; *Hamilton et al., 2014*). Fleshy macroalgae include a highly diverse group of seaweed species that act as a source of food for higher trophic levels and directly compete with corals for space (*McCook, Jompa & Diaz-Pulido, 2001*) on the reef benthos. Some fleshy macroalgae produce toxic allelochemicals which can kill corals upon contact (*Rasher et al., 2012*) while others may transmit coral disease (*Nugues et al., 2004*) or affect microbial assemblages associated with the coral holobiont via release of dissolved organic carbon (*Smith et al., 2006*; *Haas et al., 2013*; *Nelson et al., 2013*). Furthermore, the relative balance of calcifiers to fleshy macroalgae is important for reef resilience (*Hughes et al., 2010*). Increased cover of fleshy macroalgae, associated with anthropogenic disturbances such as poor water quality (*Fabricius, 2005*) and overfishing, is often used as an indicator of deteriorating reef health (*Hughes, 1994*). Given the important roles that calcareous and fleshy algae serve in the structure and function of coral reef ecosystems, it is critical to identify the potential differential effects of OA on these functionally distinct groups.

Increased CO_2 has the potential to have disparate effects on physiological processes for calcareous and fleshy algae, namely on photosynthesis and biomineralization. In terrestrial systems, rising atmospheric CO_2 can fertilize primary producers and enhance production (*Ainsworth & Long, 2005*), but in marine ecosystems, photosynthesizers have access to other relatively abundant carbon species, such as bicarbonate (HCO_3^-), that can be used for photosynthesis. The potential for CO_2 fertilization of marine primary producers is likely contingent on species–specific mechanisms of carbon acquisition, influenced by the activity of carbon concentrating mechanisms (CCMs) (*Giordano, Beardall & Raven, 2005*; *Raven et al., 2011*; *Koch et al., 2013*). Laboratory manipulations and field studies from temperate and Mediterranean ecosystems (*Hall-Spencer et al., 2008*; *Porzio, Buia & Hall-Spencer, 2011*) suggest that OA may enhance carbon fixation (*Kroeker et al., 2010*; *Cornwall et al., 2012*; *Kroeker et al., 2013*) and photosynthesis in fleshy algae resulting in increases in algal growth rates (*Gao et al., 1991*; *Kubler, Johnston & Raven, 1999*; *Cornwall et al., 2012*). However, variations in interspecific responses may depend on the extent to which a species is presently carbon-limited (*Harley et al., 2012*; *Koch et al., 2013*). The photosynthetic response of seaweeds to OA is poorly understood in part because data on the presence, absence, or activity level of CCMs is often lacking for many tropical species (*Hurd et al., 2009*; *Raven et al., 2011*). Although much of the literature on OA effects on marine algae has shown that CO_2 enrichment enhances photosynthesis in phytoplankton and phanerograms (*Riebesell et al., 1997*; *Palacios & Zimmerman, 2007*; *Gattuso & Hansson, 2011*), the photosynthetic response of seaweeds to OA has been highly variable across experiments (*Koch et al., 2013*) and sometimes negative for calcified species (*Anthony et al., 2008*; *Sinutok et al., 2011*; *Sinutok et al., 2012*).

Conversely, OA effects on skeletal production in calcareous algae have been studied in more detail and changes in carbonate chemistry (i.e., lower pH, lower carbonate availability, and decreased $CaCO_3$ saturation state) have been shown to inhibit calcification in many species. The effects of OA on calcification in marine organisms may be influenced by the ability for a species to control carbonate chemistry at the intracellular or extracellular site of calcification (*Ries, Cohen & McCorkle, 2009*). A decrease in Ω in the external environment associated with OA could make biogenic $CaCO_3$ crystal precipitation more difficult. When Ω decreases below the saturation horizon (<1) $CaCO_3$ dissolution is thermodynamically favored (*Milliman et al., 1999*). This saturation horizon is influenced by temperature, pressure, and mineralogy (*Feely et al., 2004*; *Orr et al., 2005*), and net dissolution and calcification can occur both above and below this threshold, respectively, depending on the organism and the environment (*Milliman et al., 1999*). CCA may be some of the most sensitive calcifiers to OA because they secrete high Mg-calcite, the most soluble polymorph of $CaCO_3$ (*Morse, Andersson & Mackenzie, 2006*; *Andersson, Mackenzie & Bates, 2008*). Other studies have found that OA decreases CCA calcification (*Semesi, Kangwe & Bjork, 2009*; *Johnson & Carpenter, 2012*; *Comeau, Carpenter & Edmunds, 2012*; *Comeau et al., 2013*; *Johnson, Moriarty & Carpenter, 2014*), structural integrity (*Ragazzola et al., 2012*) and increases mortality and that these effects that may be

exacerbated by warming temperatures (*Anthony et al., 2008*; *Martin & Gattuso, 2009*; *Diaz-Pulido et al., 2012*; *Martin et al., 2013*) or increased solar UV radiation (*Gao & Zheng, 2010*). The articulated calcareous green algae *Halimeda* spp. have been shown to be both sensitive (*Sinutok et al., 2011*; *Sinutok et al., 2012*) and insensitive to OA (*Comeau et al., 2013*), where the direction and magnitude of the response of *Halimeda* to OA varies among species (*Ries, Cohen & McCorkle, 2009*; *Price et al., 2011*; *Comeau et al., 2013*). Negative effects of OA on calcification of CCA and *Halimeda* spp. may have serious implications for carbonate production and framework stability on coral reefs because they are often common members of 'intact' benthic reef communities (*Sandin et al., 2008*; *Williams et al., 2013*).

The primary objective of our study was to determine if there are consistent, differential responses of fleshy and calcareous tropical marine algae to OA using parallel, replicated experimental manipulations. On Palmyra Atoll in the northern Line Islands, five common species of fleshy algae and six species of calcareous algae were exposed to CO_2 levels expected by the year 2100 under a business-as-usual carbon emissions scenario (*Meinshausen et al., 2011*). In particular, the hypotheses tested were that, even with variation in species–specific physiological responses, elevated CO_2, (1) reduces net calcification across calcareous algae but, (2) stimulates growth of fleshy algae by enhancing photosynthesis. This study provides one of the first efforts to quantify OA effects on multiple species of both calcareous and fleshy algae from a coral reef environment, and provides insight into the effects of OA on a suite of algae that are important in the structure and function of coral reefs.

MATERIALS AND METHODS

Study site and species

All experiments were conducted on Palmyra Atoll in the Northern Line Islands, central Pacific, in the recently established Pacific Remote Island Areas Marine National Monument (PRIAMNM) protected by the US Fish and Wildlife Refuge. Due to its isolation (~1,700 km south-southwest of Hawaii) and lack of permanent human residence, Palmyra's coral reefs are considered relatively healthy and are dominated by reef builders (*Sandin et al., 2008*; *Williams et al., 2013*). The remote nature of the field station limits research excursions to a few weeks at a time. Due to the absence of potentially confounding local anthropogenic impacts, Palmyra provides a unique setting for global change experiments.

To explore the effects of OA on different algal functional groups, eleven common species of algae were used in CO_2 enrichment experiments (see *Sandin et al., 2008* and *Williams et al., 2013* for relative abundances). Algae were categorized into three functional groups: fleshy macroalgae (*Acanthophora spicifera*, *Caulerpa serrulata*, *Dictyota bartayresiana*, *Hypnea pannosa*, and *Avrainvillea amadelpha*), upright calcareous algae (*Halimeda taenicola*, *Halimeda opuntia*, *Galaxaura rugosa*, and *Dichotomaria marginata*), and crustose coralline algae (CCA: *Lithophyllum prototypum*, formerly *Titanoderma prototypum*, and *Lithophyllum* sp.) (Fig. 1, Table S1). Specimens were collected via SCUBA at a depth

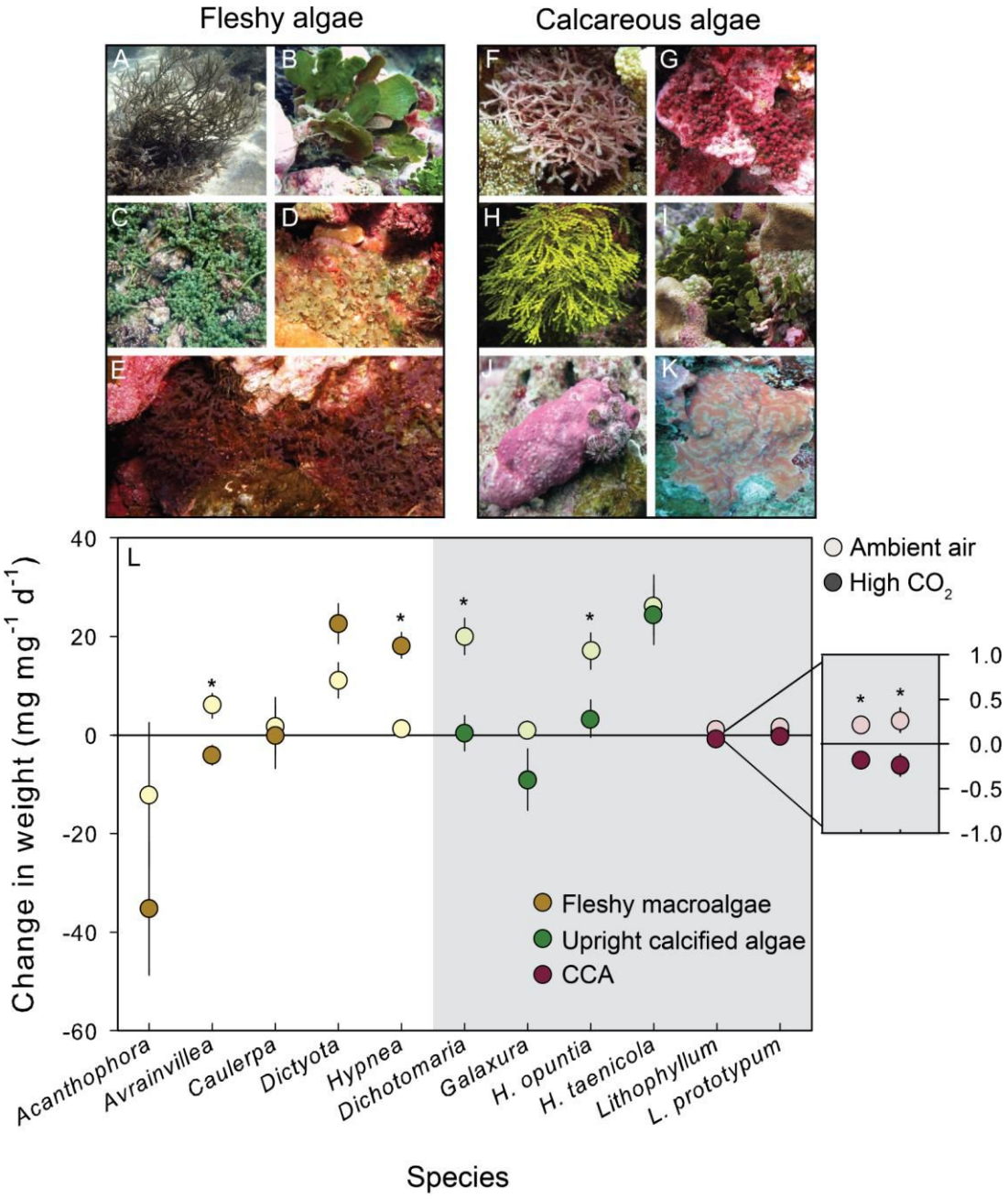

Figure 1 Growth response of fleshy and calcareous algae to treatment conditions. The eleven species of algae exposed to CO_2 enrichment experiments on Palmyra Atoll. Algae were separated by functional group. The species of fleshy macroalgae included: (A) *Acanthophora spicifera*, (B) *Avrainvillea amadelpha*, (C) *Caulerpa serrulata*, (D) *Dictyota bartayresiana*, (E) *Hypnea pannosa*. The upright calcareous algae included: (F) *Dichotomaria marginata*, (G) *Galaxaura rugosa*, (H) *Halimeda taenicola*, (I) *Halimeda opuntia*, and the CCA included: (J) *Lithophyllum* sp., (K) *Lithophyllum prototypum*. (L) The mean (±SE) change in either fleshy or calcareous biomass (highlighted in gray) following exposure to either present-day ambient air controls (open circles) or predicted OA treatments (closed circles). Fleshy macroalgae are shown in brown, upright calcareous algae in green, and CCA in red. Species tested in multiple experiments were pooled across years. * Indicates a significant difference between treatments as determined by independent *t*-tests (results reported in Table 2).

of ~5 m from the shallow western terrace (5°53.1696′N, 162°7.5756′W), excluding *L. prototypum*. *L. prototypum* was collected at a depth of ~10 m from the southern fore reef (5°53.7906′N, 162°7.6859′W) where the species is abundant. Except for the corallines, which were collected as free-living rhodoliths, individuals were removed at the holdfast or from rhizoids in order to minimize stress. Coralline rhodoliths were comprised of 100% live coralline cover, and no bare carbonate was exposed to the potentially corrosive conditions. Samples were cleaned carefully of epiphytes with a soft-bristled brush and allowed to acclimate for at least one day in fresh, ambient seawater.

Experimental conditions and seawater chemistry

To explore the effects of OA on growth, calcification and photophysiology of benthic algae, CO_2 enrichment experiments were conducted for ~2 weeks in July of 2010, and September of 2009, 2011, and 2012 (see Table S2 for experiments across years). Experimental aquariums (glass jars) held 700 mL of seawater collected from offshore and an individual alga (~2 g live tissue). Seawater was treated continuously with either air or CO_2 enriched gas for several hours prior to experimentation and changed (100%) every 48 h to prevent nutrient limitation and to maintain treatment conditions (sensu *Price et al., 2011*).

The effects of projected OA were simulated by micro-bubbling either pre-mixed air enriched with CO_2 to ~1,000 µatm into treatment aquariums (OA treatment) or ambient air into control aquariums. Clear polycarbonate lids reduced atmospheric equilibration, evaporation, and rainwater incursion. Air and CO_2 enriched gas were bubbled continuously into treatment aquariums through wooden air stones that were placed at the bottom, center of experimental replicates. The continuous bubbling within a relatively small volume facilitated thorough mixing of the seawater within the jars. It was not possible to measure water flow within the contained jars however gas was adjusted to flow into experimental aquariums at a constant rate. Sample sizes varied by experiment and the availability of samples, but ranged from 4 replicates per treatment/species in 2009 to 10 in 2012 (Table S2). Additionally, aquariums without algae were maintained in all experiments to determine if algal metabolism affected carbonate chemistry and altered treatment conditions.

Aquariums were partially submerged in flow-through seawater baths under natural sunlight with shade cloth screens to simulate *in situ* temperature and irradiance levels at 5 m depth (Table S2). Temperature and light intensity within aquariums were monitored every 15 min with data loggers (Onset, HOBO Pendant Temperature Light/Data Logger) for the duration of the experiments. Light intensity was measured in Lux, and converted to photosynthetically active radiation (PAR) with the following conversion: 1 µmol quanta (400–700 nm) m^{-2} s^{-1} = 51.2 lux (*Valiela, 1984*). This conversion was validated by additional *in situ* PAR measurements made at the collection site, using an underwater spherical quantum sensor (LICOR, LI-193). In 2009 and 2010, oxygen (O_2, polarographic electrode, ±0.2 mg L^{-1}), temperature (±0.15 °C), salinity (±0.1 psu) and pH_{SW} (±0.2) were monitored with a handheld meter (YSI Environmental Quatro). In 2011 and 2012, O_2 (±0.01 mg L^{-1}), temperature (±0.3 °C), and pH_{SW} (±0.1) were measured with a Hach

Lange HQ40 portable multi-parameter meter (IntelliCAL PHC101 Standard Gel Filled pH Electrode and IntelliCAL LDO101 Standard Luminescent Dissolved Oxygen LDO Optode). The pH probe was calibrated daily with certified Tris buffer (provided by Andrew Dickson, SIO). Using certified Tris buffer as a reference improved the accuracy of pH probe measurements to ±0.001. In each year, measurements were recorded from all aquariums in the evening (1800–2000) of each day (Table 1).

Discrete water samples for total alkalinity (A_T) and total dissolved inorganic carbon (C_T) were collected from empty aquariums (controls) and a subset of experimental aquariums from both treatment levels at multiple time points during all experiments (in 2009 only samples from empty aquariums were collected). Samples were collected by siphoning treatment water into 500 mL Corning-brand Pyrex sample bottles and fixed with 200 µL saturated $HgCl_2$, leaving a 1% head space. Water samples were transported to Scripps Institution of Oceanography (SIO) for standard carbonate chemical analyses, (SOP, sensu *Dickson, Sabine & Christian, 2007*) in the lab of Dr. Andrew Dickson. A_T was determined using an open-cell titrator (Metrohm Dosimat Model 665) and Metrohm potentiometric pH (SOP 3b), and C_T was determined with a Single Operator Multi-parameter Metabolic Analyzer (SOMMA) coulometer (SOP 2) (*Dickson, Sabine & Christian, 2007*). From the measurements of A_T and C_T, the remaining carbonate parameters were calculated using the computer program CO2SYS (Table S3) (*Pierrot, Lewis & Wallace, 2006*). The average difference (±SE) between the mean measured pH_{SW} and the mean pH_{SW} calculated from measurements of A_T and C_T was 0.1 (±0.05) ($n = 32$).

CO_2 effects on growth and calcification

Growth of fleshy algae was measured as the change in wet weight over time (to the nearest 0.01 g). Samples were spun in a salad spinner (10 revolutions) and then gently blotted dry with paper towels immediately prior to obtaining weights. Net growth and calcification were measured using the change in buoyant weight (*Davies, 1989*), where all calcareous species were weighed to the nearest 0.001 g while suspended (from the weigh-below on a balance) in a basket submerged in ambient seawater; a technique that works well for upright calcareous algae (*Price et al., 2011*). Any segments shed during the course of the experiment were weighed along with the intact thallus. Buoyant weight was converted to actual weight based on the density of seawater and the density of the respective $CaCO_3$ polymorph. Growth and calcification rates were calculated by the change in weight over the experiment, with rates normalized to initial thallus weight and number of days in treatment conditions, expressed as change in weight per day (mg g^{-1} day^{-1}).

CO_2 effects on photophysiology

To assess the effect of CO_2 enrichment on algal photophysiology, photosynthetic parameters were measured fluorometrically with a red Pulse Amplitude Modulated Fluorometer (PAM) (Walz). The fiber optic probe was clipped to the thallus halfway up the branch on an unepiphytized portion of tissue with the "dark leaf clip". Rapid light curves (RLCs) were generated by exposing algal tissue to 8 incremental steps of increasing irradiance from 0–436 µM photons m^{-2} s^{-1} in 2009, 0–533 µM photons m^{-2} s^{-1} in 2010, and

Table 1 Measured pH and dissolved oxygen of OA experiments on Palmyra Atoll. The mean (±SE) measured pH_{SW} and dissolved oxygen conditions for CO_2 enrichment experiments conducted on Palmyra Atoll from 2009–2012. Measurements were conducted at the same time of day (~2000) for the duration of the experiment in empty control (no biological material), ambient air, and high pCO_2 treatments. Daily means were calculated within a species ($n = 4$, 2009; $n = 6$, 2010; $n = 5$, 2011; $n = 10$, 2012), and then averaged across days (14 days, 2009; 9 days, 2010; 17 days, 2011; 15 days, 2012). DO, dissolved oxygen; pH_{SW}, pH seawater scale.

Treatment	Species	Temperature (°C)	DO (mg L^{-1})	pH_{SW}
2009 Experiments				
Ambient air	Control	29.31 ± 0.07	4.95 ± 0.13	8.08 ± 0.02
	H. opuntia	29.26 ± 0.08	4.57 ± 0.16	8.03 ± 0.04
	H. taenicola	29.29 ± 0.08	4.73 ± 0.12	7.99 ± 0.03
	Lithophyllum sp.	29.43 ± 0.03	5.17 ± 0.11	8.05 ± 0.01
	L. prototypum	29.38 ± 0.02	5.16 ± 0.16	8.04 ± 0.02
High pCO_2	Control	29.33 ± 0.06	4.66 ± 0.26	7.68 ± 0.04
	H. opuntia	29.23 ± 0.08	4.16 ± 0.21	7.63 ± 0.02
	H. taenicola	29.25 ± 0.07	4.38 ± 0.19	7.62 ± 0.02
	Lithophyllum sp.	29.41 ± 0.03	5.47 ± 0.10	7.68 ± 0.03
	L. prototypum	29.38 ± 0.02	4.71 ± 0.21	7.65 ± 0.02
2010 Experiments				
Ambient air	Control	29.25 ± 0.15	4.85 ± 0.05	8.06 ± 0.05
	A. spicifera	29.22 ± 0.07	4.86 ± 0.06	8.08 ± 0.04
	C. serrulata	28.95 ± 0.04	4.79 ± 0.13	8.09 ± 0.02
	G. rugosa	29.25 ± 0.02	4.92 ± 0.06	8.09 ± 0.02
	H. taenicola	29.36 ± 0.05	4.78 ± 0.07	7.98 ± 0.04
High pCO_2	Control	29.25 ± 0.05	4.70 ± 0.30	7.79 ± 0.13
	A. spicifera	29.10 ± 0.05	4.57 ± 0.18	7.88 ± 0.05
	C. serrulata	28.91 ± 0.03	4.30 ± 0.17	7.77 ± 0.06
	G. rugosa	29.21 ± 0.07	4.50 ± 0.28	7.87 ± 0.06
	H. taenicola	29.34 ± 0.06	4.69 ± 0.13	7.77 ± 0.11
2011 Experiments				
Ambient air	Control	28.46 ± 0.21	7.98 ± 0.08	7.99 ± 0.06
	C. serrulata	28.34 ± 0.06	7.93 ± 0.03	8.00 ± 0.02
	D. bartayresiana	28.35 ± 0.01	7.97 ± 0.04	8.04 ± 0.02
	H. pannosa	28.26 ± 0.18	7.68 ± 0.04	7.98 ± 0.11
	D. marginata	28.44 ± 0.10	7.86 ± 0.06	8.05 ± 0.01
	H. opuntia	28.87 ± 0.04	8.12 ± 0.04	7.97 ± 0.03
	Lithophyllum sp.	28.92 ± 0.14	7.87 ± 0.05	8.03 ± 0.06
High pCO_2	Control	28.30 ± 0.16	8.02 ± 0.08	7.76 ± 0.06
	C. serrulata	28.86 ± 0.09	7.85 ± 0.02	7.66 ± 0.03
	D. bartayresiana	28.39 ± 0.01	7.93 ± 0.03	7.76 ± 0.02
	H. pannosa	28.38 ± 0.04	7.68 ± 0.22	7.86 ± 0.04
	D. marginata	28.87 ± 0.21	7.87 ± 0.05	7.80 ± 0.03
	H. opuntia	28.87 ± 0.09	8.39 ± 0.09	7.69 ± 0.03
	Lithophyllum sp.	28.46 ± 0.08	7.82 ± 0.07	7.74 ± 0.06

(*continued on next page*)

Table 1 (*continued*)

Treatment	Species	Temperature (°C)	DO (mg L^{-1})	pH_{SW}
2012 Experiments				
Ambient air	Control	28.61 ± 0.10	7.81 ± 0.02	8.11 ± 0.03
	A. amadelpha	28.72 ± 0.18	7.84 ± 0.02	8.02 ± 0.01
	H. taenicola	28.76 ± 0.08	7.90 ± 0.05	8.01 ± 0.03
High pCO_2	Control	28.71 ± 0.07	7.81 ± 0.03	7.85 ± 0.08
	A. amadelpha	28.68 ± 0.07	7.87 ± 0.08	7.75 ± 0.05
	H. taenicola	28.72 ± 0.18	7.98 ± 0.20	7.73 ± 0.06

0–614 μM photons m^{-2} s^{-1} in 2011, with 10 s at each light step (*Saroussi & Beer, 2007*). Replicate RLCs were generated in 2009 (3 RLCs per individual) and 2010 (2 RLCs per individual), and one RLC was generated for samples in 2011. Due to variation in experimental setup and PAR conditions across experiments, RLC intensities were higher than experimental PAR intensities in 2009 and 2010 and lower than experimental conditions in 2011 (Table S2). No RLCs were conducted on *H. taenicola* and *A. amadelpha* in 2012 because of time constraints. Using this approach of short illumination interval RLCs (<1 min), we were interested in relative comparisons of photophysiological performance between treatments (*Enriquez & Borowitzka, 2010*). Photosynthetic parameters were calculated from each RLC, and where RLCs were repeated on an individual, parameters were averaged for each individual before further statistical analyses.

Statistical analyses

To explore the effects of CO_2 enrichment on growth and calcification, separate *t*-tests for each species compared responses between control and experimental treatments. Certain species were experimentally manipulated in multiple years; to examine overall effects on species independent of experimental year, data across years were pooled. Additional independent *t*-tests were run in each year for those species, because the experimental setup and sample size varied slightly from year-to-year. Prior to analysis conducted in statistical software JMP v.10, data were tested for the assumptions of normality and homogeneity of variances with the Shapiro–Wilks test and diagnostic q–q plots.

To examine photophysiological response to CO_2 enrichment, the electron transport rates (ETR) from each RLC was plotted against irradiance and fit to a three parameter model (*Frenette et al., 1993*) to estimate the initial slope of the curve (α, μM electrons μM photons^{-1}), the maximum relative electron transport rate ($rETR_{Max}$, μM electrons m^{-2} s^{-1}), and photoinhibition (β, μM electrons μM photons^{-1}) (*Platt, Gallegos & Harrison, 1980*). Mean parameter estimates were averaged across samples within a treatment level for each species. In 2009 and 2010, several RLCs were generated for an individual alga; parameters were averaged within an individual before treatment effects were explored. The analyses were conducted using the software GraphPad Prism (v.6) and in all cases the model fit the data well with $R^2 > 0.90$ and $p < 0.001$. Parameters were compared for each species between treatments using independent *t*-tests as described above.

Meta-analysis

Meta-analyses were used to combine data across independent experiments and to explore potential differences in functional group responses to OA. Each species was categorized as fleshy macroalgae, upright calcareous algae, or CCA. Species that became sexually reproductive during experiments (*A. spicifera, A. amadelpha, C. serrulata* 2011) were not included in the meta-analysis because a large portion of the algal thallus senesced, or for holocarpic species the entire thallus disintegrated, after gamete/spore release and it was not possible to differentiate between the effects of reproduction versus OA treatment on algal biomass. Species tested across multiple years were included as independent data sets, yielding 3 fleshy macroalgae, 6 upright calcareous algae, and 3 CCA representatives. A random-effects model of standardized mean differences (*Cooper, Hedges & Valentine, 1994*) was used to estimate within and across experiments variance components; effect size was weighted both by sample size and pooled standard deviation. A one-tailed *z*-test of significance (against zero) of the mean effect size of CO_2 enrichment was used for algal growth and calcification responses. OA treatments were expected to enhance fleshy algal growth (H_0: mean effect size ≤ 0) and decrease algal calcification (H_0: mean effect size ≥ 0). There was no *a priori* expectation of photosynthetic responses to OA and thus a two-tailed *z*-test was used for the meta-analyses of photosynthetic parameters (see Supplemental Information for details).

RESULTS

Experimental conditions

CO_2 enrichment treatments effectively simulated near future seawater carbonate chemistry and OA as compared to present-day ambient air controls (Table 1). Biological activity (i.e., photosynthesis and respiration) introduced variability into carbonate chemistry conditions in both ambient and high pCO_2 treatments (Table S3). Diel variability in carbonate chemistry was not characterized, however, based on previous studies photosynthesis likely caused higher pH during the day, whereas respiration reduced pH at night (*Ohde & van Woesik, 1999*). Discrete water samples and pH probe measurements were collected at approximately the same time of day (2000) during all experiments. The average difference ($\pm$SE) between the mean measured pH_{SW} and the mean pH_{SW} calculated from measurements of A_T and C_T was 0.1 ($\pm$0.05) ($n = 32$). Considering the robustness of pH probe measurements in comparison to certified Tris buffer ($\pm$0.001), the relatively small difference between measured and calculated pH, and the frequency of samples for measured pH ($n = 9$–17) (Table 1) versus calculated pH ($n = 2$–4) (Table S3), measured pH_{SW} is the most appropriate parameter to describe differences in carbonate chemistry among experimental replicates. Most other physical conditions were consistent across years, but due to changes in experimental facilities, irradiance levels were higher and more representative of shallow reef environs in 2011 and 2012; oxygen levels were also higher in those years (Table 1).

Table 2 Results of pooled growth and photosynthetic parameters in response to treatment conditions. The results of independent *t*-tests to analyze the effect of CO_2 enrichment on response variables for each species. Responses of species used in multiple experiments (different years) were pooled and averaged across years by treatment to calculate an overall mean for each species. CO_2 treatment was treated as a fixed, independent factor. Degrees of freedom (df) are the same for all photosynthetic parameters. Each experimental replicate (n) consisted of one aquarium containing one algal individual. Statistically significant differences ($p < 0.05$) are emphasized in bold.

	Growth			$rETR_{Max}$			α		β	
Species	**df**	***t***	***p***	**df**	***t***	***p***	***t***	***p***	***t***	***p***
Fleshy macroalgae										
A. spicifera	10	1.15	0.275	9	2.55	**0.031**	0.341	0.741	0.088	0.932
A. amadelpha	18	3.12	**0.006**							
C. serrulata	22	0.066	0.948	19	0.282	0.781	0.350	0.730	0.356	0.726
D. bartayresiana	8	2.13	0.066	8	1.55	0.159	0.274	0.791	1.14	0.292
H. pannosa	5	4.90	**0.004**	5	0.186	0.556	0.602	0.60	0.624	0.560
Upright calcareous algae										
D. marginata	8	3.83	**0.005**	8	0.440	0.83	0.092	0.929	0.823	0.434
G. rugosa	10	1.63	0.134	10	4.10	**0.002**	1.71	0.118	0.760	0.465
H. opuntia	16	2.59	**0.020**	16	0.046	0.964	1.43	0.171	0.223	0.827
H. taenicola	38	0.21	0.832	18	0.193	0.849	2.23	**0.039**	1.62	0.123
Crustose coralline algae										
Lithophyllum sp.	16	5.28	**<0.0001**	16	0.582	0.569	0.280	0.783	1.0	0.332
L. prototypum	6	2.79	**0.032**	6	0.357	0.733	0.404	0.700		

Species–specific effects of CO_2 enrichment on calcification and growth

High CO_2 conditions decreased net calcification rates in 4 of the 6 calcareous species, and potentially enhanced net growth in 2 of the 5 fleshy species (Fig. 1; Table 2). CO_2 enrichment significantly decreased calcification in the red calcareous macroalga *D. marginata* (by 98%), and the two CCA *Lithophyllum* sp. (by 185%) and *T. prototypum* (by 190%) relative to controls (Table 2). The response of the green calcareous algae in the genus *Halimeda* was species–specific: the effect of CO_2 enrichment on net calcification rates was negative for *H. opuntia* (when repeated experiments were pooled) but negligible for *H. taenicola* (Table 2). CO_2 enrichment significantly increased growth in the fleshy red macroalga *H. pannosa* (by 93%) relative to controls (Table 2). The fleshy brown macroalga *D. bartayresiana* showed slight but non-significant increases in growth in high CO_2 likely due to small sample size and lack of power ($\beta = 0.46$; Table 2).

In addition to across species variability in the growth response, there was intra-specific variation to CO_2 enrichment across different years of experiments (Fig. 2). The trends and absolute magnitude in growth responses remained the same for 2 of the 4 species tested over multiple years. Irrespective of year, the calcareous green alga *H. opuntia* calcified significantly less (by 14.55 mg g^{-1} d^{-1} in 2009 and 12.97 mg g^{-1} d^{-1} in 2011) under high CO_2 conditions (Table 3), although the relative response varied by year. *H. opuntia* calcified 200% less at high CO_2 than ambient conditions and even

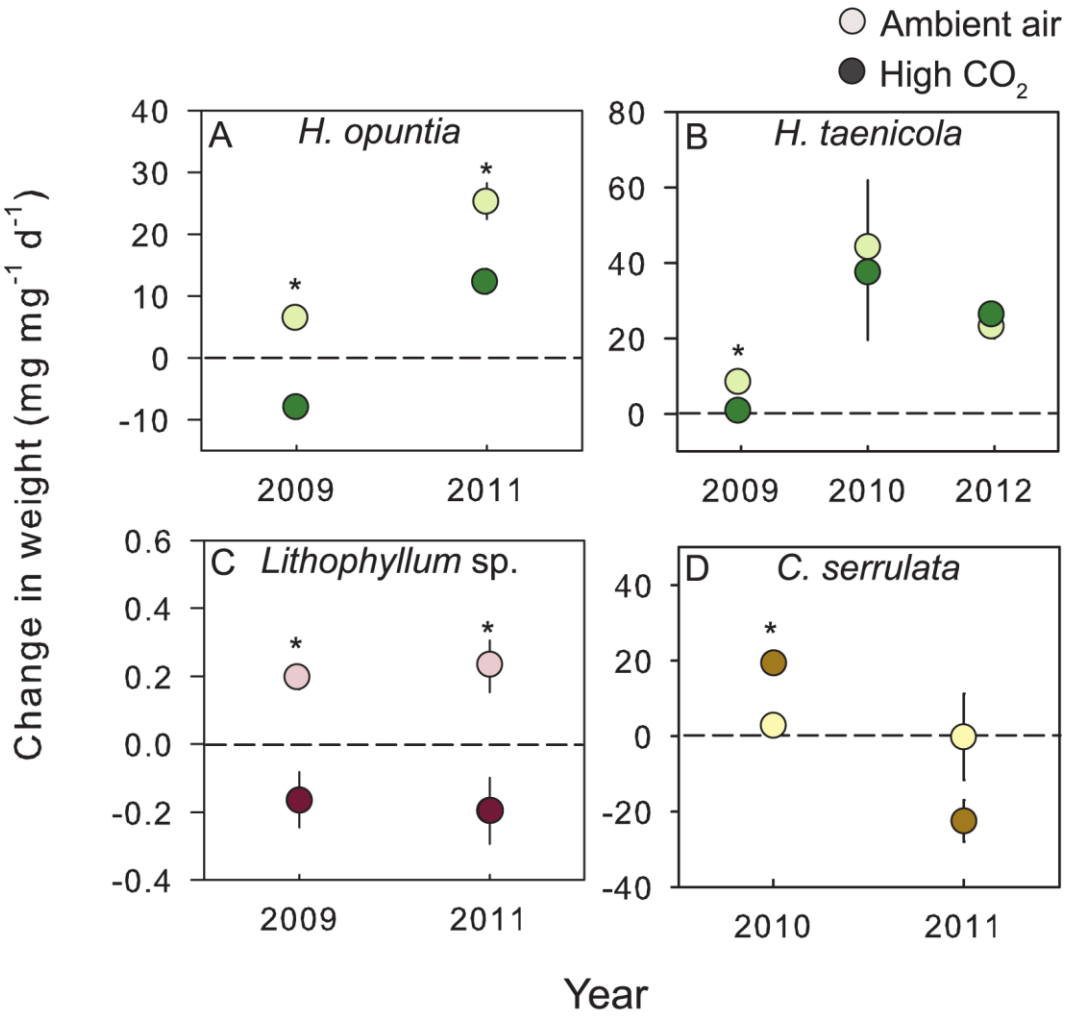

Figure 2 **Species–specific growth response to treatment conditions.** The mean (±SE) change in either fleshy or calcareous biomass following exposure to either present-day ambient air controls (open circles) or predicted OA treatments (closed circles) for species tested in multiple experiments. The dashed line is positioned at zero to indicate relative growth or loss of tissue for (A) *Halimeda opuntia*, (B) *Halimeda taenicola*, (C) *Lithophyllum* sp., and (D) *Caulerpa serrulata*. Fleshy macroalgae are shown in brown, upright calcareous algae in green, and CCA in red. * Indicates a significant difference between treatments as determined by independent *t*-tests (results reported in Table 3).

experienced net dissolution in 2009, but only calcified 50% less in 2011 and experienced net growth, despite the same high CO_2 conditions (Table S4). *Lithophyllum* sp. showed a consistent response to CO_2 treatment in direction and absolute and relative magnitude across years. *Lithophyllum* sp. calcified 185% less at high CO_2 in both 2009 and 2011 (Table S4). *H. taenicola* calcified 89% less at high CO_2 relative to controls in 2009, but there was no significant difference in calcification during the 2010 and 2012 experiments (Table 3). *C. serrulata* grew significantly more at high CO_2 in the 2010 experiment, however in 2011 *C. serrulata* grew less in the CO_2 enrichment treatment than in ambient conditions (Table 3).

Table 3 Results of growth/calcification by species and year. The mean growth and calcification rates of species tested in multiple experiments were examined using independent *t*-tests for each species by year; CO_2 treatment was treated as a fixed, independent factor. Each experimental replicate (n) consisted of one aquarium containing one algal individual. Statistically significant differences ($p < 0.05$) are emphasized in bold.

		Growth		
Species	**Year**	**df**	***t***	***p***
Fleshy macroalgae				
C. serrulata	2010	10	4.28	**0.002**
	2011	8	1.75	0.119
Upright calcareous algae				
H. opuntia	2009	6	7.32	**0.0003**
	2011	8	3.62	**0.007**
H. taenicola	2009	6	5.93	**0.001**
	2010	10	0.224	0.827
	2012	18	0.612	0.548
Crustose coralline algae				
Lithophyllum sp.	2009	6	4.10	**0.006**
	2011	8	3.43	**0.009**

Several fleshy macroalgal species became reproductive in CO_2 treatments over the course of our study, as evidenced by the presence of fertile tissue which eventually released gametes or spores leaving behind only a small portion of the vegetative thallus. All samples of *A. spicifera* and *A. amadelpha* released spores or gametes, respectively, upon exposure to treatment conditions. In 2011, *C. serrulata* also reproduced, causing tissue loss in both ambient and CO_2 treatments; 40% of *Caulerpa* individuals in the ambient treatment reproduced, whereas 100% of *Caulerpa* samples in the CO_2 enrichment treatments reproduced.

Species–specific effects of CO_2 enrichment on photophysiology

Exposure to CO_2 treatments had no detectable effect on relative photophysiology of the 9 species tested, with a few exceptions (Fig. 3). CO_2 enrichment significantly increased the maximum photosynthetic capacity ($rETR_{Max}$) in the calcareous red alga *G. rugosa* (Fig. 3A, Table 2) relative to the control. In the fleshy red alga *A. spicifera*, $rETR_{Max}$ was significantly lower following exposure to high CO_2, however, these individuals had reproduced during the experiment and the remaining vegetative tissue following gamete release was not representative of healthy algal tissue. In the calcareous green alga *H. taenicola*, the initial slope of the RLC (α) was significantly depressed after CO_2 enrichment (Fig. 3B, Table 2). There was no evidence of photoinhibition (β) in any of the species tested (Fig. 3C, Table 2).

Meta-analysis of experiments across years

Experimental effects were combined across species to assess the consistency of physiological responses to CO_2 enrichment within different algal functional groups using meta-analyses. The mean effect size for calcification and growth was significantly greater than zero for fleshy species, but significantly less than zero for both groups (upright and

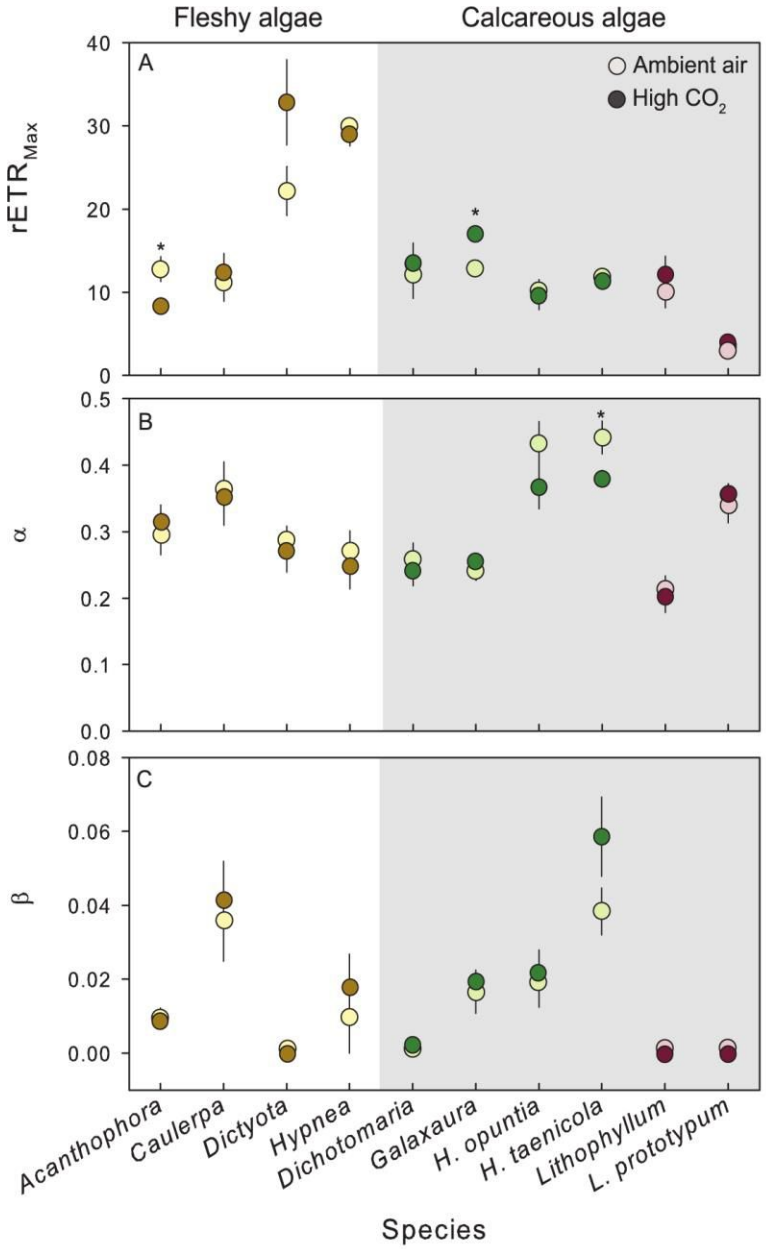

Figure 3 Photosynthetic response of fleshy and calcareous algae to treatment conditions. The mean (±SE) photosynthetic parameters from RLCs following exposure to either present-day ambient air controls (open circles) or predicted OA treatments (closed circles). Species tested in multiple experiments were pooled across years. RLCs were measured fluorometrically with a pulse amplitude modulated fluorometer (PAM), and fit to the model of *Platt, Gallegos & Harrison (1980)*. From the model we derived (A) maximum photosynthetic performance ($rETR_{Max}$), (B) photosynthetic efficiency (α), and (C) photoinhibition (β). Parameters from replicate RLCs were averaged for each individual. Fleshy macroalgae are shown in brown, upright calcareous algae in green, and CCA in red. * Indicates a significant difference between treatments as determined by independent *t*-tests (results reported in Table 2).

Table 4 Meta-analysis results. Heterogeneity (Q_T) in overall analyses and results from a random effects model of standardized mean differences for response variables pooled by functional group: fleshy macroalgae, upright calcareous algae, or crustose coralline algae (CCA). Statistically significant values ($p < 0.05$) are emphasized in bold. $rETR_{Max}$, maximum relative electron transport rate (μM photon m^{-2} s^{-1}); α, photosynthetic efficiency or initial slope of the rapid light curve (μM electrons μM $photons^{-1}$); β, photoinhibition (μM electrons μM $photons^{-1}$).

Response	df	Q_T	p	k	Mean effect size	Z	p
Fleshy macroalgae							
Growth	19	0.07	>0.05	3	16.1 ± 12.5	2.11	**0.017**
$rETR_{Max}$	12	0.18	>0.05	3	0.454 ± 6.19	0.144	0.886
α	12	0.07	>0.05	3	−0.005 ± 0.13	0.073	0.471
β	12	0.16	>0.05	3	−0.0004 ± 0.0009	0.632	0.2248
Upright calcareous algae							
Growth	4	2.04	>0.05	7	−10.8 ± 4.7	3.80	**0.0001**
$rETR_{Max}$	4	1.62	>0.05	6	0.031 ± 3.56	0.017	0.987
A	4	0.72	>0.05	6	−0.020 ± 0.06	0.722	0.470
B	4	0.60	>0.05	6	0.001 ± 0.01	0.756	0.4333
Crustose coralline algae							
Growth	6	0.08	>0.05	3	−0.405 ± 0.35	1.90	**0.029**
$rETR_{Max}$	6	0	>0.05	3	0.693 ± 27.4	0.339	0.735
α	6	0.05	>0.05	3	−0.002 ± 0.09	0.053	0.941
β	6	0	>0.05	3	−0.001 ± 0.004	0.169	0.2637

encrusting) of calcareous species (Table 4; Fig. 4A). There was no overall effect of CO_2 enrichment on photophysiology ($rETR_{Max}$, α, β) relative to the control for algal functional groups (Fig. 4, Table 4). The variation between experiments was never significantly different from 0 ($Q \leq 2.04$, $p > 0.05$ for each functional group and response variable; Table 4), indicating that the inconsistencies in PAR did not influence the overall response of fleshy versus calcareous algae to OA. Due to the significant effect of CO_2 enrichment on growth and calcification rates across experiments, and the lack of significant variation in the strength of this response, we pooled species across years to show overall trends in treatment responses (Fig. 1).

DISCUSSION

This series of experimental manipulations indicate that tropical algae respond differently to CO_2 enrichment depending on species and whether or not they are calcified. When combining data from multiple experiments, calcareous algae experienced a reduction in biomineralization while fleshy algae became more productive. The magnitude of algal growth and calcification responses to OA conditions varied by species, and occasionally, within a species over multiple experiments. In contrast, there was no effect of CO_2 enrichment on algal photophysiology relative to controls as measured by short illumination RLCs. Furthermore, exposure to OA conditions initiated sexual reproduction in 3 out of 5 species of fleshy macroalgae tested. These results support the hypothesis that OA has differential effects on the growth of fleshy macroalgae and the calcification of calcareous algae.

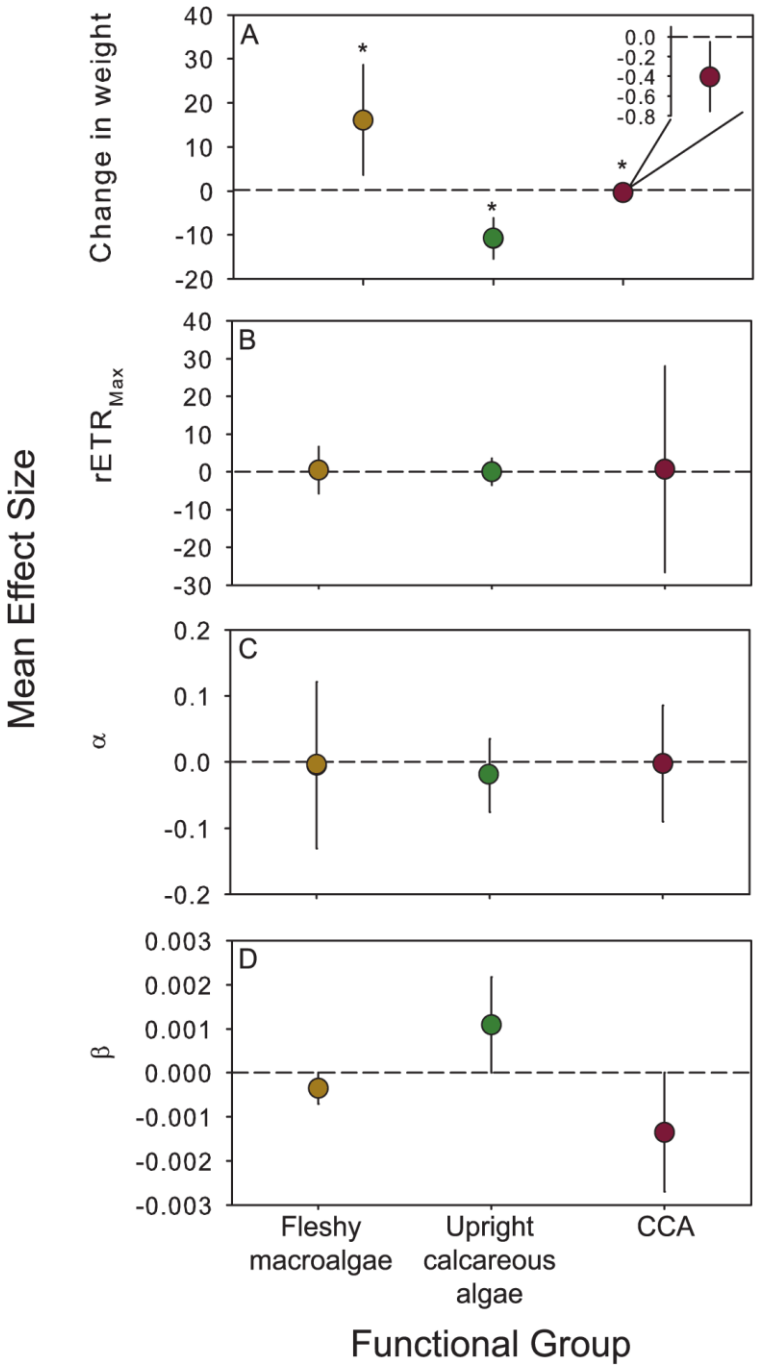

Figure 4 Functional group responses to OA. Mean (±95% CI) effect sizes were calculated to explore the cumulative effects of OA on algae categorized into functional groups (fleshy macroalgae, upright calcareous algae, and crustose coralline algae (CCA). Species that reproduced during experiments were not included in this analysis. The dashed line is positioned at zero to indicate a relative increase or decrease following exposure to OA conditions for (A) change in weight, (B) maximum photosynthetic capacity ($rETR_{Max}$), (C) photosynthetic efficiency (α), and (D) photoinhibition (β). Fleshy macroalgae are shown in brown circles, upright calcareous algae in green, and CCA in red. * Indicates an effect size different than zero as determined by meta-analysis (results reported in Table 4).

Table 5 OA effects on tropical benthic macroalgae. A summary of findings to date from experiments exploring OA effects on growth, calcification, and photosynthesis in tropical benthic macroalgae. Only business-as-usual OA experiments (800–1200 µatm) are included. +, positive effect; −, negative effect; 0, no effect.

Species	Growth/ Calcification	Photosynthesis	Reproduction	Reference
Fleshy macroalgae				
Acanthophora spicifera	0	−	+	This study
Avrainvillea amadelpha	−		+	This study
Caulerpa serrulata 2010	+	0		This study
Caulerpa serrulata 2011	0	0	+	This study
Dictyota bartayresiana	+	0		This study
Hypnea pannosa	+	0		This study
Lobophora papenfussii	−			(*Diaz-Pulido et al., 2011*)
Upright calcareous algae				
Galaxaura rugosa	0	+		This study
Dichotomaria marginata	−	0		This study
Halimeda opuntia	−	0		This study
Halimeda taenicola	0	0		This study
Halimeda cylindracea	−	−		(*Sinutok et al., 2011*; *Sinutok et al., 2012*)
Halimeda macroloba	−	−		(*Sinutok et al., 2011*; *Sinutok et al., 2012*)
Halimeda incrassata	+			(*Ries, Cohen & McCorkle, 2009*)
Crustose coralline algae				
Lithophyllum prototypum	−	0		This study
Lithophyllum sp.	−	0		This study
Hydrolithon sp.	−	+		(*Semesi, Kangwe & Bjork, 2009*)
Porolithon onkodes	−	−		(*Anthony et al., 2008*; *Diaz-Pulido et al., 2012*; *Johnson & Carpenter, 2012*; *Comeau, Carpenter & Edmunds, 2012*; *Comeau et al., 2013*)
Neogoniolithon sp.	+			(*Ries, Cohen & McCorkle, 2009*)
Mixed CCA	−			(*Jokiel et al., 2008*; *Kuffner et al., 2008*)

Biomineralization by seaweeds substantially contributes to carbonate production on tropical reefs and these results suggest that OA may decrease reef formation and cementation services provided by these often over-looked ecosystem engineers. In these experiments, OA decreased calcification of calcareous green algae (*H. opuntia* and *H. taenicola)* and caused net dissolution of calcareous red macrophytes and CCA (*D. marginata*, *G. rugosa*, *Lithophyllum* sp., and *L. prototypum*). Many other studies have reported decreased calcification as a consequence of simulated OA for tropical (Table 5) and temperate calcareous algae even in milder acidification scenarios than used in our study (see *Koch et al., 2013* for review). However, much of the previous work exploring OA effects on calcareous algae across ecosystems has focused on the crustose coralline algae (family Corallinaceae) and this study is among the first to expand to different taxonomic entities such as the lightly calcified red algae *D. marginata* and *G. rugosa* (Table 5).

The results of this study indicate that calcareous algae calcified less after two weeks of exposure to CO_2 enrichment than ambient controls, but the response varied by functional group. CCA, which deposit the more soluble high Mg-calcite (12–18% $MgCO_3$; *Milliman, Gastner & Muller, 1971*), experienced net dissolution in the OA treatments, where $\Omega_{\text{Mg-calcite}}$ was ≤ 1 (using the solubility constant estimated by *Lueker, Dickson & Keeling, 2000*), despite assuming our samples deposited the conservative lower range of 8% Mg mole fraction. Intracellular dolomite ($CaMg[CO_3]_2$), a stable form of carbonate, can be the source of Mg in other species of CCA and actually reduces net thallus dissolution at higher skeletal mole fractions (*Nash et al., 2012*). The exact mineral composition of the carbonate in our CCA species is unknown, but was not robust to our treatment conditions. The calcareous upright algae all deposit aragonite and calcified less under OA, but only experienced net dissolution in one instance. Differences in the magnitude of effects between calcareous species may be influenced by species–specific mechanisms of calcification (*Price et al., 2011*; *Comeau, Carpenter & Edmunds, 2012*; *Koch et al., 2013*), mineralogy of $CaCO_3$ deposited (*Ries, Cohen & McCorkle, 2009*), and potential compensatory or antagonistic effects of high CO_2 on photosynthesis (Table 5). Differences in within-species susceptibility to OA demonstrate the complexity of how ocean acidification may influence biological and chemical interactions in tropical marine primary producers. Within-species responses across years of experiments may have been driven by changes in dissolution versus calcification or by net growth rate, and the relative contribution of dissolution versus calcification in influencing net effects of OA or organisms should be a focus in future studies.

Understanding the effects of OA on algal physiology is difficult because photosynthesis and calcification are inextricably coupled. In the process of fixing carbon, algal photosynthesis alters the intracellular environment in favor of $CaCO_3$ precipitation (*Borowitzka & Larkum, 1976*). In the external environment, photosynthesis also has the potential to alter carbonate chemistry and to create conditions more favorable for calcification (*Gattuso, Pichon & Frankignoulle, 1995*; *Anthony, Kleypas & Gattuso, 2011*; *Smith et al., 2013*). Fleshy macroalgae that are currently carbon limited are hypothesized to be affected positively by increasing CO_2 concentrations (*Gao et al., 1991*), which is demonstrated here, but these effects are species and condition specific. Previous studies have documented both positive and negative effects of CO_2 enrichment on growth in fleshy macroalgae (Table 5). Enhanced algal growth also has been documented *in situ* in ecosystems near underwater volcanic vents where conditions of low pH and high CO_2 facilitate communities dominated by fleshy organisms (*Hall-Spencer et al., 2008*; *Fabricius et al., 2011*). It has been hypothesized that higher concentrations of dissolved CO_2 would enhance fleshy macroalgal growth by stimulating photosynthesis. However, despite the fact that fleshy algae grew more with high CO_2 there was not a concurrent response in photosynthetic parameters measured from chlorophyll fluorescence. While the fluorescence technique is used widely to monitor algal photophysiology, it can be highly variable (*Edwards & Kim, 2010*) and provides only an instantaneous snapshot of photophysiological function. Short illumination RLCs (<1 min) are not comparable

to estimates obtained using oxygen evolution from photosynthesis-irradiance curves because there is not sufficient time with RLCs for organisms to reach steady-state flow of electrons (*Enriquez & Borowitzka, 2010*). Thus, it may not be the most suitable technique to assess the cumulative effects of CO_2 enrichment on algal photophysiology and more direct measures of photosynthesis are preferred.

Predicting the response of primary producers to high CO_2 is complex and may depend on resource acquisition strategies that are species–specific and potentially plastic over time. The primary substrate for the photosynthetic enzyme Rubisco in all marine algae is dissolved CO_2. Seaweeds must compensate for the slow rates of CO_2 diffusion through seawater, as opposed to air, as well as the higher concentration of HCO_3^- compared to CO_2. Some primary producers have developed carbon concentrating mechanisms (CCM) that increase the concentration of CO_2 in the proximity of Rubisco (*Raven, 1970*). Thus, the presence or absence of CCMs may influence species–specific responses to CO_2 enrichment (*Hurd et al., 2009*; *Koch et al., 2013*), and changes to CCM activity levels may explain the mixed responses of photosynthesis in the literature, as well as the growth results documented here. One possible mechanism that may have facilitated increased algal productivity under high CO_2 in the present study, without concurrent increases in $rETR_{Max}$ or α, may have been an increase in algal energy reserves through down regulation of energetically costly CCMs, noted in another tropical green macroalga (*Liu, Xu & Gao, 2012*) and phytoplankton (*Eberlein, Van de Waal & Rost, in press*). An additional alternative hypothesis is that nitrate reductase activity, an enzyme that reduces nitrate to nitrite, can be stimulated by CO_2 (*Hofmann, Straub & Bischof, 2013*), potentially releasing seaweed from nitrogen resource limitation in oligotrophic coral reef ecosystems. Furthermore, photophysiology should be assessed using more direct techniques in addition to RLCs such as measuring oxygen evolution rates, in order to accurately quantify photosynthetic rates. Predicting changes in enzymatic activity is critical to understanding mechanisms behind species–specific responses to OA, yet basic physiological descriptions are lacking for the majority of tropical algae, including the species used in the present study.

This and other studies have documented high variability among species in response to OA. However, there also was within species variability across years, suggesting that species–specific responses to OA may be context dependent. For example, due to logistical constraints experiments conducted in 2009 and 2010 had substantially lower daily mean irradiances than in 2011 and 2012 (ESM Table 2). Although mixing rates were consistent from year to year, flow rates in experimental aquariums were relatively low. Thus, care should be taken when extrapolating these biological responses to OA under higher water flow regimes. Few studies have experimentally tested the effects of both water flow and OA on coral reef algae, although flow rate has been shown to be an important factor influencing pH gradients within the diffusive boundary layer (DBL) (*Hurd et al., 2011*) and the response of some reef calcifiers to high CO_2 (*Anthony et al., 2013*). Increasing DBL thickness, with decreasing water flow, may buffer organisms against changes in the carbonate chemistry of bulk seawater by providing a metabolically mediated microenvironment of higher pH within the DBL. Furthermore, the biological variability in

carbonate conditions introduced by algal photosynthesis and respiration in the contained, aerated volume of water likely created a diel cycle in pH that may have approximated carbonate chemistry variability on a shallow reef flat (*Hofmann et al., 2011*). Variability in pH conditions has been shown to influence growth rates of coralline algae (*Johnson, Moriarty & Carpenter, 2014*), therefore care should be taken when extrapolating the results from the present study to other systems. Diel cycles in carbonate chemistry were not characterized in this experiment, and have been infrequently included in descriptions of experimental conditions in many OA studies. However, the variability in all experimental conditions across this suite of experiments is far less than that of experiments combined in several recent meta-analyses (*Hendriks, Duarte & Alvarez, 2010*; *Kroeker et al., 2010*; *Kroeker et al., 2013*)). With the meta-analysis approach used here to explore effects of OA across experiments, we accounted for the variability within and across experiments and found that OA treatment was a significant driver of enhanced growth in fleshy macroalgae, and loss of calcified biomass in upright calcareous algae and CCA.

Some within-species variability in response to OA treatment was due to the induction of sexual reproduction following exposure to treatment conditions. Higher irradiance levels can modulate the negative effects of high CO_2 on algal responses to OA (*Sarker et al., 2013*; *Yildiz et al., 2013*), including potentially triggering reproduction and may explain the inconsistent results from year to year, specifically for *C. serrulata*. In 2011 the decrease in *C. serrulata* growth likely was a result of the loss of algal tissue in individuals that became sexually reproductive upon exposure to high CO_2 and relatively higher irradiance. Similar reproductive responses to treatment conditions were also noted for related *A. amadelpha* and for a red macroalga, suggesting that there may be an interactive effect between irradiance levels and CO_2 concentrations. Sexual reproduction in these taxa has been observed in Hawaii and the Caribbean during the spring (*Clifton & Clifton, 1999*; *Smith, Hunter & Smith, 2002*). In all of these species, a large portion of the algal thallus senesced after sexual reproduction, and for the green algae the reproduction was clearly visible due to the loss of pigmentation following release of heavily pigmented gametes or spores (*Clifton & Clifton, 1999*). The typical progression of sexual reproduction in Bryopsidales ranges from 1–2 days (*Clifton & Clifton, 1999*), and the specimens did not show signs of reproduction prior to the experiment. Furthermore, gametogenesis in *C. serrulata* has been shown to be induced either as a coping mechanism (*Williamson, 2010*) or to maximize favorable conditions (*Brawley & Johnson, 1992*). Experiments were conducted outside the potential seasonal reproductive cycle of tropical algae, and there was no evidence of sexual reproduction before experiments, thus it is likely that sexual reproduction was induced by experimental conditions. An alternative explanation is that the reproductive response may have been an artifact of experimental manipulation and stress associated with rapid exposure to high pCO_2. The rate of exposure to high pCO_2 has been shown to be an important determinant in the response of coralline algae to CO_2 enrichment (*Kamenos et al., 2013*). Future work should explore the effects of both rate and magnitude of CO_2 enrichment on reproduction in fleshy macroalgae.

OA poses an ever-increasing global threat (*Kleypas et al., 1999*; *Hoegh-Guldberg et al., 2007*) to the ecological balance and stability of tropical reef systems via disparate effects on calcareous versus fleshy taxa (*Hall-Spencer et al., 2008*; *Fabricius et al., 2011*; *Porzio, Buia & Hall-Spencer, 2011*). It is difficult to predict the specific responses of macroalgal taxa to CO_2 enrichment; however, the patterns of response presented here suggest that growth of fleshy macroalgae on coral reefs may be stimulated by OA, while calcareous species may be depressed. Given that numerous other human impacts (overfishing, pollution, warming) negatively affect corals and other calcifying reef builders while enhancing the abundance of fleshy algae, our results suggest that OA may potentially exacerbate community shifts towards fleshy macroalgal dominated states. However, little is known about how reef species or communities will respond to the interactive effects of multiple stressors including OA. Given the importance of coral reefs for supporting biodiversity (*Knowlton, 2001*), as well as human populations and economies of coastal nations (*Moberg & Folke, 1999*), it is imperative that we understand the scope of species responses to impending rapid climate change.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Maggie Dorothy Johnson and Nichole N. Price conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Jennifer E. Smith conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

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Supplemental information

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CHAPTER 4

Complex and interactive effects of ocean acidification and warming on epilithic and endolithic coral reef turf assemblages

Maggie D. Johnson, Jennifer E. Smith

ABSTRACT

Algae are critically important for organic and inorganic production in marine systems, yet are markedly understudied in the context of global change. Within the algae, even less is known about the response of turf algae assemblages, particularly on coral reefs, to environmental stressors. We conducted a mesocosm experiment in Moorea, French Polynesia to test the combined effects of CO_2 and temperature on growth of the epilithic and endolithic turf community and turf assemblage metabolic rates. These metrics relate to the roles of turf algae in production, competition, and as a resource for herbivores. Carbonate plugs covered by turf were collected from the reef and exposed to ambient and high CO_2 (1,000 µatm) conditions for three weeks. Each CO_2 treatment was replicated across six temperatures, ranging from 24-31.5°C. OA and warming had complex, and sometimes interactive, effects on turf responses. We found that epilithic and endolithic components of the turf assemblage grew significantly more under OA, but there was no effect of temperature. Further, metabolism of the turf community, including respiration and net and gross primary production, demonstrated complex and interactive responses to OA and warming. The most pronounced physiological response was a significant increase in primary production (net and gross) and a significant decrease in respiration of turfs exposed to OA at the warmest temperatures. These results indicate that global change impacts could enhance the competitive ability of turf algae over other slower growing taxa such as reef-building corals. The complex effects of CO_2 and warming across a suite of algal responses demonstrate how multiple stressors have diverse and unexpected biological effects.

INTRODUCTION

Global change is occurring at an unprecedented rate in the earth's history, with serious consequences for marine ecosystems (Doney et al. 2009; Hoegh-Guldberg et al. 2007). Increasing anthropogenic carbon dioxide (CO_2) emissions are decreasing mean ocean pH through ocean acidification (OA) (Caldeira and Wickett 2003) and increasing climate and ocean temperatures (IPCC 2013). Significant attention has been devoted to understanding the impacts of global change on marine organisms over the last decade (Gattuso and Hansson 2011), particularly in the form of single stressor controlled laboratory experiments. Through these studies we have advanced our understanding of the response of many marine organisms to decreasing ocean pH and warming temperatures separately. However, much of this work has focused on commercially valuable and calcifying species such as bivalves, corals and coralline algae (Doney et al. 2009; Fabry et al. 2008; Kroeker et al. 2010), because of the negative implications of OA and decreasing calcium carbonate ($CaCO_3$) saturation state (Ω) on biomineralization (Kleypas et al. 1999; Orr et al. 2005). Non-calcifying, or fleshy, marine species also respond to global change, but the direction and magnitude of response is often species-specific (Johnson et al. 2014b; Kroeker et al. 2010). A key group of non-calcifying photoautotrophs that are markedly understudied with respect to global change are the turf algae, one of the most ubiquitous primary producers in temperate and tropical marine ecosystems (Copertino et al. 2005; Wanders 1976). What we know of turf algae responses to global change are based on temperate turf assemblages (Connell and Russell 2010; Falkenberg et al. 2014; Russell et al. 2009), which vary substantially in form and function from tropical coral reef turf

assemblages. There remains a significant gap in our power for understanding and predicting the response of tropical turf algal assemblages to multiple global change stressors.

Much of the focus of global change experiments with photoautotrophs has been on the response of algal photosynthesis to OA (Beer and Koch 1996; Hurd et al. 2009; Koch et al. 2013), because changes in seawater carbon speciation associated with OA directly influence the availability of photosynthetic substrates. Carbon dioxide is the primary substrate for the photosynthetic enzyme RubisCo and the increased availability of dissolved CO_2 in the near-future ocean is expected to enhance photosynthesis and potentially fuel algal growth (Holbrook et al. 1988; Koch et al. 2013). A possible caveat to this is variability in algal response based on the presence and activity of carbon concentrating mechanisms (CCMs) (Giordano et al. 2005; Raven et al. 2011). Many species of algae have evolved CCMs to elevate CO_2 concentrations at the site of photosynthetic fixation in order to compensate for lower concentrations of dissolved CO_2 relative to other carbon species in seawater (e.g. bicarbonate, HCO_3^-) (Beardall et al. 1998; Giordano et al. 2005). However, some algae lack CCMs and instead rely on passive diffusion of CO_2 from seawater through algal tissues to sustain photosynthesis (Raven et al. 2005). These algae likely will respond positively to higher CO_2 concentrations because more CO_2 will be available for photosynthesis. Algae that possess CCMs may either respond positively to OA, or not respond at all, if the added CO_2 provides no additional advantage for photosynthesis. The role of CCMs in regulating the response of algae to OA is uncertain (Raven et al. 2011), but there is general consensus that most species of algae, with or without

CCMs, will respond positively to OA, while others may be unaffected (Beardall et al. 1998; Koch et al. 2013).

Many marine photoautotrophs have demonstrated positive growth responses to OA (Johnson et al. 2014b; Kroeker et al. 2010), with the exception of calcifying algae. Calcifying algae, or those that biomineralize a calcium $CaCO_3$ skeleton, generally have slower growth rates under OA, and some shift towards net dissolution (Koch et al. 2013; Kroeker et al. 2010). Biomineralization becomes more difficult, and dissolution more favorable, as acidification decreases Ω (Kleypas et al. 1999; Orr et al. 2005). Crustose coralline algae (CCA) are among the most sensitive marine organisms to OA (Kroeker et al. 2010; McCoy and Kamenos 2015), demonstrating decreased rates of recruitment (Kuffner et al. 2008) and calcification at lower pH (Johnson et al. 2014a), and net dissolution (Anthony et al. 2008; Diaz-Pulido et al. 2012; Jokiel et al. 2008). The potential stimulatory effects of added CO_2 on algal productivity are thus outweighed by the negative consequences on coralline calcification. Adding further complexity is the neutral response of some species of calcifying and fleshy algae to OA (Comeau et al. 2013; Johnson et al. 2014b). Given the considerable variability in algal response to OA, it is important to target key marine algae in OA experiments to improve our capacity to predict functional group responses to global change.

Warming ocean temperatures are another consequence of increasing CO_2 emissions that have important implications for marine organisms. Temperature influences organismal metabolism by affecting the speed of biochemical reactions (Gillooly et al. 2001), and warming temperatures have the potential to increase metabolic rates (Beer and Koch 1996). Algal photosynthesis, for example, typically

increases with increasing temperature until an optimum is reached, after which rates quickly decline (Davison 1991; Raven and Geider 1988). Direct effects of warming on algal metabolism may not directly translate to long-term rates of enhanced algal growth. At longer time scales, warming temperatures can alter the abundance of thermally sensitive macroalgae in benthic communities. For example, 2-3 °C of warming has been shown to increase the abundance of ephemeral subtidal marine algae (Sangil et al. 2012) and freshwater macrophytes (McKee et al. 2002), but a similar degree of warming decreased the abundance habitat-forming kelps and foliose red algae (Schiel et al. 2004). Changes in seawater temperature thus have the potential to alter both the metabolic rates of algae and the abundance of algae.

Ocean acidification and warming will occur simultaneously, which makes projecting the outcome of single stressors experiments challenging. Multiple-stressors experiments are important for elucidating potential synergistic effects. For example, elevated seawater temperature has been shown to increase the sensitivity of some marine taxa to OA (Kroeker et al. 2013). Warming frequently exacerbates the negative effects of OA algal calcification (Diaz-Pulido et al. 2012; Johnson and Carpenter 2012; Martin and Gattuso 2009) and percent cover of settlement substrata by algal crusts (Vogel et al. 2016). However, warming and OA do not always act synergistically. In a multiple-stressor experiment, OA and warming separately increased photosynthetic rates of 4 species of calcifying tropical algae (Scherner et al. 2016). Species-specific effects, potential complexities due to CCMs, and the paucity of multiple-stressor experiments preclude our ability to confidently predict the response of diverse algal assemblages to global change.

Turf assemblages (also referred to as algal turfs, turfs or mat-forming algae) are diverse multi-species assemblages of algae and cyanobacteria, typically less than 1 cm in height (Carpenter 1986; Steneck and Dethier 1988). Turfs are ubiquitous across temperate and tropical marine ecosystems where they can cover up to 70-80% of hard substrata (Borowitzka 1981; Copertino et al. 2005; Melville and Connell 2001). Here we refer to the turf assemblage that grows on the surface of hard substrata as the epilithic community (Fig 1). Assemblages vary widely in species composition and diversity depending on region, season, habitat, grazing pressure (Hay 1981; Scott and Russ 1987) and the spatial scale of study (Harris et al. 2015), among other factors. Due to a combination of high abundance and rates of turnover of the epilithic community due to continual grazing by herbivores, turf assemblages have some of the highest rates of primary production in temperate (Copertino et al. 2005; Westphalen and Cheshire 1997) and tropical subtidal communities (Adey and Steneck 1985; Carpenter 1985; Hatcher 1988; Marsh 1976; Odum and Odum 1955). These rapid growth and production rates enable turf assemblages to take advantage of available nutrients, open substrata, and relief from herbivore pressure (Carpenter 1986), which often facilitates the colonization and rapid expansion of turf algae into habitats that are heavily disturbed or have low water quality due to human activities (e.g. nutrient pollution, sedimentation) (Gorgula and Connell 2004). The rapid turnover of turf biomass provides an important source of energy for ecosystems (Carpenter 1985; Klumpp et al. 1990; Wanders 1976), and epilithic turf canopies facilitate algal recruitment (Ang 1985; McCook and Chapman 1993) and ameliorate negative effects of OA on calcification of underlying crustose coralline algae (Short et al. 2014).

However, turf assemblages also have numerous negative impacts by inhibiting algal recruitment (Worm and Chapman 1998), especially by canopy-forming kelps (Connell and Russell 2010; Reed 1990), and decreasing the abundance of juvenile and adult corals (Birrell et al. 2005; Birrell et al. 2008; Vermeij et al. 2010). The epilithic component of turf assemblages serve many ecological roles, contributing to primary production, altering competitive interactions and influencing the trajectory of benthic community structure.

An important component of the turf algal assemblage that is frequently overlooked is the endolithic community. Endoliths (or euendoliths) are pervasive in hard bottom ecosystems, where they colonize pores in living and dead invertebrate skeletons (Fig 1) by actively penetrating and dissolving carbonate substrates (Golubic et al. 1981). These organisms act as the principal bioeroding agents in many ecosystems, and are particularly important on coral reefs where they contribute significantly to carbonate dissolution (Chazottes et al. 1995; Tribollet 2008; Tribollet and Payri 2001). The endolithic community is concentrated immediately beneath the substratum surface, and is comprised of a diverse assemblage of microboring cyanobacteria, algae, fungi and microorganisms (Golubic et al. 1981) that varies by substratum (Le Campion Alsumard et al. 1995; Tribollet and Payri 2001). Endoliths have unique adaptations that allow them to photosynthesize in low-light conditions. Despite living within the substratum, the endolithic community provides an important source of primary production, especially in coral reef environments (Tribollet 2008; Tribollet et al. 2006). Although CCMs have not been well-studied in endolithic algae, the primary microboring endolith on coral reefs globally, chlorophyte *Ostreobium* sp.

(Chazottes et al. 1995; Tribollet 2008), is thought to lack CCMs and thus be carbon-limited under present-day conditions (Tribollet et al. 2006). As with other marine photoautotrophs, OA has the potential to increase rates of endolithic production by providing additional CO_2 for photosynthesis which may have the strongest effects on algae that lack CCMs. The role of endoliths as bioeroders and primary producers could become more significant in the near-future if OA and warming increase endolithic production and growth, thus increasing the potential for bioerosion. Considering that endolithic community composition is highly variable, it is important to explore the response of endolithic community assemblages from different types of substrata to global change.

Given the importance of the epilithic and endolithic components of turf algae assemblages to marine ecosystems, the potential for OA and warming to enhance fleshy algal growth, and the variability in algal responses to global change stressors, it is important to experimentally quantify the response of different turf assemblages to multiple global change stressors. Despite the importance of turf assemblages to primary production, community structure and bioeroson on coral reefs, relatively little is known about the biological response of coral reef turf assemblages to warming and OA. The goal of this study was to quantify the effects OA on the growth and metabolic responses of coral reef turf assemblages across a suite of temperatures that are relevant to present-day and the near-future environmental conditions. We conducted controlled laboratory experiments where we manipulated CO_2 concentrations to simulate present-day (ambient) and high CO_2 conditions (OA) expected by the end of the century in pessimistic carbon emission scenarios (~1,000

μatm) (IPCC 2013). To consider the simultaneous effect of warming, we crossed CO_2 treatments with 6 temperatures that represent the full range of temperatures naturally experienced by organisms in the field, and include a 2 °C warming treatment. We quantified the effects of CO_2 and temperature on growth of the epilithic and endolithic communities of turf assemblages from a fringing reef in Moorea, French Polynesia, and the effects on overall turf assemblage metabolic rates. By exploring OA effects across a range of temperatures, we provide new information on algal responses to multiple global change stressors and show that temperature is important for contextualizing biological responses to OA.

METHODS

Sample Collection

This study was conducted in the mesocosm facilities at the Richard B. Gump South Pacific research station in Moorea, French Polynesia from January-February 2015. Turf assemblages were collected from the fringing reef located between Cook's Bay and the Sheraton resort on the north shore of Moorea (17°29'02.07" S, 149°50'19.38" W). Intact turf communities (Fig 1) were collected as cores (5 cm diameter) from dead mounding corals at a depth at 1-2 m. A pneumatic drill with a diamond tipped hole-saw was used to drill 4-5 cm into the carbonate, and a hammer and chisel were used to dislodge the core. The carbonate cores covered by turf were then transported to the Gump station and maintained under ambient light and in flowing seawater for further processing.

Cores were first trimmed to a height of ~2 cm with a diamond band saw (Gryphon C-40) and then cut in half. The average (± SD) surface area of each half-core was 15.5 ± 1.6 cm^2. One half of the core was used for initial estimates of turf biomass and the second half of the core was used in experimental treatments (Fig 1). Visible invertebrates were removed and the exposed carbonate of each experimental core was coated with marine epoxy (Aquamend) and attached to a rigid plastic mesh (Vexar) base. Turf assemblages were allowed to recover from processing in flowing seawater and ambient light for 24 hrs.

Experimental Design

After processing, turf assemblages were randomly assigned to 12 150-L tanks, with 6 cores per tank. Each tank received a constant flow of filtered seawater at a rate of 0.3-0.4 L min^{-1}, yielding roughly 3-4 full water exchanges per day. Tanks were fitted with clear plexiglass lids to reduce gas exchange with the atmosphere. Temperature, pH and light were controlled in each tank independently.

Turfs were exposed to a combination of 6 temperature treatments and 2 CO_2 treatments. Temperature treatments ranged from 24 °C to 31.5 °C at 1.5 °C increments (24.0, 25.5, 27.0, 28.5, 30.0 and 31.5 °C), spanning the full seasonal range of temperatures experienced by organisms on the fringing reef of Moorea (Leichter 2015). The 31.5 °C treatment was chosen to represent the 2 °C increase in seawater temperature commonly predicted by the end of the century (IPCC 2013). Each temperature treatment was crossed with either an ambient CO_2 treatment (~400 µatm) or a high CO_2 treatment (~1,000 µatm) to simulate OA and the pH conditions

expected by the end of the century under increasing CO_2 emissions scenarios (RCP 8.5) (IPCC 2013). To reduce temperature shock to turfs, all tanks were initially set to the ambient temperature (~28 °C) and then temperature was incrementally changed by 0.5 °C over a period of 6 days, or until the treatment temperature was reached.

The experimental design utilizes a regression approach, with one tank per temperature and CO_2 treatment, and response variables averaged per tank. Turf assemblages were positioned at least 5 cm apart, and given the high volume and flow rate of seawater into tanks, it is unlikely they affected each other.

Treatment Conditions

To simulate effects of increased atmospheric CO_2 on seawater, all tanks were continuously bubbled with ambient air and periodically with pure CO_2 and treatments were maintained with a pH feedback system. Tank pH was continuously monitored by pH electrodes (Aquacontroller, Neptune systems) connected to a digital controller, and pure CO_2 was injected into tanks by solenoid valves to maintain the targeted pH, 8.1 and 7.7 (total scale pH), for the ambient and high CO_2 treatments, respectively. Turfs were exposed to treatment conditions for 3 weeks, following the 6 day acclimation period to temperature treatments.

The carbonate chemistry of each tank was monitored by direct measurements of total scale pH (pH_T), total alkalinity (A_T), temperature and salinity. Tank pH conditions were monitored daily (0900) with a handheld pH meter (Orion 3-stars, Thermo Scientific) and combination pH probe (Orion Ross Ultra, Thermo Scientific). The pH probe was calibrated every other day with Tris/HCl buffer following standard

protocols (Dickson et al. 2007). Accuracy of the pH probe measurements was verified by comparisons to pH of discrete samples measured with a temperature-controlled spectrophotometer and the pH indicator dye m-cresol, following Dickson et al. (2007). Spectrophotometric pH yielded values within 1% of values measured by the pH meter and probe. Potential temporal variation in tank pH was assessed once during the experiment by discrete pH measurements at 0600 and 1800 hrs. There was little variability in pH between time points (~0.5 units).

Total alkalinity was measured every 3 days on discrete water samples collected at 0900. Open-cell potentiometric titrations were conducted following the protocols of Dickson et al. (2007) with an automated titrator (T50, Mettler-Toledo) fit with a DG115-SC pH probe (Mettler-Toledo) and calibrated daily to Tris/HCl buffer. Titrations were conducted on certified reference material (Reference Material for Oceanic CO_2 Measurements, Batch 140, A. Dickson, Scripps Institution of Oceanography) before every set of titrations. The average accuracy of A_T titrations was ± 2.9 μmol kg^{-1} (n = 12).

Salinity of each tank was measured every week with a benchtop conductivity meter (YSI 3100) and temperature was measured daily with a traceable digital thermometer (Fisher-Scientific). The remaining parameters of the carbonate system were calculated with programming software R and the package *Seacarb*.

Each tank was illuminated with an LED modular light (Aquaillumination Sol) set to ~650 μmol photon m^{-2} s^{-1}, simulating the average daily irradiance on the Moorea back reef at a depth of 2 m. Lights were programmed for a 12:12 hour photoperiod. To simulate natural diel light cycles, the light intensity was gradually increased from

darkness to maximum intensity over a period of 4 h. Maximum irradiance was maintained for 4 h, and then light intensity was gradually decreased to darkness over the subsequent 4 h. Light levels in each tank were measured during periods of maximum irradiance with a submerged 4π quantum sensor (LI-193) attached to a LiCor LI-1400 meter. To reduce potential lighting effects within tanks, turf assemblages were haphazardly repositioned every other day during the experiment.

Epilithic and Endolithic Growth

Growth of the epilithic turf community on the surface of the core, and the endolithic community within the carbonate matrix of the core was determined by the change in organic biomass measured by ash free dry weight (AFDW). One half of the whole turf core was processed for initial estimates of epilithic turf biomass and endolithic biomass (initial), and the second half was exposed to experimental treatments (experimental) (Fig 1). To determine initial epilithic biomass, half of the initial core was scraped with a straight edge razor blade, without removing the underlying carbonate. The scraped turf was dried at 60 °C for 24 hrs, weighed, and then combusted at 500 °C for 4 hrs in a muffle furnace. The combusted sample was reweighed, and the difference between the dried and combusted sample is the ash free dry weight (AFDW), or the organic biomass of the epilithic turf community. The turf core was photographed with a ruler for scale, and surface area was determined using image analysis software (ImageJ). Biomass was normalized to the surface area of the scraped core. The initial core was then scraped a second time with a sharpened screw driver to remove the endolithic community within the carbonate matrix. The core was

scraped until all green pigmentation was removed, and the endolithic sample was similarly processed for AFDW.

After subsequent metabolic rates were measured, the final experimental core was processed for biomass following the same procedure as above. The difference in the AFDW of the initial core and the final experimental core represents the change in biomass, and thus growth, over the duration of the experiment. Growth rates of the epilithic and endolithic community are expressed as g AFDW cm^{-2}.

Turf Metabolism

At the end of the experiment, and prior to final biomass sampling, 3 turf assemblages were randomly selected from each tank for physiological measurements. A preliminary set of incubations were conducted to construct photosynthesis versus irradiance curves (PI curves) and determine saturating irradiance for metabolic incubations (950 µmol photon m^2 s^{-1}).

Incubation chambers were illuminated by one 7-color LED module (AquaIllumination, Hydra) set to ~950 µmol photon m^{-2} s^{-1}. Incubations under saturating irradiance (net primary production) and in darkness (respiration) were conducted on the same turf core. Gross primary production was then calculated by adding respiration rates to net primary production rates, based on the common assumption that respiration in the dark is equivalent to respiration in the light.

Metabolic incubation chambers consisted of 2-L translucent plastic storage containers with a gasket sealed lid. The lid had one hole drilled in the top to hold an optical dissolved oxygen (DO) probe (Hach IntelliCal LDO101). The probe was

wrapped in parafilm to ensure a tight seal and was connected to a portable meter (Hach HQ40d), accurate to ±0.1 mg L^{-1}. Temperature and dissolved oxygen were measured every minute for 15-20 min for each light and dark incubation. Water flow was maintained in each incubation chamber by a submersible mini aquarium pump (80 GPH, Aquatop) and temperature was maintained by a temperature controlled water bath that held two incubation chambers. Two incubations were conducted simultaneously for one temperature treatment, and additional blank incubations were conducted for each temperature x CO_2 treatment to approximate background changes in dissolved oxygen. These blank values were subtracted from production and respiration values for the respective treatments prior to subsequent analyses. Rates were calculated as the linear slope of dissolved oxygen concentration over the duration of the incubation. Metabolic rates are normalized to core surface area and are expressed as mg O_2 cm^{-2} h^{-1}.

Statistical Analyses

Response variables were averaged per tank (temperature x CO_2 treatment) and averages were used in statistical analyses. Response variables met the assumptions of normality. The effect of CO_2 on the epilithic turf community, the endolithic community, and metabolic rates were analyzed with an analysis of covariance (ANCOVA) with temperature as the covariate. Statistical analyses were conducted in JMP v.10.

RESULTS

Treatment Conditions

CO_2 enrichment and temperature control were successful at maintaining target temperatures crossed with ambient and high CO_2 (OA) treatments (Table 1). The daily average (± SE) total scale pH (pH_T) was 8.05 ± 0.02 and 7.71 ± 0.00 for the ambient and OA treatments, respectively. Due to the high rate of fresh inflowing seawater (0.3-0.4 L min^{-1}), variability in total alkalinity (A_T) among all treatment tanks was minimal for the duration of the experiment, with a daily tank average (± SE) of 2283 ± 0.66 µmol kg^{-1}. The remaining carbonate parameters were calculated at a measured salinity of 35.7, yielding a daily average pCO_2 of 387 ± 21 and 969 ± 7 µatm for the ambient and OA treatments, respectively.

Epilithic and Endolithic Growth

CO_2 enrichment significantly increased growth of the epilithic turf community (Fig 2) and the endolithic community (Fig 3). However, there was no significant effect of temperature on epilithic or endolithic growth rates, and no significant interactive effect of temperature and OA (Table 2).

High CO_2 elicited higher epilithic growth rates at all temperatures ($F_{1,1}$ = 7.8632, P = 0.023). However, there was no significant effect of temperature ($F_{1,1}$ = 1.4759, P = 0.2591) or significant interaction between temperature and OA on epilithic turf growth ($F_{1,1}$ = 1.2683, P = 0.2927). Similarly, endolithic growth was highest under OA ($F_{1,1}$ = 8.0015, P = 0.0222), with a marginally significant interactive

effect of temperature and OA ($F_{1,1}$ = 4.1283, P = 0.0766) but no main effect of temperature ($F_{1,1}$ = 0.2719, P = 0.6162).

The effects of OA on epilithic turf growth were most pronounced at ambient temperature, 28.5 °C, and at the most common seasonal thermal maximum experienced by organisms on the reef, 30 °C. At these temperatures OA increased epilithic growth by 90-100% (Fig 2). Similarly, OA had the most pronounced effects on endolithic growth at, or above, ambient temperature (28.5, 30 and 31.5 °C). Turf cores demonstrated an overall loss of endolithic biomass across virtually all treatments, except at 28.5, 30 and 31.5 °C where OA shifted endolithic communities from net biomass loss to net gain and increased endolithic growth by 80-245% relative to ambient treatments (Fig 3).

Turf Metabolism

There was a significant interactive effect of temperature and OA on turf physiological response, including net primary production and respiration, with only a marginally significant interactive effect on gross primary production (Fig 4). Turf community respiration rates linearly increased with increasing temperature, but more under ambient conditions than OA (Table 2, Fig 4B). Turf community net and gross primary production rates, however, were highest under OA, but only at the warmest temperatures, 30 and 31.5 °C (Table 2, Fig 4A,C). The significance of the interaction between temperature and OA precludes interpretation of the main effects, but indicates that temperature is important for contextualizing the effects of OA on turf metabolism.

DISCUSSION

The goal of this study was to quantify growth and physiological responses of coral reef turf algae to OA across a wide range of temperatures, thus addressing the issue of multiple stressors on coral reef turf assemblages, an understudied, but critically important functional group. The epilithic and endolithic components of a coral reef turf assemblage showed a strong positive growth response to OA, but there was no effect of temperature. Turf assemblages further demonstrated complex and interactive physiological responses to OA and warming. OA and warming elicited the highest rates of primary production (net and gross), and the lowest rates of respiration, with OA having more of an effect on metabolic rates at warmer temperatures. These results indicate that OA has potential strong effects on the growth of turf assemblages, and that temperature has additional effects on metabolic rates. By exploring a range of temperatures, we show that a full suite of temperature treatments are important for contextualizing biological responses to OA.

The present study is among the first to quantify the effect of global change stressors on coral reef turf assemblages (except see Bender et al. 2014). Our findings corroborate other studies that have shown positive growth responses of algal turfs for ecosystems outside the tropics. Temperate turf communities, for example, increased in biomass and percent cover by 2-4 times in response to OA (Connell and Russell 2010; Short et al. 2014), and the effects of CO_2 on temperate turfs is frequently exacerbated by nutrient enrichment (Falkenberg et al. 2013; Russell et al. 2009). Furthermore, turf algae are one of the few functional groups that can survive extremely low pH conditions at natural CO_2 vents in the Mediterranean, where they dominate benthic

cover and settlement tiles at low (pH 7.7) and extremely low (pH 7.0) pH sites (Kroeker et al. 2013; Porzio et al. 2013). This response is consistent across aquatic systems, as high CO_2 increased biomass of filamentous algae in freshwater lakes (Andersen and Andersen 2006). Our study provides new information on the combined effects of warming and OA, demonstrating that the OA alone influenced eplithic turf biomass across the full range of temperatures that turfs normally experience on the fringing reef of Moorea, and even at 2 °C of warming.

The present turf biomass results are not consistent with those of Bender et al. (2014), who found no effect of a warming treatment and several levels of OA on coral reef turf biomass. Further, our multiple stressor results contrast those of Connell and Russell (2010) which found significant synergistic effects of warming and OA on turf biomass. The disparity between these studies is likely due to differences in the species composition of turf communities studied. Coral reef turf assemblages have much higher diversity and variability in species composition compared to temperate turf assemblages, which makes comparing results across systems challenging. For example, over 100 eukaryotic algae are common to coral reef turf assemblages (Hatcher and Larkum 1983), but the temperate mat-forming turfs used in experiments in South Australia are dominated by one species of brown algae, *Feldmannia* (Connell and Russell 2010; Russell et al. 2009). Furthermore, coral reef turf assemblages are highly variable across multiple spatial scales, and can even vary in community composition within a reef (Harris et al. 2015). Thus, it is not unexpected for turf assemblages from different regions of the ocean, within the same ecosystem, to show variable responses to global change experiments, especially considering that algae

demonstrate species-specific responses to OA (Johnson et al. 2014b). Because of this high degree of variability across turf communities on coral reefs, it will be important to explore the response of turf assemblages from all types of reefs to global change.

While turf assemblages have received scant attention in the global change literature, even less effort has been dedicated to understanding the impacts of global change on the endolithic component of turf assemblages. Similar to the response of the epilithic community, we found that OA significantly increased the biomass of the endolithic community relative to the ambient treatment. Our findings corroborate the few previous studies showing that OA significantly increased biomass of the endolithic communities associated with coral and coralline algae substrata (Diaz-Pulido et al. 2012; Reyes-Nivia et al. 2014; Reyes-Nivia et al. 2013; Tribollet et al. 2009). The community composition and function of endolithic communities varies by substrata, and is different between living versus dead coral and coralline algae (Lecampionalsumard et al. 1995; Tribollet and Payri 2001). Our findings thus build on these previous studies, which were conducted on dead carbonate blocks (Tribollet et al. 2009), recently dead coral carbonate skeleton (Reyes-Nivia et al. 2013) or with coralline algae (Diaz-Pulido et al. 2012; Reyes-Nivia et al. 2014), by quantifying the response of endolithic communities associated with living turf algae assemblages to warming and OA.

The significant increase in epilithic and endolithic biomass in response to OA is likely driven by the increased availability of CO_2 for photosynthesis in the OA treatment. Although we expect fleshy algal photosynthesis to increase under elevated CO_2, the direction and magnitude of that response is likely linked to the presence and

activity of CCMs. CCMs vary across algal taxa (Kremer 1981; Raven et al. 2011), which makes it difficult to predict the potential response of a diverse assemblage, such as tropical turf communities, to OA. Even if CCMs are present in some species, turf assemblages may be functionally carbon limited due to the high-density of autotrophs that have high rates of primary production and compete for CO_2 within the diffusive boundary layer (Carpenter 1990; Larkum et al. 2003). Increasing the concentration of CO_2 in the surrounding seawater increases the diffusion of CO_2 across the diffusive boundary layer, and potentially relieves carbon limitation (Carpenter 1990). The increased biomass of epilithic turf algae in response to OA supports the idea that CO_2 is indeed limiting for turf assemblages under present-day conditions, although the mechanisms underlying carbon limitation should be explored further.

The endolithic community is imbedded within the carbonate matrix of the reef. It is potentially more carbon-limited than the epilithic community because it has less access to resources in the surrounding seawater. The most common endolithic alga on reefs across the globe, including Moorea, French Polynesia (Chazottes et al. 1995; Tribollet and Payri 2001) is the chlorophyte *Ostreobium* sp. In addition to being carbon limited by habit, *Ostreobium* sp. likely relies on passive uptake of dissolved CO_2 (Tribollet et al. 2009) The increase in endolithic biomass we document here matches our expectations of higher endolithic growth under higher CO_2 concentrations. Higher endolithic biomass may have negative implications for the persistence of the coral reef carbonate matrix by increasing bioerosion. By dissolving calcium carbonate through boring, the endolithic community contributes significantly to bioerosion of coral carbonates, and endolithic bioerosion rates have already been

shown to increase with higher CO_2 (Tribollet et al. 2009). Ocean acidification may further increase bioerosion by increasing overall growth rates and biomass of the microboring endolithic community. The negative implications of increased bioerosion for reef persistence are further compounded by the overall higher potential for reef dissolution due to thermodynamics of Ω and other chemical parameters associated with OA (Andersson et al. 2007; Andersson and Gledhill 2013).

In contrast to the response of eplithic and enodlithic biomass, we found synergistic effects of OA and temperature on turf assemblage metabolic rates. The effects of OA on turf metabolism were dependent on temperature, with the greatest effects at the warmer temperatures of 30 and 31.5 °C. Ocean acidification generally increased primary production rates (net and gross), and decreased respiration, and this was exacerbated at the warmest temperatures. The effects of both temperature and CO_2 match what we expected based on known temperature-effects on metabolic rates and potential CO_2 limitation of photosynthesis under present-day conditions.

Temperature controls metabolism by affecting the rate of biochemical reactions, and we expected that temperature would increase metabolic rates (Gillooly et al. 2001). Despite surpassing average temperatures that turf assemblages normally experience on the reef by ~2 °C in the warming treatment, we found no thermal threshold effect on turf primary production or respiration. Although the 8-year daily average (± SD) temperature at the fringing reef collection site on Moorea is 28.6 ± 0.5 °C (during the austral summer), temperature can range from 23.7-30.9 °C (Leichter 2015). The thermal variability of this habitat, particularly in the shallows where samples were collected (1-2 m depth), may have acclimatized turf assemblages to

warmer temperatures. By exploring a range of temperatures, we provide important context to understanding the effects of OA on turf assemblage metabolism. Ocean acidification had the most pronounced effects on metabolic rates at warmer temperatures, which was likely a result of both increased metabolic rates from warmer temperatures and increased substrate availability for photosynthesis.

Few studies have explored the effects of global change stressors on turf assemblage metabolism, particularly in coral reefs. However, our results concur with one previous study showing CO_2 and warming to have similar interactive effects on the productivity of a turf algae assemblage from the Great Barrier Reef (Bender et al. 2014). Endolithic metabolism is even less understood, even under present-day conditions. There is some indication that higher CO_2 does not affect endolithic primary production, although the authors attributed the lack of effect to nutrient or light limitation and urged for future experiments to assess these questions (Tribollet et al. 2006). We did not distinguish between epilithic versus endolithic contributions to community production, and thus we consider metabolic rates at the scale of the whole turf assemblage. The change in metabolic rates under OA and warming is, therefore, attributed to both epilithic and endolithic processes, and future experiments should tease apart the relative effects of multiple stressors on each component of the community.

We found a significant effect of OA on epilithic and endolithic biomass and no effect of temperature. At the smaller physiological scale, we found synergistic effects of OA and warming on turf metabolic rates. The decoupling of global change stressor effects on these two separate biological processes, growth and physiology, is complex.

We might expect that because photosynthesis provisions energy for growth, turf assemblage production and growth would demonstrate the same pattern of response to experimental treatments. The different patterns of response may be because our growth and physiological measurements represent biological responses at different temporal scales. For example, estimates of growth by change in epilithic and endolithic biomass represent a cumulative response to warming and OA, while our final assessment of metabolic rates represents an instantaneous response following 3-weeks of exposure to treatment conditions. Another possible explanation, is that while temperature increased metabolic rates over short periods of time, carbon was ultimately the limiting factor for turf growth and the release of carbon limitation outweighed any cumulative effects of warming on primary production.

Turf algae are important primary producers on coral reefs, and increases in growth and production with OA and warming could lead to higher rates of ecosystem primary production, creating more energy available to higher trophic levels. However, turf algae are equally important in their role as competitors with reef-building corals and algae, where they are frequently the dominant competitor (McCook et al. 2001). Higher growth rates by turf assemblages under warmer and more acidic conditions could increase the frequency of coral-turf interactions and will occur alongside decreased rates of coral (Chan and Connolly 2013) and coralline algal growth and calcification (Johnson and Carpenter 2012; Johnson et al. 2014a; Kroeker et al. 2013). In addition to turf assemblages, OA also favors growth by fleshy macroalgae (Johnson et al. 2014b) and could give these fleshy species a competitive advantage over corals and CCA (Diaz-Pulido et al. 2011). Ocean acidification and warming could, therefore,

shift coral reefs from calcifer-dominated to flesh-dominated ecosystems. This pattern has been documented in benthic cover of coral reefs along natural CO_2 vents shifts, where reefs shift from dominance by reef-building corals to fleshy macroalgae and turf with decreasing pH (Fabricius et al. 2011). Decreasing cover of calcifiers and increasing microboring endolithic biomass OA (Tribollet et al. 2009), have serious consequences for the persistence of the coral reef carbonate framework and habitat. Coupled with additional stressors, such as nutrient pollution and overfishing, the increase in turf assemblage growth under OA poses a potential threat to coral reefs, particularly on coral reefs where turf growth may already be enhanced by nutrient pollution and a loss of herbivores from overfishing.

In summary, we demonstrate that OA has strong positive effects on the growth of both epilithic and endolithic components of a coral reef turf assemblage, and that temperature and OA synergistically increase turf community primary production. We contribute new information on the effects of OA on coral reef turfs across a wide-range of temperatures, and suggest that future experiments incorporate both multiple stressors and a full range of environmental conditions in order to more accurately contextualize biological responses to global change stressors.

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Chapter 4, in part, is currently being prepared for submission for publication of the material. Johnson, Maggie D.; Smith, Jennifer E. The dissertation author was the primary investigator and author of this material.

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FIGURES

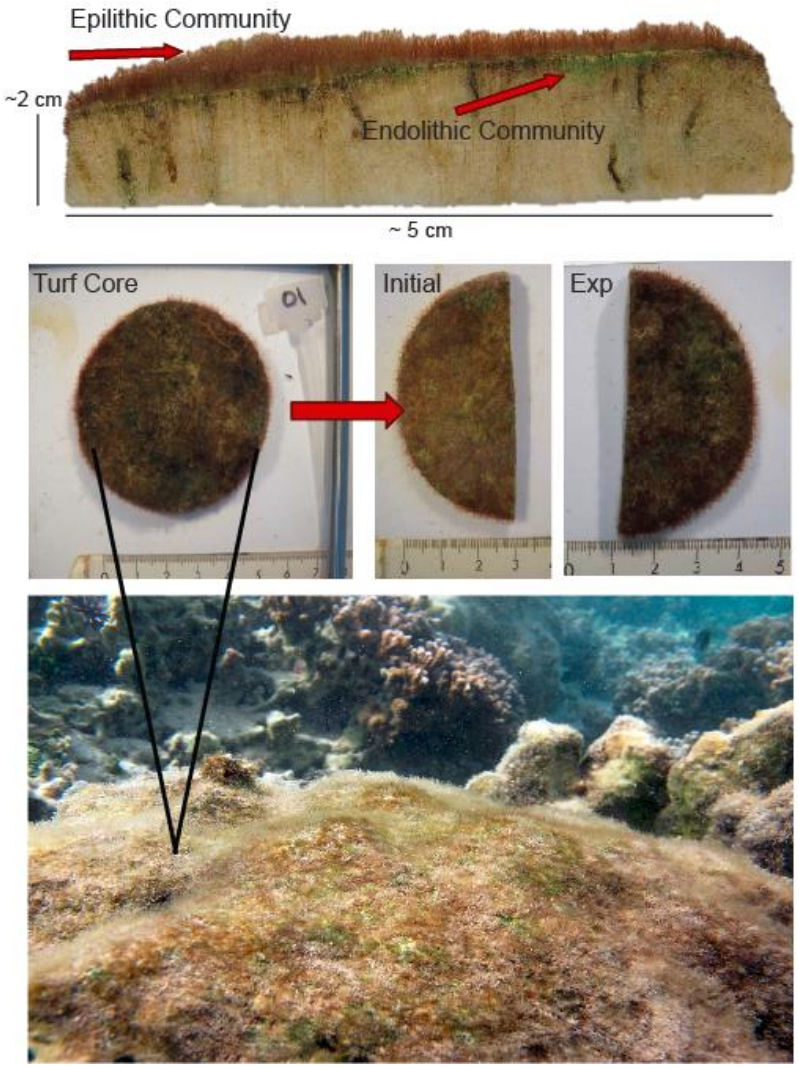

Figure 4.1. Epilithic and endolithic turf assemblages. Carbonate cores covered by turf were collected from the fringing reef on the north shore of Moorea, French Polynesia. Whole cores were cut in half, with one half used for initial estimates of biomass (initial) and the second half used in experimental treatments (exp). The epilithic community was sampled by scraping the surface community, while the endolithic community was sampled by scraping into the core until green pigmentation was no longer visible.

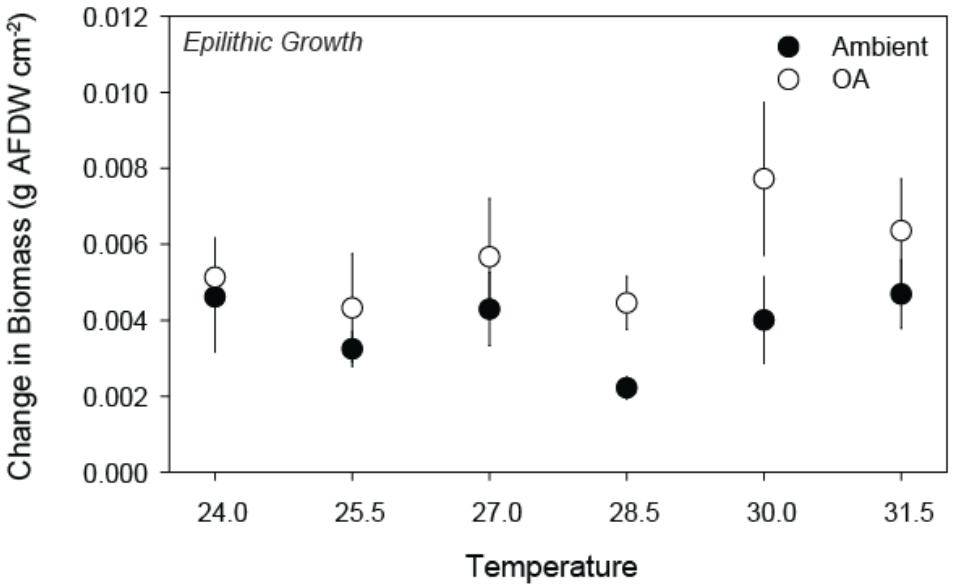

Figure 4.2. Epilithic turf response to OA and warming. Mean (±) change in epilithic turf community biomass (measured by ash free dry weight), as a proxy for growth, in response to ocean acidification (OA) and warming (n = 6). Present-day conditions (ambient) are represented by closed circles and high CO_2 conditions (OA) are represented by open circles. There was a significant effect of OA on epilithic growth, but no effect of temperature (ANCOVA).

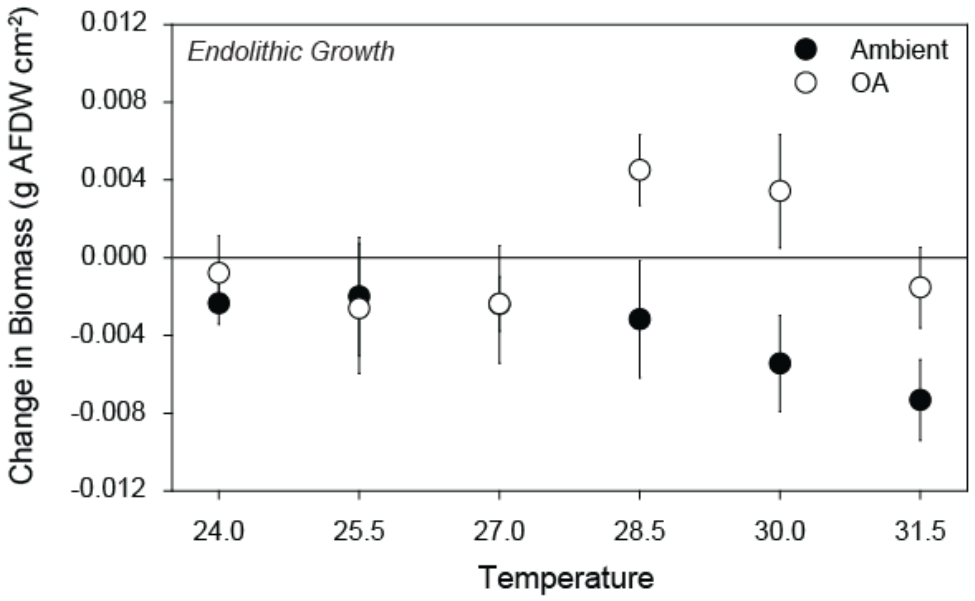

Figure 4.3. Endolithic turf response to OA and warming. Mean (±) change in endolithic community biomass (measured by ash free dry weight), as a proxy for growth, in response to ocean acidification (OA) and warming (n = 6). Present-day conditions (ambient) are represented by closed circles and high CO_2 conditions (OA) are represented by open circles. There was a significant effect of OA on endolithic growth, but no effect of temperature (ANCOVA).

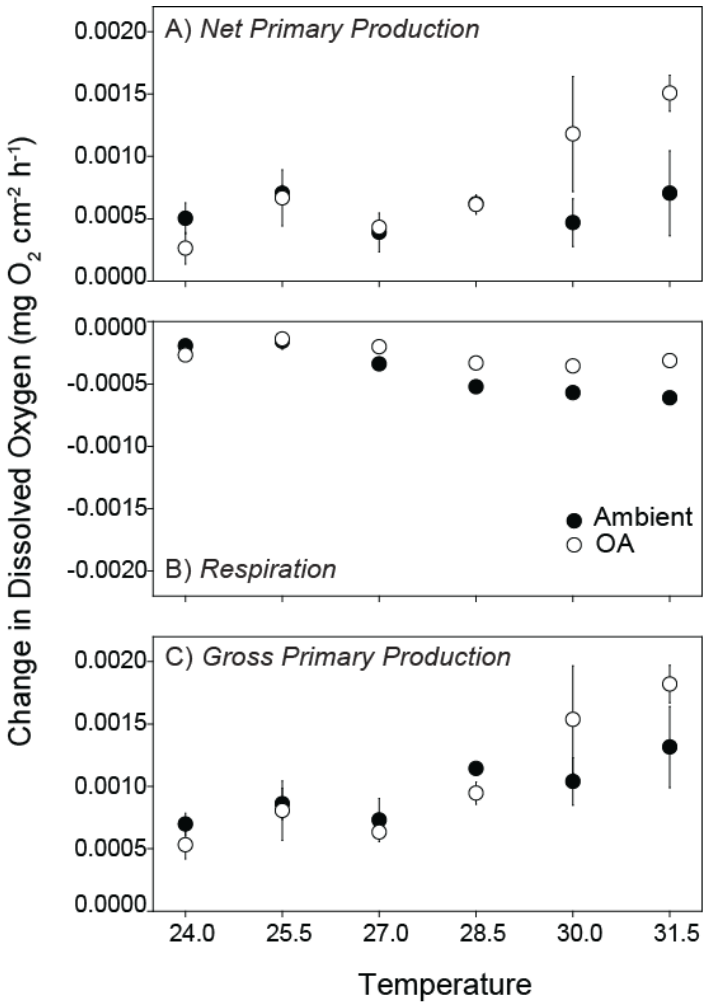

Figure 4.4. Turf assemblage metabolic rates in response to OA and warming. Mean (±) change in dissolved oxygen of the whole turf assemblage in saturating irradiance (net primary production) (A), and in darkness (respiration) (B) (n = 3). Dark and light metabolic incubations were conducted on the same turf assemblage, and gross primary production (C) was calculated by adding dissolved oxygen consumed from respiration to net primary production. Present-day conditions (ambient) are represented by closed circles and high CO_2 conditions (OA) are represented by open circles. There was a significant synergistic effect of OA and warming on metabolic rates (ANCOVA).

TABLES

Table 4.1. **Carbon chemistry measurements**. Turf assemblages were exposed to 6 temperatures crossed with 2 CO_2 treatments, ambient and high CO_2 (ocean acidification- OA). Values are the daily mean (± SE) physical parameters measured for each tank over the course of the 3-week experiment (n = 21). Measured pH_T (total scale), temperature and total alkalinity (A_T) were used to derive the remaining carbonate parameters (pCO_2 and aragonite saturation state (Ω_{Ar})) using R and the package Seacarb.

Treatment	Temperature (°C)	pH_T	A_T (µmol kg^{-1})	pCO_2 (µatm)	Ω_{Ar}
24.0-Ambient	23.99 ± 0.06	8.11 ± 0.01	2281 ± 3	326 ± 7	3.67 ± 0.04
24.0-OA	24.00 ± 0.08	7.70 ± 0.01	2284 ± 3	990 ± 19	1.70 ± 0.02
25.5-Ambient	25.41 ± 0.04	8.07 ± 0.01	2283 ± 2	348 ± 7	3.69 ± 0.04
25.5-OA	25.46 ± 0.07	7.71 ± 0.01	2287 ± 2	974 ± 18	1.82 ± 0.03
27.0-Ambient	26.97 ± 0.06	8.06 ± 0.01	2282 ± 2	374 ± 8	3.71 ± 0.04
27.0-OA	27.01 ± 0.06	7.72 ± 0.01	2281 ± 2	941 ± 12	1.97 ± 0.02
28.5-Ambient	28.42 ± 0.07	8.05 ± 0.01	2284 ± 2	388 ± 6	3.79 ± 0.03
28.5-OA	28.55 ± 0.06	7.71 ± 0.00	2281 ± 3	963 ± 12	2.05 ± 0.02
30.0-Ambient	29.87 ± 0.09	8.02 ± 0.01	2280 ± 3	420 ± 7	3.77 ± 0.04
30.0-OA	29.88 ± 0.04	7.72 ± 0.00	2283 ± 4	960 ± 8	2.16 ± 0.01
31.5-Ambient	31.33 ± 0.06	7.98 ± 0.01	2284 ± 3	466 ± 10	3.72 ± 0.05
31.5-OA	31.25 ± 0.05	7.71 ± 0.01	2289 ± 2	984 ± 13	2.24 ± 0.02

Table 4.2. ANCOVA statistical analyses. Results of the analysis of covariance (ANCOVA) for each response variable. Temperature was treated as a continuous covariate in exploring effects of CO_2 on turf assemblage growth and metabolic rates. Due to significant interactive effects of temperature and CO_2 on metabolism, the non-significant interactive term was left in all ANCOVA models for consistency. Significant values are noted in bold.

Response Variable	Term	DF	F	P
Epilithic Growth	Temperature	1	1.75	0.260
	CO_2	1	7.86	**0.023**
	Temperature * CO_2	1	1.27	0.293
Endolithic Growth	Temperature	1	0.28	0.616
	CO_2	1	8.00	**0.022**
	Temperature * CO_2	1	4.13	0.077
Net Primary Production	Temperature	1	13.78	**0.006**
	CO_2	1	3.62	0.094
	Temperature * CO_2	1	10.53	**0.012**
Respiration	Temperature	1	30.36	**0.001**
	CO_2	1	10.73	**0.011**
	Temperature * CO_2	1	9.35	**0.016**
Gross Primary Production	Temperature	1	36.62	**<0.001**
	CO2	1	0.60	0.461
	Temperature * CO2	1	5.23	**0.050**

CONCLUSION

Environmental heterogeneity and global environmental change have long been recognized as important abiotic drivers of terrestrial and marine ecosystem structure and function (Barry and Dayton 1991; Smith and Buddemeier 1992; Vitousek 1994). At the core of ecosystems are the primary producers that provide the foundation to virtually all food webs, and in marine ecosystems primary production is driven almost entirely by photosynthesis (Lieth and Whittaker 1975). Large-scale changes in ecosystems, such as shifts in community structure and changes to ecosystem function are fundamentally influenced by small-scale responses of organismal physiology to the environment. Studying the ecophysiology of primary producers in response to environmental change, whether natural or anthropogenic, therefore provides important insight into how organisms are influenced by the rapidly changing environment and has broader implications for ecosystem dynamics.

In Chapter 2, I use the Southern Line Island archipelago as a natural experiment to explore how heterogeneity in inorganic nutrient availability influences the photosynthetic potential of benthic primary producers. Photosynthetic potential of all taxa increased as a function of increasing inorganic nutrient availability. These findings suggest that photosynthesis by primary producers at the more oligotrophic islands may be limited by inorganic nutrients, and that increasing availability associated with equatorial upwelling relieved some of that limitation. The increase in photosynthetic potential by primary producers under higher inorganic nutrients has implications for energy transfer into food webs and the overall capacity for ecosystem productivity. Chapter 2 adds a new perspective to the current literature by broadening

the contentious view that inorganic nutrient enrichment has deleterious effects on coral reef ecosystems (Hughes 1994; Lapointe 1997; Szmant 2002). When coupled with healthy populations of herbivores (Edwards et al. 2014), natural fluxes of inorganic nutrients may be beneficial to the reef ecosystem by increasing the capacity for ecosystem primary production and by building a stronger food-web foundation.

Global change has far-reaching impacts on natural ecosystems, and a wide body of literature has been dedicated to understanding effects of ocean acidification (OA) and warming on marine organisms (Kroeker et al. 2010; Kroeker et al. 2013). Despite these research efforts, there remains a gap in our understanding of OA effects on coral reef algae, especially the fleshy macroalgae. In Chapter 3, I address this gap by quantifying the ecophysiology of three algal functional groups in response to OA. Ocean acidification had contrasting effects on algal functional groups. Fleshy macroalgae grew more under OA, while calcareous algae grew less or even dissolved. There were no effects of OA on algal photophysiology. The results of Chapter 3 contribute to the growing body of work on OA effects on marine organisms by quantifying effects on 11 different species of algae. By using numerous species, I can make broader conclusions about functional group responses to OA. These results indicate that fleshy and calcareous species may respond differently to OA, which has implications for ecological interactions and ecosystem dynamics. If OA facilitates growth of fleshy species and provides a competitive advantage over calcifying species, there may be a shift in community structure towards reefs dominated by fleshy "winners" (Fabricius et al. 2011).

In Chapter 4, I continue my analysis of algal functional group responses to global change. Although turf algae assemblages are the most ubiquitous primary producers on coral reefs, we know little about how tropic turfs respond to either OA or warming, let along the combination of both. Ocean acidification had the strongest effect on turf assemblage growth and increased the biomass of epilithic and endolithic components, independent of temperature. There were interactive effects of OA and warming on turf metabolism, where OA increased primary production but more so at the warmest temperatures. These results indicate that turf assemblage primary production responds positively to OA, which agrees with the findings of Chapter 3 that OA fuels growth of fleshy algae (turfs have primarily non-calcifying components).

One of the important points from Chapter 4 is that we need to improve experimental design of global change experiments in order to more accurately predict the fate of marine organisms in the near-future high CO_2 ocean. The main result of Chapter 4, however, is that OA enhances primary production of both epilithic and endolithic turf assemblages. While the increase in primary production could have positive benefits for coral reef ecosystems by increasing energy transfer into food webs, the increase in fleshy algal biomass and abundance could lead to more frequent competition with reef-building calcifiers. Further, higher growth rates by the endolithic community may lead to higher rates of bioerosion. The positive effects of global change stressors on turf assemblages, therefore, have broader implications for ecological interactions, ecosystem primary production and bioerosion of coral reefs.

The results of this dissertation highlight the importance of natural heterogeneity and global change for the ecophysiology of coral reef algae. Across all functional groups of benthic primary producers, I found that inorganic nutrients increased the potential for primary production, but OA and warming increased primary production potential only for fleshy functional groups of coral reef algae (fleshy macroalgae and turf algae assemblages). These findings contribute to our broader understanding of how environmental variability influences the physiology of organisms, and provides insight into potential changes in community structure and function coral reef environments that are increasingly vulnerable to global change.

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