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The Effects of Climate Change on Macroalgal Growth, Trophic Interactions and Community Structure

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THE EFFECTS OF CLIMATE CHANGE ON MACROALGAL GROWTH,
TROPHIC INTERACTIONS AND COMMUNITY STRUCTURE

BY
GORDON OBER

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
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UNIVERSITY OF RHODE ISLAND

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DOCTOR OF PHILOSOPHY DISSERTATION
OF
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ABSTRACT

Global climate change is threatening the structure, function, and health of ecosystems. While factors of climate change have been studied extensively over the past few decades, most research has focused on the response of single organisms or populations; as our ecosystems are comprised of complex interactions and relationships, it is of critical importance to understand how entire communities are going to be impacted by climate change. Ocean acidification (a by-product of increased atmospheric carbon dioxide, CO₂), and nutrient loading are two major forces of global change that are projected to have detrimental impacts on coastal marine species and ecosystems. Most work on ocean acidification has focused on the response of calcifying organisms, where the changes in ocean chemistry associated with acidification enhance shell dissolution and impair growth. However, while calcifying species are expected to exhibit negative responses to acidification, primary producers, like macroalgae, are expected to flourish.

Both ocean acidification and nutrient loading can stimulate the growth and productivity of opportunistic, fast-growing, ephemeral macroalgae at the expense of foundational species such as corals, seagrasses, and long-lived, perennial macroalgae (i.e. kelps). As a result, these ecosystems will likely undergo major shifts in structure, function, and diversity. Few studies have investigated the interactive effects of ocean acidification and nutrient loading, particularly in terms of community response and trophic interactions. Despite increasing the growth rates of macroalgae, the presence and diversity of herbivores within an ecosystem has the potential to control this

expected algal growth. The research described in this dissertation aims to: 1) quantify the combined effects of ocean acidification and nutrient loading on the growth, tissue quality, and competition of two abundant macroalgal species with different life histories; 2) test whether or not an abundant grazer can enhance consumption of macroalgae under future conditions of acidification and nutrients, promoting community resilience; 3) describe the impact of ocean acidification on the growth and diversity of reef-associated turf algal communities.

Using a laboratory mesocosm design, the response of *Ulva* (an ephemeral, opportunistic green alga) and *Fucus* (a long-lived, perennial brown alga) to the interaction of two levels of ocean acidification and two levels of nutrients was tested. Individual, field-collected algal thalli were placed in flow-through seawater systems with one of four experimental conditions: high pCO₂ (~1100 µatm) or background pCO₂ (~390 µatm) and high nutrients (200 µM TN) or low nutrients (10 µM TN), in a fully factorial design. Three experiments were run: the first two investigated the response of *Ulva* and *Fucus* in monocultures; the third tested the response of both species cultured together (biculture). Growth rates and tissue quality (via carbon to nitrogen ratios, C:N) were measured after 21 days of exposure to treatments. Ocean acidification and nutrient loading significantly increased the growth in *Ulva*, where growth rates under high pCO₂ and high nutrients were about 3X greater than those grown under ambient conditions, with the environmental factors appearing to have an additive impact on *Ulva* growth. Growth rates of *Fucus* were unaffected by environmental conditions. Both species exhibited an increase in tissue quality as a result of decreased C:N when exposed to high nutrients. Response variables were

compared between monoculture and biculture experiments for both species. Growth rates of *Ulva* and *Fucus* were unaffected by the treatment culture, but tissue C:N of *Fucus* was significantly higher when grown with *Ulva*, indicating potential resource competition, where *Ulva* outcompetes *Fucus*.

The enhanced growth exhibited by *Ulva* supports previous work indicating the enhanced growth of opportunistic algal species under future climate conditions. While this could be troubling for species inhabiting coastal ecosystems (such as seagrasses, non-bloom forming macroalgae, and fish), grazers may hold the key to mitigating algal growth and keeping ecosystems in balance. Consumption rates and feeding preferences of a common marine snail (*Littorina littorea*) were tested using the same experimental design and environmental parameters detailed above. Snails were placed in treatment mesocosms for seven days and were given a choice of *Ulva* and *Fucus*. High pCO₂ levels significantly reduced macroalgal consumption by about 50% and snails switched from a mixed algal diet to feeding exclusively on *Ulva*. Respiration rates for *L. littorea* were measured, and under high pCO₂ respiration was significantly reduced. Artificial food trials were run to help explain the diet preference change. No difference was found comparing consumption of the artificial food, pointing to the preference shift being driven by algal tissue toughness.

Reef ecosystems have been well studied with respect to ocean acidification. This research shows that corals are overtaken by fleshy macroalgae and fast-growing turf algae. Here, we tested the response of turf algal communities to three levels of ocean acidification. Natural turf communities growing on coral rubble from the Great Barrier Reef, Australia were collected and exposed to ambient, medium, and high

pCO₂ treatments in mesocosms for 41 days. Communities were assessed for biomass and genus diversity. Biomass of turf communities was significantly higher under high pCO₂. Turf community evenness and diversity significantly increased under high pCO₂. This change in community structure is likely due to the decline in abundance of *Polysiphonia* (a filamentous, branched red algae). The results indicate that enhanced turf growth under conditions of acidification will aid in the growth and expansion of macroalgae at the expense of corals in reef ecosystems. Changes in turf diversity should inform how larger macroalgal communities may be structured in the future.

This research highlights the success that opportunistic macroalgae and turf algae will have under future climate conditions. The success of macroalgae, however, comes at the expense of other critical and foundational species within a community. In addition, macroalgal communities are likely to undergo assemblage shifts as well, due to species-specific responses to environmental change, where species gain a competitive advantage. Top-down forces, such as grazing, may protect against changes in community structure and macroalgal assemblages, but only if grazers are not negatively impacted directly by environmental change. If grazers exhibit decreased consumption and are unable to keep up with anticipated algal growth, this will ultimately enhance macroalgal abundance in coastal ecosystems.

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DEDICATION

I would like to dedicate this dissertation to family, friends, and colleagues. To my parents, Marion and Steve Ober, thank you for putting an empty coffee tin in my hand and encouraging me to explore the natural world around me. The coffee tin, of course, was for collecting organisms. Despite it now being considered “bad advice,” you encouraged and supported me in my desire to pursue my passion. To my wife, Celeste, your patience and support throughout this process have been unbelievable. You have helped balance me and you’ve kept me sane. I can’t imagine how many times you’ve had to listen to me talk about climate change, seaweeds, and cite obscure scientific facts. Sadly, you are now in for a lifetime of it! To my sister, Leigh, I’m glad you were able to help keep my ego in check, forcing me to take a broader perspective. Sam Richards and Ariel Soscia, I know for a fact that I wouldn’t have been able to pull this off without you guys, thank you.

To friends and colleagues, both scientist and non-scientist alike, whether we had a five minute conversation at a conference or shared wave in the cold Atlantic. I have taken something from each of these experiences and interactions, they have helped guide or inspire my research or have given me fresh perspectives and balance. Thank you all.

PREFACE

This dissertation is being submitted in manuscript form. It is comprised of three chapters each of which have been submitted, or are in preparation of submission, for peer-reviewed publication. Chapter one, “Interactive effects of ocean acidification and eutrophication on the growth, nutritional quality, and competition of coastal macroalgae,” has been submitted for publication in the Journal of Experimental Marine Biology and Ecology. Chapter two, “Ocean acidification but not nutrient enrichment reduces grazing and alters diet preference in a common marine snail,” is in preparation for submission to the Journal of Ecology. Chapter three, “Ocean acidification influences the biomass and diversity of reef-associated turf algal communities,” has been submitted for publication in the journal Marine Biology.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	vi
DEDICATION.....	viii
PREFACE.....	ix
TABLE OF CONTENTS.....	x
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xiii
CHAPTER 1: INTERACTIVE EFFECTS OF OCEAN ACIDIFICATION AND EUTROPHICATION ON THE GROWTH, NUTRITIONAL QUALITY, AND COMPETITION OF COASTAL MACROALGAE.....	1
CHAPTER 2: OCEAN ACIDIFICATION BUT NOT NUTRIENT ENRICHMENT REDUCES GRAZING AND ALTERS DIET PREFERENCE IN A COMMON MARINE SNAIL.....	41
CHAPTER 3: OCEAN ACIDIFICATION INFLUENCES THE BIOMASS AND DIVERSITY OF REEF-ASSOCIATED TURF ALGAL COMMUNITIES.....	83

LIST OF TABLES

TABLE	PAGE
Chapter 1	
Table 1. Carbonate chemistry parameters and dissolved inorganic nitrogen (DIN) values for algal growth experiments.....	33
Table 2. Summary of three-way analysis of variance (ANOVA) on growth rates of <i>Ulva</i> and <i>Fucus</i> by pCO ₂ , nutrients, and culture experiment.....	34
Table 3. Summary of three-way ANOVA on the C:N, carbon concentration, and nitrogen of <i>Ulva</i> and <i>Fucus</i> tissues by pCO ₂ , nutrients, and culture experiment.....	35
Chapter 2	
Table 1. Carbonate chemistry parameters and dissolved inorganic nitrogen (DIN) values for grazer consumption experiments.....	75
Table 2. Summary of the two-way ANOVA showing the effects of pCO ₂ , nutrients, and their interaction (pCO ₂ X Nutrients) on the total algal consumption (mg snail ⁻¹ day ⁻¹) by <i>L. littorea</i>	76
Table 3. Summary of the factorial MANOVA and protected ANOVA showing the effects of pCO ₂ , nutrients, and their interaction (pCO ₂ X Nutrients) on the consumption (mg snail ⁻¹ day ⁻¹) of <i>Ulva</i> and <i>Fucus</i> by <i>L. littorea</i>	77
Table 4. Summary of two-way ANOVA showing the effects of pCO ₂ ,	

nutrients, and their interaction (pCO ₂ X Nutrients) on the respiration rates (μmol O ₂ g ⁻¹ L ⁻¹ hr ⁻¹) of <i>L. littorea</i>	78
---	----

Chapter 3

Table 1. Carbonate chemistry parameters for turf algal growth experiments, values listed for ambient, medium, and high CO ₂ treatments.....	107
--	-----

Table 2. Mean relative abundance of present genera and mean community diversity metrics by CO ₂ treatment. NC indicates negligible cover, as the genus was observed but did not contribute to the overall cover; - indicates no observation of genus.....	108
--	-----

LIST OF FIGURES

FIGURE	PAGE
Chapter 1	
Figure 1. Relative growth rate (% day ⁻¹) for <i>Ulva</i> monoculture (A), <i>Ulva</i> biculture (B), <i>Fucus</i> monoculture (C), and <i>Fucus</i> biculture (D). Values plotted are means $\pm$ 1 SE (N = 7).....	37
Figure 2. Carbon to nitrogen ratios (C:N) within tissues of <i>Ulva</i> grown in monoculture (A), <i>Ulva</i> grown in biculture (B), <i>Fucus</i> grown in monoculture (C), and <i>Fucus</i> grown in biculture (D). Values plotted are means $\pm$ 1 SE (N = 7).....	38
Figure 3. Carbon and nitrogen concentrations within the tissues of <i>Ulva</i> (A) and <i>Fucus</i> (B). Mean values $\pm$ 1 SE are plotted for species grown in monoculture (circles) and biculture (triangles), broken down by ambient pCO ₂ (black), high pCO ₂ (gray), ambient nutrients (patterned), and high nutrients (filled) (N = 7).....	39
Chapter 2	
Figure 1. Mean total algal consumption rate (live thalli) (mg snail ⁻¹ day ⁻¹ $\pm$ 1 SE) by environmental treatment.....	79
Figure 2. Mean consumption of <i>Ulva</i> and <i>Fucus</i> (live thalli) (mg snail ⁻¹ day ⁻¹ $\pm$ 1 SE) by environmental treatment.....	80
Figure 3. Mean proportion of initial count of artificial <i>Ulva</i> and	

Fucus (± 1 SE) consumed by *L. littorea* by environmental treatment.....81

Figure 4. Mean respiration rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ L}^{-1} \text{ hr}^{-1} \pm 1$ SE) of

L. littorina by environmental treatment.....82

Chapter 3

Figure 1. Turf algal community replicates for ambient CO₂ treatment

(left), medium CO₂ treatment (middle), and high CO₂ treatment (right)

at the beginning of experimentation.....110

Figure 2. Mean biomass of turf algal communities ($\text{mg cm}^{-2} \pm 1$ SE)

after 41 days of experimentation under three levels of CO₂.....111

Figure 3. MDS plot of turf communities under different CO₂

treatments: ambient (triangles), medium (circles), and high (squares).....112

Figure 4. Mean relative abundance (proportion of present genera)

of turf algal phyla and cyanobacteria (± 1 SE) by CO₂ treatment.....113

CHAPTER 1

INTERACTIVE EFFECTS OF OCEAN ACIDIFICATION AND EUTROPHICATION ON THE GROWTH, NUTRITIONAL QUALITY, AND COMPETITION OF COASTAL MACROALGAE

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Abstract:

Coastal ecosystems are subjected to global and local environmental stressors, including increased atmospheric carbon dioxide (CO₂) (and subsequent ocean acidification) and nutrient loading. Here, we tested how two common macroalgal species in the Northwest Atlantic (*Ulva* spp. and *Fucus vesiculosus* Linnaeus) respond to the combination of ocean acidification and nutrient loading. We utilized two levels of pCO₂ with two levels of nutrients in a full factorial design, testing the growth rates and nutritional quality of *Ulva* and *Fucus* grown for 21 days in monoculture and biculture. We found that the opportunistic, fast-growing *Ulva* exhibited increased growth rates under high pCO₂ and high nutrients, with growth rates increasing three-fold above *Ulva* grown in ambient pCO₂ and ambient nutrients. By contrast, *Fucus* growth rates were not impacted by either factor. Both species exhibited a decline in carbon to nitrogen ratios (C:N) with elevated nutrients, but CO₂ concentration did not alter nutritional quality in either species. Species grown in biculture exhibited similar growth rates to those in monoculture conditions, but the *Fucus* C:N increased significantly when grown with *Ulva*, indicating potential resource competition. Our results suggest that the combination of ocean acidification and nutrients will enhance abundance of opportunistic algal species in coastal systems and will likely drive macroalgal community shifts, based on species-specific responses to future conditions.

Key words: ocean acidification, eutrophication, climate change, macroalgae, nutritional quality

1. Introduction:

Increasing amounts of carbon dioxide (CO₂) in the earth's atmosphere are the driving force behind global climate change (Pachauri et al., 2014). Ocean acidification, a decrease in pH brought about by increased atmospheric CO₂, has garnered attention due to the overwhelmingly negative effects predicted for calcifying organisms (Comeau et al., 2014; Diaz-Pulido et al., 2011; Hoegh-Guldberg et al., 2007; Waldbusser et al., 2015). Changes in ocean chemistry associated with ocean acidification, such as lowered saturation states, are causing reductions in growth, increased shell dissolution, and declines in fitness and performance of many marine calcifying species (Ries et al., 2009; Waldbusser et al., 2015).

Conversely, less attention has been paid to non-calcifying autotrophic organisms. These species may benefit from ocean acidification and the subsequent change in ocean chemistry, as increased concentrations of both aqueous CO₂ and bicarbonate (HCO₃) may enhance photosynthesis and growth in primary producers. Enhanced growth rates under acidification conditions have been observed in fleshy macroalgae (Kübler et al., 1999; Olischläger and Wiencke, 2013; Diaz-Pulido et al., 2011; Zou, 2005; Kroeker et al., 2010) and seagrasses (Zimmerman et al., 1997). However, the response of primary producers to ocean acidification is highly species specific, ultimately dependent on carbon limitation and carbon acquisition ability as well as developmental stage (Gaitán-Espitia et al., 2014; Olischläger et al., 2012). As such, negative and neutral responses to ocean acidification have also been observed (Gutow et al., 2014; Rautenberger et al., 2015). Divergent responses of fleshy

macroalgae to acidification are correlated with the presence and efficiency of the carbon concentrating mechanism (CCM) (Giordano et al., 2005; Raven and Beardall, 2003). Due to highly abundant bicarbonate ions, most macroalgae rely on CCMs to convert HCO_3^- to CO_2 for use in photosynthesis (Hepburn et al., 2011). In addition, many species also have the ability to passively diffuse CO_2 and may gain an advantage under future conditions due to reduced reliance and down-regulation of CCMs. While most marine macroalgae have CCMs, a few species within the Rhodophyta rely on passive diffusion of CO_2 for photosynthesis (Giordano et al., 2005; Raven and Beardall, 2003). These species, among others, should experience enhanced growth and photosynthesis due to the increased concentration of CO_2 associated with ocean acidification.

While ocean acidification is projected to impact all marine systems, the effects will likely vary across ecosystems (Hofmann et al. 2011). The signal of ocean acidification is easy to determine in the open ocean; unlike in the open ocean, coastal pH is highly variable due to daily and seasonal shifts in photosynthesis and respiration, and coastal acidification may be driven more by eutrophication than increases in atmospheric CO_2 (Feely et al., 2010; Cai et al., 2011; Wallace et al. 2014). Nutrient loading (and potential eutrophication events) also impacts coastal bays and estuaries with low flow and low turnover (Lee and Olsen, 1985). Nutrients can enter these waterways via agricultural and urban runoff and sewage treatment discharge, pumping excess nitrogen and phosphorous into the water column (Nixon, 1995). While these nutrients are critical to algal growth, excess concentrations can facilitate harmful algal blooms (toxic or non-toxic), either composed of micro and/or

macroalgae (Anderson et al., 2002). Decomposition or respiration from large macroalgal blooms can lower oxygen levels in the water column potentially leading to hypoxic events, with detrimental impacts on coinhabitants (Granger et al., 2000; Thomsen et al., 2006; Valiela et al., 1997). Macroalgal blooms can also act as a deterrent to coastal recreation (Valiela et al., 1997; Worm and Lotze, 2006).

Our understanding of climate effects on coastal zones is critical, as these ecosystems hold high value in biodiversity as well as economic and societal importance. Increased CO₂ from acidification, combined with increased concentrations of limiting nutrients, could act in conjunction to stimulate and enhance growth in primary producers. While acidification studies are beginning to incorporate additional environmental stressors such as light intensity and warming (Olischläger and Wiencke, 2013; Rautenberger et al., 2015; Roleda et al., 2012; Sarker et al., 2013), the combined effects of acidification and nutrients on primary producers are less well understood (but see Campbell and Fourqurean, 2014; Falkenberg et al., 2013; Russell et al., 2009).

In coastal zones, the green macroalga *Ulva* and the brown macroalga *Fucus* have different life history and ecological traits. *Ulva* is a fast-growing, opportunistic, ephemeral genus that thrives in a wide range of environments. *Fucus* is a long-lived, slow growing, perennial genus that creates complex, three-dimensional habitat for other organisms. These genera, among others, form the base of coastal marine food webs in the northwest Atlantic and are commonly grazed by herbivores and omnivores (Bracken et al., 2014; Lubchenco, 1983; Watson and Norton, 1985). Both genera use CCMs (Koch et al., 2013), but exhibit divergent responses, with increased growth

rates for *Ulva lactuca* and decreased growth rates for *Fucus vesiculosus* under high CO₂ conditions (Gutow et al., 2014; Olischläger et al., 2013). Similarly, *Ulva lactuca* has increased growth rates under high nutrients (Steffensen, 1976). *Fucus vesiculosus* experiences a reduction in growth and cover due to the indirect effects of added nutrients, such as increased turbidity and increased growth of epiphytic algae (Berger et al., 2004).

The objective of our research was to quantify the impacts of the interaction of ocean acidification and nutrients on *Ulva* spp. and *F. vesiculosus*, by assessing growth rates, tissue quality (tissue C:N ratio), carbon and nitrogen content of algal tissues, and potential competitive impacts. While *U. lactuca* was chosen for this study, recent invasions of the cryptic *U. australis* have nullified our initial identification (Guidone et al., 2013; Hofmann et al., 2010). It is likely that the tested specimens are a mix of two species: *U. lactuca* and *U. australis*. We will hereafter refer to our test organisms as *Ulva* and *Fucus*. We predicted that the growth rate and tissue quality of *Ulva* would increase with increased pCO₂ and nutrients, as the combination of the two environmental factors would result in a synergistic effect on its growth rate (Neori et al., 1991; Russell et al., 2009). By contrast, we predicted that increased pCO₂ will decrease the growth rate of *Fucus*, which is likely to occur due to potential pH sensitivity of its carbon concentrating mechanism CCM (Axelsson et al., 2000; Gutow et al., 2014), but increase tissue quality of *Fucus* (as seen in Gutow et al., 2014). We expect *Fucus* growth to be unaffected by nutrient loading as this species is adapted to low-nutrient environments (Savage and Elmgren, 2004), and nutrients may indirectly reduce growth rates by promoting the growth of competitors (Hemmi et al., 2005;

Pedersen and Borum, 1996; Worm and Lotze, 2006). Growth rates and tissue quality of both *Ulva* and *Fucus* will be tested in a biculture experiment, where we expect opportunistic, fast-growing *Ulva* to outcompete *Fucus* resulting in lower growth rates and tissue quality of *Fucus* (Connell and Russell, 2010; Falkenberg et al., 2013; Worm and Lotze, 2006). We interpret our results in the context of shifting macroalgal assemblages and ecosystem structure.

2. Materials and Methods:

2.1 Algal Collection and Experimental Design

We conducted three temporally distinct experiments using the flow-through seawater facility at the US Environmental Protection Agency Atlantic Ecology Division in Narragansett, RI (*Ulva* monoculture - September 2014, biculture - October 2014, *Fucus* monoculture - November 2014). These experiments were run in succession of one another where all environmental parameters remained constant. Biculture and monoculture experiments were run separately in order to ensure the presence and abundance of the ephemeral *Ulva spp.* in the field. First, thalli of *Ulva* and *Fucus* were collected from the shallow subtidal zone at the University of Rhode Island's Narragansett Bay Campus beach (41°29'26"N, -71°25'11"W) in August 2014, September 2014, and October 2014. Non-reproductive tips of *Fucus* (~3-5cm in length) were cut from adult thalli (see Gutow et al., 2014). *Fucus* tips and *Ulva* thalli were cleaned of any epiphytes, and transferred into separate 20L glass aquaria with

flow-through seawater and aeration. Algal individuals were acclimated to lab conditions for five days prior to the start of each experiment.

To set up each experiment, we first spun algae 20x in a salad spinner (Thornber et al., 2008), removed a small piece (~10% of starting mass) of the thallus which was then dried at 38°C for 24 hours and then placed in a desiccator for C:N analysis (see below), and then recorded the initial algal wet mass of each remaining piece. We placed individuals into 20L aquaria with one individual per tank (for monocultures) or one individual of each species (for the biculture experiment). Starting wet mass was 0.50g and 0.75g for *Ulva* and *Fucus*, respectively.

Narragansett Bay has high variation in pCO₂ and DIN on both spatial and temporal scales. Annual average pCO₂ concentration is around 400 µatm but ranges from 150 – 1000 µatm (Turner, 2015). DIN in Narragansett Bay runs along a north south gradient, where water has an annual average of 70 µM DIN in the north and annual average of 4-10 µM DIN in the south, where certain parts of the bay can exceed 180 µM DIN on occasion (Krumholz, 2012). We experimentally tested the response of algae to four environmental treatments, which factorially combined two levels of acidification (ambient ~ 400 µatm pCO₂, pH 8.10, and representative concentration pathway 8.5 (RCP8.5) projections for the year 2100 ~ 1200 µatm pCO₂, pH 7.65; Moss et al., 2011) and two levels of nutrient loading (ambient ~ 5 µM dissolved inorganic nitrogen, DIN, and high ~ 200 µM DIN). Each of the four environmental treatments had a 40L headwater tank where CO₂ gas or ambient outdoor air (depending on the treatment) was bubbled in at a constant rate. We obtained high pCO₂ by bubbling in pure CO₂ gas via an Aalborg Mass Flow Controller

GFC (Aalborg Instruments and Controllers, INC) into the headwater tank. Filtered, tempered seawater (18°C, kept constant) was pumped into headwater tanks to mix with CO₂ or air. Mixing of gas and water was aided via a Hydor Circulation Fan. Treated water was pumped from the headwater tank via an Eheim 1200 submersible pump to a manifold, delivering treated water to seven experimental aquaria for each treatment. Aquaria received water at a rate of 130 ± 5 mL/min, using a flow-through design to mimic the natural variability of coastal pH that, on a daily basis, fluctuated by up to 0.4 units.

Nutrients were added individually to each experimental aquarium, as appropriate, through slow-release agar blocks (Teichberg et al., 2010). Blocks were created to meet the desired nutrient concentration (~ 200 μ M DIN for the 20L aquaria) by adding nitrate and ammonium, in the form of KNO₃ (at 2M concentration) and NH₄CL (at 2M concentration), along with 3% agar and seawater (Tate, 1990). Phosphate was added to the blocks, in the form of KH₂PO₄ (at 1M concentration). Blocks containing only agar and seawater were added to the ambient nutrient tanks in order to simulate the physical addition of the block. Seawater nutrient levels were analyzed midway through the experiment by taking 60 mL samples of filtered which was then frozen. Seawater samples were analyzed for ammonia, nitrate, and nitrite (sum of which is equal to DIN) by the URI Marine Science Research Facility, an RI NSF EPSCoR Core Facility (Table 1).

Each experiment ran for a total of 21 days, and tanks were supplemented with artificial light (Sylvania Full Spectrum) at 128.7 ± 2.7 μ mol photons m⁻²sec⁻² with a light/dark rhythm of 14:10h (L:D). Tanks were scrubbed and cleaned every two days

and any epiphytes growing on the algae were removed. Every seven days, algae were briefly removed, weighed, and a small piece (<10% of total wet mass) was removed for future C:N analysis. Mass of removed tissue was included in the calculation of total growth; however, this likely resulted in an underestimation of total algal growth. Algal tissue quality was determined by drying tissue samples for 24 hours at 38°C. All dried samples were preserved in glass vials and placed in a desiccation chamber. Samples were ground into a powder and placed in tin capsules. All samples were analyzed for carbon and nitrogen concentrations by Dr. Brad S. Moran's laboratory at the University of Rhode Island Graduate School of Oceanography.

2.2 Carbonate Chemistry

Water samples were collected and preserved, using mercuric chloride (HgCl_2), over the course of each experiment to measure dissolved inorganic carbon (DIC) and total alkalinity (TA) following the Best Practices Guide (Dickson et al., 2007). Two samples from each of the four environmental treatments were taken each week of each experiment at two points during the day. Seawater samples presented in this manuscript describe carbonate chemistry during midday sampling only. Temperature and salinity were measured directly. DIC was measured using a Shimadzu TOC-V total organic carbon analyzer. TA was determined for each sample using a Metrohm 877 Titrino Plus titrator. In determining DIC and TA, samples were standardized against Dickson seawater standards CRM Batch 138. All parameters of carbonate chemistry were calculated using the seacarb package (R software; Lavigne et al. 2011) (Table 1). WTW Profiline 3110 pH meter with SenTix 21 glass electrodes were used

to record high frequency pH measurements of seawater in each of the four treatments, this data ensured that we were able to capture the natural variability of coastal pH in our treatments (data not presented).

2.3 Statistical Analysis:

Relative growth rate (RGR, % day⁻¹) was calculated for each alga based on change in wet mass between initial and final masses for each experiment (Lüning et al., 1990). Mean RGR, final carbon and nitrogen concentrations, and final C:N ratios for each species were assessed using three-way analyses of variance (ANOVA) with pCO₂ (ambient or high), nutrient level (ambient or high), and culture (or community; monoculture or biculture) experiment as fixed factors. Initial C:N ratios of either species did not differ among environmental treatments (two-way ANOVA, p>0.05). By including culture experiment as a factor in our analysis, we were able to determine potential resource competition between the two species. All statistical analyses were performed using JMP v 11 (www.jmp.com).

3. Results:

3.1 Seawater parameters

We were able to obtain elevated and relevant environmental parameters for each of our treatments. Average midday values calculated for pCO₂ in our two acidification (high pCO₂) treatments averaged 1219.65 ± 86 µatm and $1226.52.51 \pm 51$ µatm; in our two ambient pCO₂ treatments, calculated pCO₂ levels averaged $413.33 \pm$

25 μatm and $412.40 \pm 31 \mu\text{atm}$ (Table 1). For acidification treatments, calculated $\text{pH}_{(\text{total})}$ averaged 7.58 ± 0.03 and 7.59 ± 0.03 ; while calculated $\text{pH}_{(\text{total})}$ from our ambient treatments averaged 8.00 ± 0.04 and 8.00 ± 0.03 (Table 1). DIC in acidification treatments were measured at $2055.98 \pm 91 \mu\text{mol kg}^{-1}$ and $2091 \pm 76 \mu\text{mol kg}^{-1}$; whereas ambient treatments were measured at $1888.1 \pm 101 \mu\text{mol kg}^{-1}$ and $1875.55 \pm 80 \mu\text{mol kg}^{-1}$ (Table 1). TA was measured at $2099.83 \pm 96 \mu\text{mol kg}^{-1}$ and $2136.33 \pm 83 \mu\text{mol kg}^{-1}$ under acidification treatments; under ambient treatments TA was measured at $2074.7 \pm 122 \mu\text{mol kg}^{-1}$ and $2059.62 \pm 91 \mu\text{mol kg}^{-1}$ (Table 1). For all experiments, temperature ranged from 17.95°C to 18.1°C and salinity ranged from 30.65 ppt to 30.88 ppt (Table 1).

Adding nutrients to our aquaria proved effective in increasing the concentrations of DIN above ambient levels. Measurements of DIN in high nutrient treatments averaged $187.24 \pm 2.11 \mu\text{M}$ and $207.63 \pm 6.36 \mu\text{M}$, respectively; whereas ambient nutrient treatments averaged $7.52 \pm 0.87 \mu\text{M}$ and $6.73 \pm 0.65 \mu\text{M}$ DIN, respectively (Table 1).

3.2 Algal Growth

Ulva grown in monoculture under high pCO_2 and high nutrients had nearly a threefold faster relative growth rate (RGR) than monoculture *Ulva* grown under ambient conditions ($7.5 \pm 0.33 \text{ \% day}^{-1}$ vs. $2.83 \pm 1.06 \text{ \% day}^{-1}$, respectively; Fig. 1A). *Ulva* grown in biculture under high pCO_2 and high nutrients experienced a similar threefold increase in RGR compared to individuals grown under ambient conditions ($7.6 \pm 0.55 \text{ \% day}^{-1}$ vs. $2.3 \pm 1.4 \text{ \% day}^{-1}$, respectively; Fig. 1B). RGR of *Ulva* grown in monoculture and biculture was significantly increased by elevated pCO_2 and

elevated nutrients ($p = 0.002$, $p < 0.0001$, respectively; Table 2), with no significant interaction ($p = 0.72$; Table 2).

We did not observe an effect of $p\text{CO}_2$ ($p=0.55$; Table 2) or nutrient level ($p=0.32$) on the RGR of *Fucus* grown in monoculture, as rates ranged from 1.91 ± 0.17 to $2.16 \pm 0.20 \text{ \% day}^{-1}$ (Fig. 1C), with a nonsignificant interaction ($p=0.34$). We observed a similar RGR of *Fucus* grown in biculture, where rates ranged from 1.99 ± 0.17 and $2.18 \pm 0.11 \text{ \% day}^{-1}$ (Fig. 1D).

We observed similar growth rates of *Ulva* and *Fucus* between monoculture and biculture experiments ($p = 0.53$, $p = 0.80$, respectively; Table 2).

3.3 Algal Tissue Content (C:N, C, N)

The C:N tissue content of both *Ulva* and *Fucus* was significantly lower in high nutrient treatments ($p < 0.0001$ and $p < 0.0001$, respectively; Table 3; Fig. 2). Under conditions of high nutrients, mean C:N in *Ulva* was measured at 14.81 compared to a mean of 19.82 under ambient nutrients. Mean C:N within *Fucus* grown under high nutrients was measured at 19.59 compared to a mean of 23.72 under ambient nutrient conditions. By contrast, $p\text{CO}_2$ did not significantly affect the C:N of either *Ulva* or *Fucus* ($p = 0.18$, $p = 0.183$, respectively; Table 3), with non significant $p\text{CO}_2 \times$ nutrient interactions (*Ulva* or *Fucus* ($p = 0.25$, and $p = 0.403$, respectively; Table 3). We found evidence of resource competition between the two species for *Fucus* tissue C:N; *Fucus* grown in biculture had tissues with significantly higher C:N ($p = 0.0021$; Table 3). Conversely, *Ulva* tissue C:N was not impacted by community ($p = 0.21$; Table 3).

Percent carbon of *Ulva* and *Fucus* tissues was significantly higher under high pCO₂ ($p = 0.04$, $p = 0.02$, respectively; Table 3, Fig. 3A and 3B). Similarly, both *Ulva* and *Fucus* percent nitrogen increased in high nutrient treatments ($p = 0.001$, $p = 0.01$, respectively; Table 3, Fig. 3A and 3B). Nitrogen content of *Ulva* and *Fucus* tissues were unaffected by community, pCO₂, or any interactions of factors (Table 3). There was a significant interaction between community and nutrient level influencing the carbon content in *Ulva* ($p = 0.03$; Table 3) as well as a significant three-way interaction between pCO₂, nutrients, and community ($p = 0.05$; Table 3).

4. Discussion

4.1 Algal Growth

Non-calcifying primary producers are predicted to benefit from changes in seawater chemistry due to ocean acidification (see Kroeker et al., 2013), as we found for *Ulva*, with growth rates doubled under 1200 μatm pCO₂ conditions. This matches the response of *Ulva* to ocean acidification in other systems (Olischläger et al., 2013; Xu and Gao, 2012) as well as other macroalgal species (Campbell and Fourqurean, 2014; Kübler et al., 1999; Olischläger and Wiencke, 2013; Swanson and Fox, 2007). Olischläger et al. (2013) observed a doubled growth rate of *U. lactuca* when exposed to 700 μatm pCO₂, and Xu and Gao (2012) found *U. prolifera* exhibited increased growth rates of about 40% when exposed to 1000 μatm pCO₂. These studies used concentrations of pCO₂ expected in the next 50-100 years, whereas our study focused on the more extreme pCO₂ projection for the year 2100. However, the response of

macroalgae is still highly species-specific, and neutral or negative impacts of ocean acidification on growth rate have been observed in non-calcifying macroalgae (Cornwall et al., 2012; Gutow et al., 2014; Israel and Hophy, 2002; Mercado and Gordillo, 2011). Divergent responses of macroalgae to acidification are likely due to the differences in CCM effectiveness, potentially giving certain species more independence from the environment, or CCMs are optimized for higher pH conditions and their activity is sensitive to pH (Axelsson et al., 2000; Moulin et al., 2011).

How *Ulva* takes advantage of ocean acidification conditions may be due to changes in physiological processes, such as down-regulation of CCM activity and reallocation of energy, increased nitrogen assimilation, and/or slight increases in photosynthetic activity (Olischläger et al., 2013; Xu and Gao, 2012). However, Rautenberger et al. (2015) did not observe downregulation of the CCM in *U. rigida*, but instead suggested that benefits arise from acidification may arise from increases in internal bicarbonate. Algal species that lack CCMs are predicted to have increased photosynthetic activity under high pCO₂ conditions, as these species are carbon limited (Kübler et al., 1999). *Ulva*, however, has a highly efficient CCM and does not appear carbon limited (Axelsson et al., 1999, 1995). Photosynthetic rates of *Ulva*, as determined by oxygen production, increased by a factor of 1.2 under 700 µatm pCO₂, but this increase was not statistically significant (Olischläger et al., 2013). Conversely, Rautenberger et al. (2015) found that light levels drive photosynthetic activity and growth in *U. rigida*, not high pCO₂, and light and nutrients are typically cited as the most important factors influencing growth in *Ulva spp.* (Aldridge and Trimmer, 2009; Coutinho and Zingmark, 1993).

Our prediction that the growth rate of *Fucus* would decrease under high pCO₂ (e.g. Gutow et al. 2014) was not validated. Similar to *Ulva*, *Fucus* utilizes a CCM to help convert and supply sites of photosynthesis with usable carbon, but *Fucus* species have an internal store of usable carbon and thus are not as sensitive to changes in carbon chemistry (Kawamitsu and Boyer, 1999). It is possible that long-lived species such as *Fucus* may respond more slowly (e.g. months vs. weeks) to an altered environment.

Pedersen and Borum (1996) found that fast-growing species, like *Ulva*, are nutrient limited and increase growth rates when nutrient concentrations are high, unlike *Fucus*, which is adapted to low-nutrient environments. Our *Ulva* growth rates were also significantly enhanced by the addition of nutrients, similar to prior experimental studies (Steffensen, 1976; Teichberg et al., 2010) and field observations (Díaz et al., 2005). As a fast-growing opportunistic species, *Ulva* can absorb excess nutrients in the water column and form blooms that are detrimental to community coinhabitants (Teichberg et al., 2010; Valiela et al., 1997) and can lead to eelgrass declines (Hauxwell et al., 2001; McGlathery, 2001). By contrast, we found no effect of nutrient treatment on the growth rate of *Fucus*. *Fucus* can take up excess nutrients, but at a marginal rate (~3%) that may not enhance growth rates (Savage and Elmgren, 2004).

Of several studies on the combined impacts of ocean acidification and nutrients (Campbell and Fourqurean, 2014; Falkenberg et al., 2013; Russell et al., 2009), only Russell et al. (2009) found a synergism between these two factors, with turf algal percent cover multiplied when both of these factors are increased. Campbell and

Fourquean (2014) and Falkenberg et al. (2013) found that at least one of the factors increased growth rates, but with no significant interaction. Similarly, our results do not point to a synergism, but the mean growth rates for each treatment show an additive affect of ocean acidification and nutrients for *Ulva* (Fig. 1A and 1C).

4.2 Algal Tissue Content

Overall, we found that increased pCO₂ did not affect the C:N ratio of either *Ulva* spp. or *Fucus vesiculosus*. Although adding carbon is counterintuitive to decreasing the C:N ratio, Gordillo et al. (2001) found that increased pCO₂ resulted in increased uptake of nitrate in *U. rigida*, thus lowering the tissue C:N ratio. Our results indicate that nutrient level was the primary driver of tissue C:N ratio in both *Ulva* and *Fucus*. High nutrient treatments resulted in decreased C:N ratios, which increases tissue quality. Falkenberg et al. (2013) found that increasing each factor resulted in decreased C:N ratios for turf algae, but only nutrients effectively lowered the C:N in the kelp, *Ecklonia radiata*. We did not find a significant interaction between acidification and nutrients loading on tissue C:N, similar to Falkenberg et al. (2013). Our results contradict those from Gutow et al. (2014), where high pCO₂ resulted in a decreased C:N ratio for *F. vesiculosus*.

Our findings indicate that both acidification and nutrients have the ability to alter tissue composition with respect to concentrations of carbon and nitrogen. The addition of pCO₂ significantly increased the percent carbon found in *Ulva* and *Fucus* tissues, and increased nutrient concentrations raised tissue nitrogen in both. While we observed higher concentrations of N within high pCO₂ treated individuals, the

difference was not significant and our results do not match Gordillo et al. (2001) and Xu and Gao (2012), who found that high pCO₂ facilitates nitrate uptake in *U. rigida* and *U. prolifera*, respectively.

We found evidence of resource competition in C:N ratios, which were significantly higher in *Fucus* tissues when grown with *Ulva*. This is likely due to the inability of *Fucus* to acquire nutrients in the presence of fast-growing, nutrient limited species like *Ulva* (Duarte, 1995). Intraspecific competition and interactions for space and resources drive algal community composition and function (Olson and Lubchenco, 1990; Stachowicz, 2001), and algal communities may undergo assemblage shifts under climate change (Connell and Russell, 2010). However, we did not find any evidence of competition affecting the growth rates of either species..

4.3 Impacts to Coastal Ecosystems

In coastal systems where anthropogenic nutrient loading is prevalent, algal blooms likely occur. The role of nutrients in facilitating macroalgal blooms is well established (Lapointe, 1997; Lapointe and Bedford, 2010). Both nutrients and CO₂ are both critical resources for primary producers, but few studies have assessed the role of pCO₂ in contributing to blooms. As our study shows, the interaction of nutrients and pCO₂ results in an additive growth effect on *Ulva*. As such, ocean acidification may ultimately end up contributing to the timing, frequency, and duration of macroalgal blooms. Ocean acidification has been shown to not only enhance growth rates in some algal species but also may enhance the uptake of nutrients (Gordillo et al., 2001), increasing growth rates indirectly. In addition, acidification can alleviate the cold-

driven temperature stress in a species of red algae, resulting in higher growth rates in colder waters (Olischläger and Wiencke, 2013). These factors may ultimately contribute to macroalgal bloom events in coastal ecosystems.

Seaweed takeover of critical ecosystems such as coral reefs (Diaz-Pulido et al., 2011) and the turf algal dominance in kelp forest ecosystems (Connell and Russell, 2010) highlight the prediction that many macroalgal species are expected to flourish under future climate scenarios. Our results support this, with major increases in the growth rates of an opportunistic species when pCO₂ and nutrients are high. Field studies of algal diversity at naturally low pH vent sites indicate shifting assemblages as water become more acidic (Porzio et al., 2011). Based on our results, we expect a similar shift where small, fast-growing species are able to take advantage of changing conditions, outcompeting larger, slow-growing species (Connell and Russell, 2010; Falkenberg et al., 2013). In this system, long-lived species like *Fucus* may not be directly impacted by environmental change, but they may be indirectly affected by the overgrowth of epiphytes (Berger et al., 2004) or competing species. *Fucus* dominates space in the intertidal and shallow subtidal and helps create complex, 3-D structure that is critically important as a source of refuge and habitat for other organisms in the community. Ocean acidification and nutrient loading is likely to have an indirect effect on long-lived, complex species like *Fucus*, where the growth and spread of algal epiphytes and fast-growing turf species may outcompete, overgrow, and shade out other species (Falkenberg et al., 2013; McSkimming et al., 2015). Our results do not describe a direct, negative effect of acidification or nutrients on *Fucus*, but these indirect effects may ultimately result in *Fucus* decline, altering algal community

assemblages and ecosystem services. However, to fully understand how macroalgal communities will respond to change more work needs to be done investigating how larger, more diverse communities respond. It is necessary to quantify algal community response over long periods of time, as the seasonality of algal species in temperate coastal ecosystems will likely play a role in determining community dynamics.

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Table 1. Carbonate chemistry and nutrient parameters from midday seawater samples averaged across all three experiments. Dissolved inorganic carbon (DIC), total alkalinity (TA), temperature, and salinity were measured directly. Seacarb (R software; Lavigne et al. 2011) was used to calculate $\text{pH}_{(\text{total})}$, pCO_2 , CO_2 , HCO_3^- , and CO_3^{2-} . Dissolved inorganic nitrogen (DIN) was measured from water samples that were taken weekly over the course of experimentation. All values represent means $\pm$ standard error.

<i>PARAMETER</i>	Ambient pCO_2		High pCO_2	
	Ambient Nutrients	High Nutrients	Ambient Nutrients	High Nutrients
$\text{pH}_{(\text{total})}$	8.00 ± 0.04	8.00 ± 0.03	7.58 ± 0.03	7.59 ± 0.03
pCO_2 (μatm)	413.33 ± 25	412.40 ± 31	1219.65 ± 86	1226.52 ± 51
CO_2 ($\mu\text{mol/kgSW}$)	14.46 ± 0.87	14.44 ± 1.1	42.53 ± 3	42.77 ± 2
HCO_3^- ($\mu\text{mol/kgSW}$)	1739.32 ± 86	1728.73 ± 71	1955.54 ± 86	1988.83 ± 71
CO_3^{2-} ($\mu\text{mol/kgSW}$)	134.31 ± 17	132.37 ± 12	57.92 ± 6	59.43 ± 6
DIC ($\mu\text{mol/kgSW}$)	1888.10 ± 101	1875.55 ± 80	2055.98 ± 91	2091.03 ± 76
TA ($\mu\text{mol/kgSW}$)	2074.70 ± 122	2059.62 ± 91	2099.83 ± 96	2136.33 ± 83
Temp. ($^{\circ}\text{C}$)	17.98 ± 0.01	17.95 ± 0.11	18.10 ± 0.08	18.08 ± 0.11
Salinity (ppt)	30.69 ± 0.05	30.67 ± 0.08	30.88 ± 0.15	30.85 ± 0.17
DIN (μM)	7.52 ± 0.87	187.24 ± 2.11	6.73 ± 0.65	207.63 ± 6.36

Table 2. Summary of three-way ANOVAS showing the effects of ocean acidification (pCO₂), nutrient addition (Nutrients), competition (Community, monoculture vs. biculture) and their interactions on the growth rates (RGR %g day⁻¹) of *Ulva* and *Fucus*. P-values that are significant (P ≤ 0.05) are in bold.

	<i>Ulva</i>				<i>Fucus</i>			
Model	df	SS	F	P	df	SS	F	P
Source								
RGR (%g day ⁻¹)								
<i>pCO₂</i>	1	50.20	17.42	0.0002	1	0.060	0.35	0.56
<i>Nutrients</i>	1	86.56	30.03	<0.0001	1	0.171	1.01	0.32
<i>Community</i>	1	0.082	0.03	0.87	1	0.140	0.82	0.37
<i>pCO₂ X</i>	1	0.367	0.13	0.72	1	0.153	0.91	0.35
<i>Nutrients</i>								
<i>pCO₂ X</i>	1	0.002	1.0x10 ⁻³	0.98	1	0.038	0.22	0.64
<i>Community</i>								
<i>Nutrients X</i>	1	1.082	0.38	0.54	1	4.3x10 ⁻⁵	3.0x10 ⁻⁴	0.99
<i>Community</i>								
<i>pCO₂ X</i>	1	1.138	0.40	0.53	1	0.011	0.06	0.80
<i>Nutrients X</i>								
<i>Community</i>								
<i>Error</i>	36	103.75			48	8.14		

Table 3. Summary of three-way ANOVAS showing the effects of ocean acidification (pCO₂), nutrient addition (Nutrients), competition (Community -- monoculture vs. biculture) and their interactions on the C:N (a), carbon concentration (µmol/mg) (b), and nitrogen concentration (µmol/mg) (c) within tissues of both *Ulva* and *Fucus*. P-values that are significant (P ≤ 0.05) are in bold.

	<i>Ulva</i>				<i>Fucus</i>			
Model Source	df	SS	F	P	df	SS	F	P
(a) C:N								
<i>pCO</i> ₂	1	17.62	1.87	0.18	1	13.94	1.84	0.183
<i>Nutrients</i>	1	277.0	29.38	<0.0001	1	218.3	28.77	<0.0001
<i>Community</i>	1	15.66	1.66	0.21	1	82.73	10.90	0.0021
<i>pCO</i> ₂ X <i>Nutrients</i>	1	12.62	1.34	0.25	1	5.420	0.714	0.403
<i>pCO</i> ₂ X <i>Community</i>	1	4.83	0.52	0.48	1	9.870	1.300	0.261
<i>Nutrients</i> X <i>Community</i>	1	27.40	2.91	0.10	1	18.06	2.380	0.131
<i>pCO</i> ₂ X <i>Nutrients</i> X <i>Community</i>	1	2.58	0.27	0.60	1	4.310	0.568	0.456
<i>Error</i>	36	339.4			39	295.9		
(b) C concentration (µmol/mg)								
<i>pCO</i> ₂	1	101.2	4.38	0.04	1	418.9	6.41	0.02
<i>Nutrients</i>	1	18.60	0.80	0.38	1	27.20	0.42	0.52
<i>Community</i>	1	6.71	0.30	0.59	1	6.90	0.11	0.75
<i>pCO</i> ₂ X <i>Nutrients</i>	1	1.17	0.05	0.82	1	0.04	6x10 ⁻⁴	0.98
<i>pCO</i> ₂ X <i>Community</i>	1	40.54	1.75	0.19	1	229.0	3.50	0.07
<i>Nutrients</i> X <i>Community</i>	1	120.4	5.21	0.03	1	18.32	0.28	0.60
<i>pCO</i> ₂ X <i>Nutrients</i> X <i>Community</i>	1	95.30	4.12	0.05	1	8.55	0.13	0.72
<i>Error</i>	36	832.5			39	2549.4		
(c) N concentration (µmol/mg)								
<i>pCO</i> ₂	1	0.00	0.03	0.88	1	0.57	3.80	0.06
<i>Nutrients</i>	1	2.25	12.62	0.001	1	1.11	7.44	0.01
<i>Community</i>	1	0.03	0.15	0.70	1	0.10	0.65	0.43
<i>pCO</i> ₂ X <i>Nutrients</i>	1	0.20	1.11	0.30	1	0.03	0.20	0.67

<i>pCO₂ X</i>	1	0.42	2.38	0.13	1	0.30	1.98	0.17
<i>Community</i>								
<i>Nutrients X</i>	1	0.01	0.08	0.78	1	5.4x10 ⁻⁵	4x10 ⁻⁴	0.99
<i>Community</i>								
<i>pCO₂ X Nutrients</i>	1	0.13	0.75	0.39	1	0.08	0.54	0.47
<i>X Community</i>								
<i>Error</i>	36	6.41			1			
					39	5.83		

Figure 1. RGR (% day⁻¹) for *Ulva* monoculture (A), *Ulva* biculture (B), *Fucus* monoculture (C), and *Fucus* biculture (D). Values plotted are means $\pm$ 1 SE (N = 7). We observed a significant, positive effect of nutrient addition and increased pCO₂ on the RGR of *Ulva* (* represents $p < 0.001$, ** represents $p < 0.0001$).

Figure 2. Carbon to nitrogen ratios (C:N) within tissues of *Ulva* grown in monoculture (A), *Ulva* grown in biculture (B), *Fucus* grown in monoculture (C), and *Fucus* grown in biculture (D). Values plotted are means $\pm$ 1 SE (N = 7). We observed a significant, negative effect of nutrient addition on the C:N of both *Ulva* and *Fucus* (** represents $p < 0.0001$). In addition, we observed a significant difference between the C:N of *Fucus* tissue by culture treatment where C:N was lower in monoculture ($C < D$, $p = 0.002$).

Figure 3. Carbon and nitrogen concentrations within the tissues of *Ulva* (A) and *Fucus* (B). Mean values $\pm$ 1 SE are plotted for species grown in monoculture (circles) and biculture (triangles), broken down by ambient $p\text{CO}_2$ (black), high $p\text{CO}_2$ (gray), ambient nutrients (patterned), and high nutrients (filled) (N = 7).

CHAPTER 2

OCEAN ACIDIFICATION BUT NOT NUTRIENT ENRICHMENT REDUCES GRAZING AND ALTERS DIET PREFERENCE IN A COMMON MARINE SNAIL

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SUMMARY:

1. Ocean acidification (increased pCO₂) and eutrophication (nutrient loading) have direct, positive effects on the growth rates of many marine macroalgae, potentially causing shifts in ecosystem structure and function. This enhanced growth of macroalgae, however, may be controlled by the presence of grazers, where indirect effects of acidification and eutrophication result in increased consumption.
2. We tested whether a common marine herbivorous snail, *Littorina littorea*, could increase consumption rates of macroalgae under ocean acidification and eutrophication conditions. Choice experiments were run giving snails both *Ulva* (an opportunistic, ephemeral green alga) and *Fucus* (a slow-growing, perennial brown alga). We measured total consumption rates, diet preference (live algal tissue and artificial, reconstituted algae), and respiration rates in snails.
3. High pCO₂ (acidification) resulted in a 50% reduction in the total consumption rate. Under high pCO₂ conditions, snails switched from a mixed diet to feeding almost exclusively on *Ulva*. Despite increasing the quality of both *Ulva* and *Fucus*, high nutrients did not affect the consumption rates or feeding preference of snails. Snails consumed similar amounts of artificial *Ulva* and *Fucus* across environmental treatments, indicating that physical characteristics of algal tissues were driving diet shifts. Snails also showed signs of stress as respiration rates were significantly reduced under high pCO₂ conditions.
4. *Synthesis*. This study shows an inability of *L. littorea* to increase consumption to match expected growth rates of macroalgae. In this system, decreased consumption, coupled with increased growth of macroalgae, will ultimately enhance algal growth and spread. Here, the direct, physiological effects on snails had a greater effect than the indirect effects of increased food quality. Grazer diversity will be important in determining the structure and function of coastal communities in the future, and although *L. littorea* may not be able to increase consumption, other grazers may be able to fill the void.

KEY WORDS: acidification, nutrients, grazing, consumption, respiration

INTRODUCTION:

Ecological communities are shaped and structured by an interaction of top-down and bottom-up forces (Power 1992; Hunter & Price 1992; Menge 2000; Hereu, Zabala & Sala 2008). Anthropogenic activities, such as human-induced climate change, can alter or disrupt the impact of both of these forces in communities (Jochum *et al.* 2012). Warming and nutrient loading has been linked to decrease size and body mass in predators allowing populations of lower trophic levels to flourish (Beukema 1991; Gardner *et al.* 2011; Sheridan & Bickford 2011; Jochum *et al.* 2012). Climate change can also lead to phenological changes within organisms, resulting in a trophic mismatch and a disruption of top-down forces (Edwards & Richardson 2004; Winder & Schindler 2004). However, factors of climate change may have divergent effects on species and ecosystems. For example, ocean warming may alter bottom-up forces by causing declines in growth and biomass of primary producers (Doney 2006; Wernberg *et al.* 2011), but ocean acidification and eutrophication enhance bottom-up forces by promoting increased growth and spread of many macroalgal species (Diaz-Pulido *et al.* 2011; Olischläger & Wiencke 2013; Falkenberg, Connell & Russell 2014). As such, climate change may result in major shifts in ecosystem function and trophic cascades (Hughes *et al.* 2003; Casini *et al.* 2009), but there is still uncertainty as to how these processes ultimately play out.

Ocean acidification, a byproduct of increased atmospheric carbon dioxide (CO₂), and nutrient loading have a direct effect on primary producers resulting in increased growth rates and productivity of fleshy macroalgae (Kübler, Johnston & Raven 1999; Zou 2005; Aldridge & Trimmer 2009; Russell *et al.* 2009) by increasing

the concentrations of CO₂ and nutrients in the water column. Macroalgal response to ocean acidification is highly species-specific, depending on the activity of a carbon concentrating mechanism (CCM) (Raven *et al.* 2005; Hepburn *et al.* 2011) and developmental stage (Olischläger *et al.* 2012), but many species experience a positive responses to acidification (increased pCO₂) (Kroeker *et al.* 2010; Olischläger & Wiencke 2013). Similarly, nutrient loading can alter macroalgal assemblages, as many open-coast species are adapted to low-nutrient conditions and may be adversely indirectly affected by the growth and spread of opportunistic species (Berger *et al.* 2004). Ocean acidification and nutrient loading are likely going to aide phase shifts by enhancing the growth and spread of macroalgae in critical ecosystems, resulting in significant changes to ecosystem structure and function (Hughes *et al.* 2003; Diaz-Pulido *et al.* 2011; Falkenberg *et al.* 2014). Under these climate conditions, macroalgae can outcompete and overgrow corals in reef ecosystems (Anthony *et al.* 2011; Diaz-Pulido *et al.* 2011), epiphytes threaten to overgrow and shade out seagrasses (Duarte 1995; Wear *et al.* 1999), and fast-growing turf algae may outcompete other, perennial macroalgae in kelp forest ecosystems (Connell & Russell 2010). Excess nutrients can also induce harmful macroalgal blooms, which can have detrimental impacts at the community level (Valiela *et al.* 1997; Thomsen, McGlathery & Tyler 2006).

The direct effects of ocean acidification and nutrient loading on macroalgal growth may be counteracted by an enhancement of top-down forces, such as grazing, resulting from the indirect effects of ocean acidification and nutrient loading on grazers and herbivores (Connell *et al.* 2013; Falkenberg *et al.* 2014). Grazers have the

ability to control growth and expansion of macroalgae and epiphytes (Hughes *et al.* 2003). Grazer presence and grazer diversity act to quell increased algal growth under future climate conditions (Falkenberg *et al.* 2014; Baggini *et al.* 2015). Where nutrient loading stimulates algal growth, grazing invertebrates keep algal growth in check (Nielsen 2003; Whalen, Duffy & Grace 2013; McSkimming *et al.* 2015). Increased grazing can be an indirect effect of acidification and/or nutrient loading via altered tissue quality of macroalgae (Hemmi & Jormalainen 2002; Russell & Connell 2007; Falkenberg *et al.* 2014; Duarte *et al.* 2015). Acidification and nutrient loading can alter algal quality by decreasing carbon to nitrogen ratios (C:N) within algal tissues (Gordillo, Niell & Figueroa 2001; Hemmi & Jormalainen 2002; Falkenberg, Russell & Connell 2013) resulting in higher food quality and greater levels of individual consumption (Falkenberg *et al.* 2014). Due to the species-specific nature of macroalgal response to climate change, tissue quality and consumption are not always increased (Borell, Steinke & Fine 2013; Gutow *et al.* 2014; Poore *et al.* 2016), and indirect effects of climate change may enhance grazing, but only if they are not outweighed by the negative, direct effects of climate change on herbivores and grazers (Melatunan *et al.* 2011; Stumpp *et al.* 2011; Albright *et al.* 2012; Russell *et al.* 2013).

As a suite of climate-related factors threatens coastal ecosystems, it is important to understand the interactions of these factors. While many studies have investigated response to acidification or nutrient loading, few studies have looked at their interaction on macroalgal growth (but see Russell *et al.* 2009; Falkenberg *et al.* 2014; Campbell & Fourqurean 2014, Ober & Thornber *in review*), and fewer studies have investigated this interaction on grazing (but see Falkenberg *et al.* 2014). It is

critical to quantify how acidification and nutrient loading, two factors that can enhance macroalgal growth, impact grazing organisms and their ability to impose top-down forces; these interactions will help in our understanding of community and ecosystem response to climate change.

Intertidal and shallow subtidal communities in the northwest Atlantic are supported by a diverse assemblage of macroalgae which provide both food and habitat (Watt & Scrosati 2013). For this study, we focused on the green alga *Ulva spp.* and the brown algal *Fucus vesiculosus* (referred to hereby as *Ulva* and *Fucus*), two common macroalgae with different life history and ecological traits. *Ulva* is fast-growing, opportunistic, and can readily colonize, whereas *Fucus* is long-lived and slow growing. These species have divergent responses to ocean acidification, nutrients, and their interaction, with increased growth rates of *Ulva* and decreased or null growth rates in *Fucus* (Olischläger *et al.* 2013; Gutow *et al.* 2014, Ober & Thornber *in review*). Under conditions of nutrient loading, tissue quality of both *Ulva* and *Fucus* is enhanced, as a result of decreased tissue C:N (Ober & Thornber *in review*).

Under ambient environmental conditions, *Fucus*, despite being competitively disadvantaged, dominates space as *Ulva* is the preferred food source for many grazers, including the abundant gastropod *Littorina littorea* (Lubchenco 1978, 1983; Watson & Norton 1985; Bracken, Dolecal & Long 2014). *Littorina littorea* can manipulate algal communities and competitive interactions by preferential grazing and clearing space within the intertidal and shallow subtidal (Lubchenco 1983). However, *Littorina* has exhibited reduced growth and reduced respiration when exposed to ocean acidification conditions (Bibby *et al.* 2007; Melatunan *et al.* 2011; Landes & Zimmer 2012), which

may ultimately impact their grazing ability. Therefore, while the quality of algal food may increase under future climate conditions, the direct physiological effects on herbivores may disrupt their ability compensate for excess algal growth.

Here, we investigated the interactive effects of ocean acidification and nutrients on the consumption rates, feeding preference and physiology of a common snail (*Littorina littorea*) when feeding on algae (*Ulva* and *Fucus*). We tested the hypotheses that under novel environmental conditions: (1) grazing organisms can control algal growth under these climate scenarios by consuming at higher rates (Falkenberg *et al.* 2014) and (2) altered tissue quality under high nutrients will drive diet preferences from a diet of mostly *Ulva* to a mixed diet, resulting in a partial release from grazing pressure, which may have implications in terms of growth and abundance in this species. We also tested the direct physiological effects of ocean acidification and nutrient loading on grazers by measuring respiration rates. Identifying these aspects of consumption by an abundant grazer will help determine the influence of top-down forces in structuring coastal communities in the future.

MATERIALS & METHODS:

SNAIL CONSUMPTION (LIVE ALGAL THALLI)

To test the impacts of ocean acidification and nutrients on snail consumption rates and feeding preferences, we conducted a series of consumption experiments at a flow-through seawater facility at the US Environmental Protection Agency Atlantic Ecology Division in Narragansett, RI. *Ulva*, *Fucus*, and *L. littorea* were collected from the shallow subtidal zone at the University of Rhode Island's Narragansett Bay

Campus beach (41°29'26"N, -71°25'11"W) in August 2015. Non-reproductive tips of *Fucus* (~3-5cm in length) were cut from adult thalli (Gutow *et al.* 2014). *Fucus* tips and *Ulva* thalli were cleaned of any epiphytes, and experimental organisms were transferred into separate 20L glass aquaria with flow-through seawater and aeration. Individuals were acclimated to lab conditions (ambient pCO₂ and nutrient levels, artificial lighting) for five days prior to the start of each experiment.

After five days of acclimation, algae and snails were exposed to one of four environmental treatments, based on the factorial combination of two pCO₂ levels (ambient ~ 400 µatm pCO₂, pH 8.10, and representative concentration pathway 8.5 (RCP8.5) projections for the year 2100 ~ 1200 µatm pCO₂, pH 7.65; Moss *et al.*, 2011) and two levels of nutrient loading (ambient ~ 5 µM dissolved inorganic nitrogen, DIN, and high ~ 200 µM DIN). DIN in Narragansett Bay runs along a north south gradient, where water has an annual average of 70 µM DIN in the north and annual average of 4-10 µM DIN in the south, where certain parts of the bay can exceed 180 µM DIN on occasion (Krumholz, 2012). Each of the four unique environmental treatments had a 40L headwater tank which was fed directly by filtered, tempered sea water (set at a constant 18°C). In the headwater tanks, pure CO₂ gas or ambient air (depending on the treatment) was bubbled in via helium spargers at a constant rate set by an Aalborg Mass Flow Controller GFC (Aalborg Instruments and Controllers, INC). Mixing of gas and water was aided via a Hydor Circulation Fan. Treated water was pumped from the headwater tank via an Eheim 1200 submersible pump to a manifold, delivering treated water to seven experimental aquaria for each treatment. Aquaria (N=7 per environmental treatment) received water at a rate of 130

± 5 mL/min, using a flow-through design to mimic the natural variability of coastal pH that, on a daily basis, fluctuated by up to 0.4 units.

Nutrient levels were altered by use of slow-release agar blocks placed in each experimental aquarium (Teichberg *et al.* 2008). Nitrate (in the form of KNO_3 at 2M concentration) and ammonium (in the form of NH_4Cl at 2M concentration) were mixed with 3% agar, seawater, and phosphate (in the form of KH_2PO_4 at 1M concentration to meet the Redfield ratio) to create the desired nutrient concentrations representing eutrophication events ($\sim 200 \mu\text{M}$ DIN for the 20L aquaria) (Teichberg *et al.* 2008). For ambient nutrient aquaria, blocks containing just agar and seawater were used to simulate the physical addition of the block.

Organisms were exposed to environmental treatments for one week prior to the start of consumption experiments. Based on evidence from prior experiments (Ober & Thornber, *in review*), this amount of time was sufficient for algal tissues to reflect changes in tissue C and N concentrations. *Littorina littorea* were starved during this acclimation period.

Post-acclimation to environmental treatments, algae were removed, blotted dry, and spun 20x in a salad spinner to remove excess water (Thornber *et al.* 2008). Initial algal wet mass of each individual was recorded. The average ratio of starting biomass was 2:1, *Fucus* to *Ulva*, with initial wet mass measuring 1500 mg and 750 mg, respectively.

Of the seven replicate tanks for each treatment, four were designated as consumer tanks (three snails in each, with a piece of *Ulva* and *Fucus*), with the remaining three designated as non-consumer controls (tanks with *Ulva* and *Fucus*, but

no snails). Consumption experiments were run for one week and tanks were supplemented with artificial light (Sylvania Full Spectrum) at $128.7 \pm 2.7 \mu\text{mol photons m}^{-2}\text{sec}^{-2}$ with a light/dark rhythm of 14:10h (L:D). Tanks were scrubbed and cleaned every two days and any epiphytes growing on the algae were removed. After one week, algae were removed, blotted dry, spun, and weighed for final mass.

Two trials were run for this experiment, with new individuals collected and acclimated for the second trial. In this trial, consumer tanks and non-consumer control tanks were switched, resulting in seven replicates per consumer treatment per environmental treatment. There was no significant difference in consumption between trials, therefore, we pooled our data. Total consumption, based on the total algal biomass consumed by snails (both *Ulva* and *Fucus*), was calculated as $(S_i \times C_f \times C_i^{-1}) - S_f$, where S_i and S_f were the initial and final mass of the algae exposed to consumption and C_i and C_f were the initial and final mass of the non-consumer control algae (Stachowicz & Hay 1999; Jones & Thornber 2010). These calculations were repeated for *Ulva* and *Fucus* consumption separately.

SNAIL CONSUMPTION (ARTIFICIAL FOOD)

To test the mechanisms behind potential shifts in snail feeding preference, we ran a series of consumption experiments with artificial (or reconstituted) food. By using artificial food, we were able to remove any physical characteristics of the algal tissue but retain the chemical composition of tissue (i.e. nutritional quality and secondary compounds). In October 2015, *Ulva*, *Fucus*, and *L. littorea* were collected from the shallow subtidal zone at the University of Rhode Island's Narragansett Bay Campus beach. Using the same experimental design and set-up as the live thalli

consumption experiments, algae and snails were acclimated to lab conditions for five days. Algae and snails were then acclimated to experimental conditions for one week. Post-treatment acclimation, *Ulva* and *Fucus* were removed and immediately frozen at -80C for 24 hours. Algae were freeze-dried for 48 hours before being ground separately using a Wiley Mill (mesh size 40). Powdered algae of both species was mixed with agar and seawater to reconstitute (Thornber, Stachowicz & Gaines 2006). The reconstituted food was spread over window screen onto Whatman paper (Guidone, Thornber & Vincent 2012). We cut 40mm X 15mm strips containing ~225 squares of artificial food. Strips were photographed and the initial number of artificial food squares were counted using ImageJ. Strips of both artificial *Ulva* and *Fucus* were then placed in experimental aquaria along with three snails. The experiment ran for three days and was stopped prior to the snails consuming 100% of the artificial food available. Replicates were removed from the data set if artificial food was fully consumed. Strips were removed from aquaria and photographed. The final number of artificial food squares were counted via ImageJ (Guidone *et al.* 2012). As in live thalli consumption experiments, artificial food consumption experiments were run with both consumer tanks and non-consumer control tanks, in order to quantify any potential loss of artificial food to treatment conditions. This experiment was run twice in order to obtain seven replicates per consumer treatment per environmental treatment. We observed no differences in consumption between trials and therefore pooled our data.

There was no loss of artificial foods squares in non-consumer control tanks; therefore, we determined artificial food consumption for each species by calculating percent change between initial squares count and final square count.

SNAIL RESPIRATION

To determine the individual physiological effects of environmental treatments on snails, we measured respiration rates in *L. littorea* from field-collected individuals in November 2015. Using the same experimental design as above, snails were lab acclimated for five days at 18°C before being exposed to environmental treatments. Snails were kept in one of the four environmental treatments for two weeks and were fed *Ulva* and *Fucus* throughout. To measure respiration, small oxygen optodes (PreSens Fibox 3) were placed in 50mL glass vials. Vials were filled with treated water from one of the four environmental treatments. One snail was then placed in the vial of its respective treatment and the vial was sealed. An instantaneous reading of initial oxygen percent within the vial was measured (PreSens Software). Vials were then placed in a water bath kept at a constant 18°C. After 30 minutes, a final instantaneous measurement of oxygen percent within the vial was measured. Overall, measurements of respiration were taken for five snails under each environmental treatment, with an equal number of “blank” vials run (vials without snails) in order to correct measurements of oxygen consumption. All snails were weighed. Respiration rates were calculated by determining the initial and final amounts of oxygen within the vials ($\mu\text{mol L}^{-1}$) and were standardized by snail weight and time for a final measurement of oxygen consumed ($\mu\text{mol g}^{-1} \text{ L}^{-1} \text{ hr}^{-1}$).

CARBONATE CHEMISTRY AND NUTRIENT CONCENTRATION ANALYSIS

Measuring and calculating carbonate chemistry methods were taken from Ober et al. (in review). Seawater samples were bottled and preserved, using mercuric chloride (HgCl_2), over the course of each experiment to measure dissolved inorganic carbon (DIC) and total alkalinity (TA) following the Best Practices Guide (Dickson et al., 2007). Two samples from each environmental treatment were collected on two consecutive days during each experiment. Temperature and salinity were measured directly. DIC was measured using a Shimadzu TOC-V total organic carbon analyzer. TA was determined for each sample using a Metrohm 877 Titrino Plus titrator. In determining DIC and TA, samples were standardized against Dickson seawater standards CRM Batch 151. All parameters of carbonate chemistry were calculated using the seacarb package (R software; Lavigne et al. 2011) (Table 1). Natural variation of seawater pH was observed by using WTW Profiline 3110 pH meter with SenTix 21 glass electrodes recording high frequency pH measurements in each of the four treatments (data not presented).

Nutrient levels for each treatment were analyzed at the midway through each experiment by removing and filtering 60mL water samples, which were frozen at -20°C for preservation. Samples were later analyzed for DIN concentration by the URI Marine Science Research Facility, a RI NSF EPSCoR Core Facility.

STATISTICAL METHODS

Mean total consumption rate ($\text{mg snail}^{-1} \text{ day}^{-1}$) was compared across environmental treatments using two-way fixed factor analysis of variance (ANOVA) with pCO_2 and nutrient levels as categorical, fixed factors. To determine diet preference, mean consumption of *Ulva* and mean consumption of *Fucus* were

compared across environmental treatments using a factorial MANOVA to account for non-independence of algal consumption (Roa 1992). Mean total consumption of artificial *Ulva* and *Fucus* (% of initial square count per species consumed) were arcsine transformed and analyzed using a factorial MANOVA. Protected ANOVAs were run post-hoc on MANOVA results to determine which algal species contributed to significant effects of environment. Mean respiration rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ L}^{-1} \text{ hr}^{-1}$) were compared using a two-way fixed factor ANOVA. All statistics were performed using JMP v. 11 (www.jmp.com) and R software (R Development Core Team, 2016, R-project.org).

RESULTS:

SEAWATER PARAMETERS

Our manipulation of both pCO₂ and nutrients proved effective as we were able to obtain desired environmental levels. Average values calculated for pCO₂ in our two acidification treatments averaged $1225.1 \pm 45 \mu\text{atm}$ and $1199.2 \pm 40 \mu\text{atm}$; in our two ambient pCO₂ treatments, calculated pCO₂ levels averaged $476.2 \pm 24 \mu\text{atm}$ and $461.7 \pm 23 \mu\text{atm}$ (Table 1). Calculated pH_(total) averaged 7.58 ± 0.02 and 7.59 ± 0.02 in acidification treatments; while calculated pH_(total) from our ambient treatments averaged 7.94 ± 0.02 and 7.96 ± 0.02 (Table 1). DIC measured $2060.4 \pm 20 \mu\text{mol kg}^{-1}$ and $2075.8 \pm 16 \mu\text{mol kg}^{-1}$ under acidification treatments; where under ambient treatments were measured at $1926.7 \pm 25 \mu\text{mol kg}^{-1}$ and $1912.5 \pm 24 \mu\text{mol kg}^{-1}$ (Table 1). Under acidification, TA was measured at $2105.8 \pm 21 \mu\text{mol kg}^{-1}$ and $2125.9 \pm 18 \mu\text{mol kg}^{-1}$; under ambient treatments TA was measured at $2095.3 \pm 27 \mu\text{mol kg}^{-1}$ and

$2085.2 \pm 24 \mu\text{mol kg}^{-1}$ (Table 1). Temperature ranged from 18.1°C to 18.5°C and salinity ranged from 31.08 ppt to 31.2 ppt (Table 1).

By adding nutrients to our aquaria we effectively increased concentrations of DIN above ambient levels. DIN in high nutrient treatments averaged $202.4 \pm 6.9 \mu\text{M}$ and $196.6 \pm 11 \mu\text{M}$, respectively; whereas ambient nutrient treatments averaged $6.92 \pm 0.78 \mu\text{M}$ and $6.96 \pm 0.5 \mu\text{M}$ DIN, respectively (Table 1).

CONSUMPTION (LIVE ALGAL THALLI)

Total macroalgal consumption rates by *L. littorea* were reduced by more than half under high pCO_2 compared to consumption rates under ambient pCO_2 (53.5 and $113.3 \text{ mg snail}^{-1} \text{ day}^{-1}$, respectively) (Fig. 1; Table 2). Total consumption rates were not affected by nutrient level ($p = 0.62$, Table 2, $86.5 \text{ mg snail}^{-1} \text{ day}^{-1}$ for high nutrients and $80.3 \text{ mg snail}^{-1} \text{ day}^{-1}$ for ambient nutrients), with a non-significant interaction ($p = 0.72$, Table 2).

We found a significant effect of pCO_2 on the diet preference of *L. littorea* ($p < 0.0001$; Table 3). While there was no significant effect of environment on snail consumption rates of *Ulva* (Fig. 2; Table 3), consumption rates of *Fucus* were significantly decreased ($p < 0.0001$; Table 3) and almost nonexistent under conditions of acidification, as rates dropped from $53.6 \text{ mg snail}^{-1} \text{ day}^{-1}$ under ambient pCO_2 to $8.0 \text{ mg snail}^{-1} \text{ day}^{-1}$ under high pCO_2 (Fig. 2; Table 3). Snail consumption rates of *Fucus* were not affected by nutrient level or the interaction of pCO_2 and nutrients (Table 3).

CONSUMPTION (ARTIFICIAL FOOD)

Artificial food diet of *L. littorea* was unaffected by pCO₂ ($F_{1,16} = 1.43$, $p = 0.27$), nutrients ($F_{1,16} = 0.06$, $p = 0.94$), and their interaction ($F_{1,16} = 0.18$, $p = 0.83$). The proportion of artificial *Ulva* consumed ranged between 0.45 and 0.70 while the proportion of artificial *Fucus* consumed ranged between 0.58 and 0.73 (Fig. 3). There was no significant change in algal diet across environmental treatments based on the chemical composition of tissue (i.e. nutritional quality and secondary compounds).

RESPIRATION RATES

Respiration rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ L}^{-1} \text{ hr}^{-1}$) in *L. littorea* were significantly lower under high pCO₂ than ambient pCO₂ (1.10 and 1.74 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ L}^{-1} \text{ hr}^{-1}$, respectively; Fig. 4; Table 4). Snail respiration was not significantly impacted by nutrient concentration ($p = 0.19$) or the interaction of pCO₂ and nutrients ($p = 0.97$; Fig. 4; Table 4).

DISCUSSION:

In marine ecosystems, ocean acidification and nutrient loading are predicted to enhance the growth and spread of ephemeral and turf algae at the expense of foundational species like corals, seagrasses, and perennial macroalgae (i.e. kelps), as well as reduce overall community diversity (Worm & Lotze 2006; Connell & Russell 2010; Diaz-Pulido *et al.* 2011; Hale *et al.* 2011; Koch *et al.* 2013). Increasing evidence points to the indirect effects of climate change on herbivores to exert top-down control on excessive algal growth, promoting community resilience (Russell & Connell 2005; Falkenberg *et al.* 2014; McSkimming *et al.* 2015). In this system, *Ulva* grows three times faster under high pCO₂ and nutrients compared to growth under ambient

conditions, and *Fucus* maintains constant growth or exhibits a reduced growth rate (Ober & Thornber *in review*). We found an inability of *L. littorea* to increase consumption and compensate for increased growth of macroalgae as its total algal consumption rate decreased by 50% under high pCO₂, with no effect of nutrient level. Russell *et al.* (2013) found a similar decrease in consumption of biofilms by *L. littorea* under high CO₂ but simultaneously observed consumption differences based on the exposure time of snails, where a “shock” of low pH resulted in lower consumption than snails that were acclimated to treatments for five months.

In rocky shore communities, nutrient loading can stimulate the growth of bloom-forming *Ulva* (*Enteromorpha*) *spp.*, but grazer consumption does not control or slow blooms, leading to decreases in diversity within these communities (Worm & Lotze 2006). When exposed to high nutrients, both *Ulva* and *Fucus* tissues decreased their C:N ratios (Ober & Thornber *in review*), but *L. littorea* did not exhibit altered consumption rates in response. These findings contrast those of Falkenberg *et al.* (2013 and 2014), where turf algae grown under elevated pCO₂ and nutrients had higher tissue quality (via decreased C:N) and were consumed at an increased rate by gastropods. Similarly, Hemmi & Jormalainen (2002) found that elevated nutrients increased the quality of *Fucus vesiculosus* and promoted enhanced growth rates and consumption by isopods, and Poore *et al.* (2013) observed increased feeding rates by amphipods feeding on *Sargassum spp.* as a result of exposure to high CO₂. By contrast, Duarte *et al.* (2015) found CO₂-induced decreases in organic content (the concentration of carbon within algal tissue) and proteins in the brown alga *Durvillaea antarctica*, but the loss in quality was correlated with compensatory feeding by

amphipods in which more algae was consumed in order to compensate for the decrease in quality. Decreased C:N does not always enhance consumption; Gutow *et al.* (2014) found evidence of decreased C:N in *Fucus vesiculosus* but no change in consumption by isopods. In our study system, decreased C:N does not prompt a change in consumption. Rather, increasing pCO₂ to levels expected in 2100 plays a significant role in determining algal consumption rates by *L. littorea*, where acidification reduces overall consumption.

Diet preference of marine grazers is highly correlated with both tissue quality and tissue toughness (Cruz-Rivera & Hay 2000; Watson & Norton 2009; Molis *et al.* 2015). Marine grazers, including *L. littorea*, exhibit a preference for higher quality food (Watson & Norton 1985) and early successional, ephemeral species (Lubchenco 1978). *Littorina littorea* consume both *Ulva* and *Fucus*, with a preference for *Ulva* over *Fucus*, but also preferentially feed on algal epiphytes and microalgae (Watson & Norton 1985; Russell *et al.* 2013; Bracken *et al.* 2014). Our study shows that, under high pCO₂, *Ulva* is selected over *Fucus* as a result of the physical characteristics of the algal tissue, with *Fucus* having tougher tissue (Watson & Norton 2009). Despite a lack of preference for *Fucus*, our study remains ecologically relevant as *L. littorea* can appear in such high densities along the coastline of the northwest Atlantic (greater than 200 individuals m⁻²) that even minimal consumption plays a large role in structuring the algal communities (Perez *et al.* 2009; Poore *et al.* 2012). While our study showed changes in algal preference (or choice) under acidification conditions, we did not test aspects of algal use or selection. The amount of time *L. littorina* spends using either *Ulva* or *Fucus* could provide insight as to how these two algal species are

used by snails. In addition, we did not manipulate the abundance of the algal species. In terrestrial snails, availability (abundance) is related to the proportion consumed, but preference does not rely on abundance (Speiser & Rowell-Rahier 1991).

Detrimental effects of acidification on the physiology of many marine species have been reported (Ries, Cohen & McCorkle 2009; Stumpp *et al.* 2011; Barton *et al.* 2012). We observed a significant decrease in the respiration rate of *L. littorea* when exposed to acidification, similar to Leung *et al.* (2015) and Melatunan *et al.* (2011).

Littorina littorea physiological stress under high CO₂ could influence diet shifts in one of two ways: (1) stressed snails may actively choose food of higher quality (in this case, *Ulva*), as higher quality diets reduce the quantity (and thus effort) needed (Duarte *et al.* 2015); or (2) the tissue toughness of *Fucus* would force snails to consume a higher proportion of *Ulva* (Watson & Norton 2009; Molis *et al.* 2015). We found no significant difference of the consumption of artificial *Ulva* of *Fucus* across environmental treatments, indicating that tissue toughness was likely the primary driver behind the shift in diet of *L. littorea*, matching prior studies (Watson & Norton 2009; Molis *et al.* 2015). It is possible that the preference of *Ulva* by *L. littorea* has evolved in part due to physical cues from the tissues, which are removed when testing feeding on artificial food.

Few studies have investigated the interactive effects of ocean acidification and nutrients (but see Russell *et al.* 2009; Falkenberg *et al.* 2014; Campbell & Fourqurean 2014, Ober & Thornber *in review*). While Falkenberg *et al.* (2014) found that a marine gastropod can control the excess growth of opportunistic turf algae under high CO₂ and high nutrients, there was no indication of herbivore stress. In addition, the results

presented by Russell *et al.* (2013) highlight a need for longer-term studies (> 6 months, multiple generations), as acclimation may happen within a generation and adaptation to environment may occur between generations. These factors are likely to have an impact on herbivore feeding and physiology. This will be critical when placing this work in the context of how climate change impacts Narragansett Bay ecology. Previous research has highlighted changes in phenology of species the bay (Nixon *et al.* 2009) and northward migrations of mobile marine fauna (Nye *et al.* 2009). Ocean acidification and nutrient loading are likely going to alter the macroalgal growing seasons and aid in expansion northward as recent research has highlighted the ability of increased CO₂ to alleviate temperature stress in some macroalgae (Olischläger & Wiencke 2013).

In both terrestrial and marine systems, climate change is altering the dynamics between plants and herbivores (O'Connor *et al.* 2009; DeLucia *et al.* 2012) through both direct and indirect effects. Increased CO₂ can increase the rate of plant consumption by terrestrial herbivores by decreasing time between developmental stages, as well as increasing feeding rates (Bezemer & Jones 1998; Stiling & Cornelissen 2007). Increased CO₂ can alter tissue composition of both terrestrial and marine primary producers, which can indirectly influence feeding rates of herbivores (Bezemer & Jones 1998; Falkenberg *et al.* 2014). Our study highlights the overwhelming direct effects of climate change on *L. littorea*, which lead to decreased consumption of macroalgae, despite positive indirect effects (increased food quality). Coupling decreased consumption with increased growth rates of opportunistic species under future CO₂ conditions will ultimately enhance algal growth in this ecosystem

and could lead to shifts in macroalgal assemblages, favoring ephemeral species at the expense of more long-lived species that are critical in creating suitable habitat for other organisms (Worm & Lotze 2006).

As herbivores play a major role in structuring communities (Poore *et al.* 2012), quantifying the direct physiological effects and the indirect effects (altered food quality) will be critical in determining the response of communities and ecosystems (Connell *et al.* 2013). Ultimately, community resilience will depend on a diversity of herbivores (Baggini *et al.* 2015), as climate change is likely going to affect organisms differently (Ries *et al.* 2009). While certain species might not have an ability to increase consumption under high CO₂, other herbivores in this ecosystem may be able to compensate. As such, our understanding of ecosystem response to climate change will be dictated by the species involved and the diversity of the community.

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Table 1. Carbonate chemistry and nutrient parameters, data averaged across consumptions and respiration experiments. DIC and TA were measured via water samples collected during each experiment. Temperature, and salinity were measured directly. Seacarb (R software; Lavigne et al. 2011) was used to calculate $\text{pH}_{(\text{total})}$, pCO_2 , CO_2 , HCO_3^- , and CO_3^{2-} . Dissolved inorganic nitrogen (DIN) was measured from water samples that were taken during experimentation. All values represent means $\pm$ standard error.

<i>PARAMETER</i>	Ambient pCO_2		High pCO_2	
	Ambient Nutrients	High Nutrients	Ambient Nutrients	High Nutrients
$\text{pH}_{(\text{total})}$	7.94 ± 0.02	7.96 ± 0.02	7.58 ± 0.02	7.59 ± 0.02
pCO_2 (μatm)	476.2 ± 24	461.7 ± 23	1225.1 ± 45	1199.2 ± 40
CO_2 ($\mu\text{mol/kgSW}$)	16.85 ± 0.9	16.1 ± 0.8	42.39 ± 2	41.29 ± 1.4
HCO_3^- ($\mu\text{mol/kgSW}$)	1785.2 ± 24	1769.77 ± 24	1958.8 ± 18	1972.9 ± 15
CO_3^{2-} ($\mu\text{mol/kgSW}$)	124.65 ± 5	126.65 ± 4	59.2 ± 2.4	61.39 ± 2.2
DIC ($\mu\text{mol/kgSW}$)	1926.7 ± 25	1912.5 ± 24	2060.4 ± 20	2075.83 ± 16
TA ($\mu\text{mol/kgSW}$)	2095.3 ± 27	2085.2 ± 24	2105.8 ± 21	2125.9 ± 18
Temp. ($^{\circ}\text{C}$)	18.44 ± 0.16	18.1 ± 0.08	18.3 ± 0.09	18.49 ± 0.15
Salinity (ppt)	31.1 ± 0.1	31.1 ± 0.1	31.2 ± 0.1	31.08 ± 0.09
DIN (μM)	6.92 ± 0.78	202.4 ± 6.9	6.96 ± 0.50	196.6 ± 11

Table 2. Summary of the two-way ANOVA showing the effects of ocean acidification (pCO₂), nutrient addition (Nutrients), and their interaction (pCO₂ X Nutrients) on the total algal consumption (mg snail⁻¹ day⁻¹) by *L. littorea*. (*) Represent significant p-values.

Model Source	df	SS	F	p
pCO ₂	1	25039	24.2	<0.0001*
Nutrients	1	264.5	0.25	0.62
pCO ₂ X Nutrients	1	132.7	0.13	0.72
Error	24	24876		

Table 3. Summary of the factorial MANOVA and protected ANOVA showing the effects of ocean acidification (pCO₂), nutrient addition (Nutrients), and their interaction (pCO₂ X Nutrients) on the consumption (mg snail⁻¹ day⁻¹) of *Ulva* and *Fucus* by *L. littorea*. (*) Represent significant p-values.

MANOVA Source of Variation	Wilks' λ	df	F	p
pCO ₂	0.39	2, 23	17.87	<0.0001*
Nutrients	0.98	2, 23	0.21	0.81
pCO ₂ X Nutrients	0.99	2, 23	0.04	0.96

ANOVA Response Variable	Source of Variation	df	SS	F	p
<i>Ulva</i> consumed	pCO ₂	1	1.35 x 10 ⁻³	1.95	0.18
	Nutrients	1	2.36 x 10 ⁻⁵	0.03	0.86
	pCO ₂ X Nutrients	1	4.43 x 10 ⁻⁵	0.06	0.80
	Error	24	0.017		
<i>Fucus</i> consumed	pCO ₂	1	0.015	33.5	<0.0001*
	Nutrients	1	1.61 x 10 ⁻⁴	0.37	0.55
	pCO ₂ X Nutrients	1	1.0 x 10 ⁻⁶	0.02	0.88
	Error	24	0.01		

Table 4. Summary of two-way ANOVA showing the effects of ocean acidification (pCO₂), nutrient addition (Nutrients), and their interaction (pCO₂ X Nutrients) on the respiration rates (μmol O₂ g⁻¹ L⁻¹ hr⁻¹) of *L. littorea*. (*) represent significant p-values.

Model Source	df	SS	F	p
pCO ₂	1	2.06	15.9	0.001*
Nutrients	1	0.24	1.87	0.19
pCO ₂ X Nutrients	1	1.5x10 ⁻⁴	0.0012	0.97
Error	16	2.08		

Figure 1. Mean total algal consumption rate (live thalli) ($\text{mg snail}^{-1} \text{ day}^{-1} \pm 1 \text{ SE}$) by environmental treatment. Significant decreases in consumption rate were observed under high pCO_2 ($p < 0.0001$, indicated by *).

Figure 2. Mean consumption rates of *Ulva* and *Fucus* ($\text{mg snail}^{-1} \text{ day}^{-1} \pm 1 \text{ SE}$) by environmental treatment. No significant differences were observed in the consumption of *Ulva* by environment. Consumption rates on *Fucus* were significantly reduced ($p < 0.0001$; Table 3).

Figure 3. Mean proportion consumed of artificial *Ulva* and *Fucus* by *L. littorea*, for each environmental treatment. Data are means $\pm$ 1 SE. No significant impact of environment on consumption was found for either species.

Figure 4. Mean respiration rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ L}^{-1} \text{ hr}^{-1} \pm 1 \text{ SE}$) of *L. littorina* by environmental treatment. Significant decreases in respiration rate were observed under high pCO_2 ($p = 0.001$, indicated by *).

CHAPTER 3

OCEAN ACIDIFICATION INFLUENCES THE BIOMASS AND DIVERSITY OF REEF-ASSOCIATED TURF ALGAL COMMUNITIES

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ABSTRACT:

Projected ocean acidification will have a detrimental impact on coral reef ecosystems, where fleshy algae are expected to replace corals. Of particular importance to reef ecosystems are fleshy turf algal communities, which have the potential to overgrow corals; few studies have investigated the community structure and diversity of turfs to climate change. Here, we assessed the response of reef-associated turf algal communities from the Great Barrier Reef, Australia to three levels of ocean acidification. Biomass of turf communities was positively affected by increases in carbon dioxide (CO₂), where turf communities grown under high CO₂ had the greatest biomass. No effect of CO₂ was found on mean turf organic content or genus richness. By contrast, turf community evenness and diversity (H') increased under medium and high CO₂ treatments. Our results indicate that increased turf growth under high CO₂ will aid the overall expansion and growth of fleshy macroalgae in coral reef ecosystems, as opportunistic algae may have an advantage over other reef-associated species. Changes in turf community diversity will help provide insight into how macroalgal communities may be structured in the future, highlighting genera primed to take advantage of the changes in ocean chemistry associated with ocean acidification.

Key Words: ocean acidification, climate change, turf algae, diversity, coral reef

INTRODUCTION:

As atmospheric carbon dioxide (CO_2) continues to rise, ocean pH continues to decrease (Pachauri et al. 2014). As a result, ocean acidification (OA) is happening at unprecedented rates (Feely et al. 2004; Hofmann et al. 2011). OA and associated changes in ocean biogeochemistry, specifically changes in aragonite and calcite saturation states, have been targeted as problematic and potentially lethal for many calcifying marine organisms. Under future projected OA levels, impaired calcification and shell dissolution are likely to occur (Fabry et al. 2008; Waldbusser et al. 2015). Most OA research has focused on the physiological responses of calcifying organisms (Orr et al. 2005; Hoegh-Guldberg et al. 2007; Ries et al. 2009). Concurrently, non-calcifying primary producers, like fleshy macroalgae, seem primed to take advantage of chemical changes associated with OA (Gao et al. 1991; Kroeker et al. 2013; Olischläger and Wiencke 2013).

The response of fleshy macroalgae to OA is highly species specific (Raven 1997; Raven et al. 2005), but many species have exhibited positive, or at least neutral, impacts on growth rates (Gao et al. 1991; Olischläger and Wiencke 2013; Johnson et al. 2014). OA shifts the proportion of carbon species in the water column, increasing the relative amount of bicarbonate (HCO_3) and CO_2 (Zeebe and Wolf-Gladrow 2001; Sabine et al. 2004). Overwhelmingly, macroalgae use HCO_3 to obtain carbon for photosynthesis, facilitated by the use of carbon concentrating mechanisms (CCM) (Giordano et al. 2005; Raven et al. 2005). However, many primary producers are able to utilize the excess CO_2 in the water column by passive diffusion and can reallocate

CCM energy to growth, reproduction, or defense (Johnston 1991; Magnusson et al. 1996; Hurd et al. 2009). Some species, however, are not able to take advantage of the excess CO₂, or the change in pH disrupts the activity of the CCM, and as a result macroalgae exhibit reduced growth (Swanson and Fox 2007; Gutow et al. 2014). Ultimately, however, it is expected that future ocean environments will favor the growth and spread of fleshy macroalgae (Harley et al. 2006; Diaz-Pulido et al. 2011).

The divergent responses of calcifying and non-calcifying organisms may lead to major shifts in communities and assemblages (Hall-Spencer et al. 2008; Connell and Russell 2010; Anthony et al. 2011; Porzio et al. 2011). One such community is the coral reef, where foundation species such as corals and crustose coralline algae are susceptible to OA and are predicted to be replaced by fleshy macroalgae (Hoegh-Guldberg et al. 2007; Anthony et al. 2008; Kuffner et al. 2008; Diaz-Pulido et al. 2011). Shifting from a coral dominated system to an algal dominated system will likely yield changes in ecosystem productivity and diversity (Fabry et al. 2008).

Filamentous turf algae are an important component in many coastal ecosystems (Airoldi et al. 1995). Despite morphological diversity, turf algae are low-lying (0.5-10cm), can grow rapidly and cover a wide area, and are typically good at trapping sediment (Connell et al. 2014). Turf algae act as a key food source for grazers (Carpenter 1986; Morrison 1988) and since they have a shorter life span and due to their rapid turnover, turf algae contribute significantly to nutrient cycling in reef systems (Klumpp et al. 1987; Connell et al. 2014). Turf algae are one of the most abundant benthic components in coral reefs (Klumpp et al. 1992; Vermeij et al. 2010) and are able to quickly colonize space, taking over bare rock or dead coral (Diaz-

Pulido and McCook 2002; Carilli et al. 2009). Algal turfs can directly or indirectly affect coral settlement and growth (Birrell et al. 2005; Vermeij et al. 2010; Venera-Ponton et al. 2011). Turf algal communities are expected to thrive under future OA scenarios in many ecosystems (Connell and Russell 2010), potentially blocking the settlement and growth of habitat forming kelps (Connell and Russell 2010; Falkenberg et al. 2014) and corals.

Despite a diverse species composition, turf algal communities are typically treated as one entity (Westphalen and Cheshire 1997; Connell et al. 2014; Fricke et al. 2014). Research focusing on turf algae and OA has focused on the response of the community (Connell and Russell 2010; Kroeker et al. 2013) or one dominant species (Falkenberg et al. 2014). Few studies, however, have investigated turf assemblages at different pH ranges (but see Porzio et al. 2011; Porzio et al. 2013; Bender et al. 2014). Due to the diversity of turf communities, species within the community are likely going to exhibit a wide range of responses to OA. Turf communities may exhibit similar assemblage shifts as non-turf macroalgal communities, where those species poised to take advantage of the changes in ocean chemistry. For examples, calcareous species decline and a small number of non-calcifying species are successful and increase reproduction (Hall-Spencer et al. 2008; Porzio et al. 2011).

The overall goal of this research was to investigate how turf algal communities respond to OA. Using three levels of CO₂ (ambient, medium, and high), representing three OA scenarios, we tested the response of reef-associated turf communities in the Great Barrier Reef, Australia in terms of species diversity, richness, total biomass, and organic content. We hypothesized that under medium and high CO₂ scenarios, turf

algal communities would exhibit increased growth rates and a higher organic content (an indicator of algal health; Taylor et al. 2002) than in ambient CO₂ treatments, due to the excess carbon and potential reallocation of resources. We also hypothesized that turf species diversity would be lower under higher CO₂ treatments (Porzio et al. 2011) and that turf communities exposed to higher CO₂ would become red algal dominated (Raven and Beardall 2003). We interpret our results in the context of the resilience of turf algal communities.

MATERIALS AND METHODS:

Turf algae collection

Turf algal communities were collected in the reef slope of Coral Gardens, Heron Island, Great Barrier Reef, Australia (23° 26.698' S, 151 ° 54.533' E) at a depth of 6-8m. We used turf algal communities growing on the tips of dead *Acropora* spp. coral branches. Dead coral branches were collected by hand or using cutters and were placed in plastic bags underwater. We used two criteria for selecting the turf algal communities: 1) turfs exhibited comparable densities of algal growth (Fig. 1) and 2) dead coral branches were of around 7-10cm long. Turfs were then immediately transported to the outdoor flow-through aquaria facilities of Heron Island Research Station (HIRS) and acclimatized in tanks with running seawater during one week prior to the experiment. Dead coral fragments covered in turf algae were then placed in the bottom of 10L plastic tanks and randomly assigned to CO₂ treatments.

CO₂ manipulations and experimental setup

The CO₂ treatments consisted of three CO₂-dosing /pH regimes with CO₂ target values of 400 (pH: 8.00–8.10), 560 (pH: 7.85–7.95) and 1140 µatm (pH: 7.60–7.70), representing present-day (ambient), and projected mid-century (medium CO₂) and late-century CO₂ (high CO₂) levels under the representative concentration pathways 8.5 (RCP8.5) scenario (Moss et al. 2010; Meinshausen et al. 2011). The CO₂ manipulations were conducted following methods described earlier (Diaz-Pulido et al. 2011). For the medium and high CO₂ treatment levels, pH was manipulated using a computerized control system (Aquatronica-AEB Technologies, Cavriago, Italy) that operated solenoid valves to regulate the amount of CO₂ (analytical grade) injected into the seawater, based on pH levels measured continuously (every 10 minutes) in 200-L mixing tanks (one mixing tank per CO₂ level) using Mettler-Toledo polarographic sensors (InPro4501VP; Anthony et al. 2008; Diaz-Pulido et al. 2011). Sumps were connected to the outdoor flow-through aquarium system at HIRS, which uses unfiltered seawater pumped directly from the reef flat. To dampen diurnal pH fluctuations (7.9–8.4) of the pH from the reef flat (caused by benthic carbon fluxes), and better simulate the more stable pH regime on the outer reef slope where algal turfs were collected, low and high pH extremes of the ambient level treatment were prevented by dosing CO₂ when pH exceeded 8.2 and scrubbing CO₂ (as described in Diaz-Pulido et al. 2011) when pH fell below 8.0. We used two 10L plastic tanks per CO₂ level, and each tank had 5 coral branches covered in turfs. The experiment was conducted during May and June, the Austral autumn/winter, of 2009, and run for 41 days. At the end of the experimental period, turfs growing on coral fragments were frozen (-18°C). pH, pCO₂ (partial pressure CO₂), TA (total alkalinity), TCO₂ (total

dissolved inorganic carbon) and Ω_{Arag} (aragonite saturation state) values are means of eight replicates (SE). TCO_2 was measured colormetrically and TA analyses were made by open cell potentiometric titration. The pCO_2 , Ω_{Arag} , and pH on the total seawater scale were calculated for 24-25°C from the TCO_2 and TA values. DOC (dissolved organic carbon), NH_4 , NO_2 , NO_3 and PO_4 are means of six replicates (SE). DOC was measured by high temperature combustion (680°C) using a Shimadzu TOC-5000A carbon analyzer, and dissolved inorganic nutrients were determined by standard wet chemical methods using a segmented flow analyzer. All samples were collected during the course of the experiment in May 2009.

Biomass and organic content of turf communities

Pieces of coral branches covered in turf algae were thawed and first measured for total length and circumference to standardize turf community biomass results. One half of the turf-covered coral then was scraped with a razor until all turf algae were removed. The non-scraped half of the coral was saved for assessing turf community assemblage (see *Turf community composition*). As the purpose of this study was to investigate non-calcifying turf species, scraped turf algae were decalcified with a 1M HCL solution for ten minutes in order to dissolve any existing calcifying species and coral (Bender et al. 2014). After dissolution, the HCL solution was rinsed with DI water and turf algae were blotted dry and placed in pre-weighed aluminum tins. Wet mass of each replicate was taken and then standardized to mg/cm^2 based on surface area measurements from coral rubble.

Tins containing the turf algal community replicates were then placed in a 60°C drying oven for approximately 48 hours. After 48 hours, tins were removed and weighed to determine the dry mass of the turf community. Tins were placed in a muffle furnace at 550°C for 2.5 hours and turf ash mass for each replicate was recorded. Organic content of turf communities was determined by calculating the difference between total dry mass and ash mass (inorganic tissue) (Taylor et al. 2002).

Turf community composition

Using the same pieces of coral rubble, we thawed turf algal replicates and removed a 1cm X 1cm square of turf algae from the covered half of the rubble via scraping. The removed turf algae was rinsed for 10 minutes in a 1M HCL solution to dissolve and remove any unwanted calcified algae and coral (Bender et al. 2014). Turfs were then rinsed and blotted dry. Turf algae was then placed on a glass microscope slide and spread into one thin layer, with one slide per replicate (as per, Diaz-Pulido and McCook 2002). Turf algae were stained aniline blue dye, allowing algal features to be determined, ultimately aiding in the identification of turf algal genera and cyanobacteria. Once the dye was set, slides were examined at a magnification of 40X and turf algae were identified down to the genus.

We determined the relative abundance of present genera by selecting five fields of view on each slide, recording the genera present, and calculating percent cover within the field of view. Since empty spaces on each slide were a byproduct of mounting the turf community, percent cover of empty space in each field of view was also calculated in order to determine the relative abundance of each genus. Relative abundance of each genus was averaged over the five fields of view to establish an

overall breakdown of turf community for each replicate. Genus was used as the taxonomic unit of classification as many turf algal species are cryptic and unidentifiable without molecular analysis.

Statistical analysis: Biomass and organic content

Turf community biomass was standardized to mg/cm^2 (dry mass) and organic content (%C of tissue) was calculated. Due to the nature of the experimental set-up and potential pseudoreplication, we ran our data with a nested analysis of variation (ANOVA), nesting tank within CO_2 treatment to test whether the treatment tank was a significant factor. Our nested ANOVA showed tank was not a significant factor ($F_{3,25} = 1.752$, $p = 0.182$) and we were able to proceed with our statistical analysis with each piece of coral rubble as a replicate. For each response variable, a one-way ANOVA model was performed using JMP v 11 (www.jmp.com). If the statistical model indicated a significant effect, Tukey's HSD was run post-hoc.

Statistical analysis: Diversity metrics and community structure

We calculated mean genus richness for each CO_2 treatment from the observed present genera. Genus evenness for each CO_2 treatment was determined by the observations and abundances within the five fields of view. The Shannon-Weiner Index (H') was used to assess the diversity of turf genera. H' was calculated for each replicate based on average relative abundances of each present genera in the five fields of view. We assessed the difference among treatments for mean richness, evenness and diversity (H') with one-way ANOVAs. Where significant differences occurred, we used a Tukey's HSD test as a means of post-hoc analysis.

Turf algal community structure was analyzed within and across treatments based on the relative abundance of present genera using Primer v6 (<http://www.primer-e.com>; accessed January 2013). A Bray-Curtis similarity index was used to create an MDS plot to visualize similarities among turf communities treated under the three levels of CO₂ (Clarke 1993). To determine whether there were significant differences in community structure between CO₂ treatments we ran a one-way analysis of similarity (ANOSIM) using the vegan package in R (version 2.14, R Development Core Team, 2016, R-project.org). For genera of interest, we ran one-way ANOVA on percent cover among CO₂ treatments.

RESULTS AND DISCUSSION:

Seawater parameters

Elevated CO₂ and decreased pH were realized for each one of our treatments. Ambient pH measured 8.02, our medium treatment measured 7.85, and our high pH measured 7.63 (Table 1). Measurements of pCO₂ were found at 402 ($\pm$ 20) μ atm under ambient conditions, 564 ($\pm$ 12) μ atm under our medium treatment, and 1140 ($\pm$ 52) μ atm under our high treatment (Table 1).

Turf community biomass and organic content

We observed a significant, positive affect of OA on turf community biomass ($F_{2,26} = 3.24$, $p = 0.05$ Fig. 2), where turf communities grown under the most extreme acidification scenario (the highest CO₂ treatment) had the greatest biomass. The high CO₂ treatment had a mean community biomass that was 50% and 20% greater than in the medium and control (ambient) CO₂ treatments, respectively. Post-hoc analysis

revealed a significant increase in turf biomass between medium and high CO₂ treatments ($p = 0.04$), but no significant difference between ambient CO₂ and high CO₂ treatments.

While ocean acidification is expected to have detrimental effects on calcifying organisms, the effect on non-calcifying, fleshy macroalgae appears to be more species specific (see Johnson et al. 2014). Depending on their ability to acquire carbon from the water column, some algal species have shown increased growth rates under elevated CO₂ levels (Olischläger and Wiencke 2013), while other species appear stressed and their growth rates decline (Gutow et al. 2014). Under future conditions of ocean acidification, turf algal communities flourish via enhanced growth and primary productivity (Russell et al. 2009; Connell and Russell 2010; Falkenberg et al. 2014). Our observed response of a greater mean turf biomass under high CO₂ scenarios is similar to results from Falkenberg et al. (2014) and Connell and Russell (2010). Our analysis indicates that greater community biomass only appears under the high CO₂ treatment. We interpret this as a potential threshold for turf algal communities, wherein modest increases in CO₂ are not enough to influence community growth, but as CO₂ levels continue to increase, turf communities may flourish.

Organic content was not impacted by OA treatment ($F_{2,26} = 0.8067$, $p = 0.4572$). Mean proportion organic content was found at 0.88 (± 0.006) for the ambient, 0.91 (± 0.009) for the medium, and 0.90 (± 0.012) for the high treatment. An indicator of algal health and nutritional quality, organic content would likely be higher when more resources are available (Giordano et al. 2005; Hepburn et al. 2011). Community composition has the potential to play a large role in determining collective

organic content; turf algal species have different levels of baseline organic content and respond differently to ocean acidification (Hepburn et al. 2011). For example, Falkenberg et al. (2014) found the C:N within the brown filamentous alga *Feldmannia* spp. was reduced under high CO₂ and nutrient addition. Alternatively, Gutow et al. (2014) found that nutritional quality (as determined by C:N:P) was unaffected by CO₂. These results point to resilience of turf species, where under acidification conditions, turfs are able to maintain their tissue quality and health.

Diversity and community structure

Overall, OA had no effect on genus richness ($F_{2,26} = 0.08$, $p = 0.92$). Turf communities grown under all three OA scenarios had means of ~12 genera (Table 2). Despite genus richness consistency across treatments, CO₂ had a significant effect on genus evenness ($F_{2,26} = 3.818$, $p = 0.036$), with increased evenness found with increased CO₂ (Table 2). Turf genus diversity (Shannon H') was also significantly impacted by CO₂ level ($F_{2,26} = 3.4285$, $p = 0.0495$) with medium and high CO₂ levels resulting in significantly larger H' than control communities (Table 2). Thus, despite having similar numbers of genera present, the abundance of these genera was influenced by OA.

We constructed an MDS plot (Fig. 3) to assess turf community similarities. Based on present genera and relative abundance, we expected communities of the same CO₂ treatment to group together. Despite significant differences in H' and evenness, the resulting plot yielded no strong patterns grouping communities by treatment, likely due to the overall variability within treatments. Results from our ANOSIM support the lack of difference found between treatment communities ($p =$

0.86). These results agree with Bender et al. (2014), showing no pattern of community similarity under different CO₂ treatments.

We had initially hypothesized that our turf communities would become less diverse with increased CO₂ as has been observed in other macroalgal communities (Hall-Spencer et al. 2008; Porzio et al. 2011). However, we found that increasing CO₂ results in increased turf community H'. Although CO₂ and ocean acidification act as a major source of stress for many marine organisms, fleshy macroalgae, like those comprising turf communities, are expected to either flourish or persist. Most macroalgae use CCMs to convert the abundant bicarbonate molecules for use in photosynthesis; in addition, many algal species also have the ability to passively diffuse CO₂. Species that can passively diffuse CO₂ are the ones expected to thrive under ocean acidification conditions, where CO₂ is more readily available and existing CCMs can be down-regulated (Cornwall et al. 2012). The turf community analysis in this study suggests that the dominant present genera represented algae that have CCMs, but more work needs to be done to determine the contribution of passive diffusion and/or quantifying CCM down-regulation. By investigating genus evenness of turf communities, we were able to determine that increased levels of CO₂ had a positive effect on evenness. Despite observing diverse communities across all treatments, those communities grown under ambient CO₂ were dominated by only a few of the present genera, particularly *Polysiphonia* spp. Abundances within medium CO₂ and high CO₂ communities were more evenly distributed. Similar to genus evenness, H' for medium and high CO₂ treatments was than H' in ambient CO₂ treatments. However, this may be due entirely to the abundance of *Polysiphonia* spp.,

the genus accounting for 30% of the community under ambient CO₂ treatments. Under medium and high CO₂, *Polysiphonia* abundance is decreased. Statistical analysis of *Polysiphonia* cover over CO₂ treatment reveals a trend where abundance decreases as CO₂ increases ($F_{2,26} = 2.810$, $p = 0.077$). Species within the genus *Polysiphonia* have CCMs (as inferred from carbon stable isotope ($\delta^{13}\text{C}$) signatures, Raven et al. 2002), and other studies have indicated either a lack of *Polysiphonia* in low pH environments (Porzio et al. 2013) or reduced cover under high CO₂ (Bender et al. 2015). We can thus infer that the increased evenness and diversity of our treated turf communities in low pH environments is correlated to the decrease in cover of *Polysiphonia*.

We hypothesized that red algae would dominate turf communities under future projections of OA due to the larger relative portion of species within this phylum that have the ability to diffuse CO₂ (Raven 1997; Kübler et al. 1999; Raven and Beardall 2003). We separated genus relative abundance by phyla, including cyanobacteria (Fig. 4). Our study showed a significant decline in the relative abundance of red turf algae from the ambient CO₂ treatments to the medium and high CO₂ treatments (Fig 3, $F_{2,26} = 3.428$, $p = 0.048$). The relative abundance of green turfs and brown turfs were unaffected by CO₂ treatment ($F_{2,26} = 1.021$, $p = 0.37$, $F_{2,26} = 1.115$, $p = 0.34$, respectively). We observed significantly greater abundances of cyanobacteria with increasing CO₂ ($F_{2,26} = 3.328$, $p = 0.05$), supporting evidence of Bender et al. (2014). Here, cyanobacteria abundance was significantly higher in medium than ambient CO₂ treatments ($p = 0.02$). Several studies have investigated the relative abundance of different phyla at different pH levels (Hall-Spencer et al. 2008; Porzio et al. 2011; Bender et al. 2014). Porzio et al. (2011) found higher abundance of brown algae at

extreme low pH (6.7) from field surveys but little change in community structure between normal pH zones (8.1) and slightly more acidic zones (7.8). Hall-Spencer et al. (2008) found similar success for brown algae and found some resilient green algae. Bender et al. (2014) showed a trend shifting from higher abundances of brown turf species to higher counts of red turf algae and cyanobacteria as pH decreased. Our analysis of turf algal communities supports Bender et al. (2014) findings of increased abundance of cyanobacteria with increased pH, but we found an opposite trend in red algal abundance. This discrepancy points to the need for determining species-specific responses, in addition to continued studies of entire communities for a better understanding of algal community dynamics to climate change.

Overall, we found there to be slight, but significant changes to turf communities when exposed to different levels of CO₂. These findings support the positive effect of elevated CO₂ on growth of turfs (Russell et al. 2009; Connell and Russell 2010; Falkenberg et al. 2014). Under future climate conditions, where coral cover will likely decline as a result of bleaching caused by global warming (Hoegh-Guldberg and Bruno 2010), resilient and opportunistic turf algal communities may play a large role in the phase shift expected in this ecosystem (Diaz-Pulido et al. 2011). The shift in dominance is also likely to occur in kelp forest ecosystems (Connell and Russell 2010; Falkenberg et al. 2014), where long-lived kelps are expected to be replaced by opportunistic turf communities. Turf algal communities can increase genus diversity and evenness under future ocean acidification by reducing the abundance of dominant species, opening up valuable space and resources. While the mechanism behind this shift is unclear, whether it is a result of decline of a

dominant genus or the rise of competing species, in reef-associated turf communities we expect to see increased biomass, genus evenness, and turf community H' under future climate conditions.

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or animals performed by any of the authors.

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Table 1. Summary of mean values (SE) for water carbonate chemistry parameters for CO₂ treatment levels.

CO ₂ Treatments	pH	pCO ₂ μatm	TA μmol kg ⁻¹	TCO ₂ μmol kg ⁻¹	Ω _{Arag}	DOC mg l ⁻¹	NH ₄ μmol l ⁻¹	NO ₂ μmol l ⁻¹	NO ₃ μmol l ⁻¹	PO ₄ μmol l ⁻¹
Ambient	8.02 (0.02)	402 (20)	2170 (27)	1918 (29)	2.78 (0.07)	0.68 (0.01)	0.020 (0.020)	0.032 (0.009)	0.460 (0.058)	0.238 (0.010)
Medium	7.85 (0.01)	564 (12)	2208 (22)	2012 (17)	2.26 (0.07)	0.69 (0.01)	0.072 (0.045)	0.036 (0.010)	0.524 (0.019)	0.254 (0.007)
High	7.63 (0.02)	1140 (52)	2212 (20)	2123 (24)	1.32 (0.04)	0.71 (0.03)	0.070 (0.045)	0.033 (0.002)	0.545 (0.024)	0.252 (0.007)

Table 2: The mean relative abundance of present genera and mean community diversity metrics by CO₂ treatment. NC indicates negligible cover, as the genus was observed but did not contribute to the overall cover; - indicates no observation of genus.

Taxa	Ambient CO ₂	Medium CO ₂	High CO ₂
Rhodophyta			
<i>Polysiphonia</i>	30.49	15.70	24.81
<i>Ceramium</i>	0.89	2.30	1.24
<i>Hypoglossum</i>	3.58	NC	4.11
<i>Gelidiella</i>	11.50	11.28	8.61
<i>Wurdemannia</i>	5.39	7.73	6.29
<i>Centroceras</i>	0.92	1.32	0.57
<i>Anotrichium</i>	NC	-	-
<i>Spermathamnion</i>	1.04	0.34	0.22
<i>Champia</i>	NC	NC	-
<i>Antithamnion</i>	NC	NC	NC
<i>Griffithsia</i>	NC	-	NC
<i>Laurencia</i>	NC	-	-
<i>Gelidiopsis</i>	NC	-	NC
<i>Melanamansia</i>	2.90	4.47	4.55
<i>Gelidium</i>	2.67	0.53	2.57
<i>Lejolisia</i>	NC	-	0.05
Phaeophyceae			
<i>Sphacelaria</i>	5.87	7.28	8.14
<i>Dictyota</i>	6.37	5.06	5.45
<i>Lobophora</i>	3.56	9.76	5.79
<i>Hincksia</i>	0.59	-	NC
Chlorophyta			
<i>Uronema</i>	0.38	0.45	0.57
<i>Bryopsis</i>	10.52	8.92	10.69
<i>Cladophora</i>	0.44	0.78	-
<i>Ulvella</i>	3.61	2.36	1.34
<i>Cladophoropsis</i>	6.70	10.83	11.76
<i>Ulva</i>	NC	4.248	-
<i>Derbesia</i>	0.01	-	-
Cyanobacteria	0.45	6.54	2.56
Diversity Metrics (SE)			
Richness	12 (0.65)	12.3 (0.52)	12 (0.50)

H'	1.65 (0.07)	1.88 (0.09)	1.87 (0.05)
Evenness	0.66 (0.02)	0.76 (0.04)	0.76 (0.02)

Figure 1. Turf algal community replicates for ambient CO₂ treatment (left), medium CO₂ treatment (middle), and high CO₂ treatment (right) at the beginning of experimentation.

Figure 2. Mean biomass of turf algal communities ($\text{mg cm}^{-1} \pm 1 \text{ SE}$) after 41 days of experimentation under three levels of CO_2 . Letters represent significant differences between CO_2 treatments (post-hoc).

Figure 3. An MDS plot of turf communities under different CO₂ treatments: ambient (triangles), medium (circles), and high (squares).

Figure 4. Mean relative abundance (proportion of present genera) of turf algal phyla and cyanobacteria (± 1 SE) by CO₂ treatment. A significant effect of CO₂ was observed in the relative abundance of Rhodophyta where relative abundance under medium and high CO₂ are less than that of the ambient treatment ($p = 0.048$).