EFFECTS OF NUTRIENT LOADING, ELEVATED TEMPERATURE, AND OCEAN ACIDIFICATION ON CRUSTOSE CORALLINE ALGAE

A THESIS SUBMITTED TO
THE GLOBAL ENVIRONMENTAL SCIENCE
UNDERGRADUATE DIVISION IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELORS OF SCIENCE

IN

GLOBAL ENVIRONMENTAL SCIENCE

MAY 2016

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We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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To my dog, Zooxanthellae.

Thank you for never leaving my side all those late nights writing this thesis.

Acknowledgements

First and foremost I would like to thank Chris Jury and Rob Toonen for their patience, support, and guidance. Without them I not only would not have had a project, but I would not have been able to complete this final semester. I hope that one day I can pay forward the guidance and assistance the two of you were able to offer me. I would also like to express my gratitude to Michael Guidry and Jane Schoonmaker for always helping me troubleshoot class schedules and much more when life threw me curve balls.

Thank you to all of the GES students and alumni who supported me through the years and shared in this academic adventure. And to Catalpa Kong, you never failed me when I needed last second office assistance. You are truly the best auntie the GES crew could ask for, thank you! To Natasha Griswold, my lab mate and thesis partner in crime, thank you for sharing in this incredible and huge project, it has been an amazing journey. In completing this experiment I would also like to thank SeaGrant (University of Hawaii) and the National Science Foundation for their generous funding and providing this wonderful research opportunity.

Most importantly I want to express my extreme appreciation for my husband Matt, my parents Gary and Jennifer, and grandparents for supporting me all these years in school from an ocean away. Together, you have remained the driving force to my success.

Abstract

Rising temperatures, ocean acidification, and chronic eutrophication all contribute drastic functional changes to tropical shallow water reef ecosystems. "Business-as-usual" carbon dioxide emission scenarios predict atmospheric concentrations of CO_2 will nearly double by the end of this century. The increased absorption of CO_2 in ocean surface waters contributes to lower pH and lower carbonate saturation states. This acidification raises concern as to whether marine calcifying organisms could successfully continue to build their skeletons under future conditions. The development of crustose coralline algae (CCA) is a vital component of the coral reef environment that supports the function and growth of the reef ecosystem. CCA provide settlement cues for invertebrate and coral larvae while also acting like cement, holding reef structure together. My research focused on investigating the possible synergistic effects that rising temperatures, ocean acidification, and chronic eutrophication could have on the growth rates of CCA. The experiment was held over 24 days during the summer of 2015, exposing groups of CCA nubbins to a variety of environmental stressors. Overall, no significant effects were observed to have changed the growth rates of CCA, possibly suggesting that these marine calcifiers are capable of acclimating to rapid climate change, at least for short periods of time.

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List of Abbreviations

Amb – ambient

ANOVA – analysis of variance

CO_2 – Carbon Dioxide

CCA – crustose coralline algae

g – gram

High – elevated

HST – Hawaii Standard Time

L – Liters

mg – milligram

mM – millimolar

mg/g/d –milligrams per gram per day

mg g^{-1} d^{-1} –milligrams per gram per day

N – nutrients

OA – ocean acidification

pCO_2 – partial pressure of carbon dioxide

T – temperature

µM – micro molar

Chapter 1: Introduction

Beneath the umbrella of climate change implications, three specific variables have gained particular attention when considering their impacts on coral reef health: rising temperatures, ocean acidification, and chronic eutrophication all contribute drastic functional changes to tropical shallow water reef ecosystems (De Carlo et al., 2007; Belliveau & Paul, 2002; Ordonez et al., 2014). These marine ecosystems serve as a vital ecological, social, cultural, and economic global resource. The economic value of coral reefs in Hawai'i alone is estimated to be $33.4 billion per year (Bishop et al., 2011). The building blocks of reef ecosystems rely significantly upon the calcifying organisms that provide substrate structure for primary producers (Hofmann & Bischof, 2014; Manzello et al., 2008; Ordonez et al., 2014). Unfortunately, reef calcifiers, such as corals and certain macro algae, are becoming increasingly threatened by the impacts of ocean acidification (OA), and consequently reef ecosystems are declining rapidly (Ordonez et al., 2014).

The development of crustose coralline algae (CCA) in a coral reef environment is a vital component that supports the function and growth of the reef ecosystem (Ordonez et al., 2014). CCA provide settlement cues for invertebrate and coral larvae while also acting like cement, holding reef structure together (Hofmann & Bischof, 2014; Manzello et al., 2008; Ordonez et al., 2014). Similar to results seen in many other marine organisms including corals,

coccolithophores, foraminifera, and mollusks, CCA have an impaired ability to secrete their calcium carbonate ($CaCO_3$) skeletons under elevated seawater pCO_2 (Jokiel et al., 2008; Kuffner et al., 2007; Hofmann & Bischof, 2014; Cryonak et al., 2015).

Evidence is accumulating that the impacts of climate change on reef builders is not limited to solely the influence of increased CO_2 in the ocean (Anthony et al., 2008). Additional factors that alter the sensitivity of marine calcifiers to OA include elevated temperature and nutrient enrichment. Elevated sea surface temperatures have been shown to increase the sensitivity of many corals and some calcifying macro algae to ocean acidification (Langdon & Atkinson, 2005; Chavin et al., 2011; Hofmann & Bischof, 2014; Cryonak et al., 2015). On the other hand, it has also been observed that some corals become less sensitive to ocean acidification when exposed to increased nutrient availability (Langdon & Atkinson, 2005; Chavin et al., 2011), but nothing is known as to whether CCA experience a similar, if any, change in sensitivity to OA when exposed to nutrient influx.

The availability of nutrients in coastal marine environments varies depending on factors such as freshwater flux, residence time of the water column, and upwelling (De Carlo et al., 2007; Szmant, 2002). Additionally, anthropogenic runoff may be polluted by agricultural waste, sewage, or industrial contamination. Thus, nutrient quality delivered by runoff varies depending on source, storm frequency and intensity. Some studies have found that increased

nutrient availability contributes to fleshy macroalgal proliferation (Belliveau & Paul, 2002; Thacker et al., 2001; Raven & Taylor, 2003). Other studies have shown ocean acidification depresses calcification rates of calcareous corals and coralline algae (Jokiel et al., 2008; Kuffner et al., 2007; Manzello et al., 2008), but none have thus far considered all these factors simultaneously. This study is the first to study the effects of elevated temperature, elevated pCO_2, and increased nutrient flux in a fully factorial design on treated CCA.

Our experiment was held at the Hawai'i Institute of Marine Biology (HIMB) in Kāne'ohe Bay, O'ahu, Hawai'i. Kāne'ohe Bay is a semi-enclosed embayment on the windward side of O'ahu with relatively long residence times as compared to adjacent open coastal zones and is well studied in regards to the ways natural and anthropogenically-induced events have impacted reef health and resiliency within the bay (De Carlo et al., 2007; Bahr et al., 2015b; Jury et al., 2013). The long residence time of water in the bay exposes marine residents to conditions of high temperatures, sewage spills, eutrophication, and ocean acidification for longer spans than if they were in open coastal zones (De Carlo et al., 2007). Our goal was to examine the individual as well as interactive effects of increased temperature, increased CO_2, and nutrient loading on the growth and skeletal dissolution of CCA.

Chapter 2: Methods

2.1 Mesocosm flow-through system

In order to test for both individual treatment impacts and combined treatment impacts, a factorial design was constructed to include eight individual treatments including ambient and elevated temperature (T), ambient and elevated CO_2, and ambient and elevated nutrients (N). The application of these treatments was provided to 80 live corals dispersed amongst 16 treatment tanks as described below (Figure 2.1: Mesocosm Setup).

An outdoor, flow-through experiment using sixteen 60 L plastic aquaria fed by eight 100 L header tanks was conducted with eight duplicated treatments. Seawater was pumped from the adjacent reef into the header tanks and flowed through the mesocosm system at a rate of 2.0 ± 0.2 L per minute, concluding in a turnover approximately every 30 minutes. The four treatments were generated in the header tanks on an elevated structure adjacent to the treated aquaria with the following treatments; two tanks with ambient seawater T and ambient pCO_2, two tanks with ambient T and elevated pCO_2, two tanks with elevated T and ambient pCO_2, and two tanks with elevated T and elevated pCO_2. Each header tank fed two plastic aquaria in which one of each set of aquaria was randomly selected to receive an additional treatment of daily nutrient enrichment. This resulted in a total of eight different treatments, duplicated amongst sixteen aquaria. Treatment duplication was completed to test for possible tank effects.

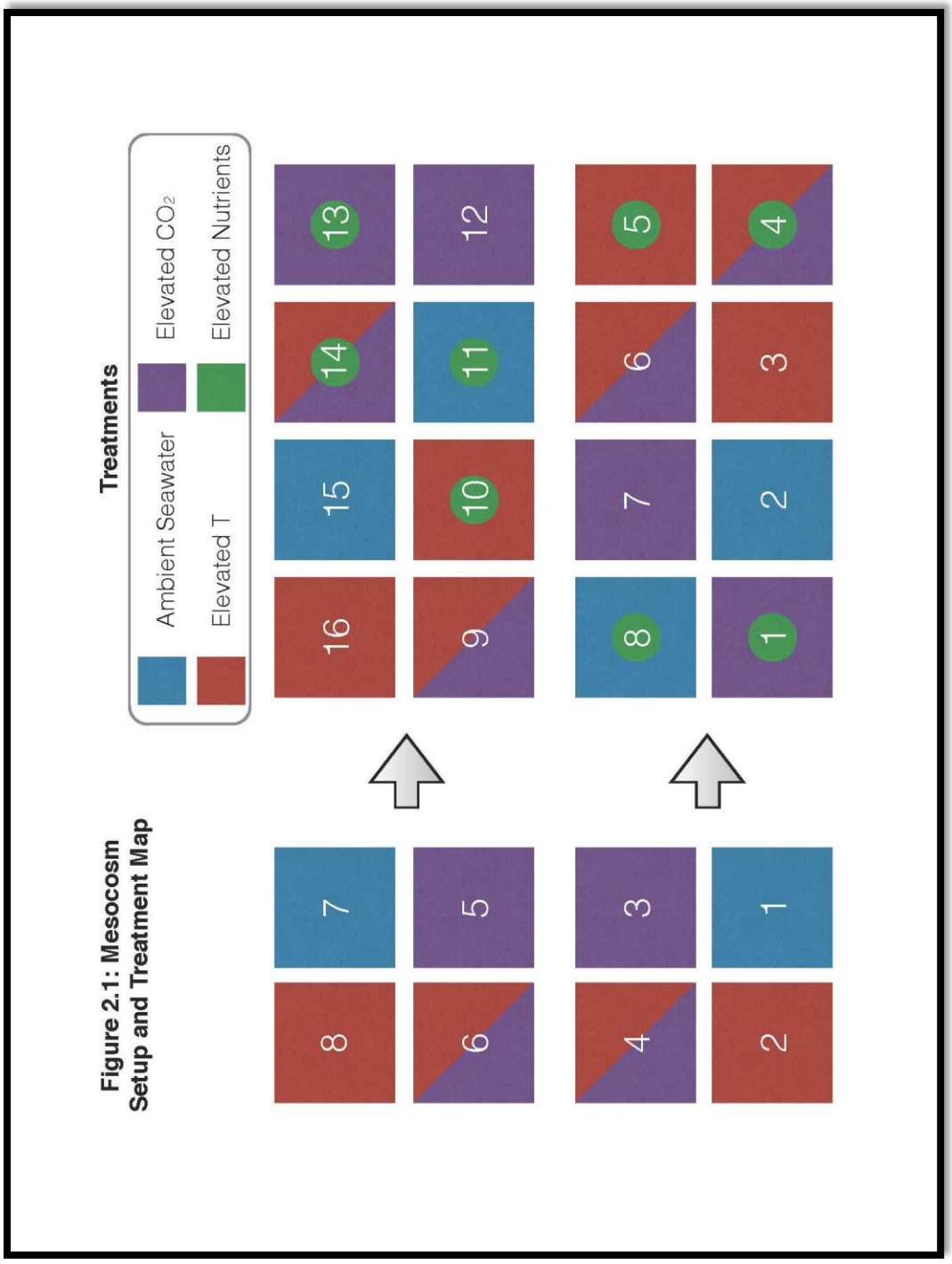

Figure 2.1: A total of 24 tanks are represented in the figure above. The eight tanks on the far left are the header tanks that provided varied temperature and CO2 treatments described on the previous page. A total of 80 CCA nubbins were dispersed amongst sixteen treatment tanks on the right side of the figure. Eight total environmental treatments were applied and duplicated randomly amongst the sixteen aquaria. Treatment types applied to individual tanks are color coded as per the treatment key in the top right of the figure.

Figure 2.2: Photo of mesocosm setup. The original tank construction included 40 treatment tanks, 16 of which were used for this experiment. The 16 treatment tanks were positioned closest to the header tanks (featured in the back of the photo under the black shade tarp). As you can see, there in an adjacent reef just a couple yards to the right of the mesocosm setup. This was the reef that was pumped to directly supply flow-through seawater for the experiment.

2.2 Treatments

Treatments of elevated T and pCO_2 were targeted to simulate conditions projected later this century due to anthropogenic CO_2 emissions. Elevated T was maintained between 30-31 °C to simulate a 2-3 °C increase over the Bay's normal summer maximum, which is known to cause extensive bleaching in Hawaiian corals (Jokiel & Brown, 2004) and a 0.3 unit pH reduction was targeted in application of CO_2. Aquaria treated with nutrient enrichment were provided 10 mL of nutrient stock solution every day for two hours at 6:00 PM. The nutrient stock solution was formulated in a 2 L container of deionized water to comprise of 6 mM sodium phosphate (Na_3PO_4) and 120 mM sodium nitrate ($NaNO_3$). The 10 mL daily treatment exposed treated aquaria to initial concentrations of approximately 1 µM Na_3PO_4 and 20 µM $NaNO_3$ to match typical enrichment conditions within Kaneohe Bay following fresh-water storm inundation (Ringuet & Mackenzie, 2005). To ensure a two-hour treatment of nutrient enrichment, flow-through water was turned off to all tanks at 6:00 PM to provide a fixed water volume, the nutrient-enriched tanks were spiked with the nutrient stock solution, and the tanks were given 2 hours to take up nutrients before the flow-through water was turned back on at 8:00 PM.

2.3 Water chemistry and environmental monitoring

Regular monitoring of temperature, salinity, total alkalinity, pH, and nitrate and phosphate concentrations of individual aquaria, including header

tanks, was conducted throughout the experiment. Daily measurements were taken every evening at 6:00 PM HST with the use of an YSI 85 conductivity meter to ensure frequent temperature calibration. Each aquarium was also provided individual HOBO loggers that recorded temperature every ten minutes throughout the experiment. Total alkalinity and pH was determined twice weekly. Total alkalinity was assessed potentiometrically with a modified Gran titration while pH was determined spectrophotometrically with m-cresol purple following standard protocols (Dickson, 2007; Kuffner et al., 2008).

2.4 CCA growth and statistical analysis

Buoyant weights were taken using methods described by Jokiel et al. (1978). Specimens were weighed while suspended in a buoyant medium of seawater. Initial weights were measured at the beginning of the experiment on July 6, 2015 and again on the final day of the experiment on July 30, 2015. Net growth rate was normalized to the initial weight of the CCA in mg g^{-1} d^{-1}.

ANOVA was used to analyze treatment effects on net calcification for each of the live and dead CCA with CO_2, nutrients, and temperature as fixed factors, and tank as a random, nested factor. Analyses were performed using R v.3.1.2 (R Core Team, 2014).

Chapter 3: Results

As shown in Table 3.1, CCA growth rates were not significantly affected by any individual treatment or combination of treatments with all ANOVA p-values ranging between 0.125 to 0.880. Although not significant, overall average growth rates were higher in individual treatment tanks compared to those with no treatment (Figure 3.1). The tanks with all possible treatments applied (High T, High N, and High CO_2) showed the lowest overall average growth rate of 0.303 ±0.303 mg g^{-1} d^{-1}. The second lowest growth rate observed was for CCA in tanks with no elevated treatments (Amb T, Amb N, and Amb CO_2) at 0.543 ± 0.226 mg g^{-1} d^{-1}.

Table 3.1: ANOVA results of growth rate averages affected by individual and combined treatments.

Factor	Df	Sum Sq	Mean Sq	F Value	p
Temp	1	0.01	0.0123	0.023	0.880
CO2	1	0.67	0.6705	1.250	0.268
Nutrients	1	0.82	0.8176	1.524	0.222
Temp:CO2	1	0.71	0.7127	1.329	0.254
Temp:Nutrients	1	1.21	1.2144	2.264	0.138
Nutrients:CO2	1	1.30	1.3003	2.424	0.125
Temp:CO2:Nutrients	1	0.30	0.2966	0.553	0.460
Temp:CO2:Nutrients:Tank	8	7.12	0.8898	1.659	0.127
Residuals	64	63.18	0.987		

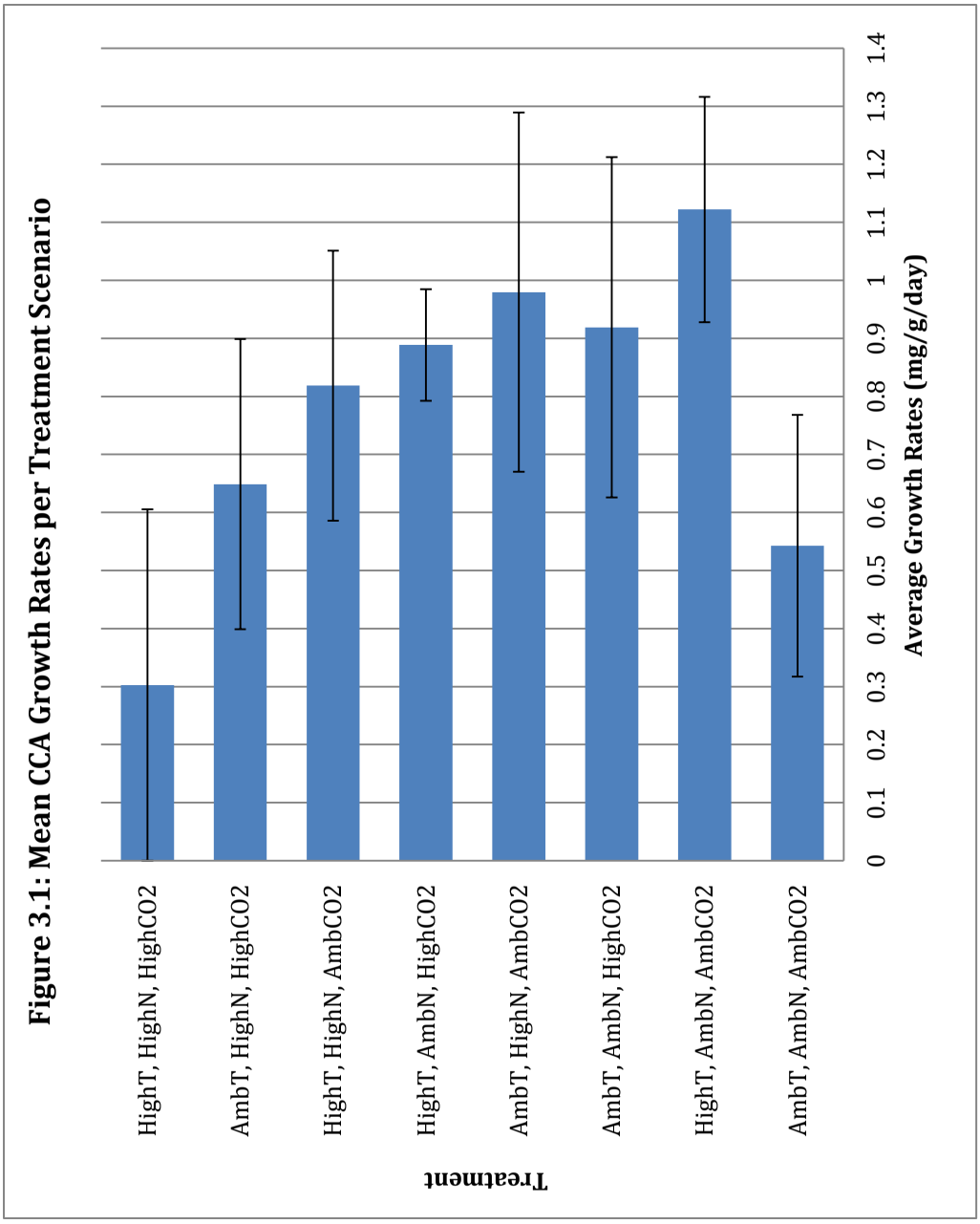

Figure 3.1: Average growth rates per individual and combined treatment applications are displayed above. Overall treatments are identified on the y-axis where "Amb" is short for ambient and "High" indicates an elevated treatment. Each prescript, Amb or High, is followed by a treatment, T= temperature, N= nutrients, and CO2= carbon dioxide. Growth rates are measured along the x-axis in mg g^{-1} d^{-1}. Sample size, n = 8-10 per treatment.

Chapter 4: Discussion

Atmospheric CO_2 concentrations are predicted to nearly double by the end of the 21st century, if anthropogenic carbon emissions remain unchanged (Caldeira & Wickett, 2005). Under such a "business as usual" scenario, not only will CO_2 emissions acidify our oceans, but also continue to heat our atmosphere as well, something being detected around the planet already (Mora et al. 2013). These global climate change factors, combined with their influence on increased evaporation and storm intensification, have led many to predict a dire future for shallow coral reef environments and their inhabitants around the globe (Jokiel et al., 2008; Kuffner et al., 2007; Hofmann & Bischof, 2014; Cryonak et al., 2015).

Crustose coralline algae (CCA) are a vital component to the overall structure and health of a coral reef. CCA hold reef structures together like cement while also acting as settlement cues for coral and invertebrate larvae (Hofmann & Bischof, 2014; Manzello et al., 2008; Ordonez et al., 2014). Although elevated temperature, ocean acidification, and nutrient loading have each been shown to have significant impacts on CCA growth, their combined influence has not been studied previously (De Carlo et al., 2007; Belliveau & Paul, 2002; Ordonez et al., 2014).

My study evaluated the individual and combined impacts of nutrient loading, elevated temperature and ocean acidification on crustose coralline algae. Previous studies have indicated that increased nutrient availability, as an

individual environmental factor in the water column, enhances CCA and other macroalgal growth (Belliveau & Paul, 2002; Thacker et al., 2001; Raven & Taylor, 2003). Although my experiment yielded one of the highest overall growth rates of CCA when exposed to only elevated nutrients, the overall difference in growth rate was not statistically significant. Likewise, all other scenarios in which CCA were exposed to elevated nutrients along with either elevated temperature or elevated CO_2, or both, did not reveal any significant impacts on CCA growth (Figure 3.1).

In contrast to known impacts of nutrient loading on CCA development, both elevated CO_2 and elevated temperature have individually and jointly shown significant impairment on CCA growth (Jokiel et al., 2008; Kuffner et al., 2007; Latham, 2008; Manzello et al., 2008; Vasquez-Elizondo & Enriquez, 2016). For example, CCA in ambient seawater mesocosms grew an average of 0.6 g of buoyant weight per year whereas CCA in acidified seawater lost an average of 0.9 g buoyant weight per year, a 250% decrease in growth rate under acidification (Jokiel et al., 2008). However, my study did not show any significant differences when pairing elevated CO_2 or elevated temperature with additional environmental treatments. In fact, of the eight overall treatments evaluated in this experiment, the CCA that were only exposed to elevated CO_2 and no other elevated treatment displayed the third highest overall growth rate. Furthermore, CCA exposed only to elevated temperature showed the highest average growth rate of the eight treatments. This result suggests that the CCA

used in this experiment may be exhibiting characteristics of resistance to climate variability.

I failed to find any significant vulnerability in the way crustose coralline algae respond to induced climate change. These results differ from the results of previous studies based on each of these parameters tested individually (Belliveau & Paul, 2002; Thacker et al., 2001; Raven & Taylor, 2003 Jokiel et al., 2008; Kuffner et al., 2007; Latham, 2008; Manzello et al., 2008; Vasquez-Elizondo & Enriquez, 2016). There are a variety of reasons why my data may have failed to find results consistent with previous research. First, the 24 day length of this experiment is much shorter than the duration of most previous studies, which may suggest that 24 days of exposure to unfavorable environmental conditions is not a long enough duration for these organisms to express any adverse growth effects. It would be worthwhile to revisit a similar experiment for a prolonged duration and reapply these compound treatments to see whether CCA can acclimate to these synergistic components for months or years at a time. Such prolonged experiments would better simulate the realistic timeframe which CCA are believed to respond and endure if scientific projections of climate change persist as predicted. A second potential source of variation in this experiment is that the CCA used in this study were identified at the genus level (*Sporolithon* spp.) but not to species . Therefore, it is necessary to consider that species variation was possible amongst the nubbin samples used in the experiment, and individual variability could be responsible for variation in how CCA responded to

treatments. Thus, if there are any differences among species in how they react to the experimental conditions, this uncontrolled diversity may have further increased variability within treatments.

However, the fact that the CCA used in my experiment did not exhibit any signs of dissolution like that seen in previous studies (Jokiel et al., 2008; Kuffner et al., 2007; Latham, 2008; Manzello et al., 2008; Vasquez-Elizondo & Enriquez, 2016), is an indication that it may be possible for these organisms to resist stressful changes in climate. In a simultaneous study to my experiment, corals treated in the same aquaria for the same timeframe exhibited substantial net loss in growth rates when exposed to elevated temperature and when competing with a fleshy macroalgal counterpart (Griswold et al., unpub. data). The combined results of CCA resistance and coral susceptibility to climate variability in this experiment may suggest that we could see a major shift in the dominate species of reef builders from corals to CCA or other macroalgae by the end of this century if predictions about coral persistence in the face of climate change are correct.

Chapter 5: Conclusion

Previous studies have identified a range in responses of crustose coralline algae (CCA) development under individual treatments of elevated nutrients, elevated temperature, and elevated concentrations of CO_2. However, no studies to date have evaluated the combined influence of these factors on CCA. This experiment seeks to fill that gap by being the first to apply a fully factorial design to assess the possible impacts these combined environmental factors may impose upon CCA, an ecologically vital reef inhabitant.

My hypothesis in starting this experiment was that CCA would be susceptible to acidification as shown in previous studies, but more resistant to the combined influence of elevated temperature and CO_2 when provided elevated nutrients. However, the data reported here did not support my hypothesis, because we find no significant differences in CCA growth among any of our treatments, likely due to small sample size or experimental duration that limit statistical power.

In conclusion, although previous individual experiments have yielded different results, my synergistic experiment does not show the same impacts. As previously mentioned, there may have been one or more experimental reasons as to why my results varied so much from previous research. If so, it may prove to

be a worthwhile investment to take another look by elongating the timeframe of the experiment and engaging in species verification.

Although it is a concern that rising anthropogenic greenhouse gas emissions are warming our oceans and infringing upon that abilities of marine calcifiers to build their skeletons, this experiment suggests that the compound effects of nutrient loading, increased temperature, and ocean acidification may have alternative impacts on the way CCA are able to respond to these environmental conditions. Considering that the CCA I sampled displayed some resistance to climatic variance, it is also possible that they may be capable of acclimating to environmental change. If acclimation is possible, then as global temperature and ocean acidification continue to intensify as predicted, we may see a shift in dominant reef building species from the less stress tolerant corals to the possibly more stress tolerant coralline algae in the near future.

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