2013

Individual And Population Responses Of The Caribbean Sponge, Aplysina Cauliformis, To Natural And Anthropogenic Stressors

Cole Gregory Easson
University of Mississippi

INDIVIDUAL AND POPULATION RESPONSES OF THE CARIBBEAN SPONGE, *APLYSINA CAULIFORMIS*, TO NATURAL AND ANTHROPOGENIC STRESSORS

A Dissertation
presented in partial fulfillment of requirements
for the degree of Doctorate of Philosophy
in the Environmental Toxicology Graduate Program
The University of Mississippi

by

Cole G. Easson

June 2013

ABSTRACT

Coral reefs are under increasing threat from a diversity of stressors. These reefs have undergone a phase shift from coral-dominated to algal-dominated ecosystems, and in many cases other functional groups such as sponges are now the predominant organisms on many reefs. This dissertation investigated the responses of a common branching sponge, *Aplysina cauliformis*, to algal contact, eutrophication, hurricanes, and disease.

The effects of algal competition, anthropogenic nutrients and a combination of these stressors on *A. cauliformis* were examined using factorial designed field experiments on Bahamian reefs. These experiments demonstrated a complex interaction between sponge and alga, in which the green alga, *Microdictyon marinum,* elicited a competitive effect on the sponge, through shading of its photosymbionts, but contact with the sponge facilitated increased algal productivity. Elevated nutrient concentrations had a positive effect on *M. marinum* by increasing algal productivity, but showed mixed effects on *A. cauliformis*, by increasing sponge symbiont abundance, while decreasing overall holobiont health. A further investigation of the sponge-algal interaction using stable isotopes of carbon and nitrogen showed that algal facilitation was likely due to nitrogen transfer from the sponge. In addition, these experiments showed that algal contact did not have acute effects on internal sponge regulation and partitioning of carbon and nitrogen resources.

The fate and dynamics of the sponge disease *Aplysina* Red Band Syndrome (ARBS) in *A. cauliformis* were investigated *in situ*. This study showed that ARBS infection decreased an

individual's chance of survival on the reef. Dynamics of ARBS were investigated using spatial pattern analysis of *A. cauliformis* populations and revealed that direct physical contact was the transmission mechanism for ARBS within a population. In the three year period of this study, hurricane effects on sponge population an disease dynamics were also investigated, and showed a dramatic loss in sponge population biomass, increased breakage, especially in diseased individuals, and randomization of ARBS distribution within the population. With current environmental conditions often favoring alternative states in which organisms such as sponges and/or algae are the dominant organisms, it is important to understand how these organisms respond to multiple environmental stressors.

DEDICATION

This dissertation is dedicated to my parents Greg and Darlene Easson. They have always been my biggest supporters and encouraged me to pursue a career that I was passionate about. I thank them for their constant support, advice, and love. This truly would not have been possible without them. I would also like to dedicate this dissertation to my biological mother Annette. Her dream for me was that I get a college education, and though she was not able to see it herself, she ensured that I would have that opportunity as her legacy. I am forever grateful to her and will always cherish the time we had together.

LIST OF ABBREVIATIONS AND SYMBOLS

δ Delta

ANOVA Analysis of variance

APE Atom Percent Excess

ARBS *Aplysina* Red Band Syndrome

ASE Accelerated Solvent Extractor

ATPase Adenine Triphosphatase

DCM Dichloromethane

EDTA Ethylenediaminetetraacetic acid

F_0 minimum fluorescence

F_m maximum fluorescence

F_v variable fluorescence

GIS Geographical Information Systems

H_2O water

HCl Hydrochloric acid

HPLC High Performance Liquid Chromatography

LSI Lee Stocking Island

MeOH Methanol

NIH National Institutes of Health

NSF National Science Foundation

PAM Pulse Amplitude Modulated

RLU Rapid Light Unit

TFA Trifluoroacetic acid

UM University of Mississippi

UV Ultraviolet

ACKNOWLEDGEMENTS

I would like to thank my advisors, Drs. Deborah Gochfeld and Marc Slattery. I am very grateful for all the opportunities you have provided for me over the course of my time in your lab. Thank you for your constant support, advice and patience as you taught me how to be a scientist and make meaningful contributions to the field. I would also like to thank my other committee members Drs. Kristie Willett and Henrique Momm for their help and advice in completing my degree. The staff of the Perry Institute for Marine Science at Lee Stocking Island in the Bahamas was instrumental in logistical support of this research, and I would like to especially thank Tori Redinger, Erich Muller, Brenda Gadd, and all the interns that provided this support. Special thanks go to Drs. Chris Freeman, Cara Fiore, and Erica Hunkin for their tremendous help in the field and lab, wonderful conversations, and most of all their friendship. I also thank Drs. Julie Olson, Robert Thacker, Michael Lesser, Anne Boettcher, John Rimoldi, and Ms. Liz Kintzing for their help and guidance with a variety of field and laboratory experiences. I have had many wonderful field and lab mates during my time here and would like to thank Lindsay O'Donahue, Dr. Haidy Kamel, Dr. Sridevi Ankisetty, Jim Weston, Sylvester Lee, Katerina Pappas, Mallory Johnson, Caroline Williams, Mindi Ansley, Dexter Lee, Jessica Jarrett, Julia Stevens, Sabrina Bradford, Adam Hawkins, Frank Booc, Crystal Warren, Cammi Thornton, Olivia Dale, and Katherine Martin. At the University of Mississippi, I thank Dr. Greg Easson and the entire staff at the UM Geoinformatics Center and Mississippi Mineral Resources Institute for their help and support in accomplishing the GIS portion of this research. I also thank Casey

Stauber for her crucial assistance in keeping me in good standing with all my university paperwork, and for her kindness and willingness to always help me out when I needed it. Stable isotope analysis was run at the Carnegie Institute of Washington, D.C. by Dr. David Baker, Dr. Marilyn Fogel and Dr. Roxane Bowden. Funding for this research was provided by NIUST NA16RU1496, NSF OCE-1214303 and NSF OCE-0727996, and salary support from the University of Mississippi Graduate School. I would again like to thank my parents Greg and Darlene, as well as the rest of my family, Lee, Carina and Mikey for their love and support throughout this experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Coral reefs have experienced dramatic changes due to several natural and anthropogenic stressors that threaten the diversity and function of these important communities (Porter and Meier 1992, Hughes 1994, Aronson et al. 2004, Ward and Lafferty 2004). Coral reefs are the most diverse ecosystems on the planet (Connell 1978, Knowlton 2001). Despite occupying 0.1-0.5% of the global oceans, they host about one third of the ocean's species (Moberg and Folke 1999). In addition to their biological importance from the standpoint of diversity, these ecosystems provide many goods and services to humans such as food, medicine, shoreline protection, and tourism (Moberg and Folke 1999). Coral reef ecosystems contribute both directly and indirectly to the livelihood of a vast number of people in the world, yet with coastal development continually increasing, these ecosystems are under threat (Moberg and Folke 1999, Bellwood et al. 2004, Hoegh-Guldberg et al. 2007). To truly understand the degree of this threat we must first understand the ecological processes that structure these immensely complex and diverse ecosystems. A combination of ecological processes such as competition, predation, pathogenesis, and periodic disturbances maintain diversity on coral reefs (Jackson and Buss 1975).

Coral reef ecosystems are characterized by high levels of interspecific and intraspecific competition. Sessile organisms in these ecosystems compete predominantly for resources such as space (Jackson and Buss 1975, Carpenter 1990, Díaz and Rützler 2001), light (Huston 1985, McCook et al. 2001), and

biochemical nutrients (McCook 1999, Lapointe et al. 2004) by employing a variety of strategies such as allelopathy or rapid growth. Allelopathy has been shown in several reef taxa including corals, sponges and macroalgae (Jackson and Buss 1975, Rasher and Hay 2010, Rasher et al. 2011). Rapid growth has classically been shown in branching corals that can overtop slower growing massive corals, reducing irradiance and water flow to them (Huston 1985). More recently this strategy has been shown in macroalgae that grow much faster than many coral species, and in some instances can overgrow and smother corals and other reef organisms (McCook et al. 2001). Non-sessile organisms such as reef fish compete for limited food sources or suitable refuge from predators. Abundances of these organisms that exceed the resources available can lead to increased vulnerability to predation or starvation.

Predation, like competition, is a powerful influence on species abundance and diversity on coral reefs. Coral reefs are characterized by very high levels of predation in complex food webs (Paine 1966). These food webs often have considerable overlap of prey for organisms that have broadly similar nutritional requirements (Knowlton 1992). A classic example on coral reefs is the regulation of macroalgal abundance through predation (herbivory). This type of regulation is often referred to as top-down regulation, whereby herbivores such as urchins, parrotfishes, surgeonfishes, and others graze down algal populations (Lewis 1985), which in turn makes suitable substrate available for colonization by corals and sponges (Hughes 1994, Hughes and Connell 1999). This process often works in conjunction with competition in that if one group of organisms becomes over abundant, they would start competing with each other for resources such as food and refuge, which may make them more vulnerable to predation.

While some ecological theory suggests that with the high levels of competition and predation on coral reefs one group of organisms would eventually emerge as dominant, coral reefs have maintained their great diversity (Connell 1978, Knowlton 1992). The key to the maintenance of this diversity is likely the occurrence of periodic disturbances (Connell 1978, Knowlton 1992). One major difference between tropical and non-tropical ecosystems is that tropical ecosystems are not subjected to routine seasonal resetting from changes in the environment (Paine 1966). Natural disturbances to coral reefs are historically infrequent allowing for large amounts of niche overlap and the success of a great number of species within these ecosystems (Connell 1978). In this way, coral reefs never reach an equilibrium state that favors the emergence of a dominant member at each trophic level. Disturbances to these systems are in the forms of periodic storm events and disease outbreaks. While these periodic disturbances help to maintain the great diversity on coral reefs, they can also in some cases lead to large scale shifts in these communities. Fossil evidence suggests that climatic occurrences such as prolonged El Nino events can dramatically shift coral reef community composition for a period of time (Aronson et al. 2004, Toth et al. 2012). These periodic natural events are rare, and despite their dramatic effects, these systems often rebound.

The biggest concern today is that through human influence, we are changing these ecosystems at a faster rate than has ever happened in history. Coral reefs have evolved to endure a certain level of disturbance, but at some threshold of human induced stress, these systems may not have the ability to recover (Connell 1978, Knowlton 1992, Hughes and Connell 1999, Nyström et al. 2000). The problem with these human-induced stressors is that they disrupt these ecosystems by affecting the ecological processes that have historically structured them. The release of anthropogenic nutrients through agricultural runoff and sewage seepage has been

shown to potentially have large impacts on coral reef communities (Lapointe 1997, Lapointe et al. 2004). Overexploitation of fisheries resources has led to community imbalances in these ecosystems leading to dramatic changes in species composition and overall ecosystem function (Hughes 1994). Marine diseases have had a profound impact on the abundance of many important functional groups on coral reefs. Two prominent examples are white band disease, which has decimated *Acropora* populations throughout the Caribbean (Aronson and Precht 2001), and an unknown pathogen that wiped out over 90% of the important Caribbean herbivore *Diadema antillarum* (Lessios 1988). More recently, the effects of human induced increases in CO_2 levels and global climate change have been observed (Hoegh-Guldberg and Bruno 2010). Increasing frequency of temperature extremes has led to wide-spread bleaching of corals, the major habitat builder in these ecosystems (Hoegh-Guldberg et al. 2007). Increased CO_2 in seawater has caused the pH of the ocean to decrease, which has been shown to have major impacts on some organisms that depend on the process of calcification (Hoegh-Guldberg et al. 2007, Hoegh-Guldberg and Bruno 2010). Many organisms that rely on calcification are essential conspicuous members of these communities and range from primary producers such as corals and calcareous algae, to predators such as urchins and sea stars (Hoegh-Guldberg 1999, Hoegh-Guldberg et al. 2007, Hoegh-Guldberg and Bruno 2010). These stressors have had profound impacts on community composition and diversity in coral reef ecosystems, shifting these communities away from hard coral domination and towards sponge, octocoral and macroalgae domination (Norström et al. 2009). While these stressors are often addressed separately in studies, the reality is that coral reefs often experience many of these natural and anthropogenic stressors simultaneously (Hughes and Connell 1999), so to truly understand these systems, we must understand the interactive effects of multiple stressors.

Two competing theories have been developed to understand the reasons for the phase-shift that has been witnessed on Caribbean coral reefs over the past couple decades. Top-down theory states that species diversity and abundance is regulated via predation control in the food web (Aronson and Precht 2000), Bottom-up control theory states that species diversity and abundances are controlled by limited nutrients in these systems (Lapointe 1997). The bottom-up theory argues that even when herbivores have been restored in some areas, they have not been able to restore community balance and return the community to a coral dominated one (Lapointe 1997). Top-down theory argues that elevated nutrient levels represent local influences and are not widespread enough to have the Caribbean basin wide effect that has been observed (Aronson and Precht 2000). Both theories have compelling data to back up their conclusions, and the answer is more likely a combination of the two processes combined with other stressors, rather than either of these theories independently.

Anthropogenic nutrients have been implicated in many negative effects on coral reef systems (Lapointe 1997). Inputs of these chemicals often come from either agricultural runoff, seepage of sewage, or a combination of the two. One prime example of the negative effects of anthropogenic nutrients can be seen annually in the Gulf of Mexico. Due to agricultural runoff of nitrogen and phosphorus along the Mississippi River valley, each year the river inputs large amounts of these chemicals into the Gulf or Mexico. These elevated nutrients stimulate plankton blooms, and when this plankton dies, they sink and decompose using up the oxygen in the seawater stratified below the freshwater layer (Bruckner 2011). The hypoxic seawater is trapped below the freshwater layer, resulting in a massive dead zone, devoid of life (Rabalais et al. 2002). While this is an extreme case, it highlights the problems with large inputs of freshwater containing high levels of nutrients. An example that pertains to coral reefs directly is that of

Kaneohe Bay in Hawaii. Terrestrial runoff led to elevated nutrient levels in this bay (Banner 1974, Maragos et al. 1985, Stimson et al. 2001). The result was massive blooms of the alga *Dictyosphaeria cavernosa*, that was able to take advantage of this suddenly non-limiting resource and out-compete corals through overgrowth (Banner 1974, Smith et al. 1981, Maragos et al. 1985, Lapointe 1997). An example in the Caribbean is that of Discovery Bay where elevated nutrient levels were associated with increases in algal cover in a variety of species (Lapointe 1997). While macroalgal blooms on coral reefs have been suggested as the main effect of elevated nutrients, there have been other effects. Elevated nutrients can causes a breakdown in the coral-zooxanthellae relationship, resulting in a reduced level of resources being translocated to the host coral cells (Falkowski et al. 1993).

While anthropogenic nutrient input is recognized as a potential human induced stressor, over exploitation of coral reef fisheries is another big problem in the world and especially in the Caribbean (Hay 1984, Hughes 1994, Mumby et al. 2007). Many coral reefs have become over-fished as both native populations continue to grow, and as tourism to these tropical locations increases (Jackson et al. 2001). Removal of key members of food webs in coral reef communities is not without consequence. When higher-level predators are removed, organisms at lower trophic levels become more abundant (Jackson et al. 2001, Mumby et al. 2006). However, when these removed species are essential herbivores, macroalgae may become more prolific components of the coral reef community (Hughes 1994). The removal of herbivores partly through overfishing, has been implicated as a top-down cause of the phase shift from a coral dominated reef community to an algal dominated reef community (Hughes 1994).

Marine diseases are recognized to have enormous impacts on coral reef communities (Aronson and Precht 2001, Hughes et al. 2003, Harvell et al. 2007). One historical disease of note ended the commercial sponge industry in the Caribbean (Galstoff et al. 1939, Smith 1941, Lauckner 1980). More recently, an unknown epizootic wiped out the population of the urchin, *Diadema antillarum*, a very abundant and important herbivore (Lessios et al. 1984). This die-off led to increased algal abundance on coral reefs due to the sudden decrease in herbivory, consistent with the top-down theory of phase shifts (Hughes 1994). Marine diseases are being reported with increasing frequency (Hughes et al. 2003, Ward and Lafferty 2004, Harvell et al. 2007), and there is increasing evidence to suggest that human impact may be affecting these diseases (Bruno et al. 2003b, Voss and Richardson 2006, Harvell et al. 2007). Many recent marine disease studies on coral reefs have focused on hard corals, and these diseases have resulted in massive declines in many prominent reef-building corals (Edmunds 1991, Aronson and Precht 2001, Richardson 2004). For example, white band and white pox diseases have decimated the populations of the two Caribbean *Acropora* species (Aronson and Precht 2001, Sutherland and Ritchie 2004). These two species were the main reef-building coral species on forereefs, but due to outbreaks of this disease, combined with other stressors, their abundance in the Caribbean is dramatically lower than it was just a few decades ago (Gladfelter 1982, Aronson and Precht 2001, Gardner et al. 2005). In some instances, human influence has been implicated in the cause of a disease, such as in the case of White pox, where researchers have suggested a causal link between White Pox and sewage contamination on the reef (Sutherland et al. 2010). Other diseases are exacerbated by human impacts, such as black band disease, which progresses faster under elevated nutrient conditions (Voss and Richardson 2006). Additionally, some diseases have shown a strong seasonal relationship, often being more prevalent when the sea

surface temperature is warmer (Edmunds 1991, Willis et al. 2004), which could be unfortunate foreshadowing of future reef conditions as average sea surface temperatures continue to rise each year. With such a wide range of confirmed and potential infectious agents, this diversity of responses is not surprising. There are many questions that remain to be answered in marine epidemiology, so to clearly understand to what extent these human related impacts will effects these communities, continued study is needed.

While coral reefs face a myriad of human induced stressors, they must also contend with some natural ones. Periodic hurricanes can have dramatic negative short-term impacts on coral reef communities, but they were historically viewed as beneficial to the long term health and diversity of the system (Knowlton 1992, Hughes 1994). When a hurricane hits a coral reef, it often breaks up the corals, and this is especially pronounced in the less calcified, faster growing, branching corals. By not allowing these systems to reach a state of equilibrium, these storms promoted diversity on coral reefs by preventing one group of species from dominating. Additionally, the storms opened up substrate on the reef for larval settlement, which also helped to promote colonization of new species in these systems (Carpenter 1990, Hughes and Connell 1999).

While historically, this has been the case, more modern periodic hurricanes have led to more negative long-term effects for a variety of reasons. One reason is that these storms have increased in average intensity recently (Elsner et al. 2008, Mann et al. 2009). This has been attributed to the elevations in sea surface temperature, and coral reefs now experience very strong storms much more often than they did in the past leading to increased destruction by each storm. Another reason for the negative impacts is that coral reefs are under stress from a variety

of human influences (Hughes and Connell 1999). These stressors, which are mentioned previously, act in synergy with hurricanes leading to the overall degradation of the reefs. An example of this phenomenon is Jamaica after Hurricane Allen (Hughes 1994). When this hurricane hit, it broke apart branching corals, and opened up new substrate for larval settlement. Instead of this substrate being settled by corals and sponges, it became colonized by macroalgae (Hughes 1994, Aronson and Precht 2001). Many factors played a role in this, including anthropogenic inputs of nutrients, overfishing of herbivorous fishes, and a disease that wiped out the *Diadema antillarum* population (Hughes and Connell 1999). All of these stressors were influencing the coral reef communities in the area at the time of the hurricane, and led to the short-term negative impacts of the hurricane being translated into long-term shift toward an algal dominated reef.

Current environmental conditions often do not favor coral dominated communities, and as a result, other groups of organisms such as sponges have become increasingly dominant (Norström et al. 2009). Sponges are important members of coral reef communities (Díaz and Rützler 2001, Pawlik 2011). This group of organisms occupies many crucial niches on coral reefs, and is becoming increasingly dominant as coral cover in the Caribbean continues to decline. On many reefs in the Caribbean, sponges are now the primary habitat forming organisms, providing the reef structure as scleractinian corals have done previously (Pawlik 2011). This structure provides important habitat for many coral reef organisms (Pawlik 2011), but sponges also provide habitat for a wide diversity of organisms within their tissue (Taylor et al. 2007, Thacker and Freeman 2012). Sponges are also an important source of primary productivity in coral reef ecosystems, as many of them host symbiotic photosynthetic microbial communities (Thacker and Freeman 2012). These photosynthetic microbial communities can be

dinoflagellates, unicellular cyanobacteria, or filamentous cyanobacteria, and studies of these interactions have shown their relationship with the sponge to be highly variable between species (Taylor et al. 2007, Thacker and Freeman 2012). Sponge-microbe associations can range from parasitism (i.e. disease) to mutualistic interactions, and there is some evidence to suggest that these relationships change under altered environmental conditions (Freeman and Thacker 2011).

Another important function of sponges on coral reefs is carbon and nitrogen cycling (Maldonado et al. 2012). Sponges cycle large amounts of particulate organic carbon and nitrogen through filter feeding. This process removes massive amounts of particulate organic matter from coral reef ecosystems (Maldonado et al. 2012). The trapping of these particulates from the water column is an important mechanism by which energy resources from the pelagic compartment are transferred to the benthos in a process called benthic-pelagic coupling, and sponges are crucial to this process on Caribbean coral reefs (Lesser 2006). In addition to the removal of particulate organic matter from the water column, many sponges are active in the cycling of dissolved carbon and nitrogen resources. Sponges that host symbiotic microbes are able to absorb dissolved inorganic sources of carbon and nitrogen that are unavailable to the sponges directly (Maldonado et al. 2012). In many cases these organisms are able to then translocate these resources to the host sponge cells in a form they are able to absorb (Freeman and Thacker 2011). This allows sponges that host symbiotic microbial communities to have access to inorganic nutrients and thrive in areas where particulate food sources may be scarce.

In addition to making these inorganic resources available to the sponge host, these microbial communities also contribute resources to the ecosystem (Southwell et al. 2008). This is especially the case for nitrogen, which is often considered a limiting resource on coral reefs

(Lapointe 1997). Sponges can harbor microbial communities capable of every step of the nitrogen cycle (Taylor et al. 2007). All of this nitrogen is not used by the host sponge in many cases, as several species have been shown to have a net efflux of either ammonium (NH_4^+) or nitrate/nitrite (NO_x) nitrogen sources (Southwell et al. 2008). This efflux of nitrogen by sponges is an important source of new nitrogen or coral reefs that is often underestimated when calculating the nitrogen budget of coral reefs (Fiore et al. 2010). Direct influence of this efflux of nitrogen on other reef organisms is limited, but some studies have shown the ability of these nitrogen resources to be transferred to other organisms, including macroalgae (Ellison et al. 1996, Davy et al. 2002, Pile et al. 2003).

As sponges become more dominant members of coral reef communities (Maliao et al. 2008), it is increasingly important to understand how these organisms respond to the suite of stressors that affect coral reefs. Studies on anthropogenic nutrient inputs have largely focused on their ability to stimulate algal growth (Lapointe 1997, Lapointe et al. 2004). Fewer studies have investigated the direct effects of elevated nutrients on sponges (Gochfeld et al. 2012a), although a shift in sponge community composition across a gradient of anthropogenic influence has been noted (Gochfeld et al. 2007). Since sponges are active in the natural cycling of nitrogen and phosphorus on coral reefs (Maldonado et al. 2012), they may be expected to be tolerant of higher nutrient concentrations. Elevated nutrients could help to stimulate productivity in symbiotic cyanobacteria as it does in macroalgae, or increase the plankton load in the water column providing more food for sponges that rely more heavily on heterotrophic feeding. Negative effects may be indirect and connected to increased algal abundance due to a combination of increased anthropogenic nutrients and reduced herbivory. Some work has shown that sponge-macroalgal interactions are detrimental to the sponge (González-Rivero et al. 2012) and that in

some cases, the sponge can benefit the algae through providing it with nitrogen resources (Davy et al. 2002). Additionally, the impact of disease on sponges is important. Diseases such as orange band disease can have dramatic effects on sponge populations in an area (Cowart et al. 2006), and with new diseases such as *Aplysina* Red Band Syndrome (Olson et al. 2006) being discovered, it is important to understand how they will affect the sponge community.

The current study investigated how several of these stressors individually and in combination affect a common Caribbean branching sponge *Aplysina cauliformis*. The first part of this study investigates the physiological effects of elevated nutrients and macroalgal contact both together and separately. The second part of this study further investigated the sponge-macroalgal interaction to determine a mechanism for algal facilitation by the sponge, and investigated algal contact effects on carbon and nitrogen cycling within the sponge. The last part of this study addresses two stressors: hurricanes and *Aplysina* Red Band Syndrome (ARBS). This section builds on previous knowledge of ARBS and investigates within-population transmission mechanisms for ARBS using spatial analysis and how the occurrence of Hurricane Irene impacted disease transmission and the *A. cauliformis* population.

CHAPTER 1: COMPLEX ECOLOGICAL ASSOCIATIONS: EVIDENCE FOR COMPETITION AND FACILITATION IN A SPONGE-ALGAL INTERACTION

Abstract

Over the past few decades, Caribbean coral reefs have undergone a phase shift from coral dominated communities to algal dominated communities. This phenomenon has been attributed to many different factors, one of which is increased nutrients from local anthropogenic inputs of fertilizers and sewage. Coral reefs typically thrive in oligotrophic conditions, but nutrient enrichment can lead to dramatic changes in community structure. With coral cover declining, sponges have become more dominant members of Caribbean coral reef communities. Increased algal and sponge dominance on Caribbean reefs has increased the frequency of interaction between these two functional groups. This study used a factorial design to assess the independent and interactive effects of contact and elevated nutrient levels on two common members of these communities, the sponge, *Aplysina cauliformis*, and the macroalga, *Microdictyon marinum*. Algal contact had a significant negative effect on *A. cauliformis*, affecting both the host sponge and its cyanobacterial photosymbionts. While elevated nutrient levels had some positive effects on the sponge photosymbionts, this only occurred in the absence of algal contact or a shading/abrasion control, and elevated nutrient levels had a negative effect on the sponge holobiont. In contrast, *M. marinum* responded positively to both elevated nutrients, and to sponge contact under natural nutrient regimes, but was not affected by sponge contact under elevated nutrient concentrations. Thus, while *A. cauliformis* facilitates increased productivity in *M. marinum*, algal contact competitively inhibits the sponge.

Keywords

Anthropogenic nutrients, Caribbean coral reefs, Competition, Facilitation, Macroalgae, Sponge

Introduction

Predation and pathogenesis are recognized as critical ecological processes shaping coral reef communities; however, competition and facilitation are gaining increasing attention as important forces in coral reef structure and function (Bruno and Bertness 2000, Connell et al. 2004). Competition occurs when shared requirements for a limiting resource force individuals to interact, leading to an increase in fitness of one individual at the expense of the other (Crawley 1986). Facilitation, on the other hand, is an interaction between organisms that benefits at least one organism without harming the other (Bruno et al. 2003a). Most macroalgal-coral interactions have been classified as competitive, with the algae negatively affecting the coral through shading, abrasion, or allelopathy (McCook et al. 2001). Allelopathy in macroalgae is well documented (Rasher and Hay 2010, Rasher et al. 2011, Shearer et al. 2012), but in many cases, the faster growing algae are able to simply overgrow and shade and/or abrade the coral colony (McCook et al. 2001, River and Edmunds 2001). Even though competition appears to be important in structuring reef communities, there is increasing evidence that facilitation is also important. Facilitation has been documented in many marine habitats (Ellison et al. 1996, Stachowicz 2001, Bruno et al. 2003a), including coral reefs (Hill 1998, Bruno et al. 2003a, Cebrian and Uriz 2006, Gochfeld 2010). With the continuing decline of coral cover in the Caribbean over the past few decades (Hughes 1994) leading to the increased dominance of algae and sponges on these reefs (Díaz and Rützler 2001, Bell 2008, Maliao et al. 2008), it is important to understand the consequences of increasingly frequent interactions between macroalgae and sponges.

Nutrients are essential to coral reefs, although they can also act as stressors and potential causes of phase shifts in reef communities (Littler and Littler 1984, McCook 1999). For

example, nitrogen is often a limiting resource on pristine coral reefs, with typical concentrations below 1 µM (Lapointe 1997). Nutrients can originate from a variety of sources, both natural and anthropogenic. Tidal bores can bring up nutrient-rich deep water to shallow reefs, providing periodic pulses of high nitrate concentrations to coral reef communities (Leichter et al. 2003). Additionally, nitrogen fixation makes a significant contribution to the amount of "new" nitrogen available to coral reefs. This process is exclusively prokaryotic, but it also occurs in microbial symbionts associated with other reef organisms such as sponges (Fiore et al. 2010). In fact, sponges that harbor nitrifying and nitrogen fixing microbial communities contribute a large part of the available nitrogen on coral reefs (Fiore et al. 2010). In some areas, anthropogenic inputs of nutrients through groundwater seepage and runoff can elevate nitrate concentrations above normal ranges, resulting in dramatic changes to coral reef community structure (Lapointe 1997, McCook 1999); notably, the algal cover observed throughout the Caribbean (Lapointe et al. 2004). While coral reefs are able to deal with periodic increases in nutrients, with human populations in coastal areas continuing to increase at a rapid rate, understanding anthropogenic influences of elevated nutrients on coral reef communities is increasingly important.

Sponges represent much of the species diversity found in coral reef communities (Díaz and Rützler 2001; Bell 2008), and with the decline in coral cover, sponges now make up a much larger proportion of the coral reef community (Maliao et al. 2008). These organisms occupy many niches on coral reefs, and they provide habitat for other reef organisms, stabilize substrata, and are sources of food (Bell 2008). Sponges also interact with other sessile organisms in a variety of ways, both as competitors (Engel and Pawlik 2005, González-Rivero et al. 2011) and facilitators (Hill 1998, Cebrian and Uriz 2006, Wulff 2006a). Many sponges are important sources of primary productivity on coral reefs due to their cyanobacterial photosymbionts (Erwin

and Thacker 2007, Freeman and Thacker 2011), and are major sources of nitrogen (Weisz et al. 2007, Southwell et al. 2008, Fiore et al. 2010). Due to their role in nitrogen cycling, some sponges survive at elevated nutrient levels (Richter et al. 2001), suggesting that they might be able to tolerate nutrient concentrations that could represent potential stressors to other reef organisms. This study examined interactions between *Aplysina cauliformis*, a common branching sponge species on Caribbean reefs, and *Microdictyon marinum*, a green alga that occupies large areas of substratum on reefs in the Bahamas during the summer months. This study further investigated the consequences of sponge-algal contact on the health of both organisms, and assessed the effect of anthropogenic nutrients on these interactions.

Methods

Study sites: This study was conducted at the Perry Institute for Marine Science on Lee Stocking Island (LSI), Exuma Cays, Bahamas, during May and June of 2009. Field experiments and surveys were conducted at Big Point (N 23˚ 47.301', W 76˚ 08.118') and Rainbow Gardens (N 23˚ 47.778', W 76˚ 08.789'), two shallow reef sites (3-5 m depth) near LSI. All sponge and algal samples were collected from North Norman's reef (N 23˚ 47.388', W 76˚ 08.273'), 1 km north of Big Point, from a depth of approximately 5 meters.

Field surveys: To measure the frequency of interaction between the sponge *A. cauliformis* and the green alga *M. marinum*, surveys along 12 band transects (10 m x 2 m) were conducted at Big Point and Rainbow Gardens in May 2009 (n = 6 per site). Along each transect, all sponges were counted, as well as discrete clumps of *M. marinum* and every organism that was in contact with this alga. The diversity of the sponge community was compared between sites from these band transects. The visible outcomes (i.e., abrasion, pigment change, tissue necrosis) of each contact

between *A. cauliformis* and *M. marinum* were also recorded. Additionally, percent cover of *M. marinum* was quantified on these transects using a point-intercept method by recording the species under the transect line every 10 centimeters.

Contact experiment: To test whether contact elicits a measureable physiological effect on the sponge or the alga, *A. cauliformis* and *M. marinum* were collected and acclimated to the laboratory at LSI in individual containers with flow through seawater for 2 days prior to the experiment. The sponges were cut into 10 cm pieces and the algae were separated into clumps of approximately 10 x 10 cm. Initial weights, measurements, and photographs were taken for both sponges and algae, and they were then randomly assigned to one of four treatment groups (n=10 per treatment): 1) sponge alone, 2) algae alone, 3) sponge-algae in contact, and 4) sponge with a shade/abrasion control. The shade/abrasion control was composed of three layers of plastic coated window screen, which resembled the mesh-like morphology and texture of the algae. This screen provided the shading equivalent of an average piece of algae in the sponge-algae contact treatment, as determined by light level measurements with a light meter (LI-COR®, USA) above and underneath algae pieces. In addition to providing shade, the contact between the sponge and window screen simulated potential abrasion by the algae.

Each algal or sponge individual, or pair, was attached to a 20 x 20 cm plastic rack using cable ties. These racks were attached to the substrate at Big Point, at a depth of approximately 5 meters, and left in the field for 4 weeks. At the end of the experiment, racks were placed in re-sealable plastic bags filled with seawater and returned to the lab at LSI, where sponges and algae were removed from the racks, weighed and measured. Small pieces of each sponge (0.5 cm thick

cross-sections) were preserved for measurements of chlorophyll *a* concentration and cyanobacterial symbiont density as measures of cyanobacterial symbiont condition, and total protein concentration as a measure of the holobiont (sponge host and cyanobacteria) condition. Algae were preserved for chlorophyll *a* concentration measurements. All samples were frozen or preserved (see below) for transport to the University of Mississippi (UM) for further analysis.

Nutrient Experiment: To determine the effect of elevated nutrient levels on the interaction between *A. cauliformis* and *M. marinum*, we performed a factorial designed field experiment that investigated the effects of contact and nutrients on algae and sponges in isolation and together. *A. cauliformis* and *M. marinum* were collected and brought back to the lab on LSI, where they were maintained as described above. Initial weights, measurements, and photographs were taken. The next day, organisms were randomly assigned to 12 treatments (n = 10 replicates for each treatment). Each of the four treatments used in the contact experiment (i.e., sponge alone, algae alone, sponge-algae contact, and the shade/abrasion control; see above) was exposed to one of three nutrient doses. Nutrients were delivered using 10 grams of 14-14-14 (N-P-K) Osmocote® slow release fertilizer (Scotts) in packets made of window screen (Thacker et al. 2001; Gochfeld et al. 2012a). Nutrient dose was regulated by varying the distance of samples attached to plastic racks from the nutrient pack. The high dose, at 5 cm from the nutrient pack , was quantified as 0.22 ± 0.11 µM NO_3 and 0.24 ± 0.08 µM PO_4 after 1 day of enrichment (Gochfeld et al. 2012a), and the medium and low doses were dilutions at 25 cm and 50 cm from the nutrient pack, respectively. Nutrient packs were replaced every 7 days for 4 weeks to maintain the approximate nutrient dose over the course of the experiment. After 4 weeks, the racks were collected and returned to the lab, where cyanobacterial symbiont population condition was assessed via Pulse

Amplitude Modulated (PAM) fluorometry (Diving-PAM; Walz, Germany) measurements on dark-adapted organisms on the night of collection. Sponges and algae were then removed from the racks, weighed, measured and photographed. As in the contact experiment, sponges and algae were processed for further analysis at UM.

Allelopathy experiment: To determine whether algal allelopathy played a role in the sponge-algal interaction, we performed an assay similar to that described in Thacker et al. (1998). *M. marinum* was collected and the volume to wet weight ratio was calculated using displacement volume. The alga was then lyophilized and extracted in 1:1 dichloromethane (DCM): methanol. We obtained a crude extract yield of 23.55 grams in 1.018 L of algal tissue (= 1.13g/ml). The extract was added at natural volumetric concentration to 50 ml of a 5% molten agar solution. An equivalent amount of the carrier solvent was added to the control agar. These solutions were poured into plastic molds backed with window screen to form 6 treatment strips and 6 control strips measuring 2 cm by 6 cm. The agar strips were allowed to harden onto the window screen, and were attached to *A. cauliformis* branches on the reef with cable ties. Each *A. cauliformis* branch (n = 6) had one control and one treated gel strip spaced at least 10 cm apart. After one week, the *A. cauliformis* branches were collected and brought back to the lab. That evening, strips were removed, PAM readings were taken on the sponge tissue under each gel (Pawlik et al. 2007), and the samples were wrapped in foil and preserved for further analysis of chlorophyll *a* at UM.

Chlorophyll a concentration: To assess photosynthetic potential of the sponges' photosymbionts, chlorophyll *a* was measured from frozen foil-wrapped samples using methods described in Erwin and Thacker (2007), except that sponge and algae pieces in this study were lyophilized prior to

extraction. Briefly, 0.25 g wet weight of *A. cauliformis* or *M. marinum* was placed in a foil-wrapped glass vial with 10 ml of 90% acetone for 18 hours at 4˚C. Extracts were then transferred to quartz cuvettes and the absorbance of each extract was quantified at 750 nm, 664 nm, 647 nm and 630 nm on an Agilent 8453 spectrometer. Chlorophyll *a* concentrations were calculated using formulas from Parsons et al. (1984) and standardized to the mass of the extracted sponge or algae (µg chlorophyll *a* / mg sponge or algal tissue).

Fluorescent yield: PAM fluorometry was used to measure photosynthetic efficiency of the algae and the sponge's photosymbionts (Gochfeld et al. 2012a). The Diving-PAM provides the organism with an actinic flash of light and measures the maximum fluorescent yield from the organisms' photo-system II. This is determined by subtracting the minimum fluorescence (F_0) from the maximum fluorescence (F_m) to calculate variable fluorescence (F_v), and, subsequently, by dividing this value by F_m to obtain the maximum quantum yield (F_v/ F_m). Three measurements were taken at a standardized distance from different locations on the sample, and the three measurements were then averaged. The samples were measured in seawater raceways at least 1 hour after dark in order to maximize the ability of the photochemical pathways to absorb light energy (Fitt et al. 2001). PAM measurements were collected after samples were retrieved from the field at the end of the nutrient experiment and the allelopathy experiment.

Cyanobacterial symbiont density: Symbiont density was quantified using the methods outlined in Freeman and Thacker (2011). Sponges were preserved in 4% paraformaldehyde in 2 ml

cryovials at 4˚C for 24 hrs. The paraformaldehyde was then removed and replaced with a 70% ethanol solution. The samples were further dehydrated and embedded in paraffin wax. Cross sections (20 µm) were cut and mounted onto glass slides. Each sample was viewed at 1000X magnification under oil immersion using an epifluorescence microscope. Ten photographs of each sample were taken on haphazardly chosen areas of the sponge sections. The number of cyanobacterial cells was counted using the "analyze particles" feature in Image J software (NIH). For each image, total cyanobacterial cell number was counted, and values from all ten images were averaged to calculate the mean number of cells in a viewing area of 2886 μm^2.

Protein concentration: Protein content was measured as a proxy for holobiont health in lyophilized sponge samples using the Bradford assay (1976). Briefly, 5 ml of 1M NaOH was used to extract 10 mg of lyophilized sponge tissue for 18 hours, after which 100 µl of each sample was added to a test tube with 5 ml of Quick Start™ Bradford Dye Reagent (Bio-Rad). The absorbance of each sample was measured at 595nm using a BioPhotometer V .032 (Eppendorf) and then plotted against a standard curve developed from a bovine serum albumin sample. Protein concentrations were then standardized to the dry weight of each sponge sample in order to calculate µg protein per mg sponge tissue.

Sponge and algae growth: Sponge and algae weights were recorded initially and at the end of each experiment. Organisms were briefly blotted with a paper towel to remove excess water before weighing. Percent change in weight was calculated using the following formula: [(final weight-initial weight)/initial weight]*100.

Data analysis: The percent of *M. marinum* in contact with *A. cauliformis*, and other organisms, as well as proportions of each contact outcome, were calculated from field surveys. Average percent cover of *M. marinum* among transects was determined from the line-intercept data, and the proportion of *A. cauliformis* in the sponge community was calculated from the sponge community band transects. Sponge communities at each site were compared with unpaired t-tests. The observed number of *M. marinum* contacts with sponge and coral species was compared to the expected number of *M. marinum* contacts with *A. cauliformis* using a Chi-square analysis. Percent change in sponge and algae weights were arcsine transformed and the treatments were compared using a one-way analysis of variance (ANOVA) for the contact experiment and a two-way ANOVA, with nutrient dose and contact treatment as the fixed factors, for the nutrient experiment. For *A. cauliformis* in the contact experiment, chlorophyll *a* concentration, cyanobacterial symbiont density, and total protein concentration were analyzed using one-way ANOVAs. These end-points, along with fluorescent yield, were analyzed for the nutrient experiment using two-way ANOVAs, with two exceptions. Due to sample loss, cyanobacterial symbiont density was analyzed using a one-way ANOVA, and within the sponge alone treatment in the nutrient experiment, chlorophyll *a* concentrations were analyzed for the effects of nutrient dose using a one-way ANOVA. Chlorophyll *a* concentrations from algae in the competition experiment were analyzed using an unpaired t-test and a two-way ANOVA in the nutrient experiment. In the allelopathy experiment, chlorophyll *a* concentrations and fluorescent yield measurements in *A. cauliformis* were analyzed using paired t-tests. For each experiment, Tukey's HSD post-hoc tests were used to detect differences.

Results

Field surveys: Percent cover of *M. marinum* was 24.0 ± 7.7% at Big Point. At Rainbow Gardens, where *M. marinum* only occurred on one transect, where its percent cover was measured as 1.2 ± 1.2 %. Surveys of the sponge populations at each study site showed a diverse community composed of at least 22 species (Supp. 1). Abundance of these species varied between sites, and *A. cauliformis* comprised 52.0 ± 3.7% (393 individuals) of all sponges at Big Point and 12.0 ± 3.2% (182 individuals) of all sponges at Rainbow Gardens. Both sites combined constituted 506 discrete patches of *M. marinum*, of which 214 were in contact with other reef organisms. Most of these contacts (92%) occurred at Big Point where abundance and percent cover of *M. marinum* was higher. Of the contacts at Big Point, 37% were with *A. cauliformis*. At Rainbow Gardens, where *M. marinum* abundance was low, 38% of all algal contacts were with *A. cauliformis*. In these surveys, *A. cauliformis* had a significantly higher number of contacts with *M. marinum* compared to all other sponge species surveyed (Chi-Square, df = 1 $P < 0.0001$) and compared to all coral species (Chi-Square, df = 1, $P < 0.0001$) except for *Montastrea annularis* (Chi-Square, df = 1, $P = 0.78$; Fig. 1). The effects of these *A. cauliformis-M. marinum* contacts varied, with 70% resulting in darker sponge pigmentation at the point of contact, 7% resulting in abrasion damage to the sponge, and 23% showing no visible effects on the sponge.

Chlorophyll a concentration: In the contact experiment, chlorophyll *a* concentrations in *A. cauliformis* were significantly affected by algal contact (ANOVA, df= 2, F= 3.52, $P = 0.044$), as

shown in Fig. 2A. Contact with *M. marinum* resulted in significantly lower chlorophyll *a* concentrations in *A. cauliformis* compared to the shading/abrasion control, and compared to the sponge alone (Tukey's HSD post-hoc test, $P < 0.05$). Chlorophyll *a* concentrations in *M. marinum* in the contact experiment were significantly affected by sponge contact, resulting in a higher chlorophyll *a* concentration (0.026 ± 0.002 µg chlorophyll *a* mg^{-1} algal tissue) than the algae alone (0.021 ± 0.0009 µg chlorophyll *a* mg^{-1} algal tissue; ANOVA, df= 1, F= 7.22, P = 0.020).

In the nutrient experiment, chlorophyll *a* concentrations in *A. cauliformis* were also significantly affected by algal contact (two-way ANOVA, df= 2, F= 8.74, P = 0.0005), as seen in Figure 3A. Sponges in the algal contact and shading/abrasion control treatments had significantly lower chlorophyll *a* concentrations than did the sponge alone (Tukey's HSD post-hoc test, $P < 0.05$). Overall, there were no significant effects of nutrients (two-way ANOVA, df= 2, F=0.93, P = 0.40) or interactions (two-way ANOVA, df= 4, F= 0.97, P = 0.43) across treatments. However, within the sponge alone treatment, there was a significant effect of nutrient dose (one-way ANOVA, df= 2, F= 4.16, P = 0.027, Figure 3A), and chlorophyll *a* content increased significantly as the nutrient dose increased (Tukey's HSD post-hoc test, $P<0.05$). Algal chlorophyll *a* concentrations in the nutrient experiment also increased significantly with increasing nutrient dose (ANOVA, df= 2, F= 9.56, P = 0.0003, Fig. 4). There was no significant effect of contact with the sponge (ANOVA, df= 1, F= 1.48, P = 0.23 for treatment) or interactive effects of contact treatment and nutrient dose (ANOVA, df= 2, F= 0.35, P = 0.71) on chlorophyll *a* concentrations of the algae.

In the allelopathy experiment, chlorophyll *a* concentrations in *A. cauliformis* showed no differences between the solvent control (0.24 ± 0.015 µg chlorophyll *a* mg^{-1} sponge tissue) and the *M. marinum* extract treatment (0.22 ± 0.002 µg chlorophyll *a* mg^{-1} sponge tissue, paired t-test df = 5 P = 0.19).

Fluorescent yield: Fluorescent yield measured in *A. cauliformis* was significantly affected by contact treatment. Fluorescent yield of *A. cauliformis* in the algal contact treatment was significantly lower than in either the shade/abrasion or sponge alone treatments (two-way ANOVA, df= 2, F= 10.90, P = 0.0001, Fig. 3B). There were no significant effects of nutrient dose (two-way ANOVA, df= 2, F= 0.46, P = 0.63) or interaction (two-way ANOVA, df= 4, F= 0.49, P = 0.74) on fluorescent yield in *A. cauliformis*. Fluorescent yield in *M. marinum* was not significantly affected by treatment (two-way ANOVA, df= 1, F= 0.63, P=0.44), nutrient dose (two-way ANOVA, df= 2, F= 0.054, P= 0.95), or their interaction (two-way ANOVA, df= 2, F= 0.79, P= 0.47). Due to an equipment malfunction, sample sizes for PAM measurements in the nutrient experiment were reduced to 5-7 in each treatment. In the allelopathy experiment, fluorescent yield of *A. cauliformis* was not affected by contact with *M. marinum* extract (mean ± 1SE = 415.6 ± 22.72 for controls, and 409.01 ± 21.01 for extract treatments: paired t-test, df = 5, P = 0.8232).

Cyanobacterial symbiont density: Cyanobacterial symbiont density in *A. cauliformis* was significantly reduced by shading and by algal contact in the contact experiment (one-way

ANOVA, df= 2, F= 9.63, P = 0.0011, Fig. 2B). Cyanobacterial symbiont density was reduced by shading and algal contact, compared to the sponge alone treatment (one-way ANOVA, df= 2, F= 4.999, P = 0.011, Fig. 3C). Cyanobacterial density was not significantly affected by nutrients (one-way ANOVA, df= 2, F= 0.6326, P= 0.536), nor were there significant interactions (one-way ANOVA, df= 4, F= 0.26, P= 0.90) across the treatments. Reduced sample sizes were utilized in all treatments and doses in both experiments due to sample loss; final sample sizes are shown in Figures 2B and 3C.

Total protein concentration: In the contact experiment, protein concentrations in *A. cauliformis* were not significantly different among treatments (mean ± 1SE = 413.5 ± 25.5 µg protein mg^{-1} sponge for the sponge alone treatment, 436.7 ± 39.9 protein mg^{-1} sponge for the shade control treatment, and 377.9 ± 15.6 protein mg^{-1} sponge for the algal contact treatment; ANOVA, df= 2, F= 1.06, P = 0.36). Total protein concentration in *A. cauliformis* in the nutrient experiment was significantly affected by contact treatment (two-way ANOVA, df= 2, F= 12.01, P < 0.0001), and nutrient dose (two-way ANOVA, df= 2, F= 26.04, P < 0.0001), but there were no interactive effects (two-way ANOVA, df= 4, F= 1.75, P = 0.15). In the nutrient experiment, the sponge alone treatment had significantly higher protein concentrations compared to the other two treatments, and sponges at the low nutrient dose had significantly higher total protein concentrations than at the high and medium doses (Tukey's HSD post-hoc test, P<0.05, Fig. 3D).

Sponge and algae growth: Percent change in sponge weight was not significantly affected by algal contact (mean ± 1SE= 0.001 ± 0.01 % for sponge alone treatment, 0.01 ± 0.02 % for shade control treatment, and 0.005 ± 0.01 % for algal contact treatment; one-way ANOVA, df= 2, F= 0.08, P= 0.92). Growth of algae in the contact experiment was not affected by sponge contact (mean±1SE = 0.47 ±0.08 percent change for the algae alone treatment and 0.39 ± 0.06 percent change in weight for the sponge contact treatment; one-way ANOVA, df= 2, F= 0.62, P= 0.44).

In the nutrient experiment, percent change in sponge weight was not affected by algal contact (two-way ANOVA, df= 2, F= 0.42, P = 0.66), nutrient dose (two-way ANOVA, df= 2, F= 0.28, P = 0.76), or their interaction (two-way ANOVA, df= 4, F= 1.34 P = 0.26). Percent growth of sponges (mean±1SE) in the sponge alone treatment was 0.08 ± 0.14 %, -0.06 ± 0.09 %, and 0.02 ± 0.03 % for the low, medium and high nutrient doses, respectively. Percent growth of the sponge (mean±1SE) in the shade control was 0.01 ± 0.01 %, 0.02 ± 0.02 %, and 0.04 ± 0.04 % for the low, medium and high nutrient doses, respectively. Sponges in the algal contact treatment had a percent change in weight (mean±1SE) of 0.02 ± 0.04 %, 0.09 ± 0.06 %, and 0.02 ±0.02 % for the low, medium and high nutrient doses, respectively. Algal growth in the nutrient experiment was also not significantly affected by sponge contact (two-way ANOVA, df= 1, F= 2.20, P = 0.14), nutrient dose (two-way ANOVA, df= 2, F= 0.86, P = 0.43), or the interaction of the two factors (two-way ANOVA, df= 2, F= 0.74, P = 0.48). Percent growth in algae (mean±1SE) from the algae alone treatment was 0.28 ± 0.15 %, 0.22 ± 0.06 %, and 0.29 ± 0.04 % for low, medium and high nutrient doses, respectively. While percent growth of algae (mean±1SE) in the sponge contact treatment was 0.14 ± 0.07 %, 0.15 ± 0.06 %, and 0.15 ± 0.06 % for the low, medium and high nutrient doses, respectively.

Discussion

This study identified a complex interaction between *A. cauliformis* and *M. marinum,* in which both competition and facilitation play a role. While the alga caused a negative, competitive effect on the sponge, the sponge appeared to facilitate increased productivity in the alga. Elevated nutrient concentrations benefited the alga, and had a positive effect on the sponge symbionts, in the absence of other stressors. However, nutrient addition eliminated the beneficial effect of the sponge on the alga, suggesting that the positive effect on the alga may be caused by nutrient transfer from the sponge to the alga. These results are summarized in Table 1.

Studies of algal interactions with other reef species have largely focused on corals, and have largely been labeled as competitive interactions (reviewed in McCook et al 2001), but sponge interactions with other coral reef organisms have been gaining attention (Davis et al. 1997, Baldacconi and Corriero 2009, González-Rivero et al. 2011, Pawlik 2011). Despite their high abundance on Caribbean coral reefs, few studies have investigated the effects of sponge-algal contact *in situ* (López-Victoria et al. 2006, González-Rivero et al. 2012). In one such study, Gonzalez-Rivero et. al. (2012) showed that contact with the brown alga *Lobophora variegata* had a negative effect on *Cliona tenuis* through reduction of its lateral growth rate. The success of algal interactions with other reef organisms appear to rely on three mechanisms; shading, abrasion, and allelopathy (McCook et al. 2001, River and Edmunds 2001), although more recently, the effects of dissolved organic matter and microbial interactions have been recognized (Smith et al. 2006, Barott et al. 2012). Many algae possess allelopathic compounds that can damage competitors (de Nys et al. 1991), and while *M. marinum* can have allelopathic effects on the hard coral *Montastraea annularis* (Easson and Gochfeld unpubl. data), an allelopathic effect

was not observed against *A. cauliformis*. This difference could be due to a temporal factor, as the allelopathy experiment in the current study lasted just one week, whereas the contact and nutrient experiments in the current study ran for a longer time period, as have other algal allelopathy studies (Rasher and Hay 2010, Rasher et al. 2011). Alternatively, *A. cauliformis* may have some resistance to allelopathic compounds from *M. marinum*, or the seasonality of the alga may provide periodic relief from any stress associated with algal contact, but to date, long term population effects of *M. marinum* contact are unknown.

Microdictyon marinum can grow as a canopy over the substrate during the summer months, enabling it to shade other reef organisms (Kramer et al. 2003; Peckol et al. 2003). In this way, *M. marinum* may be able to overgrow small and repent growth forms of *A. cauliformis*, as well as shading and possibly weakening the bases of large upright *A. cauliformis*. Because *A. cauliformis* has been documented to receive up to 75% of its energy budget from its photosymbionts (Freeman and Thacker 2011), a reduction in irradiance would likely decrease the energy resources available to the sponge. Even under shaded conditions, *A. cauliformis* maintained its relationship with its symbionts, receiving the majority of its carbon and nitrogen from them (Freeman and Thacker 2011). The maintenance of this relationship coupled with the reduction in symbiont abundance observed in this study could lead to a reduced energy budget in the host. Our survey data lend further support to shading as a competitive mechanism in this algal-sponge interaction. At Big Point, which has high algal cover, there was a significantly lower number of small and encrusting sponge species compared to Rainbow Gardens, the low algal cover site. The results of this study suggest that shading may be a major stressor resulting from algal contact, although the sponges were able to maintain high chlorophyll *a* concentrations in the shade control treatment, which is suggestive of higher productivity, despite lower

cyanobacterial abundance. Sponges were not able to compensate for this shading in the algal contact treatment, suggesting a more complex interaction between the alga and the sponge than just a shading effect. While sponge growth was not affected in the current study, many factors can affect holobiont growth rate including season, and the biochemical endpoints used in this study represent responsive proxies for sponge health.

Whereas *M. marinum* caused several negative effects on the sponge, this interaction enhanced productivity in the algae. *A. cauliformis* possesses a diversity of chemical defenses (Puyana et al. 2003, Pawlik 2011, Gochfeld et al. 2012b), yet there was no evidence that *A. cauliformis* released allelopathic compounds that damaged *M. marinum*. Instead, the sponge appears to facilitate increased productivity in the algae, potentially by leaching nutrients that the algae could absorb when in contact with the sponge (Slattery et al. 2013). Facilitation would likely lead to increased growth in the algae, which was not observed in the current study. *M. marinum* is a very brittle alga, and given this property, growth (biomass change) may not be as reliable a metric of algal health in this species as measuring algal productivity.

This study demonstrated that increased nutrient levels enhanced the condition of the algae as measured by chlorophyll *a* concentration, supporting studies that implicate nutrients as a cause of increased algal abundance on reefs (Bell 1992, Lapointe 1997, Littler and Littler 2006). In contrast, Szmant (2002) has argued that evidence for nutrient enrichment directly causing increased algal abundance and decreased coral abundance on reefs is lacking. Furthermore, published results showing nutrient effects often use concentrations that are orders of magnitude higher than ever found on a reef. The present study used nutrient concentrations documented in previous field experiments with *A. cauliformis* (Gochfeld et al. 2012a), and showed that while nutrients benefited the sponges' photosymbionts, as exhibited by increased chlorophyll *a* content

in the sponges, they also led to reduced sponge holobiont health, as demonstrated by the reduction in total protein content. This dichotomy could be due to reduced sponge metabolic activity, or it may be a symptom of nutrient stress changing the sponge-symbiont relationship. While this was not observed in *A. cauliformis* under shading stress (Freeman and Thacker 2011), the addition of nutrients may release the symbionts' dependence on host derived sources of nitrogen (reviewed in Fiore et al. 2010). However, *M. marinum* does not take over these reefs in the presence of excess nutrients. This may be partially due to localized herbivory on reefs that have not been overfished (*sensu* Hughes 1994, Burkepile and Hay 2006), but it also seems to be due to the loss of algal tissue during autumn storms and/or winter senescence (Gochfeld and Easson unpublished data).

While it is important to understand the consequences of sponge-algal interactions and of elevated nutrients, it is also important to understand how these potential stressors function together. In a coral-algal interaction, Jompa and McCook (2002) found that increased nutrient loads increased growth of the alga, which subsequently caused greater coral tissue mortality, although a high level of herbivory was able to mask the effects of elevated nutrients in this study. Slattery et al. (2013) observed a similar response in a natural experiment that assessed the impact of cave sponge nitrate addition to nearby patch reefs. Additionally, elevated nutrients can interact with other stressors that cause coral reef decline, such as disease progression (Bruno et al. 2003b, Voss and Richardson 2006, but see Gochfeld et al. 2012a). In the case of this study, presenting these stressors both separately and in combination enabled us to discern a potential mechanism for facilitation of the algae. The alga in this study benefited from contact with the sponge, but only in the absence of added nutrients. When nutrient levels were elevated, the alga exhibited no difference in productivity between the sponge contact and algae alone treatments.

This suggests that contact with the sponge may provide the alga additional nutrients that enhance algal productivity. Many sponges harbor nitrifying bacterial communities (Fiore et al. 2010) and leach nitrogen onto the reef, and *A. cauliformis* produces a net efflux of nitrogen (Southwell et al. 2008). This efflux of nitrogen from the sponge could facilitate algal productivity, whereas when additional inorganic nutrients were provided, the interaction was no longer beneficial to the algae. While the addition of nutrients did not appear to alter the algae's impact on sponge condition, elevated nutrients reduced the sponges' ability to compensate for reduced irradiance in the shade control, causing these sponges to group with the algal contact treatment rather than the sponge alone treatment in terms of chlorophyll *a* concentrations. The protein data from the nutrient experiment also suggests that nutrient addition and algal contact, both separately and in combination, elicited negative effects on the sponge. Whereas cyanobacterial abundance was unaffected by nutrient addition, protein concentration in the sponge holobiont was inversely proportional to nutrient dose. These data suggest that the observed protein reduction was likely related to sponge host condition rather than symbiont condition.

The results of this study indicate that species interactions on reefs can be very complex. The interaction between *A. cauliformis* and *M. marinum* exhibits characteristics of both competition and facilitation. While *M. marinum* clearly benefits from contact with *A. cauliformis*, our survey data suggest that sponge contact is not required for this alga to be prolific on the reef. In contrast, the alga has a detrimental competitive effect on the sponge. This study explored the consequences of a sponge-algal interaction on shallow reefs in the Bahamas, though many important questions about this interaction and other similar ones still need to be answered to truly understand their long term consequences for these organisms.

Table 1. Results Summary of sponge and algal health endpoints from the contact and nutrient experiments. Positive (facilitation) effects are shown underlined in the table. N.S. represents non-significant responses (P> 0.05), and --- represent untested responses.

	Contact Experiment	Nutrient Experiment		
	Algae/Sponge contact	Algae/Sponge contact	Nutrient dose	Interaction
Sponge				
Growth	N.S.	N.S.	N.S.	N.S.
Protein	N.S.	P <0.0001	P < 0.0001	N.S.
Symbionts				
Chlorophyll *a*	P = 0.04	P = 0.0005	N.S.	N.S.
Symbiont density	P = 0.001	P = 0.01	N.S.	---
Fluorescent yield	---	P = 0.0001	N.S.	N.S.
Protein	N.S.	P <0.0001	P < 0.0001	N.S.
Algae				
Chlorophyll a	P = 0.02	N.S.	P = 0.0003	N.S.
Growth	N.S.	N.S.	N.S.	N.S.

Figure 1. Number of individuals in contact with *M. marinum* for each sponge and coral species from surveys at Big Point and Rainbow Gardens.

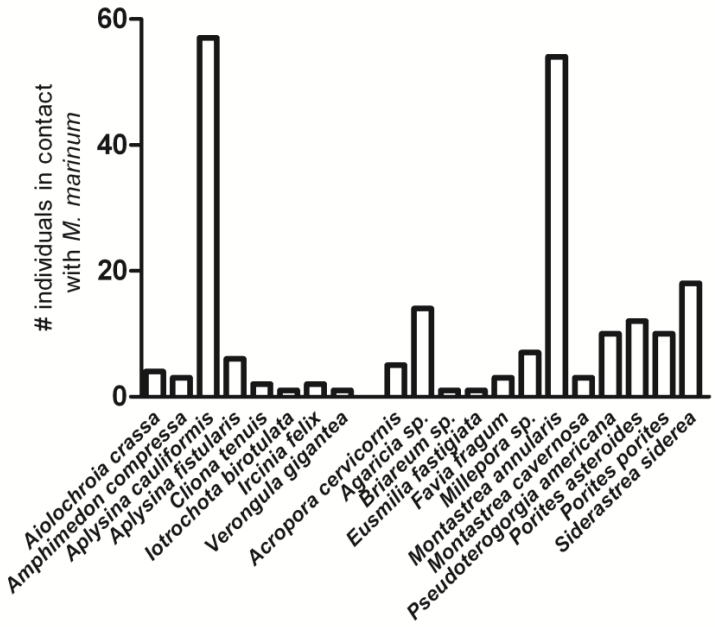


Figure 2. *A. cauliformis*. Mean (± 1SE) (A.) chlorophyll *a* concentration and (B.) cyanobacterial symbiont density after 28 days in contact with algae or a shade/abrasion control in the contact experiment. Histograms with different letter groups are significantly different by ANOVA.

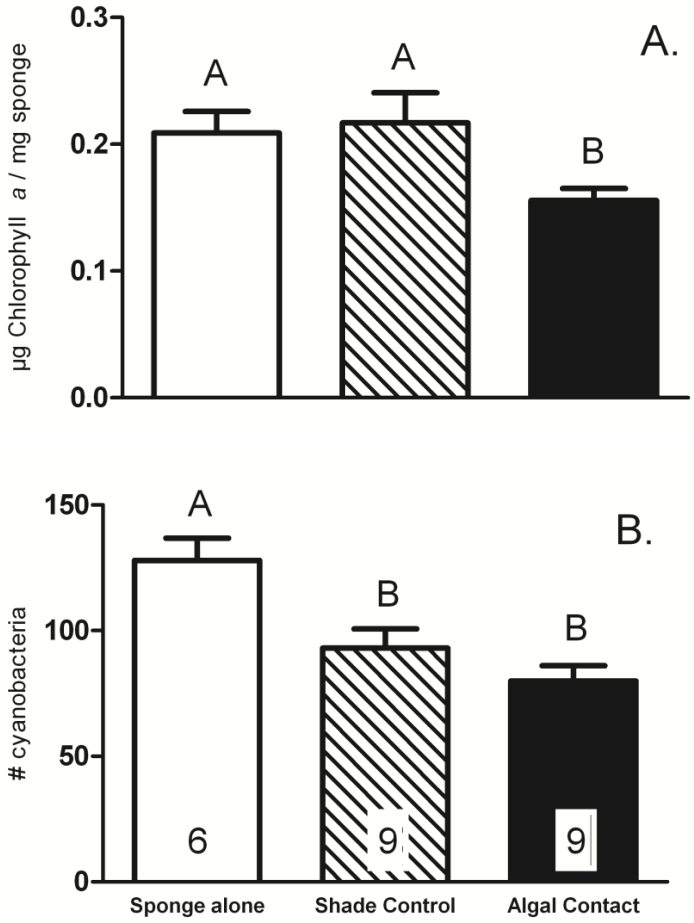


Figure 3. *A. cauliformis*. Mean (± 1SE) (A.) chlorophyll *a* concentration, (B.) cyanobacterial symbiont density, (C.) quantum yield and (D.) protein concentration under manipulations of contact and nutrient dose in the 28 day nutrient experiment. Solid white bars, dashed bars and solid black bars represent the high, medium and low nutrient doses, respectively. Histograms with different letters are significantly different by ANOVA.

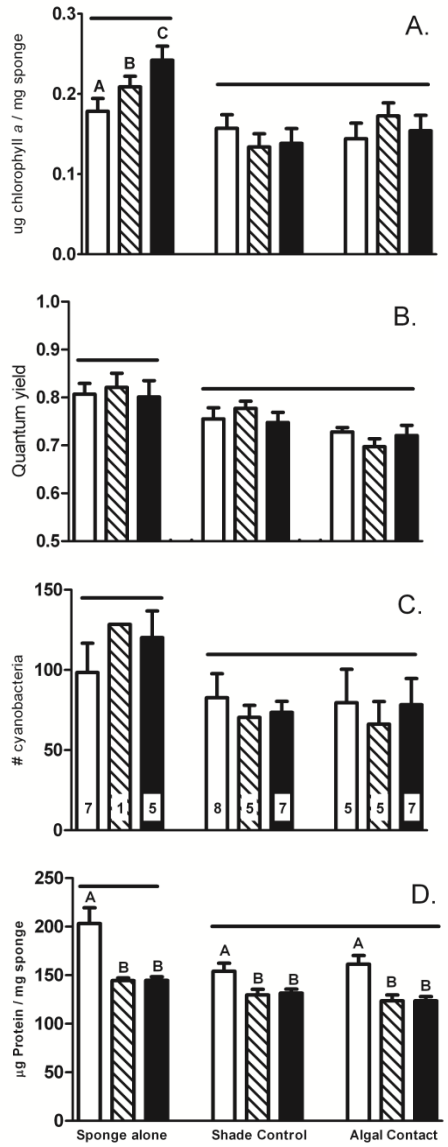
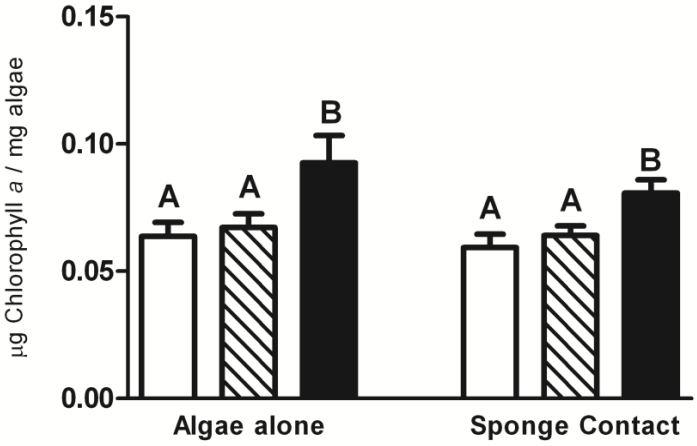


Figure 4. *M. marinum*. Mean (± 1SE) chlorophyll *a* concentration under different treatment conditions after 28 day nutrient experiment. Solid white bars, dashed bars and solid black bars represent the high, medium and low nutrient doses, respectively. Histograms with different letters are significantly different by ANOVA.



CHAPTER 2: CARBON AND NITROGEN DYNAMICS AND TRANSFER IN CARIBBEAN SPONGE-ALGAL INTERACTION

Abstract

As corals in the Caribbean continue to decline, sponges and macroalgae are becoming more dominant members of these communities. Sponges are crucial to carbon and nitrogen cycling on coral reefs, and many species have a net efflux of these nutrients. The current study investigated the carbon and nitrogen dynamics in a sponge-algal interaction as a possible explanation for algal facilitation when in contact with the sponge, as previously observed in Chapter 1. This study tested whether sponge derived carbon and nitrogen resources could be absorbed by macroalgae on the reef, and whether algal contact disrupted carbon and nitrogen dynamics within the sponge. Individuals of the sponge *Aplysina cauliformis* were enriched in seawater solutions containing 98% ^{13}C for carbon and ^{15}N for nitrogen. Pieces of the alga *Microdictyon marinum* were placed in contact with or at two distances away from the sponge in individual aquaria, and sampled at 12 and 24 hours. Each sponge was subsequently separated into bacterial cell fraction and a sponge cell fraction. Isotopic ratios of ^{13}C and ^{15}N were analyzed in each cell fraction, as well as in algal pieces. Sponge samples showed significant enrichment with the heavy isotopes of carbon and nitrogen, but enrichment in both cell fractions was not affected by algal contact over the course of the experiment. Algal samples were significantly enriched with ^{15}N only when in contact with the sponge, but no enrichment of ^{13}C was observed. These results suggest that algal facilitation is likely mediated by transfer of nitrogen resources from the sponge to the alga.

Introduction

Coral reefs have undergone a phase shift over that past couple decades (Hughes 1994). This phase shift has led to an algal dominated reef in many areas that were once dominated by corals (Hughes 1994, Lapointe et al. 2004). One suspected cause for this phase shift in many areas is the input of anthropogenic nutrients onto coral reefs (Littler and Littler 1984, Lapointe 1997, McCook 1999, Lapointe et al. 2004). The results of elevated nutrients in these communities are conditions that favor algal dominance (Lapointe 1997) and increase the opportunity for algae to interact with other reef organisms. Although nitrogen and phosphorus concentrations are typically low in these ecosystems, they do experience pulses of nutrients from natural sources such as tidal bores that bring up nutrient rich deep water onto shallow reefs (Leichter et al. 2003). Additionally, nitrogen cycling by coral reef organisms makes a significant contribution to the limited nitrogen budget on coral reefs (Fiore et al. 2010). While nitrogen cycling is a prokaryotic process, these organisms can be associated with many reef organisms such as sponges and corals, and many other reef compartments (Fiore et al. 2010, Maldonado et al. 2012). With the decline in coral cover across the Caribbean, sponges are becoming more dominant members of these communities, but their broader effects to these shifted communities remain largely unknown.

Marine sponges are an important functional group on coral reefs (Pawlik 2011). With their ability to harbor diverse microbial communities, these organisms have been shown to be important in carbon and nitrogen cycling on coral reefs (Taylor et al. 2007, Weisz et al. 2007, Fiore et al. 2010, Maldonado et al. 2012, Thacker and Freeman 2012). Studies have shown that sponges can harbor microbial communities that are active in all steps of nitrogen cycling

(nitrogen fixation, nitrification, denitrification, anamox) (Fiore et al. 2010, Maldonado et al. 2012). These microbial communities are often essential for absorption and processing many inorganic nitrogen resources (Zehr and Ward 2002, Taylor et al. 2007) and transferring them to the host (Thacker and Freeman 2012), but in some cases, sponges and their associated microbial communities can also transfer these nitrogen resources to other organisms (Ellison et al. 1996, Davy et al. 2002, Pile et al. 2003). Sponge microbial communities also help sponges to access dissolved carbon resources such as bicarbonate (HCO_3^-) (Freeman et al. 2013), and transfer these resources to the host sponge as photosynthates (Wilkinson 1979, Taylor et al. 2007). Sponges play an important role in large scale carbon cycling on reefs through benthic-pelagic coupling of these resources, which supports the increase of several groups of benthic organisms (Lesser 2006). Carbon transport to these benthic organisms could potentially boost primary productivity in this compartment but to date, this potential has not been investigated.

Sponge-macroalgal interactions are becoming more frequent, as macroalgae on coral reefs continues to increase. Coral-macroalgal interactions are well studied, and algae are often demonstrated to have a competitive advantage over the coral through a variety of mechanisms (McCook et al. 2001, Smith et al. 2006). In a previous study we found a complex interaction between a common branching sponge, *Aplysina cauliformis*, and a common green macroalga, *Microdictyon marinum* (Chapter 1). The alga elicited a negative effect on sponge health, by decreasing photosymbiont abundance and reducing circulating soluble protein content within the sponge. In contrast, the sponge had a positive effect on the algal productivity, as measured by chlorophyll *a* concentration, when in contact with the sponge (Chapter 1). Previous studies have shown the ability of marine sponges to facilitate plants and macroalgae through the transfer of nitrogen resources (Ellison et al. 1996, Trautman et al. 2000, Davy et al. 2002, Pile et al. 2003).

Aplysina cauliformis, has been shown to have a net efflux of both ammonium (NH_4^+) and nitrate/nitrite (NO_x^-) (Southwell 2007, Southwell et al. 2008), so we hypothesized that the sponge could be contributing nitrogen and potentially carbon as well, to the alga and causing this increased productivity. The goal of the current study is to investigate a possible algal facilitation mechanism by a common reef sponge, using stable isotope tracers to investigate potential carbon and/or nitrogen transfer from the sponge to the alga. Additionally, since reduced irradiance has been shown to affect carbon and nitrogen dynamics in this sponge species (Freeman et al. 2013), this study investigated whether contact with algae affected the sponge's ability to assimilate carbon and nitrogen resources.

Materials and Methods

This study was conducted at the Perry Institute for Marine Science on Lee Stocking Island (LSI). Exuma Cays, Bahamas, during June 2011. All collections of *Aplysina cauliformis* and *Microdictyon marinum* were from a depth of 5 meters at North Norman's reef (N 23˚ 47.388', W 76˚ 08.273').

NO_3^-/HCO_3^- experiment: To investigate uptake and transfer of carbon and nitrogen sources, *A. cauliformis* and *M. marinum* were collected and acclimated in the laboratory at LSI in separate containers with flow-through seawater for one day. Five individual sponges and five pieces of algae were collected and immediately frozen (t_i). An additional 37 sponges were incubated in a solution containing 1 mg/L 98% $Na^{15}NO_3$ and 98% 1 g/L $Na^{13}HCO_3$ tracers for six hours. After incubation, 5 sponges were collected and frozen (t_0), and 32 sponges were placed into individual containers with flowing seawater for 2 hours to rinse out all non-assimilated tracers from the sponge tissue. After this rinse period, sponges were randomly assigned to two treatment groups.

One group had pieces of algae attached to them using a cable tie, and the other group had pieces of algae placed at 15 cm and 30 cm from the sponge in its tank. After 12 (t_{12}) and 24(t_{24}) hours, eight tanks from each treatment group were collected, and sponges and algae were frozen for further analysis.

NH_4^+ experiment: To investigate the uptake and transfer of an ammonium nitrogen substrate, the above experiment was repeated using sponges incubated in a solution of 0.1 mg/L of $^{15}NH_4^+$.

Analysis of stable isotopes: For each *A. cauliformis* individual sponge and symbiont cells were separated from bulk sponge tissue using methods from Freeman et al. (2013). Briefly, frozen sponges were chopped into small pieces and soaked in an artificial salt water buffer with EDTA at 4°C. Sponges were then homogenized and filtered under low vacuum pressure. The filtrate was then put through several centrifugation spins that separated out the larger eukaryotic cells from the filtrate at slower speeds (2000-2500 rpm) and bacterial cells at higher speeds (4500-7000 rpm). The result was a separation of bacterial and sponge cells from the sponge holobiont. Sponge and bacterial pellets were then lyophilized and acidified with 6M HCl. After acidification, the samples were dried and then weighed out into silver capsules for isotopic analysis. Algal samples were first lyophilized before being acidified with 6M HCl, dried, and weighed into silver capsules for isotopic analysis. Stable isotope analysis was conducted at the Geophysical Laboratory at the Carnegie Institution of Washington (Washington, DC) using a Thermo Delta V Plus isotope ratio mass spectrometer coupled to a Carlo-Erba NC2500 elemental analyzer via a Conflo III open-split interface.

Enrichment of stable isotope tracers was expressed as atom percent excess (APE) for both ^{12}N and ^{13}C. Enrichment expressed as APE shows the increase in the atom percent of an isotope

compared with initial samples, which represent the atom percent of the natural population. The term "sample" refers to the experimental samples, and the term "standard" refers to the international standards of the Pee Dee Belemnite and atmospheric N2 for $\delta^{13}C$ and $\delta^{15}N$, respectively (Fry 2006, Freeman et al. 2013). The following formula shows the calculations for Atom% ^{13}C, and this same formula was used to calculate atom % ^{15}N. Once atom% is calculated, APE was calculated as the difference of the atom % of the experimental sample minus the mean atom % of the initial samples. The formula for calculating atom % of an element is as follows:

$$\text{Atom \% } {}^{13}C = [100 \times R_{standard} \times (\delta^{13}C \text{ sample } /(1000+1)] / [1 + R_{standard} \times (\delta^{13}C \text{ sample } /(1000+1)]$$

Data analysis: Initial values of ^{15}N and ^{13}C for bacterial and sponge cell fractions were compared using a one-way analysis of variance (ANOVA). To test whether sponges became significantly enriched with the ^{15}N and ^{13}C tracers in both experiments, initial isotopic ratios were compared to ratios of samples incubated in the tracer solution using a two-way ANOVA to examine difference in cell fractions, time (t_i vs. t_0) and their interaction expressed in units of $\delta^{13}C$ and $\delta^{15}N$. Sponge enrichment values post enrichment were expressed in units of APE to account for natural population abundances of the isotopes. APE ^{15}N and ^{13}C values were analyzed using a two-way ANOVA to test for differences in cell fraction, treatment (sponge alone or algal contact), and their interaction. Algal enrichment was analyzed using a one-way ANOVA for each treatment, comparing experimental ^{15}N and ^{13}C values with initial values in units of $\delta^{13}C$ and $\delta^{15}N$. Tukey's post-hoc tests were used to detect differences in each experiment.

Results

Isotopic analysis

NO_3^-/HCO_3^- experiment: Initial sponge samples showed significant differences in $\delta^{15}N$ between fractions (0.78±0.15‰ and 3.06±0.1‰ for bacterial and sponge fractions, respectively; one-way ANOVA, $F = 162.5$, $P < 0.0001$). Bacterial and sponge fractions showed no differences in $\delta^{13}C$ between fractions (-19.48±0.2‰ and -19.06±0.16‰ for bacterial and sponge fractions, respectively; one-way ANOVA, $F = 2.71$, $P = 0.13$). Analysis using a two-way ANOVA showed a significant ^{15}N enrichment of samples at t_0 (i.e. enriched samples) (12.41±3.7‰ and 1.92 ±0.39‰ for initial and enriched samples, respectively; $F = 8.89$, $P = 0.009$, Figure 5), but there was no effect of cell fraction ($F = 1.15$, $P = 0.30$) or interaction of the two variables ($F = 2.96$, $P = 0.10$). For the $NaH^{13}CO_3$ tracer, analysis revealed a significant enrichment of samples at t_i (1.51±2.81‰ and -19.27±0.14‰ for initial and t_0, respectively; one-way ANOVA, $F = 209.5$, $P < 0.0001$), but it also showed that the bacterial fraction was on average higher than the sponge fraction ($F = 25.2$, $P = 0.0001$), and a significant interaction between the two terms ($F = 28.2$, $P < 0.0001$). Tukey's HSD post-hoc test revealed that both post enrichment samples were significantly enriched with ^{13}C over the initial samples, but also that the post-enrichment bacterial fraction was more enriched than the sponge cell fraction from the same time (Figure 5). After 12 hours, there was a significant difference in APE ^{15}N between sponge fractions (two-way ANOVA, $F = 6.22$, $P = 0.02$, Figure 6a), but there was no significant difference between algal contact treatments ($F = 1.28$, $P = 0.27$) or in the interaction of the two variables ($F = 0.65$, $P = 0.43$). These same relationships were observed at 24 hours, with the bacterial fraction having a significantly higher APE ^{15}N than the sponge fraction (two-way ANOVA, $F = 5.62$, $P = 0.02$)

and no significant differences between treatments (F = 0.87, P = 0.87) or the interaction of the two variables (F = 0.13, P = 0.80, Figure 6a). APE ^{13}C was also significantly different between fractions at 12 hours (F = 58.96, P <0.0001) and 24 hours (F = 28.36, P <0.0001), showing a higher enrichment in the bacterial cell fraction. No differences in algal contact treatment (F = 0.35, P = 0.52) and no significant interaction effect (F = 1.30, P = 0.29) were observed at 12 hours or 24 hours (F = 0.002, P = 0.52 and F = 0.26, P = 0.64 for algal contact treatment and interaction term, respectively, Figure 6b).

Algal enrichment in this experiment was not significantly different than initial values. $\delta^{15}N$ values for *M. marinum* samples indicated no significant enrichment at either 12 or 24 hours, compared to initial samples (one-way ANOVA, F = 0.44, P = 0.65, Figure 7a), although results were highly variable, suggesting that some individuals may have been enriched with ^{15}N. Algae also did not absorb ^{15}N at either 12 or 24 hours when placed either 15cm (-0.33±0.21 and -0.24±0.16 for 12 and 24 hours, respectively; one-way ANOVA F = 1.88, P = 0.18), or 30cm from an enriched sponge (-0.09±0.19 and 0.28±0.55 for 12 and 24 hours, respectively; one-way ANOVA, F = 0.29, P = 0.75).

There were no differences in $\delta^{13}C$ in algal samples collected at 12 and 24 hours (one-way ANOVA, F = 2.14, P = 0.14, Figure 7b), suggesting no transfer of carbon resources from the sponge. Additionally, there was no evidence for uptake of sponge-derived carbon in algae at 15cm from the enriched sponge (-14.7±0.4 and -13.9±0.24 for 12 and 24 hour samples' one-way ANOVA F = 3.99, P = 0.04), as the $\delta^{13}C$ in fact decreased significantly from initial samples at 12 hours (Tukey's HSD post-hoc test: P< 0.05). A significant decrease in $\delta^{13}C$ was also observed at 12 hours for algae at 30 centimeters from an enriched sponge (Tukey's HSD post-hoc test P<

0.05), but algal samples at 24 hours showed no significant differences from initial samples (Mean = -13.3±0.34, -14.401±0.26 and -14.33±0.27 for initial, 12 hour and 24 hour algal samples, respectively; one-way ANOVA F = 3.95, P = 0.04).

NH_4^+ experiment: Significant differences in $\delta^{15}N$ were shown between fractions in initial (t_i)sponges (0.76±0.29 ‰ and 2.5±0.12‰ for bacterial and sponge fractions, respectively; one-way ANOVA F = 33.46, P = 0.0004). After six hours of incubation (t_0) in 0.1 mg/L of ($^{15}NH_4$) H_2PO_4, sponges became significantly enriched compared to initial samples (14.04±4.34 and 1.67±0.34 for t_0 and t_i samples, respectively; two-way ANOVA, F = 7.52, P = 0.01), but there was no effect of cell fraction type (F = 0.14, P = 0.71) or interaction of the two terms (F = 0.61, P = 0.45, Figure 8). Sponges maintained this enrichment throughout the experiment. At 12 hours (t_{12}), the bacterial cell fraction was significantly more enriched than the sponge cell fraction (F = 9.37, P = 0.005) but there were no significant effects of treatment (F = 0.43, P = 0.52), nor any interaction effect (F = 0.0, P = 0.99, Figure 9). The same trend was observed at 24 hours (t_{24}), as APE 15N was significantly higher in the bacterial cell fractions (F = 11.21, P = 0.002), but there were no differences in treatment (F = 1.85, P = 0.18), or in the interaction of the two variables (F = 0.08, P = 0.78, Figure 9).

Algae in the NH_4^+ experiment did show significant enrichment with the ^{15}N tracer (F = 6.14, P = 0.01). After 24 hours in contact with the sponge, $\delta^{15}N$ values in the algae were significantly higher than the initial values for the algae (Tukey's HSD post-hoc test, Figure 10). Algae at 15 cm from an enriched sponge showed no significant differences from initial $\delta^{15}N$ values (-0.32±0.19, -0.19 ±0.29, and -0.5±0.2 for initial, 12 hour and 24 hour samples; one-way ANOVA F = 0.45, P = 0.64), suggesting that they obtained no sponge-derived nitrogen

resources. Algae at 30 cm showed a significant decrease in $\delta^{15}N$ values at 24 hours compared to initial values, but no differences from initial values were observed at 12 hours (-0.55±0.1 and -1.7±0.12 for 12 hour and 24 hour samples, respectively; one-way ANOVA, $F = 35.11$, $P < 0.001$).

Discussion

Sponges and macroalgae can form complex interactions on coral reefs. The interaction between *A. cauliformis* and *M. marinum* showed qualities of both competition, in which sponge health was reduced, and facilitation, in which algal productivity increased (Chapter 1). The current study investigated how carbon and nitrogen dynamics function in this interaction. This study showed that algal facilitation was likely the result of nitrogen transfer from the sponge to the alga. Additionally, while the previous study noted a negative effect on the sponge by the algae, the current study saw no such effect over the short duration of these experiments.

Facilitation has gained considerable attention in studies on coral reef ecology (Bruno et al. 2003a). This study showed a potential mechanism for algal facilitation in the sponge-algal interaction in Chapter 1 through the transfer of nitrogen resources from the sponge to the alga. Examples of direct facilitation via nitrogen transfer have been shown previously (Ellison et al. 1996, Davy et al. 2002, Pile et al. 2003, Slattery et al. 2013). In one example, Davy et al. (2002) showed that sponge-derived N can be translocated to algae, a hypothesis that seems consistent with the current study, given that many algae are efficient at taking in available nutrients (Pérez-Mayorga et al. 2011). The net-like morphology of *M. marinum* maximizes its surface area to volume ratio, and even though the morphology of the alga would be expected to favor efficient

uptake of available nutrients (Pérez-Mayorga et al. 2011), the current study only saw significant uptake of the nitrogen substrate ammonium. There may be several reasons for differential uptake of nitrate and ammonium nitrogen substrates. From the standpoint of algal uptake, ammonium is a more biologically available source of nitrogen than nitrate, especially to photosynthetic eukaryotes in the marine environment (Zehr and Ward 2002). Another possible reason for differential uptake by the alga could be due to sponge processing of nitrogen resources. Previous studies have shown the ability of many sponges that host dense microbial communities to uptake nitrogen from both ammonium and nitrate, and incorporate it into their biomass (Thacker and Freeman 2012, Freeman et al. 2013). However, proteins essential for assimilation of both of these resources have only been found in the cyanobacteria and sponge associated microbial communities, and not in the eukaryotic sponge cells (Wilkinson 1979, Kramer et al. 1996, Allen et al. 2001, Zehr and Ward 2002, Taylor et al. 2007), indicating that both nitrogen substrates are assimilated by sponge microbial communities and then translocated to the sponge host in a form that can be assimilated (Taylor et al. 2007, Freeman et al. 2013). The limiting factor might be the processing of these two substrates, as ammonium can be directly assimilated into microbial and sponge biomass, whereas nitrate requires additional conversion steps for assimilation (Taylor et al. 2007). In the current study, these properties may have been important in the availability of the two nitrogen substrates to the alga, even though *A. cauliformis* has been documented to excrete both NH_4^+ and NO_x^- (Southwell 2007, but see Southwell et al. 2008).

M. marinum in this study did not absorb any of the carbon tracer from the enriched sponge. While sponges have been shown to contribute organic carbon resources to the benthic compartment (Maldonado et al. 2012), *A. cauliformis* in the current study did not transfer these carbon resources to *M. marinum*. Carbon transfer to the benthic compartment by sponges is

often through detrital particulate organic matter (Maldonado et al. 2012), which would be generated predominantly through heterotrophic feeding by the sponge. *A. cauliformis* has been shown to mostly rely on its symbiotic microbial communities for nutrient assimilation (Freeman and Thacker 2011), meaning that heterotrophic feeding is likely less important. This minimal heterotrophic feeding could have contributed to the absence of ^{13}C signal in the alga. Additionally, carbon is not often limiting in coral reef systems, so discerning a ^{13}C signal from the potentially minute transfer of carbon resources from the sponge would be difficult. This is in contrast to nitrogen, which is often limiting and may be easier to detect in small amounts since background concentrations would be quite low. Benthic algae are also known to generate large quantities of dissolved carbon resources (Haas et al. 2010, Naumann et al. 2010), and this generation of dissolved carbon through algal associated microbial communities, has even been shown to be a mechanism of stress to corals (Smith et al. 2006). Thus, assuming algal generation of dissolved carbon, reliance on sponge-derived carbon may be less important to some algal species. Some studies have even suggested that sponges may rely on benthic organisms, including algae, for greater than 50% of their carbon resources (van Duyl et al. 2011, Maldonado et al. 2012). Although this suggests that sponges may benefit from a close association with some algal species, recent research implies that this is not the case in all such interactions (González-Rivero et al. 2012, Chapter 1).

Although algal contact did not alter carbon and nitrogen dynamics within the sponge over the course of this experiment, prolonged and close association with algal communities reduced sponge photosymbiont abundance, leading to reduced sponge performance in some instances (Chapter 1). Initial $\delta^{15}N$ values of both fractions and ^{15}N and ^{13}C trends in the current study suggest that, indeed, sponge cells are obtaining significant carbon and nitrogen from their

photosymbiont communities. Thus, a reduction in photosymbiont abundance by close algal association may be particularly detrimental to these sponges, especially if sponge hosts lack the nutritional plasticity to increase their heterotrophic filter feeding under conditions of reduced symbiont abundance (Freeman and Thacker 2011). In these instances, nutrient supply to sponge cells might be significantly reduced, with a potentially drastic reduction in carbon supply due to reduced levels of carbon fixation by photosymbionts within impacted sponges (Freeman et al. 2013). Impacts on nitrogen cycling, however, might only be slightly diminished due to nitrogen processing of heterotrophic bacteria in the sponge that, under low light conditions, are still capable of assimilating low levels of inorganic substrates (Freeman et al. 2013), leading to a net efflux of nitrogen to the environment (Southwell et al. 2008). Whether heterotrophic N metabolism by symbionts ensures adequate nitrogen supply under changing conditions remains unknown, but certainly deserves future work.

The broader implications of this interaction are unknown to date. Several studies have indicated that sponges are net sources of nitrogen on coral reefs (reviewed in Maldonado et al. 2012). Because sponges are an abundant and widespread group of organisms on coral reefs, their ability to efflux nitrogen could potentially increase local concentrations of these limited resources (Maldonado et al. 2012). Assimilation of sponge-derived nitrogen by neighboring reef organisms thus may be significant, especially in closely associated organisms like the algae and sponge in the current study. Indeed, although the nitrogen budgets of only 22 Caribbean sponge species have been reported, 20 of these species were shown to have an overall net efflux of dissolved nitrogen in the form of NH_4^+ and/or NO_3^- (Maldonado et al. 2010). Because *M. marinum* interacts with several of these species (Chapter 1), interactions like those reported in the current study may be prevalent on these shallow reefs. If sponge-derived N boosts algal

productivity, then this might directly impact algal abundance on these reefs, potentially leading to circumstances supporting algal dominance on many of these reefs where overfishing, anthropogenic nutrients, and disease have already tipped the scale in favor of the algae.

Figure 5. Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ in initial (t_i) and enriched (t_0) sponges. Sponges collected from the incubation chamber were significantly enriched with $Na^{15}NO_3$ and the $Na^{13}HCO_3$ tracer ($P = 0.009$ and $P < 0.0001$ for $\delta^{15}N$ and $\delta^{13}C$, respectively). "Bac" refers to the bacterial cell fraction and "Sponge" refers to the sponge cell fraction.

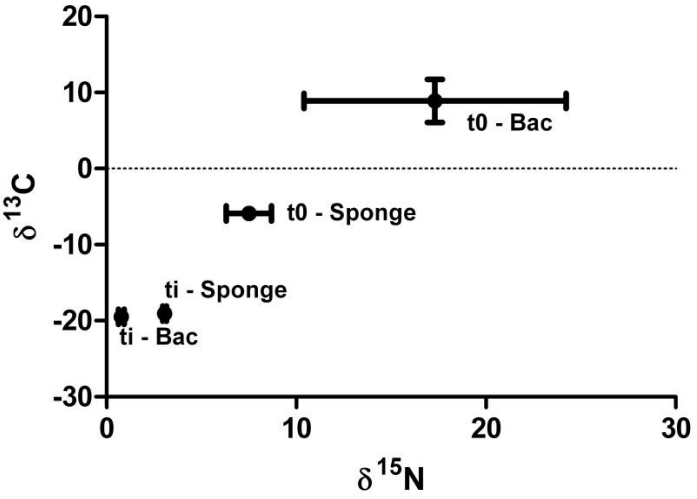
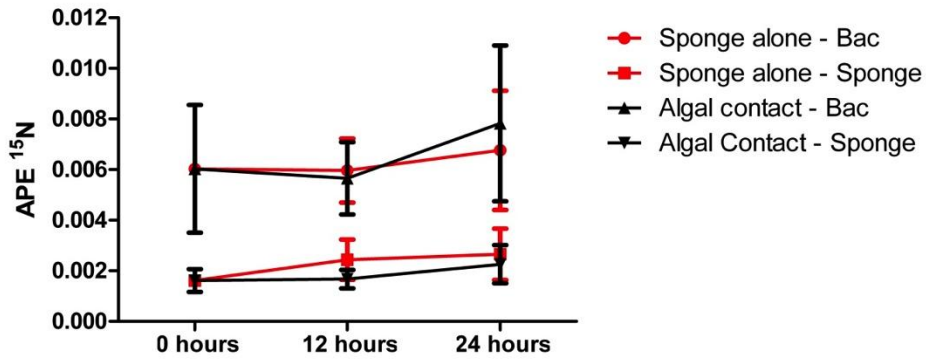


Figure 6. (A.) Mean (±SE) APE^{15}N enrichment of *A. cauliformis* samples at 0, 12 and 24 hours post incubation in $Na^{15}NO_3$ tracer. (B.) Mean (±SE) APE^{13}C enrichment of *A. cauliformis* samples at 0, 12 and 24 hours post incubation in $Na^{13}HCO_3$ tracer. Red lines indicate sponge alone treatment and black lines represent sponges in contact with algae. Bac = bacterial cell fraction and Sponge = sponge cell fraction. APE^{15}N and APE^{13}C enrichment were different between cell fractions (P =0.006, P <0.0001, respectively), but there were no differences in treatment.

A.



B.

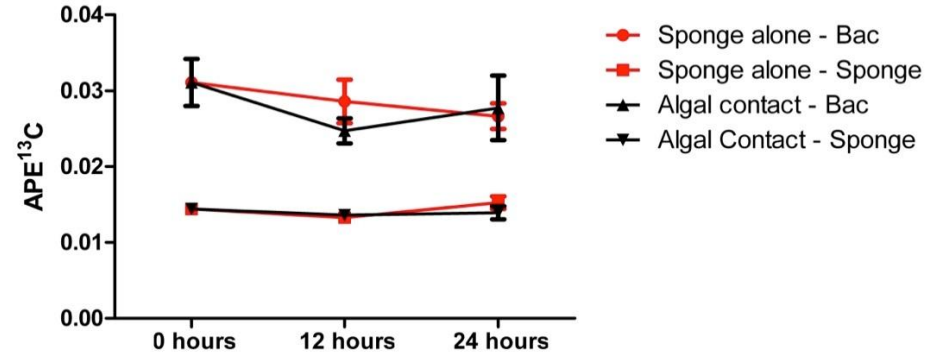
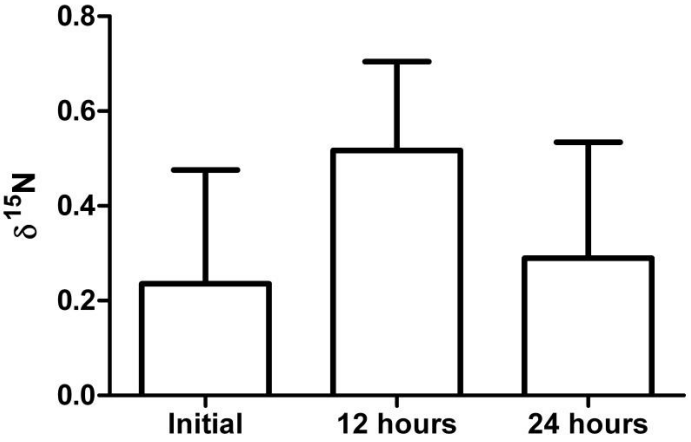


Figure 7. (A.) Mean (±SE) $\delta^{15}N$ enrichment of *M. marinum* initially and after 12 and 24 hours in contact with an *A. cauliformis* individual that was enriched with $Na^{15}NO_3$. (B.) Mean (±SE) $\delta^{13}C$ enrichment of *M. marinum* initially and after 12 and 24 hours in contact with an *A. cauliformis* individual that was enriched with $Na^{15}HCO_3$. No significant differences in $\delta^{15}N$ or $\delta^{13}C$ enrichment were detected at either time ($P > 0.05$).

A.



B.

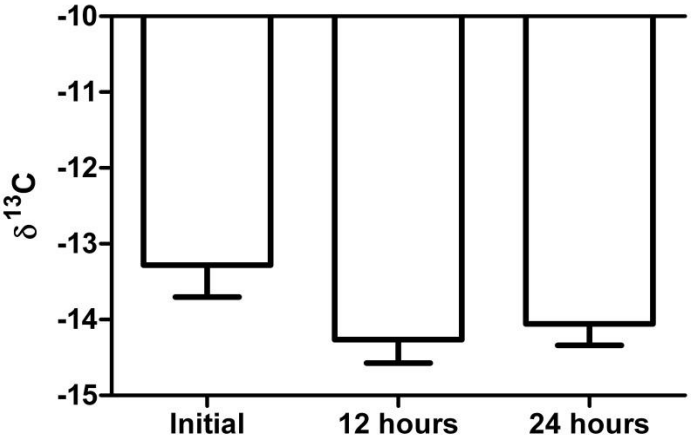


Figure 8. Mean (±SE) $\delta^{15}N$ in initial (t_i) samples and enriched (t_0) sponges. Sponges collected from the incubation chamber were significantly enriched with the ($^{15}NH_4$) H_2PO_4 tracer (P = 0.01) after 6 hours. "Bac" refers to the bacterial cell fraction and "Sponge" refers to the sponge cell fraction.

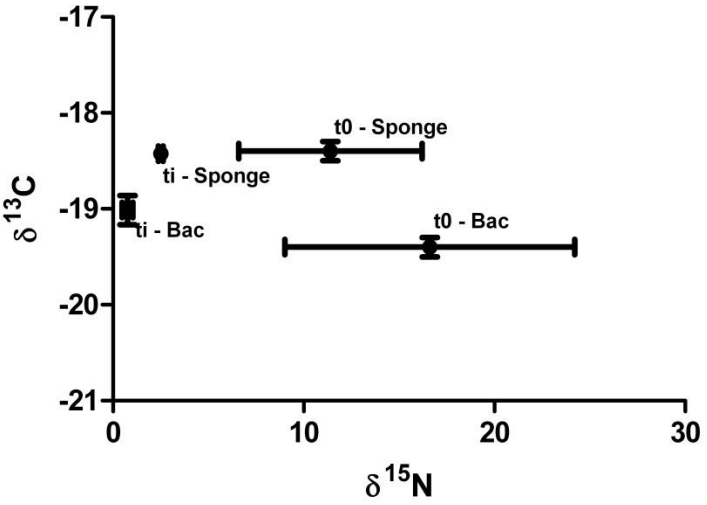


Figure 9. Mean (±SE) APE^{15}N enrichment of *A. cauliformis* samples at 0, 12 and 24 hours post incubation in $^{15}NH_4^+$ tracer. Red lines indicate sponge alone treatment and black lines represent sponges in contact with algae. Bac = bacterial cell fraction and Sponge = sponge cell fraction. APE^{15}N enrichment was different between cell fractions ($P < 0.005$), but there were no significant differences in treatment.

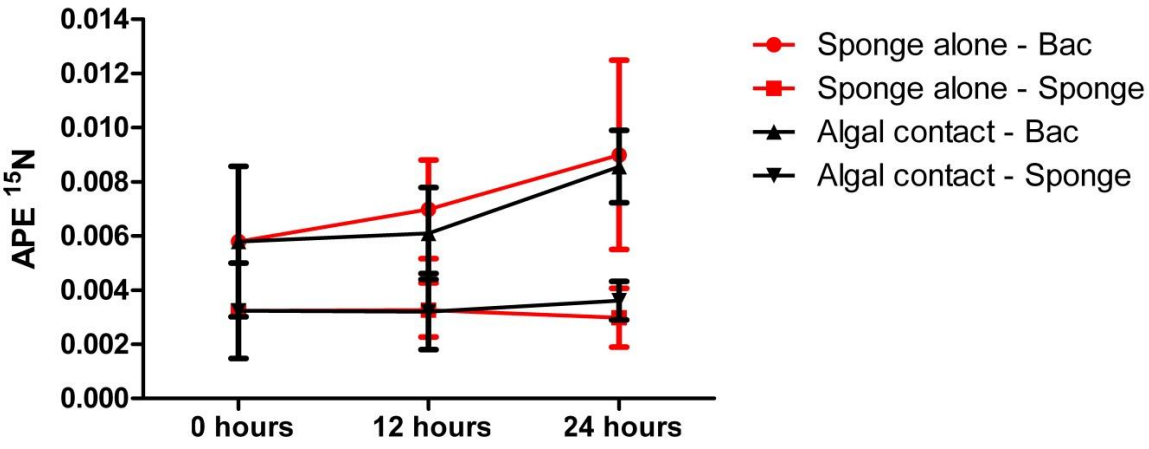
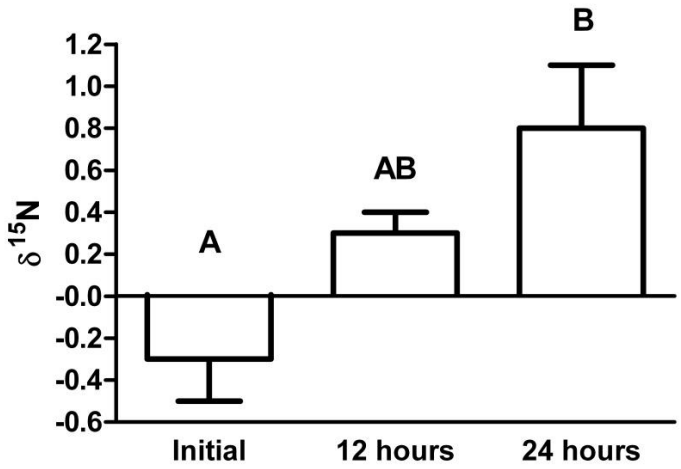


Figure 10. Mean (±SE) $\delta^{15}N$ enrichment of *M. marinum* of initial alga samples and after 12 and 24 hours in contact with an *A. cauliformis* individual that was enriched with $^{15}NH_4^+$. Significant algal enrichment with sponge-derived $\delta^{15}N$ was shown after 24 hours (P = 0.01). Histograms with different letter groups are significantly different by ANOVA.



CHAPTER 3: EXPLORING INDIVIDUAL- TO POPULATION-LEVEL IMPACTS OF DISEASE ON CORAL REEFS: FATE AND DYNAMICS OF *APLYSINA* RED BAND SYNDROME (ARBS)

Abstract

Marine diseases are of increasing concern for coral reef ecosystems, but often their causes, dynamics and impacts are unknown. The current study investigated the impacts and dynamics of *Aplysina* Red Band Syndrome (ARBS) at both the individual and population levels. The possible fates of marked healthy and ARBS-infected *Aplysina cauliformis* sponges were examined over the course of a year. Population level impacts, dynamics and transmission mechanisms of ARBS were investigated by monitoring two populations of *A. cauliformis* on shallow patch reefs over a 3 year period using digital photography and diver collected data, and analyzing these data with GIS techniques of spatial analysis. In this study, three commonly used spatial statistics (Ripley's K, Getis-Ord General G, and Moran's Index) were compared to each other and with direct measurements of individual interactions using join-counts, to determine the ideal method for investigating disease dynamics and transmission mechanisms in this system. During this 3-year period, Hurricane Irene directly impacted these populations, providing an opportunity to assess potential storm effects on *A. cauliformis* and ARBS. Infection with ARBS yielded increased loss of healthy tissue over time and a higher likelihood of an individual dying and being missed in subsequent surveys. In this study, Hurricane Irene had the most dramatic effect on the *A. cauliformis* population by greatly reducing sponge biomass on the reef, especially in diseased individuals. Spatial analysis showed that direct contact between individual *A. cauliformis* was the likely transmission mechanism for ARBS within a population, evidenced by a significantly higher number of contact-joins between diseased sponges compared to random. Of the three spatial statistics compared, the Moran's Index best represented true connections between diseased sponges in the survey area. This study showed that spatial

analysis can be a powerful tool for investigating disease dynamics and transmission in a coral reef ecosystem.

Introduction

Substantial impacts on marine populations and communities have been attributed to diseases of marine organisms (Harvell et al. 2004) affecting a wide range of taxa from commercially important fish, shellfish and corals, to less apparent species that may be disappearing without notice. Much of the marine disease literature has focused on hard corals, which have experienced massive declines in recent decades. In most cases, coral diseases are believed to be caused by microorganisms, but the specific pathogen has only been identified in a few instances (Richardson 1998, Harvell et al. 1999, Porter et al. 2001, Harvell et al. 2007, Sutherland et al. 2010, Muller and Woesik 2012). In general, the understanding of marine diseases lags behind terrestrial diseases based on functional knowledge and techniques of investigation; however, this lag is particularly striking when considering the increasing rate at which marine diseases are reported (Porter et al. 2001, Olson et al. 2006). With coral cover declining, diseases of sponges gained increasing attention (Rutzler 1988, Gaino et al. 1992, Vacelet et al. 1994, Webster et al. 2002, Olson et al. 2006, Wulff 2006b, Webster 2007, Wulff 2007a, Maldonado et al. 2010, Gochfeld et al. 2012a). One such disease is *Aplysina* Red Band Syndrome (ARBS) (Olson et al. 2006), an infectious disease of sponges in the genus *Aplysina*. ARBS has been recorded throughout the Caribbean at prevalence rates as high as 15% of the individuals within a population (Olson et al. 2006, Gochfeld et al. 2012a). This disease can result in partial or total mortality of sponges that it infects, and while it is able to spread through experimental contact, the main mechanism of transmission within a natural population remains unknown (Gochfeld et al. 2012a).

In addition to disease, periodic storm events can have major impacts on coral reefs. Strong storms do not affect all reef species equally (Hughes and Connell 1999), and in some cases, may have positive effects on coral reefs. For example, storms promote diversity by opening new substrate for larval recruitment, and by decreasing the abundance of faster growing branching coral species, allowing slowly growing, more robust coral species to survive (Carpenter 1990, McCook et al. 2001). While storms have historically had many positive effects on coral reefs, recent studies have suggested that these storms have increased in intensity and will continue to do so under conditions of rising sea surface temperatures (Elsner et al. 2008, Mann et al. 2009). In addition, these storms are affecting reefs that are already impacted by stressors such as disease, overfishing, and anthropogenic nutrients, which have reduced the biotic diversity of Caribbean reefs (Hughes and Connell 1999). When these stressors are coupled with strong storms, the consequences to coral reefs can be dramatic (Hughes 1994). For example, in Jamaica, Hurricane Allen destroyed much of the branching coral species (Acroporids), and opened up reef substrate for settlement, which was colonized by macroalgae instead of new coral recruits. This algal dominance was attributed to a lack of herbivores on Jamaican reefs due to overfishing and a marine epizootic event (Hughes 1994, Aronson and Precht 2001) . This example illustrates that in order to assess the overall impact of a stressor, one must often take into account other factors that occur on the reef simultaneously (Hughes 1994, Hughes and Connell 1999, Aronson and Precht 2001, Aronson et al. 2004, Harvell et al. 2007).

Spatial analysis offers a powerful method to study the spread of diseases within a population. Population monitoring can be used to develop a time series of disease status in individuals within a population. These spatial and temporal patterns of disease incidence can then be used to discern the process of transmission (Real and McElhany 1996). Spatial analysis

techniques have been extensively used in many fields (Anselin et al. 1997, Moraes 2005, Anselin et al. 2007), including ecology (Rossi et al. 1992, Legendre 1993, Real and McElhany 1996), where they have been used to study disease transmission in terrestrial systems (Gilbert et al. 1994, Real and McElhany 1996). Transmission of diseases via contact, vector or through the medium (air or water) can be discerned through distinct spatial relationships. (Gilbert et al. 1994, Real and McElhany 1996). In contrast to the terrestrial environment, spatial analysis techniques have seen limited applications in marine systems (Cole and Syms 1999, Jolles et al. 2002, Gardner et al. 2008, Brandt and McManus 2009, Zvuloni et al. 2009, Lentz et al. 2011, Roff et al. 2011), and the few studies that have used them have sampled at a resolution that is too coarse to adequately investigate transmission mechanisms (Real and McElhany 1996, Lentz et al. 2011, Roff et al. 2011). To date, most marine studies that have used spatial statistics have focused on those that measure clustering of attributes, such as the Ripley's K or the Getis-Ord General G statistics (Jolles et al. 2002, Gardner et al. 2008, Zvuloni et al. 2009, Lentz et al. 2011, Roff et al. 2011, Ban et al. 2012). These statistics measure the concentration of attributes in an area (Mitchell 2005), but in epidemiological studies it may be more important to investigate relationships of individuals in the population. Less frequently used statistics such as the Moran's Index capture these individual relationships by measuring spatial autocorrelation (similarity of feature values and location) between individuals (Moran 1950, Van Houtan et al. 2010, Ban et al. 2012).

This study used spatial analysis techniques to investigate the spatial and temporal dynamics of ARBS in two populations of the sponge *Aplysina cauliformis* on Bahamian patch reefs. ARBS presents a unique opportunity to investigate transmission mechanisms because it occurs on branching sponges that are able to grow upright or horizontally and physically contact

neighboring individuals (Olson et al. 2006). These growth strategies enabled us to evaluate three transmission mechanisms: contact-driven, waterborne, and vector-driven transmission. While forced physical contact has been shown to spread this disease efficiently in both laboratory and field experiments (Olson et al. 2006, Gochfeld, unpublished data), additional or alternative transmission mechanisms may be important on the reef. This study analyzed distribution patterns of ARBS over a 3 year period. In addition, this study investigated the impacts of a severe storm event (Hurricane Irene: Category 3; 27 August 2011) on the *A. cauliformis* population and ARBS infections.

Materials and Methods

Study sites

This study was conducted on two shallow reefs (3-5m) near the Perry Institute for Marine Science on Lee Stocking Island, Exuma Cays, Bahamas, from January 2008 to June 2012. Field monitoring was conducted at Big Point (N 23˚ 47.301", W 76˚ 08.118") and Rainbow Gardens (N 23˚ 47.798", W 76 ˚ 08.786"), located 1.5 kilometers apart.

Monitoring of Individually Marked Sponges

In order to track the rate of progression of ARBS in individual sponges, 18 diseased individuals and their nearest healthy neighbors were marked and monitored from 2008-2009 at Big Point. Marked sponges were photographed, number of lesions counted, and measurements were made of the healthy tissue, active red bands and necrotic tissue during March 2008, July 2008 and June 2009. These data were analyzed for differential fates by indicating health status based on the presence of ARBS, as has been done in other studies investigating qualitative effects of a treatment (Wassenberg and Di Giulio 2004). Health status rankings were: healthy =

1, diseased = 2 and missing = 3 (Wilcoxon Rank-Sum test). Healthy tissue length was compared for healthy and diseased individuals at each time (Repeated measures ANOVA).

Sponge Population Monitoring

Permanent 10 X 10 m grids were established at Big Point and Rainbow Gardens patch reefs. Within each grid, digital images representing $1m^2$ were taken using a Canon D10 underwater camera, resulting in 100 images per grid. The location of each individual *Aplysina cauliformis* within each 1 m^2 block was recorded on an underwater paper map, total sponge length was measured using a fiberglass measuring tape, and sponge health was assessed. If the sponge was diseased, the number of lesions was counted. Grids were sampled yearly in May/June from 2010-2012, and a month after hurricane Irene (27 August 2011) in September 2011. Big Point was also sampled in July 2011. Each grid contained between 133 and 340 *A. cauliformis* individuals.

Photographs from May 2011 for Big Point (BP) and July 2011 for Rainbow Gardens (RG) were georeferenced to their locations on the patch reefs and assembled into a mosaic representing the 10 X 10 m grid in the program ArcMap (Figure 11). Photographs from other time points were photographically georeferenced to these images at the two sites. A point vector file was then created for the sponges that were measured in each grid. Each point represented one sponge and was referenced to an attribute table containing records of its length, health, number of lesions and number of branches. The number of branches for each sponge was determined from the photographs. This process was repeated for each time point.

As a comparison to previous studies that used broader surveys to investigate marine diseases (Porter et al. 2001, Sato et al. 2009, Lentz et al. 2011, Roff et al. 2011), the 10 X 10 m grids containing the point vectors at each site were transformed into polygons. Each polygon

measured 0.5 m^2, and its attributes included the total number of sponges as well as the number of diseased sponges within that polygon.

To investigate whether ARBS disproportionally affected certain size classes of sponges within the population, size frequency distributions of healthy and diseased sponges were compared using a Kolmogorov-Smirnov test (Statview).

Spatial analysis

To assess the clustering of disease within a population, three spatial statistics (Ripley's K function, Getis-Ord General G, and Moran's Index) were compared using the spatial analysis toolkit in the ArcGIS toolbox. These spatial patterns within the sponge populations were analyzed on each grid at each point in time. These spatial statistics were also compared to a higher resolution technique (join-counts) that investigated the connectedness of individuals within the population using two metrics: (1) Physical contact connectedness (as an indicator of direct transmission of disease) and (2) Gabriel connectedness (as an indicator of vector mediated transmission of disease (Gilbert et al. 1994, Real and McElhany 1996).

Ripley's K function is used to analyze spatial patterns and investigate spatial dependence of features (clustering or dispersion). While many spatial statistics require selection of a specific scale, the Ripley's K function examines patterns over a range of scales to determine the appropriate one (Getis 1984, Boots and Getis 1988, Bailey and Gatrell 1995, Mitchell 2005). The Getis-Ord General G statistic measures how concentrated certain values are in a selected area. This statistic can be used to monitor high proportions of a particular attribute in an area, for example, the number of diseased sponges (Mitchell 2005, Getis and Ord 2010). The Moran's I statistic measures spatial autocorrelation, which is the similarity of features based on both their locations and values. This tool measures the spatial relationship between features of similar and

different values to determine patterns of clustering or dispersion in a population (Moran 1950, Goodchild 1986, Griffith 1987, Mitchell 2005). This differs from the Getis-Ord statistic in that while Getis-Ord measures concentration of values in an area, Moran's I determines the spatial relationship between individuals of the same and different values.

The Ripley's K statistic has been used in many marine disease studies (Jolles et al. 2002, Gardner et al. 2008, Zvuloni et al. 2009, Lentz et al. 2011, Muller and Woesik 2012). Here, a weighted Ripley's K statistic (Ripley 1981, 1988, Mitchell 2005) was used to assess non-random distribution patterns within the sponge populations and to discern the scale at which these patterns occurred. The weighted Ripley's K statistic randomly distributes an attribute (in this case, sponge health [healthy or diseased]) among the existing points in the grid. Each trial was run for 100 iterations with 100 distance bands over a scale of 10 m, resulting in each distance band measuring 10 cm. The data table containing the 10 cm distance band measurements was then examined for any areas where the observed distribution differed significantly (areas where the expected random distribution values fell outside the confidence envelope generated from the 100 iterations in the analysis) from the expected random distribution.

For the Getis-Ord General G and Moran's I statistics, the sponges on the grid, represented by point vectors, were converted to thiessen polygons to best represent the spatial relationships among sponges in the population (Lloyd 2010). Each thiessen polygon represents the point with all its attributes and its area of influence, which is one half of the distance between the point and each of its neighbors. The number of sides of the polygon reflects the number of neighbors with converging areas of influence. The Getis-Ord General G statistic was run for the attribute sponge health using calculated network spatial weights to conceptualize the spatial relationships of the sponges in the grid. No barriers within the grid were considered, so a Euclidian distance

method was used. The Getis-Ord Gi*Hot Spot Analysis was used to visualize specific areas of clustering in the grid. These grid maps showed specific thiessen polygons with significant z-scores as calculated by this statistic (Mitchell 2005, Scott and Warmerdam 2005). The *Moran's I* statistic was calculated to examine global spatial autocorrelation between sponges weighted by the attribute sponge health using the same parameters employed for the Getis-Ord statistic. These relationships were mapped onto the grid to identify local scale clusters and outliers in the spatial autocorrelation analysis using the Cluster and Outlier Analysis (Anselin Local Moran's I) tool in ArcGIS (Anselin 1995, Mitchell 2005).

To address disease transmission mechanisms between sampling times, join-counts were performed on all sponges within each population (Sokal and Oden 1978, Real and McElhany 1996). Because each sponge was assigned a specific location within the grid, each sponge had a specific Euclidian distance and spatial relationship to every other sponge in the grid. From these relationships, we were able to establish the number of "joins" (connections) between individuals. Join-counts enabled us to track the suspected origin of a ARBS-affected sponge in one sampling time to a diseased sponge in an earlier sampling time (Gilbert et al. 1994, Real and McElhany 1996).

Joins in this study were examined using two types of connectedness: physical contact and Gabriel (vector) contact. To assess physical contact connectedness in the grid, the distance between each sponge and every other sponge in the grid was calculated. Using the join tool in ArcGIS, the attributes of each individual sponge and the attributes of all of its neighbors were joined in the data table of the distances between individuals. Using the sponge length measured *in situ*, and the number of branches determined from the photographs, an algorithm was developed to account for all sponge-sponge interactions within a single sampling time. The

algorithm determined sponge interactions by selecting individuals whose Euclidian distance was less than the sum of each sponge's length divided by its number of branches. This method assumed equal branch length for each sponge, which likely underestimates sponge interactions. These interactions were grouped into four classes: total (T), healthy:healthy (H:H), healthy:diseased (H:D), and diseased:diseased (D:D) joins. This method was automated for each grid using a Python script in ArcMap to test for contact-connectedness between sponges. To test whether the frequency of these observed contacts was statistically different from a random distribution of contact frequency, another Python script was developed which took the feature layer containing all the sponges and their attributes used in calculating the observed contacts, and randomly distributed these points in a 10 x 10 m grid before recalculating the frequency of contact-joins within each class. One hundred iterations of this process were run (Real and McElhany 1996), and the averages for each class were calculated. From these data, we were able to determine what number of the 100 random iterations overestimated, underestimated or accurately estimated the observed contacts in each of the four classes. This information was then translated into a proportion by dividing the number of realizations that contained more or an equal number of joins compared to the observed values by the total number of realizations, and this proportion became the p-value, as has been outlined in previous studies (Cliff and Ord 1981, Gilbert et al. 1994, Real and McElhany 1996).

To test for vector-based transmission, we used a Gabriel connectedness scheme originally employed in studies of plant pollinators (Real and McElhany 1996). Using this scheme, two points in the grid are connected if there are no other points that occur within a circle that has a diameter equal to the distance between the points (Real and McElhany 1996). This model assumes that the vector will most likely travel to the nearest sponge, regardless of the Euclidian

distance between them. In addition, it assumes that the vector only travels between sponges of a single species, since this disease only affects *A. cauliformis* at the sites studied. To determine if the observed Gabriel connections differed from random, the points were randomized, and the connections were determined in 100 iterations and compared to the observed connections, as described previously.

The polygon-based datasets were analyzed to identify pairs of polygons sharing boundaries and containing at least one diseased individual. The number of connections between polygons containing diseased sponges was counted for each time point. Similar to previous analyses, the original attributes describing each polygon were randomly assigned, creating a different dataset in a total of 100 iterations, and the expected random number of diseased polygon connections was compared to the observed diseased polygon connections as described previously.

Results

Monitoring of Individually Marked Sponges

Analysis of individually marked sponges demonstrated differential fates for healthy and diseased individuals. At each subsequent sampling time, the mean health status of the monitored diseased individuals was different than their healthy neighbors, which resulted in more diseased individuals being missed in surveys through time (Wilcoxon Rank-Sum test, $P < 0.0001$ and 0.03 for July 2008 and June 2009, respectively, Figure 12A). Initial health status did not significantly affect the length of healthy tissue through time (Figure 12B; $P = 0.11$), but there was a significant change in length of healthy tissue over the course of the monitoring ($P < 0.001$). Length of healthy tissue was significantly influenced by an interaction between health status and time ($P = 0.04$), showing that marked diseased sponges lost more biomass over the course of the

monitoring compared to marked healthy sponges. Analysis of sizes at each time point showed that while diseased sponges were initially larger than healthy sponges (ANOVA: $P = 0.02$), by the final sampling time there was a trend towards diseased sponges being smaller than healthy sponges ($P = 0.06$).

Sponge Population Monitoring

Sponge length, total length of all sponges and the number of healthy and diseased sponges from each site and sampling time point are summarized in Table 1. The proportion of diseased individuals in the population varied greatly from year to year (3.3% - 11%); while the average sponge length remained relatively similar both between sites and time points, Big Point experienced a pronounced decline in sponge biomass, as exhibited by the reduced total sponge number and length. The main loss in sponge biomass on the reefs seems to be related to periodic storm events, as Hurricane Irene caused a pronounced reduction in sponge biomass on both reefs in September 2011.

ARBS disproportionately affected larger sponges in the population (mean length ± SE), as determined by comparing the size frequency distributions of healthy and diseased sponges (Figure 13). All pre-hurricane grids showed a significant difference in the size frequency distribution of healthy and diseased sponges (Kolmogorov-Smirnov: $P = 0.003$). The size-frequency distributions of healthy and diseased sponges remained significantly different after the hurricane at Big Point ($P = 0.01$), but became more similar at Rainbow Gardens ($P = 0.4$). This shift in size at Rainbow Gardens is indicative of increased breakage of diseased sponges compared to healthy sponges, as has been documented in previous studies (Olson et al. 2006). While many large sponges in both grids were healthy, diseased sponges were usually in the larger size range of sponges in the population. Although it is possible that ARBS targets large

sponges, it is more likely that these sponges are affected at a higher frequency because they are older, which increases their probability of coming in contact with the pathogen. Alternatively, larger sponges have longer branch lengths and are able to interact with more sponges, thereby increasing their chances of interacting with other diseased individuals within the survey area.

Spatial analysis

Ripley's K Function: This analysis showed clustering of ARBS at all sites and time points. Most grids showed only slight clustering compared to an expected random distribution. Big Point, where the *A. cauliformis* population was denser, showed clustering of diseased sponges at a scale between 0.3 and 0.5 m, with the exception of the May 2010 time point, which showed a much larger maximum clustering scale of 5.5 m. Rainbow Gardens showed maximum clustering at 2-3 m scale for all time points.

Getis-Ord General G: Significant global clustering of ARBS for the pre-storm time points of Big Point May 2011, Big Point July 2011 and Rainbow Gardens July 2011 time points ($P = 0.04$, 0.02 and 0.006, respectively, Figure 14) was found, while the sponges from Big Point May 2010 and Rainbow Gardens May 2010 did not exhibit global clustering across their respective grids ($P = 0.88$, 0.98, respectively). No post-hurricane time points at either site exhibited global clustering patterns ($P > 0.05$, Figure 15). Even though some of these grids did not exhibit global clustering patterns of ARBS, every time point at each site showed ARBS hot-spots, suggestive of some small-scale clustering within the grid (Figures 14 and 15).

Moran's Index: Tests of the Moran's I statistic revealed global spatial autocorrelation in pre-storm time points for Big Point May 2011, Rainbow Gardens May 2010, and Rainbow Gardens

May 2011 (Moran's I; P = 0.002, 0.02, 0.07, respectively, Figure 14). While immediate post-hurricane grids (September 2011) showed no significant spatial autocorrelation between diseased individuals, there was significant spatial autocorrelation by June 2012 at Big Point (P = 0.0005; Figure 15), which suggests a strong influence of a transmission mechanism in a dense *A. cauliformis* population. Cluster and Outlier (Anselin Local Moran's I) analysis revealed specific areas of each grid that exhibited significantly high spatial autocorrelation, suggesting clustering, or significantly low spatial autocorrelation, suggesting dispersion of diseased sponges (Figures 4 and 5). The Cluster and Outlier analysis indicated that in time points without significant global spatial autocorrelation, the areas of significant negative spatial autocorrelation outnumbered those with significant positive spatial autocorrelation.

Join-counts: Join-count statistics suggested contact as the likely mechanism of transmission, demonstrated by significantly higher D:D contact joins in 3 of the 5 pre-hurricane grids (Table 2). Gabriel (vector) D:D joins did not differ from random at any sample time, and in 60% of cases, sponges that were connected by Gabriel connectedness were also connected by contact connectedness. One exception was Rainbow Gardens May 2010, where there were no shared joins, yet there was a trend (P = 0.08) toward significant Gabriel connectedness. Post-hurricane grids showed no significance for either type of connectedness. While the post-hurricane grids at Big Point showed increased probability of contact connectedness from September 2011 to June 2012 (29% to 86%, respectively), the Rainbow Gardens quadrats showed no such trend. These data suggests the existence of a density-dependent effect, due to a higher overall density of sponges at Big Point than at Rainbow Gardens, further implicating contact as the primary mode of transmission.

Analysis of polygon grids demonstrated a strong dependence of these results on clustering scale. At Big Point, where maximum clustering for significant contact joins was small, the 0.5 m^2 grids did not adequately reflect the trends seen in the join-count data for individuals. However, at Rainbow Gardens, where maximum individual clustering was at a larger scale, the same trends were observed for both significant point joins and polygon joins.

Discussion

Although *Aplysina cauliformis* is an abundant sponge species on Bahamian coral reefs (Olson et al. 2006), ARBS can reduce the abundance of cyanobacterial symbionts (Gochfeld et al. 2012a) on which *A. cauliformis* relies for most of its energy budget (Freeman and Thacker 2011); infection is also associated with differences in secondary chemistry (Gochfeld et al. 2012b), which can have implications for feeding deterrence, allelopathy and other important ecological functions. Additionally, previous studies have shown that ARBS infection weakens a sponge's skeleton, making infected sponges more susceptible to breakage, especially during storm events (Olson et al. 2006). However, despite documenting many dramatic effects of ARBS on these sponges, previous studies were not able to determine whether ARBS detrimentally impacted the long-term survival of individuals on the reef. By monitoring individually marked sponges through time, our data demonstrate that ARBS infection increased a sponge's rate of tissue loss over time, due to the expansion of the ARBS lesion and an increased probability of breakage. ARBS infection also increased the probability of death/removal of an individual from the reef, suggesting that infection with ARBS leads to a differential proximate fate of individual sponges on a reef.

Biomass of *A. cauliformis* populations declined over time. At Big Point, both total biomass and mean length of sponge in the population declined. At Rainbow Gardens, after an initial increase in biomass in July 2011, overall biomass decreased while mean length of sponges in the population remained relatively stable. The greatest influence on the sponge population, as well as to the coral reef community as a whole, was a major storm event. The immediate post-storm sampling time showed dramatic decreases in sponge biomass over a very short period of time (loss of 2,095 and 1,878 cm for Big Point and Rainbow Gardens, respectively). By the following June, both populations had gained biomass and the number of individuals in the population had increased. This increase in individuals, combined with the changes in the size frequency distribution post storm to smaller sponges, suggests that fragmentation of *A. cauliformis* occurred and that some of these fragments successfully settled on the reef. Diseased sponges at Rainbow Gardens were no longer, on average, larger than healthy sponges on the reef. Fragmentation has been shown to be an important mode for reproduction for some branching sponge species (Wulff 1991, Tsurumi and Henry 1997, Wulff 2007b), but fragmentation in the current study was more pronounced in diseased sponges, agreeing with previous observations that documented increased breakage in ARBS infected sponges (Olson et al. 2006). Additionally, increased breakage of individuals with active ARBS lesions could help in spreading ARBS to other members in the population and even to other reefs in the region.

Spatial analysis

Spatial analysis is a useful tool for epidemiology in a wide variety of applications, ranging from cholera epidemics to tree fungus transmission (Gilbert et al. 1994, Cliff and Haggett 1996, Real and McElhany 1996, Jolles et al. 2002). While these techniques have been

applied to several marine systems (Porter et al. 2001, Jolles et al. 2002, LeDrew et al. 2004, Gardner et al. 2008, Sato et al. 2009, Zvuloni et al. 2009, Van Houtan et al. 2010, Lentz et al. 2011, Roff et al. 2011, Ban et al. 2012), few have used them to their full potential, which has resulted in an inability to discern mechanistic information from the data. This lack of mechanistic information has largely prevented researcher from determining the dynamics of a disease within populations, and the long term effects of a particular disease (Bolker et al. 1995). Additionally, because spatial and temporal scales of transmission patterns can vary greatly among diseases, some basic understanding of a particular disease is crucial prior to studying disease dynamics using spatial pattern analysis (Gilbert et al. 1994, Real and McElhany 1996, Jolles et al. 2002). The current study built on previous studies focused on ARBS and assessed clustering using a variety of spatial statistical techniques and also investigated specific connectedness between sponges on the reef using join-counts. This comparison between spatial statistical methods in this study highlighted the subtle differences in the relationships identified by these various spatial statistics (Table 3).

The Ripley's K statistic is a common statistic used in marine epidemiology (Jolles et al. 2002, Gardner et al. 2008, Zvuloni et al. 2009, Lentz et al. 2011), and in the current study this statistic showed some degree of clustering at all sampling times, but with a highly variable range (Table 3). In a similar study by Jolles et al. (Jolles et al. 2002), the Ripley's K statistic helped to discern the mechanism for secondary transmission of Aspergillosis in sea fans (Jolles et al. 2002). The Ripley's K statistic was used to determine the presence of clustering and the scale at which clustering occurred, and from this information, researchers were able to investigate specific physical contact connections between individuals in their sampling area based on the recorded locations for each individual (Jolles et al. 2002). In the current study, due to the wide

clustering range at some time points and variability between time points, this statistic simply suggested that there might be some degree of ARBS clustering in the population.

The Getis-Ord General G spatial statistic has also been used in marine epidemiology studies (LeDrew et al. 2004, Roff et al. 2011, Ban et al. 2012) and in the current study it showed significant ARBS clustering at some time points, as well as showing specific areas of clustering within the grid for all time points. Ban et al. (Ban et al. 2012) used this technique at a much larger scale to identify areas along the Great Barrier Reef (GBR) that showed between reef clustering of coral bleaching and white syndrome. From this information, they can now investigate causal relationships for these stressors by focusing on conditions at specific reefs. Significant clustering using this statistic did not always correspond with significant results of true connections between individuals, which would suggest that measuring clustering with the Getis-Ord may not represent true spatial relationships between individuals on the reef.

Analysis of spatial autocorrelation using the Moran's Index provided results that aligned best with the true individual connectedness results. In every case where the join-count connections were significant, the Moran's I results were also significant, and this statistic seemed more sensitive to spatial relationships than the join-count connections. The Moran's I statistic may be less affected by the possible loss of intermediate diseased sponges that may have provided the connection between two currently diseased individuals.

The three spatial statistics used here are excellent initial approaches to investigate the basics of spatial relationships in a system, but to ultimately determine a specific mechanism for disease transmission, individual connectedness must be examined. Our study investigated both direct contact connectedness and Gabriel (vector) connectedness. These two methods proved useful because they specifically examined how individuals in the population were connected to

each other, taking into account the attributes of each sponge and its location relative to all other sponges in the survey area (Gilbert et al. 1994, Real and McElhany 1996). Direct contact transmission analyses were selected based on previous studies demonstrating ARBS transmission with forced contact between diseased and healthy individuals (Olson et al. 2006) and because the morphology of *A. cauliformis* enables potential physical contact with neighboring sponges. Gabriel connectedness (vector transmission) was adapted from a terrestrial model used to investigate transmission of a plant disease by pollinating insects (Gilbert et al. 1994, Real and McElhany 1996), and in this study was used to test for transmission through spongivore feeding. The results of the join-count statistics showed significant contact joins in three out of five pre-storm time points, indicating that physical contact was the most likely form of transmission in this system. Following hurricane disturbance, we observed diseased sponge connections that were no different than random, with insignificant join-count statistic p-values; however, these observations added additional support to the theory of transmission by physical contact. Previous data (Olson et al. 2006) and post-storm size frequency data showed that diseased sponges were more susceptible to breakage. When these sponges fragmented or were removed from the substrate, they could have rolled around the reef, potentially randomly contacting other sponges in the population. Using the physical contact theory, if contact was randomized, the disease pattern should also be randomized, as we observed in the post-hurricane time points. In contrast, we would not expect a vector driven system to become randomized after the hurricane, since the feeding patterns of spongivores, for example *Canthigaster rostrata or Monacanthus tuckeri* (Easson pers obs), would not be randomized suddenly due to the hurricane. Additionally, between September 2011 and June 2012, the probability of contact connectedness at Big Point increased from 29% to 86% as the time since the storm increased. This trend was not observed

at Rainbow Gardens, where the sponge population is less dense than at Big Point. The difference may reflect a possible dependence on the density of the *A. cauliformis* population for ARBS transmission that we would expect in a contact driven system.

Multiple factors could be affecting disease and transmission within the population of *A. cauliformis*. One challenge was the ephemeral nature of the sponges themselves. As seen in the marked sponge data, the annual resample rate was 67% and 39%, for healthy and diseased individuals, respectively. Unlike corals, which are long-lived (years to decades), and leave behind a permanent skeletal record of their location, sponges have shorter life-spans (months to years), and when they die, they do not leave behind a skeleton that can be accounted for in subsequent sampling times. Thus, the ability to resample individuals and determine their proximate fate is lower than in studies investigating coral diseases. Another factor that might affect a spatial population study like the current one is the influence of sponges outside the sampling area. Our grids represent only a portion of the shallow reefs on which they are located. Thus, there could be significant influence of sponges just outside the sampling area in terms of disease dynamics. Many diseased sponges at these sites occurred along the edge of the grid throughout the sampling period, but since these outside sponges were never directly sampled, we cannot account for their influence on sponges inside the grid. These issues are potentially reflected in some of the insignificant statistical results found in this study, suggesting that additional factors may be influencing the spatial dynamics of the sponge population in these grids.

While the connectedness models fit reasonably well, each failed to meet all assumptions of the models. In the physical contact model, the sponge branch lengths were considered equal since exact branch length of every sponge was not recorded, and this may underestimate sponge

contacts. The vector model (Gabriel connectedness) assumed that the vector, in this case spongivores, presumably fed on *A. cauliformis* exclusively. While many organisms eat sponges on Caribbean reefs (Pawlik 2011), none have been documented to feed exclusively on *A. cauliformis*. This could account for the presence of ARBS-like lesions in other species of Verongids, but it may also dilute the influence of vector-driven transmission in our model, since a spongivore may move to other sponge species after feeding on a diseased *A. cauliformis* individual. Conversely, it means that any effects of vector transmission from other sponge species will be unaccounted for and therefore underestimated. Despite these imperfections, we feel that these statistical models adequately represent the dynamics of ARBS transmission due to a significant contact connectedness signal that shows up over the noise of a natural system.

In conclusion, ARBS has been shown to be a detrimental disease to individuals, but its effects at the population level were not as obvious, possibly due to the dramatic and potentially confounding effects of the hurricane. Spatial analysis techniques have tremendously increased our understanding of the dynamics of *Aplysina* Red Band Syndrome within *A. cauliformis* populations, and enabled us to propose a physical contact mechanism of ARBS transmission within populations of *A. cauliformis*. However, this study also raised questions regarding disease susceptibility of these sponges. Among sponges that were in contact, transmission rates for ARBS were typically low, suggesting that some genotypes may be more susceptible to infection than others. With the advent of many next generation sequencing techniques, we can begin to address these questions. Coupling these techniques with spatial analysis may enable us to understand the implications of ARBS on *Aplysina* populations in the Caribbean.

Figure 11: Representative photo-mosaics of *Aplysina cauliformis* population in 10 x 10 m grids at A. Big Point and B. Rainbow Gardens. Dots represent the relative locations of healthy and diseased *A. cauliformis* in the grids.

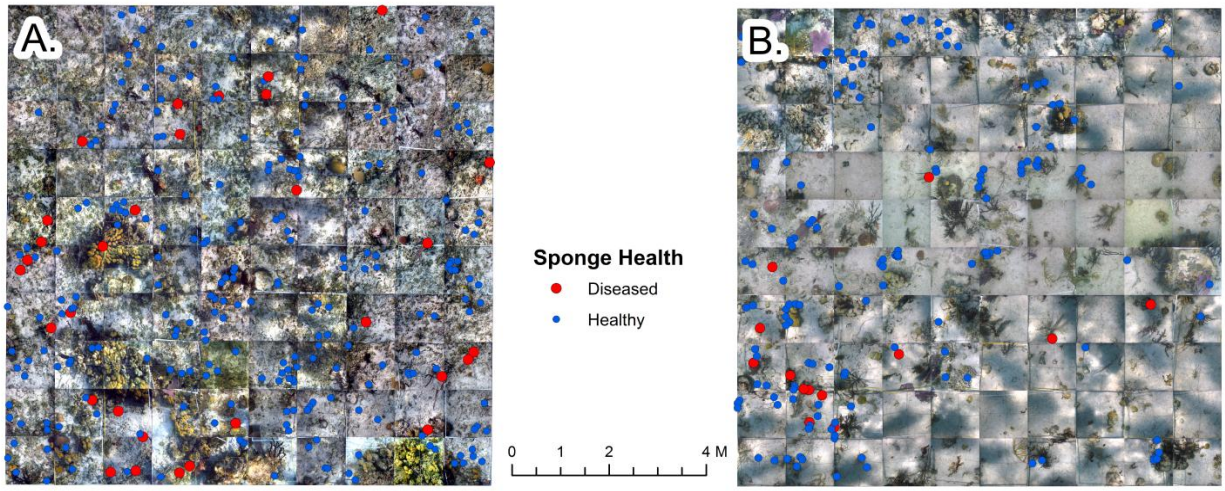


Figure 12: A. Fate of Healthy and Diseased Marked sponge. Diseased sponges were more likely to stay diseased and go missing compared to healthy sponges (Wilcoxon rank-sum analysis). **B.** Mean length of marked sponges over time. While the diseased sponges are larger than healthy sponges in March 08, their mean lengths decline at a greater rate than healthy sponges (Repeated measures ANOVA, P = 0.04).

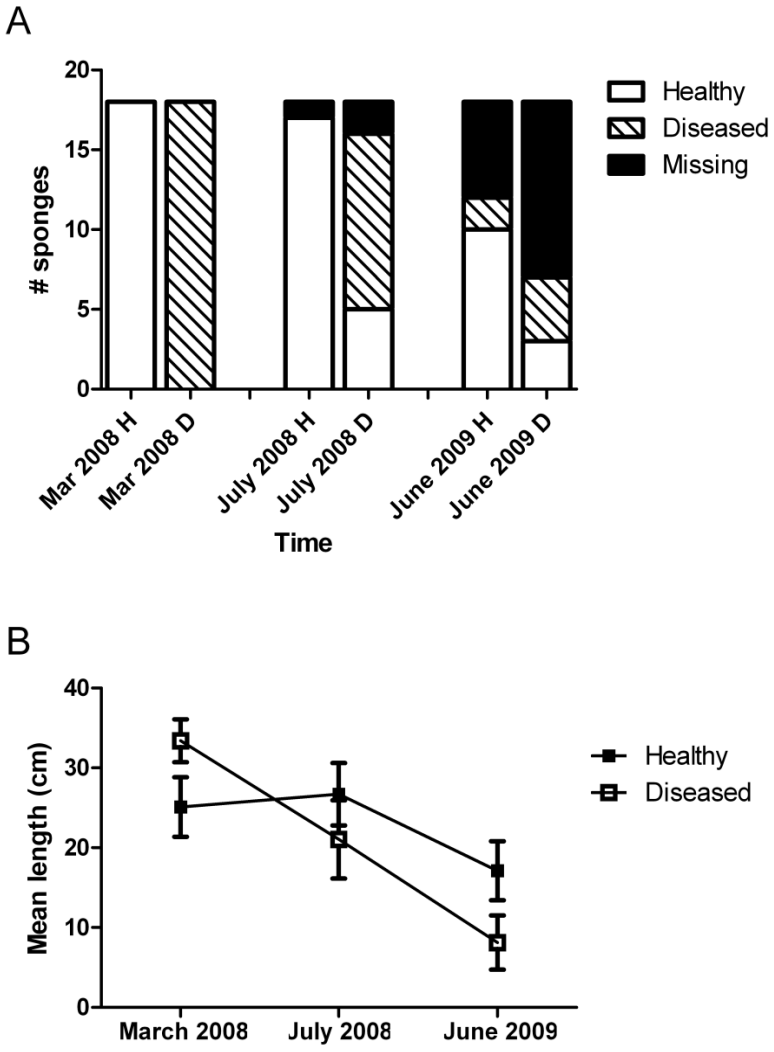


Figure 13: Size Frequency Distribution of Healthy and diseased sponges. A-E are pre-storm sampling times and F-I are post-storm sampling times. White bars represent healthy sponges in the population, and black bars represent diseased individuals in the population.

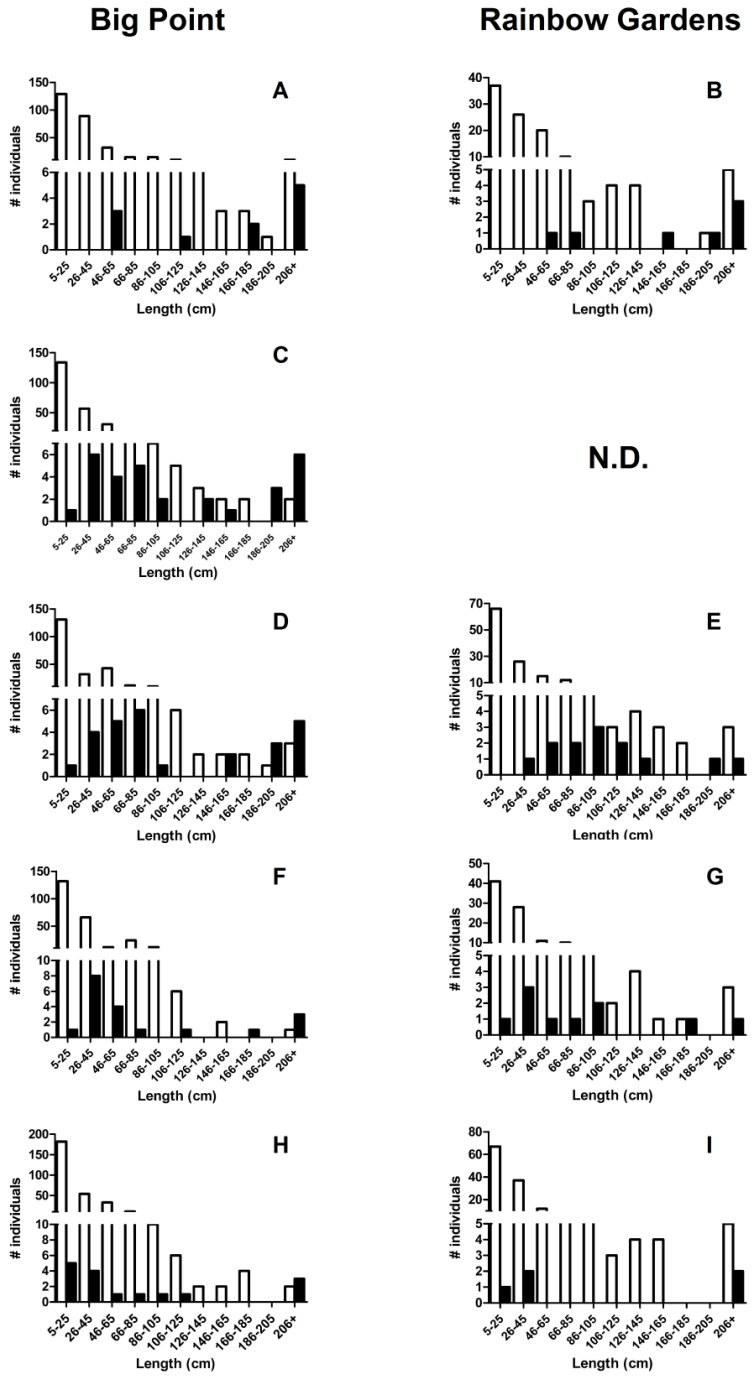


Figure 14: Comparison of areas of clustering and dispersion between the Getis-Ord General G and Moran's I statistics. Getis-Ord quadrats (on left) display areas where the General G value was significant for clustering. Moran's I quadrats (on right) display areas of significantly high clustering and significantly low clustering. The differences in the ways in which they calculate spatial relationships translate into them recognizing slightly different areas as "clustered". Also note that between sampling times, significant clusters often overlap or are immediately adjacent to one another suggesting transmission is over a relatively small scale.

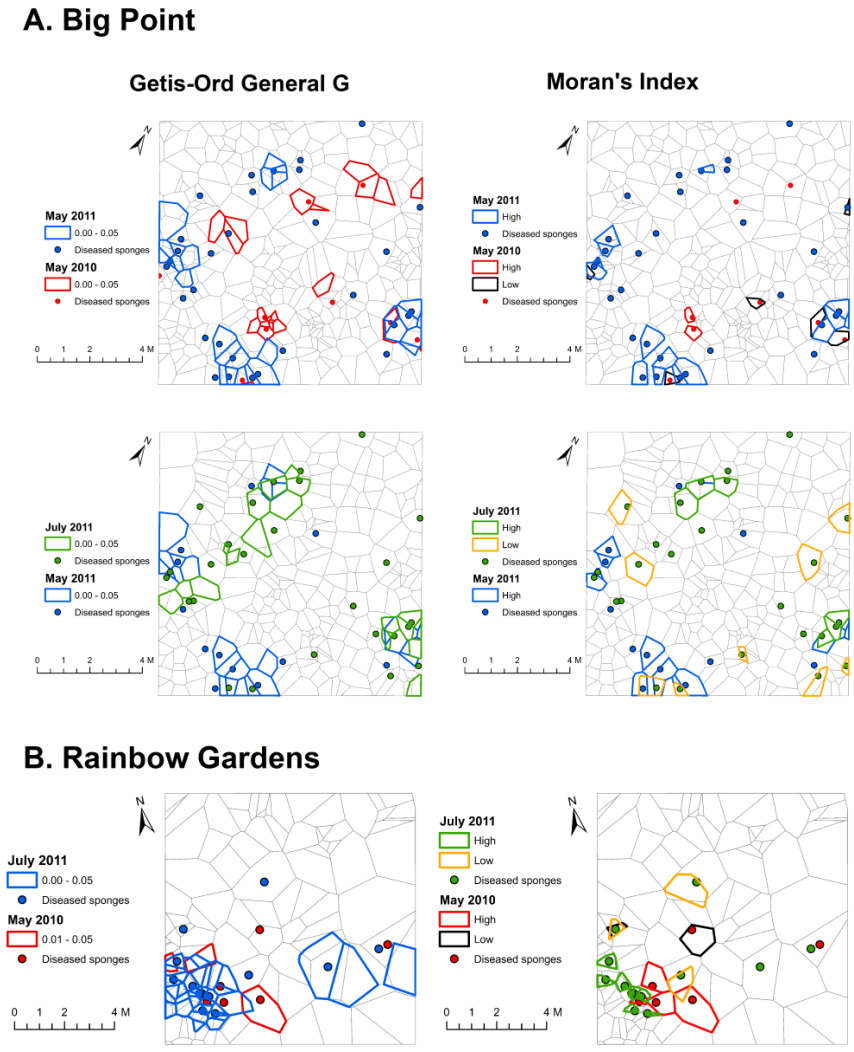


Figure 15: Post-storm sampling times showing the effects of the storm on the clustering patterns observed in the grids. Getis-Ord results are shown on left and Moran's I results are shown on the right. The hurricane randomized the observed patterns in pre-storm sampling times, but this pattern appeared to recover to some degree between September 2011 and June 2012 at Big Point.

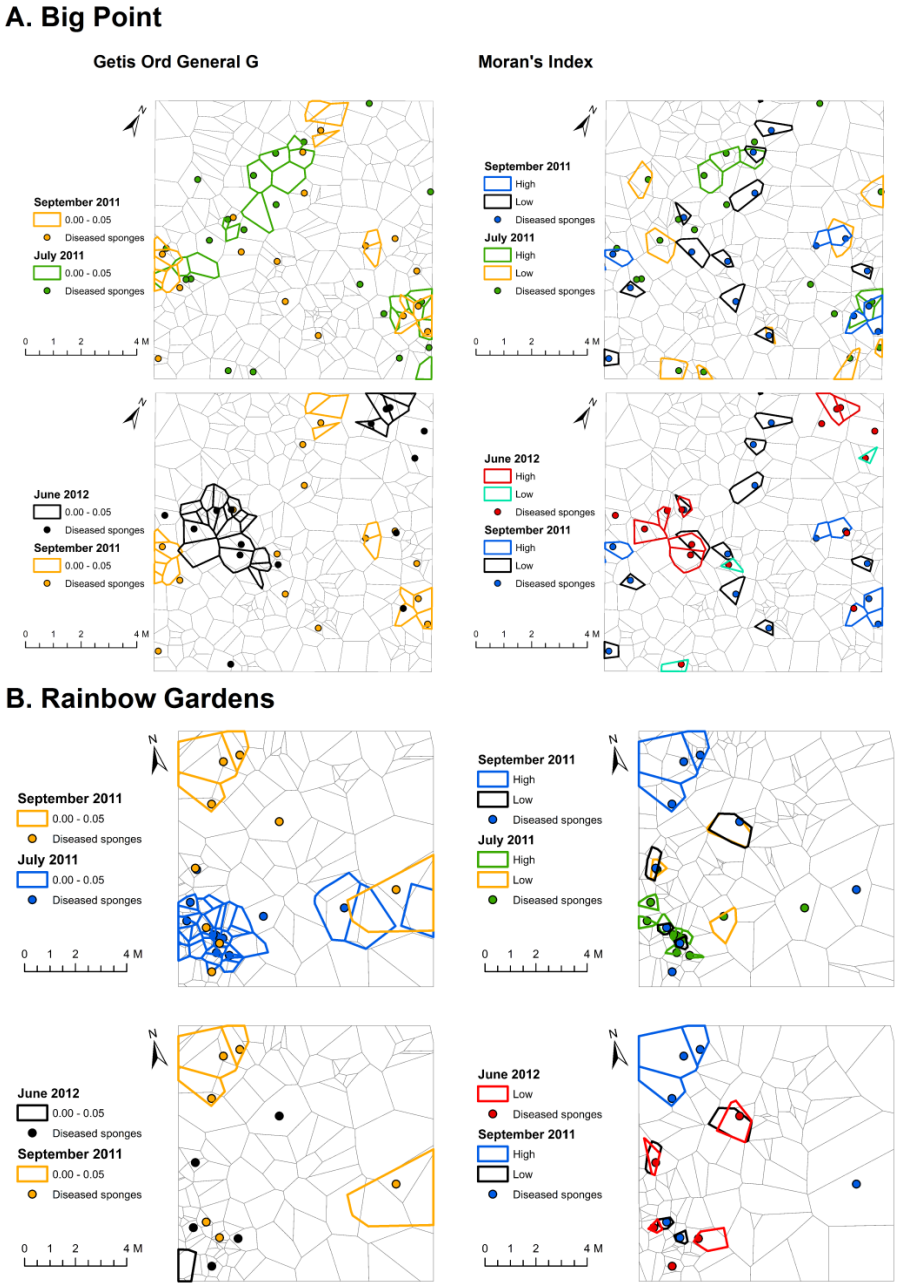


Table 2: *Aplysina cauliformis* population attributes at two shallow reef sites in the Bahamas from 2010 to 2012. The dashed line represents the occurrence of Hurricane Irene.

	Total sponges	Healthy sponges	Diseased sponges	Percent Diseased	Mean±SE length (cm)	Total length (cm)
Big Point 2010	342	330	12	3.5	52±4	17,795
Big Point May 2011	285	254	31	10.9	46±4	13,063
Big Point July 2011	270	243	27	10	47±4	12,728
Big Point September 2011	280	261	19	6.8	38±3	10,633
Big Point June 2012	320	305	15	4.7	36±2	11,529
Rainbow Gardens 2010	133	126	7	4	56±6	7,400
Rainbow Gardens 2011	187	174	13	9.8	48±4	8,283
Rainbow Gardens September 2011	118	108	10	8.5	54±5	6,405
Rainbow Gardens June 2012	152	147	5	3.3	50±5	7,599

Table 3: Join-count statistics of healthy and diseased *A. cauliformis* in 10 x 10 m grids at two sites in the Bahamas. Joins are represented as H:H = healthy:healthy, H:D = healthy:diseased, D:D = diseased:diseased; Total = total joins in a grid. P=the proportion of random diseased:diseased joins that were greater than or equal to the observed number of diseased:diseased joins. BP = Big Point and RG = Rainbow Gardens, Bold numbers = significant values and Underlined numbers = trends in the results. The dashed line represents the occurrence of Hurricane Irene.

	Contact Connectedness Observed/ Expected Joins					**Gabriel Connectedness Observed/Expected Joins**				
	H:H	H:D	D:D	Total	*P* =	H:H	H:D	D:D	Total	*P* =
BP May 2010	873/843	98/143	2/4.4	973/991	0.93	587/604	41/45	0/1	628/650	0.99
BP May 2011	393/328	106/144	24/14	524/486	**0.03**	407/425	100/109	10/7	517/540	0.50
BP July 2011	458/331	126/156	18/14	602/502	0.30	393/410	81/92	8/5	482/507	0.13
BP Sept 2011	412/341	94/92	4/4.7	510/434	0.71	424/459	60/67	2/2	486/529	0.70
BP June 2012	482/381	72/80	6/3	560/464	0.14	508/549	46/55	2/1	556/605	0.27
RG May 2010	184/83	30/20	4/1	218/104	**0.05**	197/216	23/24	2/0.5	222/241	<u>0.08</u>
RG July 2011	298/121	66/29	8/1.6	373/152	**0.03**	225/274	42/45	2/1.6	269/321	0.45
RG Sept 2011	236/71	46/18	0/1	282/90	0.99	157/176	30/33	2/1.4	189/210	0.39
RG June 2012	306/119	20/12	0/0.2	326/132	0.99	221/259	15/18	0/0.3	236/278	0.99

Table 4: Summary of Spatial statistical analyses. Comparison of the results of spatial statistics associated with disease of *Aplysina cauliformis* two populations at different time points. Each statistic tests for slightly different spatial characteristics. Contact connectedness was best able to explain clustering patterns within the study grids. In the current study, the results of the Moran's Index best represented individual connections shown by direct contact join-counts for ARBS infected sponges. *Range represents scale of significant clustering for the Ripley's K statistic. Clusters and Outliers are individual Thiessen polygons in the grids that exhibited significant values for the Getis-Ord General G and the Moran's Index Statistics. BP=Big Point; RG=Rainbow Gardens.

Statistic	Origin	What is Tested	References in Marine Epidemiology
Ripley's K function	Ripley 1981	Clustering or dispersion over a range of distances	Jolles et al. 2002, Gardner et al. 2008, Zvuloni et al. 2009, Lentz et al. 2011, Muller and van Woesik 2012
Getis-Ord General G	Getis and Ord 1992	Clustering of values in a given area	LeDrew et al. 2004, Roff et al. 2011, Ban et al. 2012
Moran's Index	Moran 1950	Clustering via measuring spatial autocorrelation: Feature similarity based on locations and values	Van Houton et al. 2010, Ban et al. 2012
Join-Count	Sokal and Oden 1978	Connections between individuals in a population (Contact, Gabriel, Nearest Neighbor)	Jolles et al. 2002, Zvuloni et al. 2009

Results Summary		BP May 2010	BP May 2011	BP July 2011	BP Sept 2011	BP June 2012	RG May 2010	RG July 2011	RG Sept 2011	RG June 2012
Ripley's K function	Max	5.5m	0.3m	0.3m	0.5m	0.3m	3.1m	2.3m	2.5m	2m
	Range*	0-7m	0-1.8, 2.3-6.4m	0-1m	0-6.2m	0-1.7, 4.4-6.1m	0-6m	0-5.9m	0-5.4m	0-6.2m
Getis-Ord General G	Clusters	23	**31**	19	17	26	8	40	4	1
	P-value	0.88	**0.04**	**0.02**	0.64	0.39	0.98	**0.0006**	0.59	0.53
Moran's Index	Clusters	2	15	6	7	8	**4**	8	3	0
	Outliers	6	10	9	12	7	2	3	4	4
	P value	0.9	**0.002**	0.47	0.72	**0.02**	0.07	**0.0005**	0.95	0.147
Join-Count - Direct Contact	Diseased joins (O/E)	**2/4**	**24/14**	18/14	4/5	6/3	**4/1**	8/2	0/1	0/0
	P-value	0.93	**0.03**	0.3	0.71	0.14	**0.05**	**0.03**	0.99	0.99
Join-Count - Gabriel (vector)	Diseased joins (O/E)	0/1	10/7	8/5	2/2	2/1	2/0.5	2/2	2/1	0/0
	P-value	0.99	0.5	0.13	0.7	0.27	0.08	0.45	0.39	0.99

CONCLUSIONS

The research in this dissertation aimed to answer some fundamental questions about how sponges, important members of the coral reef community, respond to a variety of potential environmental stressors. This research sought to not just address these stressors individually, but in environmentally relevant combinations, to better understand their true impacts. The work presented here built on previous knowledge about the ecology of sponges, how they interact with other reef organisms, how diseases are spread in a population, and how sponges respond to natural and anthropogenic stressors.

The research in Chapter 1 revealed a complex sponge-algal interaction that changed under elevated nutrient concentrations. This complex interaction exhibited properties of both competition and facilitation. Algal contact reduced sponge photosymbiont abundance and productivity. This occurred mostly through shading of these symbionts, shown by similar responses between the shade control and algal contact treatments, as well as a lack of allelopathic effects. When this experiment was repeated in a laboratory setting over a two week period (Appendix III), algal contact did not elicit a negative effect on the sponge, suggesting that it may be able to cope with shorter periods of reduced irradiance. Sponge contact positively affected the alga through increasing algal productivity. This result indicates that the alga may be receiving some nutrient from the sponge. Because *A. cauliformis* has been shown to efflux nitrogen resources (Maldonado et al. 2012), it was hypothesized that nitrogen transfer from the sponge could be the cause of increased productivity in the alga.

Elevated nutrients had some effects on the sponge-algal interaction. Nutrient enrichment had mixed effects on *A. cauliformis* individuals. The addition of nutrients enhanced symbiotic cyanobacterial productivity in the sponge, in the absence of shading. This is not surprising, as this has previously been shown to happen in closely related free-living cyanobacteria (Zehr and Ward 2002). Despite the benefit to the cyanobacterial symbionts, the sponge holobiont responded negatively to elevated nutrients, as measured by circulating soluble protein concentrations, and this same negative effect was also observed in a shorter-term laboratory experiment (Appendix III). This discrepancy between cell fractions could indicate a disruption in the tightly coupled symbiosis between the cyanobacterial symbionts and the sponge host. Because *A. cauliformis* relies on its symbionts for most of its energy budget, and has not shown the ability to adjust to more heterotrophic feeding strategies (Freeman and Thacker 2011), a disruption in this important relationship could be quite detrimental to the long-term survival of the sponge. Algal productivity increased in response to elevated nutrients, but unlike in the previous experiment, it did not respond to sponge contact. This may further suggest that the driver for increased algal productivity associated with sponge contact is nutrient transfer, because when nutrient levels were elevated above the low levels the sponge may excrete, the benefit of sponge contact was not observed.

Potential mechanisms for algal contact stress and sponge facilitation of the alga using stable isotopes of carbon and nitrogen were explored in Chapter 2. There were no effects on carbon and nitrogen dynamics within *A. cauliformis* associated with *M. marinum* contact. Long-term contact with *M. marinum* might be expected to reduce carbon and nitrogen assimilation and/or translocation, as has been seen in other studies (Freeman et al. 2013), but over the 24 hour time frame of the experiments, no effect was observed. In contrast, sponge transfer of nitrogen to

the alga was observed for the nitrogen substrate ammonium. This transfer was not observed for a nitrate substrate of nitrogen, and many factors, as discussed in chapter 2, could contribute to this differential transfer and/or availability of these two nitrogen substrates. The results of these experiments suggest that the increased productivity observed in the alga after a month in contact with the sponge was due to the transfer of low levels of nitrogen from the sponge to the alga.

To build on previous research showing that *Aplysina* Red Band Syndrome (ARBS) maintains a noticeable presence in populations of *A. cauliformis* in the Bahamas (Olson et al. 2006), has detrimental effects to the health of an individual (Gochfeld et al. 2012a) and possibly its chemical defenses (Gochfeld et al. 2012b), and can be transmitted to healthy individuals through forced physical contact (Olson et al. 2006, Gochfeld and Olson unpub), the research in the final chapter asked three fundamental questions about ARBS. (1) Does ARBS affect the long-term survival of an individual in the population? (2) How is ARBS transmitted within a natural population of *A. cauliformis*? and (3) How does the *A. cauliformis* population and ARBS transmission respond to natural disturbances to the ecosystem? This research showed that infection with ARBS significantly decreases an individual's chance of survival on the reef. Given the individual effects that were previously documented associated with ARBS infection (tissue loss and increased susceptibility to breakage; Olson et al. 2006, Gochfeld et al. 2012a), it is not surprising that these characteristics would negatively affect long-term survival of an individual.

To address transmission of the disease in a natural system, this study utilized spatial analysis techniques that have been applied to several marine disease studies, with mixed results (Jolles et al. 2002, Zvuloni et al. 2009, Roff et al. 2011, Ban et al. 2012). Spatial analysis proved

extremely useful for studying transmission of ARBS and helped us to determine that physical contact between individuals on the reef was the major driver for transmission within a population. Having the knowledge of how this disease is transmitted within a population and how it responds to population structure and periodic disturbances allows us to now model its spread and predict the long-term impacts of ARBS to the *A. cauliformis* population. It is important to note that the current research determined how ARBS is transmitted within a population, not between populations. It is unlikely that direct contact would account for the widespread occurrence of ARBS across the Caribbean basin. Determining transmission between regions and/or initial establishment in a region will likely come from identification of the etiologic agent responsible for ARBS.

The occurrence of Hurricane Irene during our study gave us the unique opportunity to investigate the effects of this storm on *A. cauliformis* populations. The storm had a dramatic effect on sponge biomass, reducing sponge biomass by approximately 19% almost overnight. This sharp decline in biomass was particularly high in diseased sponges, and the hurricane effectively randomized the disease pattern. One possible explanation of this random post-storm pattern is that when the storm removed or broke diseased sponges, these infected pieces could have rolled around the reef randomly contacting other individuals in the population. Storm effects on the greater population reduced overall biomass, but in later surveys, the number of individuals was dramatically increased, which may suggest increased fragmentation, an important reproductive mode in sponges (Wulff 2006b). In the absence of further disturbance, these new individuals, along with new recruits, would be allowed to prosper, essentially resetting the system. While historically this would be the case, the sponges in these populations now have to contend with additional stressors such as ARBS and algal contact.

Our ability to analyze the effects of multiple stressors is paramount to understanding how coral reefs of the future will be structured. While this dissertation offered some insight into how an important reef organism responds to a combination of natural and anthropogenic stressors, many questions remain unanswered. Through continued population monitoring, we could likely determine whether the seasonality of *M. marinum* gives sponges some respite from algal contact, which might allow the *A. cauliformis* population to persist despite the stress of algal contact. To determine whether internal carbon and nitrogen dynamics are disrupted and/or if nitrogen transfer is affected by long-term algal contact, a similar experiment to the chapter 1 field experiment could be done, coupled with enrichment of sponge samples and investigation of subsequent transfer to algae as in chapter 2. This would determine whether the sponge-symbiont relationship was disrupted by algal contact and/or increased nutrients, and if nitrogen contribution to the alga was affected as a result. Lastly, when investigating disease transmission, one must consider susceptibility. It is possible that some genotypes are less susceptible to ARBS infection, explaining the relatively low transmission rate in the natural population. Employing modern molecular techniques with a relevant spatial sampling scheme for the population could begin to answer the question of whether some sponges are inherently more or less susceptible to ARBS infection, allowing us to better predict the broader effects of the disease in the population.

BIBLIOGRAPHY

Allen, A. E., M. G. Booth, M. E. Frischer, P. G. Verity, J. P. Zehr, and S. Zani. 2001. Diversity and detection of nitrate assimilation genes in marine bacteria. Applied Environmental Microbiology **67**:5343-5348.

Anselin, L. 1995. Local Indicators of Spatial Association - LISA. Geogr Anal **27**:93-115.

Anselin, L., S. Sridharan, and S. Gholston. 2007. Using Exploratory spatial data analysis to leverage social Indicator databases: The discovery of interesting patterns. Soc Indic Res **82**:287-309.

Anselin, L., A. Varga, and Z. Acs. 1997. Local Geographic Spillovers between University Research and High Technology Innovations. J Urban Econ **42**:422-448.

Aronson, R. B., I. G. Macintyre, C. M. Wapnick, and M. W. O'Neill. 2004. Phase shifts, alternative states and the unprecedented convergence of two reef systems. Ecol **85**:1876-1891.

Aronson, R. B. and W. F. Precht. 2000. Herbivory and Algal Dynamics on the Coral Reef at Discovery Bay, Jamaica. Limnology and Oceanography **45**:251-255.

Aronson, R. B. and W. F. Precht. 2001. White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia **460**:25-38.

Bailey, T. C. and A. C. Gatrell. 1995. Interactive Spatial Data Analysis. Longman Scientific & Technical, Harlow, U.K. .

Baldacconi, R. and G. Corriero. 2009. Effects of the spread of the alga *Caulerpa racemosa var. cylindracea* on the sponge assemblage from coralligenous concretions of the Apulian coast (Ionian Sea, Italy). Marine Ecology **30**:337-345.

Ban, S. S., N. A. J. Graham, and S. R. Connolly. 2012. Relationships between temperature, bleaching and white syndrome on the Great Barrier Reef. Coral Reefs:1-12.

Banner, A. 1974. Kaneohe Bay, Hawaii, Urban pollution and a coral reef ecosystem.*in* Proceedings of the Second International Symposium on Coral Reefs.

Barott, K. L., B. Rodriguez-Mueller, M. Youle, K. L. Marhaver, M. J. A. Vermeij, J. E. Smith, and F. L. Rohwer. 2012. Microbial to reef scale interactions between the reef-building coral Montastraea annularis and benthic algae. Proceedings of the Royal Society B: Biological Sciences **279**:1655-1664.

Bell, J. 2008. The functional roles of marine sponges. Estuarine, Coastal and Shelf Science **79**:341-353.

Bell, P. 1992. Eutrophication and coral reefs: some examples in the Great Barrier Reef lagoon. Water Resources **26**:553-568.

Bellwood, D., T. Hughes, C. Folke, and M. Nyström. 2004. Confronting the coral reef crisis. Nature **429**:827-833.

Bolker, B. M., M. Altmann, F. Ball, N. D. Barlow, R. G. Bowers, and B. G. Williams. 1995. Group report: spatial dynamics of infectious diseases in natural populations. Cambridge University Press, Cambridge, UK.

Boots, B. and A. Getis. 1988. "Point Pattern Analysis". Sage Publications, Newbury Park, CA.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**:248-254.

Brandt, M. E. and J. W. McManus. 2009. Disease incidence is related to bleaching extent in reef-building corals. Ecol **90**:2859-2867.

Bruckner, M. 2011. The Gulf of Mexico Dead Zone. Microbial Life Educational Resources.

Bruno, J. B. and M. D. Bertness. 2000. Habitat modification and facilitation in Benthic Marine Communities. Marine Community Ecology. Sinauer Associates, Sunderland, MA.

Bruno, J. B., J. J. Stachowicz, and M. D. Bertness. 2003a. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution **18**:119-125.

Bruno, J. F., L. E. Petes, D. C. Harvell, and A. Hettinger. 2003b. Nutrient enrichment can increase the severity of coral diseases. Ecology Letters **6**:1056-1061.

Burkepile, D. E. and M. E. Hay. 2006. Herbivore vs. nutrient control of marine primary producers: context-dependent effects. Ecology **87**:3128-3139.

Carpenter, R. C. 1990. Mass mortality of *Diadema antillarum* . I. Long-term effects on sea urchin population dynamics and coral reef algal communities. Mar Biol **104**:67-77.

Cebrian, E. and M. J. Uriz. 2006. Grazing on fleshy seaweeds by sea urchins facilitates sponge *Cliona viridis* growth. Marine Ecology Progress Series **323**:83-89.

Cliff, A. D. and P. Haggett. 1996. The impact of GIS on epidemiological mapping and modeling. Pages 321-343 *in* P. Longley and M. Batty, editors. Spatial Analysis: Modeling in a GIS Environment. Pearson Professional, New York, NY.

Cliff, A. D. and J. K. Ord. 1981. Spatial processes : models & applications. Pion, London.

Cole, R. G. and C. Syms. 1999. Using spatial pattern analysis to distiguish causes of mortality: and example from kepl in north-eastern New Zealand. J Ecol **87**:963-972.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Set.

Connell, J. H., T. P. Hughes, C. C. Wallace, J. E. Tanner, K. E. Harms, and A. M. Kerr. 2004. A long-term study of competition and diversity of corals. Ecological Monographs **74**:179-210.

Cowart, J., T. Henkel, S. McMurray, and J. Pawlik. 2006. Sponge orange band (SOB): a pathogenic-like condition of the giant barrel sponge, Xestospongia muta. Coral Reefs **25**:513-513.

Crawley, M. 1986. Plant Ecology. Blackwell Scientific Publications, Oxford.

Davis, A. R., D. D. Roberts, and S. P. Cummins. 1997. Rapid invasion of a sponge dominated deep reef by *Caulerpa scalpelliformis* (Chlorophyta) in Botany Bay, New South Wales. Australian Journal of Ecology **22**:146-150.

Davy, S. K., D. A. Trautman, M. A. Borowitzka, and R. Hinde. 2002. Ammonium excretion by a symbiotic sponge supplies the nitrogen requirements of its rhodophyte partner. Journal of experimental biology **205**:3505-3511.

de Nys, R., J. Coll, and I. Price. 1991. Chemically mediated interactions between the red alga *Plocium hamatum* (Rhodophyta) and the octocoral *Sinularia cruciata (*Alcyonacea). Marine Biology **108**:315-320.

Díaz, M. C. and K. Rützler. 2001. Sponges: An essential component of Caribbean coral reefs. Bulletin of Marine Science **69**:535-546.

Edmunds, P. J. 1991. Extent and effect of black band disease on a Caribbean reef. Coral Reefs **10**:161-165.

Ellison, A. M., E. J. Farnsworth, and R. R. Twilley. 1996. Facultative mutualism between red mangroves and root-fouling sponges in Belizean mangal. Ecology **77**:2431-2444.

Elsner, J. B., J. P. Kossin, and T. H. Jagger. 2008. The increasing intensity of the strongest tropical cyclones. Nature **455**:92-95.

Engel, S. and J. Pawlik. 2005. Interactions among Florida sponges. II. Mangrove habitats. Marine Ecology Progress Series **303**:145-152.

Erwin, P. and R. Thacker. 2007. Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. Journal of Marine Biology Associations of the U.K. **87**:1683-1692.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and L. McCloskey. 1993. Population control in symbiotic corals. BioScience **43**:606-611.

Fiore, C. L., J. K. Jarrett, N. D. Olson, and M. P. Lesser. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends in Microbiology **742**:1-9.

Fitt, W., B. Brown, M. Warner, and R. Dunne. 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs **20**:51-65.

Freeman, C. and R. Thacker. 2011. Complex interactions between marine sponges and their symbiotic microbial communities. Limnol Oceanogr **56**:1577-1586.

Freeman, C. J., R. W. Thacker, D. M. Baker, and M. L. Fogel. 2013. Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance&quest. The ISME journal.

Fry, B. 2006. Stable isotope ecology. Springer.

Gaino, E., R. Pronzato, G. Corriero, and P. Buffa. 1992. Mortality of commercial sponges: incidence in two Mediterranean areas. Boll Zool **59**:79-85.

Galstoff, P. S., H. H. Brown, C. L. Smith, and F. G. W. Smith. 1939. Sponge mortality in the Bahamas. Nature **394**:137-138.

Gardner, B. G. B., P. J. S. P. J. Sullivan, S. J. M. S. J. Morreale, and S. P. E. S. P. Epperly. 2008. Spatial and temporal statistical analysis of bycatch data: patterns of sea turtle bycatch in the North Atlantic. Can J Fish and Aquat Sci **65**:2461-2470.

Gardner, T. A., I. M. Cote, J. A. Gill, A. Grant, and A. R. Watkinson. 2005. Hurricanes and Caribbean coral reefs: impacts, recovery patterns, and role in long-term decline. Ecology **86**:174-184.

Getis, A. 1984. Interactive modeling using second-order analysis. Environ Plann A **16**:173-183.

Getis, A. and J. K. Ord. 2010. The analysis of spatial association by use of distance statistics. Geogr Anal **24**:189-206.

Gilbert, G. S., S. P. Hubbell, and R. B. Foster. 1994. Density and distance-to-adult effects of a canker disease of treesin a moist tropical forest. Oecologia **98**:100-108.

Gladfelter, W. B. 1982. White-band disease in Acropora palmata: implications for the structure and growth of shallow reefs. Bulletin of Marine Science **32**:639-643.

Gochfeld, D. 2010. Territorial damselfishes facilitate survival of corals by providing an associational defense against predators. Marine Ecology Progress Series **398**:137-148.

Gochfeld, D. J., C. G. Easson, C. J. Freeman, R. J. Thacker, and J. B. Olson. 2012a. Disease and nutrient enrichment as potential stressors on the Caribbean sponge *Aplysina cauliformis* and its bacterial symbionts. Mar Eco Prog Ser **456**:101-111.

Gochfeld, D. J., H. N. Kamel, J. B. Olson, and R. W. Thacker. 2012b. Trade-Offs in Defensive Metabolite Production But Not Ecological Function in Healthy and Diseased Sponges. J Chem Ecol **38**:451-462.

Gochfeld, D. J., C. Schloder, and R. W. Thacker. 2007. Sponge community structure and diseaseprevalence on coral reefs in Bocas del Toro, Panama. Porifera Research: Biodiversity, Innovation and Sustainability:335-343.

González-Rivero, M., R. Ferrari, C. Schönberg, and P. Mumby. 2012. Impacts of macroalgal competition and parrotfish predation on the growth of a common bioeroding sponge. Marine Ecology Progress Series **444**:133-142.

González-Rivero, M., L. Yakob, and P. Mumby. 2011. The role of sponge competition on coral reef alternative steady states. Ecological Modeling **222**:1847-1853.

Goodchild, M. F. 1986. Spatial autocorrelation. Geo Books Norwich.

Griffith, D. 1987. Spatial Autocorrelation: A Primer. Association of American geographers.

Haas, A. F., C. Jantzen, M. S. Naumann, R. Iglesias-Prieto, and C. Wild. 2010. Organic matter release by the dominant primary producers in a Caribbean reef lagoon: implication for in situ O2 availability. Mari Ecol Progress Series **409**:27-39.

Harvell, C. D., K. Kim, J.M. Burkholder, R.R. Colwell, P.R. Epstein, D.J. Grimes, E.E. Hofmann, E.K. Lipp, A.D.M.E Osterhaus, R.M. Overstreet, J.W. Porter, G.W. Smith, and G. R. Vasta. 1999. Emerging Marine Diseases - Climate Links and Anthropogenic factors. science **285**:1505-1510.

Harvell, D., R. Aronson, N. Baron, J. Connell, A. Dobson, S. Ellner, L. Gerber, K. Kim, A. Kuris, H. McCallum, K. Lafferty, B. McKay, J. Porter, M. Pascual, G. Smith, K. Sutherland, and J. Ward. 2004. The rising tide of ocean diseases: unsolved problems and research priorities. Front Ecol Environ **2**:375-382.

Harvell, D., E. Jordán-Dahlgren, S. Merkel, E. Rosenberg, L. Raymundo, G. Smith, E. Weil, and B. Willis. 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography **20**:172-195.

Hay, M. E. 1984. Patterns of fish and urchin grazing on Caribbean coral reefs: are previous results typical? Ecology **65**:446-454.

Hill, M. S. 1998. Spongivory on Caribbean reefs releases corals from competition with sponges. Oecologia **117**:143-150.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshwater Res **50**:839-866.

Hoegh-Guldberg, O. and J. F. Bruno. 2010. The impact of climate change on the world's marine ecosystems. science **328**:1523-1528.

Hoegh-Guldberg, O., P. Mumby, A. Hooten, R. Steneck, P. Greenfield, E. Gomez, C. Harvell, P. Sale, A. Edwards, and K. Caldeira. 2007. Coral reefs under rapid climate change and ocean acidification. science **318**:1737-1742.

Hughes, T. and J. Connell. 1999. Multiple stressors on coral reefs: A long-term perspective. Limnol. Oceanogr. **443**:932-940.

Hughes, T. P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. science **265**:1547-1551.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. Jackson, and J. Kleypas. 2003. Climate change, human impacts, and the resilience of coral reefs. science **301**:929-933.

Huston, M. 1985. Patterns of species diversity on coral reefs. Annual review of ecology and systematics **16**:149-177.

Jackson, J. and L. Buss. 1975. Alleopathy and spatial competition among coral reef invertebrates. Proceedings of the National Academy of Sciences **72**:5160-5163.

Jackson, J. B., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, and J. A. Estes. 2001. Historical overfishing and the recent collapse of coastal ecosystems. science **293**:629-637.

Jolles, A. E., P. Sullivan, A. P. Alker, and C. D. Harvell. 2002. Disease transmission of Aspergillosis in sea fams: Inferring process from spatial pattern. Ecol **83**:2373-2378.

Jompa, J. and L. McCook. 2002. The effects of nutrients and herbivory on competition between a hard coral (*Porites cylindrica*) and a brown alga (*Lobophora variegata*). Limnology and Oceanography **47**:527-534.

Knowlton, N. 1992. Thresholds and multiple stable states in coral reef community dynamics. American Zoologist **32**:674-682.

Knowlton, N. 2001. The future of coral reefs. Proceedings of the National Academy of Sciences **98**:5419-5425.

Kramer, J. G., M. Wyman, J. P. Zehr, and D. G. Capone. 1996. Diel variability in transcription of the structural gene for glutamine synthetase (*glnA*) in natural populations of marine diazotrophic cyanobacterium *Trichodesmium thiebauttii*. FEMS Microbiology Ecology **12**:187-196.

Lapointe, B. E. 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamasica and southeast Florida. Limnology and Oceanography **42**:1119-1131.

Lapointe, B. E., P. J. Barile, C. S. Yentsch, M. M. Littler, D. S. Littler, and B. Kakuk. 2004. The relative importance of nutrient enrichment and herbivory on macroalgal communities near Norman's Pond Cay, Exumas Cays, Bahamas: a ''natural'' enrichment experiment. Journal of Experimental Marine Biology and Ecology **298**:275-301.

Lauckner, G. 1980. Diseases of Porifera. Pages 139-165 *in* O. Kinne, editor. Diseases of Marine Animals. Wiley & Sons, Chichester.

LeDrew, E. F., H. Holden, M. A. Wulder, C. Derksen, and C. Newman. 2004. A spatial statistical operator applied to multidate satellite imagery for identification of coral reef stress. Remote Sens Environ **91**:271-279.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecol **74**:1659-1673.

Leichter, J. J., H. L. Stewart, and S. L. Miller. 2003. Nutrient Transport to Florida Coral Reefs. Limnology and Oceanography **48**:1394-1407.

Lentz, J. A., J. K. Blackburn, and A. J. Curtis. 2011. Evaluating Patterns of a White-Band Disease (WBD) Outbreak in *Acropora palmata* Using Spatial Analysis: A Comparison of Transect and Colony Clustering. PLoS ONE **6**:e21830.

Lesser, M. P. 2006. Benthic-pelagic coupling on coral reefs: Feeding and growth of Caribbean sponges. Journal of Experimental Marine Biology and Ecology **328**:277-288.

Lessios, H. 1988. Mass mortality of Diadema antillarum in the Caribbean: what have we learned? Annual review of ecology and systematics **19**:371-393.

Lessios, H. A., J. D. Cubit, D. R. Robertson, M. J. Shulman, M. R. Parker, S. D. Garrity, and S. C. Levings. 1984. Mass mortality of Diadema antillarum on the Caribbean coast of Panama. Coral Reefs **3**:173-182.

Lewis, S. 1985. Herbivory on coral reefs: algal susceptibility to herbivorous fishes. Oecologia **65**:370-375.

Littler, M. M. and D. S. Littler. 1984. A relative-dominance model for biotic reefs.*in* Joint Meeting of the Atlantic Reef Committee and the International Society of Reef Studies, Miami, FL.

Littler, M. M. and D. S. Littler. 2006. Assessment of coral reefs using herbivory/nutrient assays and indicator groups of benthic primary producers: a critical synthesis , proposed

protocols, and critique of management strategies. Aquatic Conservation: Marine and Freshwater ecosystems **17**:195-215.

Lloyd, C. D. 2010. Spatial Data Analysis - An introduction for GIS Users. Oxford University Press, New York, USA.

López-Victoria, M., S. Zea, and E. Weil. 2006. Competition for space between encrusting excavating Caribbean sponges and other coral reef organisms. Marine Ecology Progress Series **312**:113-121.

Maldonado, M., M. Ribes, and F. C. van Duyl. 2012. Nutrient Fluxes Through Sponges: Biology, Budgets, and Ecological Implications. Page 113 Advances in Marine Biology.

Maldonado, M., L. Sanchez-Tocino, and C. Navarro. 2010. Recurrernt disease outbreaks in corneous demosponges of the genus Ircinia: epidemic incidence and defense mechanisms. Mar Biol **157**:1577-1590.

Maliao, R., R. Turingan, and J. Lin. 2008. Phase-shift in coral reef communities in the Florida Keys National Marine Sanctuary (FKNMS), USA. Marine Biology **154**:841-853.

Mann, M. E., J. D. Woodruff, J. P. Donnelly, and Z. Zhang. 2009. Atlantic hurricanes and climate over the past 1,500 years. Nature **460**:880-883.

Maragos, J., C. Evans, and P. Holtus. 1985. Reef corals in Kaneohe Bay six years before and after termination of sewage discharges(Oahu, Hawaiian Archipelago). Pages 189-194 *in* Proceedings of the fifth international coral reef congress, Tahiti 27 May- 1 June 1985.

McCook, L. J. 1999. Macroalgae, nutrients and phase shifts on coral reefs; scientific issues and management consequences for the Great Barrier Reef. Coral Reefs **18**:357-367.

McCook, L. J., J. Jompa, and G. Diaz-Pulido. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. Coral Reefs **19**:400-417.

Mitchell, A. 2005. The ESRI Guide to GIS Analysis. ESRI Press **2**.

Moberg, F. and C. Folke. 1999. Ecological goods and services of coral reef ecosystems. Ecological economics **29**:215-233.

Moraes, R. M. 2005. Geographic Information System Method for Assessing Chemo-Diversity in Medicinal Plants. Planta medica **71**:1157-1164.

Moran, P. A. P. 1950. Notes on continuous stochastic phenomena. Biometrika:17-23.

Muller, E. M. and R. Woesik. 2012. Caribbean coral diseases: primary transmission or secondary infection? Glob Chang Biol **18**:3529-3535.

Mumby, P. J., C. P. Dahlgren, A. R. Harborne, C. V. Kappel, F. Micheli, D. R. Brumbaugh, K. E. Holmes, J. M. Mendes, K. Broad, J. N. Sanchirico, K. Buch, S. Box, R. W. Stoffle, and A. b. Gill. 2006. Fishing, Trophic Cascades, and the Process of Grazing on Coral Reefs. science **311**:98-101.

Mumby, P. J., A. Hastings, and H. J. Edwards. 2007. Thresholds and the resilience of Caribbean coral reefs. Nature **450**:98-101.

Naumann, M., A. Haas, U. Struck, C. Mayr, M. El-Zibdah, and C. Wild. 2010. Organic matter release by dominant hermatypic corals of the Northern Red Sea. Coral Reefs **29**:649-659.

Norström, A. V., M. Nyström, J. Lokrantz, and C. Folke. 2009. Alternative states on coral reefs: beyond coral-macroalgal phase shifts. Mar Ecol Prog Ser **376**:295-306.

Nyström, M., C. Folke, and F. Moberg. 2000. Coral reef disturbance and resilience in a human-dominated environment. Trends in Ecology & Evolution **15**:413-417.

Olson, J. B., D. J. Gochfeld, and M. Slattery. 2006. *Aplysina* red band syndrome: a new threat to Caribbean sponges. Dis Aquat org **71**:163-168.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist:65-75.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press.

Pawlik, J. 2011. The chemical ecology of sponges on Caribbean reefs: Natural products shape natural systems. BioScience **61**:888-898.

Pawlik, J. R., L. Steindler, T. P. Henkel, S. Beer, and M. Ilan. 2007. Chemical warfare on coral reefs: Sponge metabolites differentially affect coral symbiosis in situ. Limnology and Oceanography:907-911.

Pérez-Mayorga, D. M., L. B. Ladah, J. A. Zertuche-González, J. J. Leichter, A. E. Filonov, and M. F. Lavín. 2011. Nitrogen uptake and growth by the opportunistic macroalga Ulva lactuca (Linnaeus) during the internal tide. Journal of Experimental Marine Biology and Ecology **406**:108-115.

Pile, A. J., A. Grant, R. Hinde, and M. A. Borowitzka. 2003. Heterotrophy on ultraplankton communities is an important source of nitrogen for a sponge–rhodophyte symbiosis. Journal of experimental biology **206**:4533-4538.

Porter, J. W., P. Dustan, W. C. Jaap, K. L. Patterson, V. Kosmynin, O. W. Meier, M. E. Patterson, and M. Parsons. 2001. Patterns of spread of coral diseases in the Florida Keys. Hydrobiol **460**:1-24.

Porter, J. W. and O. W. Meier. 1992. Quantification of loss and change in Floridian reef coral population. Am. Zool. **32**:625-640.

Puyana, M., W. Fenical, and J. Pawlik. 2003. Are there activated chemical defenses in sponges of the genus *Aplysina* from the Caribbean. Marine Ecology Progress Series **246**:127-135.

Rabalais, N. N., R. E. Turner, and W. J. Wiseman Jr. 2002. Gulf of Mexico Hypoxia, AKA" The Dead Zone". Annual review of ecology and systematics:235-263.

Rasher, D. B. and M. E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. Proceedings of the National Academy of Sciences **107**:9683-9688.

Rasher, D. B., E. P. Stout, S. Engel, J. Kubanek, and M. E. Hay. 2011. Macroalgal terpenes function as allelopathic agents against reef corals. Proceedings of the National Academy of Sciences **108**:17726-17731.

Real, L. A. and P. McElhany. 1996. Spatial Pattern and Process in Plant-Pathogen Interactions. Ecol **77**:1011-1025.

Richardson, L. L. 1998. Coral diseases: what is really known. Trends in Ecol Evol **13**:438-443.

Richardson, L. L. 2004. Black band disease. Pages 325-336 Coral Health and Disease. Springer.

Richter, C., M. Wunsch, M. Rasheed, I. Koetter, and M. Badran. 2001. Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity dwelling sponges. Nature **413**:726-730.

Ripley, B. D. 1981. Spatial Statistics. John Wiley, New York, USA.

Ripley, B. D. 1988. Statistical inference for spatial processes. Cambridge University Press, Cambridge, UK.

River, G. F. and P. J. Edmunds. 2001. Mechanisms of interaction between macroalgae and scleractinians on a coral reef in Jamaica. Journal of Experimental Marine Biology and Ecology **261**:159-172.

Roff, G., E. C. E. Kvennefors, M. Fine, J. Ortiz, J. E. Davy, and O. Hoegh-Guldberg. 2011. The Ecology of ‘Acroporid White Syndrome', a Coral Disease from the Southern Great Barrier Reef. PLoS ONE **6**:e26829.

Rossi, R. E., D. J. Mulla, A. G. Journel, and E. H. Franz. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecol Monogr **62**:277-314.

Rutzler, K. 1988. Mangrove sponge disease induced by cyanobacterial symbionts: falilure of a primitive immune system? Dis Aquat Organ **5**:143-149.

Sato, Y., D. G. Bourne, and B. L. Willis. 2009. Dynamics of seasonal outbreaks of black band disease in an assemblage of *Montipora* species at Pelorus Island (Great Barrier Reef, Austrailia). Proc Biol Sci **276**:2795-2803.

Scott, L. and N. Warmerdam. 2005. Extend crime analysis with ArcGIS spatial statistics tools. ArcUser Online **April-June 2005**.

Shearer, T., D. Rasher, T. Snell, and M. Hay. 2012. Gene expression patterns of the coral *Acropora millepora* in response to contact with macroalgae. Coral Reefs:1-16.

Slattery, M., D. J. Gochfeld, C. G. Easson, and L. R. O'Donahue. 2013. Facilitation of coral reef biodiversity and health by cave sponge communities. MEPS **476**:71-86.

Smith, F. G. W. 1941. Sponge disease in British honduras, and its transmission by water currents. Ecology **22**.

Smith, J. E., M. Shaw, R. A. Edwards, D. Obura, O. Pantos, E. Sala, S. A. Sandin, S. Smriga, M. Hatay, and F. L. Rohwer. 2006. Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecology Letters **9**:835-845.

Smith, S., W. J. Kimmerer, E. A. Laws, R. E. Brock, and T. W. Walsh. 1981. Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. Pacific Science **35**:279-402.

Sokal, R. R. and N. L. Oden. 1978. Spatial autocorrelation in biology 1. Methodology. Biol J Linn Soc Lond **10**:199-228.

Southwell, M. W. 2007. Sponge impacts on coral reef nitrogen cycling, Key Largo, Florida. P.h.D. thesis, University of North Carolina at Chapel Hill.

Southwell, M. W., J. B. Weisz, C. S. Martens, and N. Lindquist. 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography **53**:986-996.

Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of Ecological Communities. BioScience **51**:235-246.

Stimson, J., S. Larned, and E. Conklin. 2001. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga Dictyosphaeria cavernosa in Kaneohe Bay, Hawaii. Coral Reefs **19**:343-357.

Sutherland, K. P., J. W. Porter, J. W. Turner, B. J. Thomas, E. E. Looney, T. P. Luna, M. K. Meyers, J. C. Futch, and E. K. Lipp. 2010. Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, Acropora palmata. Environ Microbiol **12**:1122-1131.

Sutherland, K. P. and K. B. Ritchie. 2004. White pox disease of the Caribbean elkhorn coral, Acropora palmata. Pages 289-300 Coral health and disease. Springer.

Szmant, A. 2002. Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? Estuaries and Coasts **25**:743-766.

Taylor, M. W., R. Radax, D. Steger, and M. Wagner. 2007. Sponge associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev. **71**:295-347.

Thacker, R. W. and C. J. Freeman. 2012. Sponge-Microbe Symbioses: Recent Advances and New Directions. Page 57 Advances in Marine Biology.

Thoms, C. and P. Schupp. 2007. Chemical defense strategies in sponges: a review. Porifera Research Biodiversity, Innovation and Sustainability. Rio de Janeiro: Série Livros **28**:627-637.

Toth, L. T., R. B. Aronson, S. V. Vollmer, J. W. Hobbs, D. H. Urrego, H. Cheng, I. C. Enochs, D. J. Combosch, R. van Woesik, and I. G. Macintyre. 2012. ENSO drove 2500-year collapse of eastern Pacific coral reefs. science **337**:81-84.

Trautman, D. A., R. Hinde, and M. A. Borowitzka. 2000. Population dynamics of an association between a coral reef sponge and a red macroalga. Journal of Experimental Marine Biology and Ecology **244**:87-105.

Tsurumi, M. and M. R. Henry. 1997. Sexual versus asexual reproduction in an oviparous rope-form sponge, Aplysina cauliformis (Porifera; Verongida). Invertebr Reprod Dev **32**:1-9.

Vacelet, J., E. Vacelet, E. Gaino, and M. F. Gallisian. 1994. Bacterial attack of spongin skeleton during the 1986-1990 Mediterranean sponge disease, Balkema, Rotterdam.

van Duyl, F. C., L. Moodley, G. Nieuwland, L. van Ijzerloo, R. W. van Soest, M. Houtekamer, E. H. Meesters, and J. J. Middelburg. 2011. Coral cavity sponges depend on reef-derived food resources: stable isotope and fatty acid constraints. Marine Biology **158**:1653-1666.

Van Houtan, K. S., S. K. Hargrove, and G. H. Balazs. 2010. Land use, macroalgae, and a tumor-forming disease in marine turtles. PLoS ONE **5**:e12900.

Voss, J. and L. Richardson. 2006. Nutrient enrichment enhances black band disease progression in corals. Coral Reefs **25**:569-576.

Ward, J. R. and K. D. Lafferty. 2004. The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? PLoS Biology **2**:e120.

Wassenberg, D. M. and R. T. Di Giulio. 2004. Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A inhibitors in Fundulus heteroclitus. Environ Health Perspect **112**:1658-1664.

Webster, N. S. 2007. Sponge disease: a global threat? Environ Microbiol **9**:1363-1375.

Webster, N. S., A. P. Negri, R. I. Webb, and R. T. Hill. 2002. A spongin-boring α-proteo-bacterium is the etiological agent of disease in the Great Barrier Reef sponge *Rhopaloeides odorabile.* Mar Ecol Prog Ser **232**:305-309.

Weisz, J., U. Hentschel, N. Lindquist, and C. Martens. 2007. Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Marine Biology **152**:475-483.

Wilkinson, C. R. 1979. Nutrient translocation from symbiotic cyanobacteria to coral reef sponges. Pages 373-380 Biologie des spongiaires, Paris, France.

Willis, B. L., C. A. Page, and E. A. Dinsdale. 2004. Coral disease on the Great Barrier Reef. Pages 69-104 Coral health and disease. Springer.

Wulff, J. 2006a. Ecological interactions of marine sponges. Canadian Journal of Zoology **84**:146-166.

Wulff, J. 2006b. A simple model of growth form-dependent recovery from disease in coral reef sponges, and implications for monitoring. Coral Reefs **25**:419-426.

Wulff, J. 2007a. Disease prevalence and population density over time in three common Caribbean coral reef sponge species. J Mar Biol ASsoc **87**:1715-1720.

Wulff, J. L. 1991. Asexual fragmentation, genotype success, and population dynamics of erect branching sponges. J Exp Mar Biol Ecol **149**:227-247.

Wulff, J. L. 2007b. Disease prevalence and population density over time in three common Caribbean coral reef sponge species. J Mar Biol Assoc U. K. **87**:1715-1720.

Zehr, J. P. and B. B. Ward. 2002. Nitrogen Cycling in the Ocean: New Perspectives on Processes and Paradigms. Applied and Environmental Microbiology **68**:1015-1024.

Zvuloni, A., Y. Artzy-Randrup, L. Stone, E. Kramarsky-Winter, R. Barkan, and Y. Loya. 2009. Spatio-Temporal Transmission Patterns of Black-Band Disease in a Coral Community. PLoS ONE **4**:e4993.

LIST OF APPENDICES

APPENDIX I: CHEMISTRY RESULTS FROM CHAPTER 1: COMPLEX ECOLOGICAL ASSOCIATIONS: EVIDENCE FOR COMPETITION AND FACILITATION IN A SPONGE-ALGAL INTERACTION.

Goal

The purpose of this analysis was to determine the effects of algal contact, elevated nutrients and combinations of the two stressors on the internal chemistry of *A. cauliformis*.

Methods

Aplysina cauliformis samples from the competition and nutrient experiments were extracted and processed according to the methods in Gochfeld et al. (2012b). Briefly, 1 cm of lyophilized sponge was extracted using an Accelerated Solvent Extractor (ASE, Dionex). Each sample was exhaustively extracted in a 1:1 mixture of dichloromethane (DCM) and methanol (MeOH), followed by an 80:20 solution of MeOH and H_2O to yield organic and aqueous extracts, respectively. The organic extracts were dried and weighed. The organic extracts were then re-dissolved to a concentration of 5 mg/ml and filtered through a 0.45 µm PolyTetraFluoriEthylene (PTFE) filter. Chemical fingerprints of each organic extract were created by injecting 10 µl of filtered extract onto an analytical High Performance Liquid Chromatography (HPLC) system (Alliance 2695, Waters, Milford, MA, USA) coupled to a Waters 2998 Photodiode Array detector. Analysis was done on a Phenomenex 5 µm C18 250X4.6 mm column using a gradient system of 0.05% trifluoroacetic acid (TFA) in water to 0.05% TFA in acetonitrile. Chromatograms were analyzed at 254 nm and the areas under the curve of 5 peaks were analyzed. Peak areas were compared between samples in the competition experiment using one-way ANOVAs to test for treatment effects, and in the nutrient experiment using two-way ANOVAs to test for nutrient and competition treatment effects. Tukey's HSD post-hoc test ($P<0.05$) was used to analyze differences among treatments.

Results

Competition experiment

The concentrations of fistularin–3, aplysamine and peak 4 did not differ significantly between treatments (fistularin-3: $F = 2.5466$, $P = 0.097$, Figure 16; aplysamine: $F = 1.1693$, $P = 0.326$, Figure 17; peak 4: $F = 0.8943$, $P = 0.422$, Figure 18). Peak 5 only appeared in the algal contact treatment and in one sample from the sponge alone treatment. Due to this, we were not able to run statistics on this peak in the competition experiment. Peak 6 occurred in significantly higher concentrations in the algal contact treatment than the sponge alone treatment ($F = 4.17$, $P = 0.028$, Figure 19).

Nutrient experiment

Fistularin-3 concentration was not significantly affected by nutrient dose ($F = 0.91$, $P = 0.41$), but was significantly affected by algal contact treatment ($F = 5.94$, $P = 0.004$), and the interaction of the two variables ($F = 5.79$, $P = 0.0004$). The sponge alone treatment had significantly lower concentrations of fistularin-3 than the algal contact and shade control treatments (Figure 20). Aplysamine showed no significant effects of nutrient dose ($F = 2.09$, $P = 0.13$), algal contact treatment ($F = 0.12$, $P = 0.89$) or the interaction of the terms ($F = 2.26$, $P = 0.07$, Figure 21). Peak 4 showed no significant effects of algal contact treatment ($F = 0.37$, $P = 0.69$), but did show a significant effect of nutrient dose ($F = 12.51$, $P < 0.001$) and a significant interaction between nutrient dose and algal contact treatment ($F = 4.68$, $P = 0.0025$). The high nutrient dose samples had significantly lower concentrations of peak 4 than the low and medium doses (Figure 22). Peak 5 showed a significant effect of nutrient dose ($F = 12.66$, $P < 0.001$), but

no effect of algal contact treatments (F = 2.05, P = 0.14) and no significant interaction effect (F = 1.30, P = 0.28). Samples given the high nutrient dose had significantly higher concentrations of Peak 5 compared to the medium and low doses (Figure 23). Peak 6 showed significant effects of both algal contact treatment (F = 8.05, P = 0.001) and nutrient dose (F = 3.40, P = 0.04), and a significant interaction between the two variables (F = 6.12, P = 0.0005). The algal contact treatment showed significantly higher levels of peak 6 compared to the sponge alone and shade control treatments (Figure 24).

Conclusions

Variation in chemical fingerprints was observed in both the competition and nutrient experiments. Two peaks that were analyzed responded to algal contact treatment. Fistularin-3 in the nutrient experiment showed an increased concentration in the shade control and algal contact treatments, suggesting that this compound increases in response to reduced irradiance. Peak 6 showed an increased concentration in the algal contact treatment in both experiments. In the competition experiment, peak 6 concentration in the sponge alone treatment differed from the algal contact treatment, with the shade control as an intermediate, while in the nutrient experiment, the shade control and the sponge alone treatments were significantly different than the algal contact treatments. This suggests some role of nutrients in the concentration of this compound, but also that the concentration of this compound responds to the algal contact itself, and not just to the reduced irradiance caused by the algae. These data provide some evidence for up-regulation of a secondary metabolite in response to algal contact, suggesting the presence of an inducible defense mechanism (Thoms and Schupp 2007). Peaks 4 and 5 both showed an effect of nutrient dose on their concentrations. For Peak 4, the high nutrient dose showed significantly

lower concentrations than the low and medium doses, suggesting a stress response to high levels of nutrients. The opposite was shown for Peak 5, as its concentration increased at the high nutrient dose, possibly suggesting an up regulation of these nitrogenous compounds in response to higher concentrations of the chemical in the water column. The chemical fingerprints of these samples show that *A. cauliformis* has a variety of responses associated with algal contact and increased nutrient loads. Comparisons of these fingerprints with other endpoints from this experiment could elicit some causal relationships for the observed changes.

Figure 16: Fistularin-3 concentration among treatments in the competition experiment. No significant differences in concentration were observed after the 4 week duration of the competition experiment (one-way ANOVA P = 0.097).

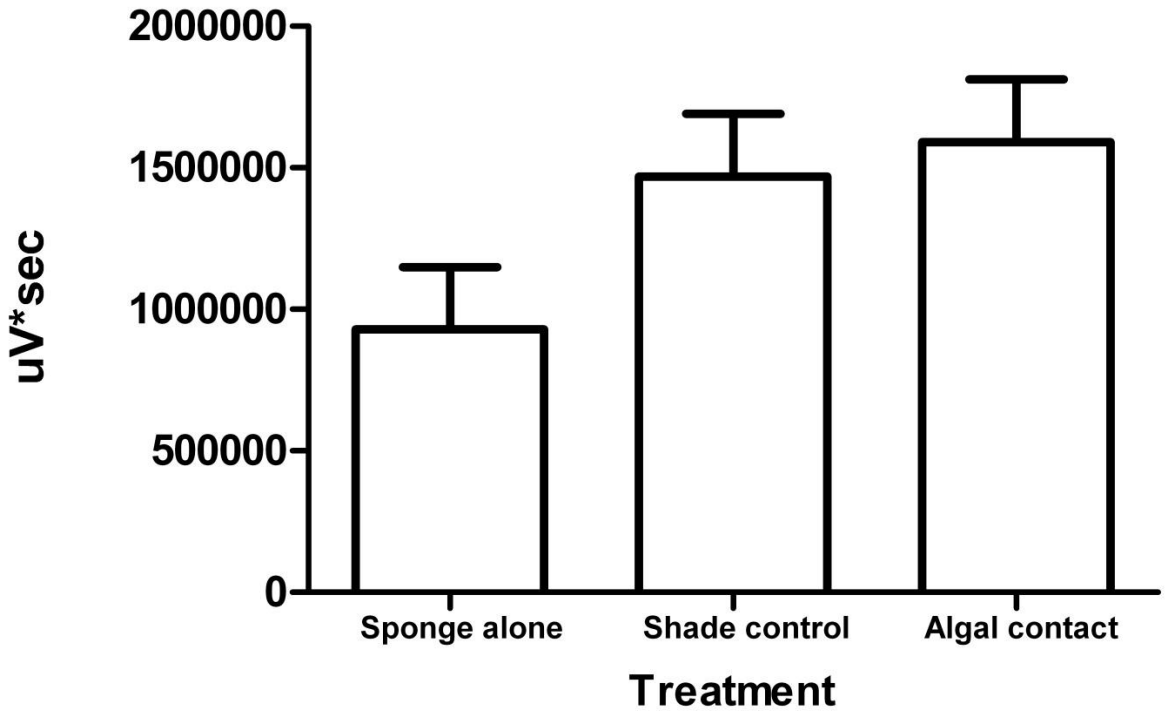


Figure 17: Aplysamine concentration among treatments in the competition experiment. No differences in concentration were observed after the 4 week experiment (one-way ANOVA P = 0.326)

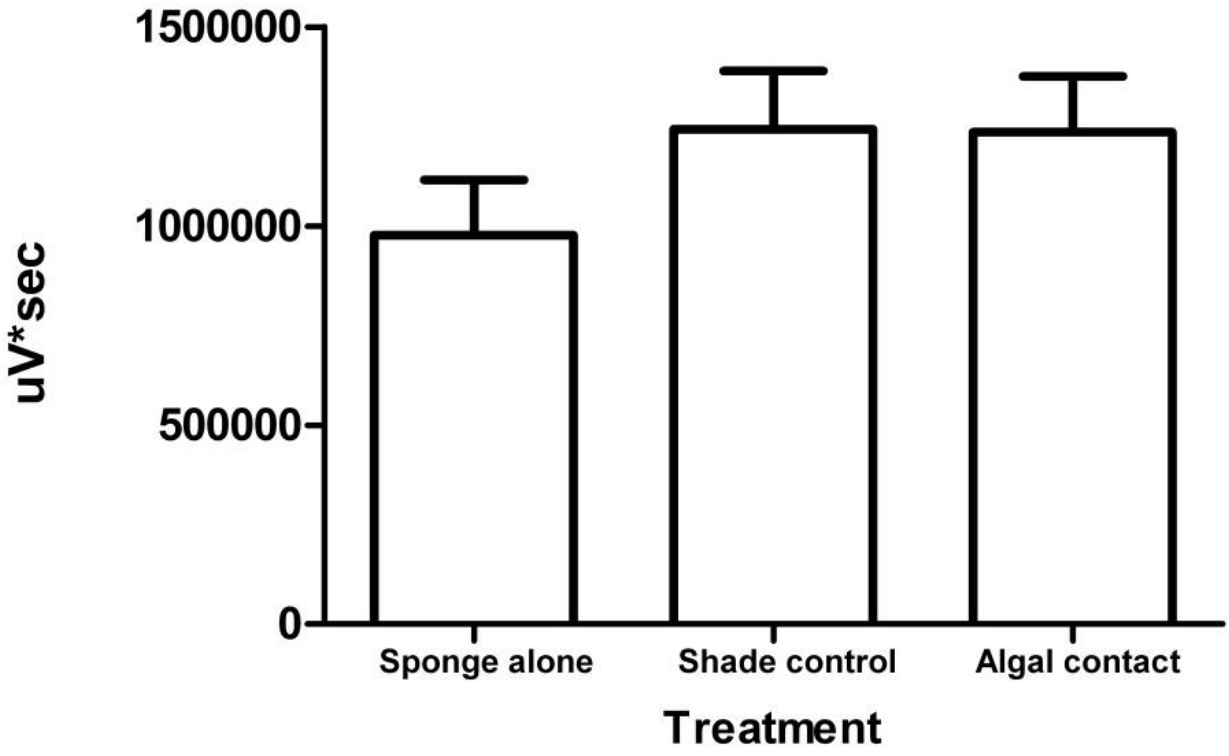


Figure 18: Concentration of peak 4 among treatments in the competition experiment. Peak 4 concentrations did not differ among treatments after the competition experiment (One-way ANOVA, P =.42)

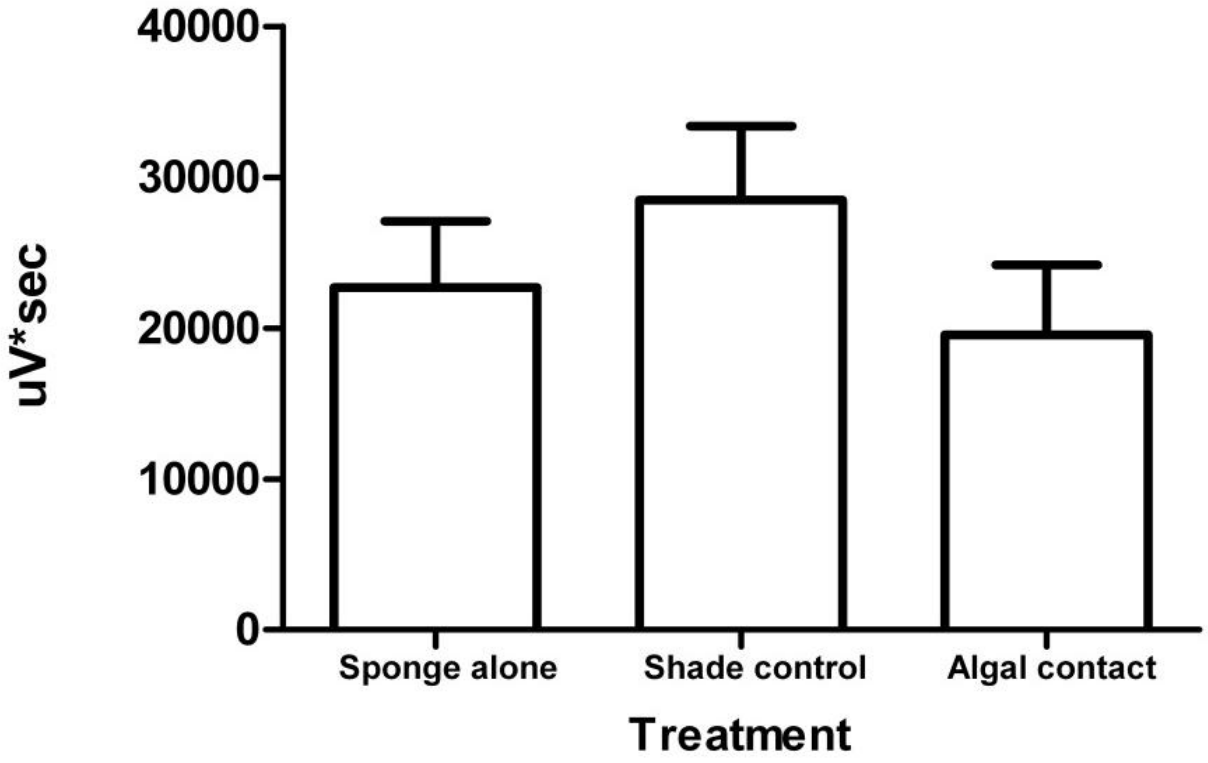


Figure 19: Peak 6 concentrations in the competition experiment. Peak 6 showed a significant effect of algal contact treatment (One-way ANOVA, P = 0.03). Significant differences among groups are shown by different letters above each bar.

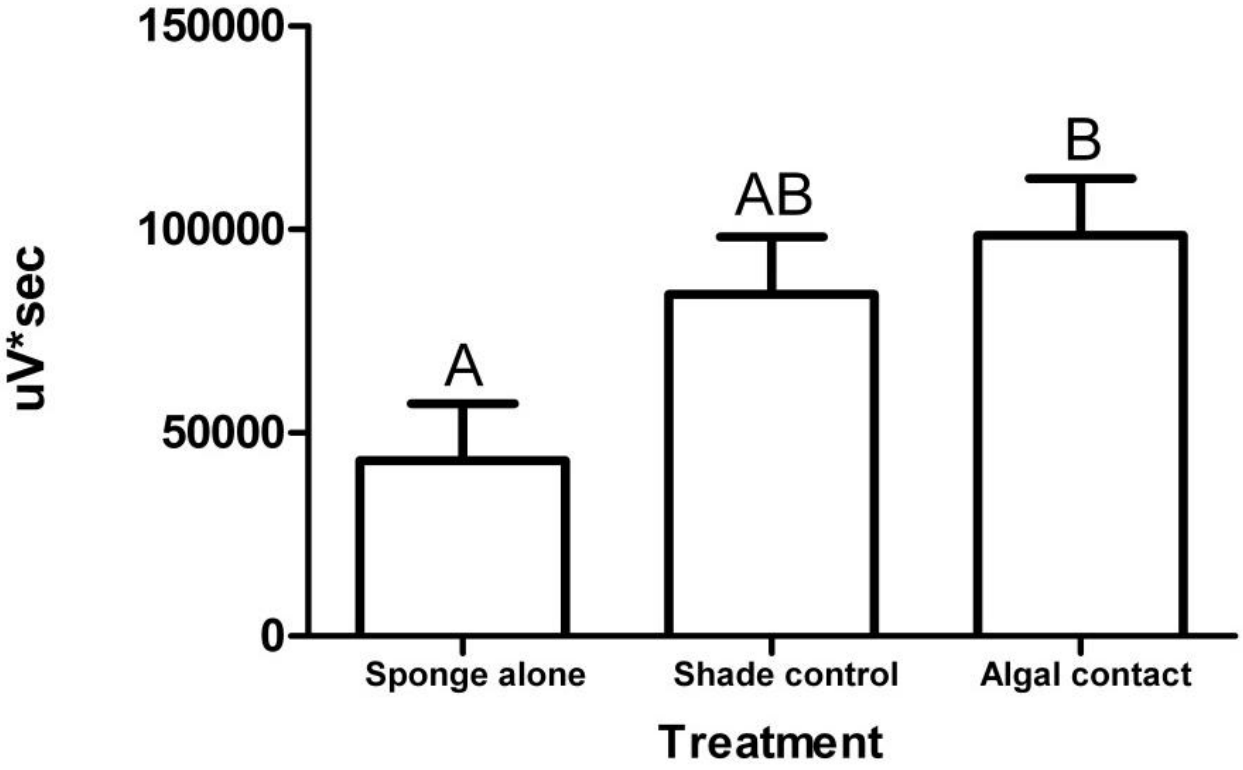


Figure 20: Fistularin-3 concentrations from the nutrient experiment. Fistularin-3 showed a significant effect of treatment (Two-way ANOVA P = 0.004) and a significant interaction between treatment and nutrient dose (P = 0.0004). Significant differences among groups are shown by different letter designations above each bar.

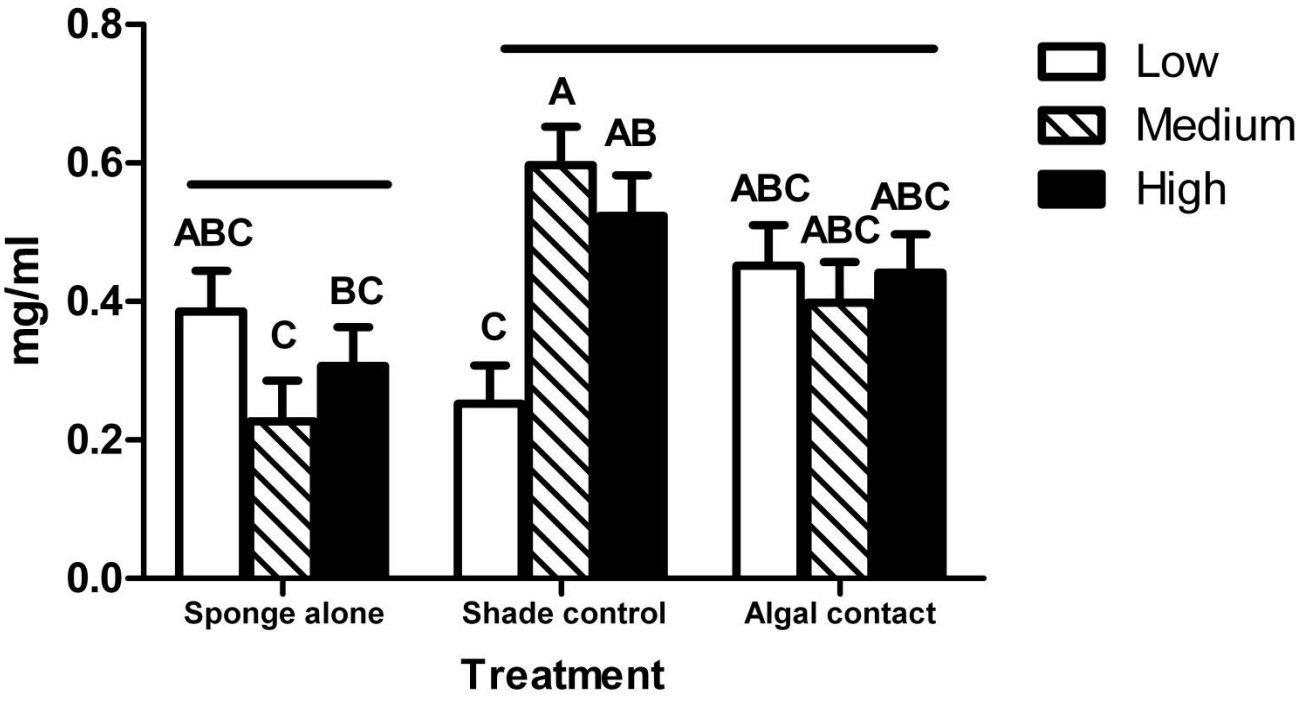


Figure 21: Aplysamine concentrations from the 4 week nutrient experiment. Aplysamine concentration did not differ among treatment groups.

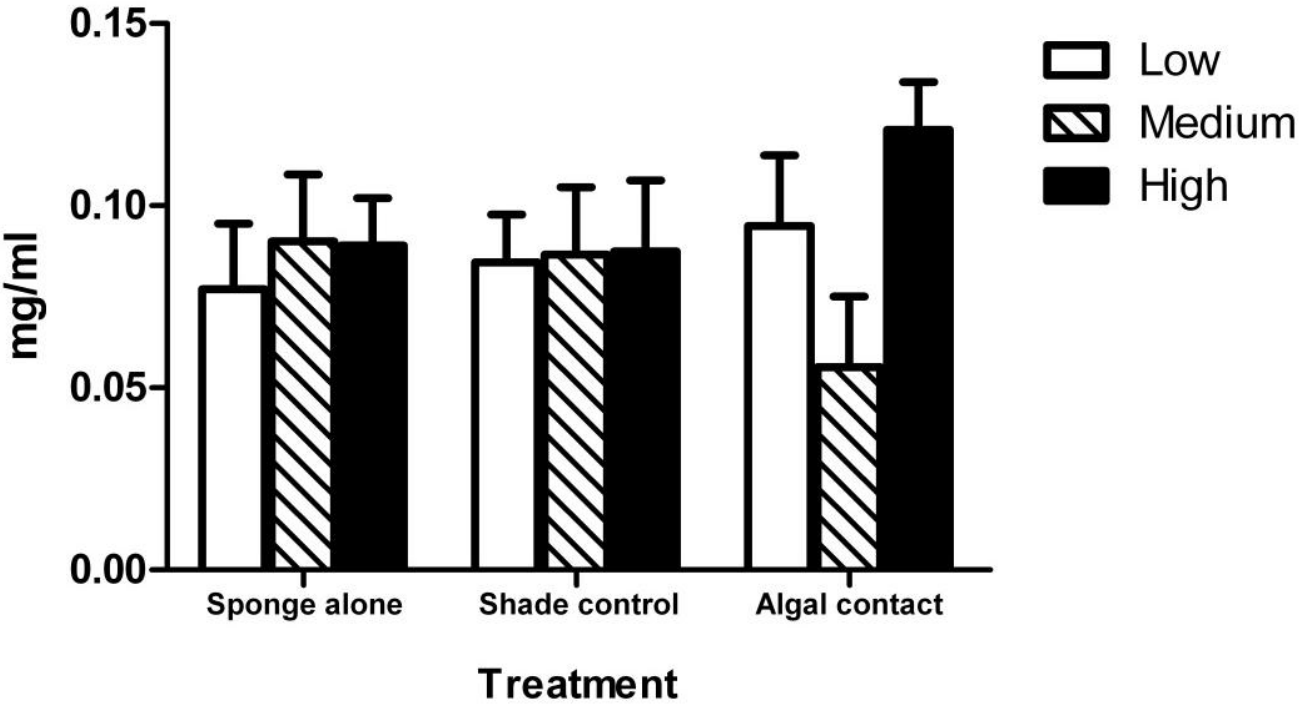


Figure 22: Concentrations of peak 4 from the nutrient experiment. Concentration of this compound showed a significant effect of nutrient dose (P< 0.0001) and a significant interaction between algal contact treatment and nutrient dose (P = 0.003). Significant differences are shown by different letter groups in the figure.

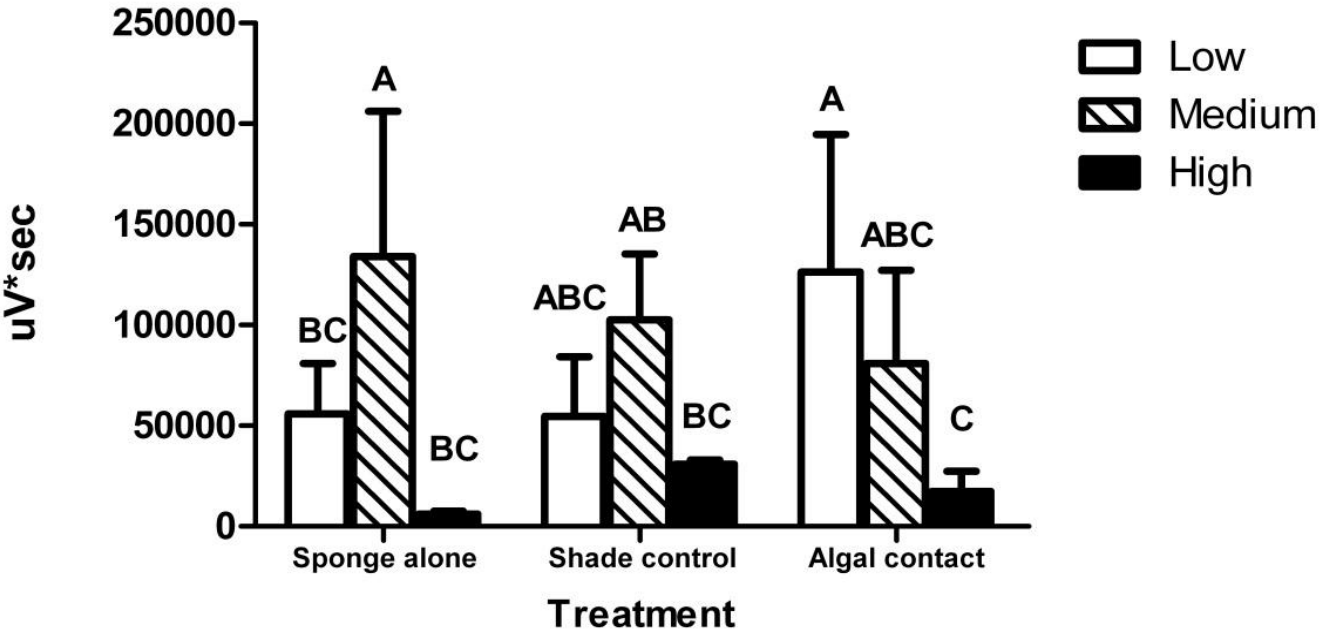


Figure 23: Concentration of peak 5 after the 4 week nutrient experiment.This peak showed a significant effect of nutrient dose (P< 0.001). Significant differences are shown by different letter groups in the figure.

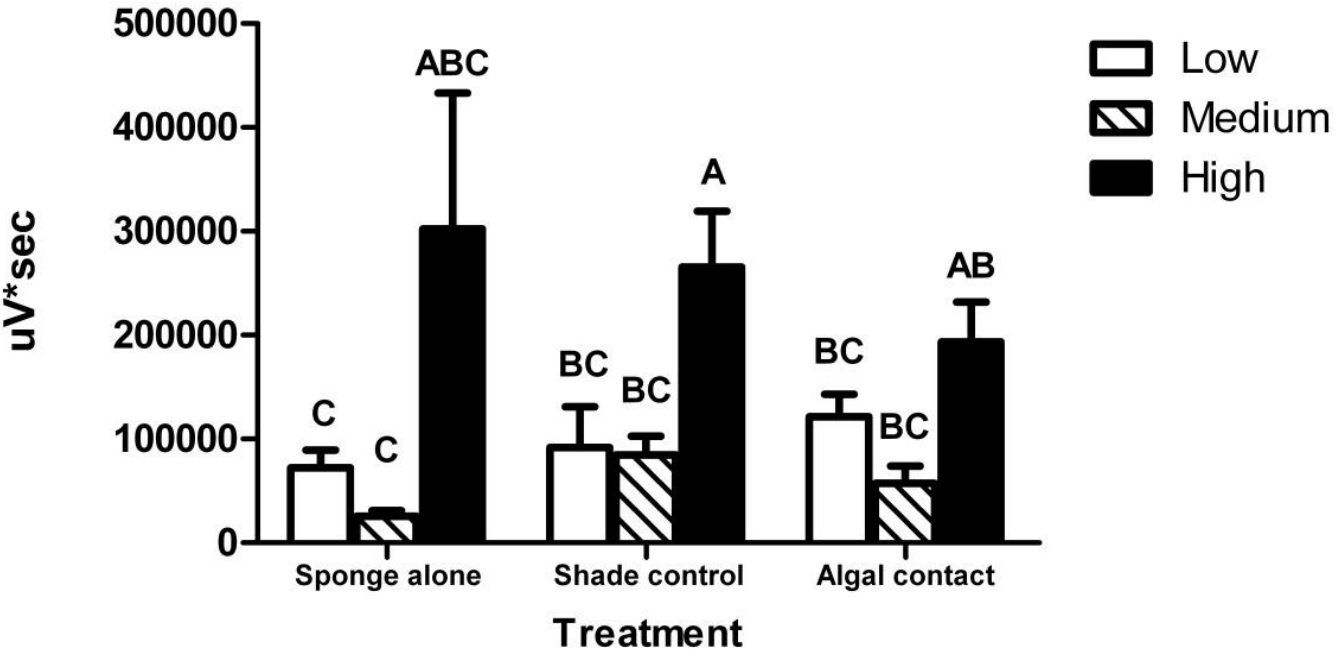
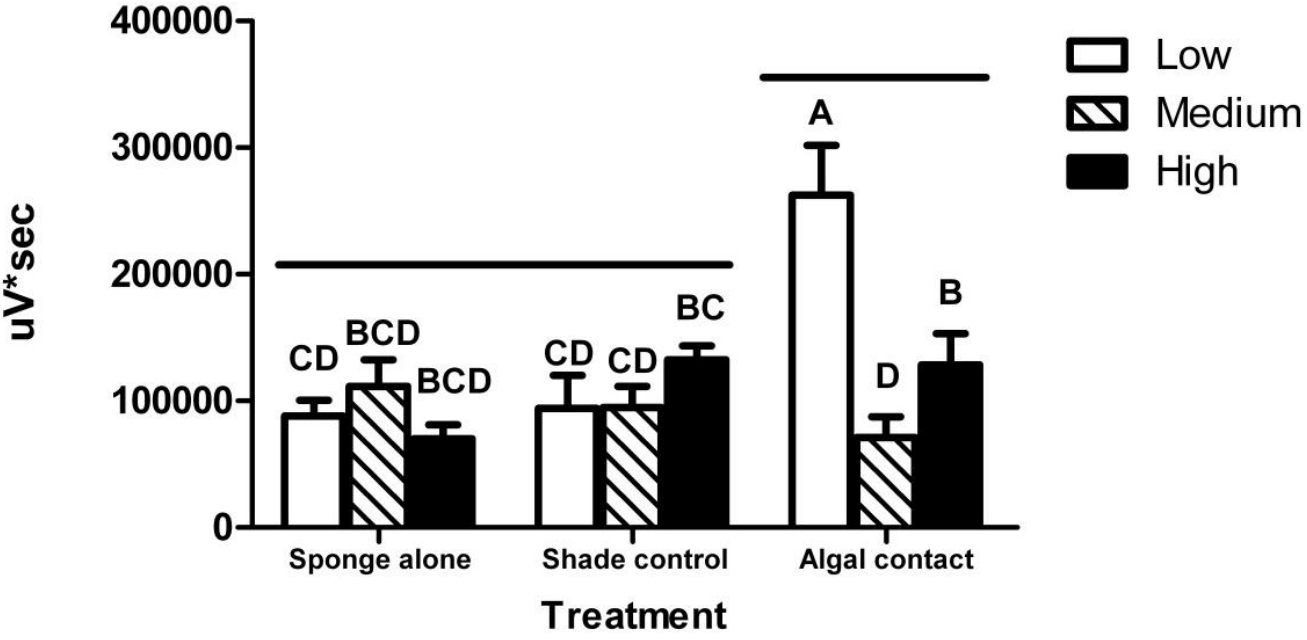


Figure 24: Concentration of peak 6 after the 4 week nutrient experiment. This peak showed a significant effect of algal contact treatment (P = 0.001) and a significant interaction of algal contact treatment and nutrient dose (P = 0.0005). Significant differences are represented by a break in the horizontal line and different letter groups in the figure.



APPENDIX II: CHLOROPHYLL *A* MEASUREMENTS FROM CHAPTER 2: CARBON AND NITROGEN DYNAMICS AND TRANSFER IN A CARIBBEAN SPONGE-ALGAL INTERACTION.

Goal

The goal of this analysis was to examine the acute effects of algal contact on *A. cauliformis* chlorophyll *a* concentrations**.**

Methods

Chlorophyll *a* concentration was analyzed for *A. cauliformis* and *M. marinum* samples in both the NO_3^-/HCO_3^- and the NH_4^+ stable isotope experiments. Chlrophyll *a* concentration was measured according to the methods outlined in Erwin and Thacker (2007), and are briefly described on pages 20-21. Chlorophyll *a* concentration data for sponges was analyzed with separate one-way analyses of variance (ANOVA) to examine differences among treatments within a time point and between time points, rather than a two-way ANOVA, due to a lack of sufficient degrees of freedom in the two-way analysis. Algal chlorophyll *a* concentrations were analyzed using one-way ANOVAs to investigate possible differences among treatment groups and time points.

Results

Chlorophyll *a* concentration in sponges from the NO_3^-/HCO_3^- experiment did not differ significantly among sampling times ($F = 2.09$, $P = 0.12$) or between treatments ($F = 0.30$, $P = 0.82$, Figure 25). Concentration of chlorophyll *a* in algae from the NO_3^-/HCO_3^- experiment did change significantly from initial values when the algae was at 15cm from the enriched sponge ($F = 0.69$, $P = 0.51$) or at 30 cm from the enriched sponge ($F = 1.35$, $P = 0.28$). When the alga was in contact with the sponge, there was significant variation in chlorophyll *a* concentration ($F = 4.81$, $P = 0.02$), but the significant differences were between the 12 hour and 24 hour samples,

with the initial samples intermediate and not significantly different than either of the other time periods (Figure 26).

Chlorophyll *a* concentration in sponges from the NH_4^+ experiment did not differ among times ($F = 0.07$, $P = 0.97$) or between algal contact treatments ($F = 0.47$, $P = 0.70$, Figure 27). Algal chloropyll *a* concentration in the NH_4^+ experiment did not differ from initial concentrations when the algae was in contact with the enriched sponge ($F = 0.81$, $P = 0.46$), at 15cm from the enriched sponge ($F = 1.25$, $P = 0.31$), or at 30 cm from the enriched sponge ($F = 1.61$, $P = 0.23$, Figure 28).

Conclusions

No differences were seen in chlorophyll *a* concentration in either the sponge or the alga. In the contact experiments performed in Chapter 1, *A. cauliformis* benefited *M. marinum* when they were in contact by enhancing productivity, as measured by chlorophyll *a* concentration. Additionally, in the contact experiments, uptake of nutrients by the sponge enhanced symbiont chlorophyll *a* concentration when the sponge was not in contact with the alga (Chapter 1). These trends were not observed in the stable isotope experiments described here. The short time frame for this experiment (1 day) may be responsible for these differing results. From the standpoint of the sponge, these results suggest that it can withstand short-term algal contact without consequence to symbiont productivity. For the alga, these results suggest that uptake of nitrogen resources may not immediately boost chlorophyll *a* concentrations, and a longer time frame may be needed to observe noticeable biochemical effects in the alga.

Figure 25: Mean (±SE) Chlorophyll *a* concentration for sponges in the NO_3^-/HCO_3^- experiment at each time point. No significant differences were observed among different time points or between treatments ($P > 0.05$).

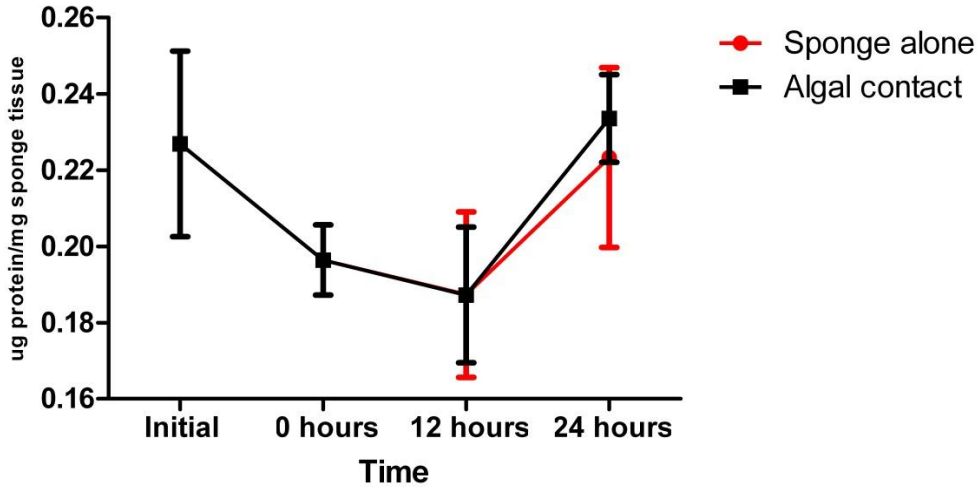


Figure 26: Mean (±SE) chlorophyll *a* concentration for *M. marinum* at different time points and distances from *A. cauliformis*. Sponge contact elicited significant variation in chlorophyll *a* concentration (P = 0.02), but the observed difference was between the 12 and 24 hour samples, with the initial samples intermediate. No differences were seen in algal samples at 15 cm and 30 cm (P >0.05).

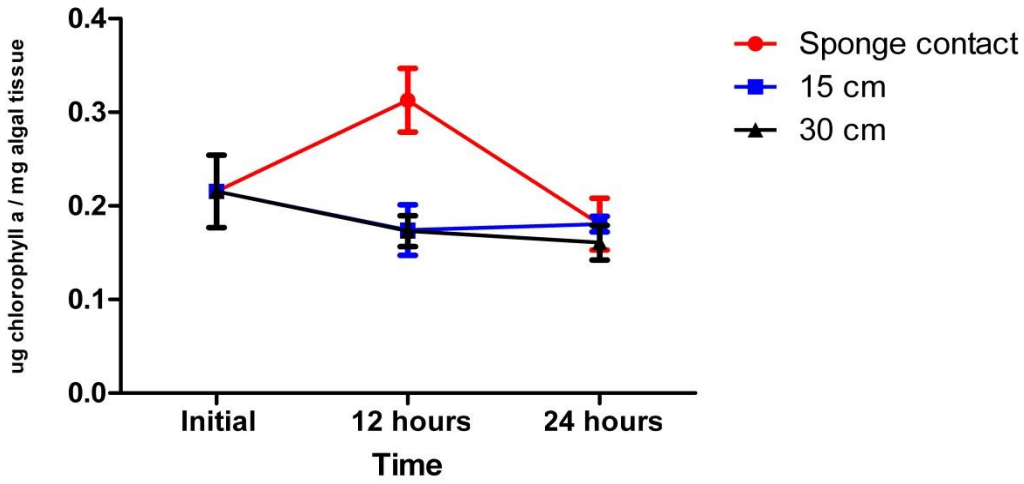


Figure 27: Mean (±SE) chlorophyll *a* concentration for *A. cauliformis* in the NH_4^+ experiment. There were no differences among time points or between treatments ($P > 0.05$).

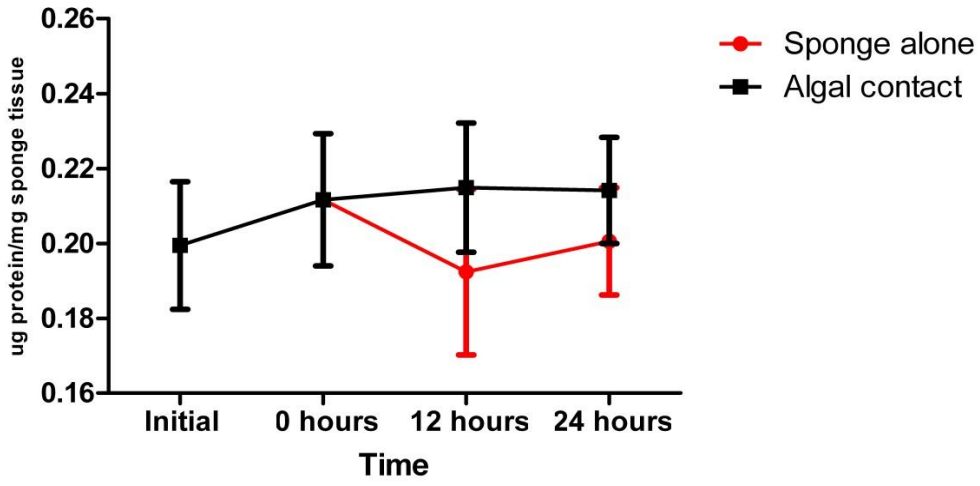
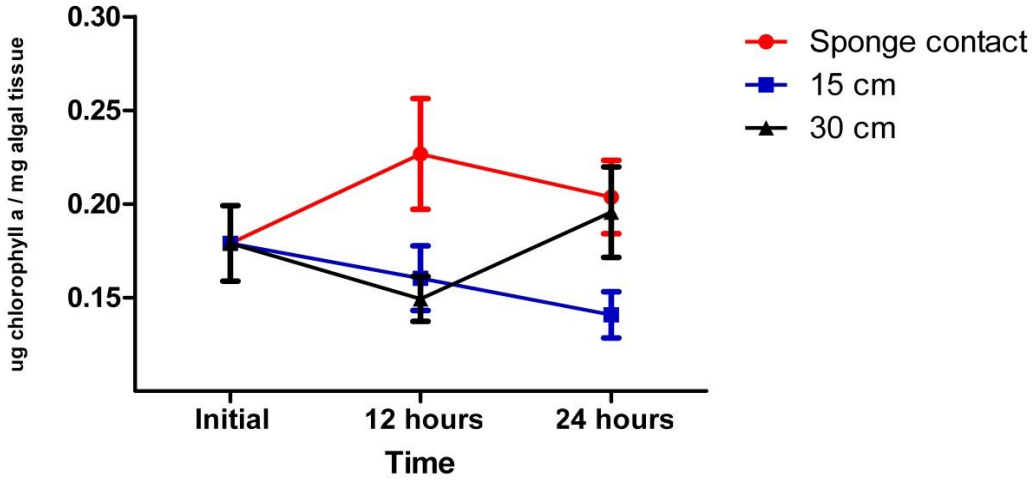


Figure 28: Mean (±SE) chlorophyll *a* concentration for *M. marinum* at different time points and distances from the sponge in the NH_4^+ experiment. No differences among treatments or among time points was observed (P >0.05).



APPENDIX III: ALGAL-MICROBIAL INTERACTION EXPERIMENT: MEDIATION OF ALGAL STRESS THROUGH THE REMOVAL OF ALGAL MICROBIAL COMMUNITIES.

Goal

The aim of this experiment was to examine the role of algal microbial communities in the interaction between *Aplysina cauliformis* and *Microdictyon marinum*.

Methods

To examine the role of algal microbes in the interaction between *A. cauliformis* and *M. marinum*, a factorial designed laboratory experiment was performed. This experiment manipulated 3 variables: algal contact, nutrient concentration and microbial abundance. Sponges and algae were collected from the shallow reef site, North Normans (N 23˚ 47.388, W 76˚ 08.273'). Samples were brought back to the lab and allowed to acclimate in flowing seawater. Each sponge was placed in an individual tank and randomly assigned to a treatment group (N = 7 per treatment). The three treatment groups were: algal contact or sponge alone, nutrient enrichment (2 grams of 14-14-14 Osmocote© fertilizer wrapped in window screen) or ambient nutrient concentration, and UV-sterilized water or non UV-sterilized water. Seawater was UV sterilized using an in-line sterilizer. Algae were sterilized by soaking in 50 ug/ml ampicillin antibiotic (Smith et al. 2006) for 3 hours, after which they were rinsed with sterile seawater before being placed in contact with a sponge. The experiment ran for 14 days, and fertilizer packs were replaced after 7 days. Sterility of the water was measured using a handheld luminometer (SystemSURE PLUS, Hygiena). Fifty milliliters of water was collected from a randomly selected tank in each treatment (UV and Ambient), and this volume was filtered through a sterile 0.2 um filter. After filtration, the filter paper was swabbed with an ATPase swab (Ultrasnap ATP test, Hygiena) and put in the luminometer to measure ATPase activity as a proxy for bacterial abundance on the filter paper.

Chlorophyll *a* concentration and total protein concentration were the most sensitive endpoints for determining sponge health (Chapter 1), and these endpoints were measured according to the methods outlined in chapter 1. Change in chlorophyll *a* and protein concentration data (Final-Initial) was analyzed using a 3-way ANOVA. When UV treatments were pooled for soluble protein concentration, the data was analyzed using a 2-way ANOVA. Tukey's HSD post-hoc test ($P<0.05$) was used for all post-hoc tests.

Results

Measurements of ATPase activity using rapid light units (RLU) showed a significant decrease in microbial abundance in the UV sterilized treatment compared to the ambient microbial abundance treatment (41.00 ± 5.54 and 11.67 ± 5.05 for ambient and UV treatments, respectively; $F = 15.31$, $P = 0.004$, Figure 29).

Chlorophyll *a* concentration for sponges in the 2 week lab microbe experiment was not affected by algal contact ($F = 0.15$, $P = 0.70$), UV treatment ($F = 0.0024$, $P = 0.96$), nutrient dose ($F = 0.09$, $P = 0.76$), or any interaction ($F = 0.82$, $P = 0.37$, $F = 0.03$, $P = 0.86$, $F = 0.07$, $P = 0.80$, and $F = 1.05$, $P = 0.31$ for algal contact and UV treatment, algal contact and nutrient dose, UV treatment and nutrient dose and all three variables, respectively, Figure 30). Soluble protein concentration for sponges in the lab microbe experiment was affected by nutrient dose ($F = 14.34$, $P = 0.0004$), but it was not affected by algal contact ($F = 3.27$, $P = 0.08$), UV treatment ($F = 1.61$, $P = 0.21$), or the interaction of any variables ($F = 0.65$, $P = 0.42$, $F = 0.00$, $P = 0.99$, $F = 0.01$, $P = 0.91$, and $F = 0.00$, $P = 0.98$ for algal contact and UV treatment, algal contact treatment and nutrient dose, UV treatment and nutrient dose, and all three variables, respectively, Figure 31) Since no effect of UV treatment was observed, samples were pooled and reanalyzed using a

two-way ANOVA for the factorial effect of nutrient dose and algal contact treatment. Pooled data showed a significant effect of algal contact treatment ($F = 6.70$, $P = 0.01$) and nutrient dose ($F = 20.11$, $P < 0.0001$), but there was no interactive effect of the two variables ($F = 0.70$, $P = 0.41$, Figure 32). The algal contact treatment showed a higher protein concentration than the sponge alone treatment. Increased nutrient concentration significantly decreased soluble protein concentration in the sponge.

Conclusion

Unlike the results of Chapter 1, chlorophyll *a* was not affected by algal contact or nutrient dose, potentially due to the shorter duration of the current experiment. Soluble protein concentration was significantly reduced under higher nutrient concentrations, which aligns with the results in chapter 1. The main goal of this experiment was to determine whether algal microbes played a role in sponge stress related to algal contact. While there is evidence to suggest that microbial abundance was reduced both in the water and in the sponge in the UV treatment, there was no difference in sponge health associated with this reduction. It is likely that the algal microbes do not play a large role in the stress associated with algal contact with a sponge, which is in contrast to what has been observed for algal-coral interactions (Smith et al. 2006). Since sponges are often mixotrophic, they may be able to cope with the algal microbial communities by increasing filter feeding activity, although there is no direct evidence for this currently.

Figure 29: Mean (±SE) for water sterilization measured in rapid light units (RLU) taken over the course of the 2 week algal microbial interaction experiment. UV sterilized water had significantly lower RLU measurements indicating lower ATPase activity and lower microbial abundance (P = 0.004).

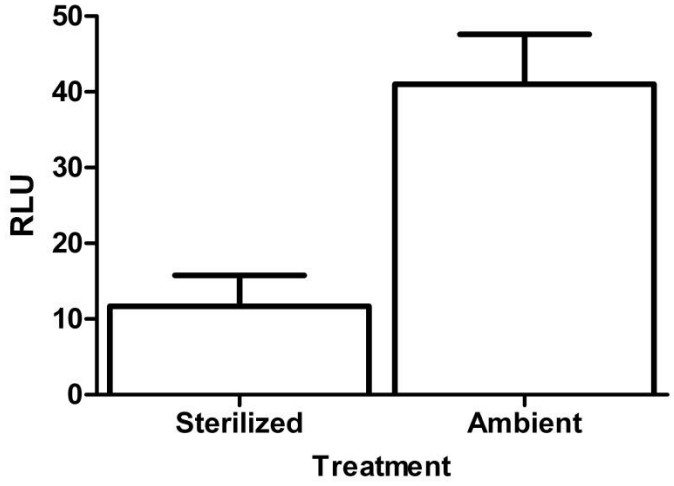


Figure 30: Mean (±SE) change in chlorophyll *a* concentration from the 2 week lab microbe experiment. There were no treatment effects on symbiont chlorophyll *a* concentration (P> 0.05). Amb refers to ambient microbial abundance, while UV refers to the UV sterilized treatment. Low is the ambient nutrient concentration and High represents increased nutrient concentration.

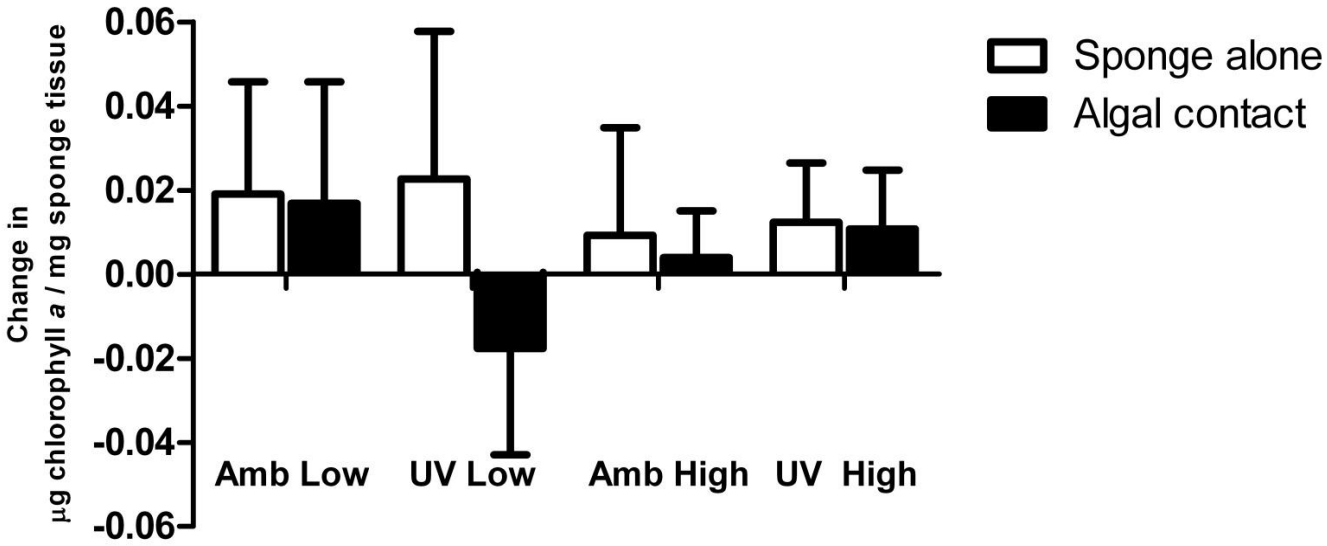


Figure 31: Mean (±SE) change in soluble protein concentration from the lab microbe experiment. There was a significant effect of nutrient dose (P = 0.0004) but no other treatments had an effect (P > 0.05). Amb refers to ambient microbial abundance, while UV refers to the UV sterilized treatment. Low is the ambient nutrient concentration and High represents increased nutrient concentration. Different letter groups represent differences among treatments.

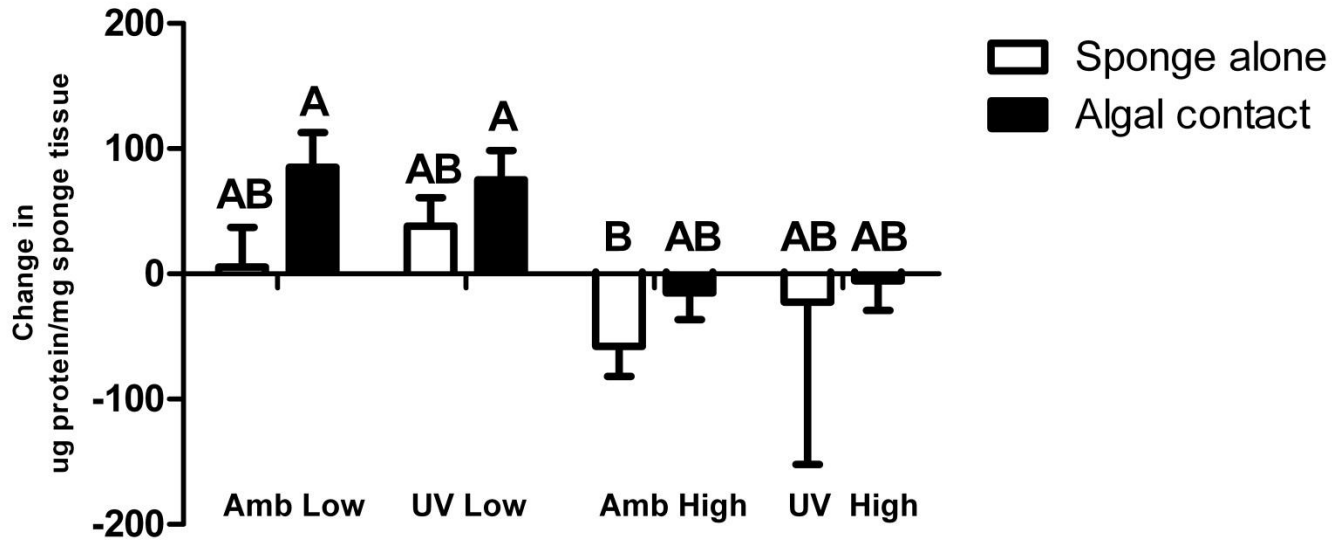
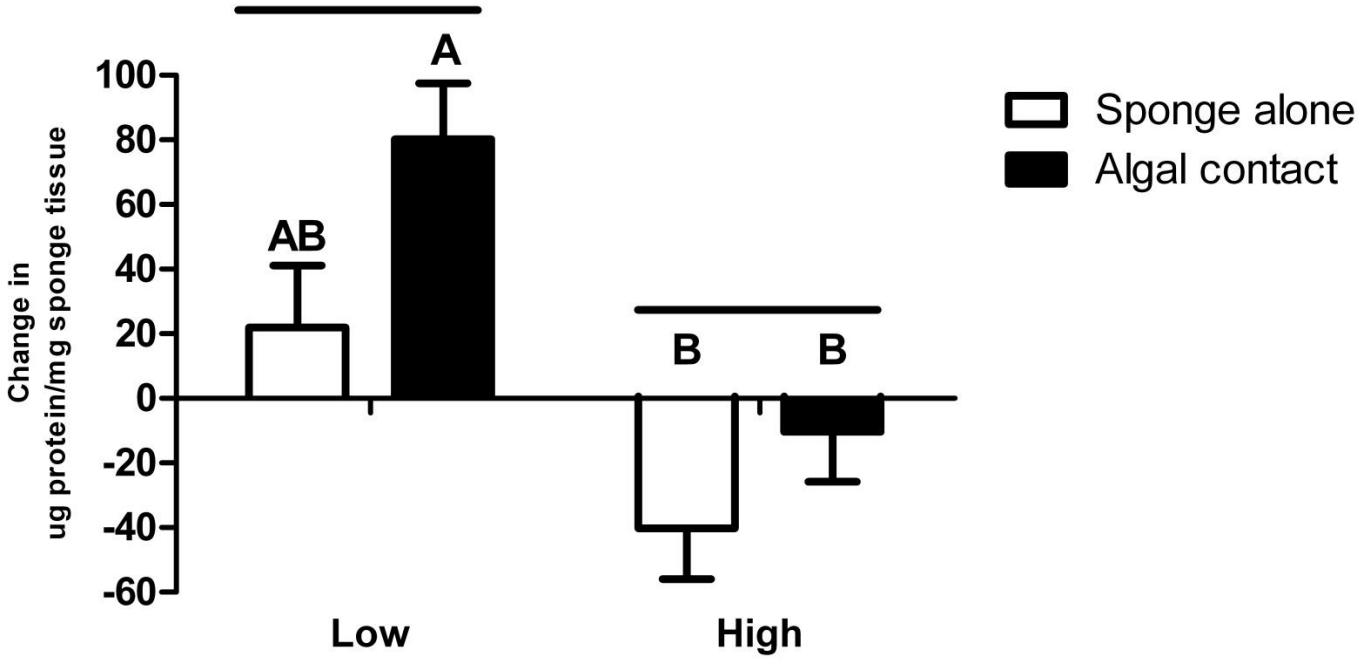


Figure 32: Mean (±SE) change in soluble protein concentration from the lab microbe experiment with UV treatments pooled. There was a significant effect of nutrient dose and algal contact ($P < 0.05$), but no effect of the interaction of the two terms ($P > 0.05$). Low refers to ambient nutrient concentration, and high refers to elevated nutrient concentration. Different letter groups indicate significant differences between treatments.



VITA

Cole Easson
Environmental Toxicology Graduate Program
cgeasson@olemiss.edu

(a) Professional Preparation

Ph.D. in Environmental Toxicology, University of Mississippi, 5/08-8/13

Individual and Population Responses of the Caribbean Sponge Aplysina cauliformis to Natural and Anthropogenic Stressors: Research focusing on sponge-algal interactions on coral reefs, including the consequences of this interaction under natural and elevated nutrient regimes, investigating the role of algal microbes in sponge stress, using stable isotopes to investigate the transfer of carbon and nitrogen between sponges and algae, and using GIS to determine the transmission mechanism of Aplysina red band syndrome using photo-mosaics of shallow patch reefs.

B.S in Biology, Minor in Mathematics, University of Mississippi, 2008

Emphasis in marine biology with study abroad classes on marine and tropical ecology in Galapagos Islands and Belize. Participated in a project using Quickbird and IKONOS image data to monitor seagrass loss in Belize.

(b) Appointments

2011-2012 Research Associate for Dr. Marc Slattery. Ocean acidification on Mesophotic Coral Reefs, and Coral Reef Landscape Responses to Ocean Acidification. Funded by NOAA and NOAA's Aquarius Reef Base, respectively.

Participated in field and laboratory projects related to ocean acidification effects on coral reef landscapes, with a focus on mesophotic reefs. This project involved a saturation diving mission in the Aquarius Underwater Habitat in 2011, as well as studies on mesophotic reefs in both the Atlantic and Pacific oceans.

2010-2011 Teaching assistant for: Coral reef Stressors: Adaptation in Tropical Marine Ecosystems, taught in the Bahamas. Funded by NOAA

Led field and laboratory activities for students. Lectured on anthropogenic stressors and their impact on coral reef ecosystems.

2008 – 2013 Research Associate for Dr. Deborah Gochfeld, *Aplysina* Red Band Syndrome on Caribbean Reefs. Funded by NSF and NOAA's National Institute for Undersea Science and Technology (NIUST).

Conducted field and laboratory projects related to understanding the dynamics of a sponge disease. Conducted studies investigating transmission of this disease and how anthropogenic inputs of nutrients affect this disease. A large part of this project has focused on monitoring large photo-quadrats and using spatial analysis tools to understand transmission within a population and propose a mechanism of transmission for this disease.

2007 –2008 Research Assistant, Dr. Marc Slattery, Department of Pharmacognosy. Processing YES Assay samples collected in Grand Bay, Mississippi. Processing MODIS satellite imagery of Grand Bay, Mississippi (working with the University of Mississippi Geoinformatics Center).

Participated in sample collection and laboratory processing of water samples to measure estradiol compounds in coastal estuaries. This project also used MODIS data to monitor seagrass cover and compare these data to other datasets such as the estradiol and polycyclic aromatic hydrocarbon measurements at these sites.

2006 –2007 Teaching Assistant, BISC 161 and 163.

Responsible for teaching lab sections for the Introduction to Biology course for majors.

2006 –2007 Teaching Assistant, BISC 445, Study Abroad, Belize.

2005 –2007 Student Worker, Dr. Murray Nabors, Chair, Department of Biology, Developed teacher resources for Dr. Nabors' textbook, Botany, and assisted in gathering and organizing material for the second edition of the textbook.

(c) Publications

Gaston GR, **C Easson**, G Easson, J Janaskie, & MA Ballas (2008) Seagrass loss in Belize: studies of turtlegrass (*Thalassia testudinum*) habitat using remote sensing and ground-truth data. *Gulf and Caribbean Research* 21:23-30.

Gochfeld, DJ, **CG Easson**, CJ Freeman, RW Thacker, JB Olson. (2012) Disease and nutrient enrichment as potential stressors on the Caribbean sponge *Aplysina cauliformis* and its bacterial symbionts. *Marine Ecology Progress Series* 456:101-111.

Gochfeld DJ, **CG Easson**, M Slattery, RW Thacker, JB Olson (2012). Population dynamics of a sponge disease on Caribbean reefs. In: Stellar DL, Lobel LK (eds) Proceedings of the American Association of Underwater Sciences 31st Scientific Symposium, Monterey, CA. pp. 125-128

Slattery, M, DJ Gochfeld, **CG Easson,** LRK O'Donahue (2013) Facilitation of coral reef biodiversity and health by cave sponge communities. *Marine Ecology Progress Series* 476:71-86

Easson, CG, H Momm, DJ Gochfeld, M Slattery, JB Olson, RW Thacker (in review) Exploring individual- to population-level impacts of disease on coral reefs: fate and dynamics of *Aplysina* Red Band Syndrome (ARBS) *PLOS ONE*

Easson, CG, M Slattery, DJ Gochfeld (in preparation) Complex ecological associations: Evidence for competition and facilitation in a sponge-algal interaction. *MEPS*

(d) Presentations

Easson, C.G., D.J. Gochfeld, M. Slattery, H.G. Momm, J.B. Olson, R.W. Thacker. Inferring process from pattern: Determining a transmission mechanism for *Aplysina* Red Band Syndrome (ARBS) in natural sponge populations using spatial analysis. Benthic Ecology Meeting, Savannah, GA, March 2013.

Olson J.B., Gochfeld D.J., **Easson C.G**., Thacker R.W. Understanding *Aplysina* Red Band Syndrome, a disease of marine sponges. Southeastern Branch Meeting of the American Society of Microbiology. October 2012, Athens, GA

Gochfeld, D.J., **C.G. Easson**, M. Slattery, R.W. Thacker and J.B. Olson. Population dynamics of a sponge disease on Caribbean reefs. American Academy of Underwater Sciences Diving for Science Symposium, Monterey, CA, September 2012.

Gochfeld, D.J., **C.G. Easson**, J. B. Olson and R.W. Thacker. Ecological implications of an infectious sponge disease on Caribbean reefs. 11th International Coral Reef Symposium, Cairns, Australia, July 2012.

Easson, C.G., D.M. Baker, D.J. Gochfeld, M. Slattery. Effects of macroalgal contact on carbon and nitrogen assimilation and transfer in sponge-macroalgal interactions. Benthic Ecology Meeting, Norfolk, VA, March 2012

Slattery, M., S. Lee, **C.G. Easson**, C. Williams, E. Hunkin and D.J. Gochfeld. Sponge diversity and acclimation in naturally-acidified marine caves. Ocean Sciences Meeting, Salt Lake City, UT, February 2012.

Easson, C.G., D.J. Gochfeld, M. Slattery. Effects of Nutrient Enrichment on Competition between the algae *Microdictyon marinum* and the sponge *Aplysina cauliformis.* Society for Integrative Comparative Biology annual meeting, Charleston, SC, January 2012.

Easson, C.G., H.G. Momm, D.J. Gochfeld, M. Slattery. Mapping impacts of disease on sponge communities using Geographic Information Systems (GIS). VII World Sponge Conference, Girona, Spain, September 2010.

Gochfeld, D.J., **C.G. Easson**, C. Freeman, R.W. Thacker and J.B. Olson. Impacts of nutrient enrichment and disease on the Caribbean sponge *Aplysina cauliformis* and its cyanobacterial symbionts. International Sponge Symposium, Girona, Spain, September 2010.

Easson, C.G., D.J. Gochfeld, M. Slattery. Effects of Nutrient Enrichment on Competition between the alga *Microdictyon marinum* and the sponge *Aplysina cauliformis*. SETAC North America, New Orleans, LA, November 2009.

Easson, C.G., J.B. Olson, A. Mobley, R.W. Thacker and D.J. Gochfeld. Impact of nutrient enrichment on the Caribbean sponge *Aplysina cauliformis*. Benthic Ecology Meeting, Corpus Christi, TX, 4-7 March, 2009.

Easson, C.G., D.J. Gochfeld, J.B. Olson, R.W. Thacker. Organismal- and community-level impacts of stressors on Caribbean coral reef sponges. SETAC North America, Tampa, FL, November 2008

Slattery, M., K.L. Willett, D.J. Gochfeld, J. Weston, **C.G. Easson**, G. Easson, J. Janaskie, A. Boettcher, B. Ehmen and C.A. May. Impacts of Hurricane Katrina on seagrass beds in Grand Bay, Mississippi. 29th SETAC North America, Tampa, FL, November, 2008.

(e) Skills

- Certified AAUS Research Diver. Certification Depth 180ft
- Technical diver training for Aquarius Undersea Research habitat
- NAUI nitrox certification
- Remote sensing skills using ERDAS Imagine and ENVI software programs.
- ESRI certified completion of 24 course hours of ArcGIS
- Completed Smithsonian course in Taxonomy and Ecology of Caribbean Sponges, Bocas del Toro, Panama, 2012
- General computer hardware and software skills
- Laboratory and field bioassay experience

(f) Service

Student representative on the University of Mississippi Diving Control Board, 2011-2013