ABSTRACT

ARCHER, STEPHANIE KRAFT. The Role of Sponges in Structuring Tropical Nearshore Ecosystems. (Under the direction of Dr. Craig A. Layman).

Foundation species are a class of organisms which create or modify habitat and promote a more diverse community through their positive interactions with other species. Despite the fact that foundation species are characterized by these positive interactions, it is important to recognize that many may be context dependent. Because foundation species have such a large impact in marine systems, determining if and when their interactions will be context dependent allows for a better understanding of the drivers of community structure and ecosystem function. Seagrasses are a group of foundation species that shape important coastal ecosystems. Sponges are a common component of seagrass bed communities, yet little is known regarding how sponges interact with seagrass to impact the structure and function of these systems. I examined the effect of two sponge species, *Ircinia felix* and *Halichondria melanadocia*, on the structure and function of tropical nearshore ecosystems. First, I used a long-term experiment to determine the effect of *I. felix* on seagrass, macroalgae, macroinvertebrate, and fish communities. I found that the presence of a sponge significantly impacted the structure of each of these groups, increasing the abundance and diversity of macroalgae and fishes. I also found that seagrass grew faster and was denser near sponges. From this I concluded that *I. felix* acts a secondary foundation species in *Thalassia testudinum*-dominated seagrass beds. Second, I used a combination of survey and experimental methods to investigate the interaction between *T. testudinum* and *H. melanadocia*, an epibiont which grows around seagrass shoots. I found that under oligotrophic conditions, the two organisms are commensalistic with the sponge benefiting

from the interaction. The net neutral effect of the sponge on the seagrass is maintained by a balance of the cost of the sponge shading the seagrass' photosynthetic tissue with the benefit of the sponge providing bioavailable forms of nitrogen and phosphorus (which limit seagrass growth in this system). However, as ambient nutrient availability increases, the cost-to-benefit ratio shifts with the sponge becoming parasitic. Using a Monte Carlo simulation model, I demonstrated that this shift towards parasitism significantly reduces the amount of carbon stored in live seagrass biomass at the ecosystem scale. Third, I explored natural variability in nutrient processing by sponges. Sponges' role in biogeochemical cycling of nearshore ecosystems is an important ecosystem function and, in part, drives the outcome of interactions between sponges and primary producers. The form and amount of nutrients released by a sponge is largely driven by the symbiotic microbial communities many species of sponge, including *I. felix*, host. I showed that nutrient processing in *I. felix* is extremely variable and strongly correlated with abiotic conditions, including ambient nutrient availability. This suggests that sponge-microbe interactions are context dependent and that the impact of sponges on biogeochemical cycles in nearshore ecosystems is more complex than previously thought. Taken together, the results of my dissertation show that sponges can have a large impact on the structure and functioning of seagrass ecosystems, and that this impact is complex and context dependent.

The Role of Sponges in Structuring Tropical Nearshore Ecosystems

by
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DEDICATION

This dissertation is dedicated to my mother, Adelina Christine Davidson Kraft, who passed away February 1st, 2013. She always encouraged me to pursue the things I loved and gave me the confidence that with hard work I could be and do whatever I wanted.

BIOGRAPHY

After graduating from high school in Missouri I left the Midwest to attend the University of Georgia. In 2004 I graduated with a BS in Ecology and a new love of Georgia football. After working for the US Forest Service and Forfar International Field Station I returned to school. In 2009 I received my MS in Ecology from Utah State University where I studied the antipredator behavior and learning capability of an endangered fish, the June Sucker, *Chasmistes liorus liorus.*

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CHAPTER 1. INTRODUCTION

Foundation species (sensu Dayton, 1972 as refined by Bruno and Bertness, 2001) are a class of organisms that create or modify structured habitat and engage in a large number of facilitative interactions with community members by providing associational defenses and alleviating stressful abiotic conditions. As a result, these species support abundant and diverse communities. Although foundation species are, in part, classified by their positive interactions, it is important to think of these as a series of both costly and beneficial interactions that sum to a net interaction outcome (Herre, et al., 1999; Schwartz and Hoeksema, 1998). For example, mutualisms are simply interactions where the benefits of the interaction outweigh the costs for both species involved. Often, the relative balance of costs and benefits is influenced by the biotic or abiotic conditions in which the interaction occurs, i.e., the interaction is said to be context dependent (Bronstein, 1994; Chamberlain, et al., 2014). When a change in the outcome of a context dependent interaction impacts a foundation species, the shift could have cascading consequences for community structure and ecosystem function (Angelini, et al., 2011; Tilman, et al., 2014). In this document, I examine how interactions involving foundation species shape communities and ecosystem function. I approached this topic by asking how interactions between sponges and seagrass shape nearshore subtropical ecosystems and how changes in abiotic conditions may influence the outcome of these interactions.

Seagrasses are foundation species which create important habitats in coastal systems worldwide (Duffy, et al., 2014). These habitats are centers of biodiversity that maintain

important trophic links with both mangrove and coral reef habitats, act as important centers of nutrient cycling, and sequester large amounts of carbon (Duffy, et al., 2014; Fourqurean, et al., 2012; Hemminga, et al., 1991; Marba, et al., 2006). Sponges are a diverse group of organisms and a common component of tropical and subtropical seagrass beds (Archer, et al., 2015; Van Soest, et al., 2012). They filter large amounts of water (Reiswig, 1971a) removing viruses (Hadas, et al., 2006) and ultra- and picoplankton (Pile, et al., 1997; Reiswig, 1971b; Yahel, et al., 2007). Sponges are also capable of using dissolved organic carbon (de Goeij, et al., 2008; Yahel, et al., 2003) and acting as sources of ammonium, nitrate/nitrite, and soluble reactive phosphorus (Maldonado, et al., 2012 and references therein). Additionally, sponges can play a strong role in structuring benthic communities (Dayton, 1972) through the provisioning of structured habitat and food resources for fish and invertebrates (Greene, 2008; Pawlik, et al., 2013; Wulff, 1995). Although sponges perform many ecosystem functions (Bell, 2008), how they interact with seagrass to influence the structure and function of seagrass beds is unknown. Therefore, my aim is to develop a mechanistic knowledge of the interactions between sponges and seagrass, shedding light on the role of sponges in processes governing the productivity of nearshore systems.

Here I investigated the impact of two species of sponge, *Ircinia felix* and *Halichondria melanadocia*, on the structure and functioning of *Thalassia testudinum*-dominated seagrass beds. In my second chapter, I describe an experiment that examined the effect of *I. felix* on the structure of seagrass bed communities and hypothesized that the sponge serves as a secondary foundation species within this system. Over 17 months I tracked the response of the seagrass, macroalgae, macroinvertebrate, and fish communities in

plots with and without a sponge. In the third chapter, I used a combination of observational and experimental methods to examine the interaction between *H. melanadocia* and *T. testudinum*. The sponge *H. melanadocia* is common in the seagrass beds surrounding Abaco Island, The Bahamas and grows around the base of *T. testudinum* shoots, sometimes covering a large proportion of the photosynthetic tissue of the seagrass. Using survey methods, I examined correlations between seagrass density and sponge abundance, as well as the impact of sponge presence on the morphology and nutrient content of seagrass shoots. I experimentally evaluated the effect of sponge presence on seagrass growth and the relationship between seagrass density and sponge growth. Finally, using incubations I evaluated nutrient release by the sponge. Based on these results, in the fourth chapter, I hypothesized that the interaction between the sponge and the seagrass may be context dependent and that ambient nutrient availability may drive the outcome of the interaction. To test this hypothesis, I experimentally manipulated ambient nutrient availability in plots with and without a sponge. I measured the response of the seagrass and used these data to inform a Monte Carlo simulation model that estimated carbon stored in live seagrass biomass under both oligotrophic and nutrient-enriched conditions. My fifth chapter explores natural variability in nutrient processing in *I. felix.* Using the InEx method developed by Yahel, et al. (2005) I sampled water entering and exiting *I. felix* on nine reefs located throughout The Bahamas and Curaçao. I looked for correlations between sponge nutrient processing and abiotic variables including ambient nutrient availability, temperature, and light intensity. Together these chapters shed light on the impact of sponges on the structure and function of seagrass ecosystems.

REFERENCES

Angelini, C., Altieri, A.H., Silliman, B.R., Bertness, M.D., 2011. Interactions among foundation species and their consequences for community organization, biodiversity, and conservation. Bioscience 61(10), 782-789.

Archer, S.K., Stoner, E.W., Layman, C.A., 2015. A complex interaction between a sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) in a subtropical coastal ecosystem. J. Exp. Mar. Biol. Ecol. 465, 33-40.

Bell, J.J., 2008. The functional roles of marine sponges. Estuarine Coastal and Shelf Science 79(3), 341-353.

Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology & Evolution 9(6), 214-217.

Bruno, J.F., Bertness, M.D., 2001. Habitat modification and facilitation in benthic marine communities. In: Bertness, M.D. (Ed.), Marine Community Ecology. Sinauer, pp. 201–218.

Chamberlain, S.A., Bronstein, J.L., Rudgers, J.A., 2014. How context dependent are species interactions? Ecology Letters 17(7), 881-890.

Dayton, P.K., 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica, Proceedings of the colloquium on conservation problems in Antarctica. Allen Press Lawrence, Kansas, USA, pp. 81-96.

de Goeij, J.M., van den Berg, H., van Oostveen, M.M., Epping, E.H., Van Duyl, F.C., 2008. Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Marine Ecology Progress Series 357, 139.

Duffy, J.E., Hughes, A.R., Moksnes, P.-O., 2014. Ecology of seagrass communities. In: Bertness, M.D., Bruno, J.F., Silliman, B.R., Stachowicz, J.J. (Eds.), Marine Community Ecology and Conservation. Sinauer Associates, Inc., Sunderland, MA, pp. 271-298.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience 5(7), 505-509.

Greene, A.K., 2008. Invertebrate endofauna associated with sponge and octocoral epifauna at Gray's Reef National Marine Sanctuary off the coast of Georgia. College of Charleston.

Hadas, E., Marie, D., Shpigel, M., Ilan, M., 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51(3), 1548-1550.

Hemminga, M., Harrison, P., Van Lent, F., 1991. The balance of nutrient losses and gains in seagrass meadows. Marine Ecology Progress Series 71.

Herre, E.A., Knowlton, N., Mueller, U.G., Rehner, S.A., 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology & Evolution 14(2), 49-53.

Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, X. (Eds.), Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology, pp. 113-182.

Marba, N., Holmer, M., Gacia, E., Barron, C., 2006. Seagrass Beds and Coastal Biogeochemistry. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands, pp. 135-157.

Pawlik, J.R., Loh, T., McMurray, S.E., Finelli, C.M., 2013. Sponge communities on Caribbean coral reefs are structured by factors that are top-down, not bottom-up. PLoS ONE 8(5), e62573.

Pile, A.J., Patterson, M.R., Savarese, M., Chernykh, V.I., Fialkov, V.A., 1997. Trophic effects of sponge feeding within Lake Baikal's littoral zone .2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography 42(1), 178-184.

Reiswig, H.M., 1971a. In-situ pumping activities of tropical Demospongiae. Marine Biology 9(1), 38-50.

Reiswig, H.M., 1971b. Particle feeding in natural populations of 3 marine Demosponges. Biological Bulletin 141(3), 568-591.

Schwartz, M.W., Hoeksema, J.D., 1998. Specialization and resource trade: biological markets as a model of mutualisms. Ecology 79(3), 1029-1038.

Tilman, D., Isbell, F., Cowles, J.M., 2014. Biodiversity and ecosystem functioning. Annual Review of Ecology, Evolution, and Systematics 45(1), 471.

Van Soest, R.W.M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N.J., Santodomingo, N., Vanhoorne, B., Kelly, M., Hooper, J.N.A., 2012. Global Diversity of Sponges (Porifera). PLoS ONE 7(4), e35105.

Wulff, J.L., 1995. Sponge-feeding by the Caribbean starfish *Oreaster reticulatus*. Marine Biology 123(2), 313-325.

Yahel, G., Marie, D., Genin, A., 2005. InEx - a direct in situ method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography-Methods 3, 46-58.

Yahel, G., Sharp, J.H., Marie, D., Hase, C., Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography 48(1), 141-149.

Yahel, G., Whitney, F., Reiswig, H.M., Eerkes-Medrano, D.I., Leys, S.P., 2007. In situ feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. Limnology and Oceanography 52(1), 428-440.

CHAPTER 2. SPONGES ACT AS SECONDARY FOUNDATION SPECIES AND INITIATE A POSITIVE FEEDBACK LOOP IN SEAGRASS BEDS

Abstract

Seagrass beds are a classic example of an ecosystem structured by the presence of foundation species. Foundation species create structured biogenic habitat and promote species diversity through positive interactions. Although seagrasses serve as the primary foundation species in these systems, foundation species rarely occur in isolation. Sponges, which act as foundation species in other systems, alter the physical environment in many ways, including provisioning of structured habitat. Although sponges are a common component of seagrass beds, there is a paucity of knowledge regarding how they may influence the structure of seagrass bed communities. In a long-term experiment (17 months), I determined the effect of a sponge, *Ircinia felix,* on the structure of a *Thalassia testudinum*-dominated seagrass ecosystem on Abaco Island, The Bahamas. In June 2013, fifteen 25m^2 plots were established; five containing a single, live *I. felix,* five with a polypropylene model sponge, and five controls. Seagrass density, community structure and growth, as well as macroalgae, macroinvertebrate, and fish abundance and diversity were measured prior to live or model sponge placement. All variables have been measured twice a year in July and November since establishment. Over the course of the experiment, *T. testudinum* growth increased in plots containing *I. felix.* The increased growth did not result in an increase in the shoot density of *T. testudinum*; however, in plots containing an *I. felix* the shoot density of two additional seagrass species, *Syringodium filiforme* and *Halodule wrightii,* increased significantly. By November 2014, plots containing live *I. felix* contained more species-rich

fish and macroinvertebrate communities. Taken together, these results indicate that sponges may act as a second foundation species leading to a more diverse and abundant seagrass bed community. More generally, I suggest that sponges may have a strong positive influence, through many mechanistic pathways, on seagrass ecosystems.

Introduction

Seagrass beds are an important coastal ecosystem worldwide. They help to attenuate wave energy (Fonseca and Cahalan 1992) and stabilize sediments (Folmer et al. 2012), store large amounts of carbon (Fourqurean et al. 2012), and are important sites for nutrient cycling (Hemminga et al. 1991). Seagrass beds also act as hot spots of productivity with diverse and abundance communities of macroalgae, invertebrates, and fish (Duffy 2006). The communities associated with seagrass beds maintain important links with other coastal ecosystems, such as coral reefs, by acting as a nursery habitat (Heck et al. 2003, Adams et al. 2006) and as feeding grounds (Meyer et al. 1983, Yeager et al. 2012). Unfortunately, seagrass habitats are under threat from myriad anthropogenic impacts that have driven significant worldwide declines (Orth et al. 2006, Waycott et al. 2009). As a result, there have been large efforts at both conservation and restoration of seagrass habitats. A primary goal of these efforts is to protect the diverse communities associated with seagrass beds. As such, a better understanding of the drivers of diversity within seagrass beds is of upmost importance.

The diversity of organisms found in seagrass beds is tightly linked to the role of seagrass as a foundation species. Foundation species (sensu Dayton 1972 as refined by Bruno and Bertness 2001) provide biogenic habitat while altering abiotic conditions in such a way that results in positive interactions with community members. The structure created by seagrasses provides refuge from predation, increased area available for epiphytic and epifaunal organisms to colonize, and higher food availability (Stoner 1980, Orth et al. 1984). Although seagrasses are undoubtedly an important driver of the diversity and structure of associated communities, other organisms that may function as foundation species occur

within seagrass beds, such as large bivalves and sponges (Duffy 2006). A second foundation species can alleviate additional stressful abiotic conditions beyond those ameliorated by the primary foundation species, and further augment structural complexity and spatial heterogeneity, ultimately increasing the diversity of associated communities (Angelini et al. 2011, Altieri et al. 2012, Angelini et al. 2015).

When Dayton (1972) first coined the term foundation species it was in reference to sponges, which can be abundant in seagrass beds (Peterson et al. 2006, Archer et al. 2015). In addition to comprising a significant portion of benthic cover in many marine systems, sponges are also capable of significantly altering abiotic conditions. For example, due to their large symbiotic microbial communities and high pumping rates, sponges can be significant sources of bioavailable nutrients (Maldonado et al. 2012 and references therein). Additionally, because of efficient filtration, sponges can significantly improve water quality (Peterson et al. 2006). There is also some evidence that sponges can strongly effect the structure of invertebrate community in seagrass beds. Butler et al. (1995) found that a die-off of sponges in the mixed seagrass and hard bottom habitat of Florida Bay had a significant negative effect on juvenile spiny lobster (*Panulirus argus*) abundance, as the lobsters rely on sponges for shelter. Although such observations support the assumption that sponges likely play an important role in seagrass beds (Duffy 2006), few studies have tested this assumption.

Here I experimentally tested the effect of a sponge, *Ircinia felix*, on the structure of subtropical beds of turtle grass, *Thalassia testudinum*. I hypothesized that *I. felix* serves as an additional foundation species within seagrass beds. Specifically, I predicted that sponges

would positively affect seagrass, as manifested through increased growth and density of seagrass, since *I. felix* is a known source of bioavailable nitrogen (Southwell et al. 2008) - a limiting nutrient for seagrass in the study system (Allgeier et al. 2010). Second, I predicted that the presence of a sponge would lead to increased macroalgal richness as a result of the bioavailable nitrogen supplied by the sponge. Third, I predicted that the presence of a sponge would increase the richness and abundance of fish and epibenthic macroinvertebrates as a result of the increased complexity and shelter provided by the sponge and associated seagrasses.

Methods

Study site and experimental design

This study was conducted in a shallow (1.1m low tide depth) seagrass bed located off of Southern Great Abaco Island, The Bahamas (26.02610 N, -077.37408 W). Fifteen 5 x 5 m plots were delineated in a continuous seagrass bed on June 9, 2013 by placing wooden stakes at the corners and center of each plot. All plots were separated >2m from each other. Prior to the establishment of the treatments, all response variables were measured (see below). After preliminary data were collected, each plot was randomly assigned to one of three treatments: control (n=5), structure control (n=5), or sponge (n=5). A polypropylene model of a sponge was placed inside a cage at the center of each structure control plot (Fig. 1.1). A single sponge (*Ircinia felix*, average volume ± standard deviation, 2.5 ± 0.75 L) was placed inside a cage in the center of each sponge plot. Control plots were not manipulated. All response

variables were measured 1, 5, 12, and 17 months after the treatments were established. Live sponges were replaced as needed with a total of 3 sponge replacements. All replaced sponges were still living, but showing overt signs of stress or die-back of tissues.

Seagrass response variables

Thalassia testudinum growth, short shoot density, and nutrient content were measured. Because they were rare at the beginning of the experiment, short shoot density was counted and combined for *Syringodium filliforme* and *Halodule wrightii*. Shoot densities were determined in one 1x1 m quadrat centered in the plot. Within the $1m^2$ quadrat four 15 cm x 15 cm quadrats were haphazardly placed and the shoots of each seagrass were counted (*S. filliforme* and *H. wrightii* were recorded together). *T. testudinum* growth was measured using the standard blade hole punching technique (Zieman, 1974) at the center of the plot, immediately next to either the sponge, or polypropylene model of the sponge. In order to minimize disturbance to the plots, the growth and morphometrics (blade length and width) of five short shoots per distance were measured *in situ*.

Community structure response variables

The response of the fish, epibenthic macroinvertebrate, and macroalgal communities were monitored throughout the course of the experiment. All macroalgae were identified to genus *in situ* and recorded. For algae where individuals were difficult to distinguish (e.g. *Laurencia spp.*), clumps of algae were recorded as individuals. Macroinvertebrates were identified to species when possible and recorded. When identification to species was not

possible the lowest possible taxanomic rank was recorded. For both macroalgae and macroinvertebrates, if identification was not possible *in situ*, a representative sample was photographed and subsequently collected for identification in the lab. Macroalgae and macroinvertebrate densities were averaged over three $1m^2$ quadrats to obtain a per m^2 estimate of abundance for each plot. The fish community was quantified by observing the plot for 5 minutes and all fish observed within the plot during the observation period were recorded (Layman et al. 2004). For analysis, small silvery pelagic fishes which form large schools (e.g. fishes in the families Atherinopsidae and Clupeidae) were excluded following Peters, et al. (2015). Both macroinvertebrate and macroalgal communities were quantified within the same $1m^2$ quadrats as seagrass shoot density.

Statistical analysis

Seagrass growth and shoot density, as well as fish, macroalgae, and macroinvertebrate abundance and species richness were analyzed using a Bayesian hierarchical framework. Seagrass growth was estimated using the following model:

$$y_i \sim gamma(shape_i, rate_i)$$

Where the shape and rate parameters are calculated from the estimated mean and variance for treatment at each time period.

Seagrass shoot density and fish, macroalgal, and macroinvertebrate abundance and species richness were estimated using the following model:

$$y_i \sim poisson(lambda_i)$$

The mean for seagrass growth and shoot density was calculated using the linear equation:

$$mu_i \sim \alpha_{i,j} + \beta 0_{i,k} + \beta 1_{i,k} \times x1_i + \beta 5_{i,k} \times x5_i + \beta 12_{i,k} \times x12_i + \beta 17_{i,k} \times x17_i$$

Where $\alpha_{i,j}$ is the random effect due to plot j, $\beta 0_{i,k}$ is the average value of the response for the plots assigned to each treatment k prior to any effect of treatment, $\beta 1_{i,k} \times x1_i$ is the effect of treatment k one month post establishment, the term $x1_i$ represents a vector so that $\beta 1_{i,k}$ is only included in the model if observation i occurred at that time, $\beta 5_{i,k} \times x5_i$ is the effect of treatment k 5 months post establishment, the term $x5_i$ represents a vector so that $\beta 5_{i,k}$ is only included in the model if observation i occurred at that time, $\beta 12_{i,k} \times x12_i$ is the effect of treatment k 12 months post establishment, the term $x12_i$ represents a vector so that $\beta 12_{i,k}$ is only included in the model if observation i occurred at that time, and $\beta 17_{i,k} \times x17_i$ is the effect of treatment k 17 months post establishment, the term $x17_i$ represents a vector so that $\beta 17_{i,k}$ is only included in the model if observation i occurred at that time. For all other response variables the mean was calculated using the same equation with the exception of the random term for plot. The model code can be found in Appendix A.

For seagrass growth, variance was larger for samples collected in summer months (i.e. preliminary, 1, and 12 months post establishment). Therefore, a different variance was assigned if the observation being estimated occurred in the summer than if it was in the winter. The prior distribution for summer variance was a gamma distribution with shape and rate parameters of 6 and 0.03, respectively. The prior distribution for winter variance was estimated from a gamma distribution with shape and rate parameters of 50 and 1, respectively. The other prior distributions for all models can be found in the supplemental material.

Fish, macroalgae, and macroinvertebrate community structure were compared among the three treatments using multi-response permutation procedure with 1,000 iterations and visualized using non-metric multi-dimensional scaling. Distance matrices were calculated using Bray-Curtis measure of similarity.

Results

Bayesian model diagnostics

All models were run for a minimum of 50,000 iterations. The minimum effective sample size for any model parameter was 714 and the maximum standard error was 0.038 (Kruschke 2010)(Appendix A Tables A1, A2). Priors used for each model are reported in Appendix A Table A3.

Seagrass response

There was strong seasonality in seagrass growth, such that measurements taken in November of 2013 and 2014 (5 and 17 months post experiment establishment respectively) were lower than the growth observed the previous summer (Fig. 2.2, 2.3a). Therefore, for ease of interpretation, I first focus on the changes in seagrass growth observed 12 months after the experiment was established, as this time period is in the summer when maximum growth for the year is expected. Prior to establishment, of the experiment the mean seagrass growth in control plots was 37.78 mm^2d^{-1} (± 20.89, sd), 27.64 mm^2d^{-1} (± 9.93, sd) in structure control plots, and 23.99 mm^2d^{-1} (± 12.33, sd) in sponge plots (Fig. 2.2). By July of

2014 the presence of sponges significantly increased seagrass growth (mean change in growth and high density interval (HDI), 9.58 mm^2d^{-1}, 2.27 to 16.66). Seagrass growth did not significantly change in control plots ($\bar{x}$ change: -2.39 mm^2d^{-1}, HDI: -8.70 to 3.95) or structure control plots ($\bar{x}$ change: 4.03 mm^2d^{-1}, HDI: -1.88 to 10.16, Fig. 2.2, 2.3a).

T. testudinum short shoot density was similar in all plots prior to the establishment of the experiment with an average of 382.26 shoots m^{-2} (± 152.64, sd). By November of 2014, or 17 months post experiment establishment, *T. testudinum* shoot density declined in all treatments (Fig. 2.3b). *T. testudinum* shoot density declined more in structure control plots than both sponge and control plots (Fig. 2.3.b). There was no difference in the change in shoot density between sponge and control plots (mean change in shoot density in sponge plots – control plots, 3.44, HDI: -38.33 to 44.22). *S. filliforme* and *H. wrightii* were rare and patchily distributed at the beginning of the experiment with an average of 67.64 shoots m^{-2} (± 77.13, sd). *S. filliforme* and *H. wrightii* increased in sponge plots one year after the experiment was established ($\bar{x}$ increase: 26.56 shoots m^{-2}, HDI: 10.89 to 42.44, Fig. 2.3c). By 17 months post establishment, the combined shoot density of these two species in sponge plots had increased by an average of 111.22 shoots m^{-2} (HDI, 90.44 to 132.44). There was also a slight increase in control plots ($\bar{x}$ change: 26 shoots m^{-2}, HDI 11.22 to 41.11); however, the increase observed in the sponge plots was significantly larger (change in shoot density in sponge plots-control plots, $\bar{x}$: 84.89 shoots m^{-2}, HDI 61.11 to 111.22). After 17 months total shoot density increased in sponge plots ($\bar{x}$ change: 56.89 shoots m^{-2}, HDI: 22.44-90.78) and decreased in both the control ($\bar{x}$ change: -48.56 shoots m^{-2}, HDI: -82.78 to -

14.56) and structure control plots ($\bar{x}$ change: -89.22 shoots m^{-2}, HDI: -122.78 to -56.22, Fig. 2.3d).

Macroalgae

Prior to the beginning of the experiment, there was no difference in macroalgal community structure between treatments (Fig. 2.4). Macroalgal taxa richness did not significantly change over the course of the experiment, but there was a positive trend in sponge plots ($\bar{x}$ change: 0.62 genera, HDI: -1.34 to 2.60) while control ($\bar{x}$ change: -1.21 genera, HDI: -2.94 to 0.50) and structure control ($\bar{x}$ change: -0.74 genera, HDI: -2.44 to 1.04) tended to have fewer genera. Macroalgal community structure in sponge plots diverged from both the control and structure control plots at 12 months (A=0.32, $p<0.001$) and remained significantly different at 17 months (A=027, p=0.003, Fig. 2.4a-d). The change in macroalgal community structure was driven by increased abundance of *Penicillus spp*. and *Acetabularia spp.* in sponge plots over time.

Fish

Prior to the beginning of the experiment, there was no difference in fish community structure between treatments (Fig. 2.4e). Both fish species richness ($\bar{x}$ change: 1.86 species, HDI: 0.25-3.57) and abundance ($\bar{x}$ change: 7.04 individuals, HDI: 4.21 to 9.98) increased in sponge plots 17 months post establishment. Fish species richness showed a significant increase after 12 months ($\bar{x}$ change: 1.85 species, HDI: 0.23 to 3.54). Fish community structure in sponge plots diverged from control and structure control plots after only 5

months (A=0.36, p<0.001) and the difference persisted at 12 (A= 0.39, p< 0.001) and 17 months post establishment (A=0.26, p<0.001, Fig. 2.4e-h). These differences were largely driven by increased juvenile grunt (Haemulidae) and Beaugregory (*Stegastes leucostictus*) abundance in sponge plots over time.

Macroinvertebrates

Prior to the experiment there was no difference in macroinvertebrate community structure between treatments (Fig. 2.4i). Macroinvertebrate species richness increased in sponge plots after 17 months ($\bar{x}$ change: 2.35 species, HDI: 0.40 to 4.49). Macroinvertebrate abundance increased in sponge plots 12 months post-establishment ($\bar{x}$ change: 4.60 individuals, HDI: 0.50 to 8.68), but this increase was no longer significant five months later (i.e., 17 months post establishment, $\bar{x}$ change: 2.29 individuals, HDI: -0.52 to 5.56). Despite inconsistent patterns in abundance and species richness, the community structure of macroinvertebrates in sponge plots was significantly different than both the control and structure control plots after 12 months (A=0.05, p=0.04) and again at 17 months (A=0.10, p<0.001, Fig. 2.4i-l).

Discussion

I show strong support that sponges serve as a second foundation species in seagrass beds. Seagrass grew faster and total seagrass shoot density increased in sponge plots. Also, as predicted, sponge presence influenced the community by increasing species richness and/or

abundance of macroalgae, fish, and macroinvertebrates. Taken together, these results suggest sponges have a strong influence on the structure of seagrass ecosystems, even at low densities. Previous studies have shown that physical structure provided by many foundation species, including seagrass, is often the most important attribute contributing to their positive effect on community structure (Lee and Fong 2001, Lindsey et al. 2006, Altieri et al. 2007). My study suggests that the effect of sponges on community structure goes beyond that simply attributable to the structure they provide.

Seagrass growth is co-limited by nitrogen and phosphorus in shallow waters of The Bahamas (Allgeier et al. 2010). The sponge used in this experiment, *I. felix*, is a known source of bioavailable nitrogen (Southwell et al. 2008) and phosphorus (Chapter 5). The sponges also induced other changes that altered nutrient supply. Namely, fishes became more abundant in sponge plots. Previous research has shown that aggregations of fish increase the supply rate of both nitrogen and phosphorus through their excretion (Allgeier et al. 2013). These combined sources of nutrients (sponge and fishes) likely drove the increase in both seagrass growth and shoot densities. I saw a shift in the seagrass community towards an increase in *S. filliforme* and *H. wrightii*. This result is consistent with increased nutrient supply in similar systems, as both Powell et al. (1989) and Fourqurean et al. (1995) found that the addition of nutrients in the form of bird guano shifted the dominant seagrass species in a Florida Bay seagrass bed from *T. testudinum* to *H. wrightii*. *T. testudinum* is known to outcompete *S. filliforme* and *H. wrightii* in low nutrient conditions (Williams 1990). Therefore, *S. filliforme* and *H. wrightii* moving into sponge plots is consistent with *I. felix* acting as a source of nutrients.

The macroalgal community structure was significantly different in sponge plots, largely driven by algae in the genera *Acetabularia* and *Penicillus*. Interestingly, macroalgae increased in sponge plots after 12 months at approximately the same time fish abundance began to increase (although the increase was not yet significant). Seventeen months post-establishment, macroalgal abundance showed a similar increase in structure control plots, also at the same time that fish abundance began to increase in these plots (again, increase was not yet significant, Figs S1 and S2). Although inferential, these results further suggest that nutrients supplied by fish via excretion may play an important role in mediating macroalgal abundance in seagrass beds (Burkepile et al. 2013).

Previous work has shown that structure alone serves to aggregate fish in seagrass beds (Yeager et al. 2012, Allgeier et al. 2013). My results suggest that massive form sponges such as *I. felix* attract fish above and beyond what is expected by their structure alone (Fig S1). It is reasonable to hypothesize that the attraction may be a result of increased food availability, as the two groups of fish driving the community-level differences observed in sponge plots (juvenile grunts and Beaugregory damselfish) both rely on small invertebrates for at least part of their diet (Cervigón 1966). Sponges shifted the invertebrate community to a more species-rich (and temporarily more abundant) community. Epibenthic macroinvertebrates increased in sponge plots one year into the experiment. However, this increase was no longer significant at 17 months. There are two potential mechanisms for increasing macroinvertebrate abundance. First, the presence of sponges increased the density of the seagrass and macroalgal communities, which is known to increase invertebrate densities (Stoner 1980, Stoner and Lewis 1985, Yeager et al. 2012). Second, sponges are also known

as hosts for large populations of endofauna (Pearse 1950, Ribeiro et al. 2003), and *I. felix* hosts particularly abundant and diverse invertebrate communities (Greene 2008). Additional work is needed to determine if food availability is the reason fish seem to prefer the structure provided by live sponges relative to the sponge models.

Despite the fact that I recorded significant shifts in the macroinvertebrate community, both the richness and abundance measured in this study is certainly an underestimate for all plot types, but particularly for sponge plots. I did not sample sediment infaunal communities nor trap for amphipods and other small invertebrates that would be difficult, if not impossible, to detect in visual surveys. Additionally, I did not account for organisms which make their home inside the canal system of the sponge, as there is no way to sample this community without destroying the host sponge. In a previous study *I. felix* was shown to host >6 species of infaunal invertebrates at densities up to 33 individuals per cm^{-3} of sponge (Greene 2008). Therefore, considering my underestimate of invertebrate richness and abundance in sponge plots, it is likely that sponges have an even larger effect on the invertebrate community than I document herein.

When two foundation species exhibit a nested distribution, as *I. felix* and *T. testudinum* do, theory predicts the formation of a facilitation cascade (Altieri et al. 2007, Angelini et al. 2011). The interaction between *I. felix* and *T. testudinum* cannot be described as a facilitation cascade, as neither the sponge or seagrass is reliant on the other to first colonize the area. However, the interaction between *I. felix* and *H. wrightii* and *S. filliforme* may constitute a facilitation cascade, with the presence of the sponge promoting the establishment of these two species of seagrass. The dominance of *T. testudinum* over *H. wrightii* and *S. filliforme* is

driven by the superior ability of *T. testudinum* to capture nutrients from sediments so that *H. wrightii* and *S. filliforme* are outcompeted in oligotrophic conditions (Williams 1990). However, *I. felix* can act as a source of bioavailable nitrogen and phosphorus, the two limiting nutrients in this system (Chatper 5, Southwell et al. 2008, Allgeier et al. 2010). Therefore, the presence of the sponge may alleviate nutrient limitation and allow for the establishment of the additional seagrass species.

For the establishment of *H. wrightii* and *S. filliforme* to constitute a facilitation cascade, the presence of these species must in turn promote the existence or increased abundance of additional community members. There is some evidence that this may be occurring; however, additional data are necessary to definitively confirm this. The presence of the sponge and the establishment of the two additional species of seagrass increases spatial heterogeneity in the system. Increasing heterogeneity should increase the available niche space in the system and lead to increased diversity (Chesson 2000). Invertebrate communities are likely the most sensitive to this spatial heterogeneity generated by sponges; however, this difference is likely manifested by infaunal invertebrates living within the sponge and epifaunal invertebrates living on the surface of seagrass and macroalgae. I did not measure either of these communities and therefore cannot confirm whether sponge presence increased their abundance of diversity.

My results show that sponges can positively influence the diversity and abundance of macroalgae, fish, and invertebrate communities within seagrass beds. This suggests that sponges act as a second foundation species within *T. testudinum* dominated seagrass beds. Additionally, I show evidence that sponges initiate may initiate a facilitation cascade,

promoting the establishment of two additional seagrass species, *H. wrightii* and *S. filliforme*. Future studies should focus on both the direct and indirect response of these invertebrate communities to the presence of sponges and their abundance is often tied to the health of seagrass beds (Orth and Van Montfrans 1984).

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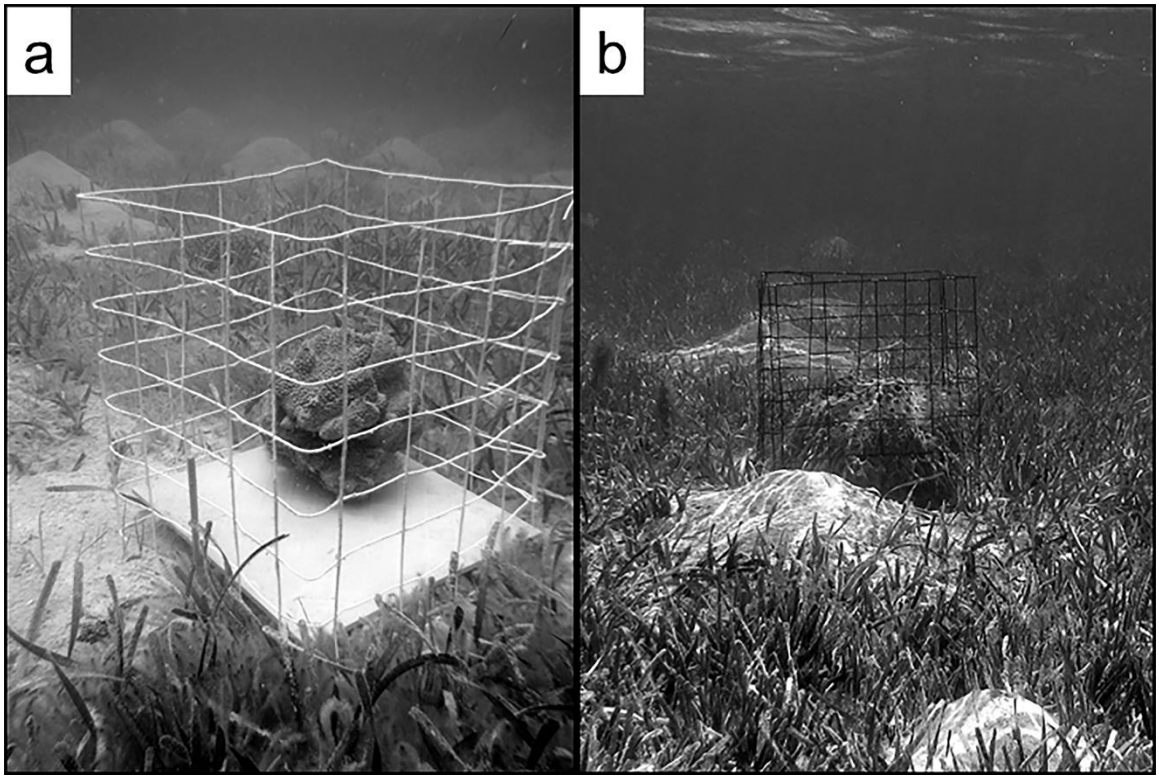

Figure 2.1 An example of a transplanted *Ircinia felix* (a) and the polypropylene model of a sponge used in the structure control treatment (b).

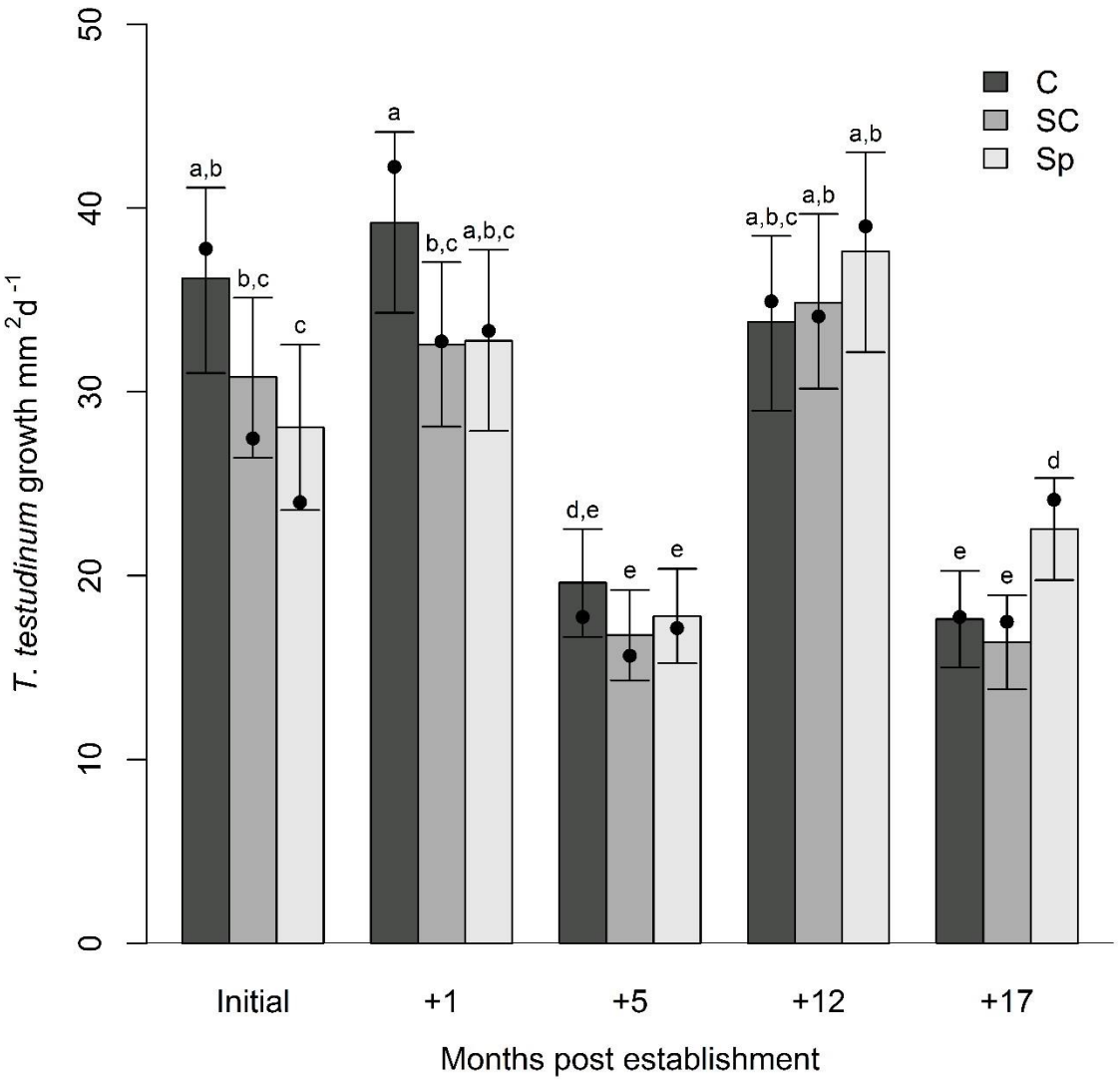

Figure 2.2 Output from Bayesian hierarchical models describing *Thalassia testudinum* growth ($mm^2\ d^{-1}$). The plotted bars represent the mean Markov chain Monte Carlo estimate of *T. testudinum* growth at each sampling point. The error bars represent the high density interval (HDI). The letters represent significantly different groups. Groups were considered significantly different if the HDI of their contrast did not include zero. The treatments are represented by C- control, SC- structure control, and Sp- sponge. The points plotted represent the observed mean *T. testudinum* growth.

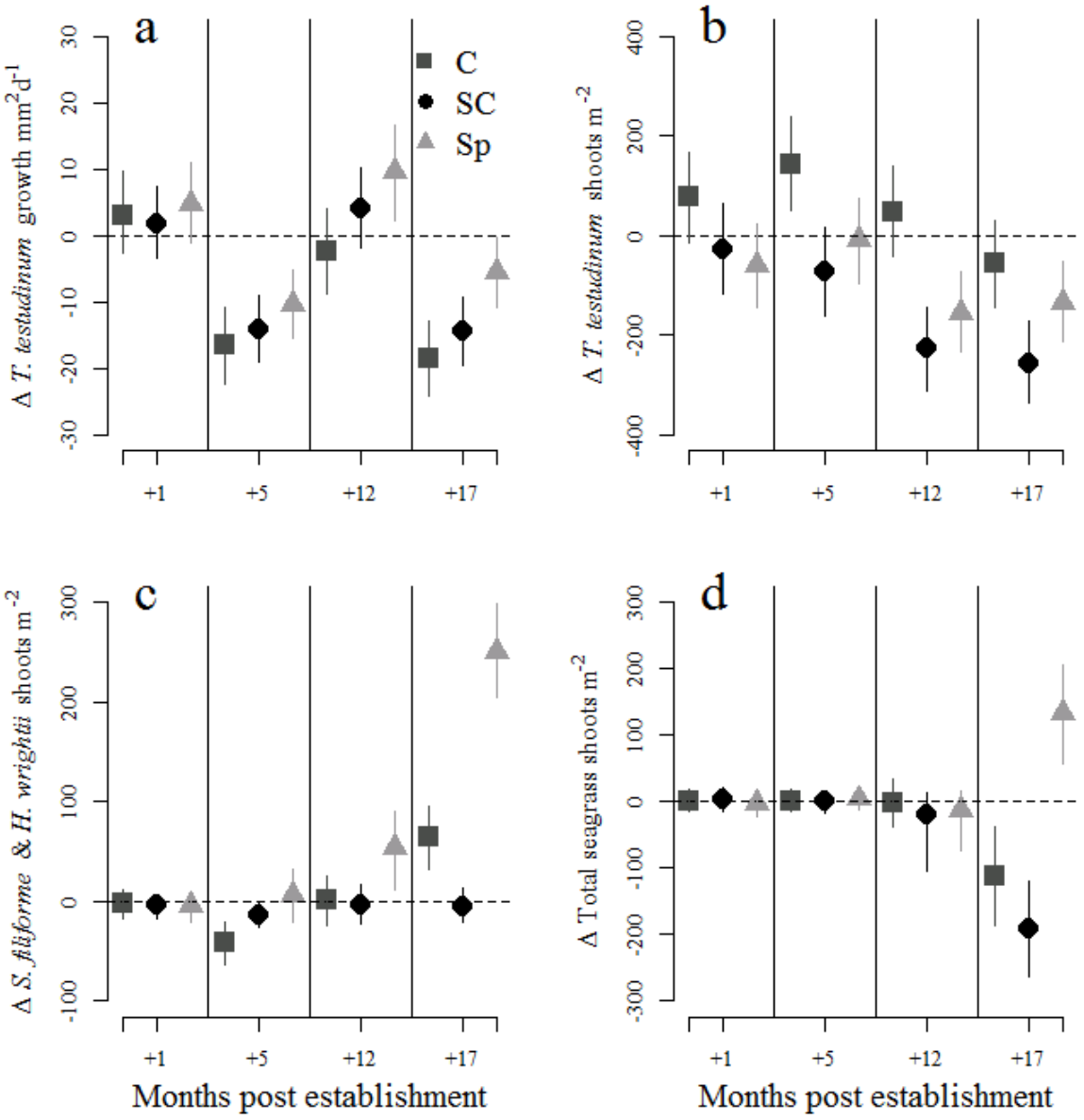

Figure 2.3. Output from Bayesian hierarchical models describing (a) *Thalassia testudinum* growth ($mm^2\ d^{-1}$), (b) *T. testudinum* short shoot density (shoots m^{-2}), (c) combined density of *H. wrightii* and *S. filiforme* short shoots (shoots m^{-2}), and (d) total seagrass short shoot density (shoots m^{-2}). The plotted points represent the mean Markov chain Monte Carlo estimate of the treatment specific change in each variable at the indicated time post experiment establishment. The error bars represent the high density interval (HDI). The change is significant if the HDI does not cross the dotted line. The treatments are represented by C- control, SC- structure control, and Sp- sponge.

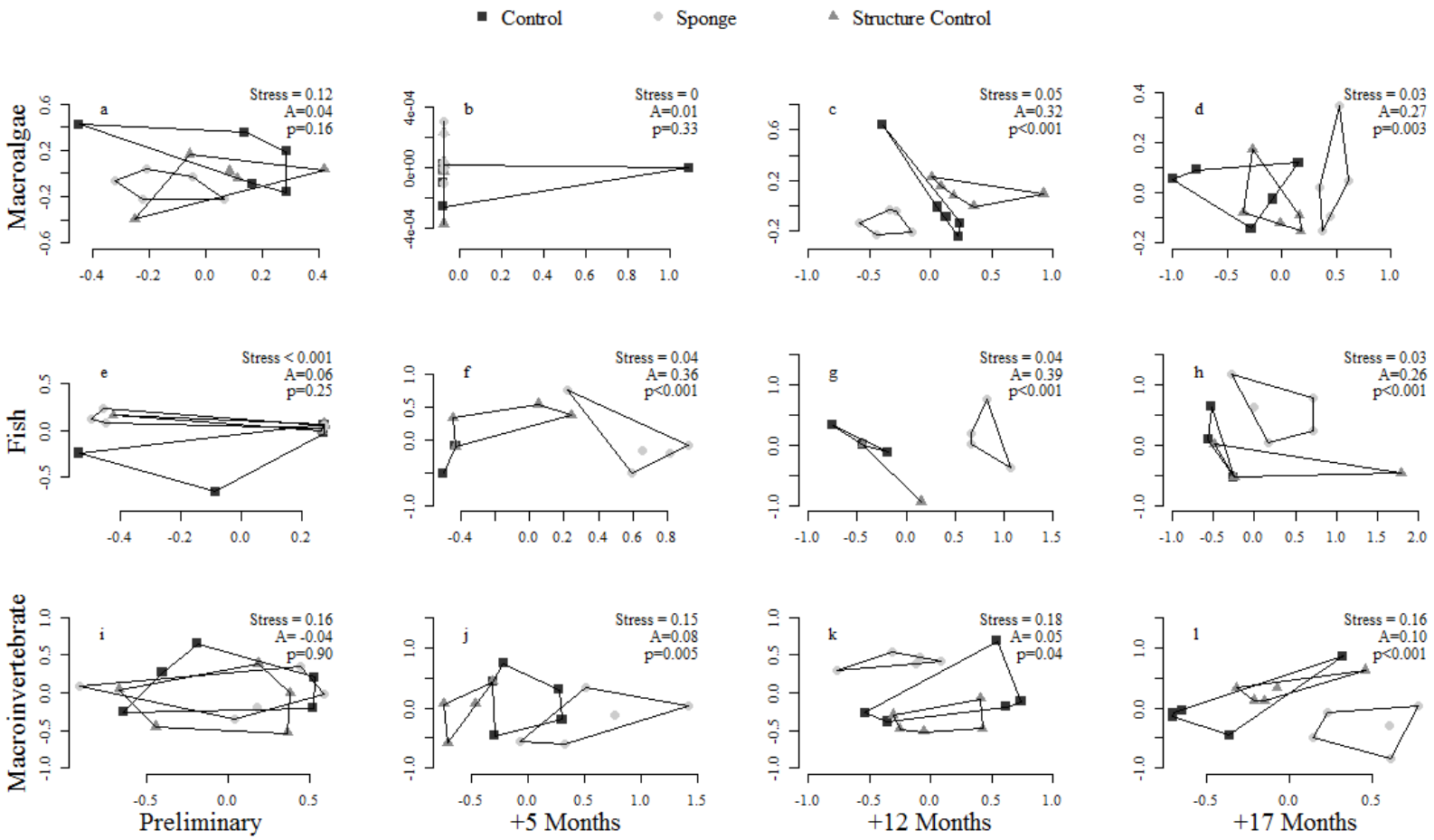

Figure 2.4. Non-metric multidimensional scaling (NMDS) plots visualizing the macroalgae (a-d), fish (e-h), and macroinvertebrate (i-l) community structure in each treatment prior to the experiment (a,e,i) and five (b,f,j), twelve (c,g,k), and seventeen month (d,h,l) post establishment. The stress for each NMDS plot is reported within the plot along with the results of a multi-response permutation procedure testing for differences between the treatments at each time period.

REFERENCES

Adams, A. J., C. P. Dahlgren, G. T. Kellison, M. S. Kendall, C. A. Layman, J. A. Ley, I. Nagelkerken, and J. E. Serafy. 2006. Nursery function of tropical back-reef systems. Marine Ecology Progress Series **318**:287-301.

Allgeier, J. E., A. D. Rosemond, A. S. Mehring, and C. A. Layman. 2010. Synergistic nutrient colimitation across a gradient of ecosystem fragmentation in subtropical mangrove-dominated wetlands. Limnology and Oceanography **55**:2660-2668.

Allgeier, J. E., L. A. Yeager, and C. A. Layman. 2013. Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. Ecology **94**:521-529.

Altieri, A. H., M. D. Bertness, T. C. Coverdale, N. C. Herrmann, and C. Angelini. 2012. A trophic cascade triggers collapse of a salt-marsh ecosystem with intensive recreational fishing. Ecology **93**:1402-1410.

Altieri, A. H., B. R. Silliman, and M. D. Bertness. 2007. Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. The American Naturalist **169**:195-206.

Angelini, C., A. H. Altieri, B. R. Silliman, and M. D. Bertness. 2011. Interactions among foundation species and their consequences for community organization, biodiversity, and conservation. Bioscience **61**:782-789.

Angelini, C., T. van der Heide, J. N. Griffin, J. P. Morton, M. Derksen-Hooijberg, L. P. Lamers, A. J. Smolders, and B. R. Silliman. 2015. Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. Page 20150421 *in* Proc. R. Soc. B. The Royal Society.

Archer, S. K., E. W. Stoner, and C. A. Layman. 2015. A complex interaction between a sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) in a subtropical coastal ecosystem. Journal of Experimental Marine Biology and Ecology **465**:33-40.

Bruno, J. F. and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. Pages 201–218 *in* M. D. Bertness, editor. Marine Community Ecology. Sinauer.

Burkepile, D. E., J. E. Allgeier, A. A. Shantz, C. E. Pritchard, N. P. Lemoine, L. H. Bhatti, and C. A. Layman. 2013. Nutrient supply from fishes facilitates macroalgae and

suppresses corals in a Caribbean coral reef ecosystem. Scientific Reports **3**:DOI: 10.1038/srep01493.

Butler, M. J., J. H. Hunt, W. F. Herrnkind, M. J. Childress, R. Bertelsen, W. Sharp, T. Matthews, J. M. Field, and H. G. Marshall. 1995. Cascading disturbances in Florida bay, USA: Cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. Marine Ecology Progress Series **129**:119-125.

Cervigón, M. F. 1966. Los peces marinos de Venezuela.(Tomo 2). Estación de Investigaciones Marinas de Margarita, Fundacioón La Salle de Ciencias Naturales. Caracas, Venezuela.

Chesson, P. 2000. General theory of competitive coexistence in spatially-varying environments. Theoretical Population Biology **58**:211-237.

Dayton, P. K. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. Pages 81-96 *in* Proceedings of the colloquium on conservation problems in Antarctica. Allen Press Lawrence, Kansas, USA.

Duffy, J. E. 2006. Biodiversity and the functioning of seagrass ecosystems. Marine Ecology Progress Series **311**:233-250.

Folmer, E. O., M. van der Geest, E. Jansen, H. Olff, T. M. Anderson, T. Piersma, and J. A. van Gils. 2012. Seagrass-Sediment feedback: An exploration using a non-recursive structural equation model. Ecosystems **15**:1380-1393.

Fonseca, M. S. and J. A. Cahalan. 1992. A preliminary evaluation of wave attenuation by four species of seagrass. Estuarine, Coastal and Shelf Science **35**:565-576.

Fourqurean, J. W., C. M. Duarte, H. Kennedy, N. Marbà, M. Holmer, M. A. Mateo, E. T. Apostolaki, G. A. Kendrick, D. Krause-Jensen, and K. J. McGlathery. 2012. Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience **5**:505-509.

Fourqurean, J. W., G. V. N. Powell, W. J. Kenworthy, and J. C. Zieman. 1995. The Effects of Long-Term Manipulation of Nutrient Supply on Competition between the Seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. Oikos **72**:349-358.

Greene, A. K. 2008. Invertebrate endofauna associated with sponge and octocoral epifauna at Gray's Reef National Marine Sanctuary off the coast of Georgia. College of Charleston.

Heck, K., G. Hays, and R. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series **253**:123-136.

Hemminga, M., P. Harrison, and F. Van Lent. 1991. The balance of nutrient losses and gains in seagrass meadows. Marine Ecology Progress Series **71**.

Kruschke, J. 2010. Doing Bayesian data analysis: A tutorial introduction with R. Academic Press.

Layman, C. A., D. A. Arrington, R. B. Langerhans, and B. R. Silliman. 2004. Degree of fragmentation affects fish assemblage structure in Andros Island (Bahamas) estuaries. Caribbean Journal of Science **40**:232-244

Lee, S. Y. and C. Fong. 2001. The effects of seagrass (Zostera japonica) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. Journal of Experimental Marine Biology and Ecology **259**:23-50.

Lindsey, E. L., A. H. Altieri, and J. D. Witman. 2006. Influence of biogenic habitat on the recruitment and distribution of a subtidal xanthid crab. Marine Ecology Progress Series **306**:223-231.

Maldonado, M., M. Ribes, and F. C. van Duyl. 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. Pages 113-182 *in* M. A. Becerro, M. J. Uriz, M. Maldonado, and X. Turon, editors. Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology.

Meyer, J. L., E. T. Schultz, and G. S. Helfman. 1983. Fish schools: An asset to corals. Science **220**:1047-1049.

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. L. Williams. 2006. A global crisis for seagrass ecosystems. Bioscience **56**:987-996.

Orth, R. J., K. L. Heck, Jr., and J. v. Montfrans. 1984. Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator: prey relationships. Estuaries **7**:339-350.

Orth, R. J. and J. Van Montfrans. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. Aquatic Botany **18**:43-69.

Pearse, A. S. 1950. Notes on the inhabitants of certain sponges at Bimini. Ecology **31**:149-151.

Peters, J. R., L. A. Yeager, and C. A. Layman. 2015. Comparison of fish assemblages in restored and natural mangrove habitats along an urban shoreline. Bulletin of Marine Science **91**:125-139.

Peterson, B. J., C. M. Chester, F. J. Jochem, and J. W. Fourqurean. 2006. Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Marine Ecology Progress Series **328**:93-103.

Powell, G. V. N., J. W. Kenworthy, and J. W. Fourqurean. 1989. Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. Bulletin of Marine Science **44**:324-340.

Ribeiro, S. M., E. P. Omena, and G. Muricy. 2003. Macrofauna associated to *Mycale microsigmatosa* (Porifera, Demospongiae) in Rio de Janeiro State, SE brazil. Estuarine Coastal and Shelf Science **57**:951-959.

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.

Stoner, A. W. 1980. The role of seagrass biomass in the organization of benthic macrofaunal assemblages. Bulletin of Marine Science **30**:537-551.

Stoner, A. W. and F. G. Lewis. 1985. The influence of quantitative and qualitative aspects of habitat complexity in tropical sea-grass meadows. Journal of Experimental Marine Biology and Ecology **94**:19-40.

Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, Jr., A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America **106**:12377-12381.

Williams, S. L. 1990. Experimental studies of Caribbean seagrass bed development. Ecological Monographs:449-469.

Yeager, L., C. Acevedo, and C. Layman. 2012. Effects of seascape context on condition, abundance, and secondary production of a coral reef fish, *Haemulon plumierii*. Marine Ecology Progress Series **462**:231-240.

Zieman, J. C. 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. Aquaculture **4**:139-143.

CHAPTER 3. A COMPLEX INTERACTION BETWEEN A SPONGE (*HALICHONDRIA MELANADOCIA*) AND A SEAGRASS (*THALASSIA TESTUDINUM*) IN A SUBTROPICAL COASTAL ECOSYSTEM

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Abstract

Foundation species, such as oysters, corals, and seagrasses, form the basis for entire ecosystems and are characterized by positive interactions with community members. However, many species interactions are context dependent, where the outcome or strength of the interaction depends on the biotic or abiotic conditions. Therefore, a mechanistic knowledge of species interactions, especially those involving foundation species, may allow for a more complete understanding of how anthropogenic changes influence nearshore ecosystems. This study describes the interaction between the seagrass *Thalassia testudinum* and the sponge *Halichondria melanadocia*, a species that grows around the base of seagrass shoots. A combination of surveys and experimental manipulations on Abaco Island, The Bahamas, revealed that the interaction between *T. testudinum* and *H. melanadocia* is a commensal relationship with the sponge benefiting from the presence of *T. testudinum* up to medium shoot densities (589-615 shoots m^{-2}). The net neutral effect of *H. melanadocia* on *T. testudinum* is likely a balance of the negative effect of the sponge shading the seagrass with the positive effect of nitrogen and phosphorus supplied by the sponge. The mechanisms

underlying the interaction between *H. melanadocia* and *T. testudinum* suggest that the interaction is likely context dependent. As such, environmental change, namely eutrophication, has the potential to shift the nature of this interaction from commensal to parasitic. A simple simulation showed that if this relationship becomes parasitic, above ground production in seagrass beds could be reduced. This study highlights the importance of a mechanistic understanding of species interactions involving foundation species when predicting human impact on the environment.

Introduction

Foundation species (sensu Dayton, 1972 as refined by Bruno and Bertness, 2001) define entire communities or ecosystems by creating habitat and altering abiotic conditions. As a result of their net positive influence on the organisms which live in and around them, foundation species are typically associated with increased abundance, diversity and distributions of community members (Bracken, et al., 2007; Stachowicz, 2001). In addition to their effect on community structure, foundation species are key mediators of ecosystem function (Duffy, 2006; Ellison, et al., 2005; McLeod, et al., 2011). Seagrasses are a globally distributed group of foundation species (Costanza, et al., 1997; Duffy, 2006; Larkum, et al., 2006) which influence processes such as nutrient cycling (Hemminga, et al., 1991; Marba, et al., 2006; Yarbro and Carlson, 2008), sediment stabilization (Folmer, et al., 2012), and carbon storage (Fourqurean, et al., 2012; McLeod, et al., 2011). Despite their importance, human activities have led to a worldwide decrease in seagrass abundance, potentially affecting their interactions with other species (Orth, et al., 2006; Waycott, et al., 2009).

Context dependent species interactions, which are common in nature, are defined as interactions where the strength or outcome differs based on the conditions (biotic or abiotic) in which they occur (Bronstein, 1994; Chamberlain, et al., 2014). For example, the effect of ulvoid macroalgae on the seagrass *Zostera marina* varies along an estuarine gradient; the effect of macroalgal blooms on the seagrass shifts from neutral at fully marine sites to strongly negative in more riverine portions of the estuary (Hessing-Lewis, et al., 2011). In areas impacted by humans, abiotic conditions are often very different from the un-impacted state, which may shift the outcome of some interactions with potential cascading effects on community structure and ecosystem function (Kiers, et al., 2010). The importance of species interactions in maintaining both species diversity within seagrass beds and seagrasses themselves is well understood (Heck and Valentine, 2006; Heck, et al., 2000; van der Heide, et al., 2012). Therefore, a mechanistic understanding of the interactions in seagrass beds can provide insight into how human activities may alter ecosystem structure and function. To this end, the goal of this study was to provide a mechanistic description of the interaction between a sponge *Halichondria melanadocia* (de Laudenfels, 1936) and a foundation species, the seagrass *Thalassia testudinum* (Banks & Sol. ex König, 1805).

Thalassia testudinum is found in the tropical and sub-tropical western Atlantic and is the dominant seagrass in the shallow waters of the Bahamian archipelago (Wabnitz, et al., 2008; Williams, 1990; Zieman, 1982). Though sponges are a common component of seagrass communities, little is known of their role in this system, despite their recognized importance in reef and hard bottom habitats (Bell, 2008; Wulff, 2006). Sponges are filter feeders which host diverse symbiotic microbial communities. As a result, many sponges are known sources

of bioavailable forms of nutrients (Corredor, et al., 1988; Diaz and Ward, 1997; Maldonado, et al., 2012; Southwell, et al., 2008) and direct mutualism involving nutrient transfer between sponges and primary producers, including mangroves (Ellison, et al., 1996) and rhodophytes (Davy, et al., 2002) have been documented. *Halichondria melanadocia*, typically considered a mangrove sponge (Diaz and Rützler, 2009), is frequently observed in Bahamian seagrass beds (Archer, this study). Unlike many sponges which grow on hard substrates within the seagrass bed, *H. melanadocia* grows surrounding one or more shoots of *T. testudinum* (Fig. 3.1).

Three potentially co-occurring mechanistic pathways through which *H. melanadocia* and *T. testudinum* may interact were hypothesized. 1) Several species of sponge are sources of bioavailable forms of both nitrogen (N) and phosphorus (P), which are limiting nutrients for seagrass growth in coastal waters of The Bahamas (Allgeier, et al., 2013; Maldonado, et al., 2012 and references therein). Although nutrient fluxes through *H. melanadocia* have not been published, it is likely that the sponge is a source of N, P or both. Therefore, it was hypothesized that the sponge may help alleviate nutrient limitation in *T. testudinum* shoots they grow around. 2) The growth of *H. melanadocia* around blades of *T. testudinum* covers a large percentage of photosynthetic tissue of the shoot (12-59%, $\bar{x}$ = 37.2, sd = 10.8, Fig. 3.1). Consequently, it was hypothesized that by shading shoots, *H. melanadocia* may lead to light limitation in *T. testudinum*. 3) Sponges, as sessile invertebrates, generally require structure for successful settlement and growth. Therefore, it was hypothesized that *T. testudinum* benefit *H. melanadocia* by providing structured habitat. These three hypothesized interaction

pathways allowed us to isolate 11 response variables (Table 3.1) that can be used to describe the nature of the interaction between *T. testudinum* and *H. melanadocia*.

Materials and Methods

Surveys

Surveys were conducted at six sites on Abaco Island, The Bahamas, in May and June 2012 (Fig. 3.2). At each site, ten, $1m^2$ plots were haphazardly selected. Plots ranged between 0.35-1.50 m ($\bar{x}$ = 0.77 ± 0.31 sd) low tide depth. Within each plot, percent cover and shoot density of *T. testudinum* were estimated and *H. melanadocia* were enumerated. *Thalassia testudinum* shoot density was determined by counting the number of shoots within four 15 cm x 15 cm quadrats haphazardly placed within the larger sampling plot. The four counts were averaged to get an estimate of shoot density for the entire plot. If more than three *H. melanadocia* were present within the plot, the *T. testudinum* shoots sponges were growing around were collected for morphometric (blade length and width, cm) and nutrient analysis. *Thalassia testudinum* shoots without *H. melanadocia* epibionts were also collected for morphometric and nutrient analysis.

Thalassia testudinum shoots collected for morphometric and nutrient analysis were immediately frozen, then transported to Florida International University for analysis. For morphometric analysis, the number of blades per shoot and blade width and length of thawed *T. testudinum* shoots were measured. Measured *T. testudinum* blades were gently scraped to remove epiphytes and then dried at 65^{o} C for 48-72 hours. Dried samples were ground into a

fine powder and stored in a desiccator until analysis. Percent carbon (C) and nitrogen (N) of the ground seagrass tissue were determined in duplicate using a Carlo Erba CHN analyzer (Fisons NA1500). Percent phosphorus (P) was determined by dry oxidation acid hydrolysis extraction followed by colorometric analysis (Fourqurean, et al., 1992).

Thalassia testudinum *growth*

Thalassia testudinum growth was evaluated at two sites, Snake Cay and Jungle Creek (Fig. 3.2), between June 22-July 6, 2013 and July 8-22, 2013 respectively using the standard blade hole punching technique (Zieman, 1974). Shoots were marked in groups of three with each group consisting of an unshaded shoot, one with *H. melanadocia* growing around the shoot, and one with a dried, dead sponge cut to cover the same percentage of the shoot as the shoot with *H. melanadocia*. Grouped shoots were located within approximately a 0.10 x 0.10 m area. For *T. testudinum* shoots with *H. melanadocia* growing around the shoot, if the sponge covered the marked area on the seagrass, the sponge tissue was gently pushed up the seagrass shoot to expose the base of the shoot. After the mark was made, the sponge tissue was gently returned to its original location. The dead sponges were dried and cleaned prior to use resulting in only structural components of the sponge remaining using techniques similar to those traditionally used to prepare sponges for commercial sale. Briefly, dead, dry sponges were collected from the beach and then held in mesh bags underwater for 3-7 days prior to placement on the seagrass. The goal of this treatment was to prevent decomposition of the sponge from creating anoxic conditions for the seagrass during the experiment. There were no visible signs of decomposition, although we did not explicitly test for decomposition.

Growth was determined for 10 and 15 groups of *T. testudinum* shoots at Snake Cay (n=30 shoots) and Jungle Creek (n=45 shoots) respectively (Fig. 3.2). Fourteen days after marking, *T. testudinum* shoots were collected and the total area of new growth was recorded. The carbon content of the new growth was determined using the same procedure described above.

Halichondria melanadocia *growth and recruitment*

Ten, 0.5 x 0.5 m, artificial seagrass units (ASUs) were constructed in each of three densities, 372, 618, and 988 shoots per m^2 representing the low, medium, and high *T. testudinum* shoot densities observed during the surveys (Fig. 3.3). ASUs were constructed using pre-soaked 3.75 mesh rug canvas (MCG Textiles®) and green polypropylene ribbon (Splendorette®) cut into 140 x 5 mm strips. Each shoot on the artificial units consisted of two ribbon strips folded in half and attached to the canvas. The length, width, and leaf number of the shoots on the ASUs corresponds to the average length, width, and leaf number observed in these surveys. *Halichondria melanadocia* were initially gathered from the Jungle Creek site on May 8, 2014 and transported to the Sandy Point site (Fig. 3.2). Although surveys were not conducted at Sandy Point, both *T. testudinum* and *H. melanadocia* were present at the site. However, the abundance of *H. melanadocia* was not sufficient to allow for the use of sponges collected at the site. The *H. melanadocia* were never held out of the water for more than five seconds. The volume, to the nearest ml, of each *H. melanadocia* was recorded, then the sponge was placed in the center of an ASU and held loosely in place with plastic zip ties. Subsequently, metal sod staples were used to attach each ASU to the benthos. The ASUs were randomly arranged in five rows, with six units per row. Twenty-one of the initially

transplanted *H. melanadocia* did not survive the transplantation. New *H. melanadocia* were gathered from the Jungle Creek site and transplanted onto the ASUs on May 14, 2014. The experiment was monitored every other day for two weeks to ensure the *H. melanadocia* survived the second round of transplantation. After the first two weeks the experiment was monitored weekly, but no additional alterations or additions were made. On July 23, 2014, *H. melanadocia* were removed from the center of each ASU and the volume of each sponge measured to the nearest ml. Any *H. melanadocia* recruits were recorded.

Halichondria melanadocia *nutrient flux*

Four *H. melanadocia* (13.8 ml ± 3.6, mean ± SD) were collected from the Jungle Creek site on July 20, 2013. The sponges were cleaned of all external algae and sediment, and their volume measured to the nearest milliliter. Five High Density Polyethylene containers were filled with 2 liters of unfiltered seawater and initial water samples were collected prior to placing a sponge into four of the containers, the fifth container served as a control. The containers were placed in a seawater bath to maintain ambient temperatures (27.2° C ± .7, mean ± SD). Throughout the incubation, dissolved oxygen concentrations were monitored and the containers were periodically stirred. Water samples were collected every 4 hours for 24 hours for the determination of ammonium, nitrate/nitrite (NO_x), and soluble reactive phosphorus (SRP). After each sampling event, the volume of water in the containers was brought back up to 2 L with unfiltered seawater.

All water samples were immediately filtered through 0.45µm Whatman nylon-membrane filter. Samples were analyzed for ammonium immediately using the fluorometric

method described by Holmes, et al. (1999) as modified by Taylor, et al. (2007). NO_x and SRP samples were frozen at -20° C in 20 mL acid washed (10% HCl) scintillation vials and transported to North Carolina State University (NCSU) for analysis. NO_x samples were analyzed by the NCSU Center for Applied Aquatic Ecology Water Quality Laboratory in Raleigh, NC. SRP samples were analyzed using a standard colormetric technique (Parsons, et al., 1984).

Statistical approach

Because abundance datasets are often zero-laden, a negative binomial regression was used to investigate the relationship between sponge abundance and several predictor variables: seagrass shoot density, survey site, and plot depth. Models including all combinations of predictor variables were tested and the best model was selected using model weights calculated using the corrected Akaike's Information Criteria (AICc). The site variable was included to allow for potential drivers of sponge abundance that were not measured in this study. An additional model, a linearized Ricker function, which allows a non-linear relationship between sponge abundance and seagrass shoot densities, was included in the model selection after visual inspection of the data (R Core Team, 2013).

To minimize differences due to sampling location, *T. testudinum* morphometric and nutrient content variables (Table 3.1) were each analyzed separately by comparing shoots with and without a sponge collected from each plot in a paired t-test. The growth of *T. testudinum*, in mg C d^{-1}, was compared between treatments using an ANOVA with treatment and group as fixed factors. When significant, differences among levels of the fixed factors

were compared post hoc using Tukey's honest significant difference (HSD). For analysis, *H. melanadocia* growth was recorded as the relative change in sponge volume per day: [(final volume-initial volume)(initial volume)$^{-1}$(number of days on the ASU)$^{-1}$]. Both *H. melanadocia* growth and the number of recruits per ASU were compared between treatments using separate ANOVAs and any significant differences between treatments were compared post hoc using Tukey's HSD.

When calculating fluxes values below detection limit were replaced with said limit (ammonium: 0.2 µg L^{-1}, SRP: 0.03 µg L^{-1}, and NO_x: 50 µg L^{-1}). As this only affected initial (i.e. those prior to introduction of the sponge) and control phosphorus values, this served to make our estimates for phosphorus more conservative. Fluxes for each solute of interest were determined by least squares regression of the concentration of the solute against time for each sponge. The coefficient of the time variable from the regression output was then normalized by sponge volume and the volume of the incubation chamber, and divided by 4 to determine hourly flux estimates (water samples were taken at four hour intervals). Fluxes reported are in µg $L^{-1}{}_{sponge}\, h^{-1}$. The mean and standard deviations reported are from the four replicate sponges incubated. For all solutes the time coefficient for the control was not significantly different than zero (see Results below), therefore we concluded *H. melanadocia* were a significant source (or sink) of each solute if the flux in sponge incubations was significantly different than zero when compared using a t-test.

Results

Surveys

The best fit model shows that *H. melanadocia* abundance is correlated with *T. testudinum* shoot density in a non-linear fashion (Table 3.2) with the highest sponge abundances predicted at *T. testudinum* densities between 589-615 shoots m^{-2} (Fig. 3.3). This *T. testudinum* density is near the mean shoot density observed in the surveys ($\bar{x}$ ± sd, 676.62 ± 201.83). Although the linearized Ricker model was clearly the best fit model (model weight = 0.86, Table 3.2), all models including *T. testudinum* shoot density as a predictor variable (other than the all-inclusive model) performed better than those including only depth, site or a combination of depth and site. There were no differences between the paired samples of seagrass with a sponge and those without for any of the *T. testudinum* morphometric or nutrient content variables (Table 3.3).

Thalassia testudinum *growth*

Thalassia testudinum growth did not differ between sites, but did differ among treatments ($F_{2,69} = 9.84$, $p < 0.001$, Fig. 3.5). The growth of unshaded seagrass shoots did not differ from shoots shaded by live *H. melanadocia* (Tukey adjusted p = 0.06), but the growth of unshaded seagrass shoots was significantly higher than shoots shaded by a dead sponge (Tukey adjusted $p < 0.0001$). There was not a significant difference in the growth of *T. testudinum* shoots shaded with live or dead sponge (Tukey adjusted p = 0.08, Fig. 3.5).

Halichondria melanadocia growth and recruitment

All but three *H. melanadocia* transplanted into the ASUs lost volume over the course of the experiment; the three which grew were on medium density ASUs. Analysis of variance (ANOVA) results show that sponges on medium density ASUs lost less volume than those on low (Tukey adjusted p = 0.008) or high (Tukey adjusted p = 0.02) density ASUs (Fig. 3.4a). There was no difference in the sponge volume lost between low and high density ASUs (Tukey adjusted p = 0.91).

Although the transplanted *H. melanadocia* did not thrive on the ASUs, recruit sponges did settle onto the seagrass mats. After the removal of one outlier (1.5x the inter-quartile range for the treatment), ANOVA results show that the number of recruits per ASU was significantly different among the treatments (with outlier removed: $F_{2,26}$= 4.12, p = 0.03, Fig. 3.4b, including outlier: $F_{2,27}$= 2.72, p = 0.08). The number of recruits was highest on medium density ASUs ($\bar{x}$ ± sd, 2.5 ± 2.4) although the difference between medium and high density ASUs was not significant (Tukey adjusted p = 0.12) or between high and low density ASUs (Tukey adjusted p = 0.81). There was, however, a significant difference in the number of sponge recruits between the medium and low density ASUs (Tukey adjusted p = 0.03, Fig. 3.4b).

Halichondria melanadocia *nutrient flux*

The time coefficient is not significant for the control for any solute (ammonium: t_5 = -0.18, p = 0.87, SRP: t_5 = -0.84, p = 0.44, NO_x: t_5 = 0.12, p = 0.91). Comparing flux values to zero revealed that *Halichondria melanadocia* are a significant source of both ammonium and

SRP ($t_3 = 5.76$, $p = 0.01$, $\bar{x}$ ± sd, 325.2 ± 113.0 µg L^{-1}_{sponge} h^{-1} and $t_3 = 3.64$, $p = 0.04$, 21.0 ± 11.5 µg L^{-1}_{sponge} h^{-1} respectively). However, *H. melanadocia* are not a significant source or sink of NO_x ($t_3 = -0.82$, $p = 0.47$, $\bar{x}$ ± sd, -4.9 ± 11.8 µg L^{-1}_{sponge} h^{-1}).

Discussion

This study shows that the sponge, *H. melanadocia* benefits from a commensal relationship with *T. testudinum*. However, the interaction appears to be complex and potentially context dependent. *Halichondria melanadocia* abundance is nonlinearly related to *T. testudinum* abundance, with the highest observed sponge abundances occurring in medium seagrass shoot densities. This nonlinearity suggests that multiple mechanisms control sponge abundance in seagrass beds. Complex dynamics also underlie the net neutral effect for seagrass. The data suggest the neutral effect results from a balance of a negative effect of sponge shading, with a positive effect of sponge nutrient fluxes which help alleviate nutrient limitation for the seagrass.

The structural complexity created by *T. testudinum* alters the environment in multiple ways. This environmental alteration is likely sufficient to explain the nonlinear relationship between *H. melanadocia* and *T. testudinum* shoot density. The increase in sponge abundance up to medium densities of seagrass can be explained by two potential mechanisms. First, structure available for *H. melanadocia* settlement and growth increases with increasing *T. testudinum* shoot density. Second, *H. melanadocia* may benefit from higher food availability in denser stands of *T. testudinum.* While food availability was not explicitly measured in this study, previous studies provide support for this hypothesis. Judge, et al. (1993) measured *in*

situ food availability for a common benthic suspension feeder, *Mercenaria mercenaria*, at varying heights above the bottom in both vegetated and unvegetated habitats. They found that food is significantly more available in seagrass beds, specifically at the measurement station closest to the bottom. In their study, the majority of food available was pennate diatoms, which sponges are known to consume (Reiswig, 1971a; Ribes, et al., 1999). Additionally, seagrasses are known to leach DOC from their leaves (Ziegler and Benner, 1999), providing another food source for sponges (Maldonado, et al., 2012 and references therein).

If the two mechanisms discussed in the previous paragraph were operating alone or in concert, the result would be a linear relationship between sponge abundance and seagrass density. However, the non-linearity of the relationship suggests that another mechanism is driving sponge abundance at high *T. testudinum* shoot densities. There is strong evidence that the structure associated with seagrass beds increases rates of sediment deposition (Gacia, et al., 1999; Gacia, et al., 2003). While the specific response of *H. melanadocia* to sedimentation is unknown, it is well established that sedimentation can reduce sponge pumping rates (Gerrodette and Flechsig, 1979). Future research is needed to specifically evaluate these potential explanatory mechanisms driving the observed nonlinear relationship between *H. melanadocia* abundance and *T. testudinum* density.

There are many factors not associated with structural complexity which are known to drive sponge abundances at large special scales. For example, both sponge species distributions and seagrass density are known to vary with depth (de Voogd and Cleary, 2007; Duarte, et al., 2006). However, the depth range covered in this study's surveys was minimal

(0.35-1.5 m). Additionally, depth was included as a potential explanatory variable in the models tested, and the highest ranked model including depth had a model weight of only 0.06 (Table 3.2), indicating that depth was not a strong predictor of sponge abundance. Patterns in sponge abundance can also be driven by predator abundance (Pawlik, et al., 2013; Wulff, 2000), prevailing currents and wave patterns (Reiswig, 1971b). While these variables were not specifically measured, they would largely vary at the site level. However, the highest ranking model tested including site as a predictor variable had a model weight of only 0.03.

The results of the ASU experiment support the assertion that seagrass density is a main driver of *H. melanadocia* abundance in this system. Transplanted *H. melanadocia* lost significantly less volume on medium density ASUs than on either the low or high density treatments. In fact, the only *H. melanadocia* to increase in volume over the course of this experiment were located on medium density seagrass units. Despite the declining volumes of *H. melanadocia* transplanted onto the ASUs, recruit *H. melanadocia* did settle on the experimental units. If availability of structure was the only mechanism driving settlement and survival of *H. melanadocia,* the highest recruit abundances would be expected on high density ASUs. However, more *H. melanadocia* recruited to medium density ASUs. This result is consistent with the hypothesis that the structure provided by *T. testudinum* shoots benefits *H. melanadocia,* while increased sedimentation resulting from structural complexity of seagrass shoots negatively impacts sponge survival. Again, while the specific response of *H. melanadocia* to sedimentation has not been reported, it is known that sedimentation can negatively affect the survival of recruit sponges (Maldonado, et al., 2008). While it is possible that what was classified as recruit *H. melanadocia* simply represent resettled

fragments of the transplanted sponges, on average more *H. melanadocia* recruited to medium density ASUs. As a result, sponges would have lost less volume and would, therefore, have necessarily either fragmented less or grown significantly since fragmentation.

Seagrasses, in general, have high light requirements and thus are susceptible to light limitation. Epiphyte and epibiont growth on seagrass blades has previously been linked to light limitation (Burkholder, et al., 2007). For example, Wong and Vercaemer (2012) found that the presence of an epibiotic sponge, *H. panacea*, led to light limitation in the seagrass *Zostera marina*. The morphological measurements and nutrient ratios used as response variables in this study (Table 3.1) are amongst the strongest indicators of light limitation in seagrasses (McMahon, et al., 2013). However, the results of the surveys comparing *T. testudinum* shoots with and without *H. melanadocia* showed no evidence of light limitation (Table 3.3). *T. testudinum* shoots with and without a live sponge grew at similar rates, but when the same percentage of a shoot was shaded with a dead sponge, there was a significant decrease in seagrass growth compared to non-shaded shoots. Consequently, although it appears as though *H. melanadocia* shade enough of the *T. testudinum* shoot to cause a decrease in growth, something is offsetting this effect.

This study shows that *H. melanadocia* are a significant source of bioavailable nitrogen (NH_4^+) and phosphorus (SRP). Several studies have documented nutrient transfer between sponges and primary producers growing in close proximity to each other (Davy, et al., 2002; Easson, et al., 2014; Ellison, et al., 1996). Because nitrogen and phosphorus co-limit seagrass growth in the study system, it is likely that the nutrients released by the sponge are taken up by the seagrass. When limiting resources are heterogeneously distributed many

clonal plant species translocate resources throughout the clone (Hutchings, 1999; Hutchings and Wijesinghe, 1997; Stuefer, 1998; Stuefer, et al., 1994). Seagrass are clonal plants and there is evidence that *T. testudinum* maintain shaded shoots by translocation of resources when shading is restricted to a small number of shoots within the clone (Tomasko and Dawes, 1989). Therefore, it is possible that seagrass shoots with the sponge growing around them transport excess N and P from the sponge to other shoots, while receiving photosynthate from nearby unshaded shoots. If this is occurring, there would be no measurable signature in the growth or nutrient content from either the nutrient supply or the light limitation, as was observed.

Taken together, the data presented suggest that the interaction between *H. melanadocia* and *T. testudinum* is likely a context dependent interaction. Specifically, for *H. melanadocia*, the interaction is likely a balance between the positive effects of increased habitat and food availability with a potential negative effect of increased sedimentation at high seagrass densities. For *T. testudinum*, the interaction ostensibly is the result of a balance between the negative effects of shading by *H. melanodocia* and the positive effect of nutrient supply. In other context dependent interactions where the benefit to at least one of the participants is dependent on nutrient transfer, the relationship will often shift from a positive interaction (e.g. mutualism, commensalism) towards parasitism with increasing ambient nutrient availability. For example, as soil fertility increases, the relationship between plants and their associated mycorrhizae will shift from mutualism to parasitism (Johnson, et al., 1997; Neuhauser and Fargione, 2004). If the effect of *H. melanadocia* on *T. testudinum* is a balance between the positive effect of nutrient supply and the negative effect of shading,

eutrophication may shift the relationship towards parasitism. It should be noted that seagrass shoots with a live sponge did grow less, although not significantly so, than unshaded shoots. This may be evidence that the interaction, even under the oligotrophic conditions under which we studied it, is bordering on parasitism. Eutrophication is characterized by increased light attenuation and ambient nutrient availability. Increased light attenuation would likely increase the consequences of the shading by *H. melanadocia,* while increased ambient nutrient availability would decrease the benefit of the nutrients supplied by the sponge. Such a shift in the cost-benefit ratio would drive the relationship over the line from commensalism to parasitism.

A simple simulation of the effect of such a shift from commensalism to parasitism suggests that this could impact the rate of above ground productivity by decreasing production by just over 1% per square meter per day. This estimate ignores below ground carbon storage by *T. testudinum,* and does not take into account the direct effect of increased light attenuation and ambient nutrient availability on seagrass productivity. Despite these caveats, the simulation suggests that a parasitic relationship between *H. melanadocia* and *T. testudinum* could contribute to reduced carbon fixation in seagrass beds beyond that predicted by only considering the direct effects of eutrophication on *T. testudinum* productivity. This simplistic simulation underscores the importance of further characterizing this, and other, species interactions involving foundation species.

Foundation species are critically important for the maintenance of biological diversity because of their positive interactions with community members and tendency to alleviate harsh abiotic conditions. As the scale and magnitude of anthropogenic impacts increase, data

allowing us to predict the response of ecosystems to abiotic alteration is paramount. If an interaction is context dependent, human alteration of the environment may result in a shift in the net outcome of previously described interactions (Chamberlain, et al., 2014; Kiers, et al., 2010). Therefore, mechanistic understandings of species interactions, rather than description of the mean net effects, will lead to a more complete description of the interaction, and its outcomes, in a changing world. Such a description, especially for interactions involving foundation species, may prove valuable for restoration and conservation efforts.

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Table 3.1. Response variables and their expected outcome for each predicted interaction mechanism if the mechanism is acting alone. X Indicates the variable is not predicted to vary directionally in response to the mechanism in question.

	Seagrass benefits from sponge derived nutrients	Sponge leads to light limitation in Seagrass	Seagrass provides structure for the sponge
Seagrass nutrient content			
%C	X	-	X
%N	+	X	X
%P	+	X	X
C:N	-	-	X
C:P	-	-	X
Seagrass morphometrics			
Blades per shoot	+	-	X
Blade length	+	-	X
Blade area	+	-	X
Abundance and growth			
Sponge abundance	X	X	+
Seagrass growth	+	-	X
Sponge growth	X	X	+

Table 3.2. AICc and model weights for all potential models predicting *H. melanadocia* abundance. SD represents *T. testudinum* shoot density, Depth is the depth of the sampling plot in m, Site represents the survey site.

Model	AICc	Model Weight
SD + ln(SD)	355.08	0.86
SD	360.24	0.06
Depth + ln(Depth)	360.34	0.06
SD + Site + SD*Site	361.58	0.03
SD+ Depth + SD*Depth	363.16	0.02
Site	363.20	0.01
Depth	363.55	0.01
Site + Depth + Site*Depth	366.49	0.00
SD + Site + Depth + SD* Site + SD* Depth + Site*Depth + SD*Depth*Site	368.54	0.00

Table 3.3. Seagrass nutrient content and morphometric response variables from the surveys conducted in the summer of 2012. Response variables were analyzed for a difference between seagrass shoots both with and without a sponge using a paired t-test with samples collected at the same site in the same plot paired.

Variable	DF	t value	p-value
%C	30	0.78	0.44
%N	30	1.04	0.31
%P	31	-0.07	0.94
C:N	30	0.62	0.63
C:P	29	0.01	0.99
Longest blade	32	-0.19	0.85
Blade area	32	0.71	0.48
Blade number	32	1.96	0.06

Figure 3.1. *Halichondria melanadocia* growing around a *Thalassia testudinum* shoot.

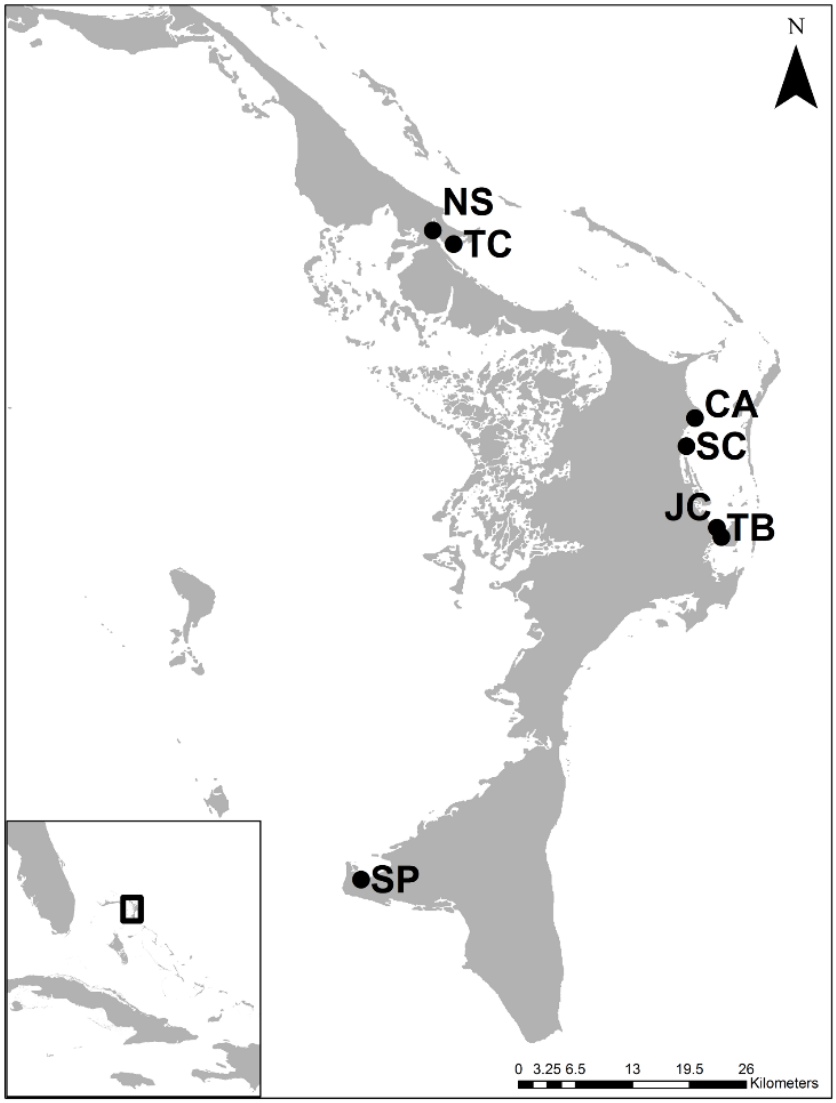

Figure 3.2. Location of sites surrounding Abaco, The Bahamas included in this study. Surveys were conducted at the following sites: NS- Nursery Site, TC- Treasure Cay, CA- Camp Abaco, SC- Snake Cay, JC- Jungle Creek, TB- Turtle Beach. *Thalassia testudinum* growth was determined at SC and JC. Artificial seagrass unit experiments to determine *Halichondria melanadocia* growth were conducted at SP- Sandy point.

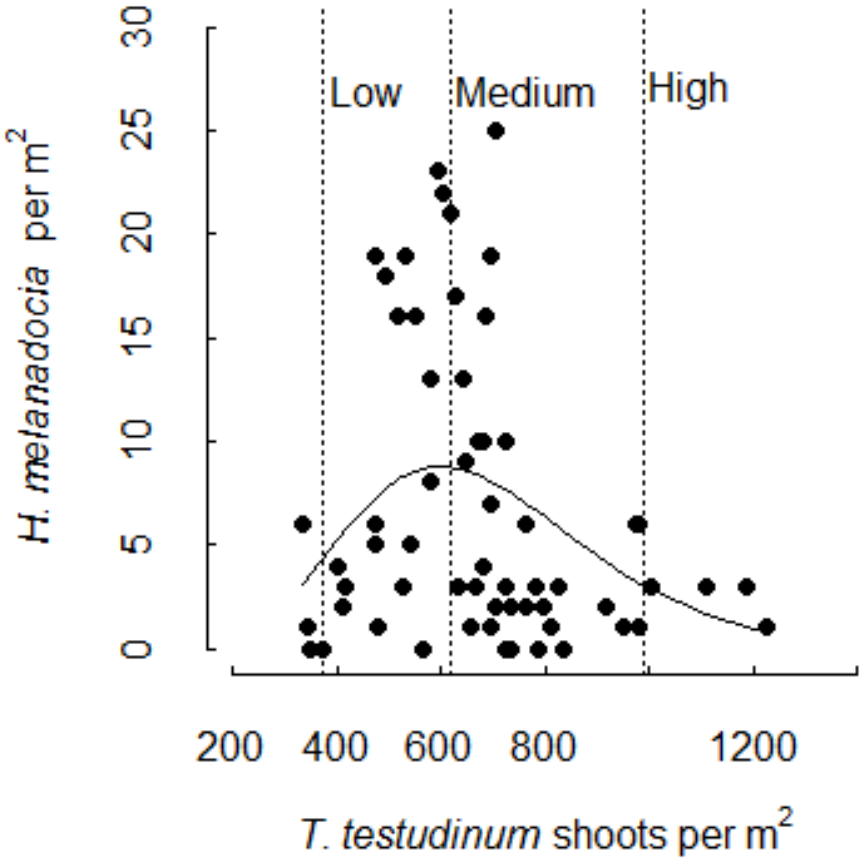

Figure 3.3. The relationship between *Thalassia testudinum* shoot density and *Halichondria melanadocia* abundance. The solid line represents predicted *H. melanadocia* abundance by the best fitting model: *H. melanadocia* per m^2 = (*T. testudinum* shoots per m^2) + ln(*T. testudinum* shoots per m^2). Dashed lines represent the shoot densities of the three artificial seagrass unit treatments.

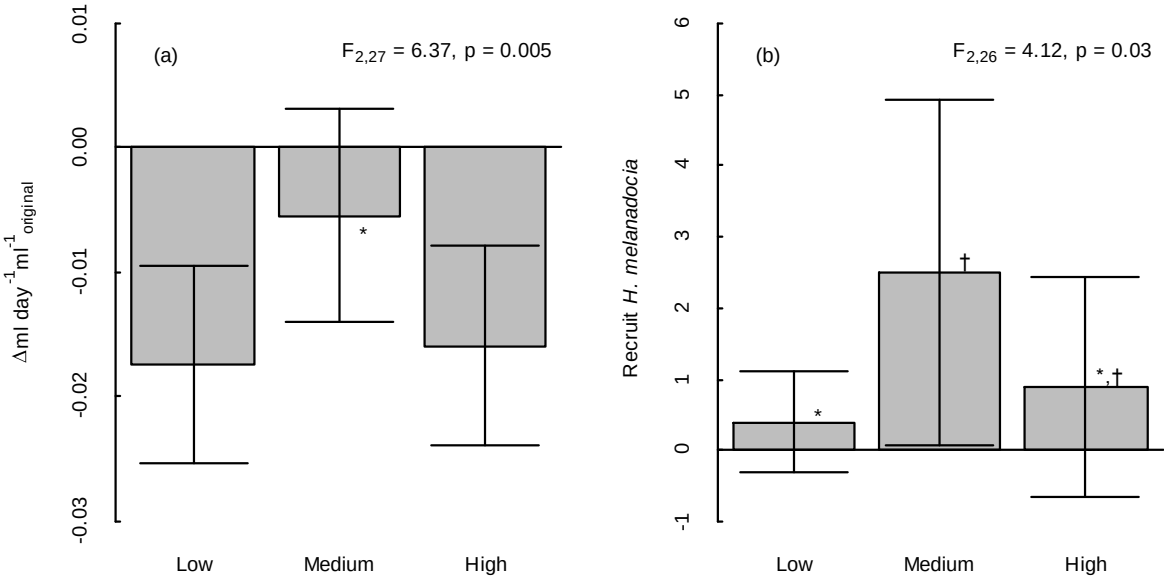

Figure 3.4. Results of the artificial seagrass unit (ASU) experiment. Panel (a) represents the change in *Halichondria melanadocia* volume over the course of the experiment standardized by the original volume of the sponge (ml day^{-1} $ml^{-1}_{original}$) for each of the three ASU shoot densities. Panel (b) represents the number of *H. melanadocia* which recruited to the ASUs in each treatment over the course of the experiment. In both panels * and † represent significantly different groups at the $\alpha = 0.05$ level after the Tukey Honest Significant Difference correction for multiple comparisons.

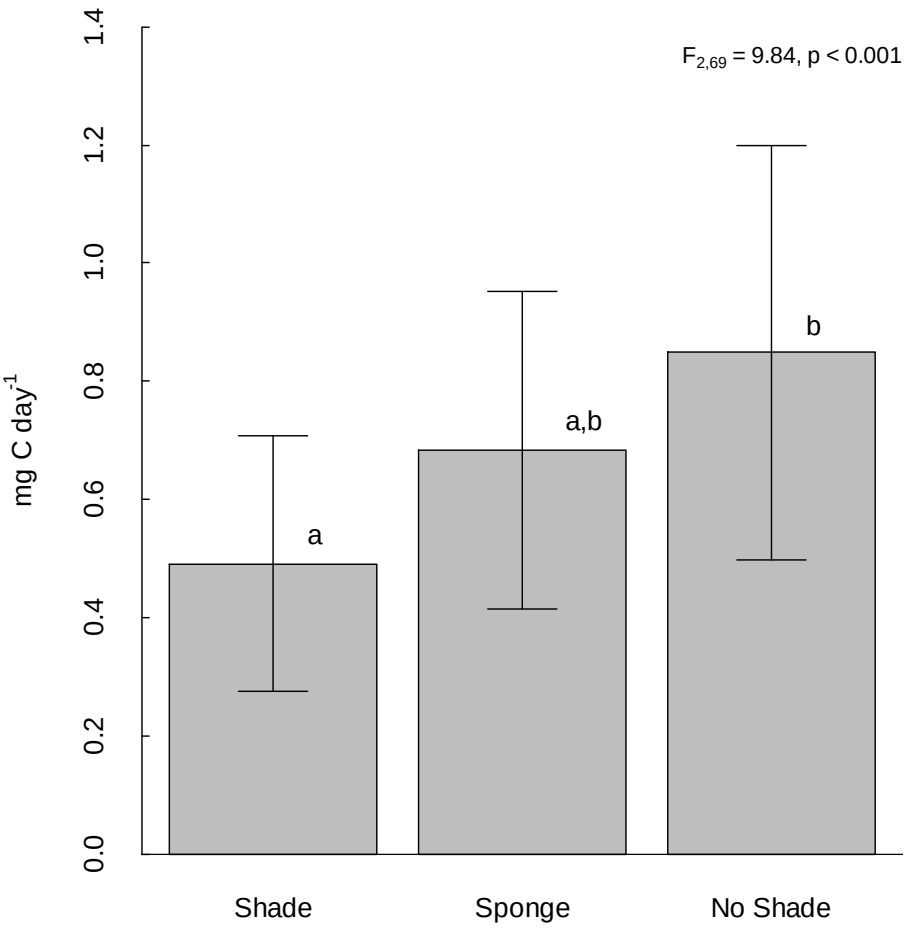

Figure 3.5. *Thalassia testudinum* growth in mg C day^{-1} for shoots shaded with a dead sponge (Shade), a live sponge (Sponge) and with No Shade. Letters represent significantly different groups at the $\alpha = 0.05$ level after the Tukey Honest Significant Difference correction for multiple comparisons.

REFERENCES

Allgeier, J.E., Yeager, L.A., Layman, C.A., 2013. Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. Ecology 94(2), 521-529.

Bell, J.J., 2008. The functional roles of marine sponges. Estuarine Coastal and Shelf Science 79(3), 341-353.

Bracken, M.E., Bracken, B.E., Rogers-Bennett, L.D., 2007. Species diversity and foundation species: Potential indicators of fisheries yields and marine ecosystem functioning.

Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology & Evolution 9(6), 214-217.

Bruno, J.F., Bertness, M.D., 2001. Habitat modification and facilitation in benthic marine communities. In: Bertness, M.D. (Ed.), Marine Community Ecology. Sinauer, pp. 201–218.

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350(1), 46-72.

Chamberlain, S.A., Bronstein, J.L., Rudgers, J.A., 2014. How context dependent are species interactions? Ecology Letters 17(7), 881-890.

Corredor, J.E., Wilkinson, C.R., Vicente, V.P., Morell, J.M., Otero, E., 1988. Nitrate release by Caribbean reef sponges. Limnology and Oceanography 33(1), 114-120.

Costanza, R., dArge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vandenBelt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387(6630), 253-260.

Davy, S.K., Trautman, D.A., Borowitzka, M.A., Hinde, R., 2002. Ammonium excretion by a symbiotic sponge supplies the nitrogen requirements of its rhodophyte partner. Journal of Experimental Biology 205(22), 3505-3511.

Dayton, P.K., 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica, Proceedings of the colloquium on conservation problems in Antarctica. Allen Press Lawrence, Kansas, USA, pp. 81-96.

de Voogd, N.J., Cleary, D.F.R., 2007. Relating species traits to environmental variables in Indonesian coral reef sponge assemblages. Marine and Freshwater Research 58(3), 240-249.

Diaz, M.C., Ward, B.B., 1997. Sponge-mediated nitrification in tropical benthic communities. Marine Ecology Progress Series 156, 97-107.

Diaz, M.C., Rützler, K., 2009. Biodiversity and Abundance of Sponges in Caribbean Mangrove: Indicators of Environmental Quality. Smithsonian Contributions to the Marine Sciences 38, 151-172.

Duarte, C.M., Fourqurean, J.W., Krause-Jensen, D., Olesen, B., 2006. Dynamics of seagrass stability and change, Seagrasses: biology ecology and conservation. Springer, pp. 271-294.

Duffy, J.E., 2006. Biodiversity and the functioning of seagrass ecosystems. Marine Ecology Progress Series 311, 233-250.

Easson, C.G., Slattery, M., Baker, D.M., Gochfeld, D.J., 2014. Complex ecological associations: competition and facilitation in a sponge-algal interaction. Marine Ecology Progress Series 507, 153-167.

Ellison, A.M., Farnsworth, E.J., Twilley, R.R., 1996. Facultative mutualism between red mangroves and root-fouling sponges in Belizean mangal. Ecology 77(8), 2431-2444.

Ellison, A.M., Bank, M.S., Barton, D.C., Colburn, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloeppel, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N.L., Sobczak, W.V., Stinson, K.A., Stone, J.K., Swan, C.M., Thompson, J., Von Holle, B., Jackson, R.W., 2005. Loss of Foundation Species: Consequences for the Structure and Dynamics of Forested Ecosystems. Frontiers in Ecology and the Environment 3(9), 479-486.

Folmer, E.O., van der Geest, M., Jansen, E., Olff, H., Anderson, T.M., Piersma, T., van Gils, J.A., 2012. Seagrass-Sediment Feedback: An Exploration Using a Non-recursive Structural Equation Model. Ecosystems 15(8), 1380-1393.

Fourqurean, J.W., Zieman, J.C., Powell, G.V., 1992. Phosphorus limitation of primary production in Florida Bay: Evidence from C: N: P ratios of the dominant seagrass Thalassia testudinum. Limnology and Oceanography 37(1), 162-171.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience 5(7), 505-509.

Gacia, E., Granata, T.C., Duarte, C.M., 1999. An approach to measurement of particle flux and sediment retention within seagrass (Posidonia oceanica) meadows. Aquatic Botany 65(1–4), 255-268.

Gacia, E., Duarte, C.M., Marbà, N., Terrados, J., Kennedy, H., Fortes, M.D., Tri, N.H., 2003. Sediment deposition and production in SE-Asia seagrass meadows. Estuarine, Coastal and Shelf Science 56(5–6), 909-919.

Gerrodette, T., Flechsig, A.O., 1979. Sediment induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. Marine Biology 55(2), 103-110.

Heck, K.L., Valentine, J.F., 2006. Plant–herbivore interactions in seagrass meadows. J. Exp. Mar. Biol. Ecol. 330(1), 420-436.

Heck, K.L., Pennock, J.R., Valentine, J.F., Coen, L.D., Sklenar, S.A., 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: An experimental assessment. Limnology and Oceanography 45(5), 1041-1057.

Hemminga, M., Harrison, P., Van Lent, F., 1991. The balance of nutrient losses and gains in seagrass meadows. Marine Ecology Progress Series 71.

Hessing-Lewis, M.L., Hacker, S.D., Menge, B.A., Rumrill, S.S., 2011. Context-dependent eelgrass–macroalgae interactions along an estuarine gradient in the Pacific Northwest, USA. Estuaries and Coasts 34(6), 1169-1181.

Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 56(10), 1801-1808.

Hutchings, M., 1999. Clonal plants as cooperative systems: Benefits in heterogeneous environments. Plant Species Biology 14(1), 1-10.

Hutchings, M.J., Wijesinghe, D.K., 1997. Patchy habitats, division of labour and growth dividends in clonal plants. Trends in Ecology & Evolution 12(10), 390-394.

Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytologist 135(4), 575-585.

Judge, M.L., Coen, L.D., Heck Jr, K.L., 1993. Does *Mercenaria mercenaria* encounter elevated food levels in seagrass beds? Results from technique to collect suspended food resources. Marine Ecology Progress Series 92, 141-150.

Kiers, T.E., Palmer, T.M., Ives, A.R., Bruno, J.F., Bronstein, J.L., 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13(12), 1459-1474.

Larkum, A.W.D., Orth, R.J., Duarte, C.M., 2006. Seagrasses : Biology, Ecology, and ConservationSpringer, Dordrecht, The Netherlands.

Maldonado, M., Giraud, K., Carmona, C., 2008. Effects of sediment on the survival of asexually produced sponge recruits. Marine Biology 154(4), 631-641.

Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, X. (Eds.), Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology, pp. 113-182.

Marba, N., Holmer, M., Gacia, E., Barron, C., 2006. Seagrass Beds and Coastal Biogeochemistry. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands, pp. 135-157.

McLeod, E., Chmura, G.L., Bouillon, S., Salm, R., Bjork, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., Silliman, B.R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. Frontiers in Ecology and the Environment 9(10), 552-560.

McMahon, K., Collier, C., Lavery, P.S., 2013. Identifying robust bioindicators of light stress in seagrasses: A meta-analysis. Ecological Indicators 30, 7-15.

Neuhauser, C., Fargione, J.E., 2004. A mutualism–parasitism continuum model and its application to plant–mycorrhizae interactions. Ecological Modelling 177(3–4), 337-352.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56(12), 987-996.

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

Pawlik, J.R., Loh, T., McMurray, S.E., Finelli, C.M., 2013. Sponge communities on Caribbean coral reefs are structured by factors that are top-down, not bottom-up. PLoS ONE 8(5), e62573.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reiswig, H.M., 1971a. Particle feeding in natural populations of 3 marine Demosponges. Biological Bulletin 141(3), 568-591.

Reiswig, H.M., 1971b. In-situ pumping activities of tropical Demospongiae. Marine Biology 9(1), 38-50.

Ribes, M., Coma, R., Gili, J.-M., 1999. Natural diet and grazing rate of the temperate sponge Dysidea avara (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology Progress Series 176, 179-190.

Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography 53(3), 986-996.

Stachowicz, J.J., 2001. Mutualism, facilitation, and the structure of ecological communities. Bioscience 51(3), 235-246.

Stuefer, J.F., 1998. Two types of division of labour in clonal plants: benefits, costs and constraints. Perspectives in Plant Ecology, Evolution and Systematics 1(1), 47-60.

Stuefer, J.F., During, H.J., Kroon, H.d., 1994. High Benefits of Clonal Integration in Two Stoloniferous Species, in Response to Heterogeneous Light Environments. Journal of Ecology 82(3), 511-518.

Taylor, B.W., Keep, C.F., Hall, R.O., Koch, B.J., Tronstad, L.M., Flecker, A.S., Ulseth, A.J., 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society 26(2), 167-177.

Tomasko, D.A., Dawes, C.J., 1989. Evidence for physiological integration between shaded and unshaded short shoots of *Thalassia testudinum*. Marine Ecology Progress Series 54(3), 299-305.

van der Heide, T., Govers, L.L., de Fouw, J., Olff, H., van der Geest, M., van Katwijk, M.M., Piersma, T., van de Koppel, J., Silliman, B.R., Smolders, A.J.P., van Gils, J.A., 2012. A Three-Stage Symbiosis Forms the Foundation of Seagrass Ecosystems. Science 336(6087), 1432-1434.

Wabnitz, C.C., Andrefouet, S., Torres-Pulliza, D., Muller-Karger, F.E., Kramer, P.A., 2008. Regional-scale seagrass habitat mapping in the Wider Caribbean region using Landsat sensors: Applications to conservation and ecology. Remote Sensing of Environment 112(8), 3455-3467.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106(30), 12377-12381.

Williams, S.L., 1990. Experimental studies of Caribbean seagrass bed development. Ecological monographs, 449-469.

Wong, M.C., Vercaemer, B., 2012. Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (Zostera marina). Aquatic Invasions 7(3), 315-326.

Wulff, J., 2000. Sponge predators may determine differences in sponge fauna between two sets of mangrove cays. Belize Barrier Reef. Atoll Res Bull 477, 251–263.

Wulff, J.L., 2006. Ecological interactions of marine sponges. Canadian Journal of Zoology-Revue Canadienne De Zoologie 84(2), 146-166.

Yarbro, L.A., Carlson, P.R., 2008. Community oxygen and nutrient fluxes in seagrass beds of Florida Bay, USA. Estuaries and Coasts 31(5), 877-897.

Ziegler, S., Benner, R., 1999. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Marine Ecology Progress Series 180, 149-160.

Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, Thalassia testudinum König. Aquaculture 4(0), 139-143.

Zieman, J.C., 1982. The Ecology of the Seagrasses of South Florida: A Community Profile. U.S. Fish and Wildlife Services, Offices of Biological Services, Washington, DC.

CHAPTER 4. ANTHROPOGENIC ALTERATION OF A FACILITATIVE INTERACTION HAS ECOSYSTEM-SCALE CONSEQUENCES FOR CARBON DYNAMICS

Abstract

Human activities are diving significant losses of seagrass habitat worldwide. This is concerning as seagrass beds provide a multitude of important ecosystem functions, including storing large amounts of carbon. Although human activities can, and do, result in mass mortality of seagrasses, they more often result in subtle chronic alterations of abiotic conditions which, by themselves, are insufficient to drive seagrass loss. Here I show that low levels of anthropogenic nutrient loading can shift the interaction between an epizootic sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) from facilitation to parasitism, significantly reducing carbon storage in this system. I found that increased nutrient availability resulted in sponges driving a significant reduction in seagrass growth, shoot density, and biomass. Subsequently, based on the data from this experiment, I showed that carbon stored in live seagrass biomass at six Bahamian seagrass beds decreased by an average of 4.8%. My model shows that if this interaction shifted to parasitism in just 1% of Bahamian seagrass beds, the net result would be equivalent to losing the standing carbon stock stored in 1,100 ha of live seagrass biomass. These findings illustrate that sponge-seagrass interaction is context dependent and affected by a common anthropogenic impact in coastal marine systems. This suggests that subtle shifts in species interactions can impact ecosystem function without triggering obvious biodiversity loss.

Introduction

Numerous studies have focused on anthropogenic alteration of ecosystem function as mediated through biodiversity loss (e.g. Cardinale, et al., 2012). However, human activities often disrupt species interactions without driving species loss (Tylianakis, et al., 2008). When human activities shift the relative costs and benefits associated with a species interaction, the outcome may shift in intensity or outcome (e.g., from facilitation to antagonism)(Bronstein, 1994; Kiers, et al., 2010). Even subtle shifts in the nature of species interactions can have large consequences for ecosystem function. For example, elevated CO_2 can result in increased carbon allocation from plants to mycorrhizal fungi in exchange for soil nutrients, increasing the strength of the interaction between the plants and fungi. This contributes to increased net primary production and the amount of carbon stored in plant biomass (Drake, et al., 2011).

Seagrass beds are a critically important, globally distributed, coastal ecosystem (Duffy, et al., 2014). Seagrasses, although occurring in less than 0.2% of the world's oceans, store a significant amount of carbon (Duarte, et al., 2005). Fourqurean, et al. (2012) estimated that live seagrass biomass stores between 75.5 and 151 Tg C globally, with significantly more carbon sequestered in the sediments of seagrass beds. However, seagrass beds are disappearing rapidly as a result of myriad anthropogenic threats (Waycott, et al., 2009). The majority of identified threats result in direct mortality of the seagrass, which include dredging, damage from boats, and threats from irresponsible coastal development. As a result, considerable attention has been focused on the areal extent of seagrass loss and concomitant loss of ecosystem functions (Orth, et al., 2006).

Eutrophication is a well-recognized threat to seagrass ecosystems. However, seagrass loss attributed to eutrophication is largely the result of dramatic increases in ambient nutrient availability which drive increases in algal and water column productivity, significantly decreasing light to the point where seagrasses are unable to survive. Additionally, increased nutrients can directly negatively impact seagrass physiology leading to seagrass loss (Burkholder, et al., 2007). Here, I test whether low levels of anthropogenic nutrient loading may drive seagrass loss through the alteration of a facilitative interaction between a sponge, *Halichondria melanadocia* and the seagrass, *Thalassia testudinum.* I tested this using a field experiment designed to evaluate the effect of nutrient addition on the sponge-seagrass interaction. I then constructed a model, parameterized with data from our experiment, to estimate carbon storage in live seagrass biomass at the six seagrass beds surveyed in Archer, et al. (2015). My results show that an increase in ambient nutrient availability can shift the sponge-seagrass interaction from facilitation to parasitism, significantly reducing carbon storage in live seagrass biomass in the system, which could have regional-scale consequences for carbon sequestration.

Methods

Study system

This study was conducted on Great Abaco Island, The Bahamas and focused on the interaction between the sponge *Halichondria melanadocia* and the seagrass *Thalassia testudinum*. The sponge grows around the base of the seagrass shoots, where it simultaneously shades the seagrass and provides bioavailable forms of both nitrogen and

phosphorus, which limit seagrass growth in The Bahamas (Allgeier, et al., 2010; Archer, et al., 2015). In the oligotrophic waters of The Bahamas, the net outcome of the interaction between the sponge and seagrass is facilitation, wherein the sponge benefits and the seagrass exhibits no measurable effect of hosting the sponge (Archer, et al., 2015). The experimental portion of this study occurred from June 2-July 3, 2014. The location of our experiment was a dense seagrass meadow of *T. testudinum* at Jungle Creek (26° 21′ 53″ N, 77° 01′ 25″W). Jungle Creek is a relatively unimpacted, sheltered, tidal system that is bounded by red mangroves (*Rhizophera mangle*) and contains a mosaic of sand flats, hard bottom, and seagrass communities. *Halichondria melanadocia* is abundant in this area (Archer, et al., 2015). The seagrass beds included in the carbon storage model are distributed throughout the Northeastern coast of Great Abaco Island and were surveyed in the summer of 2012. During the surveys seagrass shoot density and sponge abundance were recorded. Please refer to Archer, et al. (2015) for details of the survey methods and the precise location of the seagrass beds.

Experimental design and setup

I conducted a 2 x 2 factorial design with two levels of sponge (present or absent) and nutrient enrichment (ambient or fertilized) with 10 plots per treatment combination (n=40). Experimental plots were defined as a 20 cm^2 plot of *T. testudinum* and were arranged in 5 rows of 8 plots each. All plots were separated by at least 1m. This distance was deemed sufficient as the oligotrophic nature of this system results in the rapid uptake of nutrient inputs over very short distances (Layman et al. 2013). The first plot was haphazardly placed within the seagrass bed. From this first plot I searched for the presence of a sponge within

~1-3 m of the existing plot. If a sponge was found, the next experimental plot was established with the sponge in the center. If no sponge was found, the plot was established 1m from the previous plot. Ten plots for each level of the sponge treatment were randomly assigned to receive fertilizer. All plots were delineated using PVC stakes placed at the corners of the plot. Although the sponge treatment could not truly be randomly assigned, I were able to ensure that the sponge treatments were spread throughout the experimental space. For nutrient additions, I followed the protocol outlined by Ferdie and Fourqurean (2004) and Stoner, et al. (2014). This involved massaging 40 mg ($\pm$ 0.05 mg) of Plantacote slow-release fertilizer into the first 5 cm of the sediment. The initial density of *T. testudinum* short shoots was counted in each plot.

Seagrass growth rate

In mid-June, five seagrass shoots in each experimental unit were randomly chosen and marked at the base of the shoot with a surgical needle following a standard blade-hole punching technique (Zieman, 1974). For sponge treatments, the seagrass shoot that contained the sponge was intentionally marked to directly test the effect of the sponge on its host seagrass shoot following the methods described in Archer, et al. (2015). The shoots were then collected after 2 weeks and frozen for transport to North Carolina State University. Once in the lab, samples were thawed in order to measure the new growth. Because seagrass blades grow up from the base, new growth was measured as the distance up the blade the scar created by the surgical needle had traveled (Zieman, 1974). To get the total area of new growth, the width of each blade was also measured. Lastly, blades were gently scraped to

remove epiphytes and then the shoots were placed aside to be added to the appropriate biomass component during biomass processing (described below).

Seagrass biomass

At the end of the experiment, final seagrass density within each 20 cm^2 unit was recorded. Then, using a 20 cm diameter core, above- and below-ground biomass of seagrass was collected and frozen for transport to North Carolina State University. All materials within the core were collected until no more seagrass material was collected in consecutive grabs (~15-20 cm deep into the substrate). Each core was sieved in the field to remove excess sand prior to transport. Once in the lab, the samples were thawed and the core was sorted into four components: blades, sheaths, rhizomes, and roots. Sponges were gently removed from the seagrass and dried at 65º C for 48-72 hours until a stable weight was reached. Epiphytes were removed by gently scraping the blades. All components were rinsed in DI water to remove sand and other foreign particles. The samples were dried at 65º C for 48-72 hours until a stable weight was reached. The dry weight (g) was recorded and a subset of samples were then ground to a fine powder for %C determination (see below). Epiphytes removed from the seagrass blades were dried at 65º C for 48-72 hours then ashed at 500º C for 3 hours after the samples cooled the ash-free dry weight was taken.

Statistical analyses

Seagrass growth ($mm^3\ d^{-1}$), the change in *T. testudinum* shoot density (initial – final shoot density), and ephiphyte ash-free dry weight (standardized by blade biomass; $g_{epiphytes}/g_{blade}$) were each analyzed using a 2-way analysis of variance with nutrient addition

and sponge presence as the fixed factors. Total, above- and below-ground biomass, as well as their ratio, were each analyzed using analysis of covariance with initial shoot density as the covariate and nutrient addition and sponge presence as the fixed factors. In all cases post-hoc mean comparisons were conducted using Tukey's Honest Significant Difference. All statistical analyses were completed in SAS version 9.4.

Carbon simulation model

The total area (m^2) of each of six seagrass beds surrounding Abaco Island, The Bahamas, was estimated using a habitat layer developed by The Nature Conservancy in 2000 in ArcGIS. The total area of each seagrass bed was divided into 0.04 m^2 plots to match the scale of our experiment (described above). The number of plots with a sponge was randomly selected from a Binomial (n ,Θ) distribution. Where n is equal to the total number of 0.04 m^2 plots in the seagrass bed and Θ is equal to the probability of a sponge being present. Theta was modeled as a Beta (α,β) distribution. The α and β parameters were calculated by setting the expected value and variance equal to the observed mean and variance in sponge abundance as documented at each site in Archer, et al. (2015). The shoot density for each plot was assigned by randomly selecting a value for each 0.04 m^2 plot from a negative binomial distribution with the mean set to the observed value for shoot density as reported in my previous study.

In order to quantify the relationship between shoot density and biomass under ambient conditions, the dry weight of each component of the seagrass plant (blades, sheaths, roots, and rhizomes) was regressed against final shoot density in unfertilized experimental plots (both those with sponge and without). These coefficients were used to convert shoot

density to biomass in all plots under facilitative conditions. To estimate biomass after the sponge-seagrass interaction became parasitic, I first regressed final shoot density against initial shoot density in fertilized sponge plots, then used these coefficients to reduce seagrass shoot density in in the $0.04m^2$ plots the model assigned to contain a sponge. Second, I regressed the biomass of each component of the seagrass plant against final shoot density in fertilized sponge plots. I then used these coefficients to convert shoot density to biomass in these same $0.04m^2$ plots. In 0.4 m^2 plots that did not contain a sponge, the same biomass was used as under facilitative conditions. A subset of experimental plots (n=20) were randomly chosen and for these plots the total %C for each component of the seagrass plant was determined. These samples were ground to a fine powder after being dried at 60ºC for 48-72 hours and the biomass of the sample being weighed. A small subset of the ground powder (~3-6 mg) was then weighed into tin capsules and sent to the Analytical Chemistry Lab at the University of Georgia for analysis. There was no significant difference in %C in any seagrass component attributable to the presence of a sponge or fertilizer. Therefore, in order to calculate the total weight of carbon stored in live seagrass biomass for each plot, a %C was randomly selected for each seagrass component by randomly sampling for a normal distribution with mean and variance estimated from the measured %C for the appropriate component. The estimated weight of carbon was then summed across seagrass components and all 0.04 m^2 plots to get an estimate of the total carbon stored in live seagrass biomass (C_{org}) for the seagrass bed. The entire procedure was then repeated to generate 1,000 estimates of C_{org} for each seagrass bed. The model was constructed and run in R version 3.1.1 (R Core Team, 2014; code available in Appendix B).

Results

There was no difference in sponge dry weight between fertilized and non-fertilized plots (t_{16} = -1.93, p=0.07; 5.46 ± 4.08 g DW, mean ± sd). As predicted, the addition of fertilizer shifted the interaction between the sponge and seagrass from facilitation to parasitism. In fertilized sponge plots seagrass growth was significantly reduced ($F_{1,36}$ =22.12, p<0.001, Fig. 4.1a). On average, shoot density also decreased in fertilized sponge plots over the course of the experiment, while all other treatments gained shoots ($F_{1,36}$=5.06, p=0.03, Fig. 4.1b). Epiphyte biomass was not affected by the addition of fertilizer ($F_{1,36}$=0.12, p=0.74), but was significantly increased by the presence of a sponge ($F_{1,36}$=7.47, p=0.01). After accounting for the initial shoot density, the combination of fertilizer and a sponge significantly affected both total seagrass biomass ($F_{1,35}$ =7.40, p=0.01, Fig. 4.2a) and the ratio of above-to below-ground biomass ($F_{1,35}$=5.76, p=0.02, Fig. 3.2b). In both cases initial shoot density was a significant covariate (total biomass: $F_{1,35}$=11.25, p=0.002; above:below $F_{1,35}$= 8.07, p=0.007). Unfertilized sponge plots had the highest total biomass (24.77 g ± 4.45, adjusted mean ± 95% CI) while fertilized sponge plots had the lowest (16.34 g ± 4.40, Fig. 4.2a). Similarly, unfertilized sponge plots had the largest ratio of above- to below-ground biomass (1.57 ± 0.70, adjusted mean ± 95% CI). This ratio is statistically similar to that observed for the fertilized plots without a sponge (0.88 ± 0.16, adjusted mean ± 95% CI), but significantly larger than both other plot types (Fig. 4.2b). Ambient sponge plots had both the highest above- (12.77 ± 3.00, adjusted mean ± 95% CI) and below-ground biomass (12.01g ± 2.07, adjusted mean ± 95% CI) while fertilized sponge plots had the lowest biomass in both compartments (above: 7.20g ± 2.99, below: 9.15g ± 2.05, adjusted mean ± 95% CI) although these differences were not significant.

The total amount of carbon stored in live seagrass biomass varied greatly from site to site, as expected since sites varied in both seagrass and sponge densities as well as total areal extent (Table 4.1). When the sponge-seagrass interaction was facilitative, I estimated that there is between 0.22 and 0.38 kg C_{org} m^{-2} stored in live seagrass biomass. This was reduced by 1.1 to 8.8% (mean of 4.8%) when the sponge-seagrass interaction became parasitic. This reduction translated to a decrease of between 4.3 and 24.9 g C_{org} m^{-2} stored in live seagrass tissue.

Discussion

Anthropogenic nutrient loading is one of the most pervasive threats to coastal ecosystems worldwide (Vitousek, et al., 1997). Species interactions involving nutrient transfer are sensitive to changes in ambient nutrient availability; Kiers, et al. (2010) found that 60% of studies reported that nutrient transfer mutualisms were disrupted by anthropogenic nutrient loading. Importantly, there is evidence that anthropogenic nutrients impact species interactions more negatively than natural sources of nutrients. For example, Shantz and Burkepile (2014) found that fish excretion increased coral growth while anthropogenic nutrient loading resulted in a decrease in growth. They suggest that this may be a result of the cost to the symbiont of utilizing the form of nitrogen most commonly supplied by humans (nitrate) which results in decreased photosynthate being transferred to the host. Here I show that increased anthropogenic nutrients can shift the interaction between the sponge *H. melanadocia* and the seagrass *T. testudinum* from facilitation to parasitism, with significant effects on carbon storage in live seagrass biomass.

Results of this experiment corroborate those of Archer, et al. (2015); under ambient, oligotrophic, conditions the presence of *H. melanadocia* has no significant impact on *T. testudinum* growth. In non-sponge plots, the addition of fertilizer caused a non-significant increase in seagrass growth (Fig. 1a). This result is consistent with reports that seagrass growth is limited by nutrient availability in The Bahamas archipelago (Allgeier, et al., 2010; Layman, et al., 2013). However, when fertilizer was added to plots containing sponges, seagrass growth was significantly reduced. In fact, in 3 fertilized sponge plots the seagrass shoot the sponge was growing around died. Despite the fact that only three shoots with sponges died, 7 out of 10 fertilized sponge plots lost shoot density (Fig. 4.1b). Interestingly, fertilized sponge plots had both the lowest above- and below- ground biomass of any plot type. This indicates that the decrease in total biomass was not only driven by the loss of seagrass shoots but, rather, dieback of the whole plant. Increased shading by epiphytic algae does not seem to explain the decrease in fertilized sponge plots, as epiphyte load was not significantly different between fertilized and unfertilized sponge plots. Together these lines of evidence suggests that it is not the physical presence of the sponge shading the seagrass but, rather, some other mechanism (such as ammonium toxicity), driving seagrass loss in fertilized sponge plots.

The functional equilibrium model of plant growth asserts that the allocation of above- and below- ground biomass should be determined by relative availability of light and soil nutrients. If light is more limiting, or nutrients replete, the plant should allocate more biomass into above-ground tissues (Tilman, 1988). Although this breaks down somewhat for aquatic vegetation that can absorb water column nutrients through their above-ground tissues, it still provides a useful framework in which to consider patterns of above:below ground

biomass; plants should allocate biomass in such a way to maximize uptake of the most limiting resource. Duarte and Chiscano (1999) reported an average ratio of above- to below-ground biomass for *T. testudinum* of 0.89, consistent with the ratio I found in all but the ambient sponge plots. The ratio in my ambient plots is consistent with Archer et al. (2015)'s speculations on that the sponge shades the seagrass while also providing water column nutrients, both of which would result in increased allocation of above-ground biomass (Powell, et al., 1989), as I observed (Fig. 4.2b). In plots without a sponge I saw a small, non-significant, decrease in the ratio of above- to below-ground biomass in fertilized plots, again consistent with the paradigm that seagrass growth is nutrient limited in this system. The decrease in the ratio of above- to below-ground biomass for fertilized sponge plots is likely driven by a more rapid decrease in above-ground biomass rather than a shift in allocation by the plant, since both components of biomass decreased.

In the parasitism scenario, carbon stored in live seagrass biomass decreased by between 4.3 to 24.9 g C_{org} m^{-2}, corresponding to a decrease of 1.1% to 8.8%. Even a 1.1% reduction in carbon stored in live seagrass biomass can lead to a large reduction in C_{org} stored in Bahamian seagrass beds. Wabnitz, et al. (2008) estimated that seagrasses cover approximately 65,436 km^2 of the benthos in the shallow waters of The Bahamas. If I assume a shift from facilitation to parasitism results in an average loss of only 4.3 g C_{org} m^{-2}, and that this occurred in just 1% of Bahamian seagrass beds, the result is a total reduction of 2,841 Mg C_{org} stored in live seagrass biomass. This is equivalent to the loss of live biomass carbon storage for over 1,100 Ha of seagrass (Fourqurean, et al., 2012). Although carbon stored in the live seagrass biomass represents only a fraction of the total carbon stored by seagrass beds, the processes which contribute to carbon storage in sediments are strongly impacted by

seagrass shoot density (e.g. deposition of autochthonous carbon, trapping of allochthonous materials)(Gacia, et al., 2003). Therefore, it is likely that our results underestimate of the true impact of the sponge-seagrass interaction becoming parasitic on carbon dynamics.

Loss of seagrass beds, and their associated ecosystem functions is an important global conservation issue, with implications for global carbon dynamics (Duarte, et al., 2005; Fourqurean, et al., 2012). My results show that even low levels of anthropogenic nutrient loading can subtly shift a facilitative interaction to a parasitic one. Further, my models indicate that if this occurred in just 1% of Bahamian seagrass beds, the reduction in carbon storage capacity would be equivalent to 10% of the carbon storage capacity in live seagrass biomass lost each year to all other drivers of seagrass decline worldwide (Waycott, et al., 2009). These results highlight the idea that seemingly subtle changes in the relative costs and benefits associated with species interactions may correspond to large shifts in ecosystem function. By concentrating solely on habitat and biodiversity loss, we may be substantially underestimating the potential for human activities to impact key ecosystem functions such as carbon storage.

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Table 4.1. Output from a carbon simulation model. Sites, seagrass densities and sponge densities correspond to those in Archer et al. (2015). Total seagrass biomass (kg C_{org}) represents the estimated carbon stored in living seagrass tissue when the sponge-seagrass interaction is facilitative. The decrease in total seagrass biomass (kg C_{org}) represents our estimate of the total reduction in carbon stored in live seagrass tissue when the sponge-seagrass interaction becomes parasitic for each site. All values reported are means ± sd.

Site	Area (ha)	Seagrass density (shoots m^{-2})	Sponge density (sponges m^{-2})	Total seagrass biomass (kg (C_{org}))	Decrease in total seagrass biomass (kg (C_{org}))
JC	20	524 ± 130.5	11.4 ± 8.0	5614 ± 1.8	493 ± 341.0
N	30	575 ± 171.4	2.4 ± 2.3	9073 ± 2.4	172 ± 162.1
TB	47	756 ± 191.5	1.5 ± 1.4	17976 ± 3.7	203 ± 209.6
SC	193	386 ± 92.5	11.1 ± 7.6	43125 ± 4.5	3201 ± 2213.0
CA	206	629 ± 113.2	2.2 ± 2.2	67822 ± 6.6	1153 ± 1085.4
TC	227	489 ± 120.1	10.7 ± 8.1	60864 ± 5.6	4871 ± 3602.4

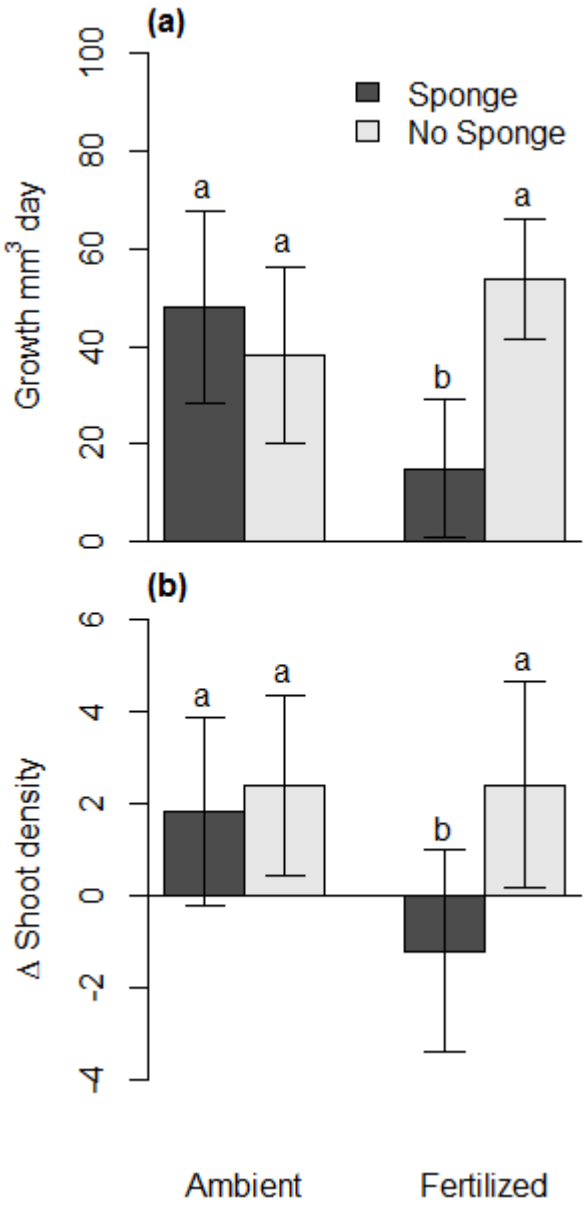

Figure 4.1. Mean and standard deviation of seagrass growth ($mm^3\ d^{-1}$, panel a) and the change in seagrass shoot density (panel b). The letters above the error bars represent statistically similar groups according to Tukey's Honest Significant Difference at $\alpha=0.05$.

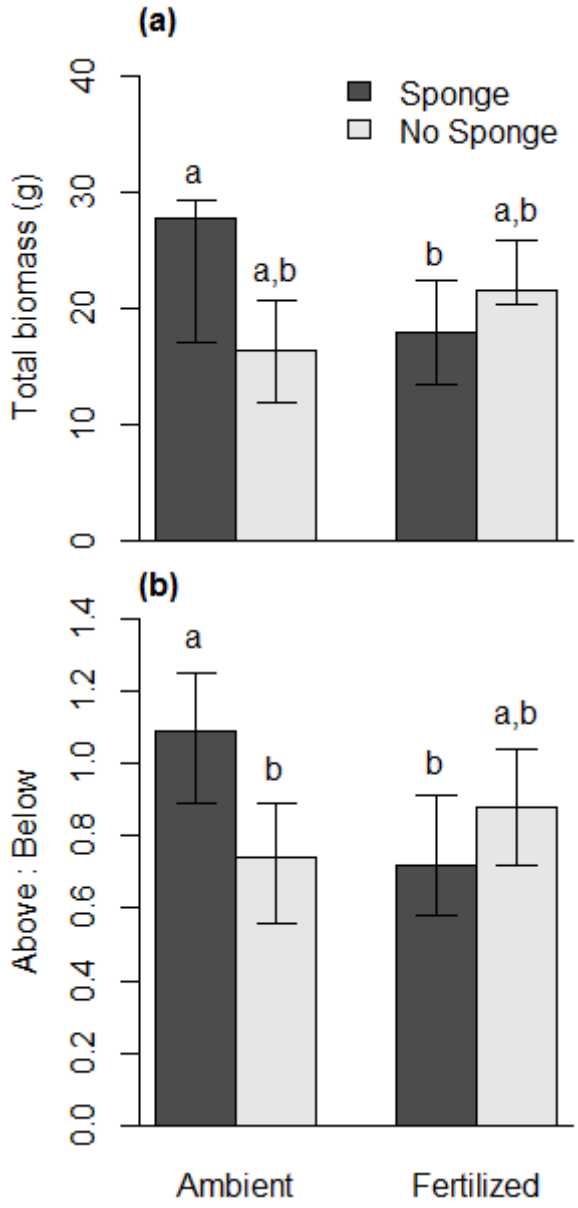

Figure 4.2. Covariate adjusted means and 95% confidence intervals for total seagrass biomass (g, panel a) and the ratio of above- to below-ground biomass (panel b). The letters above the error bars represent statistically similar groups according to Tukey's Honest Significant Difference at α=0.05.

REFERENCES

Allgeier, J.E., Rosemond, A.D., Mehring, A.S., Layman, C.A., 2010. Synergistic nutrient colimitation across a gradient of ecosystem fragmentation in subtropical mangrove-dominated wetlands. Limnology and Oceanography 55(6), 2660-2668.

Archer, S.K., Stoner, E.W., Layman, C.A., 2015. A complex interaction between a sponge (*Halichondria melanadocia*) and a seagrass (*Thalassia testudinum*) in a subtropical coastal ecosystem. J. Exp. Mar. Biol. Ecol. 465(0), 33-40.

Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology & Evolution 9(6), 214-217.

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350(1), 46-72.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486(7401), 59-67.

Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO2. Ecology Letters 14(4), 349-357.

Duarte, C.M., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65(1–4), 159-174.

Duarte, C.M., Middelburg, J.J., Caraco, N.F., 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2(1), 1-8.

Duffy, J.E., Hughes, A.R., Moksnes, P.-O., 2014. Ecology of seagrass communities. In: Bertness, M.D., Bruno, J.F., Silliman, B.R., Stachowicz, J.J. (Eds.), Marine Community Ecology and Conservation. Sinauer Associates, Inc., Sunderland, MA, pp. 271-298.

Ferdie, M., Fourqurean, J.W., 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. Limnology and Oceanography 49(6), 2082-2094.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012.

Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience 5(7), 505-509.

Gacia, E., Duarte, C.M., Marbà, N., Terrados, J., Kennedy, H., Fortes, M.D., Tri, N.H., 2003. Sediment deposition and production in SE-Asia seagrass meadows. Estuarine, Coastal and Shelf Science 56(5–6), 909-919.

Kiers, T.E., Palmer, T.M., Ives, A.R., Bruno, J.F., Bronstein, J.L., 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13(12), 1459-1474.

Layman, C.A., Allgeier, J.E., Yeager, L.A., Stoner, E.W., 2013. Thresholds of Ecosystem Response to Nutrient Enrichment from Fish Aggregations. Ecology 94(2), 530-536.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56(12), 987-996.

Powell, G.V.N., Kenworthy, J.W., Fourqurean, J.W., 1989. Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. Bulletin of Marine Science 44(1), 324-340.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Shantz, A.A., Burkepile, D.E., 2014. Context-dependent effects of nutrient loading on the coral–algal mutualism. Ecology 95(7), 1995-2005.

Stoner, E.W., Yeager, L.A., Sweatman, J.L., Sebilian, S.S., Layman, C.A., 2014. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. J. Exp. Mar. Biol. Ecol. 461, 185-192.

Tilman, D., 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press.

Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11(12), 1351-1363.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7(3), 737-750.

Wabnitz, C.C., Andrefouet, S., Torres-Pulliza, D., Muller-Karger, F.E., Kramer, P.A., 2008. Regional-scale seagrass habitat mapping in the Wider Caribbean region using Landsat

sensors: Applications to conservation and ecology. Remote Sensing of Environment 112(8), 3455-3467.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106(30), 12377-12381.

Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. Aquaculture 4(0), 139-143.

CHAPTER 5. ABIOTIC CONDITIONS DRIVE SIGNIFICANT VARIABILITY IN NUTRIENT FLUX IN A COMMON CARIBBEAN SPONGE, *IRCINIA FELIX*

Abstract

Coral reefs typically occur in oligotrophic waters, where tight recycling of energy and nutrients is essential to support their high productivity. Sponges, a common organism on coral reefs, are efficient filter feeders which host diverse and abundant microbial communities that often contain members capable of carrying out complex nutrient transformations. Sponges often act as sources of bioavailable forms of nitrogen and phosphorus while also acting as sinks for dissolved organic carbon (DOC). However, little attention has focused on variability of nutrient release by sponges, and no studies have reported how abiotic conditions may impact sponge-driven changes in nutrient concentrations. Here I show that a common Caribbean sponge, *Ircinia felix*, is capable of being both a source and a sink for DOC, ammonium, nitrate/nitrite (NO_x^-) and soluble reactive phosphoprus (SRP). Additionally, I show that abiotic conditions, particularly ambient nutrient availability, seem to explain a significant amount of the variability (R^2 range from 0.40-0.65 for SRP and NO_x^- respectively). Interestingly, for all nutrient forms measured, as ambient nutrient concentrations increased, *I. felix* transitioned from acting as a source to serving as a sink for that nutrient. These results suggest that sponges play an important role in biogeochemical cycling on reefs, particularly as human activities alter natural nutrient dynamics in coastal systems.

Introduction

Coral reefs are among the world's most valued ecosystems, providing an estimated $375 billion yr^{-1} in ecosystem services (Costanza, et al., 1997). Many of these services (e.g., food production) are linked to high productivity resulting from efficient recycling of energy and nutrients within the coral reef (hereafter reefs). Sponges, a common component of the benthic community in Caribbean reef ecosystems, contribute to this tight recycling of energy and nutrients on reefs through their efficient filter feeding (Reiswig, 1971a). Additionally, sponges host large microbial communities which are capable of capturing and transforming dissolved forms on energy and nutrients (de Goeij, et al., 2008a; Fiore, et al., 2010; Freeman, et al., 2013; Webster and Taylor, 2012). As a result, sponges play an important role in maintaining the productivity of reef systems.

Although corals and their symbiotic zooxanthellae are critical for reef productivity, they (and other primary producers) are inefficient and leach sugars into the water column in the form of dissolved organic carbon (DOC) (Haas, et al., 2010). Sponge holobionts (the sponge and their associated microbiome), unlike most organisms, can take up DOC and do so at rates sufficient to grow rapidly. However, most sponges do not grow rapidly; rather, they produce new cells and shed old cells at near equal rates, resulting in a relative steady-state with little new growth (de Goeij, et al., 2008b; De Goeij, et al., 2009). The shed cells become detritus available to the benthic feeding organisms, and thus sponges help to retain energy within the reef ecosystem (de Goeij, et al., 2013).

Sponges also transform limiting nutrients on reefs into forms that can stimulate primary production (Maldonado, et al., 2012). High microbial abundance (HMA) sponges typically host microbial communities 2-4 orders of magnitude more dense than the

surrounding seawater. These microbial communities are also significantly different than those of seawater, including microbes associated with complex nitrogen (N) and phosphorus (P) transformations (Hentschel et al. 2006, Fiore et al. 2010, Sabarathnam et al. 2010). Nutrient fluxes through sponges are partially regulated by the exchange of products (e.g. nutrients, photosynthate) between the sponge and their microbial symbionts (Fiore, et al., 2015; Freeman, et al., 2013; Thacker and Freeman, 2012). Nutrient transfer symbioses are often influenced by the abiotic conditions in which they occur (Johnson, et al., 2013; Kiers, et al., 2010; Shantz and Burkepile, 2014), and there is evidence to suggest sponge-microbe symbioses are no different (Freeman, et al., 2013). As such, it is reasonable to suspect that DOC, N, and P fluxes through the sponge holobiont will vary along with abiotic conditions. However, nearly all studies to previously quantify nutrient flux in sponges have done so at a single location and point in time (but see Fiore, et al., 2013).

In this study I quantified the change in DOC and bioavailable N and P concentrations in water passing through a common species of sponge, *Ircinia felix,* at a total of nine reefs distributed across three Caribbean and sub-tropical Atlantic Islands. I examined correlations between the change in nutrient concentrations attributable to this sponge and a suite of abiotic variables including ambient nutrient availability, water temperature, and light intensity. This study demonstrates that there can be significant variability in nutrient processing in sponges and that to truly understand sponges' role in reef ecosystem function we must gain a better understanding of the conditions governing the outcome of sponge-microbe symbioses.

Methods

Study species

Ircinia felix is a common ball-shaped sponge on reefs throughout the subtropical and tropical Atlantic and Caribbean (Diaz, 2005; Loh and Pawlik, 2014). *I. felix* is classified as a HMA sponge (Weisz, et al., 2008) and has previously been reported as a source of bioavailable nitrogen (ammonium (NH_4^+) and nitrate/nitrite (NO_x^-))(Southwell et al. 2008). To my knowledge DOC and SRP flux has not been quantified previously in *I. felix*.

Sampling sites

Sponges were sampled at nine reefs surrounding three islands: Great Abaco Island, The Bahamas, New Providence Island, The Bahamas, and Curaçao (Fig. 5.1). The islands vary greatly in their geology and location. The Bahamian islands are limestone platforms with large expanses of shallow seas, while Curaçao is an old volcanic island with fringing reefs close to shore (Bak, 1977; Buchan, 2000). Great Abaco Island is the largest of the three islands at approximately 1,681 km^2, Curaçao is the next largest at 444 km^2, and New Providence is the smallest at 207 km^2; the islands follow an opposite pattern of human density at 10, 339, and 1,190 people km^{-2} respectively (Curacao Central Bureau of Statistics, 2014; Department of Statistics of the Bahamas, 2012).

InEx sampling

Six sponges were sampled at each reef, with the exception of one reef on Great Abaco Island where three sponges were sampled (total n = 51). Both patch and fringing reefs were

sampled at depth 2-15m. The InEx sampling method accurately samples fluxes of nutrients through a sponge by simultaneously collecting water coming into the sponge (in-current) and exiting the sponge (ex-current)(Yahel, et al., 2005). Full exchange of the ex-current sampling tube is ensured by first holding an identical tube filled with fluorescein dye just above the sponge's osculum and recording the total time required to clear the tube of the dye. The ex-current sampling tube is then held just above the same osculum for 1.5x the time it took to clear the dye from the tube, while a second person slowly draws back the plunger of a syringe held near the base of the sponge to collect water entering the sponge (in-current). The difference between the in-current and ex-current values is attributable to processes occurring within the sponge (see Yahel, et al., 2005 for additional details). Three in-current and three ex-current samples were collected from each sponge. From these samples I measured DOC, dissolved inorganic nitrogen (NH4- and NO3- and NO2-, hereinafter referred to as NOx), and soluble reactive phosphorus (SRP). Samples for ammonium, NO_x^-, and SRP were filtered with a 0.45µm Whatman nylon-membrane filter into acid washed scintillation vials. DOC samples were filtered with a 0.2 µm polycarbonate filter into pre-combusted glass ampoules with 1-2 drops of concentrated H_3PO_4 (80%). All samples were placed on ice then frozen (or refrigerated in the case of DOC) for transport to North Carolina State University (NCSU). Ammonium was determined using the indophenol blue method. Soluble reactive phosphorus was analyzed using the method described by Solórzano and Sharp (1980). DOC and NO_x^- samples were sent to the NCSU Environmental and Agricultural Testing Service and University of Georgia Analytical Chemistry Lab, respectively, for analysis. In-current water samples provided ambient nutrient concentrations. HOBO® Data Loggers were placed next to

each sponge sampled to record water temperature every hour for 24 hours. At least one data logger at each site was also equipped to record light levels.

Statistical analysis

For all nutrients the response variable evaluated was the change in a nutrient attributable to processes occurring within the sponge, for the remainder of the manuscript I will refer to this as the change in the nutrient (e.g. change in DOC). This was calculated as the ex-current – in-current concentration for each nutrient (DOC, NH_4^+, NO_x^-, SRP). As a result, positive values of the response variables indicate that the sponge is a source of that nutrient while a negative value indicates the sponge is a sink.

Predictor variables (Table 5.1) were inspected for correlations and any two variables with a coefficient of correlation higher than 0.50 were considered correlated. All possible combinations of predictor variables (excluding combinations including correlated variables) were evaluated and the best fit model was selected using corrected Akaike's Information Criteria (AICc) and model weights. The best fit model was evaluated for overall fit using adjusted R^2. The effects of individual predictor variables were evaluated using both the coefficients from the linear model as well as partial R^2 values, designated throughout the remainder of the manuscript as η^2.

Results

Across all sites on average *I. felix* was a sink for DOC and NH_4^+ and a source of NO_x^- and SRP. However, the change in nutrients were extremely variable for all nutrients

measured (Table 5.2) and sponges acting as either a source or a sink were observed on all islands for all nutrients measured (Fig 5.2).

The best fit models adequately explained variance in the change in all nutrients; adjusted R^2 values ranged from 0.40 for SRP to 0.66 for NO_x^- (Table 5.3; top 20 models for each response variable can be found in Appendix C Tables C1-C4). No single predictor variable was important for all nutrients measured. However, for all nutrients the ambient availability of a nutrient was the strongest predictor for the change in that nutrient. Also in all cases *I. felix* transitioned from being a source to a sink of the nutrient as ambient nutrient concentrations increase (Table 5.3, Appendix C Figs C1-C4). Interestingly, light and temperature at the time of sample collection did not correlate with change in any nutrients I evaluated. Rather median light (NO_x^-), minimum temperature (NH_4^+), and temperature variance (DOC and NH_4^+) over the 24 hours surrounding sampling were correlated with nutrient processing (Table 5.3). Sponge-specific variables were not universally correlated with change in nutrient concentrations. Sponge volume was an important predictor of both DOC and NO_x^- with larger sponges acting as larger sources of these nutrients. Sampling tube clearance time, which I used as a proxy for sponge pumping rate, was included in the best fit model for both SRP and NO_x^- but was only significant for SRP. As tube clearance time increased (slower sponge pumping) *I. felix* went from being a source to a sink for SRP. Although it was not significant, the same pattern holds for NO_x^-.

Discussion

Many sponges host abundant and diverse microbial communities that include members capable of complex chemical transformations (Fiore, et al., 2010; Thacker and

Freeman, 2012). This, in addition to sponges' ability to pump large amounts of water (Reiswig, 1971b), results in sponges having a large impact on picoplankton (Peterson, et al., 2006; Reiswig, 1971a) and virus abundance (Hadas, et al., 2006), as well as nutrient dynamics (Maldonado, et al., 2012; Southwell, et al., 2008). Despite recording large variances, previous studies have concentrated on mean sponge nutrient fluxes when discussing sponges' impact on nutrient cycling on reefs (Corredor, et al., 1988; Diaz and Ward, 1997; Southwell, et al., 2008 but see Fiore et al. 2013). However, I suggest that variability in nutrient processing in sponges is an extremely important process to understand, as the individuals within the same species of sponge are capable of acting as both a source and a sink for a wide range of nutrients. For the first time I show that variability in sponge nutrient processing is correlated with both abiotic conditions and sponge specific variables.

Although many of our abiotic variables differed between sites, location was not a primary predictor of sponge nutrient processing for any of the nutrients measured (Table 5.3). Instead, abiotic variables including ambient nutrient availability, light, and temperature were significantly correlated with the change in nutrient concentrations attributable to *I felix*. It is important to remember that my study is observational and correlation does not imply a causal relationship. However, my study does reveal some interesting patterns that warrant further investigation.

My results show a similar pattern for all forms of nutrients I investigated; as an ambient nutrient concentration increased *I. felix* transitioned from being a source of that nutrient to acting as a sink (Appendix C Figs C1-C4). This result suggests a context dependency in either the composition of the active members of the sponge microbiome, or in sponge-microbial interactions as the microbial community residing within the sponge is

primarily responsible for nutrient processing (Fiore, et al., 2010; Maldonado, et al., 2012; Thacker and Freeman, 2012). Traditionally the microbial community within sponges has been considered spatially and temporally stable (Erwin, et al., 2012; Simister, et al., 2012). However, recent work has shown that the active members of sponges' microbial communities can change, sometimes over short temporal scales (Fiore, et al., 2015; Zhang, et al., 2014). A shift in the active microbial community could help explain the pattern of increased nutrient uptake with increased ambient availability. Further work is needed to determine if the patterns observed in this study represent a true cause and effect relationship. Additionally, more studies are needed to determine if this pattern exists in other species of sponge. If widespread, this could have important implications for reef biogeochemistry, as well as for ecosystem responses to anthropogenic nutrient loading (Vitousek, et al., 1997).

The changes in both DOC and SRP observed were largely driven by ambient nutrient concentrations (DOC and SRP respectively) with less variation explained by sponge volume (DOC) and pumping rate (SRP). Temperature variability was also an important predictor of the change in DOC, although a mechanism for this is unclear. Both changes in DOC and SRP within the sponge can be driven by similar processes, i.e., largely microbial consumption (increase uptake) and metabolic waste (decrease uptake). Although from my data it is impossible to determine the ultimate cause of increased DOC and SRP uptake by sponges, a plausible hypothesis is that the microbial community capable of utilizing these nutrients is stimulated by their availability. For example, increased SRP could stimulate primary production by the sponge's photosymbionts, as phosphorus is often a limiting nutrient in oligotrophic systems. If the pattern I observed of increased DOC uptake with increasing DOC availability is a true pattern, this could have interesting implications for reef energy

cycling and productivity as increased algal cover will likely lead to increased DOC in the water column.

Ammonium and NO_x^- processing in sponges are inextricably linked. Both NH_4^+ and NO_x^- can be used by photosynthetic symbionts or converted to N_2 gas through anaerobic ammonium oxidation (anammox) or denitrification, respectively. Ammonium can be converted to NO_x^- by microbial symbionts through nitrification. Finally, ammonium can be generated by microbes within the sponge via nitrogen fixation and through ammonification during regular metabolic activity. Although all of these processes have been documented within sponge holobionts (Fiore, et al., 2010; Han, et al., 2013; Hoffmann, et al., 2009; Schlappy, et al., 2010b; Zhang, et al., 2014; Zhang, et al., 2013), oxygenation of sponge tissue at any given time will determine the relative rates of the various nitrogen transformation. Nitrification, ammonification, and uptake by photosymbionts typically (or obligately) occur in oxic environments while nitrogen fixation, denitrification, and anammox must occur in anoxic environments. There is evidence that sponges can simultaneously have oxygenated and anoxic portions of their mesohyl (where the majority of microbial symbionts reside), creating diverse microhabitats that favor different nitrogen transformations (Schlappy, et al., 2010a). However, the sponge's pumping rate is also correlated with oxygen levels with tissues rapidly becoming anoxic as pumping ceases (Hoffmann, et al., 2008). Therefore, I expected to see a decrease in NO_x^- released by *I. felix* as pumping rates slowed. I did see this, as the time taken to clear the sampling tube increased (slower pumping rate) the change in NO_x^- decreases with *I. felix* transitioning from source to sink. This is consistent with increased denitrification; however, I cannot conclusively determine the mechanism with my data.

I did not observe a relationship between sponge pumping rate and ammonium output. This is not surprising, as processes contributing to increased (ammonification, fixation) and decreased (nitrification, anammox) ammonium output occur in both oxygenated and anoxic conditions. The two strongest predictors of the change in ammonium concentrations were minimum temperature and ambient NH_4^+ concentration. As minimum temperature increased *I. felix* released increasing amounts of ammonium. A source of ammonium with the sponge, ammonification, is the result of sponge metabolic processing, which should increase with temperature. There are several potential drivers of the observed negative correlation between ambient ammonium and the release of NH_4^+ by *I. felix*. These include increased activity by photosymbionts (nitrogen is often limiting for primary production in oligotrophic environments), increased nitrification or anammox, or a decrease in nitrogen fixation. These potential drivers are not mutually exclusive and, unfortunately, here I cannot conclusively distinguish among them.

Because of the conversion of ammonium to NO_x^- that occurs during nitrification, and the fact that *I. felix* had been previously reported as a nitrifying sponge, I expected to observe a correlation between ambient ammonium concentrations and the change in NO_x^- concentrations. Bayer, et al. (2008) reported just such a correlation for *Aplysina aerophoba*; when they increased ambient ammonium values NO_x^- release increased considerably. However, they found large seasonable variation in NO_x^- release and were only able to stimulate NO_x^- production via ammonium additions when sponges were already producing NO_x^-. Interestingly, when one restricts analysis to *I. felix* individuals observed acting as sources of NO_x^- I also noted a strong relationship between ambient ammonium and the change in NO_x^- concentrations (Fig 3). However, this correlation only extended up to

ambient ammonium concentrations of ~1.2 μmol l^{-1}. I recorded a small number of observations with ambient ammonium concentrations above 1.2 μmol l^{-1}, therefore it is difficult to tell if this is a true threshold or an artifact of our data. Regardless, the similarity of my results with those from Bayer, et al. (2008) are intriguing. It appears as though something other than ammonium availability controls whether or not the community of nitrifying microbial symbionts within sponges are active and when the nitrifying community is active increasing ammonium also increases NO_x^- output. Future research should focus on determining the drivers of activity in the nitrifying community within sponges as understanding this will shed light on sponges' true impact on nitrogen cycling.

My results show that the change in nutrient concentrations attributable to sponges are extremely variable and correlated with abiotic conditions. These results are consistent with previously drawn conclusions that sponges play a critical and underappreciated role in nutrient cycling on reefs (Maldonado, et al., 2012). However, I show that using mean nutrient flux measurements greatly oversimplifies the potential impact of sponge nutrient processing on reef biogeochemical cycles. Most importantly my results suggest sponge nutrient processing may be strongly context dependent. Understanding the relationship between observed changes in nutrient concentrations and abiotic conditions is imperative if we hope to understand how reef functioning will respond to global environmental change, particularly as sponges are predicted to become a more prevalent component of reef ecosystems (Bell, et al., 2013; McMurray, et al., 2015).

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Table 5.1. Potential explanatory variables included in model selection. Variables with matching symbols were correlated. No models were run containing both variables. Light and temperature summary statistics were calculated from values recorded over 24 hours surrounding sampling, unless otherwise indicated. Many variables were correlated with both Island and Reef, therefore the only model considered with either Island or Reef as a predictor variable was the model with location as the only predictor variable.

Light (Lux)	**Ambient nutrients ($\mu mol\ l^{-1}$)**	**Temperature (^0C)**	**Sponge**	**Location**
Maximum$^+$	DOC	Maximum*	Tube clearance rate (proxy for pumping rate)	Island
Median$^+$	NH_4^+	Median*		Reef
Light at time of sampling	NO_x^-	Minimum*	Volume	
	SRP	Variance$^+$		
		Temp at time of sampling*		

Table 5.2. Mean and standard deviation (μmol l^{-1}) for the change in nutrients attributable to processes occurring within the sponge. A negative value indicates the sponge is a sink for the nutrient while a positive value indicates the sponge is a source.

	DOC	NH_4^+	NO_x^-	SRP
Overall				
mean	-36.68	-0.07	0.06	0.08
SD	85.74	0.80	0.26	0.91
Abaco				
mean	-40.46	-0.02	-0.001	0.41
SD	114.61	1.17	0.28	1.08
Curacao				
mean	-1.94	-0.36	0.17	0.04
sd	41.69	0.64	0.29	0.96
New Providence				
mean	-68.27	0.17	0.002	-0.17
SD	81.96	0.43	0.18	0.61

Table 5.3. Overall model fit statistics and parameter coefficients, p-values, and η2 in the best fit model for each response variable.

Response variable		Light	Ambient nutrients				Temperature		Sponge		Model Statistics			
		Median	DOC	NH_4^+	NO_x^-	SRP	Min	Var	Pumping	Volume	DF	F	p-value	R^2
DOC	Coefficient		-0.88					114.85		12.35				
	p-value		<0.0001					0.02		0.01	3,47	12.38	<0.0001	0.41
	η^2		0.39					0.10		0.13				
NH_4^+	Coefficient		-0.003	-1.19			0.39	1.05						
	p-value		0.04	<0.0001			<0.0001	0.01			4,46	20.91	<0.0001	0.61
	η^2		0.09	0.60			0.47	0.13						
NO_x^-	Coefficient	-0.0001			-0.98				-0.001	0.02				
	p-value	0.002			<0.0001				0.11	0.05	4,46	24.3	<0.0001	0.65
	η^2	0.19			0.65				0.04	0.07				
SRP	Coefficient					-0.86			-0.01					
	p-value					0.0001			0.003		2,48	17.35	<0.0001	0.40
	η^2					0.27			0.17					

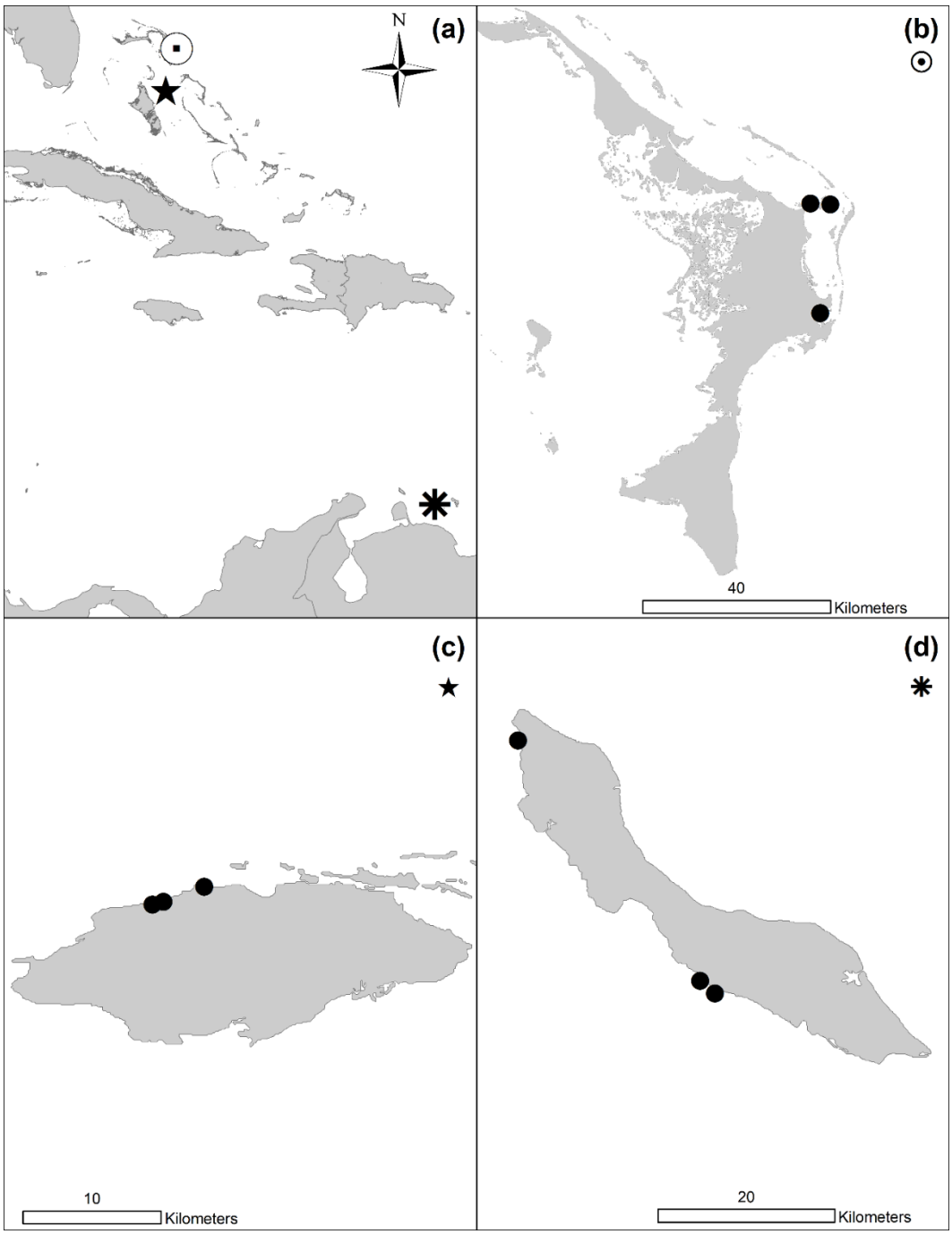

Figure 5.1. Overview (a) and island specific map of sampling locations on Abaco (b, ⊙), New Providence (c, ★), and Curacao (d, ✱).

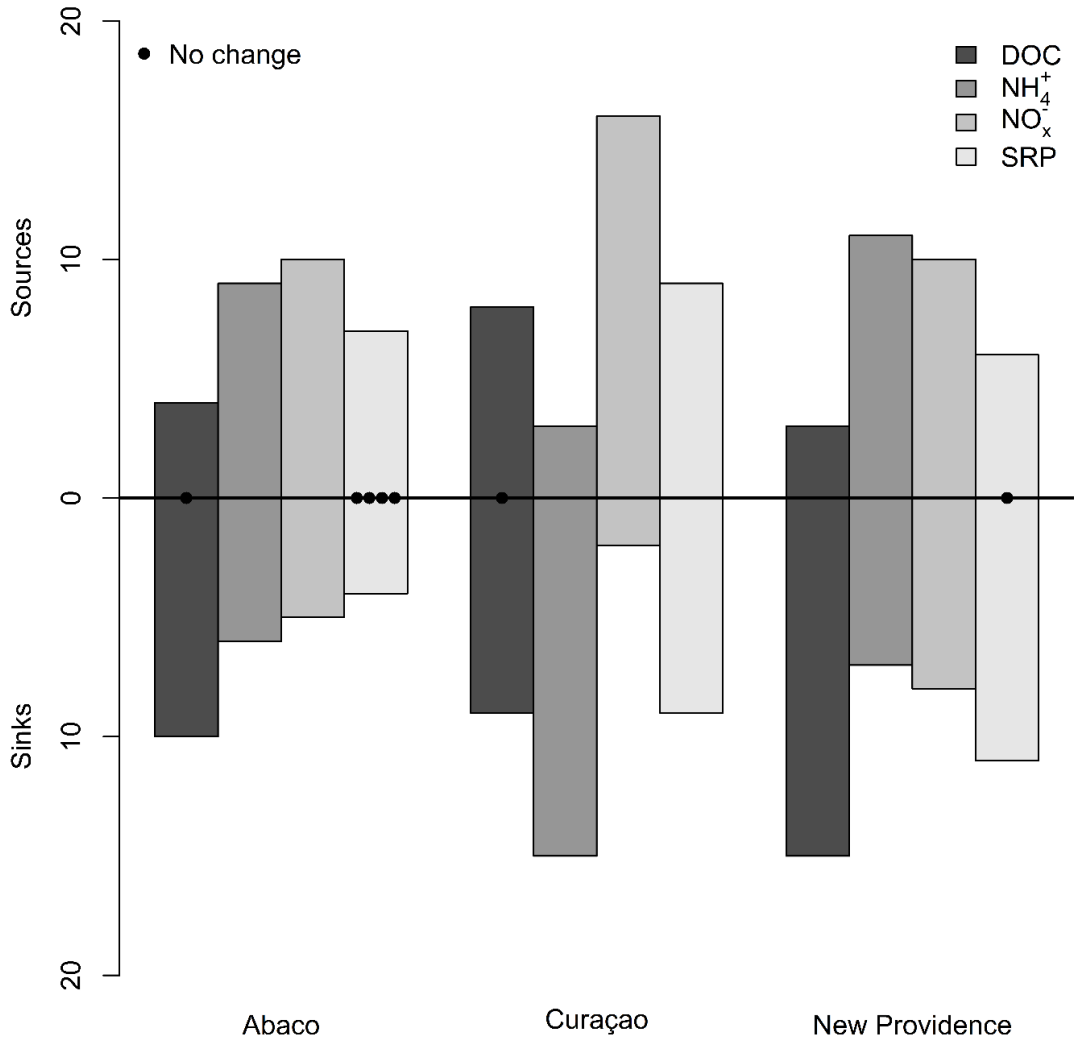

Figure 5.2. Number of sponges at each island acting as a source (above the zero line) or a sink (below the zero line) for each form of nutrients I measured. Points at the zero line indicate sponges where no change in nutrient concentration was observed. The total number of sponges sampled was 15 for Abaco and 18 for both Curaçao and New Providence.

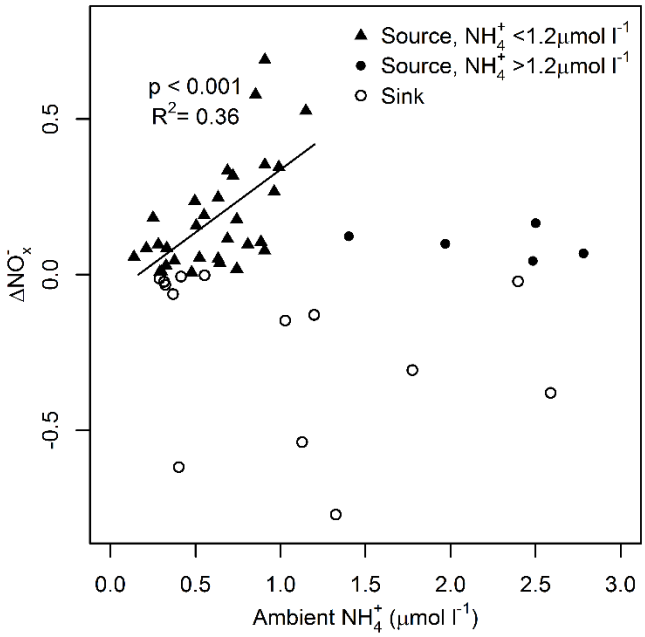

Figure 5.3. Correlation between ambient ammonium (μmol l^{-1}) and the change in NO_x^-. When analysis is restricted to *Ircinia felix* individuals acting as sources of NO_x^- there is a strong correlation between change in NO_x^- and ambient ammonium concentrations up 1.2 NH_4^+ μmol l^{-1}.

REFERENCES

Bak, R.P., 1977. Coral Reefs and Their Zonation in Netherlands Antilles: Modern and Ancient Reefs.

Bayer, K., Schmitt, S., Hentschel, U., 2008. Physiology, phylogeny and in situ evidence for bacterial and archaeal nitrifiers in the marine sponge Aplysina aerophoba. Environmental Microbiology 10(11), 2942-2955.

Bell, J.J., Davy, S.K., Jones, T., Taylor, M.W., Webster, N.S., 2013. Could some coral reefs become sponge reefs as our climate changes? Global Change Biology, 2613–2624.

Buchan, K.C., 2000. The Bahamas. Marine Pollution Bulletin 41(1-6), 94-111.

Corredor, J.E., Wilkinson, C.R., Vicente, V.P., Morell, J.M., Otero, E., 1988. Nitrate release by Caribbean reef sponges. Limnology and Oceanography 33(1), 114-120.

Costanza, R., dArge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vandenBelt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387(6630), 253-260.

Curacao Central Bureau of Statistics, 2014. Demography of Curacao, Willemstad, Curacao.

de Goeij, J.M., Moodley, L., Houtekamer, M., Carballeira, N.M., van Duyl, F.C., 2008a. Tracing 13C enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding. Limnology and Oceanography 53(4), 1376.

de Goeij, J.M., van den Berg, H., van Oostveen, M.M., Epping, E.H., Van Duyl, F.C., 2008b. Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Marine Ecology Progress Series 357, 139.

de Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., de Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science 342(6154), 108-110.

De Goeij, J.M., De Kluijver, A., Van Duyl, F.C., Vacelet, J., Wijffels, R.H., De Goeij, A., Cleutjens, J.P.M., Schutte, B., 2009. Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding. Journal of Experimental Biology 212(23), 3892-3900.

Department of Statistics of the Bahamas, 2012. Land Area and Density of Population by Island, Census Years 1901-2010, Nassau, Bahamas.

Diaz, M.C., 2005. Common sponges from shallow marine habitats from Bocas del Toro region, Panama. Caribbean Journal of Science 41(3), 465-475.

Diaz, M.C., Ward, B.B., 1997. Sponge-mediated nitrification in tropical benthic communities. Marine Ecology Progress Series 156, 97-107.

Erwin, P.M., Pita, L., Lopez-Legentil, S., Turon, X., 2012. Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. Applied and environmental microbiology 78(20), 7358-7368.

Fiore, C.L., Baker, D.M., Lesser, M.P., 2013. Nitrogen biogeochemistry in the Caribbean sponge, *Xestospongia muta*: A source or sink of dissolved inorganic nitrogen? PLoS ONE 8(8), 1-11.

Fiore, C.L., Jarett, J.K., Olson, N.D., Lesser, M.P., 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends in Microbiology 18(10), 455-463.

Fiore, C.L., Labrie, M., Jarett, J.K., Lesser, M.P., 2015. Transcriptional activity of the giant barrel sponge, Xestospongia muta Holobiont: Molecular Evidence for Metabolic Interchange. Frontiers in Microbiology 6.

Freeman, C.J., Thacker, R.W., Baker, D.M., Fogel, M.L., 2013. Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? Isme Journal 7(6), 1116-1125.

Haas, A.F., Naumann, M.S., Struck, U., Mayr, C., el-Zibdah, M., Wild, C., 2010. Organic matter release by coral reef associated benthic algae in the Northern Red Sea. J. Exp. Mar. Biol. Ecol. 389(1-2), 53-60.

Hadas, E., Marie, D., Shpigel, M., Ilan, M., 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51(3), 1548-1550.

Han, M.Q., Li, Z.Y., Zhang, F.L., 2013. The Ammonia Oxidizing and Denitrifying Prokaryotes Associated with Sponges from Different Sea Areas. Microbial Ecology 66(2), 427-436.

Hoffmann, F., Røy, H., Bayer, K., Hentschel, U., Pfannkuchen, M., Brümmer, F., De Beer, D., 2008. Oxygen dynamics and transport in the Mediterranean sponge Aplysina aerophoba. Marine Biology 153(6), 1257-1264.

Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H.T., Schlappy, M.L., Schleper, C., Kuypers, M.M.M., 2009. Complex nitrogen cycling in the sponge Geodia barretti. Environmental Microbiology 11(9), 2228-2243.

Johnson, N.C., Angelard, C., Sanders, I.R., Kiers, E.T., 2013. Predicting community and ecosystem outcomes of mycorrhizal responses to global change. Ecology Letters 16(s1), 140-153.

Kiers, T.E., Palmer, T.M., Ives, A.R., Bruno, J.F., Bronstein, J.L., 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13(12), 1459-1474.

Loh, T., Pawlik, J.R., 2014. Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. Proceedings of the National Academy of Sciences 111(11), 4151-4156.

Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, X. (Eds.), Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology, pp. 113-182.

McMurray, S.E., Finelli, C.M., Pawlik, J.R., 2015. Population dynamics of giant barrel sponges on Florida coral reefs. J. Exp. Mar. Biol. Ecol. 473, 73-80.

Peterson, B.J., Chester, C.M., Jochem, F.J., Fourqurean, J.W., 2006. Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Marine Ecology Progress Series 328, 93-103.

Reiswig, H.M., 1971a. Particle feeding in natural populations of 3 marine Demosponges. Biological Bulletin 141(3), 568-591.

Reiswig, H.M., 1971b. In-situ pumping activities of tropical Demospongiae. Marine Biology 9(1), 38-50.

Schlappy, M.L., Weber, M., Mendola, D., Hoffmann, F., de Beer, D., 2010a. Heterogeneous oxygenation resulting from active and passive flow in two Mediterranean sponges, Dysidea avara and Chondrosia reniformis. Limnology and Oceanography 55(3), 1289-1300.

Schlappy, M.L., Schottner, S.I., Lavik, G., Kuypers, M.M.M., de Beer, D., Hoffmann, F., 2010b. Evidence of nitrification and denitrification in high and low microbial abundance sponges. Marine Biology 157(3), 593-602.

Shantz, A.A., Burkepile, D.E., 2014. Context-dependent effects of nutrient loading on the coral–algal mutualism. Ecology 95(7), 1995-2005.

Simister, R., Taylor, M.W., Tsai, P., Webster, N., 2012. Sponge-microbe associations survive high nutrients and temperatures. PLoS ONE 7(12).

Solorzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnology and Oceanography 25(4), 754-758.

Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography 53(3), 986-996.

Thacker, R.W., Freeman, C.J., 2012. Sponge-microbe symbioses: Recent advances and new directions. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, X. (Eds.), Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology, pp. 57-111.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7(3), 737-750.

Webster, N.S., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: love and other relationships. Environmental Microbiology 14(2), 335-346.

Weisz, J.B., Lindquist, N., Martens, C.S., 2008. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia 155(2), 367-376.

Yahel, G., Marie, D., Genin, A., 2005. InEx - a direct in situ method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography-Methods 3, 46-58.

Zhang, F., Vicente, J., Hill, R.T., 2014. Temporal changes in the diazotrophic bacterial communities associated with Caribbean sponges Ircinia stroblina and Mycale laxissima. Frontiers in Microbiology 5, 561.

Zhang, X., He, L.M., Zhang, F.L., Sun, W., Li, Z.Y., 2013. The Different Potential of Sponge Bacterial Symbionts in N-2 Release Indicated by the Phylogenetic Diversity and Abundance Analyses of Denitrification Genes, nirK and nosZ. PLoS ONE 8(6).

CHAPTER 6. FUTURE DIRECTIONS

I have shown that sponges can act as a foundation species in seagrass beds, increasing the diversity of both fish and invertebrates. Additionally, sponges impact the seagrass community by promoting seagrass growth and the establishment of additional seagrass species. Further I have shown that the impact of sponges on seagrass beds and other nearshore ecosystems is context dependent, ranging from beneficial to parasitic with shifting abiotic conditions. This work expands our understanding how sponges function within seagrass ecosystems and the first to suggest that sponges may act as foundation species in this system. In this chapter I will detail directions of future research I will undertake based on the findings detailed in previous chapters.

To date my work has largely focused on the effect of sponges on the members of the seagrass community. A next step in my research will be to determine the effect of seagrass on sponges. In particular, I will investigate the impact of seagrass density on the quantity and quality of food available to sponges and the consequences for survival, growth, and reproduction of sponges. The results of this work has suggested the potential for positive feedbacks between sponges and the structure and function of seagrass ecosystems. However, to understand these dynamics, the effect of the seagrass on sponge growth and reproduction must be quantified. Additionally, addressing questions of how sponges are impacted by seagrass will help clarify the mechanisms behind the non-linear relationship between *Halichondria melanadocia* abundance and *Thalassia testudinum* density (chapter 3).

I am also interested in determining how the context dependent nature of the *H. melanadocia-T. testudinum* interaction effects the rate of carbon storage in seagrass beds (building on the snapshot estimate I presented in chapter 4). In order to estimate the rate of carbon storage I will need several more pieces of information, including a measure of sponge effects on carbon storage in the sediments; *H. melanadocia* could impact sediment carbon storage in multiple ways including a direct effect of the production of sponge detritus (see below) or indirectly through the sponge's effect on seagrass shoot density. Seagrass shoot density impacts sediment carbon storage in two ways. The first is through the direct input of organic carbon through dead plant material. The second is through wave attenuation and particle deposition with denser seagrass causing high sedimentation rates (Gacia and Duarte, 2001; Gacia, et al., 1999). Additionally, in order to develop a dynamic model, I need to understand how the sponge-seagrass interaction in impacted by anthropogenic nutrient loading over longer temporal scales. Particularly, I need to determine if the system reaches a new equilibrium state with sponges and seagrass still present but at lower densities, effectively reducing the carbon storage capacity of the seagrass bed over the long term.

The results in the fifth chapter showed that *Ircinia felix* can serve as a sink for dissolved organic carbon (DOC), in the future I will investigate if this pathway significantly impacts energy cycling in seagrass ecosystems. A novel pathway of energy cycling, coined the "sponge loop" was described de Goeij, et al. (2013). This loop is formed by sponges' ability to feed on an energy source unavailable to the majority of the benthic community, DOC (de Goeij, et al., 2008; Yahel, et al., 2003). This source of energy, combined with viruses (Hadas, et al., 2006), ultra and picoplankton (Reiswig, 1971a), and photosynthate

provided by photosymbionts residing within some sponges (Freeman, et al., 2013; Wilkinson, 1979), provide the sponge with sufficient energy to grow rapidly. The majority of sponges do not grow rapidly, instead they have rapid cell turnover (de Goeij, et al., 2009) shedding their old cells which become detritus available to benthic detritivores. These detritivores are then preyed upon by consumers whose excretion provides nutrients that stimulate primary production in the system (Allgeier, et al., 2013; Burkepile, et al., 2013; Meyer, et al., 1983). These primary producers then act as a source of DOC consumed by sponges (Haas, et al., 2010; Haas, et al., 2011). Many of the links in the loop described above were shown using a pulse-chase experiment on a coral reef (de Goeij, et al., 2013). To date, no one has investigated whether a sponge loop is operating in other nearshore ecosystems despite rapid cell turnover (Alexander, et al., 2014) and DOC uptake (chapter 5), capabilities documented for common sponges in mangrove and seagrass ecosystems. Sediments in mangrove and seagrass ecosystems are richer in organic matter than those on coral reefs (Duarte, et al., 2005; Fourqurean, et al., 2012). Therefore, it is possible that shed sponge cells (hereafter sponge detritus) would not contribute significantly to the detrital food web in these systems. However, sponge detritus is high in both nitrogen and phosphorus content (Jasper de Geoij *personal communication*) making it a potentially high value food source for detritivores. Additionally, DOC availability is significantly higher in seagrass beds and mangrove ecosystems than on coral reefs (Mueller, et al., 2014; Ziegler and Benner, 1999). The results of my fifth chapter suggest that at least one common sponge in these systems (*Ircinia felix*) removes increasing amounts of DOC as ambient DOC increases. This, along with the report of high cell turnover in mangrove sponges compared to reef sponges

(Alexander, et al., 2014) suggests that the input of sponge detritus may be higher in mangrove and seagrass ecosystems than on reefs. If so, it is possible that the sponge loop could be an important, and to date undescribed, pathway of energy cycling in these ecosystems.

The strong correlation between ambient nutrient availability and the change in nutrients attributable to *Ircinia felix* reported in the fifth chapter suggests context dependence in the sponge-microbe interactions within the sponge. This context dependency is either being driven by shifts in the active microbial community, a change in the rate of transfer of products between the sponge and the microbial symbionts, or both. Because sponges can process such large volumes of water and are often such a large component of the benthic community, they are capable of strongly impacting biogeochemical cycles (Maldonado, et al., 2012; Pile, et al., 1997; Reiswig, 1971b). For example, Southwell, et al. (2008) reported a dissolved inorganic nitrogen (DIN) flux rate of 15 ± 3.0 mmol m^{-2} d^{-1} for the non-encrusting sponge community of a Florida Key's coral reef, a rate which is higher than all other measured benthic substrates on reefs. However, if sponge nutrient processing is strongly controlled by abiotic conditions, these results may not always accurately capture the impact of the sponge community on reef nitrogen cycling. The result of increasing nutrient uptake with increasing ambient nutrient availability suggests that, instead of always acting as a source of DIN, sponges may become a sink at high ambient nutrient conditions.

The next step will be to experimentally validate the observations presented in the fifth chapter. The correlations between ambient nutrient availability and the change in nutrient concentrations chapter 5 are observational, as a result this correlation could prove to be

spurious or the result of both variables being correlated with another unmeasured variable. By experimentally manipulating ambient nutrient availability, e.g., using labelled isotopes, will allow for a more detailed determination of the fate of nutrients within this species of sponge as ambient conditions change (Freeman, et al., 2013). Additionally, quantifying the active microbial community at the time of water sampling will shed light on the nature of the context dependent nature of the sponge-microbe symbioses (Fiore, et al., 2015). In particular, tracing the transfer of products with an isotope labelling study while simultaneously quantifying the active microbial community will allow me to determine to what degree the changes in nutrient output are a result of a shift in the active microbial community or simply the result of a change in the rate of product transfer between the microbial community and the sponge.

The results of my dissertation show that sponges can have a strong, but context dependent effect on tropical nearshore communities. This result is consistent with previous findings in these (Butler, et al., 1995; Peterson, et al., 2006; Slattery, et al., 2013; Southwell, et al., 2008; Wulff, 1984) and other systems (Dayton, 1972; Leys, et al., 2007). Sponges are a diverse group of organisms with over 8,000 species worldwide (Van Soest, et al., 2012). Sponges are predicted to become even more dominant as oceans become warmer and more acidic (Bell, et al., 2013) and overfishing reduces densities of spongivorous fishes (Loh, et al., 2015; Pawlik, et al., 2013). Therefore, there is an increasing urgency for developing a mechanistic understanding of how sponges impact the functioning of these systems.

REFERENCES

Alexander, B.E., Liebrand, K., Osinga, R., van der Geest, H.G., Admiraal, W., Cleutjens, J.P.M., Schutte, B., Verheyen, F., Ribes, M., van Loon, E., de Goeij, J.M., 2014. Cell Turnover and Detritus Production in Marine Sponges from Tropical and Temperate Benthic Ecosystems. PLoS ONE 9(10), e109486.

Allgeier, J.E., Yeager, L.A., Layman, C.A., 2013. Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. Ecology 94(2), 521-529.

Bell, J.J., Davy, S.K., Jones, T., Taylor, M.W., Webster, N.S., 2013. Could some coral reefs become sponge reefs as our climate changes? Global Change Biology, 2613–2624.

Burkepile, D.E., Allgeier, J.E., Shantz, A.A., Pritchard, C.E., Lemoine, N.P., Bhatti, L.H., Layman, C.A., 2013. Nutrient supply from fishes facilitates macroalgae and suppresses corals in a Caribbean coral reef ecosystem. Scientific Reports 3, DOI: 10.1038/srep01493.

Butler, M.J., Hunt, J.H., Herrnkind, W.F., Childress, M.J., Bertelsen, R., Sharp, W., Matthews, T., Field, J.M., Marshall, H.G., 1995. Cascading disturbances in Florida bay, USA: Cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. Marine Ecology Progress Series 129(1-3), 119-125.

Dayton, P.K., 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica, Proceedings of the colloquium on conservation problems in Antarctica. Allen Press Lawrence, Kansas, USA, pp. 81-96.

de Goeij, J.M., van den Berg, H., van Oostveen, M.M., Epping, E.H., Van Duyl, F.C., 2008. Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Marine Ecology Progress Series 357, 139.

de Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., de Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science 342(6154), 108-110.

de Goeij, J.M., de Kluijver, A., Van Duyl, F.C., Vacelet, J., Wijffels, R.H., de Goeij, A., Cleutjens, J.P.M., Schutte, B., 2009. Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding. Journal of Experimental Biology 212(23), 3892-3900.

Duarte, C.M., Middelburg, J.J., Caraco, N.F., 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2(1), 1-8.

Fiore, C.L., Labrie, M., Jarett, J.K., Lesser, M.P., 2015. Transcriptional activity of the giant barrel sponge, Xestospongia muta Holobiont: Molecular Evidence for Metabolic Interchange. Frontiers in Microbiology 6.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience 5(7), 505-509.

Freeman, C.J., Thacker, R.W., Baker, D.M., Fogel, M.L., 2013. Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? Isme Journal 7(6), 1116-1125.

Gacia, E., Duarte, C.M., 2001. Sediment retention by a mediterranean Posidonia oceanica meadow: The balance between deposition and resuspension. Estuarine Coastal and Shelf Science 52(4), 505-514.

Gacia, E., Granata, T.C., Duarte, C.M., 1999. An approach to measurement of particle flux and sediment retention within seagrass (Posidonia oceanica) meadows. Aquatic Botany 65(1–4), 255-268.

Haas, A.F., Naumann, M.S., Struck, U., Mayr, C., el-Zibdah, M., Wild, C., 2010. Organic matter release by coral reef associated benthic algae in the Northern Red Sea. J. Exp. Mar. Biol. Ecol. 389(1-2), 53-60.

Haas, A.F., Nelson, C.E., Kelly, L.W., Carlson, C.A., Rohwer, F., Leichter, J.J., Wyatt, A., Smith, J.E., 2011. Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. PLoS ONE 6(11).

Hadas, E., Marie, D., Shpigel, M., Ilan, M., 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51(3), 1548-1550.

Leys, S.P., Mackie, G.O., Reiswig, H.M., 2007. The biology of glass sponges. Advances in Marine Biology 52, 1-145.

Loh, T.-L., McMurray, S.E., Henkel, T.P., Vicente, J., Pawlik, J.R., 2015. Indirect effects of overfishing on Caribbean reefs: Sponges overgrow reef-building corals. PeerJ 3, e901.

Maldonado, M., Ribes, M., van Duyl, F.C., 2012. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. In: Becerro, M.A., Uriz, M.J., Maldonado, M., Turon, X. (Eds.), Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology, pp. 113-182.

Meyer, J.L., Schultz, E.T., Helfman, G.S., 1983. Fish schools: An asset to corals. Science 220(4601), 1047-1049.

Mueller, B., de Goeij, J.M., Vermeij, M.J.A., Mulders, Y., van der Ent, E., Ribes, M., van Duyl, F.C., 2014. Natural Diet of Coral-Excavating Sponges Consists Mainly of Dissolved Organic Carbon (DOC). PLoS ONE 9(2), e90152.

Pawlik, J.R., Loh, T., McMurray, S.E., Finelli, C.M., 2013. Sponge communities on Caribbean coral reefs are structured by factors that are top-down, not bottom-up. PLoS ONE 8(5), e62573.

Peterson, B.J., Chester, C.M., Jochem, F.J., Fourqurean, J.W., 2006. Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Marine Ecology Progress Series 328, 93-103.

Pile, A.J., Patterson, M.R., Savarese, M., Chernykh, V.I., Fialkov, V.A., 1997. Trophic effects of sponge feeding within Lake Baikal's littoral zone .2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography 42(1), 178-184.

Reiswig, H.M., 1971a. Particle feeding in natural populations of 3 marine Demosponges. Biological Bulletin 141(3), 568-591.

Reiswig, H.M., 1971b. In-situ pumping activities of tropical Demospongiae. Marine Biology 9(1), 38-50.

Slattery, M., Gochfeld, D.J., Easson, C.G., O'Donahue, L.R.K., 2013. Facilitation of coral reef biodiversity and health by cave sponge communities. Marine Ecology Progress Series 476, 71-+.

Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography 53(3), 986-996.

Van Soest, R.W.M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N.J., Santodomingo, N., Vanhoorne, B., Kelly, M., Hooper, J.N.A., 2012. Global Diversity of Sponges (Porifera). PLoS ONE 7(4), e35105.

Wilkinson, C., 1979. Nutrient translocation from symbiotic cyanobacteria to coral reef sponges. Biologie des spongiaires 291, 373-380.

Wulff, J.L., 1984. Sponge-mediated coral reef growth and rejuvenation. Coral Reefs 3(3), 157-163.

Yahel, G., Sharp, J.H., Marie, D., Hase, C., Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography 48(1), 141-149.

Ziegler, S., Benner, R., 1999. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Marine Ecology Progress Series 180, 149-160.

APPENDICES

APPENDIX A. SUPPLEMENTAL MATERIAL FOR CHAPTER 2

JAGS code for Bayesian Heirarchical model.

Seagrass growth:

```
model{
for (i in 1:length(y)){
y[i]~dgamma(a[i],b[i])
mu[i]<-
alpha[p[i]]+beta0[trt[p[i]]]+beta1[trt[p[i]]]*x1[t[i]]+beta2[trt[p[i]]]*x2[t[i]]+beta3[trt[p[i]]]*
x3[t[i]]+beta4[trt[p[i]]]*x4[t[i]]
tau1[i]<-summervar*varx[season[i]]+wintervar*varx1[season[i]]
a[i]<-(mu[i]^2)/tau1[i]
b[i]<-mu[i]/tau1[i]
}
for(j in 1:np){
alpha[j]~dnorm(0,1/tau2^2)
}
for (l in 1:ntrt){
beta0[l]~dnorm(45,1/b0^2)
beta1[l]~dnorm(0,1/b1^2)
beta2[l]~dnorm(0,1/b2^2)
beta3[l]~dnorm(0,1/b3^2)
beta4[l]~dnorm(0,1/b4^2)
}
summervar~dunif(1,1000)
wintervar~dunif(1,100)
tau2~dunif(1,100)
b0~dunif(1,100)
b1~dunif(1,100)
b2~dunif(1,100)
b3~dunif(1,100)
b4~dunif(1,100)
}
```

Seagrass shoot density and fish, macroinvertebrate, and macroalgae richness and abundance:

```
model{
for (i in 1:length(y)){
y[i]~dpois(lambda[i])
```

```
lambda[i]<-
alpha[p[i]]+beta0[trt[p[i]]]+beta1[trt[p[i]]]*x1[t[i]]+beta2[trt[p[i]]]*x2[t[i]]+beta3[trt[p[i]]]*
x3[t[i]]+beta4[trt[p[i]]]*x4[t[i]]
}
for(j in 1:np){
alpha[j]~dnorm(0,tau1)
}
for (l in 1:ntrt){
beta0[l]~dnorm(meany,b0)
beta1[l]~dnorm(0,b1)
beta2[l]~dnorm(0,b2)
beta3[l]~dnorm(0,b3)
beta4[l]~dnorm(0,b4)
}
tau1~dgamma(agam[1],agam[2])
b0~dgamma(agam[1],agam[2])
b1~dgamma(agam[1],agam[2])
b2~dgamma(agam[1],agam[2])
b3~dgamma(agam[1],agam[2])
b4~dgamma(agam[1],agam[2])
}
```

Table A1. Effective sample sizes for all model parameters for all models.

Parameter		Response Variable									
		Shoot density				Richness			Abundance		
		Growth	*T. testudinum*	*H. wrightii* & *S. filliforme*	total seagrass	Fish	Invert	Algae	Fish	Invert	Algae
Alpha	1	28395	12562	5565	14250	-	-	-	-	-	-
	2	16360	16830	2555	15742	-	-	-	-	-	-
	3	24807	11779	9482	14775	-	-	-	-	-	-
	4	24671	12507	8689	14441	-	-	-	-	-	-
	5	15965	15128	3245	14346	-	-	-	-	-	-
	6	20459	13294	1033	20810	-	-	-	-	-	-
	7	19168	8963	2402	17622	-	-	-	-	-	-
	8	21535	16675	780	22905	-	-	-	-	-	-
	9	16085	11552	1794	19584	-	-	-	-	-	-
	10	22314	13478	2256	21472	-	-	-	-	-	-
	11	16800	16329	12260	17151	-	-	-	-	-	-
	12	21428	14476	13913	15272	-	-	-	-	-	-
	13	23188	20587	11340	17265	-	-	-	-	-	-
	14	26076	13846	13007	13956	-	-	-	-	-	-
	15	22319	14962	10685	14239	-	-	-	-	-	-
Beta0	Control	4251	5462	2748	6151	9536	20265	22651	13293	11829	5647
	S. Control	4815	5319	714	10560	12012	18372	21553	20425	7900	26167
	Sponge	4527	8668	6762	6823	25708	21825	26714	32685	7148	14431
Beta1	Control	7290	37951	12484	42121	19636	37582	43519	29525	17343	4026
	S. Control	10877	46331	12168	34316	32580	34658	40732	41491	15693	33683
	Sponge	6709	41228	18868	27287	41430	36689	45056	49028	8811	5078

Table A1. Continued

Parameter		Response Variable									
			Shoot density			Richness			Abundance		
		Growth	*T. testudinum*	*H. wrightii* & *S. filliformе*	total seagrass	Fish	Invert	Algae	Fish	Invert	Algae
Beta2	Control	5194	15248	4295	38471	13375	33367	37735	18258	14719	5526
	S. Control	6302	36007	4861	48824	28278	26176	35135	36138	8961	10477
	Sponge	5501	39982	18747	27618	43127	38529	41084	48317	10119	29659
Beta3	Control	8962	33729	11279	53012	17050	35399	42921	22523	22212	13742
	S. Control	10850	28651	13788	14018	16386	33465	35949	31794	14653	42717
	Sponge	7931	33347	16111	18846	42700	33418	47454	45336	9231	32892
Beta4	Control	5152	29065	9672	46390	19548	29843	35172	27351	21929	11291
	S. Control	6325	28763	12170	32406	30304	37854	36742	46806	11114	39848
	Sponge	5714	36218	19949	40731	45700	32873	46113	57761	9954	30222

Table A2. Standard error for all model parameters for all models.

Parameter		Response Variable									
		Shoot density				Richness			Abundance		
		Growth	*T. testudinum*	*H. wrightii* & *S. filliforme*	total seagrass	Fish	Invert	Algae	Fish	Invert	Algae
Alpha	1	6.99E-05	1.31E-04	1.28E-04	1.22E-04	-	-	-	-	-	-
	2	1.13E-04	1.12E-04	2.15E-04	1.23E-04	-	-	-	-	-	-
	3	7.23E-05	1.35E-04	1.01E-04	1.22E-04	-	-	-	-	-	-
	4	7.81E-05	1.25E-04	1.13E-04	1.24E-04	-	-	-	-	-	-
	5	1.16E-04	1.18E-04	1.66E-04	1.26E-04	-	-	-	-	-	-
	6	8.38E-05	1.26E-04	4.80E-04	7.89E-05	-	-	-	-	-	-
	7	8.99E-05	1.62E-04	3.19E-04	8.82E-05	-	-	-	-	-	-
	8	8.16E-05	1.10E-04	5.76E-04	7.79E-05	-	-	-	-	-	-
	9	1.10E-04	1.40E-04	3.79E-04	8.45E-05	-	-	-	-	-	-
	10	7.95E-05	1.26E-04	3.30E-04	8.12E-05	-	-	-	-	-	-
	11	1.11E-04	9.78E-05	5.58E-05	1.01E-04	-	-	-	-	-	-
	12	8.60E-05	1.02E-04	6.11E-05	1.10E-04	-	-	-	-	-	-
	13	7.54E-05	8.12E-05	6.10E-05	1.02E-04	-	-	-	-	-	-
	14	6.73E-05	1.05E-04	6.12E-05	1.17E-04	-	-	-	-	-	-
	15	7.93E-05	1.04E-04	6.06E-05	1.17E-04	-	-	-	-	-	-
Beta0	Control	6.71E-04	1.70E-04	1.39E-04	1.74E-04	3.38E-05	3.38E-05	2.53E-05	2.53E-05	2.56E-05	8.00E-05
	S. Control	5.28E-04	1.75E-04	5.52E-04	8.43E-05	2.50E-05	2.50E-05	2.83E-05	2.83E-05	2.64E-05	6.88E-05
	Sponge	5.76E-04	1.01E-04	5.88E-05	1.45E-04	1.98E-05	1.98E-05	2.61E-05	2.61E-05	2.33E-05	6.96E-05
Beta1	Control	4.39E-04	1.82E-05	2.28E-05	1.89E-05	2.43E-05	2.43E-05	2.24E-05	2.24E-05	2.08E-05	7.08E-05
	S. Control	2.54E-04	1.49E-05	1.93E-05	2.41E-05	1.64E-05	1.64E-05	2.15E-05	2.15E-05	2.15E-05	3.61E-05
	Sponge	4.98E-04	1.70E-05	1.70E-05	3.28E-05	1.75E-05	1.75E-05	2.16E-05	2.16E-05	2.08E-05	5.96E-05

Table A2. continued

Parameter		Response Variable									
		Shoot density				Richness			Abundance		
		Growth	*T. testudinum*	*H. wrightii* & *S. filliforme*	total seagrass	Fish	Invert	Algae	Fish	Invert	Algae
Beta2	Control	5.73E-04	8.17E-05	1.01E-04	2.16E-05	3.12E-05	3.12E-05	2.18E-05	2.18E-05	2.35E-05	4.97E-05
	S. Control	4.05E-04	2.40E-05	5.11E-05	1.58E-05	1.72E-05	1.72E-05	2.70E-05	2.70E-05	2.50E-05	3.74E-05
	Sponge	4.79E-04	2.07E-05	2.86E-05	3.31E-05	1.79E-05	1.79E-05	2.03E-05	2.03E-05	2.31E-05	2.72E-05
Beta3	Control	3.59E-04	4.25E-05	4.49E-05	2.13E-05	2.66E-05	2.66E-05	2.22E-05	2.22E-05	2.10E-05	7.04E-05
	S. Control	2.85E-04	5.09E-05	2.83E-05	1.22E-04	2.43E-05	2.43E-05	2.29E-05	2.29E-05	2.43E-05	7.87E-05
	Sponge	4.59E-04	3.98E-05	4.98E-05	7.08E-05	1.99E-05	1.99E-05	2.88E-05	2.88E-05	2.04E-05	8.91E-05
Beta4	Control	5.63E-04	4.90E-05	6.87E-05	3.38E-05	2.48E-05	2.48E-05	2.52E-05	2.52E-05	2.50E-05	7.49E-05
	S. Control	4.07E-04	4.87E-05	2.86E-05	4.70E-05	1.72E-05	1.72E-05	2.42E-05	2.42E-05	2.41E-05	8.46E-05
	Sponge	4.71E-04	3.61E-05	4.75E-05	3.86E-02	1.86E-05	1.86E-05	3.21E-05	3.21E-05	2.17E-05	8.20E-05

Table A.3. Priors.

	Response Variable									
		Shoot density			Richness			Abundance		
Parameter	**Growth**	***T. testudinum***	***H. wrightii* & *S. filliforme***	**total seagrass**	**Fish**	**Invert**	**Algae**	**Fish**	**Invert**	**Algae**
α_{ij}	~norm(0,1/τ_1^2)	~norm(0,1/τ_1^2)	~norm(0,1/τ_1^2)	~norm(0,1/τ_1^2)						
τ_1	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)
β0	~norm(0,1/b_0^2)	~norm(y_0,1/b_0^2)	~norm(y_0,1/b_0^2)	~norm(y_0,1/b_0^2)	~norm(y_0,sd_0)	~norm(y_0,sd_0)	~norm(y_0,sd_0)	~norm(y_0,sd_0)	~norm(y_0,sd_0)	~norm(y_0,sd_0)
β1	~norm(0,1/b_1^2)	~norm(0,1/b_1^2)	~norm(0,1/b_1^2)	~norm(0,1/b_1^2)	~norm(0,b_1)	~norm(0,b_1)	~norm(0,b_1)	~norm(0,b_1)	~norm(0,b_1)	~norm(0,b_1)
β5	~norm(0,1/b_5^2)	~norm(0,1/b_5^2)	~norm(0,1/b_5^2)	~norm(0,1/b_5^2)	~norm(0,b_5)	~norm(0,b_5)	~norm(0,b_5)	~norm(0,b_5)	~norm(0,b_5)	~norm(0,b_5)
β12	~norm(0,1/b_{12}^2)	~norm(0,1/b_{12}^2)	~norm(0,1/b_{12}^2)	~norm(0,1/b_{12}^2)	~norm(0,b_{12})	~norm(0,b_{12})	~norm(0,b_{12})	~norm(0,b_{12})	~norm(0,b_{12})	~norm(0,b_{12})
β17	~norm(0,1/b_{17}^2)	~norm(0,1/b_{17}^2)	~norm(0,1/b_{17}^2)	~norm(0,1/b_{17}^2)	~norm(0,b_{17})	~norm(0,b_{17})	~norm(0,b_{17})	~norm(0,b_{17})	~norm(0,b_{17})	~norm(0,b_{17})
b_0	~gamma(1,.1)	~gamma(6,.1)	~gamma(9,1)	~gamma(7,.1)						
b_1	~gamma(1,.1)	~gamma(6,.1)	~gamma(9,1)	~gamma(7,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)
b_5	~gamma(1,.1)	~gamma(6,.1)	~gamma(9,1)	~gamma(7,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)
b_{12}	~gamma(1,.1)	~gamma(6,.1)	~gamma(9,1)	~gamma(7,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)
b_{17}	~gamma(1,.1)	~gamma(6,.1)	~gamma(9,1)	~gamma(7,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)	~gamma(1,.1)
summer variance	~gamma(6,.03)									
winter variance	~gamma(50,1)									
y_0		30	15	35	2	2	2	2	4	30
sd_0					0.4	0.4	0.4	0.4	0.4	10

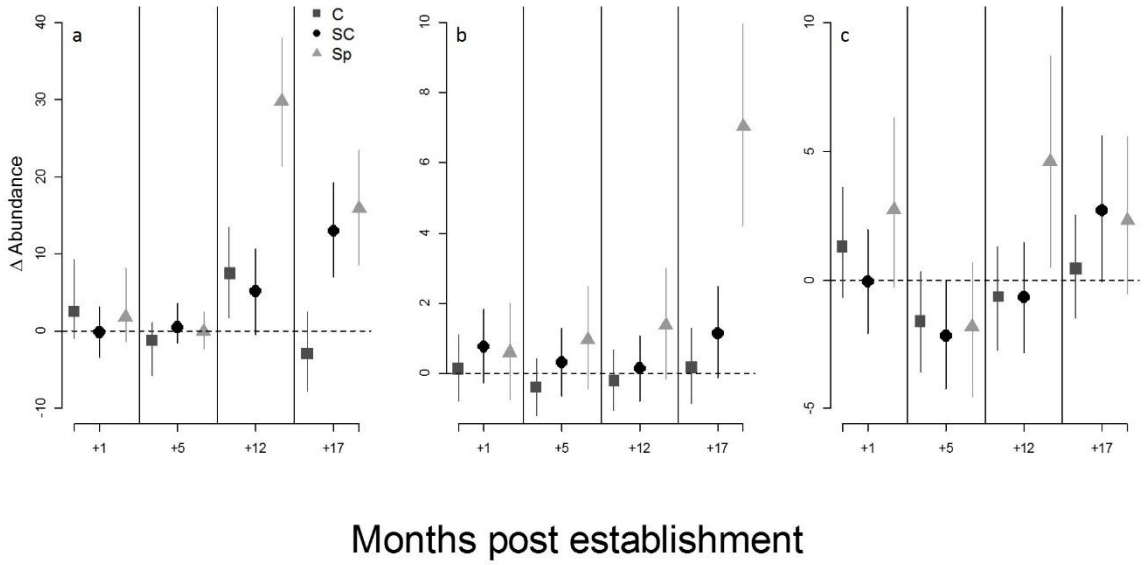

Figure S1. Output from Bayesian hierarchical models describing (a) macroalgae (b) fish and (c) macroinvertebrate abundance (per m^2). The plotted points represent the mean Markov chain Monte Carlo estimate of the treatment specific change in each variable at the indicated time post experiment establishment. The error bars represent the high density interval (HDI). The change is significant if the HDI does not cross the dotted line.

APPENDIX B. SUPPLEMENTAL MATERIAL FOR CHAPTER 4

Model code for carbon simulation model.

```
carbon.sim.parasite<-function(data.sponge,reps,size,data.sd){
ca.ab<-a_b_function((data.sponge$Sponge*.04))
plots<-rep(-99,reps)
for(i in 1:reps){
plots[i]<-plots.sponge(ca.ab,size)
}
blades<-rep(-99,size)
sheaths<-rep(-99,size)
rhizomes<-rep(-99,size)
roots<-rep(-99,size)
total<-rep(-99,reps)
total.commensal<-rep(-99,reps)
blades.c<-rep(-99,size)
sheaths.c<-rep(-99,size)
rhizomes.c<-rep(-99,size)
roots.c<-rep(-99,size)
for(i in 1:reps){
shoots<-rnbinom(n=size,mu=data.sd$Shoots,40)
end<-plots[i]
end.1<-plots[i]+1
shoots.sponges<-shoots[1:end]
shoots.sponge<-round(shoots.sponges+(deltasd[1,1]+shoots.sponges*deltasd[1,2]))
shoots.nosponge<-shoots[end.1:length(shoots)]
blades.sponge<-parasitesponge.biomass[1,1]+parasitesponge.biomass[1,2]*shoots.sponge
sheaths.sponge<-parasitesponge.biomass[2,1]+parasitesponge.biomass[2,2]*shoots.sponge
rhizomes.sponge<-parasitesponge.biomass[3,1]+parasitesponge.biomass[3,2]*shoots.sponge
roots.sponge<-parasitesponge.biomass[4,1]+parasitesponge.biomass[4,2]*shoots.sponge
blades<-parasite.biomass[1,1]+parasite.biomass[1,2]*shoots.nosponge
sheaths<-parasite.biomass[2,1]+parasite.biomass[2,2]*shoots.nosponge
rhizomes<-parasite.biomass[3,1]+parasite.biomass[3,2]*shoots.nosponge
roots<-parasite.biomass[4,1]+parasite.biomass[4,2]*shoots.nosponge
rhizomes.carbon<-rnorm(size,carbon.data[1,1],carbon.data[1,2])
blades.carbon<-rnorm(size,carbon.data[2,1],carbon.data[2,2])
roots.carbon<-rnorm(size,carbon.data[3,1],carbon.data[3,2])
sheaths.carbon<-rnorm(size,carbon.data[4,1],carbon.data[4,2])
blades<-c(blades.sponge,blades)
sheaths<-c(sheaths.sponge,sheaths)
```

```
rhizomes<-c(rhizomes.sponge,rhizomes)
roots<-c(roots.sponge,roots)
r.carbon<-rhizomes*rhizomes.carbon
b.carbon<-blades*blades.carbon
rt.carbon<-roots*roots.carbon
s.carbon<-sheaths*sheaths.carbon
total[i]<-sum(r.carbon+b.carbon+rt.carbon+s.carbon)
}
total<-total/1000
return(total)
}

carbon.sim<-function(data.sponge,reps,size,data.sd){
ca.ab<-a_b_function((data.sponge$Sponge*.04))
plots<-rep(-99,reps)
for(i in 1:reps){
plots[i]<-plots.sponge(ca.ab,size)
}
blades<-rep(-99,size)
sheaths<-rep(-99,size)
rhizomes<-rep(-99,size)
roots<-rep(-99,size)
total<-rep(-99,reps)
total.commensal<-rep(-99,reps)
blades.c<-rep(-99,size)
sheaths.c<-rep(-99,size)
rhizomes.c<-rep(-99,size)
roots.c<-rep(-99,size)
for(i in 1:reps){
shoots<-rnbinom(n=size,mu=data.sd$Shoots,40)
blades.c<-commensal.biomass[1,1]+commensal.biomass[1,2]*shoots
sheaths.c<-commensal.biomass[2,1]+commensal.biomass[2,2]*shoots
rhizomes.c<-commensal.biomass[3,1]+commensal.biomass[3,2]*shoots
roots.c<-commensal.biomass[4,1]+commensal.biomass[4,2]*shoots
rhizomes.carbon<-rnorm(size,carbon.data[1,1],carbon.data[1,2])
blades.carbon<-rnorm(size,carbon.data[2,1],carbon.data[2,2])
roots.carbon<-rnorm(size,carbon.data[3,1],carbon.data[3,2])
sheaths.carbon<-rnorm(size,carbon.data[4,1],carbon.data[4,2])
r.carbon<-rhizomes.c*rhizomes.carbon
b.carbon<-blades.c*blades.carbon
rt.carbon<-roots.c*roots.carbon
s.carbon<-sheaths.c*sheaths.carbon
total.commensal[i]<-sum(r.carbon+b.carbon+rt.carbon+s.carbon)
```

```
}
total.commensal<-total.commensal/1000
return(total.commensal)
}
#################################
### actual code to build model###
#################################
############################
# get linear relationships#
############################
setwd("C:\\Users\\Stephanie\\Dropbox\\Manuscripts\\SGSP_factorialexperiment")
require(xlsx)
grass<-read.xlsx("coredata.xlsx",sheetName="Sheet1")
sponge<-subset(grass,grass$Sponge=="sponge")
no.sponge<-subset(grass,grass$Sponge=="no sponge")
sponge.fert<-subset(sponge,sponge$Fertilizer=="Fert")
nosponge.fert<-subset(no.sponge,no.sponge$Fertilizer=="Fert")
nofert<-subset(grass,grass$Fertilizer=="No Fert")
#change in shoot density
delta.sd.sponge<-lm(deltaSD~Initial_ShootDensity,data=sponge.fert)
delta.sd.nosponge<-lm(deltaSD~Initial_ShootDensity,data=nosponge.fert)
matrixnames<-list(c("Sponge","No Sponge"),c("Intercept","Slope"))
deltasd<-
matrix(dimnames=matrixnames,nrow=2,ncol=2,byrow=T,c(delta.sd.sponge$coefficients,delt
a.sd.nosponge$coefficients))
#commensalism biomass
blades<-lm(blades_dw~Final_ShootDensity,data=nofert)
sheaths<-lm(sheaths_dw~Final_ShootDensity,data=nofert)
rhizomes<-lm(rhizomes_dw~Final_ShootDensity,data=nofert)
roots<-lm(roots_dw~Final_ShootDensity,data=nofert)
summary(blades)
summary(sheaths)
summary(rhizomes)
summary(roots)
coefficient.list<-
c(blades$coefficients,sheaths$coefficients,rhizomes$coefficients,roots$coefficients)
matrixnames<-list(c("blades","sheaths","rhizomes","roots"),c("Intercept","Slope"))
commensal.biomass<-
matrix(nrow=4,ncol=2,dimnames=matrixnames,byrow=T,coefficient.list)
#parasite sponge biomass
blades<-lm(blades_dw~Final_ShootDensity,data=sponge.fert)
sheaths<-lm(sheaths_dw~Final_ShootDensity,data=sponge.fert)
rhizomes<-lm(rhizomes_dw~Final_ShootDensity,data=sponge.fert)
```

```
roots<-lm(roots_dw~Final_ShootDensity,data=sponge.fert)
summary(blades)
summary(sheaths)
summary(rhizomes)
summary(roots)
coefficient.list<-
c(blades$coefficients,sheaths$coefficients,rhizomes$coefficients,roots$coefficients)
parasitesponge.biomass<-
matrix(nrow=4,ncol=2,dimnames=matrixnames,byrow=T,coefficient.list)
#parasite no sponge biomass
blades<-lm(blades_dw~Final_ShootDensity,data=nosponge.fert)
sheaths<-lm(sheaths_dw~Final_ShootDensity,data=nosponge.fert)
rhizomes<-lm(rhizomes_dw~Final_ShootDensity,data=nosponge.fert)
roots<-lm(roots_dw~Final_ShootDensity,data=nosponge.fert)
summary(blades)
summary(sheaths)
summary(rhizomes)
summary(roots)
coefficient.list<-
c(blades$coefficients,sheaths$coefficients,rhizomes$coefficients,roots$coefficients)
parasite.biomass<-matrix(nrow=4,ncol=2,dimnames=matrixnames,byrow=T,coefficient.list)
rhizome.carbon<-
c(36.99,31.09,33.29,31.51,34.91,31.65,36.75,36.38,33.18,35.06,38.52,31.51,36.15,37.05,32.
40,34.03,36.02,32.44,35.76,31.63,30.74)
blade.carbon<-
c(37.50,36.52,32.23,39.53,42.95,40.10,38.80,39.70,39.74,41.04,38.64,39.85,38.35,37.41,38.
47,40.42,36.13,38.54,37.95,37.88,36.69,31.84)
roots.carbon<-
c(31.25,32.32,29.26,29.70,30.81,29.55,34.28,33.04,29.69,33.57,32.94,32.73,30.21,31.26,32.
15,32.99,34.14,32.01,30.85,32.40,29.11)
sheaths.carbon<-
c(35.16,30.31,29.75,34.35,31.90,33.68,36.59,33.09,34.91,34.14,31.74,36.44,35.10,32.89,36.
99,30.53,33.23,34.30,33.62,33.01)
rhizome.carbon<-rhizome.carbon/100
blade.carbon<-blade.carbon/100
roots.carbon<-roots.carbon/100
sheaths.carbon<-sheaths.carbon/100
rc.mean<-mean(rhizome.carbon)
bc.mean<-mean(blade.carbon)
rtc.mean<-mean(roots.carbon)
sc.mean<-mean(sheaths.carbon)
rc.sd<-sd(rhizome.carbon)
bc.sd<-sd(blade.carbon)
```

```
rtc.sd<-sd(roots.carbon)
sc.sd<-sd(sheaths.carbon)
carbon.data<-
matrix(nrow=4,ncol=2,c(rc.mean,bc.mean,rtc.mean,sc.mean,rc.sd,bc.sd,rtc.sd,sc.sd))
##############
# simulation #
##############
simdata.sponge<-read.xlsx("SGSP_site.xlsx",sheetName="Sponge")
simdata.sd<-read.xlsx("SGSP_site.xlsx",sheetName="ShootDensity")
summary(simdata.sponge)
summary(simdata.sd)
CA.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Camp Abaco")
CA.sd<-subset(simdata.sd,simdata.sd$Site=="Camp Abaco")
JC.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Jungle Creek")
JC.sd<-subset(simdata.sd,simdata.sd$Site=="Jungle Creek")
N.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Nursery")
N.sd<-subset(simdata.sd,simdata.sd$Site=="Nursery")
SC.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Snake Cay")
SC.sd<-subset(simdata.sd,simdata.sd$Site=="Snake Cay")
TC.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Treasure Cay")
TC.sd<-subset(simdata.sd,simdata.sd$Site=="Treasure Cay")
TB.sponge<-subset(simdata.sponge,simdata.sponge$Site=="Turtle Beach")
TB.sd<-subset(simdata.sd,simdata.sd$Site=="Turtle Beach")
source("sponge_plot_functions.R")
source("carbon_sim_functions.R")

######################
###running the sim###
######################
TC.area<-226800
N.area<-29700
CA.area<-206100
JC.area<-19800
TB.area<-36900+9900
SC.area<-192600
TC.size<-TC.area/.04
TC.parasite<-carbon.sim.parasite(TC.sponge,1000,TC.size,TC.sd)
write.csv(TC.parasite,"TCparasite.csv")
TC.commensal<-carbon.sim(TC.sponge,1000,TC.size,TC.sd)
par(mfrow=c(2,1))
hist(TC.parasite)
hist(TC.commensal)
N.size<-N.area/.04
```

```
N.parasite<-carbon.sim.parasite(N.sponge,1000,N.size,N.sd)
write.csv(TC.commensal,"TCcommensal.csv")
write.csv(N.parasite,"Nparasite.csv")
N.commensal<-carbon.sim(N.sponge,1000,N.size,N.sd)
CA.size<-CA.area/.04
CA.parasite<-carbon.sim.parasite(CA.sponge,1000,CA.size,CA.sd)
write.csv(N.commensal,"Ncommensal.csv")
write.csv(CA.parasite,"CAparasite.csv")
CA.commensal<-carbon.sim(CA.sponge,1000,CA.size,CA.sd)
write.csv(CA.commensal,"CAcommensal.csv")
JC.size<-JC.area/.04
JC.parasite<-carbon.sim.parasite(JC.sponge,1000,JC.size,JC.sd)
write.csv(JC.parasite,"JCparasite.csv")
JC.commensal<-carbon.sim(JC.sponge,1000,JC.size,JC.sd)
write.csv(JC.commensal,"JCcommensal.csv")
TB.size<-TB.area/.04
TB.parasite<-carbon.sim.parasite(TB.sponge,1000,TB.size,TB.sd)
TB.commensal<-carbon.sim(TB.sponge,1000,TB.size,TB.sd)
hist(TB.parasite)
hist(TB.commensal)
write.csv(TB.parasite,"TBparasite.csv")
write.csv(TB.commensal,"TBcommensal.csv")
SC.size<-SC.area/.04
SC.parasite<-carbon.sim.parasite(SC.sponge,1000,SC.size,SC.sd)
SC.commensal<-carbon.sim(SC.sponge,1000,SC.size,SC.sd)
write.csv(SC.parasite,"SCparasite.csv")
write.csv(SC.commensal,"SCcommensal.csv")
```

APPENDIX C. SUPPLEMENTAL MATERIAL FOR CHAPTER 5

Table C1. The top 20 best fit models for ΔDOC and coefficients for the predictor variables

	Light			Ambient nutrients				Temperature				Sponge		Location				
Rank	Max	Median	Light at time of sampling	DOC	NH_4^+	NO_x^-	SRP	Max	Median	Min	Var	Temp at time of sampling	Tube clearance time	Volume	Island Reef	AICc	Δ AICc	Weight
1				-0.88							114.85			12.35		579.38	0.00	1.00
2			0.02	-0.89							128.73			12.33		580.17	0.79	0.67
3				-0.88		54.78					115.26			11.96		580.36	0.98	0.61
4			0.02	-0.89		64.67					131.49			11.86		580.60	1.21	0.54
5				-0.94						6.56	136.93			11.08		580.96	1.57	0.46
6				-0.87							117.89		-0.28	12.21		581.11	1.73	0.42
7				-0.93							126.76	5.33		11.29		581.21	1.83	0.40
8			0.02	-0.88							136.06		-0.39	12.12		581.22	1.83	0.40
9				-0.92					4.48		125.26			11.51		581.46	2.07	0.36
10			0.02	-0.88		67.17					139.30		-0.41	11.62		581.51	2.13	0.34
11				-0.90	9.32						128.50			12.67		581.55	2.16	0.34
12				-0.91				2.88			115.65			11.87		581.73	2.35	0.31
13				-0.88			-3.96				115.25			12.19		581.91	2.53	0.28
14				-0.87		55.11					118.33		-0.28	11.81		582.17	2.78	0.25
15				-0.93		50.00				5.59	134.04			10.91		582.32	2.94	0.23
16				-0.65										8.55		582.44	3.06	0.22
17			0.01	-0.92						3.82	139.30			11.59		582.57	3.18	0.20
18				-0.91		49.88					124.51	4.16		11.17		582.61	3.22	0.20
19			0.01	-0.91							133.92	3.05		11.72		582.64	3.26	0.20
20				-0.91		51.94			3.63		123.68			11.30		582.72	3.34	0.19

Table C2. The top 20 best fit models for ΔNH_4^+ and coefficients for the predictor variables

	Light			Ambient nutrients				Temperature				Sponge		Location				
Rank	**Max**	**Median**	**Light at time of sampling**	**DOC**	**NH_4^+**	**NO_x^-**	**SRP**	**Max**	**Median**	**Min**	**Var**	**Temp at time of sampling**	**Tube clearance time**	**Volume**	**Island Reef**	**AICc**	**Δ AICc**	**Weight**
1				-0.0027	-1.19					0.39	1.05					-39.29	0.00	0.022
2				-0.0028	-1.19		-0.14			0.38	1.04					-38.46	0.82	0.015
3				-0.0028	-1.14					0.37	1.14			0.04		-38.27	1.02	0.013
4					-1.21					0.33						-38.18	1.11	0.013
5					-1.16					0.34	0.47					-37.90	1.38	0.011
6				-0.0028	-1.18					0.40	1.06		0.0008			-37.85	1.43	0.011
7			0.000008	-0.0027	-1.19					0.39	1.05					-37.85	1.44	0.011
8				-0.0027	-1.19	0.01				0.39	1.04					-37.68	1.60	0.010
9					-1.12				0.31							-37.34	1.95	0.008
10				-0.0015	-1.18				0.34							-37.18	2.11	0.008
11					-1.10							0.30				-37.13	2.16	0.008
12	0.00				-1.16					0.34						-37.06	2.22	0.007
13				-0.0028	-1.13				0.36		0.59					-37.00	2.29	0.007
14	0.00			-0.0018	-1.17					0.37						-37.00	2.29	0.007
15		0.00			-1.18					0.32						-36.92	2.37	0.007
16				-0.0029	-1.14		-0.13			0.36	1.12			0.03		-36.89	2.39	0.007
17					-1.20		-0.14			0.32						-36.88	2.41	0.007
18					-1.11					0.32	0.55			0.03		-36.84	2.45	0.007
19				-0.0030	-1.18		-0.16			0.39	1.05		0.0013			-36.84	2.45	0.007
20					-1.16		-0.11			0.33	0.45					-36.81	2.48	0.006

Table C3. The top 20 best fit models for ΔNO_x^- and coefficients for the predictor variables

	Light			Ambient nutrients				Temperature				Sponge	Location				
Rank	Max	Median	Light at time of sampling	DOC	NH_4^+	NO_x^-	SRP	Max	Median	Min	Var	Temp at time of sampling	Tube clearance time	Volume	Island Reef	AICc	Δ AICc	Weight
1		-0.00008		-0.0006		-0.98								0.02		-39.29	0.00	0.022
2		-0.00012				-0.99								0.02		-38.46	0.82	0.015
3		-0.00009		-0.0006		-0.96	-0.06							0.02		-38.27	1.02	0.013
4		-0.00008		-0.0006		-0.98							-0.0009	0.02		-38.18	1.11	0.013
5		-0.00010		-0.0006		-0.94	-0.07									-37.90	1.38	0.011
6		-0.00012				-0.98							-0.0010	0.02		-37.85	1.43	0.011
7				-0.0009		-0.96								0.02		-37.85	1.44	0.011
8		-0.00010		-0.0006		-0.97										-37.68	1.60	0.010
9		-0.00013				-0.97										-37.34	1.95	0.008
10		-0.00008	-0.00002	-0.0006		-0.99								0.02		-37.18	2.11	0.008
11		-0.00013				-0.97	-0.05							0.02		-37.13	2.16	0.008
12		-0.00014				-0.95	-0.07									-37.06	2.22	0.007
13		-0.00009				-0.96		-0.02					-0.0012	0.02		-37.00	2.29	0.007
14		-0.00013				-0.97							-0.0010			-37.00	2.29	0.007
15		-0.00008		-0.0006		-0.97			-0.01					0.02		-36.92	2.37	0.007
16		-0.00008		-0.0006	-0.02	-0.97								0.02		-36.89	2.39	0.007
17		-0.00009		-0.0005		-0.97							-0.0009			-36.88	2.41	0.007
18		-0.00010				-0.96			-0.02				-0.0013	0.02		-36.84	2.45	0.007
19				-0.0006		-0.94		-0.02					-0.0012	0.03		-36.84	2.45	0.007
20		-0.00008		-0.0006		-0.97				-0.01				0.02		-36.81	2.48	0.006

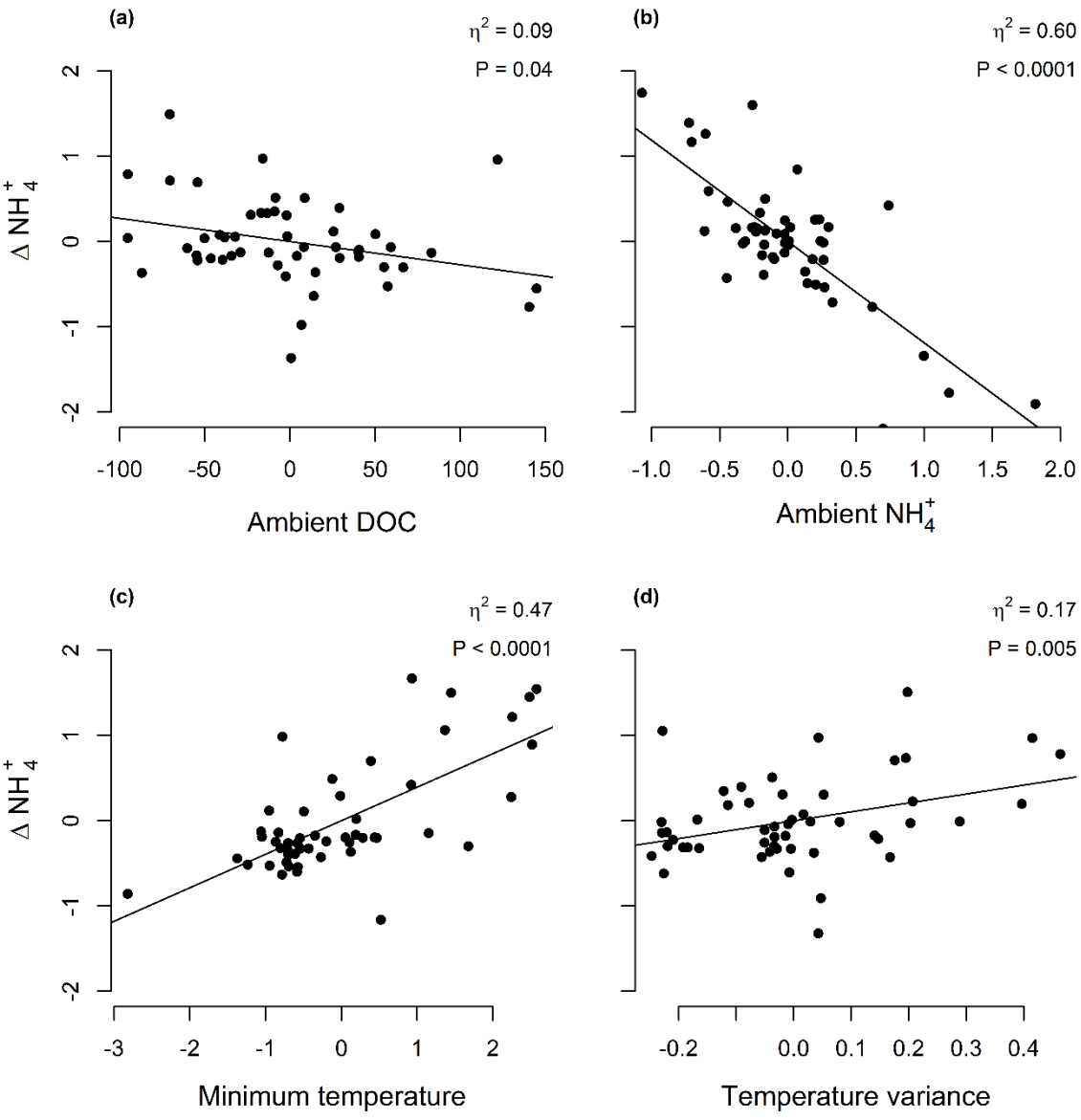

Figure C1. Partial regressions for the best fit ammonium model.

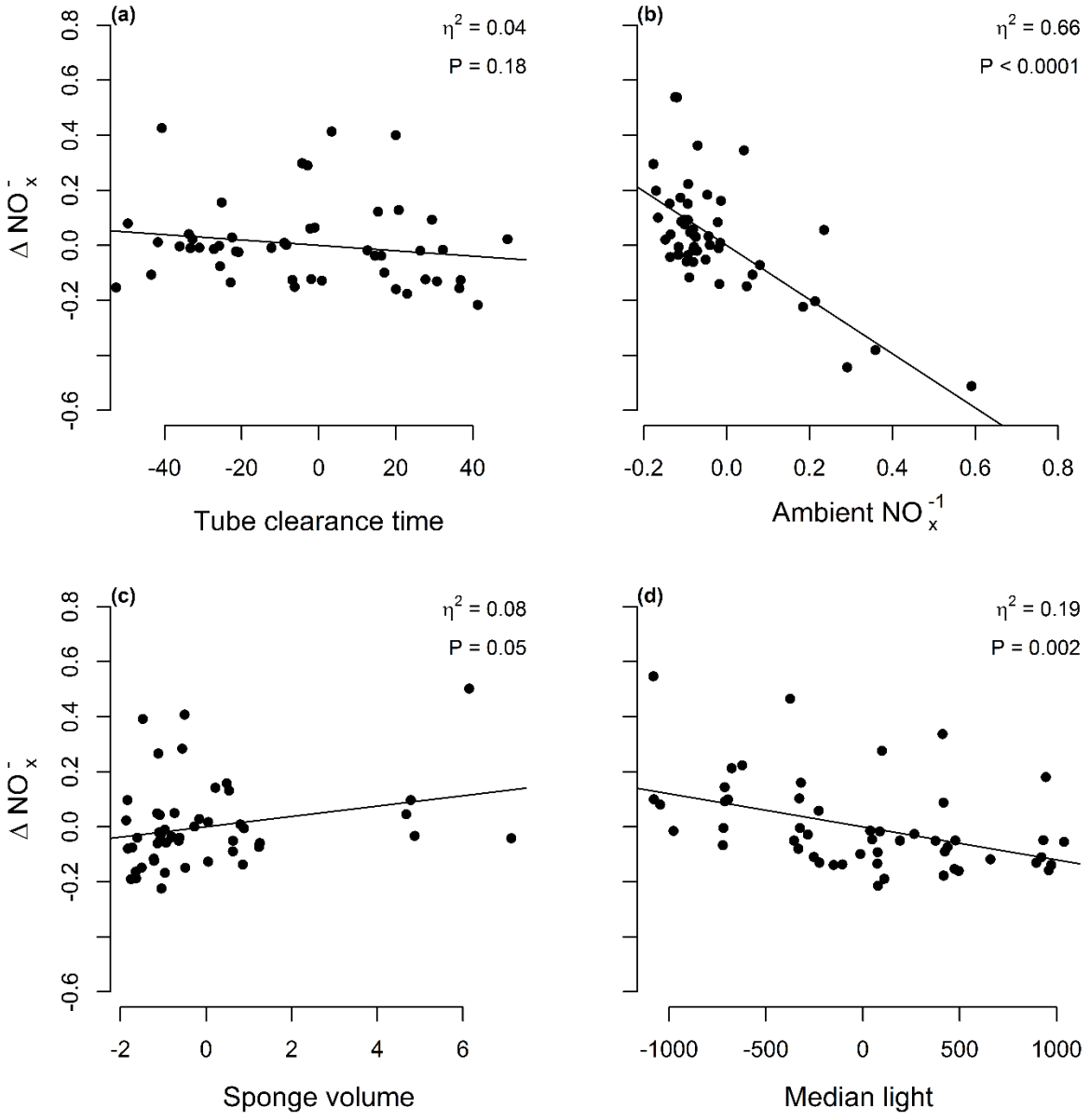

Figure C2. Partial regressions for the best fit nitrate/nitrite model.

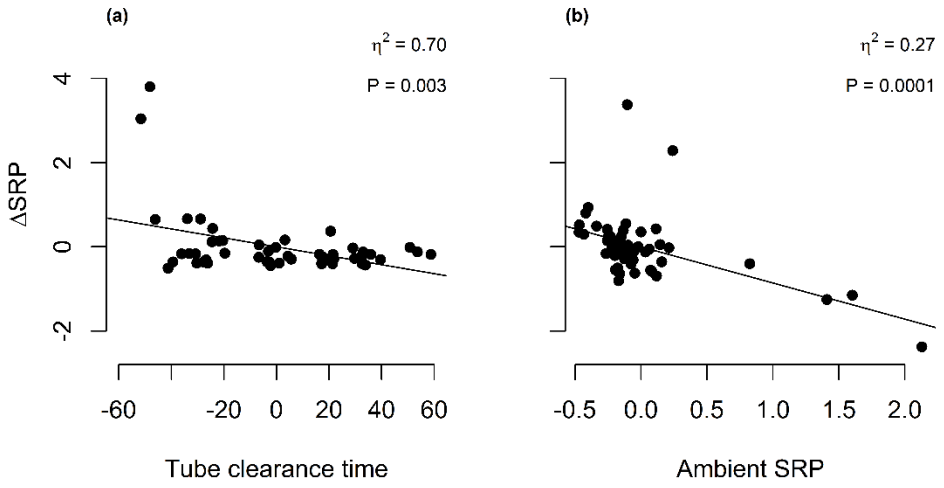

Figure C3. Partial regressions for the best fit soluble reactive phosphorus (SRP) model.

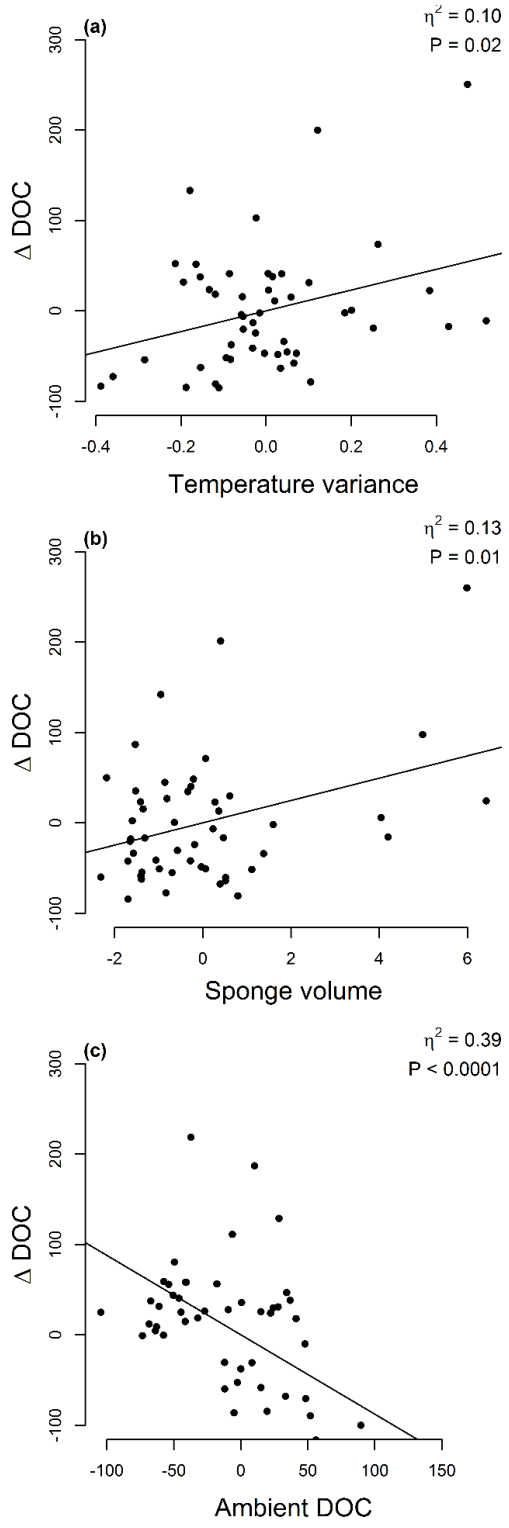

Figure C4. Partial regressions for the best fit dissolved organic carbon (DOC) model.