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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

HUMAN-DRIVEN BENTHIC JELLYFISH BLOOMS: CAUSES AND CONSEQUENCES FOR COASTAL MARINE ECOSYSTEMS

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Elizabeth W. Stoner

To: Interim Dean Michael R. Heithaus College of Arts and Sciences

This dissertation, written by Elizabeth W. Stoner, and entitled Human-driven Benthic Jellyfish Blooms: Causes and Consequences for Coastal Marine Ecosystems, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: June 10, 2014

The dissertation of Elizabeth W. Stoner is approved.

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> Dean Lakshmi N. Reddi University Graduate School

Florida International University, 2014

The following chapters have been published in peer-reviewed journals. Copyright release has been obtained from the journals for inclusion in this dissertation.

Chapter II © Copyright 2011 by Elsevier

Stoner, E.W., C.A. Layman, L.A. Yeager, and H.M. Hassett. 2011. Effects of anthropogenic disturbance on the abundance and size of epibenthic jellyfish *Cassiopea* spp. Marine Pollution Bulletin 62: 1109-1114.

Chapter IV © Copyright 2014 by Eagle Hill Institute

Stoner, E.W., L.A. Yeager, and C.A. Layman. 2014. Effects of epibenthic jellyfish,*Cassiopea* spp., on faunal community composition of Bahamian seagrass beds. CaribbeanNaturalist 12: 1-10.

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DEDICATION

To my dad, David Stoner, for teaching me to be a naturalist, for opening my eyes to the world of science, and for being the best jellyfish wrangler a daughter could ask for.

To my mom, Linda Stoner, for being a constant source of emotional support and for her unconditional love and guidance.

To my brother, Chris Stoner, for being my friend and role model.

And to my husband, Chris Bossert, for his unwavering love, friendship, patience, and encouragement.

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ABSTRACT OF THE DISSERTATION HUMAN-DRIVEN BENTHIC JELLYFISH BLOOMS: CAUSES AND CONSEQUENCES FOR COASTAL MARINE ECOSYSTEMS

by

Elizabeth W. Stoner

Florida International University, 2014

Miami, Florida

Professor Craig A. Layman, Major Professor

Coastal marine ecosystems are among the most impacted globally, attributable to individual and cumulative effects of human disturbance. Anthropogenic nutrient loading is one stressor that commonly affects nearshore ecosystems, including seagrass beds, and has positive and negative effects on the structure and function of coastal systems. An additional, previously unexplored mechanistic pathway through which nutrients may indirectly influence nearshore systems is by driving blooms of benthic jellyfish. My dissertation research, conducted on Abaco Island, Bahamas, focused on elucidating the role that benthic jellyfish have in structuring systems in which they are common (i.e., seagrass beds), and explored mechanistic processes that may drive blooms of this taxa.

To establish that human disturbances (e.g., elevated nutrient availability) may drive increased abundance and size of benthic jellyfish, *Cassiopea* spp., I conducted surveys in human-impacted and unimpacted coastal sites. Jellyfish were more abundant (and larger) from human-impacted areas, positively correlated to elevated nutrient availability. In order to elucidate mechanisms linking *Cassiopea* spp. with elevated

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nutrients, I evaluated whether zooxanthellae from *Cassiopea* were higher from humandisturbed systems, and whether *Cassiopea* exhibited increased size following nutrient input. I demonstrated that zooxanthellae population densities were elevated in humanimpacted sites, and that nutrients led to positive jellyfish growth.

As heightened densities of *Cassiopea* jellyfish may exert top-down and bottom-up controls on flora and fauna in impacted seagrass beds, I sought to examine ecological responses to *Cassiopea*. I evaluated whether there was a relationship between high *Cassiopea* densities and lower benthic fauna abundance and diversity in shallow seagrass beds. I found that *Cassiopea* have subtle effects on benthic fauna. However, through an experiment conducted in a seagrass bed in which nutrients and *Cassiopea* were added, I demonstrated that *Cassiopea* can result in seagrass habitat modification, with negative consequences for benthic fauna.

My dissertation research demonstrates that increased human-driven benthic jellyfish densities may have indirect and direct effects on flora and fauna of coastal marine systems. This knowledge will advance our understanding of how human disturbances shift species interactions in coastal ecosystems, and will be critical for effective management of jellyfish blooms.

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PREFACE

CHAPTERS II and V have been published as detailed on the Copyright Page. These chapters have been formatted according to journal specifications.

The following chapters are currently in review with peer-reviewed journals and are formatted specifically for those journals:

CHAPTER III

Stoner, E.W., S. Sebilian, and C.A. Layman. Zooxanthellae densities in upside-down jellyfish, *Cassiopea* spp., from coastal habitats of The Bahamas. Caribbean Naturalist.

CHAPTER IV

Stoner, E.W., and C.A. Layman. Does nutrient loading affect growth of a benthic jellyfish species? Bulletin of Marine Science.

CHAPTER VI

Stoner, E.W., L.A. Yeager, J.L. Sweatman, S. Sebilian, and C.A. Layman. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. Journal of Experimental Marine Biology and Ecology. CHAPTER I

INTRODUCTION

The health of marine ecosystems in the face of increasing global environmental change is a primary concern (Vitousek et al. 1997; Worm et al. 2006; Jackson 2008). Anthropogenic disturbances, including overexploitation of plants and animals, eutrophication, habitat modification, introduction of non-native taxa, ocean acidification and temperature increases associated with global warming have all fundamentally affected marine ecosystems (Jackson 2001; Lotze et al. 2006; Worm et al. 2006; Diaz and Rosenberg 2008; Halpern et al. 2008; Jackson 2008). Specifically, these disturbances, often occurring concomitantly, have driven marine biodiversity loss, population declines, and shifts in valuable ecosystem processes (Vitousek et al. 1997; Jackson 2001; Worm et al. 2006).

Coastal ecosystems, such as salt marshes, mangroves, coral reefs, and seagrass beds, are some of the most productive systems in the world, yet they are also the most vulnerable to human activities as ~75% of the global human population live in the coastal realm (Costanza et al. 1997; Emeis et al. 2001; Millenium Ecosystem Assessment 2005; Lotze et al. 2006; Jackson 2008). Anthropogenic eutrophication is one of the most common stressors to these coastal ecosystems, with ecological responses largely context dependent (Nixon 1995). For instance, in salt marsh ecosystems, acute nutrient enrichment may increase cordgrass productivity and aboveground biomass, while chronic enrichment can result in reduced belowground roots and rhizomes resulting in collapsed creek banks (Deegan et al. 2007; Deegan et al. 2012).

Seagrass ecosystems are especially susceptible to anthropogenic nutrient enrichment (reviewed in Burkholder et al. 2007). Though nutrient enrichment can, in

some circumstances, benefit seagrass (i.e., enhanced growth), it is widely viewed that elevated nutrient availability has deleterious effects on seagrass ecosystems, including shifts in seagrass species composition and seagrass die-off (Powell et al. 1989; Fourqurean et al. 1995; Ferdie and Fourqurean 2004; Burkholder et al. 2007; Layman et al. 2013). Since the late 1800's, an estimated 29% of the areal extent of seagrass has been lost globally, with eutrophication as a primary cause (Waycott et al. 2009). Elevated nutrient concentrations may affect community composition and ecosystem function through several well-established mechanistic processes, including increased epiphyte loads which can reduce light and nutrient availability, ammonium (and nitrate) toxicity to seagrasses, increased susceptibility to slime mold, sulfide intrusion, and intensified megagrazer and fish grazing rates (McGlathery 1995; Short and Burdick 1996; Borum et al. 2005; Larkum et al. 2006; Burkholder et al. 2007; Fourqurean et al. 2010; Holzer et al. 2013).

However, one additional, previously unexplored, mechanistic pathway through which seagrass systems may be affected by nutrient enrichment, is an increased abundance (i.e., "blooms") of benthic jellyfish. Once thought to be "trophic dead-ends", jellyfish are now considered to be critical components in marine food webs (Mianzan et al. 2001; Purcell and Arai 2001). Although there is a paucity of historic jellyfish population density data, the abundance of many jellyfish has increased since the 1970's, attributable to major human-impacts including eutrophication (Arai 2001; Condon et al. 2013; Purcell 2012). While many effects of pelagic jellyfish blooms have been elucidated, much less is known about benthic jellyfish blooms.

For instance, taxa of the epibenthic zooxanthellate jellyfish, *Cassiopea* spp. (also called upside-down jellyfish because of their relatively-sessile nature and bell orientation; hereafter *Cassiopea*) are globally-distributed in sub-tropical and tropical environments, and are found in several habitat types including mangrove forests, seagrass beds, and coral reefs (Holland et al. 2004; Niggl and Wild 2010). These animals may increase in human-disturbed benthic environments through several mechanistic pathways, yet ecological responses to heightened benthic jellyfish abundance are unclear. My dissertation research focused on examining the role of benthic jellyfish in structuring systems in which they are common (i.e., seagrass beds), as well as to elucidate mechanistic processes that may drive blooms of this taxa.

In CHAPTER II, entitled the "*Effects of anthropogenic disturbance on the abundance and size of epibenthic jellyfish Cassiopea* spp.", I examined epibenthic jellyfish abundance and size across human-impacted and relatively-pristine coastal areas using a survey approach. My study was the first to establish that epibenthic jellyfish abundance and size are positively influenced by human disturbance and are correlated with elevated anthropogenic nutrient availability.

Research in the next two chapters explored mechanisms that may drive the observed increase in *Cassiopea* abundance and size in human-impacted sites. Specifically, CHAPTER III entitled "*Zooxanthellae densities in upside-down jellyfish, Cassiopea spp., from coastal habitats of The Bahamas*" focused on a hypothesized mechanism facilitating *Cassiopea* blooms: enhanced autotrophic nutrition from zooxanthellae. In this study, I collected *Cassiopea* from the same human-impacted and relatively-pristine sites from CHAPTER II, and found that zooxanthellae densities (and gut content) were elevated in human-impacted areas, possibly attributed to elevated nutrient availability.

In CHAPTER IV, entitled "*Does nutrient loading affect growth of a benthic jellyfish species?*" I conducted an experiment in which I manipulated anthropogenic nutrient availability to see whether it resulted in positive jellyfish growth. My study was the first to identify that human-mediated nutrients are explicitly linked with increased jellyfish size, and indicated that elevated nutrient availability may compensate for deleterious abiotic and biotic conditions (e.g., decreased autotrophic nutrition), potentially affecting the ecological role of *Cassiopea*.

In CHAPTER V, entitled "*Effects of epibenthic jellyfish, Cassiopea* spp., *on faunal community composition of Bahamian seagrass beds*" I conducted surveys in two, relatively unimpacted, nearshore seagrass beds to evaluate faunal community composition in areas with naturally varying jellyfish densities (i.e., high and low densities). My study provided baseline information on the effects of high densities of Cassiopea jellyfish on faunal seagrass communities, and found that Cassiopea may have subtle effects on seagrass fauna.

Finally, in CHAPTER VI, entitled "*Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment*", I experimentally tested some of the hypotheses generated in the other chapters. In this experiment, I explored effects of anthropogenic nutrients and epibenthic jellyfish on floral and faunal structure in a seagrass bed. Results from this study identified that these stressors have deleterious effects on seagrass, and variable effects on benthic fauna. All five data chapters evaluate the importance of benthic jellyfish blooms in nearshore systems, and substantially contribute to our knowledge on the ecological role of these oft-underappreciated taxa, as well as how these jellyfish are linked with human disturbance. Better understanding how benthic jellyfish structure ecological systems through top-down and bottom-up processes is critical, as these blooms will likely intensify with increasing global change. Identifying the ecological responses to these blooms, and how they interact with additional stressors, will be important to predict ecological consequences.

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CHAPTER II

EFFECTS OF ANTHROPOGENIC DISTURBANCE ON THE ABUNDANCE AND SIZE OF EPIBENTHIC JELLYFISH CASSIOPEA SPP.

Abstract

Jellyfish blooms in pelagic systems appear to be increasing on a global scale because of anthropogenic factors, but much less is known about the link between human activities and epibenthic jellyfish abundance. The aim of this study was to investigate whether the epibenthic jellyfish, *Cassiopea* spp., were found in greater abundance, and attained larger sizes, in coastal habitats areas with high human population densities compared to sites adjacent to uninhabited areas on Abaco Island, Bahamas. *Cassiopea* spp. were found to be significantly more dense and larger in areas with high human population densities. Ambient nutrient levels and nutrient content of seagrass were elevated in high human population density sites, and may be one mechanism driving higher abundance and size of *Cassiopea* spp. Greater abundance of *Cassiopea* spp. may have important effects on community structure and ecosystem function in critical coastal ecosystems (e.g., seagrass beds), and their impacts warrant further study.

Keywords: Bottom-up effects, Caribbean, Estuaries, Nutrient loading, *Thalassia testudinum*, Zooxanthellae.

Introduction

Jellyfish blooms appear to be increasing on a global scale, fundamentally affecting ecosystem functioning and services provided by these affected systems (Arai 2001, Graham 2001, Mills 2001, Purcell and Arai 2001). For example, the annual catch of one of the largest jellyfish in the world, *Nemopilema nomurai*, increased 250% from 2000 to 2003 in the East China and Yellow Seas (Dong et al. 2010) devastating fisheries in those areas. Similarly, the bloom of jellyfish within the Bering Sea region has been so severe that the Alaskan Peninsula has been dubbed the "Slime Bank" because of the large numbers of jellyfish in fishery hauls. Further, it is now estimated that jellyfish consume an average of 5% of the annual crop of zooplankton in the Bering Sea, leading to a distinct shift in food web structure (Brodeur et al. 2002).

While it is sometimes difficult to ascertain mechanisms driving pelagic jellyfish blooms, it has been suggested that several anthropogenic disturbances are likely involved. These include overfishing (Purcell and Arai 2001, Lynam et al. 2006), nutrient loading (Arai 2001, Lo and Chen 2008), marine construction (Lo et al. 2008, Hoover and Purcell 2009), introduction of exotic species (Mills 2001), increased sedimentation (Arai 2001), and global climate change (Brodeur et al. 2008). Many of these disturbances may interact synergistically to drive jellyfish population blooms (Purcell et al. 2007, Jackson 2008).

Little is known, however, about links between anthropogenic disturbances and epibenthic jellyfish populations. *Cassiopea* spp. are an epibenthic jellyfish, endemic to sub-tropical and tropical ecosystems and are sessile as medusae. *Cassiopea* spp. are nicknamed 'upside-down jellyfish' because they lie flat on their bells on soft-bottom

substrates using photosynthetic zooxanthellae that live in *Cassiopea* tissues to provide the host jellyfish with a substantial source of energy (Fitt and Costley 1998, Jantzen et al. 2010). *Cassiopea* spp. have been linked anecdotally to synergistic human impacts such as eutrophication and marine construction (Arai 2001), and may invade new habitats through ship and live rock transportation (Holland et al. 2004, Bolton and Graham 2006). However, while there is some information regarding the distribution and abundance of *Cassiopea* spp. in coastal ecosystems (Collado-Vides et al. 1988, Holland et al. 2004, Niggl and Wild 2009), there is very little quantitative information regarding specific mechanisms that may influence the size of *Cassiopea* spp. populations.

Here we examine the abundance and size of epibenthic *Cassiopea* jellyfish across a gradient of human population densities. We hypothesized that *Cassiopea* spp. densities, as well as the size of individuals, would be greater in coastal areas adjacent to human population centers than those adjacent to uninhabited areas. As such, we attempted to link human population densities to the distribution and characteristics of an epibenthic jellyfish, an organism that may play an important role in shallow coastal ecosystems of the tropics and sub-tropics.

Materials and Methods

Study Site

The study was conducted in nearshore habitats on Abaco Island, Bahamas (26°25'N, 77°10'W) from June-July 2009. Sites were chosen *a priori* and assigned to one of two categories: (1) adjacent to relatively high-density human population centers or (2) adjacent to uninhabited watersheds. Sites adjacent to human population centers were

considered to be disturbed by activities from high human population densities. Likely anthropogenic disturbances include nutrient loading through point (e.g., sewage outfalls) and non-point sources (e.g., waste water run-off), construction of artificial structures (e.g., docks), and sedimentation driven by land-use practices. As a proxy for human population size, we estimated the number of buildings within a 3 km radius of the mid-point of each site (counted in Google Earth© v 5.1). Second, large tracts of many Bahamian islands, including those on Abaco, remain undeveloped and uninhabited, allowing sites with relatively little human impact to be included for comparative purposes (Layman et al. 2007, Allgeier et al. 2010).

Ten systems were chosen: 5 high human population density sites (84-1712 buildings; Cherokee, Hopetown, Little Harbour, Marsh Harbour, Treasure Cay) and 5 low human population density sites (0-10 buildings; Barracuda Creek, Cross Harbour, North Bight of Old Robinson, Snake Cay, Sucking Fish Creek) (Fig. 1). For each site, one hundred points within 100 m of shore were randomly generated using ArcMap GIS v 9.3.1 (ESRI 2008) and The Nature Conservancy (TNC) habitat layers. From these points, 6 'sub-sites' were selected within each of the ten main sites. Sub-sites were visited sequentially in the randomly-generated order and the first six sites that met two criteria were selected: 1) a low tide water depth of 2 m or shallower, as *Cassiopea* spp. are typically found in shallow water (Arai 2001), and 2) substrate comprised of silty-sandy sediment (~0.05 mm particle size as determined by the USDA soil classification triangle; Schoeneberger et al. 1998), i.e., a proxy for flow velocity, as *Cassiopea* spp. typically occur in low energy areas (Arai 2001). As such, all sites were in shallow water in areas without significant current flow.

Study sites were within tidal creeks, embayments, and along low energy coastlines. Tidal creek channels are formed by scouring of the calcium carbonate substrate and are typically lined with red mangrove, *Rhizophora mangle*. Moving landward, the creeks open to broad, shallow flats that often support extensive beds of *Thalassia testudinum* seagrass (Hammerschlag-Peyer and Layman 2010, Layman et al. 2007, Valentine-Rose et al. 2007). Substrate in tidal creeks varies from hard bottom to biogenic sand; in this study, sites ranging in silt to course biogenic sand substrate were selected. Semi-enclosed embayments in this system are typically shallow (< 3 m), and are comprised of seagrass (predominately *T. testudinum*) and sandy substrate (Yeager et al. In Press). Sites selected within semi-enclosed embayments and low-energy coastlines were typically in close proximity to shorelines because of the depth criterion, and thus were often adjacent to *R. mangle* or sandy beaches.

Sampling and Analytical Methods

Surveys and sampling were conducted during diurnal low tides. *Cassiopea* spp. were enumerated in 10 m x 10 m plots at each of the six pre-determined sub-sites. From these plots, the first 30 *Cassiopea* spp. were measured (bell diameter). Five, 1 m x 1 m quadrats were haphazardly placed in each plot, and percent cover of submerged aquatic vegetation (SAV) was estimated. Water samples were taken to determine ambient nutrient concentrations. Water samples were immediately filtered with Whatman 0.45µM and 0.20µM nylon membrane filters and frozen for later analysis. Seagrass (*T. testudinum*) was also collected, if present, in each plot for nutrient analysis. Seagrass nutrient concentrations (Duarte 1990, Allgeier et al. 2010). Near-surface

water temperatures and salinity were measured with a portable multi-parameter water quality meter (YSI 85-10), and water depth was recorded. If boats were present, they were enumerated to provide an additional proxy for human impact at each site.

Analysis of nutrient concentrations of water and seagrass were conducted at Florida International University. *Thalassia testudinum* blades were scraped to remove epiphytes and dried at 80°C (n=15 per site, if present). Dried *T. testudinum* was ground into a fine powder and % nitrogen (N) was evaluated by analyzing duplicate samples of the seagrass using a Carlo Erba CN analyzer. Percent phosphorus (P) of *T.testudinum* blades were analyzed using dry-oxidation acid hydrolysis extraction in addition to the use of a colorimetric analysis, using a CHN analyzer (Fisons NA1500) (Fourqurean et al. 1992). Total phosphorus (TP) of sample water was analyzed using the same methodologies as for % P of *Thalassia* blades. The analytical detection limit for the CHN analyzer for %P of seagrass and TP of water was 0.02μM. Water samples were processed for ammonium (NH₄+) and soluble reactive phosphorus (SRP) following the Indophenol blue method, using a CHN analyzer (Fisons NA1500) with a machine analytical detection limit of 0.05μM.

To compare the number of buildings, our proxy for human population densities between high and low human population density sites, we used a Kruskall-Wallis test, as data did not meet assumptions of normality (P < 0.05, SAS v 9.2). Number of boats, salinity, % seagrass cover, and nutrient concentrations (SRP, TP in water, NH₄+, %P in *T. testudinum*, and %N of *T. testudinum*) were compared between high and low human population density sites using t-tests (SPSS v 14.0).

In order to determine the relationship between *Cassiopea* spp. bell diameter and dry weight, *Cassiopea* spp. collected from the sampling sites were dried for 24 hours at 70°C (Lucas 2008). Log₁₀ transformed *Cassiopea* spp. dry weight were regressed against log₁₀ transformed bell diameter measurements (log₁₀ (dry weight) = $2.09*log_{10}$ (bell diameter) - 4.09, R² = 0.72; n = 149 individuals). This relationship was used to estimate the dry weight (biomass) of the 30 *Cassiopea* spp. individuals from each sub-site for which bell diameter was measured. We compared mean *Cassiopea* spp. density between high and low human population density sites using a t-test (SPSS v 14.0). We compared the size distribution of *Cassiopea* spp. between high and low human population density sites using a Kolmogorov-Smirnov test (SPSS v 14.0). Median *Cassiopea* spp. size was then compared between high and low human population density sites using a Kruskal-Wallis test, as data did not meet assumptions of normality (*P* < 0.001 in both cases) (SPSS v 14.0).

Nutrients in pelagic systems can be taken up by jellyfish, potentially driving enhanced jellyfish growth (Richardson et al. 2009). Therefore, Pearson correlations (bivariate) were run to test for relationships between nutrients (SRP, %P, %N, TP in water, NH₄+) and both (1) *Cassiopea* spp. density, and (2) *Cassiopea* spp. size (SPSS 14.0). Correlations between *Cassiopea* spp. density and nutrient concentrations are reported in this paper. Correlations between *Cassiopea* spp. size and nutrients were comparable to those reported for density, and thus are not included here.

Results

Consistent with our initial site selections, the 5 sites with high human population densities had a greater number of buildings, our proxy for population density within the watershed ($\chi^2 = 6.98$, df = 1, P = 0.008). The number of boats was also greater in high human population density sites, indicating human presence and activity (t = 2.75, df = 58, P = 0.008, Table 1). Salinity and % seagrass cover were not significantly different between high and low human population density sites (Table 1). However, water concentrations of TP, NH₄+, and %P in *T. testudinum* were significantly higher at high human population density sites (Table 1). SRP and %N from *T. testudinum* tissue did not differ between high and low human population density sites. Overall, nutrient concentrations were low, consistent with other nearshore sites in The Bahamas (Koch and Madden 2001, Allgeier et al. 2010).

Mean *Cassiopea* spp. density was greater in sites with high human population densities than with low human population densities (t = 4.57, df = 58, P < 0.001, Fig. 2). The size distribution of *Cassiopea* spp. also differed between high and low human population density sites (Z = 5.43, df = 1, P < 0.001, Fig. 3), with median size being more than two times greater at high human population density sites ($\chi^2 = 161.07$, df = 1, P <0.001). Little Harbour had both the highest density of *Cassiopea* spp. (mean = 6.9 jellyfish/m²) and the largest *Cassiopea* spp. mean size (mean diameter = 12.4 cm, mean dry weight = 3.5g). Furthermore, Little Harbour had the greatest number of jellyfish in a sub-site with 1,340 jellyfish in a 10m x 10m plot, as well as the largest individual jellyfish collected in any site (diameter = 22 cm, dry weight = 11g). Interestingly, Little Harbour had the fewest number of buildings for a high human population density site: however, point sources of pollution were observed at this site indicating direct and acute human impact. Two of the five low human population density sites had no jellyfish present at any sub-site sampled (Sucking Fish Creek and Cross Harbour), and Barracuda had only two jellyfish present within one sub-site.

Cassiopea spp. density was found to be positively correlated to TP (r = 0.58, P < 0.001, Fig. 4). No other individual correlations between *Cassiopea* spp. density and nutrients were significant (P > 0.05, Fig. 4).

Discussion

Our results suggest that Cassiopea spp. are more abundant, and are larger, in areas adjacent to relatively high human population density centers. These findings are some of the first to demonstrate such a pattern, and suggest that some aspect of anthropogenic disturbance may affect densities and size of this epibenthic organism. Anthropogenic nutrient loading in particular may affect *Cassiopea* spp. Because *Cassiopea* spp. host symbiotic zoothanthellae in their tissue, they may be able to capitalize on increased nutrient availability in nutrient-enriched areas. Zooxanthellae in *Cassiopea* spp. tissues supply much of the carbon requirements to the host, and are critical to the metamorphosis of ephyrae and the survival of the jellyfish (Fleck and Fitt 1999, Pitt et al. 2009). In zooxanthellate corals, uptake of nutrients from water has been shown to increase the population density of zooxanthellae (Bythell 1990, McAuley 1994). In a similar manner, nitrogen (primarily ammonium) and phosphorous in the water column may be taken in by *Cassiopea* spp. Various systems in The Bahamas, including tidal creeks and embayments, have been found to be extremely oligotrophic (Koch and Madden 2001, Lapointe et al. 2004, Allgeier et al. 2010). Therefore, any input of

anthropogenically-introduced nutrients may help support productivity of the zooxanthellae, which would release additional photosynthate to the host, potentially enhancing its growth (Belda and Yellowlees 1995, Fitt and Costley 1998, Jantzen et al. 2010). The positive correlation between TP and *Cassiopea* spp. density, and increased concentrations of TP in sites adjacent to high population densities, supports the hypothesis that nutrient loading can lead to blooms of epibenthic jellyfish.

While there was a fairly clear relationship between *Cassiopea* spp. densities and TP, the relationship with ammonium was less straightforward. In this study, there was greater variation in *Cassiopea* spp. densities in sites with low ammonium concentrations, whereas sites with high ammonium concentrations had consistently low densities of *Cassiopea* spp. While there was not a linear correlation between NH₄+ and *Cassiopea* spp. density, the relationship does suggest that at high densities, *Cassiopea* spp. may locally depress ammonium in the water (Fig. 4). Ammonium specifically has been suggested to be limiting to tropical zooxanthellae populations (Falkowski et al. 1993, Fitt and Cook 2001). Therefore, zooxanthellae living within the tissues of *Cassiopea* spp. may preferentially uptake ammonium from seawater. However, it is necessary to mechanistically-test this hypothesis to provide further insight into this potential relationship.

A higher abundance of *Cassiopea* spp. associated with human disturbance may have substantial implications for community structure and functioning of seagrass ecosystems through a variety of mechanistic pathways. First, *Cassiopea* spp. may compete with benthic flora for light, essentially covering seagrasses and other submerged aquatic vegetation. Second, because *Cassiopea* spp. feed on zooplankton, they may
directly compete with other filter-feeding consumers for food. Third, *Cassiopea* spp. have few predators (e.g., nudibranchs and sea turtles, Brandon and Cutress 1985, Arai 2005), so energy assimilated by these animals may be locked in a dead-end trophic pathway that may not be transferred to upper levels of the food web. Finally, reduced species richness and diversity in benthic marine habitats resulting from the previous mechanisms may affect the nature of nutrient cycling, and thus ecosystem functioning, within these habitats (Bracken et al. 2008). As such, further studies on the effects of increased epibenthic jellyfish in coastal ecosystems are needed to explore potential ways they may alter community structure and ecosystem function.

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TABLE 2.1. Nutrient concentrations and site characteristics for high and low human population density sites. All nutrient concentrations are reported in μ M, salinity was measured in ppt. Results of t-tests comparing all variables except # of buildings between high and low human population density sites are given. Statistical values for # of buildings are reported in text due to a difference in statistical test used. SRP = soluble reactive phosphorus in water, TP = total phosphorus in water, %P = percent phosphorus in *Thalassia testudinum* tissues, NH₄+ = ammonium in water, %N = percent nitrogen in *Thalassia testudinum* tissues, %SG cover = percent cover of *Thalassia testudinum*. N/A=samples were not collected for that site. See text for detailed explanation of all variables.

	Site							%SG	#	#
Site Name	Code	SRP	TP	%P	NH_4+	%N	Salinity	Cover	Boats	Buildings
High Human Population Density										
Cherokee	С	0.13	0.83	N/A	8.56	N/A	32	8	18	165
Hopetown	HT	0.08	0.96	0.10	1.15	1.8	32	6	16	362
Little Harbour	LH	0.08	1.16	0.13	0.41	1.9	35	20	3	84
Marsh Harbour	MH	0.08	0.67	0.13	3.90	2.3	29	8	13	1712
Treasure Cay	тс	0.08	0.74	0.11	7.69	1.9	27	12	0	492
Mean Values		0.09	0.09	0.12	4.36	2.0	31	14	10	563
Standard Deviation (±)		0.03	0.11	0.02	5.60	0.1	3	32	8	662
Low Human Population Density										
Barracuda	BR	0.07	0.52	0.06	4.36	1.9	32	2	0	0
Cross Harbour	СН	0.08	0.53	0.07	1.46	1.7	35	20	0	0
North Bight of Old Robinson	NB	0.11	0.47	0.07	0.51	1.8	33	29	0	7
Snake Cay	SC	0.10	0.51	0.09	1.91	2.5	18	3	0	10
Sucking Fish	SF	0.10	0.50	0.11	0.42	1.7	36	4	0	0
Mean Values		0.38	0.51	0.07	1.73	1.9	31	12	0	3.4
Standard Deviation (±)		0.01	0.11	0.01	2.30	0.15	7	27	0	4.77
Test statistic (t)		0.04	6.58	3.91	2.35	0.19	0.03	0.59	2.74	
Degrees of freedom		58	55	13	57	13	58	58	58	
P-value		0.97	<0.001	0.002	0.022	0.85	0.97	0.55	0.008	



FIGURE 2.1. Study sites on Abaco, Bahamas designated by polygons. Five sites were in high population density areas (C, HT, LH, MH and TC) and five sites were in locations with low human population densities (BR, CH, NB, SC, and SF). See Table 1 for key site codes.



FIGURE 2.2. Mean *Cassiopea* spp. density (# individuals/100m²) from sites with high and low human population densities. Sites shown without bars indicate that no jellyfish were present within these locations.



FIGURE 2.3. Size-frequency distribution of *Cassiopea* spp. from sites with high and low human population densities.



FIGURE 2.4. Correlations between *Cassiopea* spp. densities and (a) soluble reactive phosphorous concentration in water, (b) total phosphorus concentration in water, (c) percent phosphorus in *Thalassia testudinum* tissue, (d) ammonium concentration in water and (e) percent nitrogen in *Thalassia testudinum* tissue. r = Pearson correlation coefficient and P = p-value for Pearson correlation. A trendline was added for correlations where P < 0.05.

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CHAPTER III

ZOOXANTHELLAE DENSITIES IN UPSIDE-DOWN JELLYFISH, *CASSIOPEA* SPP., FROM COASTAL HABITATS OF THE BAHAMAS

Abstract

Anthropogenic disturbances may drive jellyfish blooms, including zooxanthellate jellyfish such as *Cassiopea* spp. (Upside-down Jellyfish). We show that *Cassiopea* spp. had higher zooxanthellae densities in human-impacted areas on Abaco Island, The Bahamas. Nutrient loading in impacted sites may be one factor driving zooxanthellate jellyfish blooms. Additionally, gut contents from *Cassiopea* medusae were positively correlated to zooxanthellae densities, suggesting that heterotrophicallyderived nutrition may be an important factor in facilitating increased zooxanthellae population densities. Understanding such mechanisms driving jellyfish blooms is crucial for developing effective management strategies in impacted coastal ecosystems.

Keywords: anthropogenic nutrient loading, epibenthic, facilitation, heterotrophy, mutualism, symbiont.

Introduction

Jellyfish blooms have been suggested to increase in both magnitude and frequency in certain parts of the world (Purcell 2012). Jellyfish blooms may increase as a function of anthropogenic disturbances such as nutrient loading, overfishing, global climate change, development of artificial marine structures, introduction of nonindigenous jellyfish species, and sedimentation (Brodeur et al. 2008, Bayha and Graham 2014, Graham et al. 2001, Hoover and Purcell 2009, Purcell and Arai 2001, Purcell 2012, Riisgard et al. 2012). Recently, Stoner et al. (2011) suggested that populations of the epibenthic, zooxanthellate, jellyfish, *Cassiopea* spp. Péron and Lesueur (Upside-down Jellyfish) (hereafter *Cassiopea*) are more abundant and larger in human-impacted coastal systems in The Bahamas. Increased *Cassiopea* abundance and size suggests that humans may be initiating or facilitating blooms of this relatively little-studied epibenthic jellyfish.

A mechanism suggested to contribute to *Cassiopea* blooms in anthropogenicallydisturbed habitats may be increased nutrient availability driving higher zooxanthellae densities in jellyfish tissues (Stoner et al. 2011). Because zooxanthellae are nutrient limited (by both nitrogen and phosphorus), increased nutrient supply may result in increased primary production, thereby providing higher levels of photosynthate (i.e., autotrophic nutrition) to their hosts (Falkowski et al. 1994, Koop et al. 2001, Muscatine et al. 1989). Higher zooxanthellae densities may enhance host growth and fitness for *Cassiopea*, similar to patterns that are well-documented between coral and zooxanthellae (Berkelmans and van Oppen 2006, Muscatine and Porter 1977, Yellowlees et al. 2008).

In this study, we explored if there was a difference in zooxanthellae densities in *Cassiopea* tissue between anthropogenically-disturbed and relatively-pristine coastal sites

on Abaco Island, The Bahamas. We hypothesized that zooxanthellae densities would be higher in sites adjacent to human population centers, specifically as a function of elevated nutrient concentrations.

Field Site Description

This study was conducted in nearshore habitats (26°25'N 77°10'W) from June to July 2011. Study sites were located within mangrove wetlands, embayments or low energy coastlines <2m in depth at low tide. All sites were characterized by silty-sandy sediment (~0.05mm particle size, as classified by the USDA soil classification triangle; Schoeneberger et al. 2002), which suggests low localized water velocities. Sites were *a priori* categorized as being adjacent to high or low human population densities, following Stoner et al. (2011). In short, the number of buildings within a 3km radius from the midpoint of each site was estimated using Google Earth v. 5.1 (Google Inc., 2010). High human population density sites (hereafter referred to as HP sites) had a mean number of 563 buildings (range of 84-1712 buildings; Little Harbour, Marsh Harbour, Treasure Cay, Cherokee and Little Abaco South), while low human population density sites (hereafter referred to as LP sites) had a mean number of 4 buildings present (range of 0-10 buildings; Snake Cay, North Bight of Old Robinson, Twisted Bridge, and Little Abaco South) (Stoner et al. 2011).

Methods

For each site, 15 jellyfish (8-10 cm in bell diameter) were collected. *Cassiopea* of this size were comparable to the range of bell diameters of *Cassiopea* examined for zooxanthellae densities in previous studies (Table 1). All jellyfish had eight oral arms,

with no tears or deformities on the bell, as any injury to the animal may affect zooxanthellae population densities or the ability of the animal to acquire prey.

After jellyfish were collected, they were transported in saltwater and processed within 1-5 hours from the initial sampling time. Zooxanthellae densities were evaluated by cutting off the "outpocketings" or secondary and tertiary mouths from the primary oral arms following protocol outlined by Estes et al. (2003). We selected oral arms as they have been found to have the highest zooxanthellae densities in *Cassiopea xamachana* Bigelow (Mangrove Jellyfish) (Estes et al. 2003). Oral arms were then homogenized using a mortar and pestle. We extracted 10µL of jellyfish homogenate, which we added to 1ml of seawater and mixed for 45 seconds using a vortex. Zooxanthellae densities in each sample were determined from three aliquots (10µL per sample), which were viewed using an improved Neubauer hemocytometer under a plain light microscope at 400x. Zooxanthellae density data were averaged across replicates per specimen for each site. Zooxanthellae density across LP and HP sites were analyzed using a nested univariate analysis of variance (ANOVA) with site nested within disturbance regime (i.e., anthropogenically-impacted or relatively-pristine conditions) (IBM SPSS v. 20.0).

Nutrient concentrations (NH₄+, Total Phosphorus, %P) were also evaluated in this study. To analyze ammonium (NH₄+) and total phosphorus (TP) concentrations, water samples were collected and filtered with 0.45μ M and 0.2μ M nylon membrane filters. We also analyzed %P from seagrass tissue in this study. We evaluated seagrass nutrient concentrations because they may reflect nutrient availability to a system over a longer time period (weeks), while ambient water nutrient concentrations represent a "snapshot" of nutrient availability (Allgeier et al. 2010, Stoner et al. 2011). To analyze blade tissue

%P, *Thalassia testudinum* (Banks ex König) (Turtle Grass) leaves were harvested, then scraped to remove epiphytes and dried at 80° C. Dried seagrass was ground into a fine powder, and both water (TP) and seagrass (%P) samples were analyzed using dry oxidation hydrolysis extraction in addition to the use of a colorimetric analysis using a CHN analyzer (Fourqurean et al. 2012). Ammonium samples were processed following the Indophenol blue method using a CHN analyzer.

Gut content weight (i.e., the dry weight of food in the gut) was also examined, as we wanted to explore the potential relationship between zooxanthellae densities and the amount of food that individual *Cassiopea* had consumed. It has been suggested that heterotrophically-derived nutrition may affect zooxanthellae densities and cell division due to increased energy translocated to the symbionts (McAuley and Cook 1994, Szmant-Froelich and Pilson 1984). In other words, nutrients acquired heterotrophically may be utilized by the zooxanthellae, potentially stimulating zooxanthellae productivity. Gut contents were removed from the stomach of each jellyfish by carefully removing the bell from the oral arms and picking out gonad tissue. Gut contents were then analyzed to obtain wet and dry weight for each specimen. Gut content dry weight across LP and HP sites was analyzed using a nested ANOVA (IBM SPSS v. 20.0). Pearson correlations (bivariate) were then run to evaluate the relationship between mean zooxanthellae densities, mean gut dry weight (g d^{-1}) and nutrient concentrations across sampling locations (IBM SPSS v. 20.0).

Results

Mean zooxanthellae densities from both site types (HP and LP) in this study were found to be lower than previously reported for *Cassiopea* in other systems (Table 1). We found a significant difference between zooxanthellae densities between LP and HP sites $(F_{8,125} = 8.0, P < 0.001;$ Fig. 1) in which the mean zooxanthellae density from LP sites was $2.3 \times 10^6 \pm 1.6$ (cells g⁻¹ ww), while the mean zooxanthellae density from HP sites was $3.3 \times 10^6 \pm 1.90$ (cells g⁻¹ ww). Cherokee, an HP site, had the highest zooxanthellae density (9.64 x 10⁶) while North Bight (an LP site) had the lowest zooxanthellae density (3.54×10^5) of any site. Zooxanthellae densities were found to be negatively correlated to %P concentrations in seagrass tissue (r = -0.40, P = 0.003; Fig. 2C), but were not correlated to any water column nutrient concentrations (Fig. 2A, 2B).

Mean dry gut content weight did not differ between HP and LP sites ($F_{8,125} = 1.4$, P = 0.19; Fig. 1); however, the highest gut content weight (0.02g per jellyfish) came from an HP site (Marsh Harbour). There was a significant positive correlation between zooxanthellae densities and gut content weight (r = 0.26, P = 0.003). Neither zooxanthellae densities nor gut weight values in jellyfish were correlated to jellyfish bell diameter (r = -0.35, P = 0.39 and r = -0.34, P = 0.39, respectively).

Discussion

Our results suggest that zooxanthellae densities from *Cassiopea* medusae are substantially lower in LP sites than HP sites. Human-disturbances (e.g., nutrient loading) in coastal areas of the Bahamas have been shown to drive increased densities and size of *Cassiopea* (Stoner et al. 2011). Herein we extend these findings by showing that impacted areas also may support higher densities of zooxanthellae in *Cassiopea* tissue. Further, we found that jellyfish tissues from Abaco had lower zooxanthellae densities than medusae collected from the Florida Keys (Table 1; Estes et al. 2003, Verde and McCloskey 1998, Vodenichar 1995). The Florida Keys are more heavily populated than Abaco, so it is conceivable that higher zooxanthellae densities from *Cassiopea* sampled in Florida reflect increased nutrient availability derived from human activities. Water column nutrient availability in nearshore areas of The Bahamas has been found to be extremely low (Allgeier et al. 2010, Koch and Madden 2001, Stoner et al. 2011), which may relate to lower zooxanthellae densities in The Bahamas.

We did not observe correlations between ambient nutrient concentrations (NH₄+ and TP) and zooxanthellae densities. We did, however, find a negative correlation between %P of seagrass and zooxanthellae densities. It is unclear as to what mechanism may drive this correlation, but competition between jellyfish and seagrass is one possibility (i.e., zooxanthellae rapidly uptake nutrients, reducing nutrient availability to other autotrophs). However, because of the complex nature of coastal ecosystems in The Bahamas and the lack of a relationship between zooxanthellae densities and ambient water nutrient concentrations, it is clear that more work is needed to elucidate the extent to which nutrients are responsible for elevated zooxanthellae densities in HP sites.

Elevated zooxanthellae densities may also be an indirect function of heterotrophically-derived sources of nutrition (e.g., external food resources for jellyfish). Although we did not detect a difference in gut weight between LP and HP sites, we did find that there was a strong, positive, correlation between zooxanthellae densities and gut weight. This is consistent with the idea that increased heterotrophically-derived nutrition

may stimulate zooxanthellae population growth. It is also possible that higher ambient nutrient concentrations may support greater zooplankton biomass in some coastal areas, providing a readily available food source for jellyfish.

In some parts of the world, jellyfish blooms are increasing, however, little empirical evidence exists to support specific mechanisms driving these blooms in humanimpacted environments. Our results suggest that increased human impacts, possibly nutrient loading, may be one cause of jellyfish blooms in parts of The Bahamas. These blooms may have a variety of impacts on ecosystem processes in seagrass beds where *Cassiopea* are abundant. For example, seagrass systems with high jellyfish abundance may support lower levels of biodiversity. Ultimately, understanding mechanisms driving jellyfish blooms, as well as the resultant impacts of coastal ecosystems, is crucial for developing effective management strategies.

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Source	Zooxanthellae densities (cells g ⁻¹ ww)	Bell diameter size range (cm)	Collection Site	
Verde & McCloskey (1998)	9.6x10 ⁶	1-10	Key Largo, FL	
Vodenichar (1995)	4.5x10 ⁶	4-13	Marathon, FL	
Estes et al. (2003) (oral arms only)	~4.9x10 ⁶	10-14	Marathon, FL	
Present Study- HP sites	3.2x10 ⁶	8-10	Abaco, The	
Present Study- LP sites	2.2x10 ⁶	8-10	Bahamas	

TABLE 3.1. Comparison of reported zooxanthellae density values (cells g⁻¹ ww) from *Cassiopea* medusae sampled in the Florida Keys and The Bahamas.



FIGURE 3.1. Mean gut content dry weight values from *Cassiopea* spp. medusa (A) and mean zooxanthellae density values (B) collected from relatively-pristine sites (black bars) and human-impacted sites (grey bars) on Abaco, The Bahamas.



FIGURE 3.2. Correlations between zooxanthellae densities and (A) ammonium concentrations in water, (B) total phosphorus concentrations in water, and (C) percent phosphorus in *Thalassia testudinum* tissue. r = Pearson correlation coefficient and P = p-value for Pearson correlation. A trendline in included for correlations where P < 0.05.

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CHAPTER IV

DOES NUTRIENT LOADING AFFECT GROWTH OF A BENTHIC JELLYFISH

SPECIES?

Abstract

Jellyfish abundance is increasing in many parts of the world, often attributable to human impacts. Size structure of jellyfish populations may also be affected, with implications for how they influence population, community and ecosystem dynamics in marine environments. Nutrient enrichment is one possible driver that may alter jellyfish size through indirect and direct mechanistic pathways. In this experiment, we manipulated nutrient concentrations to test if this affected growth of the zooxanthellate, benthic, jellyfish taxa, *Cassiopea*. Estimated body mass of jellyfish in the nutrientenriched treatment increased on average $0.24 \pm 0.29\%$ d⁻¹, likely as a result of elevated autotrophic carbon supply to jellyfish. Conversely, jellyfish in the ambient nutrient treatment shrank, possibly reflecting reduced light or food availability attributable to enclosures jellyfish taxa, and more broadly provide an example of how environmental conditions mediate the nature of symbiotic relationships in marine systems.

Keywords: Caribbean, *Cassiopea* spp., eutrophication, food webs, jellyfish blooms, seagrass, The Bahamas.

Introduction

Jellyfish are pervasive marine organisms that are increasingly recognized as critical components of marine food webs (Niggl et al. 2010, Condon et al. 2011, Riascos et al. 2012). Jellyfish can regulate population and community dynamics, as well as ecosystem processes, by acting as predators on zooplankton, competing with zooplanktivores for food, and excreting nutrients which are utilized as a resource primary producers and microbes (Purcell and Arai 2001, Carr and Pitt 2008, West et al. 2009, Uye 2011). Over the past two decades, increased population densities (i.e., "blooms") of jellyfish have been common, perhaps as a consequence of elevated ocean temperatures, overfishing, nutrient loading, introduction of invasive jellyfish species, and habitat modification (Arai 2001, Mills 2001, Purcell and Arai 2001, Richardson et al. 2009, Duarte et al. 2013).

In addition to proliferations of jellyfish, human disturbances may also drive shifts in jellyfish size structure. Jellyfish are comprised of >95% water and low carbon content; enabling them to allocate energy into rapid growth. Jellyfish also grow much larger than non-gelatinous zooplankton with equivalent carbon content (Lowndes 1942, Pitt et al. 2013). Under favorable environmental conditions (e.g., elevated nutrients), jellyfish may grow to larger sizes (Stoner et al. 2011, but also see Grove and Breitburg 2005). Larger jellyfish may affect ecosystem processes differently, with increased clearance rates (and consumption of larger prey), heightened efflux of nutrients (e.g., sloppy feeding), and the potential for sexual maturation at an earlier life history stage and increased reproductive capacity (Graham and Kroutil 2001, Hirst et al. 2003, Pitt et al. 2013).

Cassiopea spp. (also known as "upside-down jellyfish", hereafter referred to as *Cassiopea*) are a relatively-sessile, benthic, jellyfish taxa, and are globally distributed in tropical environments including seagrass beds, coral reefs, lagoons, and mangrove habitats (Fleck and Fitt 1999, Holland et al. 2004, Niggl and Wild 2010). *Cassiopea* derive their nutrition from heterotrophic sources (i.e., zooplankton), as well as obtain photosynthetically-fixed carbon (photosynthate) from zooxanthellae (Verde and McCloskey 1998). Stoner et al. (2011) found that *Cassiopea* are substantially more abundant and larger in human-impacted coastal areas in The Bahamas. Increased abundance and size of *Cassiopea* is presumably mediated through increased nutrient loading which supports enhanced photosynthate supplied to jellyfish via heightened zooxanthellae densities (Muscatine et al. 1989, Falkowski et al. 1993, Koop et al. 2001).

Despite mounting evidence that jellyfish increase in human impacted areas, there has been little research on how human-mediated stressors affect jellyfish growth. In the present study, we evaluated *Cassiopea* growth rates under ambient and elevated nutrient conditions, with the hypothesis that jellyfish growth rates would be higher in elevated nutrient treatments.

Materials and Methods

We conducted a field experiment in an unimpacted coastal embayment (adjacent to Jungle Creek, 26°21′ 53"N, 77°01′ 25" W) on Abaco Island, Bahamas. *Cassiopea* are naturally present in this embayment, but at a relatively low density (<2 jellyfish medusa/m²). Water depth at the site was shallow (<0.5 m in depth at low tide). Substrate was characterized by silty-sandy sediment (~0.05mm particle size, as classified by the USDA soil classification triangle (Schoeneberger et al. 2002)), which suggests low water

flow. Nearshore Bahamian systems are extremely oligotrophic, with nutrients from external sources rapidly taken up by plants, algae or microbial communities (Allgeier et al. 2011, Stoner et al. 2011). Mean ambient nutrient concentrations from the region in which this study was conducted are low (NH₄+ = 0.51 μ M, Total Phosphorus = 0.47 μ M), compared to nutrient concentrations from human-impacted embayments on the island (Mean ± SD: NH₄+ = 4.36 ± 5.6 μ M, Total Phosphorus = 0.90 ± 0.11 μ M) (Stoner et al. 2011).

Two treatments were employed: ambient nutrients (-N), and nutrient additions (+N). We designated ten, $1m^2$, plots, separated by 5m and surrounded with Landware[®] plastic garden fencing (2x2cm mesh size). Though *Cassiopea* are a relatively-sessile taxa and move < $1m^2$ over the course of a few days (Jantzen et al. 2010), fencing was utilized to prevent loss of any individuals. Five of the plots were randomly chosen for nutrient additions. To simulate eutrophication, we added Plantacote slow-release fertilizer (N:P molar ratio = 19:6, Scotts, Columbus, Ohio, USA) by massaging the fertilizer (1,000g/m²) into the top 5cm of sediment, allowing for chronic nutrient release over the duration of the experiment, following protocol outlined in Ferdie and Fourqurean (2004). Fertilizer was added to sediment in order to enable diffusion of nutrients through sediment porewater, which *Cassiopea* (and specifically their zooxanthellae) uptake as a result of their bell pulsations (Jantzen et al. 2010).

We collected 100 apparently healthy (i.e., no tears or rips in bell, no visible deformities in the bell or oral arm), 8-14 cm (medium-large size) bell diameter *Cassiopea* medusae. Jellyfish growth is negatively related to body size; in our experiment, jellyfish had relatively larger initial bell diameter which may be associated with slower growth

rates (Hirst et al. 2003, Pitt et al. 2013). Initial bell diameter (D_o) was evaluated by flattening the bell of the jellyfish, and measuring the distance between 2 opposite rhopalial lappets (i.e., clefts in the umbrella margin). In addition, we recorded detailed characteristics of jellyfish to identify possible change in growth exhibited by individual jellyfish at the end of the experiment. Identifiers included color of appendages (also commonly referred to as frills), number of appendages on each oral arm, number of rhopalia, bell color, number and general size of vesicular appendages in the center of jellyfish manubrium, and any other information which may help with later identification of each animal (Fig.1).

We haphazardly placed jellyfish in plots (N = 10 jellyfish/plot) across both treatments. Our experimental density represents the 75th percentile of jellyfish densities from human-impacted sites on the island (Stoner et al. 2011). We detected no difference in initial bell diameter of jellyfish placed in plots (N=5 plots/treatment) between treatments (mean \pm SD) (-N: 11.0 \pm 3.5cm, +N: 11.1 \pm 1.6cm; nested ANOVA, SPSS v.14.0, $F_{1,8}$ = 0.18, P = 0.8). We also identified whether jellyfish were visibly brooding fertilized eggs, as it is likely that this life-history stage is related to size (Lucas 2001). To avoid any potential influence of placing a disproportionate number of large brooding females in some plots over others, we added the same number of brooding females (N = 3/plot) in both –N and +N plots, and *Cassiopea* were left undisturbed in enclosures for 7 days.

After this period, each jellyfish was re-identified using initial identifying characteristics (e.g., number of vesicular appendages), and final bell diameter (D_t) was measured. To express as body dry weight (W, g) using D_o and D_t (cm) measurements, we

used the equation W = 1.1534(D)-2.1722 in which D = bell diameter (Fig.2). This equation was generated from a regression using *Cassiopea* dry weights (n =58) and bell diameters from a previous study conducted from a similar coastal area (Stoner et al. 2011), and all measurements were square-root transformed to better meet assumptions of normality and homoscedasticity. We could then calculate daily specific growth rate (μ , % d⁻¹) using the equation, $\mu = [ln(W_t/W_o)](t_t-t_o)^{-1}(100)$ in which W_o and W_t are the body dry weight at time t_o and t_t following protocol outlined in Olesen et al. (1994) and Frandsen and Riisgard (1997). We did not include 9 jellyfish in our analyses because these jellyfish were visibly damaged (i.e., ripped bells), which could affect growth rates. We evaluated any differences in percent change in jellyfish growth and mean daily growth rate between treatments by conducting nested ANOVA in which plot (i.e., N = 5 replicate plots/treatment) was a random factor nested within treatment (SPSS v. 14.0).

Results

We found that jellyfish growth was significantly different between treatments $(F_{1,8} = 7.0, P = 0.02)$, in which jellyfish shrank in –N plots (Mean ± SE: -5.9% ± 1.2) and grew in +N plots (Mean ± SE: 0.06% ± 1.1) (Fig.3A). Daily specific growth rate also differed significantly between both treatments ($F_{1,8} = 5.0, P = 0.04$; Fig. 3B); mean daily rate (± SE) for jellyfish from the -N treatment was $-1.0 \pm 0.22\%$ d⁻¹, while the mean daily growth rate for jellyfish from the +N treatment was $0.24 \pm 0.29\%$ d⁻¹. Accordingly, the frequency of larger jellyfish (within the 8-14cm range) in -N plots decreased over the duration of the experiment, while the frequency of larger jellyfish in +N plots increased over the experiment (Fig.4A, B). The maximum growth rate exhibited by an individual

jellyfish was 4.8% d⁻¹ from +N treatment, and the maximum mean growth rate for a plot was 1.1% d⁻¹ from the +N treatment. Growth rates exhibited by jellyfish were within the range of those reported for other scyphozoans from temperate locations (Table 1).

Discussion

Our findings indicated that elevated nutrient availability had a positive effect on epibenthic jellyfish growth. While we did not explore specific mechanisms driving this pattern, it is likely that endosymbionts in host tissue utilized nutrients, increasing photosynthetic activity, transferring additional carbon to the jellyfish. Increased *Cassiopea* growth as a function of elevated nutrient availability parallels other taxa that host zooxanthellae; for instance, enhanced nutrients under certain conditions can increase coral fitness (Allgeier et al. 2014, Shantz and Burkepile 2014). Our data provide another example of how the nature of symbiotic relationships can be mediated by local environmental conditions (Trench 1993, Chamberlain et al. 2014).

Cassiopea in –N plots exhibited a reduction in bell diameter size. Jellyfish shrinking under natural conditions is common, and may be an evolutionary adaption to survive when food availability is low. There are no long-term deleterious effects to jellyfish shrinking, and gonadal tissue (which is resorbed during shrinkage), re-develops and becomes fertile again (Lucas 2001). In our study, it is possible that enclosures around the jellyfish impeded normal water exchange, altering food availability (zooplankton or particulate organic matter) to jellyfish, as well as shifting abiotic conditions (e.g., increased water temperature) which may negatively influence jellyfish condition (Lucas 2001, Hirst et al. 2003). It is also possible that the cages shaded jellyfish, thereby reducing photosynthetic activity under ambient nutrient concentrations. Despite apparent

negative effects of enclosures on jellyfish, individuals in +N plots were still able to exhibit positive growth. To this end, it is likely that the growth rates observed in this study by jellyfish in +N plots underestimated what would have been observed had enclosures not been utilized.

It is important to highlight the context dependency of our findings. Jellyfish growth may vary on the basis of spatial and temporal availability in resources, reproductive output, and size (Lucas 2001, Hirst et al. 2003). First, jellyfish growth is often considered to be limited by food availability (Lucas 2001). As *Cassiopea* obtain nutrition from heterotrophically and autrotrophically-derived sources, natural and human-induced variability may affect resource availability in multiple ways. For instance, elevated nutrient concentrations are utilized by zooxanthellae directly, or may alter zooplankton abundance, either of which may affect jellyfish growth. Second, reproduction could obscure simple growth patterns, e.g., some jellyfish species will shrink after reproductive output (e.g., *Aurelia aurita*) (Lucas 2001). In the present study, inclusion of brooding females may have obscured growth patterns between treatments. Third, as growth rate is related to body size, it is possible that with smaller jellyfish (perhaps following a bloom event in which ephyrae are abundant) growth rates would be greater under elevated nutrient conditions.

Jellyfish are highly flexible taxa, able to successfully adapt to a wide range of environmental conditions (reviewed in Lucas and Dawson 2014). As global change accelerates, jellyfish abundance and size may both increase, potentially increasing their impacts on marine ecosystems. Understanding shifts in response of jellyfish to human impacts will allow for effective management of jellyfish blooms.

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Source	Jellyfish species	Bell diameter size range (cm)	Daily specific growth rate range (µ) (%d⁻¹)	Collection Site
Hansson	Aurelia	1-18	11-16	Gullmarsfjorden,
et al. 1997	aurita			Sweden
Uye and	Aurelia	~1-30	4.9-8.3	Inland Sea of
Shimauchi,	aurita			Japan
2005				
Møller and	Aurelia	~3.6-223.3	-3-17	Limjorden,
Riisgård	aurita			Denmark
2007				
Olesen et	Aurelia	~0.5-5.5	-3-9	Kertinge Nor,
al. 1994	aurita			Denmark
Lucas et	Aurelia	~0.2-5	-3-7	Horsea Lake,
al. 1996	aurita			England
Present	Cassiopea	8-14	-5.6-4.8	Abaco, The
study	spp.			Bahamas

TABLE 4.1. Comparison of reports of daily specific growth rates of scyphozoan jellyfish taxa sampled from wild populations.


FIGURE 4.1. Photo of (A) adult *Cassiopea* medusae, (B) a close-up of colored appendages which was one trait used to identify jellyfish individuals, and (C) rhopalial lappets which were flattened when measuring bell diameter.



FIGURE 4.2. Linear regression of square root-transformed jellyfish bell diameter (cm) and body weight (g dry weight) for jellyfish used to calculate daily specific growth rate (μ) .



FIGURE 4.3. Change in (A) jellyfish bell diameter and (B) daily specific growth rate (μ). Each jellyfish is represented with open circles, measured at the start and end of experiment (N = 10 per plot) between ambient nutrient treatment (-N) experimental plots and nutrient enrichment treatment (+N) experimental plots. Black circles in each panel represent mean values. Statistical values are significant at the α = 0.05 level.



FIGURE 4.4. Comparison of the size-frequency distribution of *Cassiopea* spp., from (A) the start of the experiment and (B) the end of the experiment in ambient nutrient treatment (-N) and nutrient enrichment (+N) experimental plots.

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CHAPTER V

EFFECTS OF EPIBENTHIC JELLYFISH, *CASSIOPEA* SPP., ON FAUNAL COMMUNITY COMPOSITION OF BAHAMIAN SEAGRASS BEDS

Abstract

Pelagic jellyfish blooms have been observed frequently in many parts of the world, which can affect various aspects of marine ecosystems. While specific effects of jellyfish blooms on pelagic marine communities are increasingly well-described, there is little information on the effects of epibenthic jellyfish on benthic marine fauna. *Cassiopea* spp. (Upside-down Jellyfish) are relatively-sessile, epibenthic, jellyfish that inhabit seagrass habitats, and may be found in high densities in shallow coastal water bodies. In this study, we surveyed seagrass beds on Abaco, The Bahamas, that had similar seagrass cover but naturally-varying densities of *Cassiopea*. We found that benthic taxa richness was lower in plots with high jellyfish densities; however, overall benthic animal densities and biomass did not differ between plots. Benthic community composition differed between high and low jellyfish density plots. Our findings suggest that *Cassiopea* jellyfish may have subtle effects on benthic fauna communities, perhaps mediated through several mechanistic pathways.

Keywords: Caribbean, coastal ecosystem, food webs, jellyfish blooms, *Thalassia* testudinum.

Introduction

Gelatinous zooplankton, (e.g., jellyfish), are conspicuous and ecologically important organisms in marine environments. Although there is a paucity of long-term jellyfish density data, there is compelling evidence to suggest that some jellyfish populations are increasing in certain parts of the world, likely due to anthropogenic disturbances (Condon et al. 2012, Purcell 2012, Richardson et al. 2009). These jellyfish 'blooms' can have substantial effects on pelagic community composition and ecosystem function (Arai 2001, Lynam et al. 2006, Purcell and Arai 2001, West et al. 2009a).

Jellyfish blooms may affect pelagic ecosystems through several mechanistic pathways. For example, predation by jellyfish can affect the abundance of targeted prey (zooplankton) directly (Carr and Pitt 2008, Purcell and Arai 2001, Uye 2011). Jellyfish blooms may also lead to intensified interspecific competition with zooplanktivores for shared food resources (Brodeur et al. 2008). Jellyfish blooms may contribute increased inorganic nutrients via excretion that can stimulate planktonic primary production, and dead jellyfish may act as food for benthic fauna (i.e., jellyfish "falls") (Pitt et al. 2009, West et al. 2009a, West et al. 2009b, Yamamoto et al. 2008).

While our understanding of effects of jellyfish blooms on pelagic communities has improved in recent years, there has been little research regarding potential impacts of epibenthic jellyfish on benthic faunal communities. Jellyfish belonging to the genus *Cassiopea* Péron and Lesueur (Upside-down Jellyfish, hereafter referred to as *Cassiopea*) are relatively-sessile, epibenthic jellyfish, and contain photosynthetic dinoflagellates (zooxanthellae) that provide energy to the jellyfish, in addition to acquiring

heterotrophically-derived sources of nutrition (Verde and McCloskey 1998). *Cassiopea* are globally distributed in tropical environments and are ubiquitous in seagrass beds, reefs, lagoons, and mangrove habitats (Fleck and Fitt 1999, Holland et al. 2004, Niggl and Wild 2010).

Cassiopea may play an important ecological role by exerting top-down and bottom-up controls on nearshore food webs. For instance, *Cassiopea* may reduce macrophyte cover primarily via shading, diminishing food and habitat availability for benthic fauna. *Cassiopea* may also compete for space with other benthic flora and fauna, and some fauna (e.g., fishes) may avoid areas with high jellyfish densities as a result of nematocysts that are released regularly by *Cassiopea* (Stoner et al. 2011). Further, *Cassiopea* contribute organic nutrients (C and N) through mucoid exudate, and they may facilitate the release of inorganic nutrients (N and P) from the sediment-water interface via chronic bell pulsation. Finally, *Cassiopea* may consume zooplankton and particulate organic matter (POM), affecting food availability to benthic fauna (Jantzen et al. 2010, Larson 1997, Niggl et al. 2010). *Cassiopea* may also be an important food resource for some marine consumers, though to date, only one nudibranch species in Puerto Rico has been definitively reported to consume *Cassiopea* oral arms (Brandon and Cutress 1985).

In The Bahamas, *Cassiopea* are abundant in seagrass beds, systems which are critical habitats for numerous other benthic organisms (Antón et al. 2011, Heck et al. 2008, Nagelkerken et al. 2000). Recently, Stoner et al. (2011) demonstrated that *Cassiopea* are over 95% more abundant (and larger) in human-impacted coastal areas in The Bahamas, potentially affecting benthic fauna in nearshore systems including seagrass beds. Our objective was to evaluate whether seagrass beds with naturally-varying

densities of *Cassiopea* were associated with different assemblages of benthic animals. We hypothesized that there would be lower taxa richness, animal density and biomass, as well as different community composition, in areas with high jellyfish densities.

Field-site Description

Benthic surveys were conducted in two nearshore seagrass beds (sites referred to as Snake Cay 26°27′22" N, 77°03′ 27" W and Jungle Creek, 26°21′ 53"N, 77°01′ 25" W) on Abaco Island, The Bahamas, in May and June 2011 (Fig. 1). Study sites were directly adjacent to mangrove habitat, and each survey site was dominated by >50% *Thalassia testudinum* Banks ex König (Turtle grass) cover, <2 m in depth at low tide, and were characterized by silty-sandy sediment (~0.05 mm particle size), which suggests lower water velocities. Sediment was classified by collecting sediment samples (N = 3/site) from each site, visually estimating percent sediment contributions in each sample, and classifying sediment type using the USDA soil classification triangle (Schoeneberger et al. 2002). Both Snake Cay and Jungle Creek are coastal areas that were identified by Stoner et al. (2011) as relatively unimpacted by human activities. This was inferred from the low number of buildings in areas adjacent to these sites, which we used as a proxy for human population density (buildings within a 3 km radius from the mid-point of each site: SC = 0.3 buildings/km², JC = 0.24 buildings/km²).

Methods

Prior to conducting surveys, we identified areas with high and low jellyfish densities within each of the sites. High and low jellyfish density plots at each site were interspersed, and each plot was 16 m² in size and at least 5 m apart. 'High' jellyfish density plots (HD, N = 7 for both sites) were defined as areas with >3 jellyfish/m², which was based on the approximate mean number of jellyfish in human-impacted sites in Stoner et al. (2011). In the present study, the mean number (\pm SD) of jellyfish found within HD plots was 4.5 \pm 1.4 jellyfish/m². 'Low' jellyfish density plots, hereafter referred to as LD plots (N = 6 in Snake Cay, N = 7 in Jungle Creek), contained an average of 0.3 \pm 0.31 jellyfish/m². *Cassiopea* are relatively-sessile and move infrequently, typically only when disturbed. Research by Jantzen et al. (2010) on *Cassiopea* mobility suggested *Cassiopea* will remain within a 1m² area for an average of ~3 days. As such, we expected that areas with high or low jellyfish densities had similar density levels over a timespan of days to weeks, a period of time long enough to influence local floral and faunal traits.

One potential pathway in which *Cassiopea* may affect benthic seagrass community composition is through altered seagrass density. Seagrass characteristics (e.g., shoot biomass, shoot density, and leaf morphology) have been previously shown to be important factors affecting composition of benthic animal communities (Ansari et al. 1991, Heck and Wetstone 1977, Orth et al. 1984). In an attempt to isolate the direct effects of *Cassiopea* on benthic fauna independent of seagrass density, we selected sites *a priori* with apparently similar seagrass cover. We estimated % seagrass cover with $1m^2$ quadrats (N = 5/plot). In order to assess seagrass characteristics in more detail, a seagrass

core (plastic bucket without bottom, diameter = 23 cm, depth = 22.5 cm) was used to collect and quantify above- and belowground *T. testudinum* biomass (N = 4/plot).

To sample benthic fauna, we haphazardly placed a cylindrical, plastic, throw trap $(\text{area} \sim 0.75 \text{ m}^2)$ in each plot. The enclosed area was sampled using two mesh dip nets (10 mm and 0.5 mm mesh size) until three consecutive sweeps yielded no additional animals (following protocol in Hammerschlag-Peyer et al. 2013). Animal specimens collected were >0.5 mm as that was the size of mesh for the smallest dip net used. Although some benthic infauna were collected (i.e., clams), most of the taxa collected were motile benthic epifauna. Animals were then placed on ice in the field and frozen for later processing. *Cassiopea* found within each plot were enumerated.

We sorted and identified animal samples in the laboratory to the lowest taxonomic level practical, then dried them at 60°C to ascertain dry biomass. For mollusks, as well as *Phascolion* spp. (Sipunculan worms found in Cerith snail shells), shells were removed and only soft tissue dried and weighed. Seagrass samples were cleared of mud and debris by gently rinsing them under deionized water, then separated into aboveground biomass (all attached green leaves of short shoots), dead seagrass, and belowground biomass (rhizomes and roots). Any adhered epiphytes were removed from intact short shoots by carefully scraping blades with a razor. Seagrass above- and belowground biomass was dried at 60°C for 48-72 hours and weighed in order to obtain dry weight (g).

Statistical analyses

Seagrass percent cover, and aboveground and belowground *T. testudinum* biomass from cores, were averaged by plot. Three plots (low jellyfish density plots from Jungle Creek) were discarded from analysis as they did not adhere to our initial seagrass

criteria (>50% seagrass cover). A two-way ANOVA was used in order to determine whether seagrass percent cover and biomass differed between HD and LD plots and/or between sites (IBM SPSS v. 20.0).

Animal communities were evaluated based on taxa richness, density (i.e., the number of animals found per m^2), biomass (dry weight of animals found per m^2), diversity, and community composition. We calculated species diversity using the Shannon-Weiner Diversity Index (H'). A two-way ANOVA was used to determine whether univariate response variables (taxa richness, density, biomass, and diversity) varied between HD and LD plots and between sites (Snake Cay or Jungle Creek). In the event of a significant interaction between jellyfish density category and site, a Tukey post-hoc test was used to evaluate which groups differed from one other (SAS® software v.9.2).

Benthic animal densities were square-root transformed to down-weight the influence of dominant taxa (Clarke 1993) and a Bray-Curtis similarity matrix was calculated for all species contributing at least 1% to the total animal density. A two-way Analysis-of-Similarity (ANOSIM) was used to determine if there were differences in community composition between sites and/or treatments (PRIMER© v6) (Clarke 1993). Post-hoc one-way ANOSIMs were used to test for a difference in community composition between HD and LD plots for each site, and a SIMPER analysis was used to evaluate which taxa may be driving differences. Non-metric multidimensional scaling (nMDS) plots were used to visualize similarities/dissimilarities in benthic animal communities between HD and LD plots.

Results

Initial estimates of percent seagrass cover did not vary between HD and LD plots (mean HD ± SD = $67.2 \pm 15.2\%/m^2$, LD = $70.9 \pm 7\%/m^2$; $F_{1,20} = 1.0$, P = 0.32), but did differ between the two sites (mean JC ± SD = $63.2 \pm 11.4\%/m^2$, SC = $75.4 \pm 10.3\%/m^2$; $F_{1,20} = 7.0$, P = 0.01). Aboveground seagrass biomass from core samples indicate that there was no difference in seagrass biomass between HD and LD plots ($F_{1,20} = 3.6$, P = 0.07), nor between sites ($F_{1,20} = 0.30$, P = 0.58). Likewise, belowground seagrass biomass did not vary between plot type ($F_{1,20} = 0.51$, P = 0.60), and there was no difference in belowground biomass between sites ($F_{1,20} = 0.39$, P = 0.53).

A total of 51 animal taxa were collected, representing 45 families and seven phyla. The most abundant and frequently observed animals were molluscs, crustaceans, and polychaetes (Table 1). Mean taxa richness was lower in HD plots (mean HD \pm SD = 10.2 ± 2.6 , LD = 14.3 ± 3.5 ; $F_{1,20} = 8.5$, P = 0.009), and was lower in Jungle Creek (mean JC \pm SD = 10.2 ± 3.5 , SC = 13.4 ± 3.3 ; $F_{1,20} = 4.9$, P = 0.04; Fig. 2A). Taxa diversity (*H'*) did not differ between plot type (mean HD \pm SD = 1.9 ± 0.40 , LD = 2.2 ± 0.45 ; $F_{1,21}$ = 1.5, P = 0.22), but was lower in Jungle Creek (mean JC \pm SD = 1.7 ± 0.46 , SC = $2.3 \pm$ 0.23; $F_{1,21} = 12.1$, P = 0.002). Animal density and biomass did not significantly differ between HD and LD plots (P > 0.05; Fig. 2B, Fig. 2C). Total benthic animal density differed between sites ($F_{1,20} = 4.4$, P = 0.04), being higher at Jungle Creek (JC \pm SD = 57.4 ind./m² ± 29.3 , SC = 39.5 ind./m² ± 11.8 , Fig. 2B). Animal biomass was lower in Jungle Creek (JC \pm SD = $0.20g/m^2 \pm 0.05$, SC = $0.35g/m^2 \pm 0.07$; $F_{1,20} = 25.3$, P <0.001; Fig. 2C). Community composition differed marginally between HD and LD plots (global R = 0.17, P = 0.053) and differed between sites (global R = 0.33, P = 0.001). In Snake Cay, benthic animal community composition differed between HD and LD plots (global R = 0.28, P = 0.006; Fig. 3A). The observed difference in community composition between HD and LD plots were likely driven by a few taxa. In LD plots, clams (*Tellina listeri* Roding and *Parvilucina* spp. Dall) and *Cerithium* spp. (i.e., several species of cerith, hereafter collectively referred to as "Cerith snails") were some of the most abundant taxa, while in HD plots, *Bulla umbilicata* Roding (Common West Indian Bubble snails), were more abundant (Table 1). No difference in community composition was found between plot types in Jungle Creek (global R = 0.02, P = 0.3; Fig. 3B).

Discussion

Our findings demonstrate that epibenthic jellyfish have subtle effects on benthic fauna. As we hypothesized, we found lower taxa richness in HD plots than LD plots, and a difference in faunal community composition between HD and LD plots at one of the two study sites. We did not detect a significant difference in animal density or biomass, though many individual taxa were less abundant in HD plots than LD plots, likely attributable to a higher abundance of some opportunistic taxa that may benefit in some way from high jellyfish densities. We discuss several possible mechanisms that may have driven these findings below.

Lower fauna richness and differences in faunal community composition may be attributed to direct and indirect effects that *Cassiopea* have on seagrass faunal communities. First, some taxa may actively avoid areas with high *Cassiopea* densities,

perhaps due to *Cassiopea* mucus, filled with nematocysts, which is regularly released by the jellyfish (Niggl and Wild 2010). Observations from seagrass beds with high jellyfish densities suggest that there are fewer mesograzer and small, scarid parrotfish grazing scars on seagrass leaves compared to areas with no jellyfish present, suggesting avoidance of *Cassiopea* (E. Stoner, unpublished data). Second, high densities of *Cassiopea* may also limit available habitat for several animals, including sessile invertebrates (e.g., clams) and slow-moving animals that require space to successfully forage (e.g., sea cucumbers). Third, consumption by *Cassiopea* of POM may limit food availability to dominant benthic suspension feeders (e.g., molluscs). In addition to filtering POM, *Cassiopea* ingest zooplankton (e.g., copepods, ostracods, tanaids, nematodes, fish eggs and mollusk veligers) (Larson 1997) driving declines in food resources available to other benthic fauna found in this study. Another set of indirect effects on fauna may be mediated through seagrass cover, although that was not explored here because we attempted to isolate plots with similar levels of seagrass cover.

Although taxa richness and overall community composition differed between HD and LD plots, we did not observe any difference in total animal density or biomass. This is largely due to an increased abundance of opportunistic taxa that might be responding to jellyfish presence. For instance, the Common West Indian Bubble snail is a nocturnal, herbivorous, gastropod which feeds in seagrass beds (Malaquias and Reid 2008). We have frequently observed bubble snails adjacent to *Cassiopea*, perhaps because they utilize *Cassiopea* as refugia, potentially to minimize predation risk. This example underscores how little is known about interactions of *Cassiopea* with other species,

information that is clearly needed to elucidate their role in the structure and function of seagrass ecosystems.

It is important to note that jellyfish densities found in HD plots are lower than what has been observed in many human-impacted seagrass beds. For instance, in one bay on Abaco, *Cassiopea* densities were found to be over $>13/m^2$, 3 times the density in HD plots from these surveys (Stoner et al. 2011). It is possible that more substantial effects would be apparent at much higher *Cassiopea* densities. Experimental manipulation of jellyfish densities is a logical next step to further describe the role of *Cassiopea* blooms in structuring seagrass communities. This understanding will be critical, as the frequency and magnitude of jellyfish blooms may be increasing in anthropogenically-disturbed marine systems.

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Snake Cay

Scientific name	Common Name	Low <i>Cassiopea</i> Density	High <i>Cassiopea</i> Density
<i>Bulla umbilicata</i> Roding	Common West Indian Bubble (snail)	1.4 ± 1.4	4.2 ± 4.5
<i>Phascolion</i> spp.(in Cerith Shells)	Sipunculan Worms	8.6 ± 4.5	6.3 ± 4.7
Ophionereis reticulata (Say)	Reticulated Brittle Star	1.9 ± 2.6	1.3 ± 1.0
Pitho mirabilis (Herbst)	Pitho Crab	1.9 ± 1.7	1.3 ± 2.3
Tellina listeri Roding	Speckled Tellin Clam	6.1 ± 3.0	2.5 ± 2.1
Hermodice carunculata (Pallas)	Bearded Fireworm	1.3 ± 1.4	0.4 ± 0.9
Holothuria mexicana Ludwig	Donkey Dung Sea Cucumber	3.8 ± 2.9	0.6 ± 1.4

Jungle Creek

Scientific name	Common Name	Low <i>Cassiopea</i> Density	High <i>Cassiopea</i> Density
Phascolion spp. (in Cerith Shells) Bulla umbilicata Roding Hermodice carunculata (Pallas)	Sipunculan Worms	1.3 ± 2.0	3.6 ± 4.6
	Common West Indian Bubble Snail	16.3 ± 9.1	18.1 ± 13.3
	Bearded Fireworm	2.3 ± 3.3	1.3 ± 3.0
Parvilucina spp. Dall Prunum apicinium (Menke) Cerithium spp.	Lucinid Clam	8.3 ± 9.5	3.6 ± 7.0
	Common Atlantic Marginella	2.3 ± 1.0	0.9 ± 1.6
	Cerith Snails	12.9 ± 6.7	3.3 ± 3.3
Pitho mirabilis (Herbst)	Pitho Crab	1.5 ± 2.2	0.2 ± 0.3



FIGURE 5.1. Map of Abaco Island, Bahamas (A) indicating the two survey sites represented by squares, in nearshore seagrass beds in Snake Cay (B) and Jungle Creek (C). Both sites are directly adjacent to shallow red mangrove (*Rhizophora mangle*) habitat.



FIGURE 5.2. Mean (\pm SD) taxa richness (A), total animal density (B), and total animal biomass (C) between high and low jellyfish density plots for Snake Cay (SC) and Jungle Creek (JC). * denotes a significant effect of jellyfish density. Different letters above bars denote a significant site effect at $\alpha = 0.05$.



FIGURE 5.3. Non-metric multi-dimensional scaling plots representing similarities/dissimilarities in community composition between high and low jellyfish density plots for Snake Cay (A) and Jungle Creek (B). Note that the stress value for panel A is relatively high, and therefore the figure should be interpreted with caution.

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CHAPTER VI

MODIFICATION OF A SEAGRASS COMMUNITY BY BENTHIC JELLYFISH BLOOMS AND NUTRIENT ENRICHMENT

Abstract

Anthropogenic activities are increasing the number and intensity of stressors, often acting in concert, in ecosystems across the globe. One stressor in many marine ecosystems is an increased abundance of jellyfish (jellyfish blooms), which have garnered recent attention for their detrimental ecological and economic impacts. We conducted a field experiment to determine effects of proliferations of benthic jellyfish, *Cassiopea* spp., and another common stressor, anthropogenic nutrient enrichment (via fertilizer additions), on a shallow seagrass community in Abaco, Bahamas. Results suggested a reduction in seagrass abundance and habitat complexity in both jellyfish and nutrient enrichment treatments, with the results being generally additive. Jellyfish additions were associated with reduced faunal densities; nutrient enrichment drove shifts in faunal community composition. Grazing frequency was substantially higher in nutrient-enriched plots and lower in plots with jellyfish alone or jellyfish combined with nutrients, suggesting that jellyfish act as a deterrent to grazers. These findings highlight the inherent complexities in predicting ecological changes within shallow seagrass ecosystems to multiple, interacting, anthropogenic stressors.

Keywords: *Cassiopea* spp.; eutrophication; global change; habitat complexity; jellyfish blooms; *Thalassia testudinum*.

Introduction

As anthropogenic disturbances intensify globally, a fundamental concern for resource managers is predicting how multiple stressors will interact (Crain et al., 2008; Darling and Côte, 2008; O'Gorman et al., 2012; Williams et al., 2013). Environmental stressors can have independent and cumulative effects on reproduction (Folt et al., 1999), disease susceptibility (Lenihan et al., 1999), food web structure (Breitburg et al., 1999, O'Gorman et al., 2012), and biodiversity (Fitch and Crowe, 2012; Williams et al., 2013). Anthropogenic disturbance can also facilitate proliferations of certain taxa, often exotic species, which may interact with other human-induced stressors to affect community and ecosystem dynamics (Stachowicz et al., 2002; Silliman et al., 2005; Didham et al., 2007; Crain et al., 2008; Martone and Wasson, 2008). Marine ecosystems may be particularly susceptible to multiple human-driven stressors acting in concert (Halpern et al., 2008).

Gelatinous zooplankton (hereafter referred to as jellyfish) exhibit rapid and extreme increases in population densities (i.e., jellyfish blooms). Although there is a paucity of historic jellyfish population density data, the abundance of many jellyfish has increased since the 1970's (Condon et al., 2013). These jellyfish blooms have been linked to various anthropogenic disturbances including climate change (Brodeur et al., 2008), overfishing (Purcell and Arai, 2001; Lynam et al., 2006), anthropogenic nutrient loading (Arai, 2001; Stoner et al., 2011), introduction of invasive jellyfish species (Mills, 2001; Baya and Graham, 2014), and habitat modification (Lo et al., 2008; Hoover and Purcell, 2009; Duarte et al., 2012). In human-impacted systems in which jellyfish are abundant, jellyfish may exert strong top-down controls on community structure and ecosystem function. For example, increased predation by jellyfish can reduce the abundance of

zooplankton such as fish larvae (Purcell and Arai, 2001). For example, in the early 1980's, a decline of over 50% of the larval herring population took place during weeks when *Aurelia aurita* abundance exceeded 75ml per 100m³ in the Kiel Fjord, Western Baltic Sea (Möller, 1984). Jellyfish blooms may also affect ecosystems through bottom-up pathways. Following a bloom of *Crambionella orsini* off of the coast of Oman in 2002, moribund and dead jellyfish carcasses covered >90% of the seabed in some areas. The associated carbon input associated with these carcasses exceeded the annual organic carbon inputs into this region by an order of magnitude, and created localized "hot spots" of nutrient availability (Billett et al., 2006).

These examples pertain to pelagic jellyfish, but much less is known about how epibenthic jellyfish affect benthic communities and ecosystem dynamics. For instance, taxa of epibenthic jellyfish, *Cassiopea* spp. (also called upside-down jellyfish because of their relatively-sessile nature and bell orientation; hereafter *Cassiopea*) are globallydistributed in sub-tropical and tropical environments, and are found in several habitat types including mangrove forests, seagrass beds, and coral reefs (Holland et al., 2004; Niggl and Wild, 2010; Stoner et al., 2011). These animals host populations of endosymbiotic dinoflagellates (i.e., zooxanthellae) which provide fixed carbon to the jellyfish, though they do still require heterotrophically-derived sources of nutrition (Verde and McCloskey, 1998; Mortillaro et al., 2009). *Cassiopea* have been previously shown to be more abundant (and larger) in human-impacted systems in The Bahamas, likely as a result of elevated nutrient availability which is beneficial for zooxanthellae productivity (Stoner et al., 2011). However, little is known about how an increased

abundance of these animals affect other community and ecosystem attributes in shallow coastal environments.

Seagrass ecosystems, systems where *Cassiopea* are often found, may be especially vulnerable to high densities of benthic jellyfish. Nutrient loading and jellyfish may act in concert to affect seagrass and fauna through several direct and indirect mechanistic pathways (conceptual model depicted in Figure 1). For instance, elevated nutrients may drive reductions in seagrass as a result of increased epiphytic loads and algal shading (Burkholder et al., 2007). Conversely, elevated nutrient concentrations have also been shown to benefit seagrass, usually in oligotrophic systems, by enhancing productivity and growth (Allgeier et al., 2013). High jellyfish densities may drive declines in seagrass primarily by shading photosynthetic tissue (i.e., resting on seagrass and inhibiting photosynthesis), physically disturbing seagrass shoots, and by preventing clonal development of seagrass via space reduction. Nutrient and jellyfish-driven declines in seagrass may also have cascading effects on fauna, as seagrass abundance and associated structural complexity (i.e., shoot density and leaf morphology) have been shown to positively influence benthic faunal densities (Orth, 1984). Alternatively, jellyfish may directly lead to declines in benthic fauna, as fauna may avoid areas with high jellyfish densities. It is also possible that nutrients may have positive effects on some benthic fauna, namely grazers that benefit from nutrient-enriched seagrass (McGlathery, 1995; Valentine and Heck, 1999; Heck and Valentine, 2006; Holzer et al., 2013). Grazing can serve to reduce seagrass biomass directly, or benefit seagrass by suppressing epiphyte growth, depending on the grazer species and other environmental conditions (Valentine and Heck, 1999).

Our goal was to examine these mechanistic pathways in a shallow, subtropical, seagrass ecosystem. We manipulated jellyfish density and sediment nutrient availability to simulate conditions that are found across gradients of human impact in Bahamian coastal systems. We examined how these stressors may affect a number of seagrass characteristics, as well as benthic fauna that utilize the seagrass bed as habitat. These data are the first from an experimental manipulation of benthic jellyfish density and nutrient availability, providing an important step toward a more thorough understanding of how human activities may affect the structure and function of seagrass ecosystems.

Materials and Methods

Site description and experimental design

We conducted a 2x2 factorial field experiment in a relatively unimpacted nearshore seagrass bed (known as Jungle Creek, 26°21′ 53"N, 77°01′ 25" W) on Abaco Island, Bahamas. *Cassiopea* are naturally present in this seagrass bed, but at a relatively low density (<2 jellyfish medusa/m²). The benthos was dominated by >50% *Thalassia testudinum* (turtle grass) cover, <2 m in depth at low tide, and is characterized by siltysandy sediment (~0.05 mm particle size, as classified by the USDA soil classification triangle (Schoeneberger et al., 2002)), which suggests low water velocities.

The experiment was conducted over a 53 day period from May-July in 2012, the period when seagrass productivity is at an annual high in this system. There were four experimental treatments $(1m^2 \text{ plots})$: controls (C), nutrient enrichment (N), jellyfish addition (J), and jellyfish and nutrient additions (J+N), with 10 replicates for each (n = 40), randomly assigned among plots. We used an open (i.e., no cage) experimental setup to better simulate natural conditions. Plots were separated by ~5 m. The average low tide

depth of 0.68 ± 0.02 m (range = 0.38 m-1.35 m) within plots; there was no difference in mean depth across plot type (one-way ANOVA, $F_{3,36} = 0.52$, P = 0.67, SPSS IBM v. 20.0).

Prior to the start of the experiment, we visually estimated % seagrass (*T. testudinum*) cover using a single, $1m^2$ quadrat in each plot. We detected no difference in % seagrass cover across treatments (one-way ANOVA: $F_{3,36} = 0.90$, P = 0.44). Ambient nutrients in the water column in this system are extremely low, with nutrients taken up rapidly by plants, algae and microbial communities. Therefore, water column nutrients may not be the best measure of nutrient availability (Allgeier et al., 2011). Alternatively, nutrient content from macrophytes (e.g., seagrass) reflects ambient nutrient concentrations over a longer time frame (i.e., months), and provides a more reliable estimate of nutrient availability in nearshore ecosystems (Layman et al., 2013). As such, we harvested seagrass shoots immediately adjacent to each plot (to avoid disturbing the seagrass within each plot), to assess initial seagrass nutrient concentrations (%P and %N). *Experimental manipulation of jellyfish and nutrients*

For jellyfish addition plots (J, J+N), we added 10, medium-sized (7-10 cm diameter), jellyfish medusae, a density representing ~ 75^{th} percentile from humanimpacted sites on the island (Stoner et al., 2011). Each week, jellyfish were enumerated, and then added or removed as necessary to maintain 10 jellyfish per plot. To simulate eutrophication, we added Plantacote slow-release fertilizer (N:P molar ratio = 19:6, Scotts, Columbus, Ohio, USA), a compound frequently used in marine enrichment studies (Heck et al., 2000, Heck et al., 2006). We elevated nutrients by massaging the fertilizer (1,000g/m²) into the top 5 cm of sediment, which allows for chronic nutrient release over the duration of the experiment, following protocol outlined in Ferdie and Fourqurean (2004). Fertilizer was added to sediment in order to enable diffusion of nutrients through sediment porewater and into the water column. Nutrient loading rates were estimated to be $0.81 \text{ g N m}^{-2} \text{ d}^{-1}$ and $0.25 \text{ g P m}^{-2} \text{ d}^{-1}$ over the duration of the experiment. We determined rates of N and P loading by filling two fine mesh laundry bags with 1,000 g of fertilizer, securing the bags with wooden stakes in 1 m^2 plots ~100 m from the experimental site, and calculating total loss of N and P over the course of the experiment. These loading rates were comparable to those reported by Ferdie and Fourqurean (2004), a similar carbonate system in the Florida Keys.

Seagrass and fauna sampling

Several seagrass and fauna characteristics were sampled to assess impacts of jellyfish and nutrient enrichment. We analyzed seagrass % cover, biomass, shoot densities and leaf morphometrics (leaf length and width). Seagrass cover, shoot densities and nutrients in seagrass tissue were measured at the start and end of the experiment, as they required no destructive sampling; the other variables were sampled only at the end of the experiment. Shoot densities were enumerated using 4, 900 cm², quadrats, haphazardly placed within each plot. Samples for biomass and morphometrics were taken using a seagrass corer (diameter = 23 cm, n = 3 per plot). To assess grazing intensity, we extracted 7 additional seagrass shoots from each plot and froze them for later processing in the laboratory.

Benthic fauna (infauna within the top \sim 5 cm of sediment and all epifauna) were sampled using a cylindrical, plastic, throw trap (Area = 0.75 m). The enclosed area was sampled using two mesh dip nets (10 mm and 0.5 mm mesh size) until three consecutive

sweeps yielded no additional animals (following protocol outlined in Hammerschlag-Peyer et al., 2013). Although some benthic infauna were collected, most of the taxa collected were motile epifauna. Animals were then placed on ice in the field and frozen for later processing. *Cassiopea* found within each plot were enumerated.

Sample processing

To analyze %N and %P concentrations, *T. testudinum* leaves were gently scraped to remove epiphytes, dried at 80°C, and ground into a fine powder. %N in leaf tissue was evaluated from duplicate samples using a Carlo Erba CN analyzer. Percent P in seagrass leaf tissue was analyzed using dry-oxidation acid hydrolysis extraction in addition to the use of a colorimetric analysis using a CHN autoanalyzer (Fisons NA1500) (Fourqurean et al., 1992). The analytical detection limit for %P was 0.02 µm.

Seagrass leaves were measured for total length and width (mm). Cores of seagrass biomass were separated into aboveground biomass (all attached green leaves of short shoots), dead seagrass, and belowground biomass (rhizomes and roots). All seagrass tissue was then dried at 60°C for 48-72 hours and weighed. To analyze grazing intensity, we measured the total number of grazing scars on seagrass shoots made by small, scarid, parrotfishes and amphipods (Alcoverro et al., 1997; Peterson et al., 2013). Grazing by parrotfishes were characterized as small crescent shaped bite marks; scars by amphipods were identified as thin strips of missing leaf material from the epidermis of one side of the leaf, as observed in a mesocosm experiment containing only seagrass (*Thalassia testudinum*) and gammaridean amphipods (J. Sweatman. *personal observation*). Though we quantified parrotfish and amphipod grazing scars separately, we added all grazing scars together to establish total grazing by small herbivores on seagrass from each plot.
Epiphytes were quantified on these same blades by removing any adhered epiphytes (including inorganic carbonates) by scraping blades with a razor blade, drying epiphytes at 70°C, and obtaining their weight.

Faunal samples were sorted and identified to the lowest taxonomic level possible, then dried at 60°C to ascertain dry biomass. For mollusks, as well as sipunculid worms found in cerith snail shells, shells were removed and only soft tissue dried and weighed. *Statistical analyses*

We calculated proportional change for %P (seagrass), %N (seagrass), % seagrass cover, and shoot densities from the start to the end of the experiment. Proportional change in %P and %N were arcsine-transformed to meet assumptions of normality and homoscedasticity. Seagrass biomass (above-and belowground), epiphyte dry weight (values+1), and total grazing intensity (values+1) were natural log-transformed to meet assumptions of normality and homoscedasticity. All aforementioned nutrient and seagrass metrics, in addition to leaf length and width, were analyzed independently using fixedfactor 2-way ANOVAs to compare main effects of nutrient enrichment and jellyfish additions (SPSS IBM v.20.0). To evaluate specific pairwise differences across each experimental treatment (C, N, J, J+N) for total grazing intensity, Tukey post-hoc tests were conducted for all response variables (SPSS IBM v. 20.0).

Differences in jellyfish densities (enumerated at the end of the experiment in each plot) among treatments were analyzed using a 2-way ANOVA (SPSS IBM v.20.0). Benthic fauna density, richness, and biomass (g dry weight/m²) (square-root transformed to better meet assumptions of normality and homoscedasticity) were also analyzed using 2-way ANOVA's (SPSS IBM v.20.0). One driver of faunal change may have been

mediated through differences in seagrass densities (Fig. 1 – see text in Introduction). To this end, we employed separate linear regressions with seagrass shoot density as the predictor, and fauna density, richness, and biomass as response variables (SPSS IBM v.20.0).

To evaluate benthic animal community composition, a Bray-Curtis similarity matrix was calculated for all species contributing at least 1% to the total animal density. *Cassiopea* were not included in any of the faunal community analyses. Data were 4th root transformed to down-weight influence of dominant taxa prior to calculation of similarity metrics (Clark, 1993). A permutational multivariate analysis of variance (PERMANOVA) was used to determine if there were differences in community composition among treatments (PRIMER© v6) (Anderson, 2001).

Results

At the end of the experiment, jellyfish densities were significantly higher from jellyfish addition plots (J, J+N; $F_{1,36} = 42.6$, P < 0.0001, 4.9 ± 0.4 jellyfish/m²) than non-jellyfish addition plots (N, C, 0.9 ± 0.3 jellyfish/m²) (Fig.2). %P in seagrass tissue was affected by nutrient enrichment, but not jellyfish additions (Table 1). There was no significant effect of either factor on %N in seagrass tissue (Table 1).

Mean % seagrass cover and shoot densities were significantly lower at the end of the experiment as a result of both nutrient enrichment and jellyfish additions (Table 1, Fig.3a, 3b). Nutrient enrichment reduced mean seagrass aboveground biomass and leaf length, but jellyfish had no significant effect on either of these variables (Table 1, Figs.3c, 3e). Nutrient enrichment and jellyfish additions did not have a significant effect on belowground seagrass, leaf width, or epiphyte dry weight (Table 1, 3d, 3f, 3g).

Grazing intensity (i.e., frequency of total bites by parrotfishes and amphipods on seagrasss shoots) was strongly influenced by both factors (Table 1, Fig.3h). We found a significant jellyfish × nutrient interaction in which grazing intensity was substantially lower on seagrass shoots from J plots and significantly higher on seagrass from N plots (Table 1). Specifically, grazing scars were over 472% more abundant on shoots from N plots than in J plots, and 296% more abundant on shoots in N plots than in J+N plots (Fig.4).

A total of 83 different benthic animal taxa were collected, representing eight phyla; some of the most common taxa included mollusks, crustaceans, and marine worms (Table 2). Jellyfish, but not nutrient enrichment, reduced mean animal density (Table 1, Fig. 5a). Mean animal richness and biomass were not affected by either factor (Table 1, Fig.5b, 5c). Seagrass density was positively related to animal richness ($R^2 = 0.10$, $F_{1,37} =$ 4.1, P = 0.04) and density ($R^2 = 0.10$, $F_{1,37} = 4.3$, P = 0.04), but not biomass ($R^2 = 0.03$, $F_{1,38} = 1.4$, P = 0.24). Changes in animal community composition were associated with nutrient enrichment (pseudo- $F_{1,33} = 2.7$, P = 0.01), but not jellyfish (pseudo- $F_{1,33} = 1.4$, P = 0.21). Several species of cerith snails, collectively referred to as *Cerithium* spp., as well as sipunculan worms living in empty cerith snail shells, were most abundant from C plots. *Bulla occidentalis* (Common West Indian bubble shell snail) were more abundant from nutrient enrichment plots (N and J+N plots) (Table 2).

Discussion

Experimental manipulations of nutrients and jellyfish suggested that both stressors influence flora and fauna, as well as some species interactions. Modification of seagrass habitat complexity was perhaps the most notable result from the experiment. Mean *T. testudinum* cover and shoot density declined substantially as a result of both nutrient and jellyfish additions, while leaf length and aboveground biomass were reduced solely as a function of nutrient enrichment. One frequently cited cause of seagrass mortality is reduced light availability, driven by excessive epiphytic growth on seagrass (Burkholder et al., 2007). However, in this experiment, we did not observe any difference in epiphytic loads on seagrass from fertilized plots, suggesting seagrass loss was not related to this mechanism. These findings mirror results from a recent nutrient enrichment experiment conducted in Mobile Bay, Alabama in which reductions in *Halodule wrightii* abundance and structural complexity occurred, with no evidence of increased epiphyte loads on seagrass leaves in fertilized plots (Antón et al., 2011).

One possibility for the observed reduction in seagrass abundance and complexity is that nutrient enrichment intensifies low oxygen concentrations mediated through increased respiration in sediment porewater, leading to sulfide intrusion into seagrass rhizomes (Borum et al., 2005; Antón et al., 2011). Sulfide has negative effects on seagrass productivity, photosynthesis and metabolism (Burkholder et al., 2007). Previous work in tropical seagrass systems has also indicated that elevated water temperature (typical in the summer months in our study system when the experiment was conducted), can interact with high sulfide concentrations to drive *T. testudinum* mortality (Koch and Erskine, 2001).

Jellyfish were also shown to have deleterious effects on seagrass. One possible reason may be that high densities of relatively sessile *Cassiopea* prevent sunlight from reaching seagrass blades. High densities of *Cassiopea* may also limit space for seagrass, indirectly affecting seagrass by preventing clonal seagrass development in areas in which

seagrass would otherwise grow. In addition, *Cassiopea* may negatively affect seagrass by competing for nutrients, as zooxanthellae in *Cassiopea* may uptake nitrogen and phosphorus, providing higher levels of photosynthate to their hosts (Falkowski et al., 1994). *Cassiopea* may also act as sources of nutrients to the sediment-water interface by releasing nutrients from porewater (via bell pulsations), potentially exacerbating effects of anthropogenic nutrients on seagrass (Jantzen et al., 2010). Further, respiration by *Cassiopea* at night depletes dissolved oxygen concentrations at the sediment-water interface (Verde and McCloskey, 1998), possibly resulting in further sulfide intrusion into seagrass rhizomes. Finally, chronic bell pulsation by *Cassiopea* visibly disturbs seagrass shoots, likely reducing the stability of shoots in the sediment (E. Stoner. *personal observation*).

Grazing intensity by herbivores (parrotfishes and amphipods) on seagrass was also strongly affected by nutrient enrichment and jellyfish, and may have contributed to the shifts we observed in seagrass abundance and complexity. Grazing scar frequency was highest on leaves from N plots, perhaps because herbivores such as small parrotfishes preferentially consume nutrient-enriched seagrass (Heck and Valentine, 2006). One common outcome of increased grazing on nutrient-enriched seagrass is a reduction in seagrass density (McGlathery, 1995; Fourqurean et al., 2010; Holzer et al., 2013). For example, Heck et al. (2006) observed that *Halodule wrightii* aboveground biomass was substantially lower following experimental nutrient enrichment, which they suggested was a result of increased grazing by large pinfish and crustaceans on nutrientenriched leaves. Conversely, under higher ambient nutrient conditions, mesograzers have been shown to control epiphyte growth on seagrass leaves, benefitting seagrass (Whalen

et al., 2013). Consistent with this idea, one hypothesis for similar epiphyte loads across treatments in our experiment could be enhanced grazing by amphipods on epiphytes in nutrient addition plots. Despite any possible reductions in epiphytic loads by amphipods, it is likely that frequent grazing on seagrass in nutrient addition plots, combined with other factors associated with nutrient enrichment (e.g., sulfide intrusion), resulted in the modification to seagrass habitat complexity we observed.

Responses of benthic fauna to jellyfish and fertilizer additions were also complex. Specifically, we found that jellyfish additions drove a reduction in faunal densities, but we observed no effects of jellyfish on taxa richness or biomass. There are various possible explanations for declines in fauna from jellyfish addition plots. As discussed above, many of the taxa sampled in our experiment were mobile epifauna that may avoid jellyfish. Consistent with this hypothesis, grazing intensity was lowest in J and J+N plots, perhaps because fishes (including herbivores) may avoid areas with high jellyfish densities, as *Cassiopea* release mucus filled with nematocysts. For example, we have observed yellowfin mojarra (Gerres cinereus) rapidly die after swimming through mucus released from *Cassiopea* (E. Stoner. *personal observation*). High densities of jellyfish may also limit available habitat for epifauna, and chronic bell pulsations by *Cassiopea* may disturb remaining habitat for fauna (e.g., via physical movement of seagrass leaves). In fact, we found that seagrass density was positively related to both fauna density and richness, suggesting that a reduction in seagrass mediated through jellyfish (and nutrient) additions could affect faunal densities.

We observed no significant effects of nutrient enrichment on faunal univariate response variables. This result is surprising, largely because a reduction in seagrass

habitat would have led to fewer fauna (as described above). For example, a nutrient enrichment study found that a reduction in seagrass (Halodule wrightii) structural complexity resulted in lower benthic faunal abundance (Antón et al., 2011). Additionally, elevated nutrients may have indirect, negative, effects on benthic fauna abundance in seagrass beds, mediated through shifts in oxygen dynamics (Deegan et al., 2002). However, moderate increases in nutrient concentrations can also lead to higher epifaunal densities, usually grazers, which will consume nutrient-enriched seagrass (Gil et al., 2006). It is possible that we did not observe lower faunal densities and richness as a result of nutrient enrichment because some opportunistic taxa proliferated from elevated nutrients, offsetting declines in more sensitive taxa. For example, the common West Indian bubble snail Bulla occidentalis, one of the most abundant taxa in across all treatments, was most prevalent in N and J+N plots. Little is known about the ecology of this herbivorous gastropod, but one hypothesis for their heightened abundance in nutrient-enriched plots is that increased microphytobenthos on the sediment following nutrient additions (and concomitant declines in seagrass) provided enhanced algal food resources.

It is important to recognize that our findings may vary on the basis of the number of stressors, system location and type, and number of abiotic and biotic factors present. For instance, it is possible that in seagrass beds with higher ambient nutrient concentrations, anthropogenic nutrient loading (and jellyfish blooms) would exacerbate seagrass loss. In one study in which nutrients were experimentally manipulated in a eutrophic Bermudian seagrass (*T. testudinum*) system, filamentous mat macroalgae increased by an estimated 60-100%, driving declines in seagrass abundance (McGlathery,

1995). In addition, the spatial scale of our plots was small ($\sim 1 m^2$), perhaps not adequately representing jellyfish bloom events and nutrient loading that occur at the scale of entire ecosystems. Further, most nutrient enrichment experiments of seagrass (including ours) do not simulate the chronic (i.e., years) nutrient loading that actually occurs in many coastal ecosystems. One notable exception was a press manipulation of low nutrient concentrations over 27 months in a southern Australia seagrass bed, in which *Amphibolis antarctica* and *Posidonia sinuosa* seagrass biomass substantially declined, presumably from elevated epiphyte loads (Bryars et al., 2011). Prolonging our experiment may have revealed other patterns that were not manifest here.

The results from our study provide another example of the inherent complexity in evaluating ecological responses to multiple stressors (Crain et al., 2008). The role of jellyfish in structuring ecological systems through top-down and bottom-up processes will likely intensify, as anthropogenic disturbances increase the magnitude and frequency of jellyfish bloom events. Understanding the ecological responses to these blooms, and how they interact with additional stressors, will be important to predict ecological consequences.

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TABLE 6.1. Summary of two-way ANOVA of nutrient enrichment and jellyfish on nutrient, seagrass, and faunal response variables. Nutrient concentrations, as well as % seagrass cover and shoot densities, represent change in values from the start to the end of the experiment. All reported nutrient and seagrass values reflect data averaged across treatments, and seagrass and faunal data were transformed if necessary. Significant values (at $\alpha = 0.05$) are highlighted in bold.

Variable	df	F	Р	Variable	df	F	Р		
Nutrient									
concentrations	(0)			N#4 1					
Phosphorus in seagrass	Phosphorus in seagrass (% dry weight)			Nitrogen in seagrass (% dry weight)					
Nutrient enrichment	1	6.4 0.56	0.01		1	0.07	0.93		
	1	0.50	0.45		1	0.37	0.54		
J X IN Posidual	1 21	0.14	0.71	J X N Residual	28	0.47	0.49		
Residual	51			Residual	20				
Seagrass									
% cover	cover			Shoot density (# shoots/cm ²)					
Nutrient enrichment	1	7.2	0.01	Nutrient enrichment	1	26.0	<0.0001		
Jellyfish	1	4.5	0.04	Jellyfish	1	31.7	<0.0001		
J x N	1	0.16	0.68	J x N	1	0.15	0.69		
Residual	36			Residual	36				
		(2)							
Aboveground biomass (g aw/	m) 62	0.04	Belowground blomass (g aw/n	1) 1 E	0.01		
	1	0.3	0.01		1	0.60	0.21		
	1	2.9	0.09		1	0.00	0.44		
J X IN Posidual	36	2.2	0.14	J X IN Residual	36	0.52	0.00		
Residual	Residual 30				50				
Leaf length (mm)				Leaf width (mm)					
Nutrient enrichment	1	4.5	0.04	Nutrient enrichment	1	0.59	0.44		
Jellyfish	1	0.54	0.46	Jellyfish	1	1.4	0.24		
JxN	1	0.42	0.52	JxN	1	0.16	0.69		
Residual	29			Residual	28				
Epiphyte dry weight (g dw/shoot)			Herbivore grazing intensity (# bites/shoot)						
Nutrient enrichment	1	1.8	0.17	Nutrient enrichment	1	7.9	<0.0001		
Jellyfish	1	1.6	0.20	Jellyfish	1	28.7	<0.0001		
JXN	1	0.00	0.92	JXN	1	7.7	0.007		
Residual	36			Residual	265				
Bonthic Fauna									
Density (# animals/ m^2)				Richness (# taxa/ m^2)					
Nutrient enrichment	1	33	0.07	Nutrient enrichment	1	21	0 15		
Jellyfish	1	4.8	0.07	Jellyfish	1	11	0.10		
JXN	1	1.3	0.24	JXN	1	0.72	0.40		
Residual	36		0.2	Residual	36	•=	01.0		
	-								
Biomass (g dw/m²)									
Nutrient enrichment	1	0.58	0.44						
Jellyfish	1	0.16	0.68						
JxN	1	0.03	0.85						
Residual	36								

TABLE 6.2. Mean densities \pm standard error for the most abundant benthic fauna/m² for each experimental treatment (C, control; N, nutrient enrichment; J, jellyfish addition; J+N, jellyfish + nutrients). The highest density for each taxa is in bold.

	Scientific Name	Common Name	С	N	J	J+N	
	Alpheus heterochaelis	Bigclaw snapping shrimp	5.2 ± 0.2	4.4 ± 0.4	4.9 ± 0.2	4.3 ± 0.2	
	<i>Tellina</i> spp.	Tellin clams (multiple species)	3.5 ± 0.2	2.5 ± 0.5	2.7 ± 0.4	3.4 ± 0.3	
-	Phascolion spp.	Sipunculan worms in cerith shells	3.1 ± 0.4	1.8 ± 0.4	1.5 ± 0.3	2.5 ± 0.3	
	Cerithium spp.	Cerith snails (multiple species)	2.9 ± 0.2	2.1 ± 0.5	2.6 ± 0.3	1.4 ± 0.2	
	Bulla occidentalis	Common West Indian bubble	1.9 ± 0.3	2.8 ± 0.5	1.0 ± 0.3	2.6 ± 0.3	
	Panopeus spp.	Mud crab	1.7 ± 0.2	1.0 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	



FIGURE 6.1. Conceptual diagram of generalized interactions between nutrients, jellyfish, seagrass, and benthic fauna. Arrows with plus (+) or minus (-) signs represent a positive or negative effect, respectively. The gray arrow from nutrients to jellyfish represents how anthropogenic nutrient loading may facilitate jellyfish blooms, which were not evaluated in this study. See Introduction for a detailed explanation of mechanistic pathways depicted here.



FIGURE 6.2. *Cassiopea* medusae resting in a jellyfish addition only (J) plot. The seagrass, *Thalassia testudinum*, surrounds the jellyfish.



FIGURE 6.3. Main treatment effects (nutrient and jellyfish additions) on (a) change in % seagrass cover, (b) change in seagrass shoot density, (c) seagrass aboveground biomass, (d) seagrass belowground biomass, (e) leaf length, (f) leaf width, (g) epiphyte dry weight, and (h) total grazing intensity (by parrotfishes and amphipods). Values are mean \pm SE. Seagrass biomass (above-and belowground), epiphyte dry weight (values+1), and total grazing intensity (values+1) were natural log-transformed to meet assumptions of normality and homoscedasticity. Experimental treatments are C, control; N, nutrient enrichment; J, jellyfish addition; J+N, jellyfish + nutrients. *P* values indicate comparisons of main treatment effects in each panel.



FIGURE 6.4. Frequency of herbivorous fish and mesograzer grazing scars per short shoot across experimental treatments (mean \pm SE; C, control; N, nutrient enrichment; J, jellyfish addition; J+N, jellyfish + nutrients). Treatments designated with the same letter did not differ significantly (based on Tukey post-hoc tests).



FIGURE 6.5. Main treatment effects (nutrient and jellyfish additions on (a) animal densities, (b) animal richness, and (c) animal biomass. Values are mean \pm SE and were square-root transformed to better meet assumptions of normality and homoscedasticity. Experimental treatments are C, control; N, nutrient enrichment; J, jellyfish addition; J+N, jellyfish + nutrients. *P* values indicate comparisons of main treatment effects in each panel.

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CHAPTER VII

CONCLUSION AND FUTURE DIRECTIONS

A primary goal in conducting research on global change is to better predict and manage human-mediated stressor effects on biodiversity, populations, and the resilience of ecosystems (Vitousek et al. 1997; Chapin et al. 2000; Sala et al. 2000). Proliferations in opportunistic (native and non-native) taxa, often due to human disturbance, can exert strong controls on structuring communities and affecting ecosystem function. As such, careful study of these is needed to predict future environmental change, and potentially mitigate undesired impacts (McKinney and Lockwood 1999; Scott and Helfman 2001; Olden et al. 2004).

Jellyfish are one such taxa, though they have been somewhat underrepresented in the literature, largely because of the difficulty in studying a gelatinous animal that is not easily collected by common sampling techniques (Purcell 2009). In the 1980's-1990's, jellyfish started to gain more attention, and were widely perceived to be a taxa that benefit from human disturbance. It is possible that globally, an increase in the frequency of jellyfish blooms is the result of natural population oscillations, yet some jellyfish populations appear to be increasing in certain human-impacted environments, warranting more careful study (Condon et al. 2013). The results of my research are the first to show that benthic jellyfish taxa in sub-tropical ecosystems benefit from human disturbances.

In CHAPTER II, I demonstrated that *Cassiopea* are more abundant, and are larger, in areas adjacent to relatively high human population density centers. Though this study established a relationship between epibenthic jellyfish and human disturbance, it was unclear as to what specific mechanisms drove the observed patterns; this provided the context for further research presented in this dissertation. One caveat of this work is that I did not attempt to identify how *Cassiopea* polyps and ephyrae may be influenced

by human disturbances, neglecting two major life-history stages of *Cassiopea* jellyfish. Evaluating how polyps, in particular, respond to human disturbances is increasingly being recognized as an important next step in better understanding jellyfish blooms and their effects (Graham 2001; Richardson et al. 2009). For instance, some reports indicate that polyps exhibit increased asexual reproduction (i.e., budding), when temperatures are elevated, with obvious implications for increased temperatures associated with global climate change (Purcell et al. 2007; Purcell et al. 2012). This is one obvious future research direction that I did not have the opportunity to study in the course of my dissertation research.

In CHAPTER III, I identified one of the possible mechanisms driving heightened *Cassiopea* abundance and size in human-impacted sites, by evaluating zooxanthellae densities across sites with varying human population densities adjacent to coastal water bodies. I found that zooxanthellae densities were greater in jellyfish tissues collected from human-impacted sites, and that elevated zooxanthellae densities were positively correlated to gut weight. This is consistent with the idea that increased heterotrophically-derived nutrition may stimulate zooxanthellae population growth (Szmant-Froelich and Pilson 1984; McAuley and Cook 1994). This research provided an example of how a mutualism between host and symbiont may be sensitive to human-driven environmental change.

In CHAPTER IV, I examined jellyfish growth under conditions of increased nutrient availability. This was, to my knowledge, the first experiment that has evaluated how jellyfish size responded to elevated anthropogenic nutrient enrichment. I found that *Cassiopea* grew in treatments with elevated nutrient availability, and conversely, shrank

under ambient nutrient conditions. Though zooxanthellae densities were not evaluated in this study, I suspect that positive growth was attributable to elevated photosynthate provided by zooxanthellae. Shrinkage of jellyfish in the ambient nutrient treatment was likely an experimental artifact, mediated through reduced food availability or shading caused by experimental cages (Lucas 2001). The results of this study have implications for how jellyfish structure marine systems. For instance, larger jellyfish (due to faster growth) may prey on greater quantities (and diversity) of plankton, potentially reducing food resources to other zooplanktivores (Graham and Kroutil 2000). Perhaps even more importantly, larger jellyfish have increased gonad weight and reproductive output, potentially facilitating jellyfish bloom events (Lucas 2001; Hirst et al. 2003).

In CHAPTER V, I used a survey-based approach to examine if benthic faunal communities differ in areas with high and low *Cassiopea* abundance (in areas with relatively little human impact). I demonstrated that taxa richness was lower in high jellyfish density areas compared to low jellyfish density areas, and found that faunal community composition differed between the two plot types in one site. However, I observed no other effects of high jellyfish densities on benthic fauna (e.g., densities). It is possible that more significant differences would have been detected if I would have included sites with much higher jellyfish densities (i.e., in areas adjacent to human settlements).

As global change intensifies, it is becoming increasingly important that we understand effects of multiple, interacting, stressors (Crain et al. 2008; Darling and Côte 2008; O'Gorman et al. 2012). In CHAPTER VI, I examined how both elevated *Cassiopea* densities and nutrient enrichment may affect seagrass communities using an

experimental approach. I found that both stressors influence flora and fauna, as well as some species interactions, at a shallow seagrass site. Modification of seagrass habitat complexity was perhaps the most notable result from the experiment, specifically with seagrass cover and density declining as a result of both stressors, while leaf length and aboveground biomass were reduced as a function of nutrient enrichment. Similar to findings from CHAPTER V, I found that jellyfish had variable effects on benthic fauna, chiefly that jellyfish additions resulted in reduced taxa densities, but not for taxa density or biomass, and that nutrients were only responsible for shifts in faunal community composition. I also demonstrated that grazing intensity on seagrass by parrotfish and amphipods varied as a function of both stressors. Specifically, I found that nutrient additions resulted in elevated grazing intensity on seagrass, a well-described response to nutrient-enriched seagrass (McGlathery 1995; Fourqurean et al. 2010; Holzer et al. 2013). However, in the presence of jellyfish, grazers were deterred from consuming seagrass. Together, these findings suggest that benthic jellyfish blooms in nutrient enriched systems may exert a strong influence on structuring seagrass communities through indirect and direct mechanistic pathways.

All five of my data chapters provide support that nutrient-driven benthic jellyfish blooms affect nearshore seagrass ecosystems through several mechanistic pathways. Not only does this research advance our ecological understanding of species interactions in seagrass beds, it more specifically has contributed to our knowledge of the ecological role of human-driven jellyfish blooms in coastal marine systems. Though my research has addressed a major knowledge gap, there is still a necessity to elucidate the various mechanisms driving ecological responses by seagrass communities to benthic jellyfish

blooms. More generally, it is important that future research efforts explicitly evaluate relationships with other natural, jellyfish populations and human-impacts. This work will be increasingly valuable, as global change drivers such as ocean acidification, a disturbance that has been reported to benefit jellyfish (making jellyfish one of the only possible marine taxa to benefit from reduced pH), intensify (Attrill et al. 2007). Finally, it is also important that experiments manipulating jellyfish abundance and human impacts be conducted in a variety of settings (and with different stressors), in order to identify trends or deviations from the patterns I observed in my research. Ultimately, having a more in-depth understanding of the effects of human-driven stressors on jellyfish populations and their effects on marine flora and fauna will be critical in more appropriate management of jellyfish blooms.

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PUBLICATIONS AND PRESENTATIONS

- Stoner, E.W., Yeager, L.A., Sweatman, J.L., Sebilian, S.S., and Layman, C.A. In Review. Modification of a seagrass community by benthic jellyfish blooms and nutrient enrichment. *Journal of Experimental Marine Biology and Ecology*.
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