ABIOTIC STRESS, GRAZING AND DISEASE: IMPLICATIONS OF GLOBAL CHANGE ON *Zostera marina* SEAGRASSES

By

GABRIELA MAXINE BLOHM

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER IN SCIENCE

UNIVERSITY OF FLORIDA

2008

To the strongest women in my life: my mother, for teaching me how to cultivate my strengths and to my grandmother, for always reminding me to stop and smell the flowers

ACKNOWLEDGMENTS

I thank Craig W. Osenberg, an inspiring and brilliant mentor and advocate. I thank Bob Holt for all of his support and positive reinforcement; Marta Wayne for empowering me to push through and grow from my experience; Benjamin Bolker for his constant and indispensable help; Michelle Mack for sparking my interest in ecosystem ecology and allowing me to use her lab space, the SOB lab for all of their helpful feedback on my manuscript; Lindsey Albertson for all of her help in the field; Brian Silliman for establishing the contact with the Duffy lab and Ondi Crino for her help in every aspect of these last 3 years.

TABLE OF CONTENTS

page

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

ABIOTIC STRESS, GRAZING AND DISEASE: IMPLICATIONS OF GLOBAL CHANGE ON *Zostera marina* SEAGRASSES

By

Gabriela Maxine Blohm

August 2008

Chair: Craig W. Osenberg
Cochair: Robert D. Holt
Major: Zoology

The effects of anthropogenic activities go beyond the non-living components of ecosystems; humans are directly altering biological communities. Understanding the implications of changing abiotic regimes on the strength of biological interactions and rates of primary production is important for predicting the ecological effects of climate change. Seagrass ecosystems are of particular interest, given their high rates of primary productivity and simultaneous exposure to a number of factors associated with climate change. Ecosystems undergo simultaneous changes in their natural environment. Extrapolating the effects of single stressors from controlled laboratory settings to natural settings has often proven insufficient; changes in abiotic regimes often occur simultaneously in the environment. Seagrass ecosystems are ideal for conducting multi-factor studies at the mesocosm scale through replicated experimental manipulation.

We investigated the effects of increased temperature, nutrient enrichment and grazing pressure on *Zostera marina* disease, biomass and senescence in a replicated mesocosm experiment at the Virginia Institute of Marine Science in Gloucester Point, VA. We found that an average daily temperature increase of 1.7°C results in a reduction of biomass and an increase in

senescence. Contrary to previous studies, which showed that increased temperature gives rise to an increase in disease-driven necrosis, we found that nutrient enrichment increased the proportion of grass that exhibited necrosis due to disease. We found no grazer effect and no significant interaction effects, although we caution that the statistical power of the experiments was low. Our results suggest that the grazing, increased temperature and nutrient enrichment operate through different pathways, given that they did not show synergies or antagonisms. In the context of climate change and seagrass disease, our results do not support the previous studies that have reported seagrass diebacks that were associated with disease outbreaks due to warmer water temperatures.

CHAPTER 1
INTRODUCTION

The biotic structure of ecosystems and the abiotic context in which they function have undergone a suite of rapid modifications due to climate change (IPCC 2007, Vitousek 1997, Harvell 2002). Although the causes of these changes are difficult to identify, global mean temperatures and N-fixation rates have increased and will continue to do so as human economic demands increase (IPCC 2007). The effects of anthropogenic activities go beyond the non-living components of ecosystems; humans are directly altering biological communities. For example, in many communities the systematic removal of species (mainly predator and large herbivore species) has cascaded down to alter primary productivity (Deegan 2007, Ripple 2007, Frank 2005, Daskalov 2002).

Biological alterations are not limited to trophic cascade studies that took hold in the 1960s when Hairston, Smith and Slobodkin (1960) posed the classic question of why the world is green. “Hidden players,” which include pathogens and decomposers, have been recognized as an important component of community structure. For example, in many ecosystems –from coral reefs and temperate marshes to tropical montane amphibian communities - disease outbreaks associated with increased UV, drought and increased temperatures greatly altered species diversity and primary production (Harvell 2002, Silliman 2005 and Pounds 2006). Understanding the dynamics of hidden players and their response to ecological changes is important for predicting how communities will respond to climate change.

Forecasting the effects of changing temperature, nutrient regimes and community structure is a challenging task, especially if they interact non-additively. Multi-factor experiments can facilitate the identification of key ecological players and possible synergisms, but are limited by the required spatial scale of the experiments. Mesocosm experiments are useful for predicting

biological responses to climate change (e.g., Kercher 2004, Koch 2007, Christensen 2006). This approach allows the manipulation of multiple factors using replicated experiments in an environment that resembles field conditions more closely than a laboratory setting. In this study, we manipulate three factors related to global change (temperature, nutrients and grazers) in a seagrass mesocosm array to determine their effects on primary producer biomass, disease and senescence.

We expect that increased temperature will become an important factor for seagrass survival as global climate change progresses, having likely effects on *Z. marina* biomass, allocation and growth. Stress due to increased herbivory and pathogen loads has been studied in the terrestrial literature and is expected to increase plant allocation to anti-herbivore chemicals (possibly changing C and N demands), resulting in tradeoffs on important life history characteristics such as reproductive allocation (Clay 1996). It has been widely documented in the terrestrial literature that physical damage due to grazing can increase susceptibility to disease by providing an entry for pathogens, as well as by altering plant allocation to anti-herbivore chemicals (Clay 1996). The effects of nitrogen on seagrasses are complex. There are indirect effects due to increased epiphyte load and reduced light, and direct effects on *Z. marina* specifically, due to NH_4^+ toxicity (Van Katwijk 1997, Touchette 2000). Although temperate seagrasses are considered N-limited during the early growing season, water-column ammonium and nitrate increase as the summer progresses, giving rise to temporal differences in nutrient limitation. Pulsed agricultural inputs of NH_4^+ in *Z. marina* ecosystems results in the accumulation of NH_4^+ that can become toxic due to changes in cell membrane potential (Van Katwijk 1997).

Seagrasses serve important roles in the global C and N cycles; thus their feedbacks on global climate change are of interest. They store an estimated 15% of the ocean's carbon. Net

production in these systems is approximately 0.6 x 10^{15} g C/yr (Duarte and Chiscano 1999), 24.3% of which is exported to adjacent terrestrial and marine ecosystems (Duarte and Cebrián 1996). Seagrasses also reduce the flow of fertilizer into coral reefs and other coastal systems that are highly susceptible to eutrophic conditions (Touchette 2000, Hemminga and Duarte 2000). Commercially and ecologically important species such as blue crab, shrimp, red drum and scallops require seagrass habitat during at least part of their life history (Hemminga and Duarte 2000).

The services provided by seagrasses may diminish with the effects of global change. Indeed, sixty percent of global seagrass habitat loss has been attributed to anthropogenic activities (Short and Wylie-Escheverria 1996). Additionally, worldwide seagrass declines have been associated with higher temperatures (Seddon and Cheshire 2000), eutrophication from agricultural runoff (Short and Wylie-Escheverria 1996) and wasting disease (Muehlstein 1992). Higher average daily temperatures will increase respiration rates (Short and Neckles 2002), and projected reductions in inorganic C in the water column (Terrados 1999) should reduce photosynthetic rates. Nutrient inputs increase algal growth, leading to low oxygen conditions and reduced light availability (Touchette 2000). They also can enhance periphyton production, leading to shading, and increase grazer populations, possibly enhancing herbivory rates on the seagrass (Touchette 2000).

These changes are expected to alter distributions and rates of ecological interactions in seagrasses (Duarte 2002). Interactions among plants and microbes, which include disease and decomposers, have not been well-described. Previous studies suggest that this group of interactions can potentially have a large effect on the extent and cover of seagrasses (Renn 1936, Muehlstein 1997). For example, a series of seagrass diebacks were reported as early as 1930,

coincident with a warm water event (Cottam 1933). The leaves of the seagrass, *Z. marina*, were brown and necrotic, suggesting the seagrass had been infected with a wasting disease (Muehlstein 1992). Although the ecological mechanisms that caused these diebacks were not experimentally elucidated, *Labyrinthula zosterae,* a marine protistan wasting disease, was later found in these necrotic wounds and described as a pathogen using Koch's postulates (Muehlsein 1990). Since then, diebacks associated with wasting disease and warm water temperatures were documented in the 1980s (Muehlstein 1991) and early 2000s (J. J. Orth, *personal communication*). Laboratory experiments have shown that *L. zosterae* thrives at higher salinities (Vergeer 1995); however, its responses to higher temperatures and nutrient regimes in the water column have not been determined experimentally. As a result, it is difficult to predict how *L. zosterae* will respond to abiotic factors, and how its possible effect on *Z. marina* may depend on other biotic and abiotic stressors that also are expected to occur in these ecosystems.

Studies that investigate the effects of multiple factors related to climate change and anthropogenic effects can inform conservation efforts while also strengthening our understanding of how simultaneous changes can affect ecosystems. Seagrass ecosystems are ideal for studying the effects of multiple factors; they are experiencing coincident changes in temperature, fertilizer and community structure (Duarte 2002, Hemminga and Duarte 2000); comprise an ecologically and economically important system (Hemminga and Duarte 2000, Lippson and Lippson 1984); and can be studied in mesocosm settings.

Few studies have investigated the effects of global climate change (or its various components) on seagrass biomass or wasting. The goal of this study was to determine the effects of three putative stressors on seagrass: increased temperature, increased water column fertilizer, and the presence of herbivorous crustaceans that feed on seagrass. We explore the single and

combined effects of these stressors on seagrass biomass, allocation, wasting disease and senescence. These results shed light on whether the past documented diebacks were due to increased temperature, nutrient inputs or increased physical damage due to grazing, and provide insight about future effects that might be expected with continued climate change.

CHAPTER 2
METHODS

Experimental Design

We applied three putative stressors: increased temperature (by ~1.7°C using heaters), grazers (via the addition of *A. valida*) and NH_4^+ (via addition of Osmocote NPK fertilizer) in a 6-week outdoor mesocosm experiment to determine their effects on 1) two separate types of *Z. marina* browning: disease-induced necrosis and senescence; and 2) *Z. marina* biomass. We conducted a fully factorial experiment (2 levels of each factor; 8 replicates/treatment; yielding 64 mesocosms) at the Virginia Institute of Marine Science in a flow-through system with water supplied from the York River estuary. Sixty four 5-gallon buckets, were arranged along the middle of 4 cattle tanks, with two replicates of each treatment per tank (i.e., tanks were treated as blocks in the design).

Mesocosm Set-up

The river water (salinity approx 15-20 ppt) was pumped through a sand filter and a mesh filter (500 microns) to remove larvae and other mesofauna. Five three-inch diameter holes covered in 250 µm mesh lined the upper edge of each bucket to allow water flow out of the buckets without the loss of grazers. To prevent backflow into the buckets from the tanks, we placed a standpipe in each tank that prevented the external water level from exceeding that of the bottom edge of each mesh hole. We lined the bottom 3 inches of each bucket with a combination of 4 parts sand to 1 part dried organic matter from the Goodwin Island salt marsh. We allowed the sediment to settle for 1 day before planting the grass. The tanks were covered with opaque mesh to reduce UV light penetration into the buckets, which are shallower than most field conditions.

We collected *Z. marina* shoots from Goodwin Island. Before transplanting, we counted sixty four groups of 15 green shoots each and placed them in small mesh bags that were floated in a tank with flow-through seawater. To ensure that all microcosms were starting with similar amounts of fouling organisms (e.g., epiphytes), *Labyrinthula sp.* spores, and tunicate larvae that could colonize the mesocosms and reach abnormally high densities, we removed the grass from the mesh bags and exposed each group of shoots to four consecutive 5-minute freshwater washes. We manually removed all visible fouling organisms such as the invasive tunicates *Botryllus sp.* and *Molgula sp* and brown shoots. We then removed excess water from the grass using a salad spinner (20 rotations) and recorded its wet mass, ensuring that each group had approximately the same mass and the same relative amounts of root and aboveground biomass (total wet mass averaged 47 g per bucket; range 42-53 g). We haphazardly arranged the grass in each bucket and planted it so the roots were buried and the blades were floating in the water column. We allowed the grass to acclimate for 1 week before applying the treatments. We then populated all buckets with two species of crustaceans (an amphipod, *Gammarus mucronatus*, and an isopod, *Erichsonella attenuata*) to control epiphyte growth on the grass throughout the duration of the experiment.

Treatments

We increased temperature by a mean of 1.7°C above ambient levels (using VisiTherm brand aquarium heaters in the +Heat treatment buckets only). During the first 11 days, we monitored temperature twice daily using a YSI brand temperature and DO meter. During the remainder of the experiment, we used 10 HOBO loggers to record temperature at 15-minute intervals. We rotated the loggers into new buckets 4 times throughout the duration of the experiment.

To increase water column fertilizer levels (specifically NH_4^+), we prepared 25 g of Osmocote brand fertilizer (19-6-12 NPK) in nylon mesh bags, which we then placed inside 20-cm PVC pipes that contained holes to allow the slow release of fertilizer into the water column. Each bucket contained either a PVC pipe with fertilizer or an empty pipe as a control. We collected water samples from each bucket on days 1, 2 and 3 after the introduction of the nutrient sticks, filtered the water through 1 µm filters, and followed the Koroleff method to obtain the concentration of NH_4^+.

We introduced an additional species of amphipod (*Ampithoe valida*) to all buckets in the "+Grazer" treatment. Unlike the former 2 species, which feed primarily on detritus and epiphytes that foul *Z. marina* blades, *A. valida* feeds directly on *Z. marina* aboveground tissue (Lippson and Lippson 1984). All buckets contained the same initial number of individuals (30 crustaceans), but the grazer levels differed in composition. The grazer treatment received 10 *A. valida,* 10 *E. attenuata* and 10 *G. mucronatus,* while the grazer controls received 15 *E. attenuata* and 15 *G. mucronatus. A. valida* feeds on epiphytes until the grass is exposed; then it switches to feeding on *Z. marina.* The initial number of crustaceans was kept the same for all treatments to ensure that the epiphyte load was controlled similarly across all treatments.

Sampling

We conducted three surveys: week 2 (non-destructive), week 4 (non-destructive) and week 6 (destructive: end of experiment) to measure the abundance of crustaceans and the abundance of browning.

Surveys during Experiment

For the first two crustacean surveys, we swept the water column and seagrass following three figure-8 sweeps using a small mesh net, counted all individuals of each species and returned them to their mesocosms (except for contaminants: *A. valida* in non-grazer treatments

were noted and removed). For the first two browning surveys, we haphazardly chose five blades from each bucket and recorded the total length, percent cover of brown (using the Wasting Index published by Burdick 1997), number of grazer scars and if possible, leaf age. Blade age was calculated from the location of the seagrass blade on the shoot, where 1 is the innermost blade and 3 is the outermost blade. Brown tissue was divided into two types based on its morphology: 1) "senesecent" tissue was identified visually as translucent light brown, originating at the tip of the blade and when cultured in the laboratory (surface-sterilized with peroxide and grown in fetal bovine serum), it contained mainly fungus and yeast (although some contained *Labyrinthula sp.*); and 2) "necrotic" tissue was opaque dark brown, displaying a mottled pattern that originated in center of the blade and expanded out towards the edges. When observed under the microscope, the cells in necrotic tissue were partially digested and disfigured and when grown in culture (surface-sterilized with peroxide and grown in fetal bovine serum), necrotic tissue contained mainly *Labyrinthula sp.* and some fungi.

Final Survey

At the end of the experiment (day 42 after initiating the treatments), we siphoned the water out of each bucket through a 500 µm sieve. We removed all algae and amphipods from the edge of the bucket and interstices of the nutrient sticks. We then uprooted the grass and rinsed it in saltwater to remove excess sediment. We froze the samples of grass and crustaceans in opaque plastic bags at -10°C in the dark for later processing at the University of Florida. We subdivided each sample of crustaceans 2 or 4 ways using a plankton splitter, identified and counted all amphipods and isopods in the subsample, and scaled counts up to the entire sample. To minimize additional browning on the grass due to light-induced breakdown of chlorophyll, we covered the grass in foil and defrosted it under running water. We then carefully separated each shoot and recorded the length, percent cover of brown, type of brown, number of scars and leaf age. We

then separated aboveground, litter and belowground biomass, placed each component in aluminum bags, and dried them at 60°C for approximately 5 days. We then weighed the dried seagrass and placed it in a muffle furnace (at 500°C), reweighed the sample, and obtained ash-free dry mass as the difference between the two masses.

Statistical Analyses

Crustacean density and total biomass were analyzed using ANOVA. Total *Z. marina* biomass was analyzed using ANOVA. *Z. marina* biomass allocation (above, below and litter) was analyzed using MANOVA. Litter was included in the analysis as part of total biomass because all buckets contained no litter initially, and decomposition rates were presumed to be low given the hypoxic conditions of the sediment and short duration of the experiments. The proportion of brown tissue was arcsine square root transformed and analyzed using Repeated Measures ANOVA in two separate models: 1) for senescence and 2) for necrosis. Models were reduced by sequentially eliminating non-significant terms ($P>0.05$). All analyses were conducted in R (C-Ran R Project, 2007).

CHAPTER 3
RESULTS

Effectiveness of Treatments

Temperature

The average daily temperature difference between the ambient and heated treatments was 1.7°C (Figure 1-1). This difference persisted during the first three weeks of the experiments. Due to a decrease in flow rates of seawater into the tanks, however, the mean difference increased to approximately 5°C on June 9-10 and on June 17-19. On both occasions, the heaters were adjusted to reduce the temperature differential. The sand filter was cleaned to restore normal water flow rates within 2 days.

Fertilizer

Although we added N, P and K, the target stressor was NH_4^+ because it can be directly toxic to *Z. marina* cells. [NH_4^+] declined with time and was significantly greater in the nutrient addition treatments ($F_{1,8}$=18.72,p=0.0001)*;* there was no effect of heat or grazers on the rate of nutrient release during the first three days of nutrient addition.

Crustacean Density

A. valida was present in all of the grazer treatments and essentially absent from the non-grazer treatments (details are summarized below), thus the grazer addition treatment had a significant effect on *A. valida* density ($F_{1,8}$ = 94.42, p=1.3x10^{-13}). *G. mucronatus* was the most abundant crustacean, having near 10 times the density of *E. attenuata,* the least abundant species.

The average number of *A. valida* was greater in the grazer addition treatment than in the treatments without grazers ($F_{1,8}$=94.4, p=1.28*10^{-13}) by approximately 120 individuals in each bucket. *A. valida* was essentially absent from all non-grazer treatments except for those with NPK, in which two of the eight replicates were contaminated with more than 30 *A. valida*

individuals. Exclusion of these replicates had a minimal effect on the results and did not change the statistical conclusions, so we left these two replicates in the final analyses.

G. mucronatus was the most abundant crustacean and its densities were significantly affected by fertilizer addition ($F_{1,8}$= 35.09, p=2.01*10^{-7}) but not heat ($F_{1,8}$ = 0.84, p= 0.36). There were approximately 200 more individuals in the fertilizer addition treatments than all treatments without fertilizer.

A qualitatively similar pattern was found in the total number of individuals for all species pooled together (effect of fertilizer: $F_{1,8}$= 37.28, P=1.02*10^{-7}), although the effect of heat was significant ($F_{1,8}$= 4.66, p= 0.04). This pattern was driven by *G. mucronatus*, which was approximately eight times more abundant than the other two species.

E. attenuata was the least abundant crustacean, which can be explained by the species' large body size (up to 4-5x the size of the other two species) and slower generation time relative to that of the other two species. *E. attenuata* abundances were significantly reduced in the heated treatment ($F_{1,8}$=14.46, p=0.0004)suggesting that increased thermal stress might reduce *E. attenuata* abundance in *Z. marina* beds.

Response Variables

Increased temperature, fertilizer and grazing had additive effects on *Z. marina* senescence, necrosis and biomass; however each response variable was affected differently by each of the treatments. Increased temperature best explained the variation in the majority of the results (*Z. marina* senescence and biomass), followed by NPK, which explained *Z.marina* necrosis and allocation to a small extent (0.1>p>0.05). The predictive power of grazing was lowest and therefore did not remain in the final models for *Z. marina* senescence, necrosis and biomass. We observed some interesting patterns as we monitored the temperature and grazer treatment data.

Z. marina Biomass

Total seagrass biomass was greatest at ambient temperature, grazer and nutrient levels. Increased average daily temperatures led to a lower mean total *Z. marina* biomass of approximately 30% when compared to all treatments that had ambient temperatures ($F_{1,8}$=5.3, p=0.025). There was no significant effect of grazing or fertilizer addition on total *Z. marina* biomass ($F_{1,8}$=.0321, p=.86 and $F_{1,8}$=0.5, p=0.48, respectively).

Z. marina proportional allocation did not vary significantly among treatments (Grazers: $F_{1,54}$= 0.26, p=0.9; Heat: $F_{1,54}$= 1.08, p=0.3; Fertilizer: $F_{1,54}$= 2.25, p=0.093). These results from the MANOVA suggested that further analysis of proportional allocation might reveal an effect of fertilizer on proportional allocation. Thus, we conducted individual ANOVAs on the proportion of total biomass that was allocated to each type of plant material. We found a significant effect of fertilizer addition on *Z. marina* belowground allocation ($F_{1,8}$=4.18, p=0.046).

Increased temperature led to a significant increase in the proportion of attached tissue (which is separate from the detached litter that was discussed in the previous section) that was senescent ($F_{60,18}$ =4.56, p=0.037) (Figure 1-3). Grazers explained some of the variation in senescence ($F_{60,18}$ =2.25, p=0.13) and exhibited some interesting trends in response to heat (see results for crustaceans). Fertilizer had little effect on the proportion of senescence ($F_{60,18}$ =0.058, p=0.8).

At higher temperatures, *Z. marina* senescence increased steadily through time. At the end of the experiments, the proportion of senescent tissue in the heated treatments was higher than those that experienced ambient temperature levels. A sharp increase in the proportion of senescent tissue during the middle of the experiment, coupled with an increase in the sample variance for the heated treatments occurred. This could be explained by the temperature spike

that occurred during the same time when the water flow rates decreased, further suggesting a strong effect of increased temperature on senescence.

Z. *marina* Necrosis

The best model included only the main effect of fertilizer (Figure 1-4). There were no significant interactions among the three factors ($p>0.3$ in all cases) on necrosis, which is the browning associated with *L. zosterae*. The addition of fertilizer increased the proportion of necrotic tissue ($F_{60, 18}= 17.06$, $p=0.0001$), suggesting that high levels of NH_4^+ increased *Z. marina* susceptibility to disease and may provide favorable conditions for the growth of *L. zosterae.* Neither grazers ($F_{60, 18}= 0.91$, $p=0.35$) nor heat ($F_{60, 18}=0.86$, $p=0.36$) had demonstrable effects, suggesting that disease outbreaks are better explained by increases in water column fertilizer than by temperature.

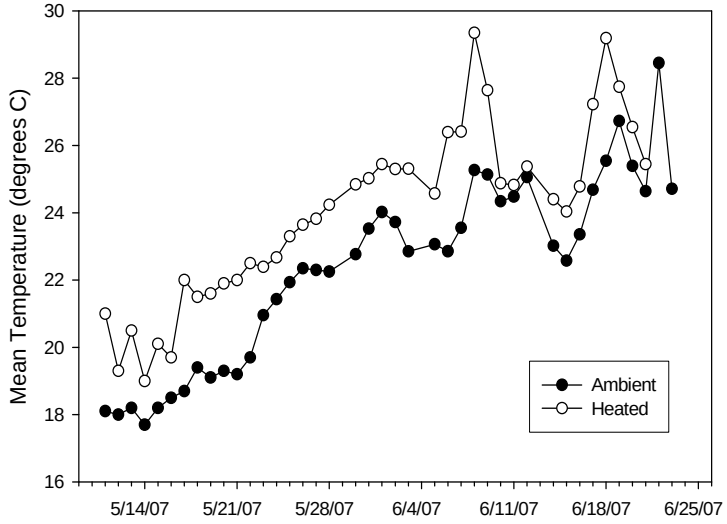

Figure 1-1. Average daily temperature of heated and ambient mesocosms. Average daily water temperature. Readings taken from twice-daily monitoring (maximum and minimum) using a YSI brand meter between 5/11/07 and 5/21/07. Readings between 5/22/07 and 5/25/07 were recorded using HOBO dataloggers, which monitored temperature at 15-minute intervals.

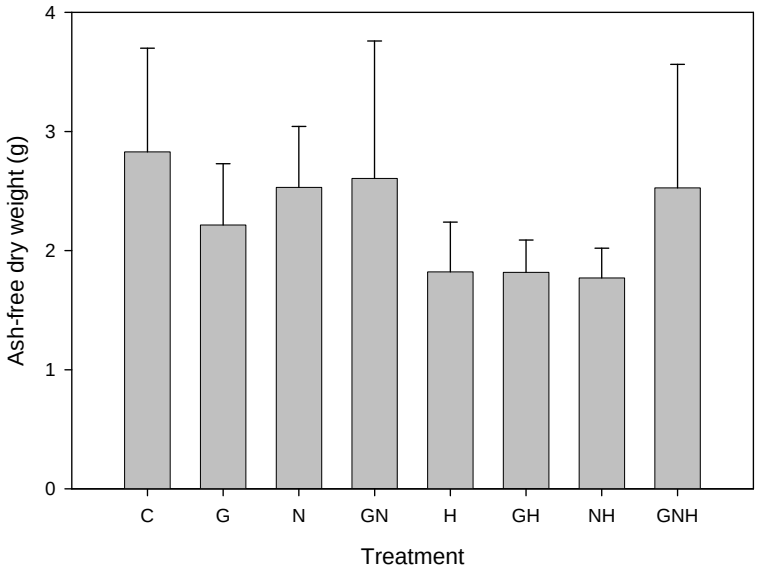

Figure 1-2 Ash-free dry mass of aboveground, belowground and litter of *Z. marina* in each treatment. C is the control; G represents *A. valida* (grazer) addition; H represents heat; N represents the addition of fertilizer. Plotted are the means +/- 2 SE.

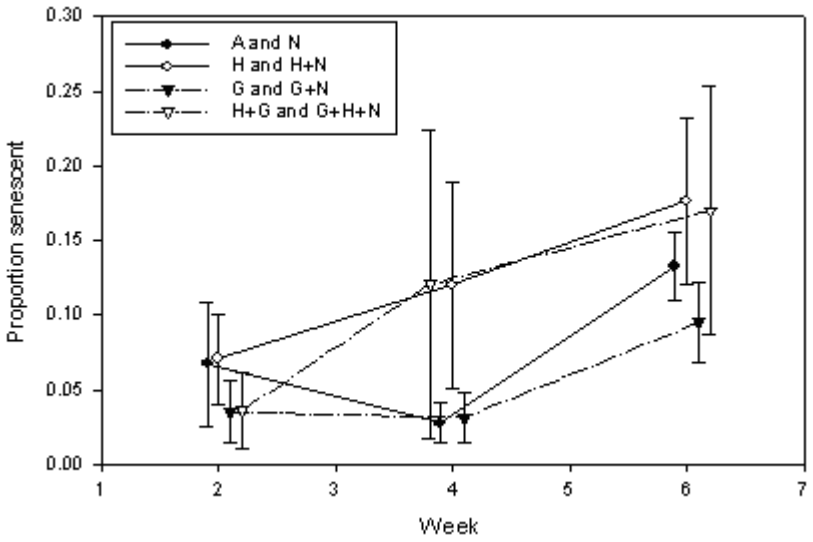

Figure 1-3 Proportion of senescent tissue, calculated as (% cover)*(leaf area)/(total leaf area). Because the effect of N was negligible (p=0.81), the data were pooled (thus, N=16). Plotted are the means +/- 2 SE.

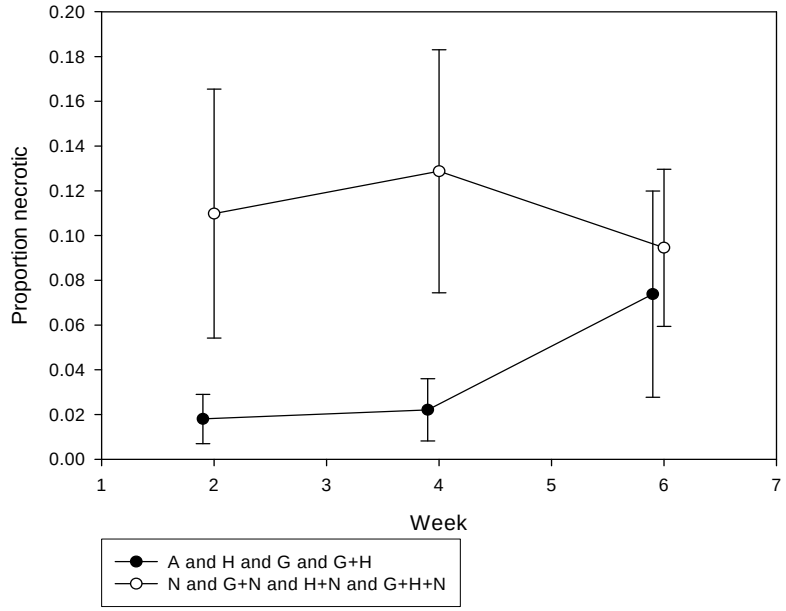

Figure 1-4 Proportion of necrotic tissue, calculated as (% cover)*(leaf area)/(total leaf area). Because the effects of Heat and Grazers were negligible (p=0.34 and p=0.35, respectively), the data were pooled for the graph (thus, N=32). Plotted are the means +/- 2 SE.

CHAPTER 4
DISCUSSION

Temperature, nutrients, and grazers affected the *Z. marina* community differently. Increased temperature led to a reduction in *Z. marina* biomass and an increase in senescence. The addition of fertilizer increased the proportion of necrosis associated with *L. zosterae*. Grazers had no demonstrable effects. The effects of increased temperature, fertilizer and grazing pressure on *Z. marina* were additive, although the data are highly variable (leading to low statistical power), so the absence of interactions should be taken with caution.

The reduction in total *Z. marina* biomass due to heat, coupled with the positive effect of heat on senescence suggests that increased temperature led to higher rates of litterfall that were not compensated by seagrass growth. Given that the proportion of litter was not significantly affected by increased temperature, it is also possible that heat directly reduced *Z. marina* growth. A reduction in growth could have been caused by a reduction in photosynthetic yield, as has been observed in other temperate seagrass species (Campbell 2006). The upper thermal tolerance limit for most temperate seagrass species is 35°C (Bulthius 1983, Ralph 1998). When temperatures exceed this limit, carbon production can be reduced because higher temperatures can increase respiration rates (Bulthius 1983, Ralph 1998) and can denature photosynthetic enzymes, as was found in some terrestrial plant species (Bruggeman 1992). Studies on the effects of thermal stress on photosynthesis and productivity in temperate seagrasses highlight that individual responses to increased temperature depend on the duration of exposure, history of thermal stress, light levels and leaf age (Bulthuis 1987, Seddon and Cheshire 2000). In tropical seagrass ecosystems, net primary productivity begins decreasing at 30°C (Fong and Harwell 1994). For example, in *Thalassia testudinum* ecosystems, standing crop was reduced at temperatures between 3 to 4°C above ambient levels. In a study of seven temperate seagrasses

(*Z. marina* was not included), Campbell (2006) found that a reduction on photosynthetic yield at high temperature pulses (15-30 minute exposures to 35-45°C) gave rise to reductions in photosynthetic yield, explaining the decreased aboveground biomass that was reported with El Niño warm water events (Seddon and Cheshire 2000). Although the results of this study do not show a significant reduction in aboveground biomass due to increased temperature, a reduction in total biomass was found, suggesting that higher temperatures might affect belowground growth as well. It is also possible that decomposition rates were greater at higher temperatures, giving rise to a reduction in the litter that was collected and measured at the end of the experiment; however the litter that accumulated was relatively in tact and decomposition did not appear to have been appreciable (Blohm, pers. obs.).

Past studies on *L. zosterae* suggest that it is an endemic and facultative pathogen found in decomposer community (Muehlstein 1988, Muehlstein 1990), as opposed to a novel pathogen that arrived via a specific vector. To understand and predict the future extent of *L. zosterae*, it is important to identify the factors (whether genetic or environmental) that most affect its growth. At least three large-scale diebacks associated with a wasting disease were attributed to warmer temperatures (Renn 1936, Muehlstein 1988); however the results of these studies were not corroborated by experimental evidence. *L. zosterae* was identified as the causative agent of seagrass wasting disease using Koch's postulates (Muehlstein 1991); however the results of our experiments suggest that temperature did not increase necrosis (an indicator of L. zosterae); instead nutrients increased the incidence of necrosis. Recent unpublished studies have shown that *L. zosterae*is less tolerant of increased temperature than is *Z. marina* (Erica Smith and Gabriela Blohm, *unpublished data*): *L zosterae* dies at temperatures above 26°C, while *Z. marina* does well up to 30°C. This suggests that past diebacks were not caused by disease outbreaks

related to high temperatures, but rather high nutrient loads or other possible factors that were not measured.

The results of our experiments suggest that nutrient inputs better explain the abundance of *L. zosterae* in seagrasses, although the mechanisms that underlie our results are unclear. Nutrient addition has a suite of direct and indirect effects on *Z. marina* growth and survival (Duarte 2002, Touchette 2000): increased epiphyte growth can give rise to light limitation; increased respiration in the water column can reduce oxygen availability, especially at night; and NH_4^+ can be directly toxic to the leaf tissue. It would follow that if *Z. marina* is stressed (due to light limitation, O limitation or NH_3^+ toxicity), it would be more susceptible to *L. zosterae.*

Developing a theoretical framework for the study of multiple factors is challenging when the physiological responses to single and multiple factors is unknown. Scaling up to the ecological level to explain changes in interaction strengths, community structure and ecosystem function will require that we understand how stressors affect individual physiology. To understand how future changes in disease and senescence will affect seagrass ecosystem function (C sequestration, N provision for adjacent ecosystems, nutrient buffering), we need to experimentally determine how this ecosystem will respond to increased microbial (disease *and* decomposer) activity, with the understanding that the dynamics underlying the cause and response of each group of decomposers are different. The results of these experiments (coupled with the documented severity of past diebacks) suggest that previous studies may have reported both senescence and necrosis as the drivers of seagrass diebacks, and that separating the two types of leaf loss is important for understanding how to manage seagrass ecosystems in the context of climate change.

LIST OF REFERENCES

Bruggeman, W., Van Der Kooij, T.A.W., Van Hasselt, P.R., 1992. Long-Term chilling of young tomato plants under low light and subsequent recovery: II. Chlorophyll fluorescence, carbon metabolismand activity of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plants 186, 179– 187.

Bulthuis, D.A., 1983. Effects of temperature on the photosynthesis-irradiance curve of the Australian seagrass, *Heterozostera tasmanica*. Marine Biology Letters 4, 47– 57.

Bulthuis, D.A., 1987. Effects of temperature on the photosynthesis and growth of seagrass. Aquatic Botany 27, 27– 40.

Campbell, S. J., L.J. McKenzie and S. P. Kerville. 2006. Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. Journal of Experimental Marine Biology and Ecology 330, 455-468.

Christensen, M.R., M.D. Graham, R.D. Vinebrooke, D.L. Findlay et al. 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Global Change Biology 12, 2316-2322.

Clay, K. 1996. Interactions among fungal endophytes, grasses and herbivores. Researches on Population Ecology 38, 191-201.

Daskalov, G.M. 2002. Overfishing drives a trophic cascade in the Black Sea. Marine Ecology Progress Series 225, 53-63.

Deegan, L.A., J.L. Bowen and D. Drake. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. Ecological Applications 17, 542-563

Duarte, C. M. 2002. The future of seagrass meadows. Environmental Conservation 29, 192-206.

Duarte, C.M. & Cebrián, J. 1996. The fate of marine autotrophic production. Limnology and Oceanography 41, 1758–66.

Fong, P., Harwell, M.A., 1994. Modelling seagrass communities in tropical and subtropical bays and estuaries: a mathematical model synthesis of current hypotheses. Bulletin of Marine Science. 54 (3), 757– 781.

Frank, K. T., B. Petrie, J.S. Choi. 2005. Trophic cascades in a formerly cod-dominated ecosystem. Science 308, 1621-1623.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community Structure, Population Control and Competition. American Naturalist 94

Harvell, C.D., C.E. Mitchell, J.R. Ward, S. Altizer, A.P Dobson, R.S. Ostfeld and M.D. Samuel. 2002. Climate Warming and disease risks for terrestrial and marine biota. Science 296, 2158-2162.

Hemminga, M. and Duarte, C.M. 2000. Seagrass Ecology. Cambridge, UK: Cambridge University Press.

Hoeksema, J.D. and E. Bruna. 2000. Pursuing the big questions about mutualism: a review of theoretical approaches. Oecologia 125, 321-330

Intergovernmental Panel on Climate Change. 2007.

Koch, M.S., S.A. Schopmeyer, M. Holmer et al. *Thalassia testudinum* response to the interactive stressors hypersalinity, sulfide and hypoxia. Aquatic Botany 87, 104-110.

Lippson, A. J. and R. L. Lippson. 1984. Life in the Chesepeake Bay. Baltimore, MD. Johns Hopkins University Press.

Muehlstein, L.K. 1992. The host-pathogen interaction in the wasting disease of eelgrass, *Zostera marina.* Canadian Journal of Botany 70, 2081-2088.

Muehlstein, L. K., D. Porter and F. T. Short. 1991. *Labyrinthula zosterae:* the causative agent of wasting disease of eelgrass, *Zostera marina.* Mycologia 83, 180-191.

Muehlstein, L. K., D. Porter and F. T. Short. *Labyrinthula sp.,* a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina.* 1995. Marine Biology 99, 465-472.

Pounds J.A., Bustamante M.R., Coloma L.A., Consuegra J.A., Fogden M.P.L., Foster P.N., La Marca E., Masters K.L., Merino-Viteri A., Puschendorf R., Ron S.R., Santiago-Azofeifa G.A., Still C.J., Young B.E. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature. 439, 161–167.

Ralph, P.J., 1998. Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress. Marine Ecology Progress Series 171, 123– 130.

Renn, C. E. 1936. The wasting disease of *Zostera marina.* A phytological investigation of the diseased plant. Biological Bulletin. 70, 148-158.

Ripple, W. J. and R. L. Beschta. 2007. Hardwood tree decline following large carnivore loss on the Great Plains, USA. Frontiers in Ecology and the Environment 5, 241-246.

Short, F.T. and D. M. Burdick. 1996. Quantifying seagrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. Estuaries 19, 730–739.

Silliman, B.R., J. van de Koppel and M.D. Bertness. 2005. Drought, snails, and large-scale die-off of southern U.S. salt marshes. Science 310, 1803-1806.

Terrados, J., Duarte, C.M., Kamp-Nielsen, L., Borum, J., Agawin, N.S.R., Fortes, M.D., Gacia, E., Lacap, D., Lubanski, M. and Greve, T. (1999) Are seagrass growth and survival affected by reducing conditions in the sediment? Aquatic Botany 65, 175–197.

Touchette, B. W. and J. M. Burkholder. 2000. Review of nitrogen and phosphorous metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250, 133-167.

Short, F.T. and H. A. Neckles. 1999. The effects of global climate change on seagrasses. Aquatic Botany 63, 169–196.

Short, F.T. and S. Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. Environmental Conservation 23, 17–27.

Seddon, S., Cheshire, A., 2000. Photosynthetic responses of *Amphibolis antartica* and *Posidonia australis* to temperature and desiccation using chlorophyll fluorescence. Marine Ecology Progress Series 220, 119– 130.

Van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W. & Roelofs, J.G.M. 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157, 159–73.

Vergeer, L.H.T., T.L. Aarts and J.D. Degroot. 1995. The wasting disease and the effect of abiotic factors (light-intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. Aquatic Botany 52, 35-44.

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

BIOGRAPHICAL SKETCH

Gabriela Blohm was born in the beautiful country of Venezuela, whose people are kind yet unaware of the fragility of their nation's social and ecological stability. She lived with her family in Caracas until the age of fourteen, when the country's political climate became volatile and the crime rate escalated to a point where their everyday lives were being affected. She moved to the United States at the age of fourteen.

During her time in South America, she spent my weekends snorkeling and SCUBA diving in places that had not suffered some of the impacts of human development. This is when her aspiration in life became to promote the research and preservation of these quickly and silently disappearing coasts. She grew up in a home where conversations at the lunch table were about her grandfather's program on the reintroduction of the endangered Orinoco crocodile, her grandmother's work with the conservation of sea turtles, and the polarized and unstable socioeconomic divide that their country was experiencing. Her grandparents were founders of Venezuela's first environmental NGO, and they always spoke of the challenge of spreading an environmental awareness throughout Venezuela. There are cultural, political and scientific challenges to conservation efforts, and all are interwoven.

As an aspiring scientist, she is thrilled by the pursuit of good questions and objective answers. She majored in Wildlife Ecology at the University of Florida, and upon graduating with a BS in 2005, she decided to pursue her MS in Zoology at the University of Florida. She hopes to continue working as a researcher and educator, with the goal of spreading a sense of ethical responsibility for natural resource conservation, human rights preservation and education