

CONSUMERS MITIGATE EFFECTS OF HEAT STRESS AND NUTRIENT ENRICHMENT
ON EELGRASS (*ZOSTERA MARINA* L.) COMMUNITIES AT ITS SOUTHERN RANGE
LIMIT

Michelle Claire Brodeur

A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Marine Sciences.

Chapel Hill
2014

Approved by:

F. Joel Fodrie

Charles H. Peterson

Michael F. Piehler

© 2014
Michelle Claire Brodeur
ALL RIGHTS RESERVED

ABSTRACT

MICHELLE CLAIRE BRODEUR: Consumers mitigate effects of heat stress and nutrient enrichment on eelgrass (*Zostera marina* L.) communities at its southern range limit
(Under the direction of F. Joel Fodrie)

At the southern end of its range, eelgrass (*Zostera marina*) meadows in North Carolina are vulnerable to multiple stressors. These include eutrophication, which can promote the overgrowth of algal epiphytes, and heat stress that causes significant seasonal die-offs during the summer. To quantify the relative influences of these ecosystem stressors on eelgrass biomass, we conducted a mesocosm experiment investigating interactive effects of heat stress (+1.5°C) and nutrient loading (+10µM NH₄NO₃/1µM KH₂PO₄) on an amphipod-eelgrass system. Additionally, we manipulated consumer pressure from an abundant omnivorous fish (pinfish, *Lagodon rhomboides*) to understand how top-down pressure interacted with bottom-up nutrient additions and warming. Pinfish consumed nearly all amphipods, which had no effect on epibiont load, but pinfish prevented macroalgal accumulation and mitigated eelgrass loss due to heat stress. Notably, when pinfish were absent from mesocosms, macroalgal biomass was high even though grazers were allowed to reach high densities >600 individuals per 18-L mesocosm and warming caused an 80% reduction in eelgrass aboveground biomass. Macroalgal biomass was also positively correlated with water temperature and had a significant correlation with eelgrass aboveground biomass. Although nutrient concentrations were similar to nutrient pulses from local storm events, nutrient enrichment only increased epibiont load when combined with warming, and we observed no discernible interactive effects on aboveground biomass. We conclude that omnivorous consumers can play an important role in preventing macroalgal

accumulation from exacerbating heat stress effects, although continued warming may cause significant regional habitat loss regardless of consumer presence as eelgrass reaches extreme physiological limits.

To Norma L. Holmberg Simard, who cultivated my love of science.
You will always be with me in the garden.

AKNOWLEDGEMENTS

I would first like to thank my advisor and mentor, Dr. Joel Fodrie, for his extensive guidance and support. I am grateful he provided me with this opportunity and for always challenging me to be a better scientist. This study would also not be possible without guidance and suggestions from my committee members, Drs. Pete Peterson and Michael Piehler.

Thanks to past and present members of the Fisheries Ecology Lab for their help and support, especially A. Poray-Morley, S. Coleman, E. Voigt, S. Fuller, C. Baillie and L. Alexander for lab assistance. I would also like to thank everyone at the Institute of Marine Sciences all staff members, especially C. Lewis and A. Witchard, for helping in all ways possible. Additionally, I am indebted to discussions with R. Gittman, L. Yeager, S. Coleman, D. Keller and SB Scyphers that greatly improved my research, including this thesis. This project was funded by a North Carolina Division of Marine Fisheries Coastal and Recreational Fishing License Grant.

To Mum, Papa, Matt and the entire Brodeur-Simard clan - thank you for your persistent encouragement and love. Lastly, I would like to thank Justin Ridge for valuable discussions, last minute field assistance, patience, and constant love and support.

TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
INTRODUCTION	1
METHODS	5
RESULTS	8
Mesocosm water quality	8
Secondary consumer	9
Grazer density	9
Epibiont load	10
Macroalgal biomass	10
Eelgrass biomass & nutrient content	11
DISCUSSION	11
REFERENCES	27

LIST OF TABLES

Table 1. Analysis of the response of community and ecosystem properties.....	18
Table 2. Nutrient content of above and belowground biomass	19
Table 3. Analysis of nutrient content	20

LIST OF FIGURES

Figure 1. Average daily temperature	21
Figure 2. Final pinfish growth, amphipod density, epibiont load, macroalgal biomass, and eelgrass aboveground biomass	22
Figure 3. Nutrient content of above- and belowground biomass	23
Figure 4. Effects of secondary consumers, increased temperature, and nutrient enrichment on an eelgrass community	24
Figure 5. Macroalgal light attenuation	26

INTRODUCTION

Across marine systems, the interactive effects of multiple stressors are frequently more severe than the predicted cumulative effects when each stressor is considered independently (Crain et al. 2008). However, the magnitude and type of interaction (additive, agonistic, or synergistic) is dependent on which community or trophic levels are being examined in addition to the unique stressor combination (e.g., warming, eutrophication, salinity, habitat loss, acidification, etc.). In particular, individuals at the southern edge of a given species' range are already near the species' thermal tolerance and interactions between warming and anthropogenic stressors may accelerate local extinctions (Harley et al. 2006, Carilli et al. 2010). The resulting ecosystem effects may be most dramatic when a foundation species becomes locally extinct because individuals that rely on habitat forming species will be displaced or go extinct (Doney et al. 2012).

Eelgrass, *Zostera marina*, is a foundation species that is widely distributed across estuaries the northern hemisphere and between 33°N and 65°N along the Western Atlantic (Thayer et al. 1984, Green & Short 2003). Coastal ecosystems are being affected by climate-driven range shifts, eutrophication (due to agriculture, silvaculture and/or coastal development), and loss of top consumers (caused by overfishing), all of which may have negative effects on eelgrass biomass and distribution (Orth et al. 2006, Ralph et al. 2006). Considering the key ecosystem services seagrass meadows provide (Larkum et al. 2006) and that seagrass cover has declined over global scales in recent decades

(Waycott et al. 2009, Anton et al. 2011), it is important to understand the potential synergistic effects of these multiple stressors on eelgrass meadows.

At its southern range limit, the optimal temperature for eelgrass growth is 22°C (Penhale 1977, Lee et al. 2007) and since water temperatures are above 25°C for most of July and August (Kenworthy 1981, Thayer et al. 1984), heat stress drives the near complete loss of aboveground biomass in many eelgrass meadows (Thayer et al. 1984). When water temperature exceeds the thermal optimum, photosynthetic rates decline and respiration rates increase, which reduces net photosynthesis, inhibits leaf growth and eventually causes mortality (Marsh et al. 1986). Seagrass at higher temperatures also need more light to maintain a positive carbon balance (Lee et al. 2007). With increasing durations of water temperature exceeding 30°C due to warming in the mid-Atlantic, models of eelgrass meadows indicate that seasonal declines in eelgrass cover will be magnified (Carr et al. 2012) and it will exacerbate the loss of essential nursery habitat for fish and crustaceans (Micheli et al. 2008).

Simultaneously, nutrient enrichment, another major stressor that works through bottom-up pathways, has been found to reduce eelgrass cover (Valiela et al. 1997, Hughes et al. 2004, Lotze et al. 2006, Burkholder et al. 2007, Waycott et al. 2009). Although a slight increase in nutrients can initially increase the biomass of eelgrass meadows in nutrient limited systems, further enrichment (especially water column enrichment) increases the biomass of algal epiphytes, reduces light penetration to the blades and ultimately reduces eelgrass biomass (Burkholder et al. 2007). However, if present, herbivorous mesograzers such as amphipods can control epiphyte growth,

mitigating these negative effects of nutrient enrichment through top-down control (Hughes et al. 2004, Spivak et al. 2009, Blake & Duffy 2010, 2012, Myers & Heck 2013).

Multiple stressors have been shown to alter the balance between bottom-up and top-down control and can have interactive effects on habitat forming species and associated fauna (Crain et al. 2008). Seagrass systems, including eelgrass meadows at the southern extent of its range, are a good model for exploring interactive effects of environmental stressors and top-down mitigation of bottom-up impacts because the effects of individual stressor on eelgrass biomass and food web interactions within eelgrass systems have already been established (Hughes et al. 2004). Burkholder et al. (1992) examined the varying seasonal effects of eutrophication on eelgrass in experimental mesocosms in North Carolina, and found that even low levels of nitrate enrichment ($3.5 \mu\text{M NO}_3^- \text{-N}$) promoted the decline of eelgrass. Also, Burkholder et al. (1992) found that this loss was more pronounced in the summer months when water temperatures were highest. Further work indicated that increasing the average temperature by 3-4°C intensified the effects of water-column nitrate inhibition on eelgrass growth (Touchette & Burkholder 2002), suggesting that the effects of increasing temperature interact with nutrient enrichment. These studies are valuable for understanding the physiological effects of nutrient enrichment and warming within seagrass systems, but do not incorporate biological interactions (i.e. trophic top-down control).

Top-down regulation also plays an important role in structuring eelgrass ecosystems. Several mesocosm studies have investigated whether increases in blue crab density reduce herbivore densities and have negative cascading effects on seagrass

biomass (Duffy et al. 2005, Canuel et al. 2007, Douglass et al. 2007). Previous work has found that crab presence can reduce amphipod abundance through consumption (Spivak et al. 2009) or suppress amphipod grazing (Duffy et al. 2005), both of which increase epiphyte biomass. However, there were no cascading effects of grazer abundance or changes in grazer behavior on eelgrass biomass. Pinfish (*Lagodon rhomboides*) are another secondary consumer that are extremely prevalent in seagrass habitats during summer months (>80% of fishes in North Carolina seagrass trawl surveys; Baillie et al. 2014). Juvenile pinfish diet consists predominantly of amphipods, and pinfish are thought to drive dramatic, seasonal amphipod declines. In deed, as pinfish and other juveniles recruit to nursery eelgrass meadows, amphipod densities decline dramatically and remain low throughout the summer months (Nelson 1979). Since pinfish become omnivorous and consume algal epiphytes as juveniles grow larger (Stoner 1979), they may also have a positive effect on seagrass biomass and productivity (Heck et al. 2000). The high densities pinfish, and other omnivorous secondary consumers, may play a critical role in controlling epiphyte loads when amphipods are at very low densities in eelgrass meadows in the summer months.

Extensive research has been conducted on the independent effects of nutrient enrichment, temperature, and food-web interactions on eelgrass, but there remains an opportunity to explore the cumulative effects of all three factors, particularly in explaining the future geographic distribution of this foundation species. To investigate the effects of top-down (secondary consumer pressure), bottom-up (nutrient enrichment) and abiotic stressors (warming) we asked: (1) How do heat stress and nutrient loading affect eelgrass biomass? (2) How do the effects of heat stress and nutrient loading change

with the removal of a secondary consumer pressure? And, (3) are there any interactive or reciprocal effects between secondary consumer pressure and stressors in eelgrass meadows? We expected elevated water temperatures to enhance heat stress and nutrient enrichment to increase epiphytic shading of the blades (both are processes that reduce aboveground biomass). Also, we anticipated that removing the secondary consumer would release grazers from top-down control and in turn reduce epiphytic cover and shading. This would in our predictions, mitigate the effects of nutrient enrichment on eelgrass and prevent nutrient enrichment from exacerbating extreme heat stress effects.

METHODS

We conducted a three-factor mesocosm experiment to explore how elevated temperature and nutrient loading affect eelgrass meadows, and whether removing secondary consumer pressure influences the direction or magnitude of these effects. To mimic natural eelgrass systems, we included a diverse community of amphipods found in local eelgrass meadows as our grazers, and manipulated the presence of a regionally dominant secondary consumer, pinfish. Temperature (ambient, +1.5°C), nutrient loading (ambient, +10μM NH₄NO₃/1μM KH₂PO₄) and consumer pressure (grazers + secondary consumers, grazers only) were crossed in a fully orthogonal design with six replicates.

We collected eelgrass cores (~26-cm diameter) from a monospecific eelgrass meadow in Middle Marsh, Back Sound, North Carolina in late July 2010. Cores were transplanted into 18-L experimental mesocosms in an outdoor flowing seawater system at the University of North Carolina at Chapel Hill's Institute of Marine Sciences in Morehead City, NC. We rinsed cores with fresh water, wiped blades to remove all mobile

invertebrates and macroalgae, and counted shoot density (29.2 ± 1.3 shoots $\mu \pm \text{SE}$) before placing eelgrass mesocosms in a continuous-flow, sand-filtered seawater system to prevent outside colonization of small invertebrates. A community of 50 amphipods, collected from water table cultures that mimic local eelgrass grazer communities (predominately *Gammarus* spp. and *Ampithoe* spp.; Nelson 1979), were added to each mesocosm.

To understand how warming will affect eelgrass biomass and its associated community, we conducted this experiment from August 10th through September 21, 2010, when seasonal temperatures and physiological heat stress are at peak levels. Heaters were added to mesocosms to obtain elevated temperatures and mock heaters were used in treatments with ambient water temperature to maintain consistent habitat complexity. Water temperature of mesocosms and adjacent Bogue Sound was monitored three times daily with a hand-held thermometer to quantify difference between heated and ambient water treatments and to ensure that ambient mesocosm water matched water temperature at the same depth in the adjacent Sound.

Mesocosm nutrient concentrations were enriched by an additional $10\mu\text{M}$ NH_4NO_3 and $1\mu\text{M}$ KH_2PO_4 (20:1 N:P) every three days to mimic storm nutrient pulses. These concentrations represent nutrient measurements following stormwater runoff events from long-term monitoring of coastal streams in the area (Piehler, unpublished data). Because our system experiences elevated nutrients following storm events opposed to a constant supply of elevated nutrients, we chose to use a pulse design for water column nutrient enrichment instead of slow release fertilizer. NH_4NO_3 and KH_2PO_4 were dissolved in mesocosm water and the concentrated solution was added to the mesocosms to increase

the nutrient concentration by $10\mu\text{M NH}_4\text{NO}_3$ and $1\mu\text{M KH}_2\text{PO}_4$. Water flow was shut off for 30 minutes immediately following the additions to allow nutrients to mix in the water column.

Finally, we added one pinfish (52 ± 1.1 mm standard length, 3.7 ± 0.2 g) to half of the mesocosms and ran the experiment for six weeks. This allowed time for amphipod abundance to approach carrying capacities representative of in situ conditions (Duffy & Harvilicz 2001) and for eelgrass to respond to heat stress in late summer. At the end of the experiment, we removed the pinfish and re-measured each individual's standard length and weight to quantify growth throughout the experiment. Amphipods were filtered from the water, sorted from eelgrass blades, and enumerated to quantify final densities.

To determine how elevated temperature, nutrient pulses, and pinfish presence affected eelgrass biomass; eelgrass was uprooted, shaken to gently remove grazers, and rinsed through a 0.5-mm sieve. We sorted eelgrass into above- and belowground portions after scraping each blade to remove all epibionts growing on blades. We also separated all macroalgae that accumulated at the top of the mesocosm, spun it to remove excess water before measuring wet weight. All portions of the eelgrass were then dried in an oven at 60°C for three days and weighed. Finally, we homogenized all above- and belowground portions the eelgrass from each mesocosm into a fine powder for C:N analysis to understand the effects of nutrient enrichment on nitrogen and carbon content of eelgrass.

We analyzed pinfish growth (change in standard length and weight) and final amphipod density, epibiont load, macroalgal biomass, aboveground biomass, and

belowground biomass using Generalized Linear Models (GLM) in JMP 10.0. These analyses tested for the fully crossed effects of water temperature, nutrient enrichment, and pinfish presence, in addition to a block effect (mesocosm position on water table) on our independent variables with significance set at an alpha <0.05. Three pinfish were lost at an unknown time during the experiment, thus we excluded these mesocosms from the analyses. Pinfish growth, amphipod density (Box-Cox power transformed), aboveground and belowground biomass met the assumptions of normal distribution therefore we used an identity link function for each GLM. Macroalgal biomass fit the Poisson distribution with a log link function. When the water table block effect was significant (macroalgal, aboveground, and belowground biomass) we determined if there were any interactive effects of block and our manipulated factors. However, there was never an interaction between water table block and temperature, nutrient, or consumer manipulation. Finally, we ran correlations to understand the relationships between macroalgae, average mesocosm temperature, and aboveground biomass.

RESULTS

Mesocosm water quality

Throughout the six-week experiment, the water temperature in control mesocosms did not differ from Bogue Sound water (average control mesocosm and Bogue Sound temperature difference: $0.1 \pm 0.9^{\circ}\text{C SE}$). Water temperature ranged from 22.8°C to 36.6°C in ambient treatment mesocosms and 25.2°C to 39.7°C in heated mesocosms across the whole experiment (Fig. 1). The difference between ambient and heated temperature treatments varied according to time of day. In the morning the water

temperatures were on average $1.7 \pm 0.1^{\circ}\text{C}$ warmer in the $+1.5^{\circ}\text{C}$ treatment and by only $0.8 \pm 0.1^{\circ}\text{C}$ warmer in the evening. Salinity averaged 33.7 PSU, but Hurricane Earl affected our experiment when it impacted eastern North Carolina on September 2, 2010, which resulted in large fresh water influx and power outage (less than three hours) that disrupted our water flow. Although this led to a system-wide decrease in water temperature, heated temperature treatments remained higher than control temperatures following the storm and we did not observe any mass mortality of eelgrass, amphipods, pinfish or macroalgae.

Secondary Consumer

To understand how secondary consumer pressure, nutrients and warming may interact and affect eelgrass biomass we first examined if there were any effects of the two stressors on pinfish. Although there were no differences in growth measured as change in standard length (final standard length 57.8 ± 0.8 mm, growth 1.5 ± 0.2 mm), nutrient enrichment significantly increased the growth measured in weight of pinfish ($\chi^2 = 3.95$, $p = 0.047$; Table 1) from 1.19 ± 0.21 g in ambient nutrient treatments to 1.76 ± 0.27 g in nutrient enrichment treatments (Fig. 2A). Increased water temperature had no effect on growth (standard length or weight).

Grazer Density

Removing secondary consumer pressure released amphipods from top-down control. Pinfish consumed nearly all amphipods when present (4.0 ± 1.5 amphipods remaining at the experiment's end), but with no secondary consumer present, amphipod densities reached 607.5 ± 113.0 amphipods per mesocosm ($\chi^2 = 93.11$, $p < 0.001$; Fig.

2B). Nutrient enrichment ($\chi^2 = 1.16$, $p = 0.281$) and warming ($\chi^2 = 0.09$, $p = 0.759$) had no effect on final amphipod density.

Epibiont Load

We expected the increase in amphipod densities due to pinfish removal to reduce the epibiont load, but pinfish presence, had no effect on epibiont load ($\chi^2 = 2.54$, $p = 0.111$). However, nutrient enrichment and temperature had an interactive effect on epibiont load ($\chi^2 = 7.75$, $p = 0.005$, Fig. 2C). When mesocosms did not receive nutrient enrichment, increasing water temperature had no effect on epibiont load (0.515 ± 0.090 in ambient treatment), but under nutrient loading conditions warming increased epibiont load to 0.888 ± 0.237 (Fig. 2C). Epibiont community composition was not quantified, but we observed no obvious trends across treatments. Epiphytes consisted predominantly of fleshy algae (both turf microalgae and *Ulva intestinalis*), however some calcareous algae were also present.

Macroalgal Biomass

Although removing pinfish and releasing amphipods from top-down control did not increase epibionts, removing pinfish did cause an increase in the macroalgae, *Ulva intestinalis*, which overgrew seagrass in mesocosms (Fig. 2D). Macroalgae was almost completely absent (0.03 ± 0.02 wet g) when pinfish were present, but biomass reached 34.11 ± 10.10 wet g when pinfish were removed ($\chi^2 = 1010.11$, $p < 0.001$; Fig. 2D). Additionally, macroalgal biomass was positively correlated (significant at $\alpha < 0.10$) with the average mesocosm water temperature ($\rho = 0.258$, $p < 0.087$).

Eelgrass Biomass & Nutrient Content

There was a significant interaction between water temperature and pinfish presence on aboveground eelgrass biomass ($\chi^2 = 11.22$, $p = 0.001$; Fig 2E), with the loss of pinfish exacerbating effects of heat stress on aboveground biomass (Fig. 2E). Increased water temperature had no significant effect on aboveground biomass when pinfish were present, but significantly reduced biomass from 0.24 ± 0.089 g when pinfish were present to 0.08 ± 0.035 g when pinfish were absent. Aboveground biomass was also negatively correlated with macroalgal biomass ($\rho = -0.365$, $p = 0.014$). There were no significant differences in belowground biomass across treatments.

Although we did not find an effect of nutrients on final epibiont load, macroalgal biomass, or eelgrass biomass, nutrient enrichment significantly increased the nutrient content of the eelgrass blades (Fig 3A; Table 3), decreasing the C:N ratio from 24.03 to 21.75 ($\chi^2 = 4.32$, $p = 0.038$). This was due to an increase in nitrogen content ($\chi^2 = 4.01$, $p = 0.045$; Table 2) in the blades from $1.85 \pm 0.10\%$ to $2.06 \pm 0.07\%$ N.

Discussion

In this study water temperature was the key factor in controlling eelgrass biomass (Fig. 4). Elevating water temperature by approximately 1.5°C (averaged over a diel cycle) magnified seasonal heat stress and led to a significantly greater loss of eelgrass biomass than in ambient temperature treatments. Nutrient enrichment alone did not cause an increase in epibiont or macroalgal biomasses as expected, but did increase the nutrient content of the eelgrass blades and interactively, with warming, increased epibiont loads. Changes in blade nutrient content did not result in discernible effects propagating up the

food chain through amphipod density. Dramatic differences in grazer densities caused by pinfish consumption had no effect on epibiont or macroalgal biomass, however we did find that pinfish played a critical role in eelgrass systems by directly preventing macroalgal accumulation. Pinfish consumed almost all macroalgae in the mesocosms, and when pinfish were absent, macroalgae proliferated and presumably further reduced eelgrass biomass through shading (Sand-Jensen 1977). Our results suggest that warming could reduce seasonal eelgrass habitat through heat stress, which would be compounded by an increase in macroalgae. However, as our study demonstrated, there is also potential for secondary consumers to mitigate macroalgal accumulation through top-down pressure.

We conducted this experiment when abiotic stress was greatest at the end of the growing season where, at the southern limit of the range, eelgrass meadows annually die-off in the summer (Kenworthy 1981, Thayer et al. 1984). Since all treatments were undergoing heat stress, low eelgrass biomass may have prevented us from seeing clear interactions between stressors because heat-stress dominated the response of aboveground biomass. However, since a small increase in water temperature enhanced the decline of eelgrass biomass dramatically, it is likely warming will continue to cause declines, shift the growing season earlier, and eventually move the species range poleward. Recent studies have found that for every 1°C increase results in a 5-6 day forward shift in the eelgrass growing season (Clausen et al. 2014). Some eelgrass meadows at the southern range limit have already experienced a reduction in biomass in the last 20-30 years as temperatures have increased by 1.4°C and water quality has been degraded between 1984 and 2005 (Micheli et al. 2008). Shoal grass, *Halodule wrightii*, a tropical species with its northern range limit also in North Carolina, may be able to

survive in warmer summers, but there has been no change in density in the last few decades and it has not begun to colonize eelgrass barrens (Micheli et al. 2008). Continued eutrophication of coastal estuaries may facilitate shoal grass colonization of old eelgrass meadows as was seen in former turtle grass meadows following bird guano enrichment in southern Florida (Fourqurean et al. 1995). *Ruppia maritima* (widgeon grass) may also do well under eutrophication because it has a physiological mechanism to prevent excessive nitrate uptake and grows well in enriched systems (Burkholder et al. 1994). However, even if shoal or widgeon grass are able to colonize eelgrass meadows, there will be significant shifts in the nursery value of seagrass meadows in North Carolina because these grasses support a different community of fishes and crustaceans in relation to eelgrass (Micheli et al. 2008, Baillie et al. 2014).

Increased temperatures have also been shown to strengthen herbivore-algae interactions (O'Connor 2009) because metabolic rates of consumers increase at a greater rate than that of primary producers and result in a trophic skew defined by increased consumer:producer biomass (López-Urrutia et al. 2006, Carr & Bruno 2013). However, the balance between herbivores and primary producers in response to increased temperatures can be dependent on absolute temperature and nutrient supply. For instance, recent work has found that top-down interactions only strengthen until temperatures reach an organisms thermal optimum where predator metabolism is greatest, after which metabolic rates and therefore interaction rates weaken (Englund et al. 2011). Furthermore, experimental work has shown that increasing temperatures only shift phytoplankton-zooplankton systems toward greater relative abundance of consumers in eutrophic environments (O'Connor et al. 2009). When examining our pinfish-amphipod-epibiont

system, we also observed an interaction between warming and nutrients, however, found that warming increased epibiont load under eutrophic conditions opposed to strengthen the top-down interaction. Additionally, because temperatures were above optimum (30-33°C for pinfish; Muncy 1984) reduced metabolic rates may have weakened the top-down interaction and resulted in greater epibiont loads (Englund et al. 2011).

Unlike the dramatic effects of warming, we only found moderate effects of nutrient enrichment on the eelgrass community. We expected nutrient loading to exacerbate the effects of heat stress on aboveground biomass through algal overgrowth or nitrate induced carbon limitation. Burkholder et al. (1992) documented 75-95% shoot mortality relative to un-enriched controls when under pulsed nitrate enrichment, and as there were no differences in epiphyte load between treatments, they attributed die-off to nitrate toxicity that was exacerbated by heat stress. We did not see any effects of nutrient toxicity compounding heat stress, but rather nutrient enrichment had positive effects on eelgrass nutrient content, increasing the nitrogen content in the blades. The pulse design of our experiment, reflecting measured nutrient concentrations from local stormwater runoff events, prevented eelgrass blades from constantly having to reduce nitrate and maintain carbon stores in the blades as in previous experiments that used a press design and a higher nutrient concentrations (Burkholder et al. 1992, 1994).

Across marine systems, positive effects of grazing in reducing nuisance macroalgae can be equal in magnitude to the negative effects of water column nutrient enrichment on seagrass biomass (Hughes et al. 2004). In both eelgrass mesocosms and field experiments, epiphyte loads have rapidly responded to water column nutrient enrichment, but amphipods have been found to control fast epiphyte growth, which

prevents nutrient loading from reducing eelgrass biomass (Neckles et al. 1993, Douglass et al. 2007, Spivak et al. 2009). Unfortunately, we did not successfully quantify epibiont loads early in the experiment (2-4 weeks), so we do not know if nutrient enrichment caused high initial epiphyte loads before consumers grazed down epiphytes (as found previously). The limited response in epibiont load may also be caused by having a full community of grazers that has been found to increase stability in response to stressors such as nutrients and warming (Spivak 2009, Blake & Duffy 2010, 2012, Alsterberg et al. 2013). Additionally, our system was more complex than previous work because there were multiple consumers (pinfish, amphipods) feeding on multiple resources (epibionts, macroalgae, etc.). Spreading consumption across multiple consumers may have also limited differences in final epibiont load across treatments and prevented epibionts from shading and reducing eelgrass aboveground biomass.

Previous studies have found that omnivorous pinfish dampen trophic cascades because they consume both mesograzers and macroalgae (Bruno & O'Connor 2005). We collected pinfish that were representative of the sizes present in the sounds in July when the experiment began. The average pinfish length (52 ± 1.1 mm) was within the omnivorous size class, where 30% of their diet likely consists of epiphytes in addition to mesograzers (Stoner 1979), however, their diet is also determined by the relative abundance of macrophytes (Stoner 1979). Instead of having large effects on epibionts through cascading effects of amphipod reduction, pinfish had the strongest impact on eelgrass by consuming the macroalgae growing on top of the eelgrass in the mesocosms (Hughes et al. 2004, Heck & Valentine 2007). Continued nutrient enrichment (Hauxwell et al. 2003) and warming (Blake & Duffy 2012) will favor ephemeral and epiphytic algae

over eelgrass biomass. Large ephemeral algal mats are present within eelgrass meadows and amphipod densities are high in spring and early summer before juvenile fishes (>80% pinfish in eelgrass meadows) arrive, after which amphipod density and macroalgal biomass quickly decline and remain low until pinfish have migrated out of coastal sounds in winter. Our study suggests that pinfish may play an important, possibly underappreciated, role in controlling macroalgal accumulation because even at high densities, amphipods in our experiment were unable to graze as much macroalgae as a single omnivorous pinfish.

Conducting a mesocosm experiment may have produced some artifacts that must be considered when making any extrapolations to natural eelgrass meadows. For instance, mesocosms may have exacerbated the quantity of macroalgae beyond what could feasibly accumulate *in situ*. In eelgrass meadows, macroalgae would be swept away by tidal currents, while lower flushing rates, higher temperatures, and increased attachment area may have facilitated macroalgal accumulation in our experimental mesocosms. Although laboratory measurements of light attenuation found that macroalgae reduced light penetration and presumably reduced photosynthesis, light levels at the highest macroalgal biomass were above 25% of ambient light and, therefore, did not completely prevent eelgrass photosynthesis (Fig. 5). We do note that pinfish are very abundant in North Carolina eelgrass meadows where macroalgal accumulation is rare in the summer, and therefore the macroalgae we observed in pinfish-absent treatments may simply highlight that in the field, this consumer provides strong top-down control, consistent in space and time, against macroscopic producers such as *Ulva*. We are also confident that macroalgal consumption by pinfish was not simply an artifact of starvation, as mesocosms contained

ample epibiont biomass available for grazing and we did not see any evidence of pinfish bites on eelgrass. These observations suggest a preference for macroalgae by omnivorous pinfish in our mesocosm trials.

Among top-down control, nutrient enrichment, and warming, our mesocosm results imply that warming is the primary factor controlling eelgrass biomass at its southern limit. Continued warming may cause die-backs to occur earlier, persist longer, and ultimately result in a local loss of essential nursery habitat. We also found that top-down control can reduce algal overgrowth of eelgrass by consuming macroalgae, preventing macroalgal shading from exacerbating heat stress. Future studies should test these findings in situ and further quantify how warming may affect the timing of the seasonal die-off and the corresponding negative effects on eelgrass communities due to loss of the foundation species.

Table 1. Analysis of response of community and ecosystem properties to the individual and interactive effects of consumer presence (Pin), nutrient enrichment (Nut), and warming (Temp) using generalized linear models (d.f. = 1 for treatments and d.f= 2 for block for all analyses). Significant treatment effects at alpha <0.05 are indicated in bold.

Factors	Pinfish				Amphipod		Biomass							
	Weight		Length		Density		Epibiont		Macroalgae		Aboveground		Belowground	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Pin					93.11	<0.001	2.54	0.111	1010.11	<0.001	1.63	0.201	0.00	0.953
Nut	3.95	0.047	1.37	0.243	1.16	0.281	2.56	0.110	0.00	0.999	0.07	0.794	0.01	0.926
Pin*Nut					0.87	0.352	0.17	0.681	0.00	1.000	0.81	0.369	0.50	0.478
Temp	2.12	0.145	0.94	0.331	0.09	0.759	0.08	0.773	0.00	1.000	11.22	0.001	0.12	0.728
Pin*Temp					2.42	0.120	0.09	0.764	0.00	0.999	7.49	0.006	0.19	0.659
Nut*Temp	2.22	0.136	0.84	0.358	1.10	0.294	7.75	0.005	0.64	0.424	0.14	0.707	0.12	0.725
Pin*Nut* Temp					0.11	0.743	2.89	0.089	0.57	0.452	0.00	0.988	3.75	0.053
Block	5.32	0.070	1.79	0.409	1.16	0.561	0.95	0.623	992.47	<0.001	22.95	<0.001	11.42	0.003

Table 2. Nutrient content ($\mu \pm 1$ SE) of eelgrass measured as percent carbon and nitrogen of aboveground biomass averaged within each treatment.

Treatment	Aboveground				Belowground			
	%C	St Err	%N	St Err	%C	St Err	%N	St Err
Ambient	37.76	0.21	1.85	0.10	31.15	0.90	0.93	0.02
Ambient, Pinfish	37.51	0.31	1.93	0.07	30.74	1.29	0.93	0.03
+1.5°C	37.25	0.16	1.88	0.09	30.77	1.61	0.81	0.04
+1.5°C, Pinfish	36.90	0.38	1.76	0.06	32.11	0.61	0.81	0.05
Nutrients	38.23	0.22	2.06	0.07	30.37	1.41	0.88	0.03
Nutrients, Pinfish	37.73	0.21	2.08	0.11	31.31	0.86	0.88	0.08
+1.5°C Nutrients	36.59	1.25	1.89	0.08	30.63	0.87	0.91	0.07
+1.5°C, Nutrients, Pinfish	37.77	0.14	1.91	0.03	32.82	1.42	0.84	0.01

Table 3. Analysis of the response of above- and belowground eelgrass carbon and nitrogen content to the individual and interactive effects of consumer presence (Pin), nutrient enrichment (Nut), and warming (Temp) using a generalized linear model. Significant treatment effects at $\alpha < 0.050$ are indicated in bold and d.f. = 1 for all treatments and d.f. = 2 for block analyses.

Factors	Aboveground						Belowground					
	C:N		% Carbon		% Nitrogen		C:N		% Carbon		% Nitrogen	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Pin	0.21	0.645	0.00	0.971	0.21	0.646	3.39	0.066	1.87	0.172	0.61	0.434
Nut	4.32	0.038	0.49	0.483	4.01	0.045	0.00	0.999	0.01	0.914	0.02	0.882
Pin*Nut	0.01	0.934	1.19	0.276	0.07	0.790	0.89	0.347	0.77	0.380	0.21	0.645
Temp	2.07	0.150	5.94	0.015	4.37	0.037	6.64	0.010	1.41	0.235	3.99	0.046
Pin*Temp	2.19	0.139	1.85	0.174	1.23	0.268	1.33	0.248	1.35	0.245	0.37	0.541
Nut*Temp	0.84	0.360	0.35	0.552	1.19	0.275	3.51	0.061	0.01	0.935	3.74	0.053
Pin*Nut* Temp	0.33	0.565	2.53	0.112	0.48	0.486	0.02	0.896	0.07	0.786	0.59	0.442
Block	2.39	0.303	0.96	0.617	2.85	0.240	7.93	0.019	6.30	0.043	20.62	<0.001

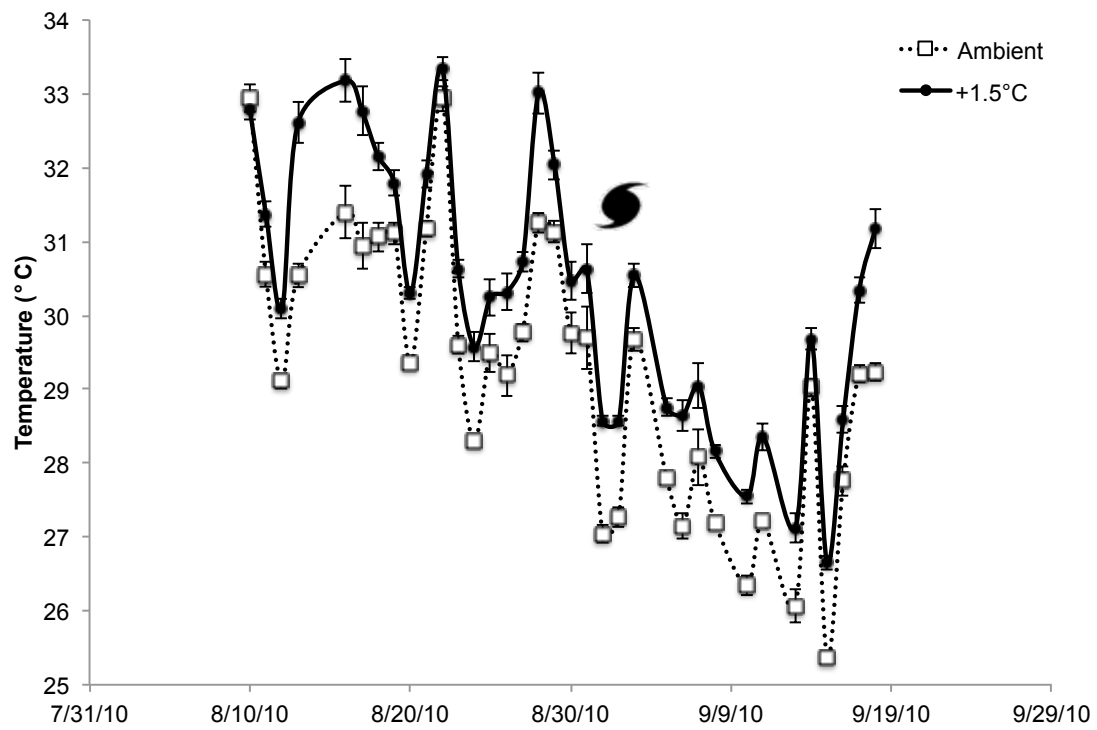


Figure 1. Average daily temperature of heated +1.5°C and ambient water treatments across the duration of the experiment. Hurricane symbol represents the arrival of Hurricane Earl on September 2, 2011.

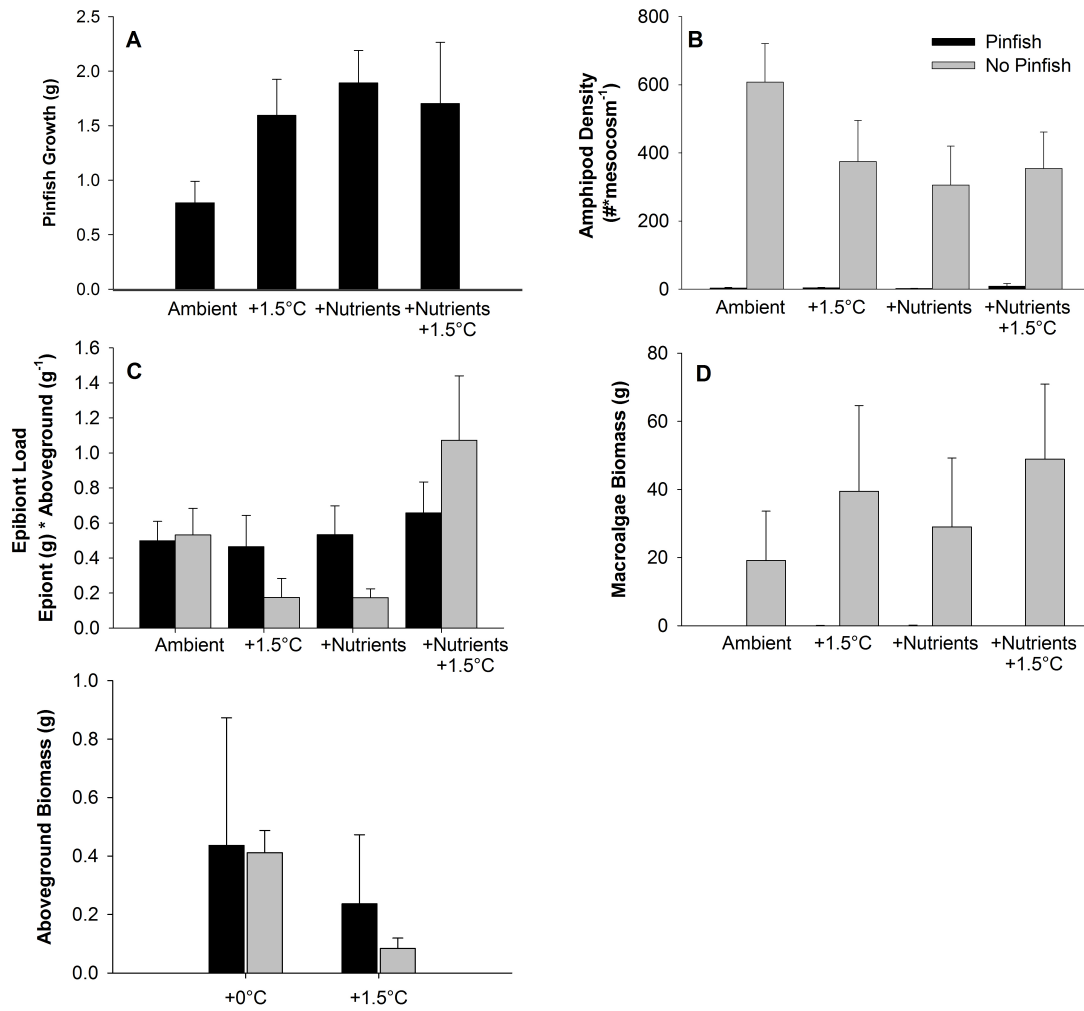


Figure 2. Final (A) pinfish growth, (B) amphipod density, (C) epibiont load, (D) macroalgae biomass, and (E) aboveground biomass at the end of the six-week mesocosm trial ($\mu \pm 1$ SE) in mesocosms with pinfish (black), without a pinfish (gray), or pooled pinfish treatments (white) under ambient temperature and nutrients (Ambient), elevated water temperature (+1.5°C), elevated nutrients (+Nutrients) or both elevated temperature and nutrients (+1.5°C, +Nutrients). Values were pooled when a factor was not significant at alpha 0.05.

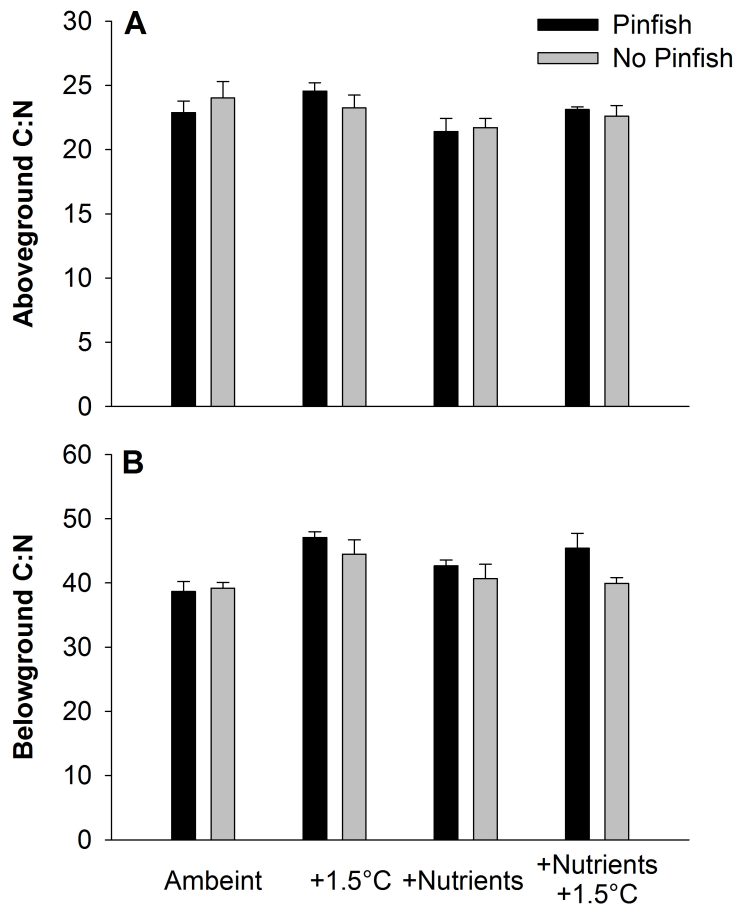


Figure 3. Nutrient content of (A) above- and (B) belowground eelgrass at the end of the six-week mesocosm trial ($\mu \pm 1$ SE) with (black) or without a pinfish (gray) under ambient temperature and nutrients (ambient), elevated water temperature (+1.5°C), elevated nutrients (+Nutrients) or both elevated temperature and nutrients (+1.5°C, +Nutrients).

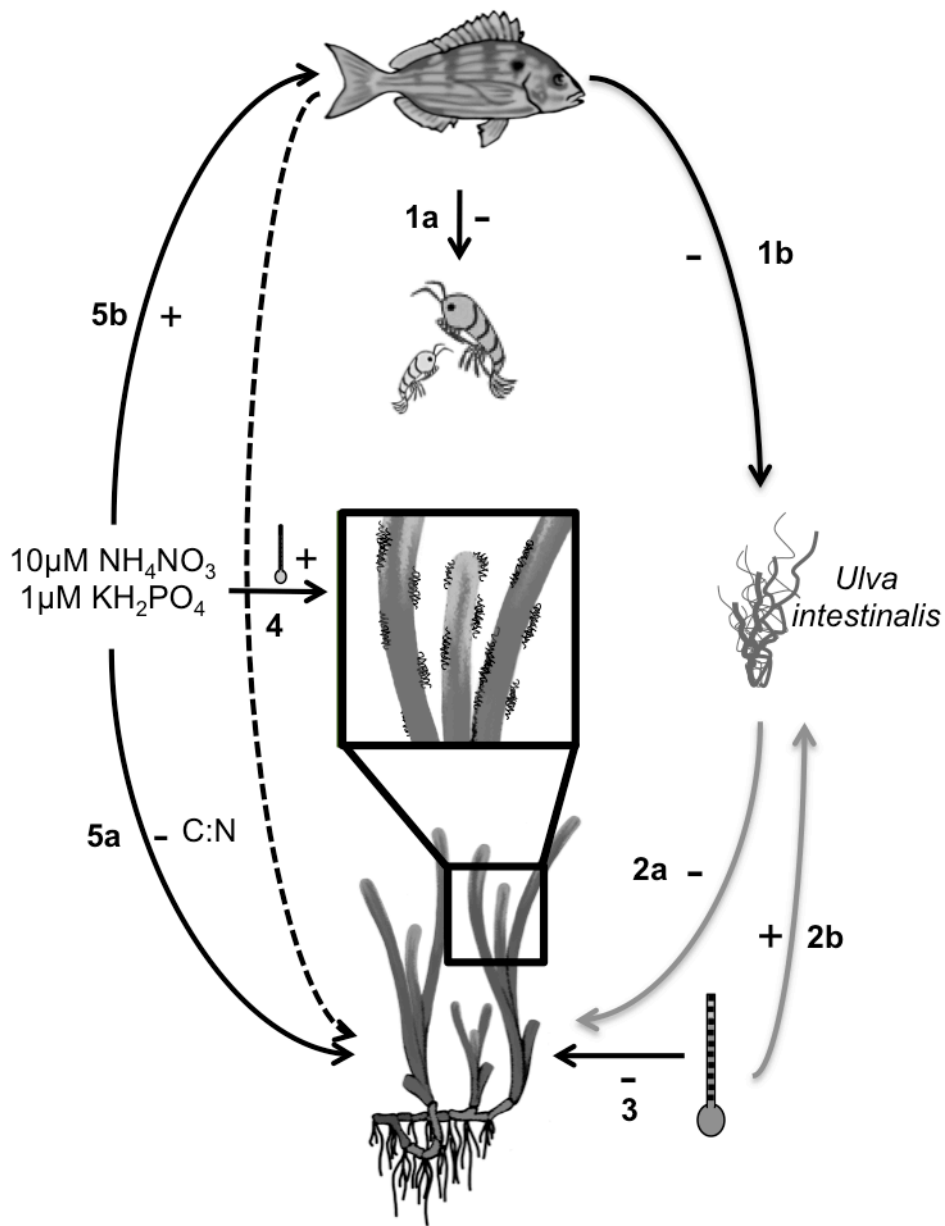


Figure 4. Effects of secondary consumers, increased temperature, and nutrient enrichment on an eelgrass community. Pinfish removal released both (1a) amphipods and (1b) macroalgae from consumer control. Increases in amphipod density had no cascading effects on eelgrass biomass, while increases in macroalgal biomass was correlated with lower aboveground biomass ($p < 0.1$). The quantity of macroalgal biomass that

accumulated when pinfish were absent was correlated with individual mesocosm average water temperature ($p < 0.05$). (3) Increased water temperature reduced aboveground biomass, but heat stress and biomass reduction was greatest when pinfish were absent. (4) Pulsed nutrient enrichments increased epibiont load only when temperature was elevated and had no discernable cascading effects on eelgrass biomass. Nutrient enrichment also (5a) decreased the C:N ratio of eelgrass blades and (5b) increased pinfish biomass. Solid black lines represent significant effects at $\alpha < 0.05$, gray lines represent correlations, and dotted lines represent indirect effects.

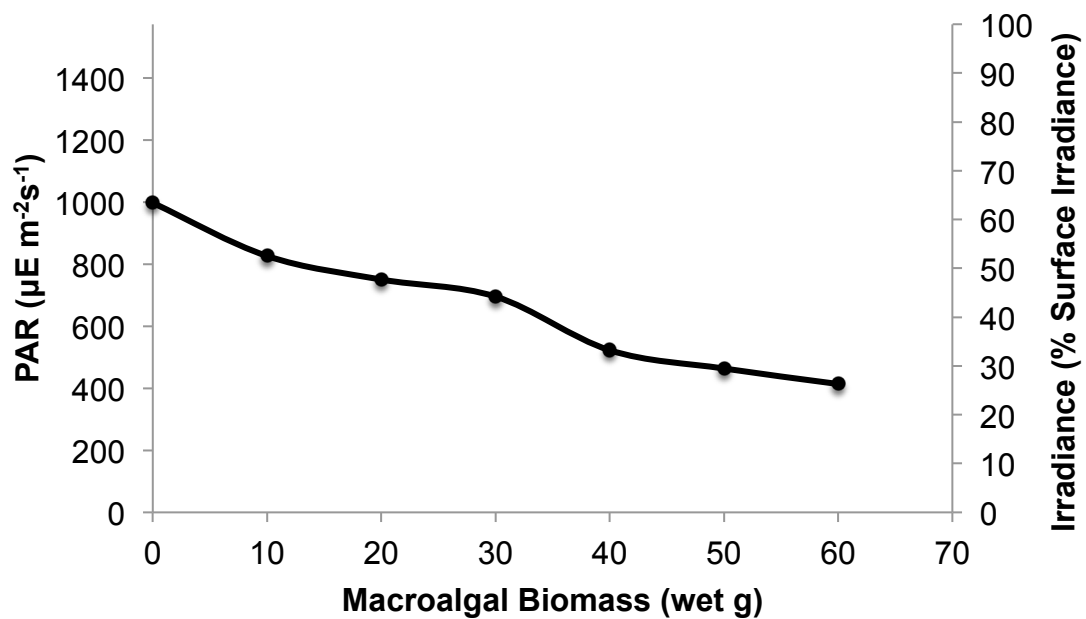


Figure 5. The relationship of PAR ($\mu\text{E m}^{-2}\text{s}^{-1}$) and irradiance with increasing macroalgal biomass (wet g) in experimental mesocosms measured in July 2014.

REFERENCES

- Alsterberg C, Eklof JS, Gamfeldt L, Havenhand JN, Sundbäck K (2013) Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proceedings of the National Academy of Sciences of the United States of America* 110:8603–8608
- Anton A, Cebrián J, Heck KL, Duarte CM, Sheehan KL, Miller M-EC, Foster CD (2011) Decoupled effects (positive to negative) of nutrient enrichment on ecosystem services. *Ecological Applications* 21:991–1009
- Baillie CJ, Fear JM, Fodrie FJ (2014) Ecotone Effects on Seagrass and Saltmarsh Habitat Use by Juvenile Nekton in a Temperate Estuary. *Estuaries and Coasts*
- Blake RE, Duffy JE (2010) Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem. *Oikos* 119:1625–1635
- Blake RE, Duffy JE (2012) Changes in biodiversity and environmental stressors influence community structure of an experimental eelgrass *Zostera marina* system. *Mar Ecol Prog Ser* 470:41–54
- Bruno JF, O'Connor MI (2005) Cascading effects of predator diversity and omnivory in a marine food web. *Ecology Letters* 8:1048–1056
- Burkholder J, Mason K, Glasgow HB Jr (1992) Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: Evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series* 81:163–178
- Burkholder JM, Glasgow HB Jr, Cooke JE (1994) Comparative effects of water-column nitrate enrichment on eelgrass *Zostera marina*, shoalgrass *Halodule wrightii*, and wideongrass *Ruppia maritima*. *Marine Ecology Progress Series* 105:121–138
- Burkholder JM, Tomasko DA, Touchette BW (2007) Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 350:46–72
- Canuel EA, Spivak AC, Waterson EJ, Duffy JE (2007) Biodiversity and food web structure influence short-term accumulation of sediment organic matter in an experimental seagrass system. *Limnology and Oceanography* 52:590–602
- Carilli JE, Norris RD, Black B, Walsh SM, McField M (2010) Century-scale records of coral growth rates indicate that local stressors reduce coral thermal tolerance threshold. *Global Change Biology* 16:1247–1257
- Carr JA, D'Odorico P, McGlathery KJ, Wiberg PL (2012) Modeling the effects of climate change on eelgrass stability and resilience: future scenarios and leading indicators of collapse. *Mar Ecol Prog Ser* 448:289–301
- Carr LA, Bruno JF (2013) Warming increases the top-down effects and metabolism of a

subtidal herbivore. PeerJ 1:e109

Clausen KK, Krause-Jensen D, Olesen B, Marbà N (2014) Seasonality of eelgrass biomass across gradients in temperature and latitude. *Marine Ecology Progress Series* 506:71–85

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* 11:1304–1315

Doney SC, Ruckelshaus M, Emmett Duffy J, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2012) Climate Change Impacts on Marine Ecosystems. *Annu Rev Marine Sci* 4:11–37

Douglass JG, Duffy JE, Spivak AC, Richardson JP (2007) Nutrient versus consumer control of community structure in a Chesapeake Bay eelgrass habitat. *Mar Ecol Prog Ser* 348:71–83

Duffy JE, Harvilicz AM (2001) Species-specific impacts of grazing, amphipods in an eelgrass-bed community. *Marine Ecology Progress Series* 223:201–211

Duffy JE, Richardson PJ, France KE (2005) Ecosystem consequences of diversity depend on food chain length in estuarine vegetation. *Ecology Letters* 8:301–309

Englund G, Öhlund G, Hein CL, Diehl S (2011) Temperature dependence of the functional response. *Ecology Letters* 14:914–921

Fourqurean JW, Powell GV, Kenworthy WJ, Zieman JC (1995) The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos* 72:349–358

Green EP, Short FT (Eds) (2003) *World Atlas of Seagrasses*.

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. *Ecology Letters* 9:228–241

Hauxwell J, Cebrián J, Valiela I (2003) Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* 247:59–73

Heck K, Valentine J (2007) The primacy of top-down effects in shallow benthic ecosystems. *Estuaries and Coasts* 30:371–381

Heck KL, Pennock JR, Valentine JF, Coen LD, Sklenar SA (2000) Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology & Oceanography*

- Hughes AR, Bando KJ, Rodriguez LF, Williams SL (2004) Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. *Marine Ecology Progress Series* 282:87–99
- Kenworthy WJ (1981) The interrelationship between seagrass *Zostera marina* and *Halodule wrightii*, and the physical and chemical properties of sediments in a mid Atlantic coastal plain estuary near Beaufort, North Carolina. University of Virginia, Charlottesville, VA
- Larkum AWD, Orth RJ, Duarte C (2006) *Seagrasses: Biology, Ecology and Conservation*. Springer
- Lee K-S, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology* 350:144–175
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312:1806–1809
- López-Urrutia Á, San Martín E, Harris R, Irigoien X (2006) Scaling the metabolic balance of the oceans. *Proceedings of the National Academy of Sciences* 103:8739
- Marsh JA Jr., Dennison WC, Alberte RS (1986) Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *Journal of Experimental Marine Biology and Ecology* 101:257–267
- Micheli F, Bishop M, Peterson C, Rivera J (2008) Alteration of seagrass species composition and function over two decades. *Ecological Monographs* 78:225–244
- Muncy RJ (1984) Life histories and environmental requirements of coastal fishes and invertebrates- pinfish. U.S. Fish & Wildlife Services FWS/OBS-82/11.26
- Myers JA, Heck KL Jr (2013) Amphipod control of epiphyte load and its concomitant effects on shoalgrass *Halodule wrightii* biomass. *Mar Ecol Prog Ser*
- Neckles HA, Wetzel RL, Orth RJ (1993) Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* 93:285–295
- Nelson WG (1979) An analysis of structural pattern in an eelgrass (*Zostera marina* L.) amphipod community. *Journal of Experimental Marine Biology and Ecology* 39:231–264
- O'Connor MI (2009) Warming strengthens an herbivore-plant interaction. *Ecology* 90:388–398
- O'Connor MI, Piehler M, Leech D, Anton A, Bruno J (2009) Warming and resource

availability shift food web structure and metabolism. PLoS biology 7:1–6

- Orth R, Carruthers T, Dennison W, Duarte C, Fourqurean J, Heck K Jr, Hughes A, Kendrick G, Kenworthy W, Olyarnik S (2006) A global crisis for seagrass ecosystems. BioScience 56:987–996
- Penhale PA (1977) Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) Community. Journal of Experimental Marine Biology and Ecology 26:211–224
- Ralph PJ, Tomasko D, Moore KA, Seddon S, Macinnis-Ng C (2006) Human Impacts on Seagrasses: Eutrophication, Sedimentation, and Contamination. In: Larkum AWD, Orth RJ, Duarte CM (eds), (null) (tran) Seagrasses: Biology, Ecology and Conservation. Springer, p 567–593
- Sand-Jensen K (1977) Effect of epiphytes on eelgrass photosynthesis. Aquatic Botany 3:55–63
- Spivak AC, Canuel EA, Duffy JE, Richardson JP (2009) Nutrient enrichment and food web composition affect ecosystem metabolism in an experimental seagrass habitat. PLoS One 4:e7473–e7473
- Spivak AC, Canuel EA, Duffy JE, Douglass JG, Richardson JP (2009) Epifaunal community composition and nutrient addition alter sediment organic matter composition in a natural eelgrass *Zostera marina* bed: a field experiment. Mar Ecol Prog Ser 376:55–67
- Stoner A (1979) Species-specific predation on amphipod crustacea by the pinfish *Lagodon rhomboides*: mediation by macrophyte standing crop. Marine Biology 55:201–207
- Thayer GW, Kenworthy WJ, Fonseca MS (1984) The ecology of eelgrass meadows of the Atlantic coast: community profile. US Fish and Wildlife Service FWS/OBS-82/02:147
- Touchette BW, Burkholder JM (2002) Seasonal variations in carbon and nitrogen constituents in eelgrass (*Zostera marina* L.) as influenced by increased temperature and water-column nitrate. Botanica Marina 45:23–34
- Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42:1105–1118
- Waycott M, Duarte CM, Carruthers TJ, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences 106:12377–12381