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Effects of Anthropogenic Stressors and Changes in Biodiversity on Lower Chesapeake Bay, VA USA Seagrass Systems

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Effects of Anthropogenic Stressors and Changes in Biodiversity on Lower Chesapeake Bay, VA USA Seagrass Systems

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By

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This dissertation is submitted in partial fulfillment of

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ABSTRACT

Anthropogenic stressors are increasingly changing conditions in coastal areas and impacting important habitats. But, when multiple stressors act simultaneously, their effects on ecosystems become more difficult to predict. In Chesapeake Bay, USA, predictions suggest that anthropogenic stressors from climate change, such as warming temperatures, may increase the frequency and severity of storm events, leading to increased freshwater, nutrient, and sediment inputs. Coastal development, another source of anthropogenic stressors, continues to increase with growing coastal populations, and may lead to altered sediment characteristics, habitat fragmentation, altered food webs, and loss of vegetated habitats. Community processes may interact with stressors, for example, immigration of propagules between habitat patches may alter diversity, and modify community response to stressors. Changes in biodiversity might alter ecosystem functioning and services, but diverse ecosystems may be more stable especially in the face of multiple stressors. Many habitats are vulnerable to anthropogenic stressors, including seagrass systems, which provide many valuable ecosystem services. Understanding how multiple stressors and community processes interact now is key to maintaining ecosystem services in the future.

Using a model seagrass (*Zostera marina*) system, I tested the effects of multiple stressors and their interactions with crustacean grazer immigration and diversity on ecosystem properties in a series of fully-factorial mesocosm experiments. I found that despite grazer species having varied responses to individual stressors, grazing pressure was largely maintained in spite of multiple stressors. More diverse grazer assemblages generally stabilized epiphytic algal biomass in the face of stressors, thereby increasing the resistance stability of this important component of the fouling community. Immigration of crustacean grazers did not interact significantly with stressors, and had little effect overall except to increase grazer biomass. Stressors generally reduced primary producer biomass, although in some cases they favored epiphytic algae. Generally, I did not find interactions among stressors and grazer immigration or diversity, even though diverse grazer assemblages stabilized epiphytic algal biomass.

To assess potential impacts of coastal development, I surveyed twenty seagrass beds in lower Chesapeake Bay, VA and assessed local shoreline development effects on adjacent seagrass beds. I sampled primary producers, consumers, water quality, and sediment characteristics in seagrass beds, and characterized development along the adjacent shoreline. I found that development significantly affected sediment characteristics, while epifaunal and epiphytic algal biomass was significantly higher on the Western versus the Eastern side of the bay. Grazer and predator biomass did not differ with either development or bay region. Thus, in seagrass communities, various factors appear to drive sediment and biological community properties on different spatial scales. This may be an important consideration for management, because efforts that incorporate spatial scales of ecosystem processes will likely have more impact.

Overall, these results suggest that stressor impacts in seagrass ecosystems generally do not interact but are sometimes context specific, while grazer diversity may have a limited but potentially important role in buffering certain ecosystem properties again stressors. Different factors appear to influence ecosystem properties at various spatial scales, an important consideration for predicting future impacts of multiple anthropogenic stressors in submerged vegetated systems.

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The primary research chapters of this dissertation were written in a format appropriate for submission to scientific journals. Therefore, these chapters were written in the third person to represent the contributions of my co-author.

CHAPTER 1
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CHAPTER 2
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CHAPTER 3
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Effects of Anthropogenic Stressors and Changes in Biodiversity on Lower Chesapeake Bay, VA USA Seagrass Systems.

INTRODUCTION

All ecosystems are potentially vulnerable to the negative impacts of stressors. A stressor is an environmental factor that detrimentally affects a population or ecosystem property (Folt et al. 1999; Darling and Cote 2008). Many stressors affect the functioning and persistence of ecological systems through their impacts on populations of ecologically important organisms, but anthropogenic stressors often exceed the range of natural environmental stressors. For example, experimental trace element additions to estuarine food webs have reduced the biomass, production and growth of phytoplankton, and may mask responses to other stressors (Breitburg et al. 1999). In Caribbean coral reef systems, slow recovery following hurricanes was attributed to overfishing and disease (Hughes and Connell 1999). Amphibian mortality increased about two fold when they were exposed to pesticides and predation, two important stressors (Sih et al. 2004). Predation, parasites, and pesticides all reduced growth and reproduction of the water flea (Daphnia magna) in an experimental freshwater system (Coors and De Meester 2008), while in boreal lakes, primary producers were reduced by the combined effects of drought and acidification (Christensen et al. 2006). Thus, anthropogenic stressors can reduce ecosystem resistance, resilience, and biodiversity (Folke et al. 2004; Darling and Cote 2008).

Predicting the impacts of multiple simultaneous stressors becomes difficult if their combined effect is different from the sum of their individual effects (Paine et al. 1998; Vinebrooke et al. 2004). Responses to multiple stressors can be classified relative to the effects of individual stressors in three simple ways: additive effects, where the multi-stressor effect equals the sum of the individual stressor effects; synergistic effects, where the impact of multiple stressors is greater than the sum of individual stressor effects; and antagonistic effects, where the impact of multiple stressors is less than predicted by the sum of the individual stressor effects. Previous studies have assumed synergisms to be widespread (Sala et al. 2000; Sala and Knowlton 2006) because species already influenced by a single stressor are expected to be more susceptible to the effects of additional stressors. Yet recent reviews suggest that antagonistic effects may be more common in some circumstances (Crain et al. 2008; Darling and Cote 2008). Predicting the responses of communities to multiple stressors is an increasingly important part of understanding how global climate change and coastal development will affect biodiversity and ecological services around the world (Sala et al. 2000).

As the speed and magnitude of global environmental change increases, more stressors are simultaneously acting in habitats around the globe (Sala et al. 2000), including estuaries and other coastal ecosystems (Breitburg et al. 1998). Many stressors are associated with climate change including acidification and climate warming, which is predicted to increase both global temperature averages and extremes. Air temperatures in the Chesapeake Bay region are predicted to warm by 2 - 6 °C by the end of the 21st century (Najjar et al. 2010), and because most of Chesapeake Bay is shallow, water temperatures will continue to closely follow increases in air temperatures (Najjar et al. 2000). Warming temperatures can impact sea level, ocean circulation, precipitation, nutrient and energy cycling, life histories and ranges of resident and transient organisms, fisheries production, and important habitats such as salt marshes, oyster reefs, and seagrass beds (Harley et al. 2006; Doney et al. 2012).

Climate warming may also increase freshwater, suspended sediment, and nutrient inputs to coastal waters as a result of predicted increases in rainfall, storm frequency, and storm intensity (Najjar et al. 2000; Najjar et al. 2008). In Chesapeake Bay, these pulsed inputs of rainfall and runoff will occur in addition to base-level increases in stream flow of tributaries such as the Susquehanna River (Najjar 1999). The predicted increase in fine sediment inputs with this runoff will likely decrease light availability in coastal waters, while eutrophication from increased nutrient inputs will promote excessive algal blooms which reduce both light and oxygen availability. Coastal habitats may be simultaneously impacted by these and other anthropogenic stressors, which may change ecosystems faster than organisms can adapt new conditions and lead to deteriorated ecosystems and loss of ecosystem functions and services (Doney et al. 2012).

Human populations in coastal areas continue to increase and by 2025, 75% of the global population is projected to live in coastal areas (Bulleri and Chapman 2010). Growing coastal populations will continue to develop coastal areas, converting vegetated pervious systems to unvegetated impervious areas by constructing buildings, paving roads, and armoring shorelines with riprap and bulkheads. Hundreds of miles of shoreline along Chesapeake Bay VA, USA, have been armored in the last several decades (Titus 1998; VIMS 2004), and in some sub-watersheds, 50% of shorelines are now hardened with armoring (Berman et al. 2000). This coastal development has thus changed a large portion of Chesapeake Bay shoreline from sandy, muddy soft sediments to hard surfaces such as rocks and concrete, and has the potential to impact important intertidal and subtidal communities by fragmenting habitats, changing movement of animals between habitats, altering wave energy and sediment composition, and increasing nutrient inputs to coastal systems (Irlandi and Crawford 1997; Bertness et al. 2002; Jennings et al. 2003; Groffman et al. 2004; Goforth and Carman 2005).

Many anthropogenic stressors results from coastal development, including increased sediment and nutrient inputs. Coastal development frequently increases the amount of fine sediments input to adjacent waters (Jennings et al. 2003), and decreases the sediment stability adjacent to developed shorelines (Goforth and Carman 2005). These fine-grain, unstable sediments often support lower infaunal densities in unvegetated habitats (Goforth and Carman 2005; Seitz et al. 2006), but their effects on submerged vegetated communities remain unclear.

Another important stressor in coastal systems is excessive nutrient loading. Changes in land-use during the last century have doubled the amount of nitrogen that is biologically available, leading to estuarine eutrophication (Vitousek et al. 1997). Eutrophication is a problem for estuaries with developed watersheds such as Chesapeake Bay, because nitrogen inputs to coastal waters are significantly higher when watersheds are dominated by development (Groffman et al. 2004). These nitrogen inputs can lead to simplified food webs, low dissolved oxygen, and losses in ecosystem functioning (Vitousek et al. 1997; Lerberg et al. 2000). Though eutrophication is widely known to negatively impact submerged vegetated habitats at watershed and basin-wide scales (Burkholder et al. 2007), local impacts in these habitats are less well understood but may be as important as in saltmarshes (Bertness et al. 2002).

An important question about the impacts of multiple stressors is whether their effects may be altered by community processes such as immigration, which can influence such ecosystem properties as diversity, abundance, and biomass (Loreau and Mouquet 1999; Mouquet et al. 2004). In unsaturated communities containing fewer than the maximum number of coexisting species that can be supported, ecological theory predicts that immigration will increase species diversity through resource partitioning and supply of novel or rare species (Loreau 2000; Chase 2003; Lee and Bruno 2009), while in saturated communities containing the maximum number of coexisting species that can be supported, immigration will maintain diversity by sustaining populations of inferior competitors that otherwise would be outcompeted (Loreau 2000). Thus, changes in immigration could alter diversity, abundance, and biomass, and result in communities that may be more or less able to cope with multiple stressors.

Movement of animals between habitat patches via immigration is an ecosystem process that can alter biodiversity. Biodiversity supports ecosystem processes, properties, and functions that are ecologically and economically important, such as water and nutrient cycling, and fisheries production. Previous experiments have shown that higher diversity among and within trophic levels tends to increase stability (Tilman 1996; Tilman et al. 1998; Griffiths et al. 2000; Caldeira et al. 2005), biomass (Allison 2004; Balvanera et al. 2006; Cardinale et al. 2006; Cardinale et al. 2011), and resource use in many ecosystems, leading to overall higher ecosystem performance. For example, increased biomass within a trophic level as a result of greater diversity may increase ecosystem processes, such as grazing which plays an important role in marine systems (Worm et al. 1999; Nystrom et al. 2000; Duffy et al. 2003; Valentine and Duffy 2006; Stachowicz et al. 2007). However, biodiversity on both global and local scales is threatened, largely by human activities that cause rapid changes in ecological systems (Sala et al. 2000). These changes in biodiversity are of concern because they can result in losses of important ecosystem functions (Tilman 1999). Thus, changes in biodiversity also act as a stressor in ecological systems, and may interact with anthropogenic stressors, or be as important as, or more important than, other stressors in marine systems (Stachowicz et al. 2007).

Theory predicts that the ecological stability of ecosystem properties should increase as species richness increases because functional redundancy among species insures against loss of functions. Model simulations and experiments confirm the theoretical role of this insurance hypothesis mechanism (Yachi and Loreau 1999; Thebault and Loreau 2005; Griffin et al. 2009). Ecological stability can be defined several ways: temporal stability is measured as the inverse of variance through time, spatial stability is measured as the inverse of variance between habitat patches within a certain area, while resistance stability is measured as the ability to withstand stressor impacts, and resilience stability is measured as the speed of recovery from stressor impacts (MacArthur 1955; Pimm 1984; Tilman 1996; Griffin et al. 2009). Of particular interest and relevance to global climate change, resistance stability measures the tendency of ecosystem properties to remain unchanged in the face of environmental stressors (Morin 1999). The biological insurance, via diversity, of ecosystem properties depends on two components: functional effect redundancy and functional response diversity (Lavorel and Garnier 2002; Elmqvist et al. 2003; Hooper et al. 2005; Griffin et al. 2009). Functional effect redundancy refers to the concept that multiple species contribute to an ecosystem function, such that loss of one species leaves others that fulfill that same function. Functional response diversity refers to the different responses of co-occurring species to a given perturbation, such that the presence of multiple species ensures that at least one will be unaffected and continue to provide the function. In other words, in a community where species affect ecosystem properties similarly but respond to stressors in diverse ways, ecosystem properties may be more stable. *The degree of biological insurance provided depends upon several factors* including interaction strength, the form of species responses to stressors, and the degree of asynchronicity of responses to stressors (Yachi and Loreau 1999; Thebault and Loreau 2005). Biological diversity often declines simultaneously with the imposition of stressors. This is an increasing concern because we know little about how biodiversity change interacts with other stressors in marine systems, especially in the context of global climate change.

Seagrass systems in Chesapeake Bay are vulnerable to climate change impacts, which is of concern because these important habitats provide refuge for juvenile fish and crabs, stabilize sediments and coastlines, and sequester carbon (Duarte 2000; Heck et al. 2003; Duarte et al. 2005; Polte et al. 2005; Canuel et al. 2007). Historically the dominant seagrass in Chesapeake Bay (Orth and Moore 1983; Orth and Moore 1988; Orth et al. 2002), eelgrass (*Zostera marina*, hereafter referred to as *Zostera*) may be especially susceptible to stressors in the bay because it is near the southern end of its range (Short and Neckles 1999). *Zostera* has been declining at an accelerated pace in recent years due in part to reduced light availability, continued eutrophication, and record summer temperatures (Moore et al. 1996; Moore et al. 1997; Orth et al. 2006; Moore and Jarvis 2008). Elevated temperatures, especially in the summer months, can lead to eelgrass die-offs (Bintz et al. 2003; Oviatt 2004). These declines are attributed to higher respiration than production, internal carbon stress, and reduced growth rates in eelgrass under higher temperature conditions (Short and Neckles 1999; Touchette and Burkholder 2000; Bintz et al. 2003; Touchette et al. 2003). Reductions in available light also cause eelgrass decline, especially in estuaries where turbidity is high due to suspended materials. *Zostera* cannot maintain a positive carbon balance when available light falls below compensating levels, leading to decreased growth and survival (Moore et al. 1997). Eutrophication has negative impacts on *Zostera*, both direct and indirect. Nutrient enrichment impacts eelgrass physiology similarly through carbon limitation, but also through ammonium toxicity, both of which lead to reduced shoot production and overall growth (Touchette and Burkholder 2000; Touchette et al. 2003). Indirect impacts of nutrients on *Zostera* include overgrowth by micro and macroalgae, which can block light and reduce growth and shoot density (Neckles et al. 1993; Hauxwell et al. 2001; Deegan 2002; Valentine and Duffy 2006; Burkholder et al. 2007).

In temperate systems, grazing by amphipods and isopods on epiphytic algae can help maintain *Zostera* (Neckles et al. 1993; Duffy et al. 2003; Duffy et al. 2005; Valentine and Duffy 2006; Whalen 2011). Thus, crustacean grazers facilitate persistence of the competitively inferior, slower growing eelgrass (Valentine and Duffy 2006). Diet and feeding rates vary slightly among mesograzer species (Duffy and Hay 2000; Duffy and Harvilicz 2001), but experiments show that more diverse grazer communities can be more effective in controlling epiphytic biomass and maintaining eelgrass dominance in some temperate systems (Duffy et al. 2003; Duffy et al. 2005). While the greater feeding efficiency of diverse grazer assemblages has been shown, the potential role of grazer diversity in stabilizing grazing processes in the face of environmental change has not been investigated. Understanding the potential role of community diversity in buffering ecosystem properties against multiple stressors is a key problem in knowing how biodiversity affects ecosystem functioning, and may lead to better predictions and management of the ecological impacts of stressors on eelgrass communities and estuarine food webs.

The goal of this research was to understand the ecological impacts and interactions of anthropogenic stressors stemming from climate change and coastal development on the persistence and functioning of seagrass systems in Chesapeake Bay. In particular, I wanted to assess the effects and interactions of multiple anthropogenic stressors, including changes in biological diversity, on the structure and functioning of model eelgrass systems, and to examine the impacts of local coastal development on the composition and structure of adjacent seagrass communities. Thus, I conducted a series of factorial mesocosm experiments to test the effects of multiple stressors and crustacean grazer immigration and diversity on ecosystem properties. I chose to manipulate water temperature, salinity, nutrients, and light availability because these stressors may affect coastal food webs, and are predicted to be increasingly important in coastal systems with climate change. I also surveyed twenty seagrass beds in lower Chesapeake Bay, VA and assessed local shoreline development to examine potential impacts of coastal development on adjacent seagrass beds. Specifically, I asked the following questions: 1) Do grazer species respond differently to stressors? 2) Does the presence and species richness of grazers affect the variance of responses to stressors, i.e., the resistance stability of the system? 3) Are stressor effects on response variables additive, synergistic, or antagonistic? 4) Are grazing effects of similar magnitude as stressor effects? 5) Do stressors interact to affect primary producer community structure and composition? 6) Does crustacean grazer immigration alter the structure of the grazer community or the effects of stressors? 7) Are there differences in seagrass-associated animal abundance and diversity with coastal development? 8) Are seagrass beds adjacent to developed shorelines nutrient enriched? 9) Does shoreline development correspond with an altered physical environment?

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CHAPTER 1

Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem

Abstract

When multiple stressors act simultaneously, their effects on ecosystems become more difficult to predict. In the face of multiple stressors, diverse ecosystems may be more stable if species respond differently to stressors or if functionally similar species can compensate for stressor effects on focal species. Many habitats around the globe are threatened by multiple stressors, including highly productive seagrass habitats. For example, in Chesapeake Bay, USA, regional climate change predictions suggest that elevated temperature and freshwater inputs are likely to be increasingly important stressors. Using seagrass mesocosms as a model system, we tested whether species richness of crustacean grazers buffers ecosystem properties against the impacts of elevated temperature and freshwater pulse stressors in a fully factorial experiment. Grazer species responded to pulsed salinity changes differently; abundance of *Elasmopus levis* responded negatively to freshwater pulses, whereas abundance of *Gammarus mucronatus* and *Erichsonella attenuata* responded positively or neutrally. Consistent with the hypothesis that biodiversity provides resistance stability, biomass of epiphytic algae that form the base of the food web was less affected by stressors in species-rich grazer treatments than in single-species grazer treatments. Stochastic (among-replicate) variation of sessile invertebrate biomass within treatments was also reduced in more diverse grazer treatments. Therefore, grazer species richness tended to increase the resistance stability of both major components of the seagrass fouling community, algae and invertebrates, in the face of environmental stressors. Finally, in our model system, multi-stressor impacts suggested a pattern of antagonism contrary to previous assumptions of synergistic stressor effects. Overall, our results confirm that invertebrate grazer species are functionally diverse in their response to environmental stressors, but are largely functionally redundant in their grazing effects leading to greater resistance stability of certain ecosystem properties in diverse grazer assemblages even when influenced by multiple environmental stressors.

Introduction

Theory predicts that the ecological stability of ecosystem properties should increase as species richness increases because of functional redundancy, and model simulations confirm the role of the insurance hypothesis mechanism (Yachi and Loreau 1999, Thebault and Loreau 2005). Ecological stability can be defined several ways: temporal stability is measured as the inverse of variance through time, while resistance stability is measured as the ability to withstand stressor impacts, and resilience stability is measured as the speed of recovery from stressor impacts (MacArthur 1955, Pimm 1984, Tilman 1996, Griffin et al. 2009). Of particular interest and relevance to global climate change, resistance stability measures the tendency of ecosystem properties to remain unchanged in the face of environmental stressors (Morin 1999). The biological insurance, via diversity, of ecosystem properties depends on two components: functional effect redundancy and functional response diversity (Elmqvist et al. 2003, Hooper et al. 2005, Griffin et al. 2009). Functional effect redundancy refers to the concept that multiple species contribute to an ecosystem function, such that loss of one species leaves others that fulfill that same function. Functional response diversity refers to the different responses of co-occurring species to a given perturbation, such that the presence of multiple species ensures that at least one will be unaffected and continue to provide the function. In other words, in a community where species affect ecosystem properties similarly but respond to stressors in diverse ways, ecosystem properties may be more stable. The degree of biological insurance provided depends upon several factors including interaction strength, the form of species responses to stressors, and the degree of asynchronicity of responses to stressors (Yachi and Loreau 1999, Thebault and Loreau 2005). Since biological diversity often declines simultaneously with the imposition of stressors, there remains a need to more thoroughly test the effects of species richness on community resistance to multiple environmental stressors (Hooper et al. 2005).

A stressor is an environmental factor that detrimentally affects a population or ecosystem property (Folt et al. 1999, Darling and Cote 2008). Many stressors affect the functioning and even the persistence of ecological systems through their impacts on populations of ecologically important organisms. However, predicting the impacts of simultaneous multiple stressors becomes difficult if their combined effect is different from the sum of their individual effects (Paine et al. 1998, Vinebrooke et al. 2004). Responses to multiple stressors can be classified relative to the effects of individual stressors in three simple ways: additive effects, where the multi-stressor effect equals the sum of the individual stressor effects; synergistic effects, where the impact of multiple stressors is greater than the sum of individual stressor effects; and antagonistic effects, where the impact of multiple stressors is less than predicted by the sum of the individual stressor effects. Previous studies have assumed synergisms to be widespread (Sala et al. 2000, Sala and Knowlton 2006) because species already influenced by a single stressor are expected to be more susceptible to the effects of additional stressors. Yet recent reviews suggest that antagonistic effects may be more common in some circumstances (Crain et al. 2008, Darling and Cote 2008). Predicting the responses of communities to multiple stressors is an increasingly important part of understanding how global change will affect biodiversity and ecological services around the world (Sala et al. 2000).

As the speed and magnitude of global environmental change increases, more stressors are simultaneously acting in habitats around the globe (Sala et al. 2000), including estuaries and other coastal ecosystems (Breitburg et al. 1998). Air and ocean temperatures have been rising over the last several decades as a result of global climate change (Watson 2001, Preston 2004, Levitus et al. 2005). For example, regional predictions for the large estuary Chesapeake Bay, USA, indicate expected increases in water temperature of 4.7 1 2.0°C by the end of the current century (Najjar et al. 2000, 2008). Elevated summer water temperatures have been shown to severely reduce populations of the important foundation species eelgrass *Zostera marina* (Oviatt 2004, Moore and Jarvis 2008), raising concern about future extirpation of this species from such locations. Climate change is also predicted to increase the frequency and severity of storm events (Watson 2001), sending large pulses of freshwater into the estuary. These pulses would be in addition to predicted increases in base-level stream flow of tributaries such as the Susquehanna River (Najjar 1999). While the impacts of these and other environmental stressors have been demonstrated individually in seagrass systems (Hauxwell et al. 2001, Bintz et al. 2003, Oviatt 2004), the interactive effects of multiple stressors and the potential mediating influence of faunal communities in this system remain understudied.

Seagrass beds are important marine habitats that sequester carbon and sediments (Duarte et al. 2005), contribute organic matter to the sediments (Canuel et al. 2007), harbor large and productive communities of algae, invertebrates, and fishes (Duarte 2000), and offer refuge for a wide diversity of organisms. Consequently seagrass beds foster ecosystem functions such as high productivity, trophic transfer, and nutrient recycling (Heck and Orth 1980, Orth and Heck 1980, Summerson and Peterson 1984, Duarte 2000, Heck et al. 2003). *Zostera marina* is the dominant seagrass of higher salinity estuaries and coastal areas and an important foundation species of vegetated coastal ecosystems throughout the northern hemisphere. In Chesapeake Bay, VA, near the southern end of its range, eelgrass may be especially susceptible to changes in temperature, light, physical stress and habitat (Short and Neckles 1999). During the past several decades, coverage of Z. marina in Chesapeake Bay has decreased significantly (Orth and Moore 1983, Orth et al. 2002), with recent data showing an accelerated rate of loss (Orth et al. 2006). Vulnerable to this loss of habitat are numerous commercially important species of nekton such as crabs and fish as well as invertebrate forage species. In temperate eelgrass beds, the dominant invertebrate grazers are amphipod and isopod crustaceans (Orth and van Montfrans 1984, Jernakoff and Nielsen 1997, Valentine and Duffy 2006). These small mesograzers are important food items for fishes, crabs and shrimp (Klumpp et al. 1989, Edgar and Shaw 1995a, 1995b), are found in high densities, and feed on epiphytic algae and recruits of sessile invertebrates growing on eelgrass leaves (Duffy and Harvilicz 2001). These mesograzers are also important in controlling fast-growing epiphytic algae, which otherwise can overgrow seagrass leaves. Thus, mesograzers facilitate persistence of the competitively inferior, slower growing eelgrass (Valentine and Duffy 2006). However, diet varies slightly among mesograzer species (Duffy and Hay 2000, Duffy and Harvilicz 2001), and experiments show that more diverse grazer communities can be more effective in controlling epiphytic biomass and maintaining eelgrass dominance in some temperate systems (Duffy et al. 2003, 2005). While the greater feeding efficiency of diverse grazer assemblages has been shown, the potential role of grazer diversity in stabilizing grazing processes in the face of environmental change has not been investigated. Understanding the potential role of community diversity in buffering ecosystem properties against multiple stressors is a key problem in knowing how biodiversity affects ecosystem functioning, and may lead to better predictions and management of the ecological impacts of stressors on eelgrass communities and estuarine food webs.

The goals of this study were to test the hypothesized role of grazer species richness in providing resistance stability against environmental change, that is, in buffering against multiple interacting stressor impacts, and to examine the effects of multiple stressors on mesograzer species in a model eelgrass community. To do this, we conducted a fully factorial mesocosm experiment that manipulated two environmental stressors, temperature and salinity, as well as grazer species composition and richness. We chose these stressors because of predicted increases due to climate change (Najjar et al. 2000, 2008, Watson 2001), and because of potential impacts of these stressors on the composition of the invertebrate grazer assemblage (Maranho and Marques 2003, Douglass et al. 2010). We addressed the following questions: 1) do grazer species display different responses to stressors? 2) does the presence and species richness of grazers affect the variance of responses to stressors, i.e., the resistance stability of the system? 3) are stressor effects on response variables additive, synergistic, or antagonistic?

Methods

Experimental Design

We factorially manipulated grazer species richness and composition, water temperature, and salinity in a set of 100 outdoor mesocosms at the Virginia Institute of Marine Science, Gloucester Point, VA, USA (37°24'7653"N, 76°50'0809"W) during summer 2006. Mesocosms were 19-l buckets set up in a water bath in a flow-through tank array supplied with filtered estuarine water from the adjacent York River. York River water initially passed through a coarse sand filter and then through 150-micron mesh, in order to prevent unintentional introduction of additional mesograzer species, but allow for propagules of algae and sessile invertebrates to recruit to the mesocosms. Approximately 2.5 l of sand and 0.5 l of organic mud were added to each mesocosm as substrate. Thirty shoots of defaunated *Zostera marina* were then planted into each mesocosm. Screens were erected to shade each tank in order to reduce light levels to approximate field conditions.

Five grazer treatments were established using the isopod *Erichsonella attenuata*, and the amphipods *Elasmopus levis* and *Gammarus mucronatus*; each species in monoculture, all three species in polyculture, and a grazer-free control (grazer species hereafter referred to by genus). Thirty grazers were added to each mesocosm, with the three-species treatments receiving ten individuals of each species. These three grazer species are among the five most common mesograzer species in local eelgrass beds during July and August (Douglass et al. 2010). The experiment ran for six weeks, allowing the grazer species to have 1–2 generations and to reach carrying capacity in the mesocosms (Duffy and Harvilicz 2001). Two temperature treatments, heated and unheated, were applied throughout the duration of the experiment. Heated treatments used one 200 W aquarium heater per heated mesocosm and increased temperatures 3 to 4°C above ambient conditions. Salinity treatments, involving presence or absence of freshwater pulses, were applied after two and four weeks. Freshwater was allowed to flow into freshwater-pulse mesocosms until the salinity reached ~0–2. After 15–20 min. at this reduced salinity, mesocosms were then reconnected to the flow-through system and allowed to return to ambient salinity (20–22). Mesocosms not receiving freshwater pulse treatment were also disconnected from estuarine water for 15–20 min. These freshwater pulse treatments were meant to simulate the increased freshwater inputs expected in estuarine systems as stream flow and storm frequency and intensity increase with climate change. Although the duration and scale of climate-induced freshwater pulses will differ in nature, we chose these conditions because they were logistically feasible, we could standardize the nature of pulse treatments, and preliminary data suggested that this treatment would negatively affect some but not all species in our system.

Artificial seagrass ribbons were placed in each mesocosm at the start of the experiment to provide a standardized substratum for accumulating and measuring epiphytic algal growth (four units of two ribbons each). During the experiment, epiphytic chlorophyll a was sampled from the artificial seagrass after two, four and six weeks (one unit removed per sampling point) as a proxy for epiphytic algal biomass (Parsons et al. 1984). Data are presented as mass of chlorophyll a normalized to area of eelgrass leaf blade (μg chl a cm−2). Grazer density was estimated before and after each freshwater pulse event and at the end of the experiment by collecting grazers in each mesocosm in three standardized sweeps of a small dip-net, identifying and counting them, and returning them to their mesocosm. At the end of the experiment, all plant, animal and algal biomass was harvested from each mesocosm and frozen. Samples were processed by sorting all biomass retained on a 500 micron sieve, separating and identifying organisms by taxon, drying at 60°C until mass was stable, and then combusting to obtain ash-free dry mass (AFDM) of each taxon. Grazers were sorted by size, identified to species, and counted to determine biomass using the method of Edgar (1990). This method calculates the biomass of animals retained by different size sieves (8.0, 5.6, 4, 2.8, 2.0, 1.4, 1.0, 0.71, 0.05 mm) according to the empirical equation, $\log B = -1.01 + 2.64 \times \log S$, where B is AFDM (mg), and S is the sieve size (mm) on which the animal was retained (Edgar 1990).

Statistical Analysis

Contamination of grazer treatments necessitated the removal of 9 of the 100 mesocosms from final analysis for all response variables. Each treatment retained at least three replicates for final analysis. We used three-way ANOVAs to test the main and interactive effects of temperature, freshwater pulses, and grazer identity and richness on the response variables *Zostera* biomass, algal biomass, epiphytic chlorophyll a, grazer biomass and sessile invertebrate biomass. ANOVAs were done using SAS 9.1. Untransformed data were used because all data met the assumption of homogeneous variances (Levene's test in R 2.7.2). Two sets of orthogonal a-priori contrasts were conducted to test the effects of grazer presence (all treatments with grazers vs no-grazer controls) and grazer richness (all grazer monocultures vs multi-grazer species treatments). We estimated the strength of the effects and the amount of variance explained by each factor in the ANOVAs, by calculating ω2 values for all factors and responses using the generalized equation (Olejnik and Algina 2003),

$$\omega^2 = \left[SS_{effect} - DF_{effect} \bullet MS_{error}\right] / \left[SS_{effect} + \left(N - DF_{effect}\right) \bullet MS_{error}\right] .$$

To examine whether grazer species richness stabilized ecosystem properties in the face of multiple stressors, we needed to test whether multi-species grazer treatments varied less in response to stressors than did single-species grazer treatments. We looked at two components of this stability: 1) community resistance to stressors (i.e. variance in response among stressor treatments), and 2) stochastic variation (i.e. variance in response among replicates within stressor treatments). Because testing for effects of species richness required us to pool single-species grazer treatments, we could not use the coefficient of variation (CV) analysis as a measure of variability. Instead we used a variation of Levene's test (Levene 1960) called the Brown–Forsythe test (Brown and Forsythe 1974), that uses the median rather than the mean to calculate deviations and test for equality of variances. Several comparative tests and simulations (Schultz 1985, Olejnik and Algina 1987) have shown the Brown–Forsythe test to be conservative but statistically robust and powerful, especially with data such as ours that has a tail to the distribution and small sample sizes. Therefore, we employed the Brown–Forsythe test to calculate the variation between single-species and multi-species grazer treatments. First, we tested how variance among stressor treatments differs with species richness (i.e. community resistance to stressors) by calculating, for each grazer treatment (single- or multi-species assemblage), the median value of a given response variable (e.g. algal biomass) across the four stressor treatments. For example

$$\mathrm{Er}_{\mathrm{Med}} = \mathrm{median}(\mathrm{Er}_{\mathrm{H}}, \mathrm{Er}_{\mathrm{F}}, \mathrm{Er}_{\mathrm{HF}}, \mathrm{Er}_{\mathrm{C}})$$

where Er = *Erichsonella attenuata*, $\mathrm{Er}_{\mathrm{Med}}$ = median of Er treatments, Er_{H} = heated treatment mean, Er_{F} = freshwater pulse treatment mean, $\mathrm{Er}_{\mathrm{HF}}$ = heat plus freshwater pulse treatment mean, and Er_{C} = no-stressor control mean. Then we took the absolute value of the deviation of each stressor treatment mean from the grazer species median to get the absolute deviations of the stressor treatments for that grazer species. For example

$$\mathrm{AbsDev}(\mathrm{Er}_{\mathrm{H}}) = \left| \mathrm{Er}_{\mathrm{H}} - \mathrm{Er}_{\mathrm{Med}} \right|$$

where $\mathrm{AbsDev}(\mathrm{Er}_{\mathrm{H}})$ = absolute deviation of Er_{H}. We also divided the absolute deviation by the median to calculate the relative deviation, a dimensionless measure of variation scaled to an average (similar to the coefficient of variation and reported by others) (Cottingham et al. 2001). For example

$$\mathrm{RelDev}(\mathrm{Er}_{\mathrm{H}}) = \left| \mathrm{Er}_{\mathrm{H}} - \mathrm{Er}_{\mathrm{Med}} \right| / \mathrm{Er}_{\mathrm{Med}}$$

where $\mathrm{RelDev}(\mathrm{Er}_{\mathrm{H}})$ = relative deviation of Er_{H}. A t-test was then performed on these deviations (single species n = 12 = three grazer species X four stressor treatments, multi-species n = 4) to test whether the deviation from the grazer species median was greater for single-species treatments than for multi-species treatments, as predicted by theory. A similar approach was used to test whether variance among replicates within stressor treatments (i.e. stochastic variation among replicates) was influenced by species richness. First, we calculated the median of the five replicates for each of the 20 grazer X stressor treatment combinations. For example

$$\mathrm{GrazerbyStressorMedian}_{\mathrm{ErC}} = \mathrm{median}(\mathrm{Er}_{\mathrm{C}})$$

Then, for each replicate mesocosm of a given treatment (grazer by stressor combination), we calculated the absolute deviation by taking the absolute value of the difference between that mesocosm and the median for that grazer by stressor treatment. The relative deviation was calculated as before. For example

$$\mathrm{AbsDev}(\mathrm{Er}_{\mathrm{C5}}) = \left| \mathrm{Er}_{\mathrm{C5}} - \mathrm{median}(\mathrm{Er}_{\mathrm{C}}) \right|,$$

$$\mathrm{RelDev}(\mathrm{Er}_{\mathrm{C5}}) = \left| \mathrm{Er}_{\mathrm{C5}} - \mathrm{median}(\mathrm{Er}_{\mathrm{C}}) \right| / \mathrm{median}(\mathrm{Er}_{\mathrm{C}})$$

where AbsDev(Er_{C5}) = absolute deviation of Er_C replicate number 5, and RelDev(Er_{C5}) = relative deviation of Er_C replicate number 5. We then conducted a t-test to test whether the mean of the single-species treatment absolute deviations was greater than multi-species treatment deviations (single species n = 52, multi-species n = 20). This was done for all response variables. Finally, in order to examine the effects of multiple stressors acting simultaneously, we calculated the expected combined impact of heat and freshwater stress from the single stressor treatments and compared these expectations with the observed impacts from the two-stressor treatments. The expected stressor impacts were calculated as simple additive effects using one of three equations (Crain et al. 2008); 1) for cases where the responses (e.g. algal biomass) of both single-stressor treatments were less than the control, we used the equation,

$$\mathrm{Stress}_{\mathrm{Exp}} = \mathrm{C} - (\mathrm{C} - \mathrm{Stress}_{\mathrm{A}}) - (\mathrm{C} - \mathrm{Stress}_{\mathrm{B}})$$

where $\mathrm{Stress}_{\mathrm{Exp}}$ = expected stressor effect, C = no-stressor control, $\mathrm{Stress}_{\mathrm{A}}$ = stressor treatment A, and $\mathrm{Stress}_{\mathrm{B}}$ = stressor treatment B. 2) For cases where the responses of both single-stressor treatments were greater than the control, we used the equation:

$$\mathrm{Stress}_{\mathrm{Exp}} = \mathrm{C} + (\mathrm{Stress}_{\mathrm{A}} - \mathrm{C}) + (\mathrm{Stress}_{\mathrm{B}} - \mathrm{C})$$

3) For cases where stressor response was mixed, with one stressor response less than the control and the other greater, we used the equation:

$$\mathrm{Stress}_{\mathrm{Exp}} = \mathrm{C} + (\mathrm{Stress}_{>\mathrm{C}} - \mathrm{C}) - (\mathrm{C} - \mathrm{Stress}_{<\mathrm{C}})$$

where $\mathrm{Stress}_{>\mathrm{C}}$ = stressor response greater than control, and $\mathrm{Stress}_{<\mathrm{C}}$ = stressor response less than control.

We chose which equation to use based on the mean response variable values for each treatment. The loss of replicates due to contamination during the experiment made it necessary to re-sample the data in order to obtain confidence limits for the estimated means. Response values were randomly re-sampled from within each treatment with replacement 10,000 times. The appropriate additive effects equation (chosen from the three detailed above) was used for all rows of re-sampled data to give us 10,000 expected additive stressor effect values per treatment.

We then took the mean and 95% confidence intervals (CI) of the re-sampled values for the expected additive and observed stressor effects data. If the 95% CI of the expected and observed stressor effects overlapped (were not significantly different), the observed stressor effect was considered additive. For treatments where both stressor responses were less than control or were mixed, observed CI below expected CI were considered synergistic while those above were considered antagonistic. When both stressor responses were greater than the control, observed CI below expected CI were considered antagonistic, and those above synergistic (Crain et al. 2008, Darling and Cote 2008).

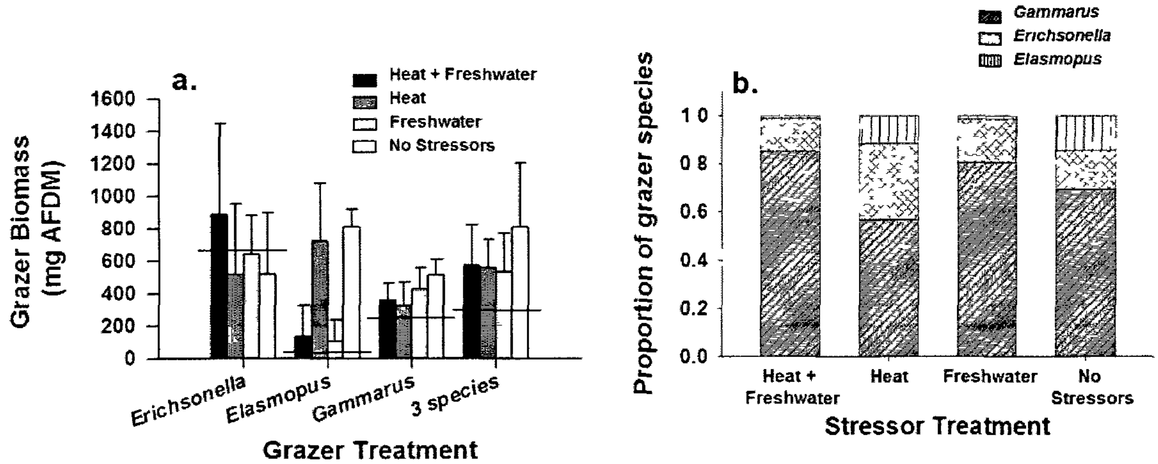

Figure 1. (a) Final biomass (mean + 1 SD) of grazer species in the different treatments. The horizontal line across each group of bars indicates the expected biomass if the stressor effects were additive. Cases where the additive model line is greater than the no-stressor treatment bar indicate that at least one of the single-stressor treatments had more biomass than the no-stressor treatment. Thus, the sum of the individual stressor effects could be greater than the no-stressor treatment. Statistical analysis in Tables 1–3. (b) Proportion of grazer species in the three-species treatments (the more diverse grazer community)

Results

Effects of grazer identity and diversity

Final grazer biomass differed strongly among grazer treatments (Fig. 1, p = 0.021, Table 1), with a trend towards higher biomass, on average, in the multi-species treatments than in the average single-species treatment (richness contrast p = 0.097, Table 2), as found previously in this system (Duffy et al. 2003, 2005). The freshwater pulse stressor treatment impacted grazer species strongly (p = 0.038), but its effects differed among grazer species (Species X Fresh, p < 0.001, Table 1). Specifically, *Elasmopus* abundance was severely reduced by freshwater and *Gammarus* abundance decreased slightly if at all, but *Erichsonella* abundance tended to increase with freshwater, especially in the presence of heat (Fig. 1, 4b). Grazers strongly reduced epiphytic algal biomass (chl *a*), on average (Fig. 2a), particularly in the absence of stressors. Across all treatments, grazer biomass was weakly but significantly inversely related to epiphytic algal biomass (r^2 = 0.06, p = 0.045). In the absence of grazers, chl *a* was also strongly reduced by heating and freshwater and was nearly absent from the multi-stressor treatment (Fig. 2a). Grazer species differed significantly in their impacts on chl *a* (p = 0.002, Table 1), but grazer species richness had no significant impact on chl *a* (richness contrast, p = 0.403, Table 2). The biomass of epiphytic chl *a* was also influenced by an interaction between grazer treatment and freshwater pulse treatment (p = 0.006, Table 1).

Table 1. Results of three-way ANOVAs and estimates of effect size (ω^2) for each response variable. Negative ω^2 values are shown as zeros. Values significant at $p = 0.05$ are in bold. Calculation of the MS used the type III SS.

	Response																	
	Grazer biomass			Sessile invertebrate biomass			*Zostera* shoot biomass			Macroalgae biomass			Primary producer biomass			Epiphytic chl a		
Factor	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2	MS	p	ω^2
Grazer species (Sp.)	267646	**0.021**	0.09	0.35	**<0.001**	0.25	0.60	**<0.001**	0.22	1.62	**0.004**	0.12	2.29	**0.005**	0.12	17.85	**0.002**	0.14
Temperature (Temp.)	21732	0.597	0.00	0.05	0.292	<0.01	0.05	0.418	0.00	2.46	**0.014**	0.06	2.50	**0.040**	0.04	7.15	0.175	0.01
Freshwater (Fresh.)	345765	**0.038**	0.05	0.27	**0.014**	0.06	0.01	0.680	0.00	0.50	0.259	<0.01	0.44	0.381	0.00	3.72	0.327	0.00
Sp. × Temp.	60891	0.502	0.00	0.003	0.989	0.00	0.21	0.052	0.06	0.52	0.261	0.02	1.26	0.077	0.05	3.46	0.465	0.00
Sp. × Fresh.	542626	**<0.001**	0.20	0.02	0.710	0.00	0.07	0.541	0.00	0.35	0.475	0.00	0.45	0.531	0.00	14.99	**0.006**	0.11
Temp. × Fresh.	156699	0.158	0.01	0.13	0.083	0.02	0.003	0.843	0.00	0.95	0.123	0.02	0.56	0.325	0.00	0.43	0.738	0.00
Sp. × Temp. × Fresh.	8362	0.954	0.00	0.04	0.404	<0.01	0.09	0.372	<0.01	0.46	0.324	0.01	0.46	0.525	0.00	3.83	0.411	0.00
Error	76651			0.04			0.08			0.39			0.57			3.81		

Table 2. Results from a-priori contrasts for effects of grazer presence (no-grazer versus pooled grazer treatments) and richness (three-species versus pooled one-species treatments) on each response variable. Values significant at $p = 0.05$ are in bold.

Response	Contrast for grazer presence	Contrast for grazer richness
Grazer biomass	n/a	0.097
Sessile Invertebrate biomass	**<0.001**	**0.024**
Zostera shoot biomass	**0.001**	0.684
Macroalgal biomass	0.227	**0.001**
Primary producer biomass	**0.009**	**0.005**
Epiphytic chl a	**0.005**	0.403

Macroalgal biomass was affected differently by each grazer species treatment, but was, on average, lower in the three-species grazer treatments than in grazer monocultures (Fig. 2b, grazer richness contrast, p = 0.001, Table 2). Like epiphytic chl *a*, macroalgal biomass was also generally reduced in heated treatments (p = 0.014, Table 1).

In general, grazers facilitated eelgrass biomass (Fig. 2c). Final *Zostera* shoot biomass varied significantly between grazer treatments (p < 0.001, Table 1); it was significantly reduced in the absence of grazers (p = 0.001, Table 2) and was highest in *Gammarus* treatments, but grazer richness did not affect eelgrass biomass (Table 2). Heat affected *Zostera* differently depending on grazer treatment, but the interaction effect was marginally non-significant (Species X Temp interaction, p = 0.052, Table 1).

When biomasses of *Zostera* shoots, periphyton, and macroalgae were pooled as aggregate primary producer biomass, the significant patterns in macroalgae and *Zostera* biomass generally remain visible. Primary producer biomass was lower in the absence than in the presence of grazers (p = 0.009, Table 2), and was also lower in the three-species than in single-species grazer treatments (richness contrast, p = 0.005, Table 2). As was true for macroalgal biomass, heat reduced aggregate primary producer biomass, on average (p = 0.040, Table 1, Fig. 2d). The opposite was true in the three-species grazer treatment, perhaps explaining the trend towards a Species X Temp interaction (p = 0.077, Table 1).

During the experiment, eelgrass blades became fouled by sessile invertebrates, including barnacles and tunicates (Fig. 3). On average, grazers substantially reduced sessile invertebrate biomass (grazer presence contrast, p < 0.001, Table 2), and did so most effectively in treatments with three grazer species (grazer richness contrast, p = 0.024, Table 2). The freshwater pulse tended to increase sessile invertebrate biomass in grazer monocultures, accounting for the significant main effect of freshwater pulse (p = 0.014, Table 1). Although heat had no significant impact, sessile invertebrate biomass was generally higher than the additive expectation in the multi-stressor treatments because the two stressors tended to have antagonistic effects (Fig. 3).

Effects of grazer species richness on resistance stability

Grazer species richness significantly stabilized epiphytic algal biomass (as chl *a*) in the face of multiple stressors. Chl *a* varied little among stressor treatments in the three-species grazer community, compared with single-species and no-grazer treatments (richness effect, p = 0.049, Table 3, Fig. 2a, 4). In fact, epiphytic algal biomass (as chl a) was the only response variable we measured that was significantly stabilized by increased grazer species richness. However, grazer diversity did not affect stochastic variance of epiphytic chl *a* (i.e. between replicates within treatments, Table 3).

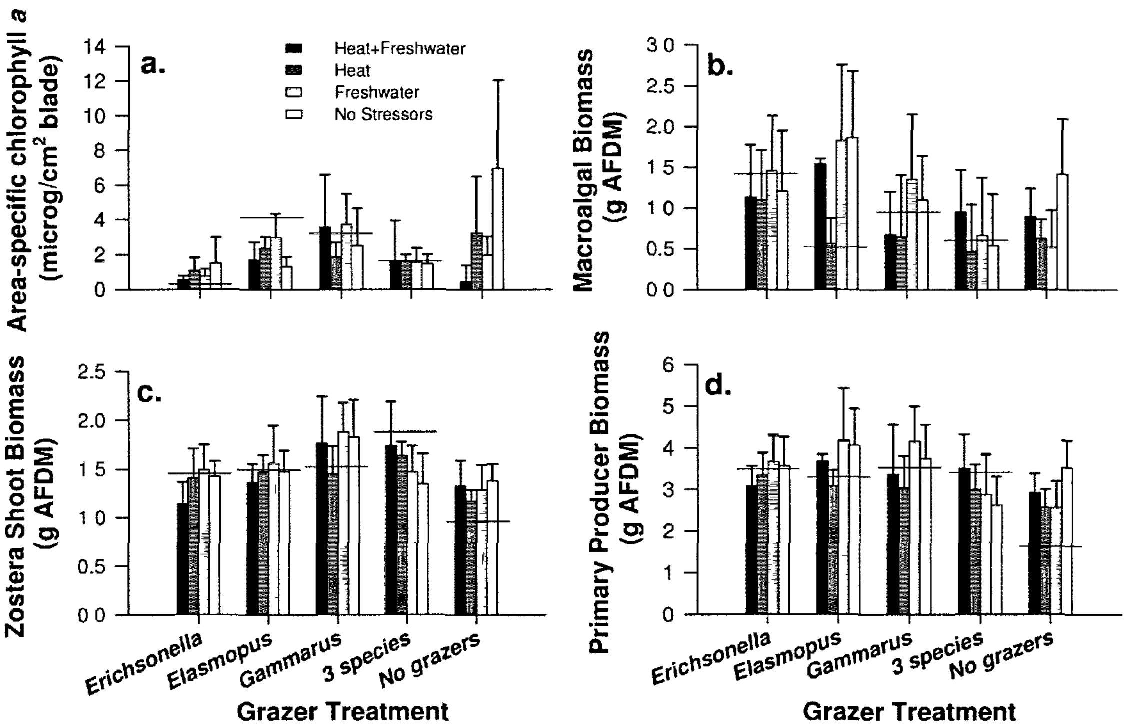

Figure 2. Final biomass of primary producers (mean + 1 SD). (a) epiphytic chlorophyll a, (b) macroalgae, (c) eelgrass shoots, (d) aggregate primary producers. The horizontal line across each group of bars indicates the expected biomass or chlorophyll if the stressor effects were additive. When the predicted additive model was less than zero, we constrained it to zero. Cases where the additive model line is greater than the no-stressor treatment bar indicate that at least one of the single-stressor treatments had more biomass than the no-stressor treatment. Thus, the sum of the individual stressor effects could be greater than the no-stressor treatment. Statistical analysis in Table 1–3.

Grazer species richness did stabilize stochastic variation (i.e. variation among replicates within treatments) for sessile invertebrate biomass, the only response variable affected. Biomass of sessile invertebrates varied less among replicates within three-grazer species treatments than among replicates of mono-specific and no-grazer treatments (grazer richness effect, p = 0.040, Table 3).

Stressor effects

The low replication of treatments after contaminated replicates were removed from analysis left little statistical power to rigorously test whether stressor impacts were significantly different from additive. However, if just the mean expected and observed stressor impacts are examined, a pattern begins to appear. Of the 34 responses examined (seven response variables, five grazer treatments for each except grazer biomass), 80% (n = 27) showed an antagonistic pattern, 18% (n = 6) indicated a synergistic pattern, and 2% (n = 1) were clearly additive.

Discussion

Grazing on epiphytic algae is a key interaction that facilitates dominance of seagrasses and the productive ecosystems they support, and is also an important process in transferring primary production up the food web to fishes. Our results show that grazer species richness can stabilize this important interaction in the presence of multiple environmental stressors. Specifically, increased grazer species richness increased the resistance stability of mean epiphytic algal biomass (chl a) in the face of heat and freshwater-pulse stressors, and reduced stochastic variation (within treatments) in sessile invertebrate biomass. We also found a pattern of mostly antagonistic multi-stressor impacts, although we did not have enough power to test this statistically.

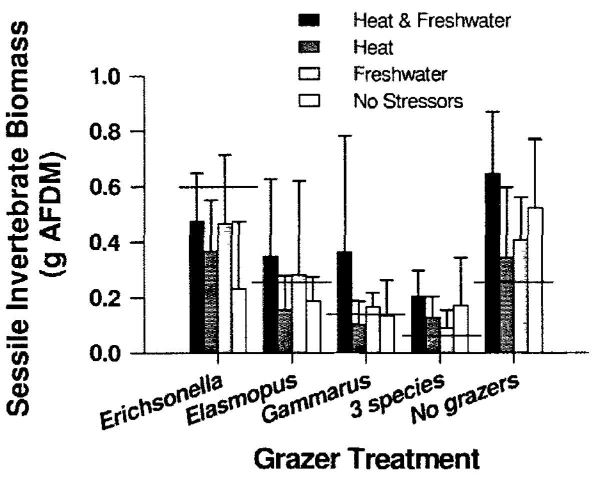

Figure 3. Final biomass (mean + 1 SD) of sessile invertebrates (*Molgula manhattensis, Botryllus schlosseri, Balanus improvisus*) that recruited into the mesocosms. The horizontal line across each group of bars indicates the expected biomass if the stressor effects were additive. Cases where the additive model line is greater than the no-stressor treatment bar indicate that at least one of the single-stressor treatments had more biomass than the no-stressor treatment. Thus, the sum of the individual stressor effects could be greater than the no-stressor treatment. Statistical analysis in Table 1–3.

The three crustacean grazer species responded differently to stressors in this experiment, confirming the functional response diversity suggested by field data. This response diversity also cascaded to affect the grazing pressure on epiphytic primary producers (Table 1). For example, biomass of the grazing isopod *Erichsonella attenuata* increased slightly in freshwater-pulse treatments, indicating that it is capable of living at lower salinities and therefore may be less impacted by the lower salinity regime predicted for Chesapeake Bay, VA as a result of climate change in the next century (Najjar et al. 2000). In contrast, biomass of the grazing amphipod *Elasmopus levis* was strongly reduced in freshwater-pulse treatments, which is consistent with field evidence of low abundance of this species in seagrass beds in the lower Chesapeake Bay, VA during periods of low salinity over the last ten years (Douglass et al. 2010). The amphipod *Gammarus mucronatus* was the least impacted by stressors of the three grazer species in this experiment and dominated the three-species assemblages. Given the wide geographic range of this species, from the Gulf of Mexico to the St. Lawrence River (Bousfield 1973), and its tolerance of many environmental conditions, it is likely to remain an abundant part of the invertebrate grazer community in spite of climate change. These results suggest that projected changes in temperature and salinity regimes may cause a shift in the composition of the grazer community in seagrass beds of lower Chesapeake Bay, with potential effects on other parts of the food web (Duffy and Harvilicz 2001, Duffy 2006).

Table 3. Results of t-tests for differences in stochastic variation (within-treatment) and resistance to stressors (among treatment variance) as a function of grazer species richness for each response variable. t-test results were calculated using both the absolute deviation (Abs. dev.) and the relative deviation (Rel. dev.). bsolute deviation is the absolute value of the difference between a response value and the group median, while relative deviation is the absolute deviation divided by the group median (see Methods for details of statistical analysis). Values significant at $p = 0.05$ are in bold.

	Grazer richness effect on stochastic variation		Grazer richness effect on resistance to stressors	
Response	Abs. Dev.	Rel. Dev.	Abs. Dev.	Rel. Dev.
Grazer biomass	0.798	0.190	0.191	0.067
Sessile Invertebrate biomass	**0.040**	0.128	0.116	0.451
Zostera shoot biomass	0.892	0.970	0.684	0.675
Macroalgal biomass	0.780	0.067	0.284	0.834
Primary producer biomass	0.991	0.462	0.482	0.668
Epiphytic chl a	0.397	0.702	**0.049**	0.107

Our experimental design allowed us to test the hypothesis that biodiversity provides resistance stability (Yachi and Loreau 1999, Pfisterer and Schmid 2002, Thebault and Loreau 2005); in other words, that species richness acts to stabilize ecosystem properties in the face of both stochastic variation and environmental stressors. We found positive but limited support for this hypothesis, in that the multi-species grazer community buffered the important ecosystem properties of epiphytic algal biomass (chl *a*) and sessile invertebrate biomass by decreasing the variance among and within stressor treatments respectively. All three grazer species used in this experiment consume micro- and macroalgae that grow on seagrass blades (Duffy and Hay 2000, Duffy and Harvilicz 2001, Douglass et al. 2010). However, previous work with these grazers (Duffy and Harvilicz 2001) has shown that some grazer species can facilitate certain algal species. Overall, more macroalgae was removed by the three-species grazer assemblage than by the average grazer species in monoculture. In the three-species treatments, epiphytic algae (chl *a*), which forms the base of the food web for many seagrass animals (Valentine and Duffy 2006), was not only reduced to lower levels as shown previously (Duffy et al. 2003, 2005, France and Duffy 2006), but was also more consistently buffered against environmental stressors. In addition to algae, sessile fouling invertebrates (*Molgula manhattensis, Botryllus schlosseri, Balanus improvisus*) grow on eelgrass blades and other mesocosm surfaces. Sessile invertebrates that grow on eelgrass blades can be removed by grazing along with algae when they are small, which may explain the buffering of sessile invertebrate variance by grazer richness (Duffy and Harvilicz 2001). However, we can't rule out the potential role of a mean-variance correlation due to low sessile invertebrate biomass. Our results suggest that, as climate change causes more variability in environmental conditions, maintaining grazer diversity may facilitate a more stable supply of ecosystem services in this eelgrass system (Naeem and Li 1997, Hooper et al. 2005).

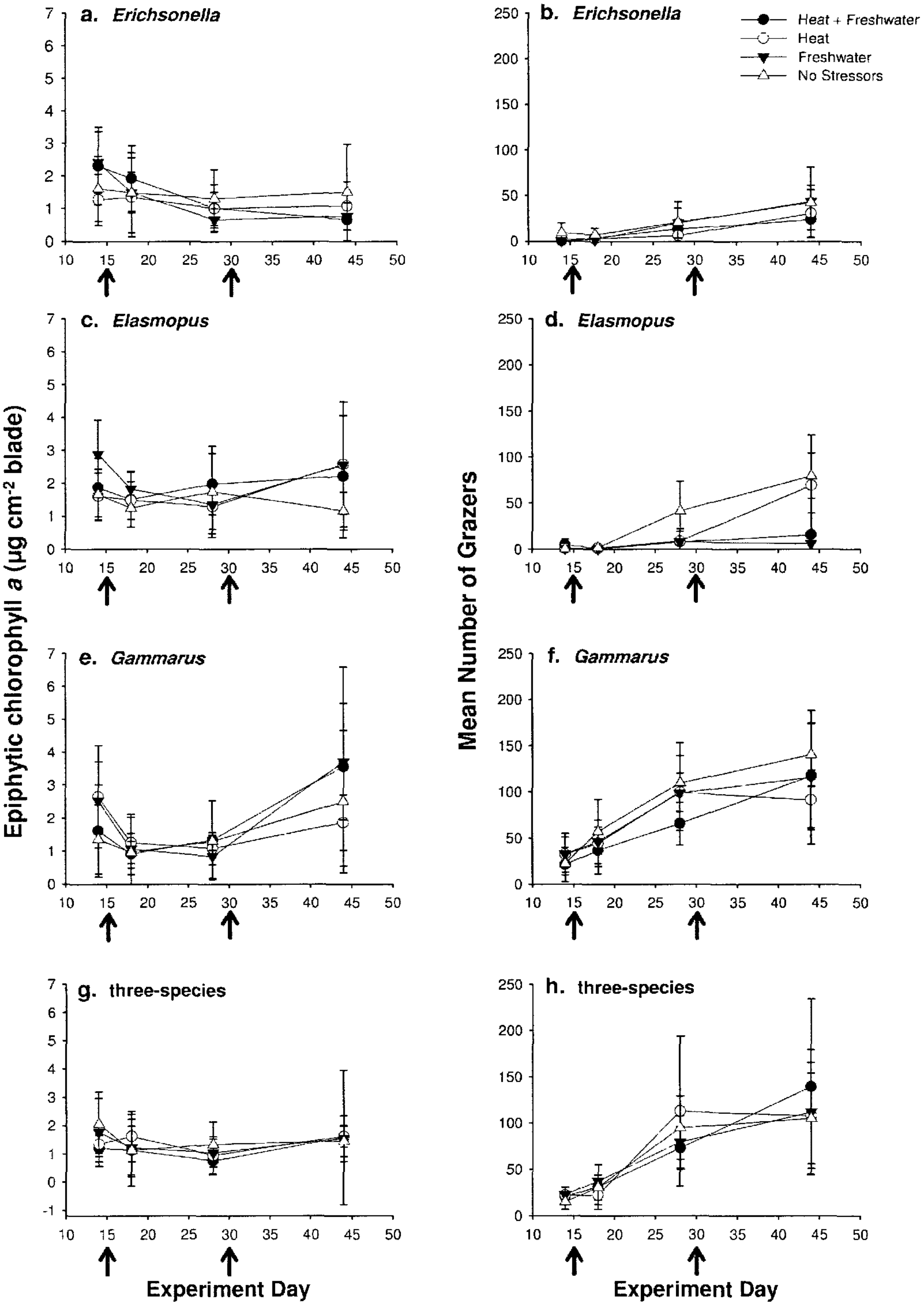

Figure 4. Time course of epiphytic chlorophyll *a* concentration and estimated population during the experiment using dipnet census technique. Arrows at experiment day 15 and 30 indicate when freshwater pulse treatments were delivered. Error bars are one standard deviation.

While the evidence for response diversity among grazer species seems clear, the mechanisms behind the stabilizing effect of grazer species richness on biomass of epiphytic algae and sessile invertebrates are less so. The stabilization of algal biomass by grazer species richness may be due in part to more stable numbers of grazers in three-species assemblages (Fig. 1), although the variance in grazer biomass among stressor treatments in three-species assemblages and monocultures did not differ significantly (p 5 0.097, Table 2). Previous work with these grazers (Duffy and Harvilicz 2001) has shown that *Gammarus* facilitates the growth of the red alga *Polysiphonia harveyi* by grazing other fouling algae and releasing *P. harveyi* from competition. This is likely why grazer presence had no significant effect on macroalgal biomass, but grazer richness did (Table 2, Fig. 4e). Despite the dominance of *Gammarus* in the three-species assemblages (Fig. 1b), the overall reduction in algal biomass in these treatments indicates that in addition to dominance via the selection effect (Huston 1997, Steiner et al. 2006), the portfolio effect (Doak et al. 1998, Tilman et al. 1998) may also be an important mechanism. In other words, it is possible that diverse grazers were better able than single grazer species to crop different types of algae that flourished under different conditions, but our data are not of sufficient resolution to test this hypothesis. The reduced stochastic (among-replicate) variation in sessile invertebrate biomass in the presence of diverse grazer assemblages is also curious. We suspect the most likely explanation is that higher overall grazing pressure on recruiting invertebrates in this treatment (Fig. 3) also reduced the total variance among replicates, compared with single grazer species treatments.

We expected eelgrass biomass to decline in heated treatments, as has been shown in the field (Moore and Jarvis 2008); however, the eelgrass in the mesocosms did not show any clear stressor impacts. This may be explained by the intensity of light reaching the eelgrass blades, which was likely enough to allow the eelgrass to maintain strong growth despite elevated temperatures (Moore and Wetzel 2000). Light levels at mid-day in the mesocosms averaged 883.6 $\mu E\ m^{-2}\ s^{-1}$. Although we shaded the mesocosm tanks to achieve approximately a 40% reduction in light, the light levels in the mesocosms were likely still higher than in the field due to a shallower water column, less turbidity, and a grazer community at or near carrying capacity due to lack of predation (not typical at most field sites, Fig. 4).

Our qualitative result indicating mostly antagonistic stressor interactions generally fits the mean pattern shown in recent multi-stressor meta-analyses (Crain et al. 2008, Darling and Cote 2008). These reviews indicate that non-additive effects of stressors are common, but that synergies are rarer than previously predicted (Sala et al. 2000, Sala and Knowlton 2006). In our experimental system, multi-stressor impacts may be less severe than would be expected with synergistic or additive stressor effects. Predicting multi-stressor effects remains difficult, but may adhere to the following patterns: 1) additive effects may occur when stressors act through similar pathways, 2) synergistic effects may occur when stressors act through alternative but dependent pathways, and 3) antagonistic effects may occur when stressors mitigate each other (Crain et al. 2008). It is possible that the stressors temperature and freshwater were able to mitigate each other in our experimental system, but we could not rigorously test this. Multi-stressor effects may vary with the identity of the stressor pairs (Crain et al. 2008), but the mechanisms behind the expected multi-stressor effects remain equivocal. Thus, more careful examination of the interactions of multi-stressors is needed in order to better predict how stressors may interact in this and other estuarine systems.

Our results corroborate previous findings that multi-species grazer assemblages more effectively reduce epiphytic algae in this system (Duffy et al. 2003, France and Duffy 2006), but more importantly, they show that invertebrate grazer species exhibit response diversity in the face of environmental stressors. Most notably, *Elasmopus* was negatively affected by the freshwater pulse, while *Erichsonella* increased in the multi-stressor treatment, and *Gammarus* was hardly affected. This suggests that the total response of the invertebrate grazer community to stressors is an aggregate of individual responses, rather than being driven by the response of any particular species.

In summary, this study shows that multi-species invertebrate grazer assemblages in eelgrass systems have the potential to buffer some important ecosystem properties against increasingly common stressor impacts because they affect ecosystem properties in a similar manner, yet display differential responses to stressors. That is, the grazers are largely functionally redundant in their grazing effects but functionally diverse in their response to stressors. Such response diversity may become increasingly important as managers seek to maintain ecosystem properties in the face of climate and other environmental change impacts. Our experiment also corroborates the pattern from previous studies that the effects of multiple stressors likely do not follow a simple additive model, making it more difficult to predict how multiple stressors will impact these systems, and underscoring the importance of maintaining the insurance provided by biodiversity. There remains a key need to better understand how multiple stressors interact in a variety of systems and how they are influenced by trophic interactions, especially as stressors are predicted to increase as climate change progresses.

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CHAPTER 2

Changes in biodiversity and environmental stressors influence community structure of an experimental eelgrass (*Zostera marina*) system.

Abstract

Changes in biodiversity are of concern because they can result in decreased ecosystem functioning and loss of ecological interactions important for ecosystem services. Ecological systems with high biodiversity are frequently very productive, which stabilizes ecosystem processes and provides resources to higher trophic levels. However, changes in biodiversity are only one stressor impacting ecosystems. In estuaries such as Chesapeake Bay, USA, climate warming and nutrient inputs are increasingly important stressors. Increased nutrients and temperatures negatively impact eelgrass (*Zostera marina*) in Chesapeake Bay, an important habitat-forming species, but the interaction of these stressors with changes in biodiversity is poorly understood. Therefore, we used experimental eelgrass communities in a fully-factorial manipulation to test how changes in biodiversity, warming, and nutrient enrichment stressors interact to impact ecosystem properties. We found that grazer presence and species richness had a larger effect on grazer, algal, and sessile invertebrate biomass than warming or nutrient enrichment. This demonstrates the importance of grazers, and emphasizes the role of biodiversity in maintaining ecosystem properties by increasing biomass and stability. Grazing in species-rich treatments stabilized epiphytic algal biomass in the face of stressors, despite stressors favoring increased epiphytic algal biomass. Conversely, stressors negatively impacted macroalgal and eelgrass biomass. Although we did not find interactions among stressor and diversity treatments, grazer presence and diversity were comparable to, or more important than, nutrient enrichment or warming treatments, which favored small-celled algae over larger macrophytes. These results, on relative impacts and interactions of stressors and changes in biodiversity, could inform restoration and management priorities for estuaries such as Chesapeake Bay.

Introduction

Biodiversity supports ecosystem processes, properties, and functions that are ecologically and economically important, such as water and nutrient cycling, and fisheries production. Diversity among and within trophic levels has benefits, including increased stability (Tilman 1996; Tilman et al. 1998; Griffiths et al. 2000; Caldeira et al. 2005; Blake and Duffy 2010) and biomass (Duffy et al. 2003; Allison 2004; Balvanera et al. 2006), that lead to overall higher ecosystem performance. For example, increased biomass within a trophic level as a result of diversity has the potential to maximize important ecosystem processes, such as grazing, that play important roles in marine systems (Worm et al. 1999; Nystrom et al. 2000; Duffy et al. 2003; Valentine and Duffy 2006; Stachowicz et al. 2007). However, biodiversity on both global and local scales is threatened, largely by human activities that cause rapid changes in ecological systems. These changes in biodiversity are of concern because they can result in losses of important ecosystem functions (Tilman 1999). Change in biodiversity also acts as a stressor in ecological systems, and may be as important as or more important than other stressors in marine systems (Stachowicz et al. 2007). The impacts of biodiversity change and other stressors in marine systems are an increasing concern because we know little about their interactions, especially in the context of global climate change.

One important stressor associated with climate change is climate warming. Climate warming is predicted to increase temperature extremes in both directions, but the Chesapeake Bay region is predicted to warm by 2 - 6°C by the end of the 21st century (Najjar et al. 2010). Because most of Chesapeake Bay is shallow, water temperatures will continue to follow changes in air temperatures very closely (Najjar et al. 2000). This warming of bay waters may have impacts on nutrient and energy cycling, lifecycles and ranges of resident and transient organisms, fisheries production, and important habitats such as salt marshes, oyster reefs, and seagrass beds (Short and Neckles 1999, Doney et al. 2012).

Increasing pressure is also being put on coastal habitats as human populations in the coastal zone continue to rise (McGranahan et al. 2007). Anthropogenic impacts along coasts include shoreline hardening and development in the coastal zone for residential, commercial, and recreational use. This development leads to decreased filtration of runoff through loss of vegetation buffers, and increased direct inputs of sewage, fertilizer, and pollutant runoff to coastal waters (Vitousek et al. 1997; Kemp et al. 2005). These impacts have led to the eutrophication of estuaries, such as Chesapeake Bay, which are now characterized by increased occurrence of bloom forming micro- and macroalgae (Hauxwell et al. 2001; Zimmerman and Canuel 2002; Burkholder et al. 2007; Najjar et al. 2010). The conditions that favor blooms of ephemeral algae are, at the same time, detrimental to important habitats such as seagrass meadows.

Seagrass systems are important habitats in Chesapeake Bay that provide habitat for juvenile fish and crabs, stabilize sediments and coastlines, and sequester carbon (Duarte 2000; Heck et al. 2003; Duarte et al. 2005; Polte et al. 2005; Canuel et al. 2007). Historically the dominant seagrass in Chesapeake Bay, eelgrass (*Zostera marina*) has been declining at an accelerated pace in recent years due largely to continued eutrophication and record summer temperatures (Orth and Moore 1983; Orth and Moore 1988; Orth et al. 2002; Orth et al. 2006; Moore and Jarvis 2008; J.E. Duffy unpublished data). Elevated temperatures, especially in the summer months, can lead to eelgrass die-offs (Bintz et al. 2003; Oviatt 2004). These declines are attributed to higher respiration than production, internal carbon stress, and reduced growth rates in eelgrass (hereafter referred to as *Zostera*) under higher temperature conditions (Short and Neckles 1999; Touchette and Burkholder 2000; Bintz et al. 2003; Touchette et al. 2003). Eutrophication has negative consequences for *Zostera*, both direct and indirect. Nutrient enrichment impacts eelgrass physiology similarly through carbon limitation, but also through ammonium toxicity, both of which lead to reduced shoot production and overall growth (Touchette and Burkholder 2000; Touchette et al. 2003). Indirect impacts of nutrients on *Zostera* include overgrowth by micro and macroalgae, and associated light limitation (Hauxwell et al. 2001; Burkholder et al. 2007). However, previous work has shown that grazing by amphipods and isopods in temperate seagrass beds plays an important role in structuring this community (Duffy et al. 2003; Duffy et al. 2005; Valentine and Duffy 2006), and has effects of similar or greater magnitude to water column nutrient enrichment (Neckles et al. 1993; Hughes et al. 2004). While grazing has the potential to counter the effects of water column nutrient enrichment in some circumstances (Neckles et al. 1993; Hays 2005), *Zostera* habitats in Chesapeake Bay are increasingly faced with multiple simultaneous stressors whose interactions are poorly understood and difficult to predict (Orth et al. 2006).

In this study, we examined the effects of crustacean grazer diversity, warming, and nutrient enrichment on the ecosystem properties of experimental seagrass communities. We planted outdoor mesocosms with *Zostera marina*, stocked crustacean grazers (*Elasmpous levis, Erichsonella attenuata, Gammarus mucronatus*) alone and in mixture, and assessed the final abundance and biomass of the communities. Specifically, we wanted to test whether 1) grazing effects were of similar magnitude as stressor effects, 2) stressors affected primary producer community composition, 3) change in biodiversity interacted with stressors to exacerbate or buffer against stressor impacts. We show that grazing effects are large, and stressors facilitate microalgae, but that there were no interactions between loss of species richness and stressor impacts.

Methods

Experimental Design

We manipulated crustacean grazer species richness, water temperature, and water column nutrients in a factorial mesocosm experiment during the summer of 2007.

Located outdoors at the Virginia Institute of Marine Science, Gloucester Point, VA, USA (37°24’7653”N, 76°50’0809”W) in a flow-through tank system, 100 19-liter experimental mesocosms were supplied with filtered estuarine water from the adjacent York River. Estuarine water first passed through a coarse sand filter and then through 150-micron mesh, in order to exclude crustacean grazer recruits but allow algal and sessile invertebrate propagules to recruit to the mesocosms. Each mesocosm sat in a water bath to buffer temperature changes, contained a mixture of approximately 2.5 liters of sand and 0.5 liters of mud as substrate, and was planted with 30 shoots of defaunated *Zostera marina*. Mesocosms were covered with shade screens to approximate light levels in local seagrass beds.

We used two amphipod species (*Gammarus mucronatus and Elasmopus levis*) and one isopod species (*Erichsonella attenuata*) to establish five grazer treatments: each species as a monoculture, a multi-species assemblage, and a grazer-free control (grazer species hereafter referenced by genus). These grazer species are among the five most abundant crustacean grazers present in local eelgrass beds in the summer season (Douglass et al. 2010). Grazers were added to mesocosms in a replacement design, with monocultures receiving 30 individuals of one species, and multi-species assemblages receiving 10 individuals of each species. Mesocosms were either left at ambient water temperature (un-warmed treatments), or were heated approximately 3°C above ambient water temperature using a 200-watt aquarium heater (warmed treatments). This warming treatment simulated a realistic climate warming scenario for Chesapeake Bay (Najjar et al. 2010). Slow-release pelletized fertilizer (Osmocote®, N:P:K = 3:1:2) was used to enrich nutrient levels approximately four times ambient levels in the nutrient-addition treatments; the fertilizer (15 - 20 grams) was contained in fine mesh inside a perforated PVC tube and suspended in the nutrient-addition mesocosms. Fertilizer was replaced every four days to maintain elevated nutrient levels (Spivak et al. 2009). Water column nutrients were sampled once during the experiment to evaluate the nutrient treatment. Both the temperature and nutrient treatments were applied continuously, and grazers had sufficient time to reach carrying capacity (having 1 - 2 generations) during the six-week duration of the experiment.

During the experiment, epiphytic chlorophyll *a* was sampled from standardized artificial seagrass placed in each mesocosm at the start of the experiment (four units of two polypropylene curling ribbons each), as a proxy for epiphytic algal biomass (Parsons et al. 1984). One artificial seagrass unit was removed at weeks two, four, and six, and processed using standard procedures (Parsons et al. 1984). Grazer density was also estimated non-destructively three times during the experiment (at weeks two, four, six) by catching grazers in three standardized dip-net sweeps, counting, identifying, and returning them to their original mesocosm.

At the conclusion of the experiment, all biomass was harvested from each mesocosm and frozen until samples could be processed. Thawed biomass retained on a

500-micron sieve was sorted to the lowest taxonomic level possible, dried in a 60°C oven until the mass was stable, and then combusted at 450°C to obtain ash-free dry mass (AFDM). Grazers were sorted by size (using sieves with mesh sizes of 8.0, 5.6, 4, 2.8, 2.0, 1.4, 1.0, 0.71, 0.05 mm), identified to species, and enumerated. Biomass of grazers was calculated using the empirical equation, log B = -1.01 + 2.64 x log S, where B is AFDM (mg), and S is the sieve size (mm) on which the animal was retained (Edgar 1990).

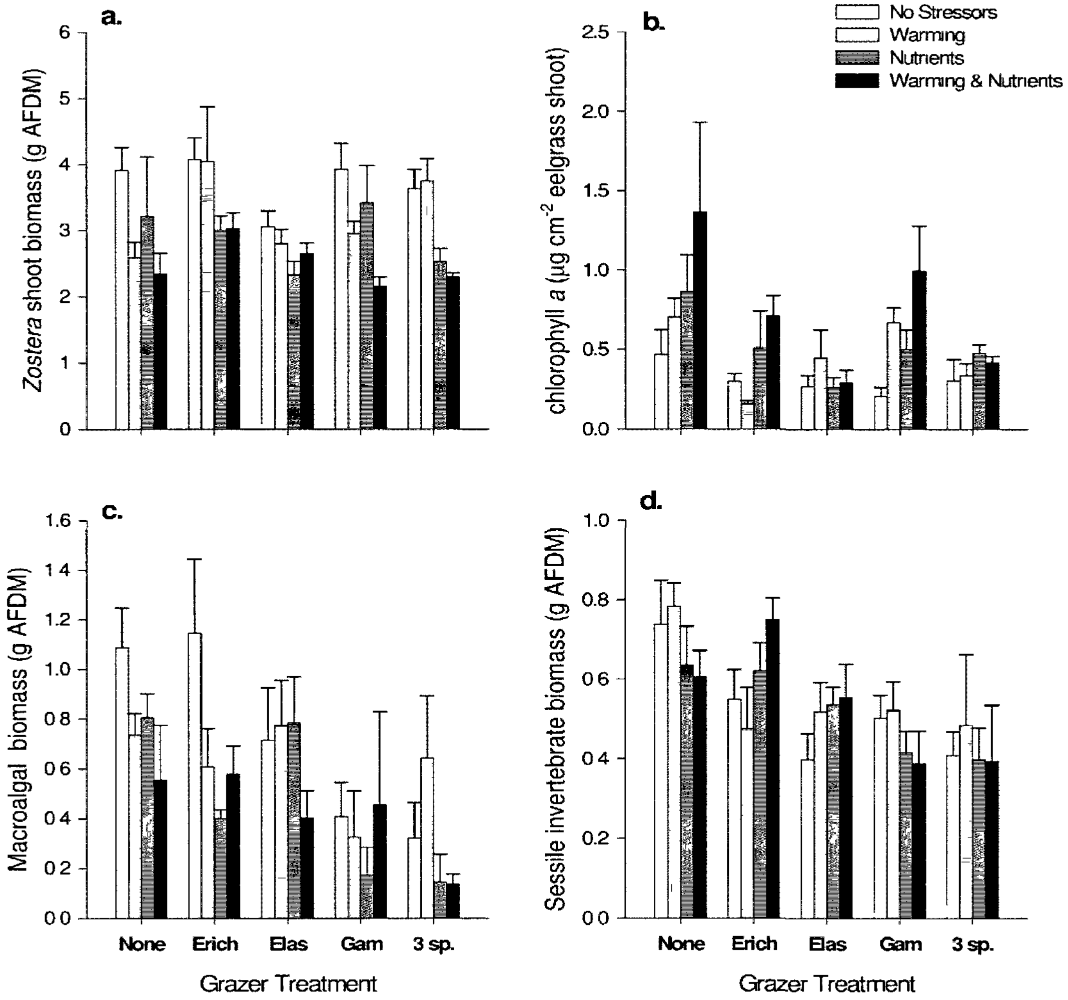

Figure 1. Final biomass (+ 1 SE) of a.) *Zostera* shoots (aboveground biomass), b.) Epiphytic algae (as chlorophyll *a*), c.) Macroalgae (primarily *Cladophora sp., Ulva sp., Ceramium sp., Polysiphonia sp.*), d.) Sessile invertebrates (*Botryllus schlosseri, Molgula manhattensis, Balanus improvisus*). Grazer treatment abbreviations: None = no grazers, Erich = *Erichsonella attenuata* alone, Elas = *Elasmopus levis* alone, Gam = *Gammarus mucronatus* alone, 3 sp. = three species assemblage. Statistical analysis located in Table 1.

Statistical Analysis

Contamination of crustacean grazer treatments occurred in 4 of the 100 mesocosms; thus, data from these mesocosms were removed from all statistical analysis. All treatments retained a minimum of four, and a maximum of five replicates.

We analyzed this factorial mesocosm experiment using three-way ANOVAs to test for main and interactive effects of warming, nutrients, and crustacean grazer species richness on *Zostera* shoot biomass, epiphytic algal biomass, macroalgal biomass, and sessile invertebrate biomass (SAS Enterprise Guide 4.3). All data were untransformed with the exception of the epiphytic chlorophyll a data which were log transformed to improve normality. We also used a-priori orthogonal contrasts to test for the effects of grazer presence (all treatments with grazers vs. no-grazer controls) and grazer species richness (grazer monocultures vs. three-species assemblage treatments). To estimate the size of effects in the ANOVA analyses, we calculated the ω2 values for each main effect and interaction using the generalized equation from Olejnik and Algina (2003):

$$\omega^2 = \left[SS_{effect} - DF_{effect} \bullet MS_{error}\right] / \left[SS_{effect} + \left(N - DF_{effect}\right) \bullet MS_{error}\right]$$

We also wanted to examine whether crustacean grazer species richness had a stabilizing effect on ecosystem properties in the face of environmental stressors, using the Brown-Forsythe test as used previously by Blake & Duffy (2010).

We also used multivariate non-metric multi-dimensional scaling (NMDS) to examine which factors most influenced the similarity and composition of communities among all mesocosms in the experiment. We conducted the analysis in R 2.13.0 (R Development Core Team 2011), using the metaMDS function in the vegan package (Oksanen et al. 2011) and a Bray-Curtis dissimilarity matrix. Instead of using untransformed data as suggested by the default settings in metaMDS, we square-root transformed the data to reduce the influence of very abundant species. We plotted the results in 2-dimensions, and used the envfit procedure, also in vegan (Oksanen et al. 2011), to overlay species vectors (determined by Principal Components Analysis) on the final NMDS plot. To statistically test the results of the NMDS ordination, we conducted PERMANOVA using the adonis function, again in the vegan package in R. This routine carries out an analysis of variances test using a dissimilarity matrix, and permutation test to determine significance. We also examined the multivariate homogeneity of the treatments using the function betadisper in the vegan package, which is analogous to Levene's test (Levene 1960) for equality of variances.

Structural equation modeling (SEM) is a multivariate framework for graphical modeling and analysis that draws from both path and factor analysis techniques (Grace 2006). SEM allows for the exploration of direct and indirect causal relationships between observed and hypothesized (latent and composite) variables through paths relations, and are well suited to studying systems with multiple simultaneous processes (Grace et al. 2010). We used maximum-likelihood estimated structural equation models created with Amos 18.0.0 (Arbuckle 2011) to examine the direct and indirect effects of warming, nutrients, and crustacean grazer species richness on primary producer biomass and sessile invertebrate biomass using the same experimental data as above (2007 experiment data). Models were evaluated based on criteria described in Grace (2006). Finally, it is important to note that we used SEM in the "model generating" mode. We first specified an initial model based on theory and previous knowledge of our experimental system, after which we modified and re-tested this model until the model fit the data well from both a statistical and ecological point of view.

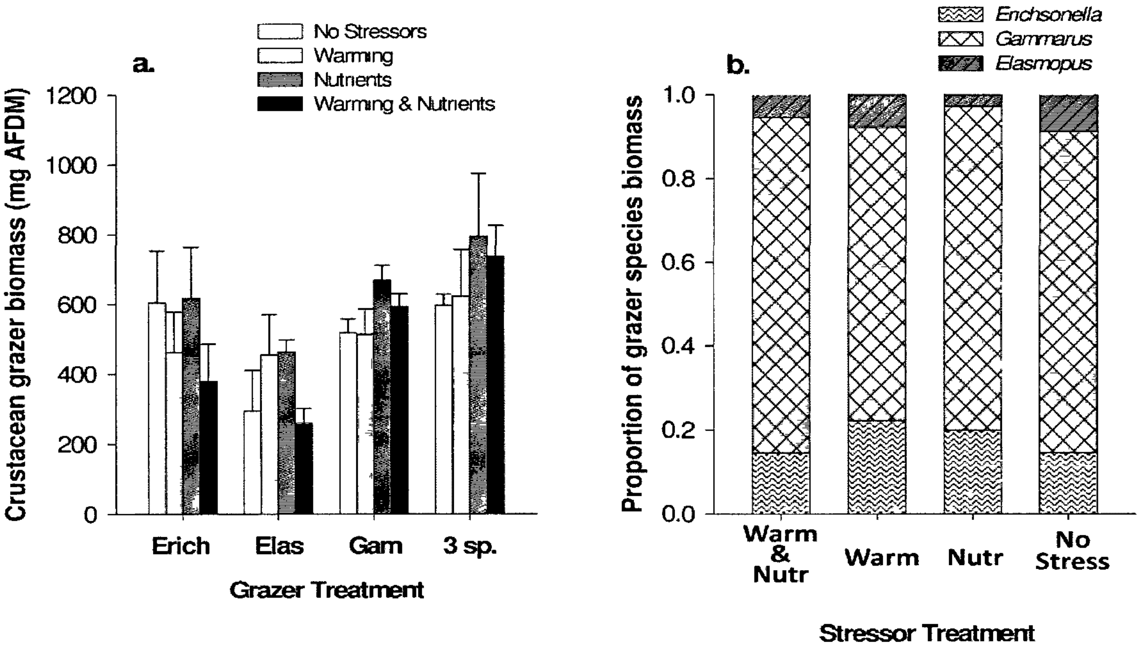

Figure 2. a) Final crustacean grazer biomass (± 1 SE) for all experimental treatments. Grazer treatment abbreviations: None = no grazers, Erich = *Erichsonella attenuata* alone, Elas = *Elasmopus levis* alone, Gam = *Gammarus mucronatus* alone, 3 sp. = three species assemblage. Statistical analysis is in Table 1; b) Final proportion of crustacean grazer species biomass (g AFDM) in the three-species assemblage treatments. Stressor treatment abbreviations: Warm & Nutr = warming and nutrient enrichment, Warm = warming, Nutr = nutrient enrichment, No Stress = no stressors.

Results

Species richness and multiple stressors

Crustacean grazer treatments had the largest effect of any experimental treatment in this study (Table 1). Crustacean grazer presence significantly reduced the biomass of epiphytic algae, macroalgae (*Ceramium spp., Polysiphonia spp., Enteromorpha spp., Cladophora spp.*), and sessile invertebrates (*Molgula manhattensis, Botryllus schlosseri,* Barnacles) in experimental communities ($p < 0.0001$, $p = 0.0052$, $p = 0.0002$ respectively, Figure 1, Table 1). Grazer species richness likewise caused significant reductions in macroalgal biomass ($p = 0.0255$, Figure 1c., Table 1). Conversely, biomass of crustacean grazers themselves was significantly greater in species-rich grazer treatments ($p = 0.0014$, Figure 2a., Table 1) compared to single-species grazer treatments, and the composition of the grazer community was dominated by *Gammarus* (Figure 2b.). Overall, grazer presence and species richness had strong significant effects in this experiment.

Stressors also significantly influenced all primary producers. Elevated temperatures significantly decreased *Zostera* biomass ($p = 0.013$, Figure 1a., Table 1), but significantly increased epiphytic algal biomass ($p = 0.0116$, Figure 1b., Table 1). There was no effect of warming on grazers, macroalgae, or sessile invertebrates. Nutrient enrichment had the strongest effect on *Zostera*, causing a significant biomass reduction in all enriched treatments ($p < 0.0001$, Figure 1a., Table 1). Ammonium in enriched treatments averaged 11 μmol, and 2 μmol in unenriched treatments. Macroalgal biomass was also lower in the nutrient enriched treatments ($p = 0.0087$, Figure 1c., Table 1). On the other hand, epiphytic algal biomass was greater in the presence of nutrient enrichment ($p = 0.0011$, Figure 1b., Table 1). Despite the strong effects of stressors on epiphytic algae, epiphytic algal biomass remained more stable in the face of stressors in species-rich treatments relative to single-species treatments ($p = 0.045$, Figure 1b., Table2). In this experiment, warming and nutrient enrichment had similarly strong effects on primary producers, but the direction of the effect differed among producers.

Community Structure

To better examine changes in community structure, we used non-metric multi-dimensional scaling (NMDS). Experimental communities (mesocosms) were significantly structured by all three treatments (grazer species, warming, nutrient enrichment). Crustacean grazer species treatments structured the experimental communities, not only by their presence, but also by their identity ($p = 0.0009$, see Figure 3c., Table 3). Species-rich and *Gammarus*-only treatments were similar (Figure 3c.), reflecting the dominance of multi-species grazer assemblages by this species, and contained more red algae (*Ceramium spp. and Polysiphonia spp.*) (Figure 3a.) but less green algae (*Enteromorpha spp. and Cladophora spp.*) than other treatments. Alternatively, no-grazer and *Elasmopus*-only treatments were similar (Figure 3c.), and contained more green algae and sessile invertebrates (Figure 3a.) but less red algae than other treatments. *Erichsonella*-only treatments were intermediate, containing approximately equal amounts of red and green algae, and average numbers of sessile invertebrates.

Communities were also structured by nutrient enrichment (p = 0.0009, Figure 3b., Table 3), which resulted in lower biomass of *Zostera* (see Figure 3a.). But community variation was reduced in nutrient-enriched treatments, as compared to non-enriched treatments (p = 0.029, betadisper test). Warming also significantly influenced experimental community structure (p = 0.004, Figure "3c., Table 3), but to a slightly lesser degree than nutrients or grazers. Warmed treatments generally had lower *Zostera* biomass, a pattern similar to nutrient-enriched communities.

Stressors and species identity

To explore complex interactions at the species level not captured by univariate analysis, we employed structural equation modeling (SEM). The SEM model (Figure 4) showed that final grazer species abundances were influenced by initial grazer presence and by stressors. Presence of both *Elasmopus* and *Gammarus* reduced *Erichsonella* abundance in three-species treatments, and together with the negative impacts of warming, explained 70% of the variation in *Erichsonella* abundance (Figure 4b., and Table 4). Our model also indicates that *Gammarus* and *Elasmopus* had direct positive effects on each other and explained about 70% of the variation in abundance of each species (Figure 4b., and Table 4), but this is likely an artifact of the experimental design (see Discussion). *Gammarus* was the only grazer species whose final abundance had a direct, negative impact on primary producers, specifically macroalgal biomass. As shown in the analysis of community structure, warming and nutrient enrichment positively affected epiphytic algal biomass (R2 = 0.21, Figure 4b.), while nutrients negatively affected *Zostera* and macroalgal biomass (R2 = 0.29 and R2 = 0.21 respectively, Figure 4b.).

Discussion

General ecological theory predicts that changes in biodiversity may alter the effects of anthropogenic stressors, because diversity is important in providing temporal, resistance, and resilience stability in ecosystems (Tilman 1996; Tilman 1999; Crain et al. 2008). We did not observe any interactions in this mesocosm experiment, meaning the effects of changes in diversity, nutrient enrichment, and warming may be understood independently in this experimental system. However, we observed increased stability of epiphytic algae in the face of stressors in higher diversity communities, demonstrating that diversity can buffer certain ecosystem processes against stressor impacts. If these results are general, ecological consequences of these stressors will still need to be evaluated for each ecosystem process of interest, but interpretation and prediction of ecosystem changes may be more straight-forward for conservation planners and managers.

Table 1. Results from three-way factorial ANOVAs, and estimates of effect size (ω^2) for each response variable. Any negative ω^2 values are reported as zero. Calculation of the MS used the type-III SS. A-priori contrasts for the effects of grazer presence (no-grazer treatments vs. pooled grazer treatments) and grazer richness (three-species treatments vs. pooled one-species treatments) are included. NA means the contrast was not applicable. Values significant at p = 0.05 are in bold.

	Response														
	Grazer biomass			***Zostera* shoot biomass**			**Epiphytic algal biomass (log chl *a*)**			**Macroalgal biomass**			**Sessile Invertebrate biomass**		
Factor	**MS**	**P**	**ω^2**	**MS**	**P**	**ω^2**	**MS**	**P**	**ω^2**	**MS**	**P**	**ω^2**	**MS**	**P**	**ω^2**
Grazer species (Sp.)	333741.72	**0.0007**	0.166	1.663	0.0718	0.049	0.0490	**0.0002**	0.177	0.966	**0.0004**	0.168	0.229	**0.0003**	0.172
Sp. presence contrast	NA	NA		0.107	0.7045		0.1400	**<0.0001**		1.365	**0.0052**		0.579	**0.0002**	
Sp. richness contrast	580662.52	**0.0014**		0.064	0.7691		0.0020	0.6559		0.855	**0.0255**		0.135	0.0642	
Warming (Warm.)	84917.46	0.2050	0.008	4.785	**0.0130**	0.054	0.0530	**0.0116**	0.056	0.113	0.4094	0.000	0.018	0.4988	0.000
Nutrients (Nut.)	60068.63	0.2855	0.000	14.357	**<0.0001**	0.161	0.0900	**0.0011**	0.098	1.196	**0.0087**	0.061	0.001	0.8484	0.000
Sp.*Warm.	32343.70	0.6015	0.000	1.747	0.0608	0.054	0.0130	0.1564	0.029	0.202	0.3061	0.009	0.004	0.9821	0.000
Sp.*Nut.	42856.36	0.4834	0.000	0.636	0.4925	0.000	0.0130	0.1699	0.026	0.110	0.6149	0.000	0.085	0.0747	0.048
Warm.*Nut.	112549.66	0.1454	0.015	0.046	0.8036	0.000	<0.0001	0.9367	0.000	0.027	0.6891	0.000	0.003	0.7936	0.000
Sp.*Warm.*Nut.	23518.48	0.7150	0.000	0.214	0.8842	0.000	0.0050	0.6767	0.000	0.261	0.1871	0.024	0.019	0.7445	0.000
Error	51737.82			0.740			0.0080			0.165			0.038		

Table 2. Results of two-sample Welch t-tests examining the effects of species richness on stochastic (with-in treatment) and resistance (among treatment) stability. Tests were performed on deviations from the median, and were conducted using R 2.13.0. P-values significant at 0.05 are in bold.

	Stochastic stability		**Resistance stability**	
Response	**Absolute Deviation**	**Relative Deviation**	**Absolute Deviation**	**Relative Deviation**
Grazer biomass	0.3432	0.2964	0.9528	0.4565
Zostera shoot biomass	0.1394	0.7743	0.1062	0.0736
Epiphytic algal biomass (log chl *a*)	0.1862	0.4333	0.06579	**0.04519**
Macroalgal biomass	0.9529	0.6675	0.8005	0.5598
Sessile Invertebrate biomass	0.0854	0.6297	0.4483	0.9227

Grazer presence and species richness had the largest overall effect on these experimental mesocosm communities, larger than effects of warming or nutrient enrichment on all response variables except *Zostera* biomass (Table 1). As found previously (Bell 1991; Duffy and Hay 2000; Duffy and Harvilicz 2001; Moksnes et al. 2008), grazers reduced biomass of epiphytes, macroalgae, and sessile invertebrates (Duffy and Harvilicz 2001) (Figure 1, Table 1). This reduction in sessile invertebrate biomass with grazing is likely due to indirect removal (non-consumptive) of newly-settled barnacles and tunicates from the *Zostera* blades and tank surfaces by grazing amphipods and isopods (Osman and Whitlatch 1995; Osman and Whitlatch 2004). We also found that grazer assemblages with higher species richness had higher grazer biomass, as suggested by ecological theory (Carlander 1955; Mulder et al. 2001; Gamfeldt and Hillebrand 2008) and demonstrated by prior research in this system (Duffy et al. 2003). This may be because the experimental stressors did not impact grazer biomass directly, or interact with species richness mechanisms (facilitation, niche complementarity, sampling effect) (Mulder et al. 2001). Grazer species richness also increased the resistance stability (Griffin et al. 2009) of epiphytic algal biomass (p = 0.04519, Table 2, Figure 1), buffering against stressor impacts, as shown previously in this system (Blake and Duffy 2010). We saw this stabilizing effect of grazer species richness despite strong stressor effects on primary producers. This may be because

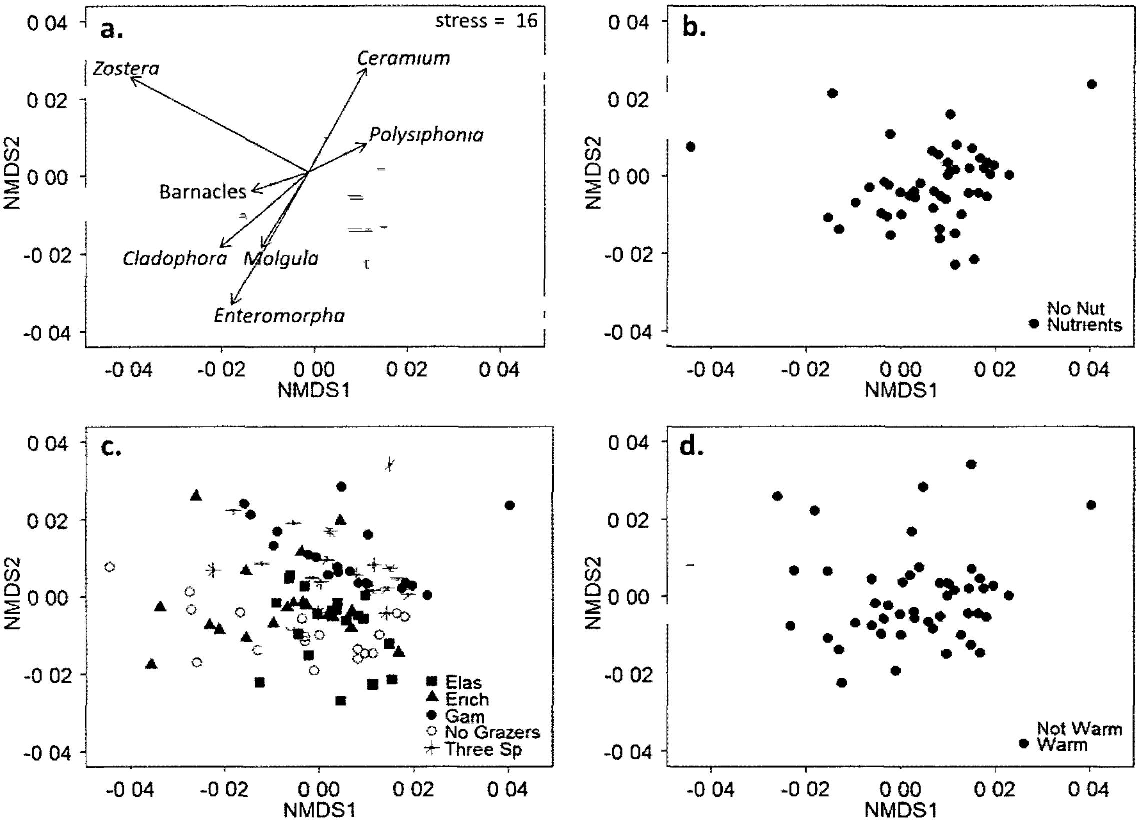

Figure 3. Plots from non-metric multi-dimensional scaling (NMDS) ordination of all invertebrate and algal species; each point represents the community in a single mesocosm. a) plot of all mesocosm communities (as points), and species with the most influence (as vectors). b) same plot as a.) color coded by nutrient addition treatment (nutrients(●) and no nutrients(◌)). c) same plot as a.) with shapes representing the crustacean grazer treatments (*Elasmopus levis*(■), *Erichsonella attenuata*(▲), *Gammarus mucronatus*(●), No Grazers(○), Three species assemblage(✱)). d) same plot as a.) color coded by warming treatment (warmed(●) and not warmed(◌)).

interaction strengths between grazers and epiphytic algae remain high in the 3-grazer-species communities (Thebault and Loreau 2005) due to the dominance of the strong consumer *Gammarus* (Zimmerman et al. 1979; Duffy and Harvilicz 2001; Douglass et al. 2010). Finally, grazer identity and composition structured the mesocosm communities through differing preferential consumption of macroalgal species (Figure 3a, c). Green alge (*Enteromorpha sp.*, and *Cladophora sp.*) were consumed by all grazers, but red alge (*Polysiphonia sp.*, and *Ceramium sp.*) were not consumed by *Gammarus* (Duffy and Harvilicz 2001). Overall, the dominant effect of grazer presence and species richness suggests that grazing is an important ecosystem process, and that changes in mesograzer species composition and diversity may have impacts comparable to or greater than environmental stressors.

Table 3. Results from PERMANOVA analysis, testing for differences in community structure with experimental treatment. This analysis used square-root transformed data and a Bray-Curtis dissimilarity matrix, as used for the NMDS ordination in Figure 3.

Factor	DF	SS	MS	F	P
Grazer Species (Sp.)	4	0.0034	0.0008	8.5577	**0.001**
Warming (Warm.)	1	0.0004	0.0004	4.5097	**0.004**
Nutrients (Nuts.)	1	0.0018	0.0018	18.0309	**0.001**
Sp.*Warm.	4	0.0003	0.0001	0.8806	0.604
Sp.*Nuts.	4	0.0003	0.0001	0.8722	0.588
Warm.*Nuts.	1	0.0000	0.0000	0.1726	0.904
Sp.*Warm.*Nuts	4	0.0002	0.0001	0.5363	0.901
Error	76	0.0075	0.0001		

Environmental stressors had the largest effect on *Zostera* biomass (Table 1, Figure 1). The experimental addition of nutrients to the water column reduced *Zostera* biomass by 22% on average (Table 1), as shown in many previous studies (Hughes et al. 2004). This direct, negative, physiological effect of nutrients could have been due to increased nitrate uptake and resulting carbon limitation (Touchette and Burkholder 2000), but may have co-occurred with shading from epiphytic algal over-growth in no-grazer treatments, exacerbating the negative effects of nutrient addition. Nutrient additions also had a smaller but significantly negative effect on macroalgal biomass (Table 1, Figure 1, Table 4, Figure 4). This may be because grazers consumed almost all standing algal biomass, primarily palatable green algae, in nutrient addition treatments (Burkepile and Hay 2006). Warming treatments, which increased temperatures approximately 3°C and doubled the number of days with temperatures above 30°C (Figure 5), significantly increased epiphytic algal biomass but reduced *Zostera* biomass (Table 1). This 30°C threshold appears to be near the physiological limit of *Zostera*, and likely increases the compensating light requirements above available light levels in Chesapeake Bay (Moore et al. 1997), leading to summer-time *Zostera* die-offs (Moore and Jarvis 2008).

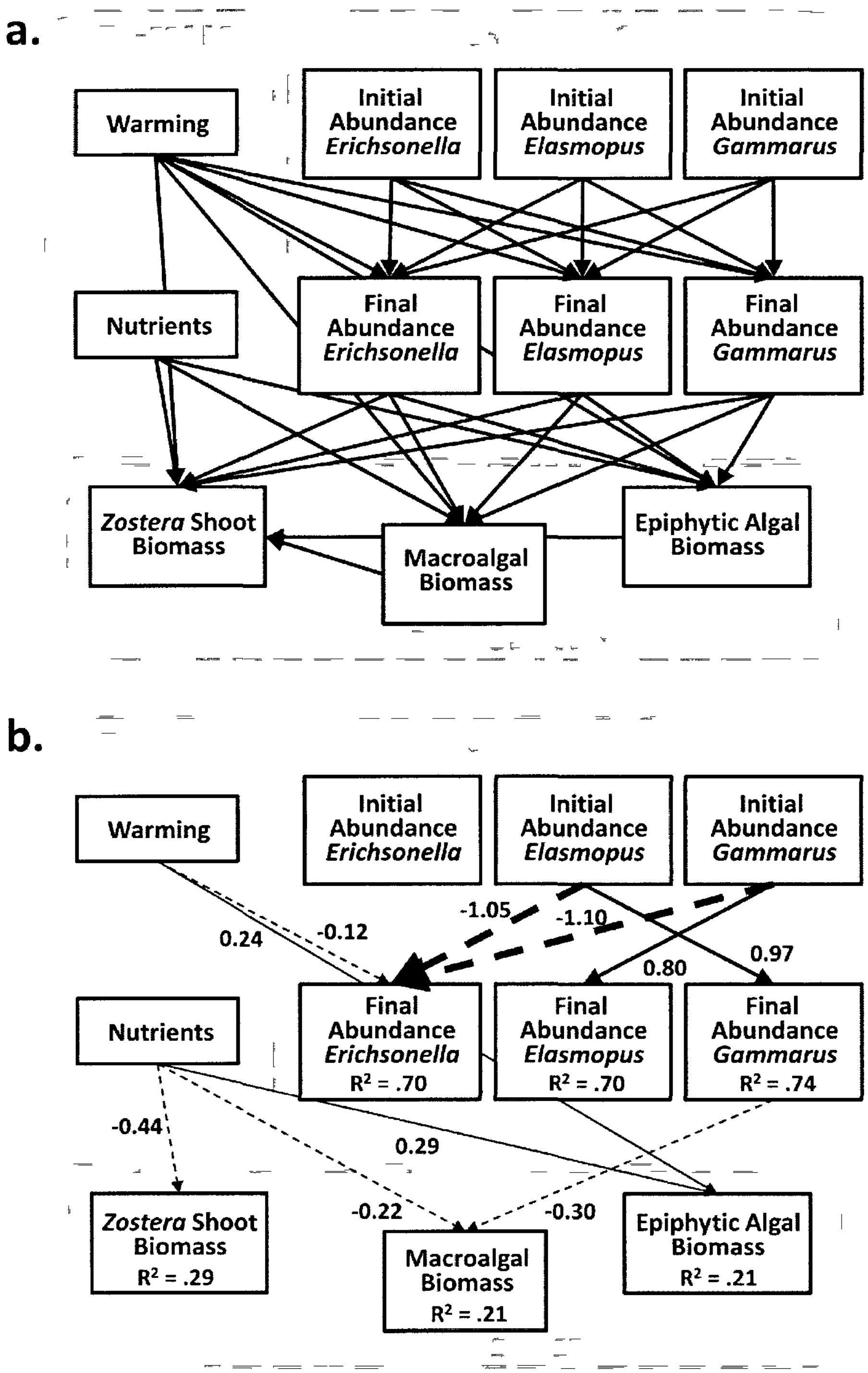

Figure 4 a) Full structural equation model (SEM) that was estimated for the experiment conducted in 2007, with warming and nutrients as stressors b) Graphical results from analysis of model shown in a) All paths significant at p = 0 05, path coefficients are standardized Dashed and solid lines represent positive and negative relationships respectively, line thickness indicates the explanatory power of a path Results in Table 4

Overall, stressors produced conditions favoring higher standing biomass of microalgae at the expense of macroalgae or eelgrass. If this holds true at an ecosystem scale, the impacts of climate warming in eutrophied systems such as Chesapeake Bay may be especially large.

Structural equation modeling (SEM) corroborated many results from the other analyses, but also provided additional insights into the structure of and effects within these experimental communities. The positive effects of warming and nutrient enrichment on epiphytic algae, and the negative effects on *Zostera* and macroalgae were again evident. In this analysis, we chose to model grazer species individually in order to examine stressor effects on each species. The isopod *Erichsonella* was significantly less abundant under warmed conditions, which follows a trend of reduced biomass in the warmest month (August) in York River, VA field collections over several years (Douglass et al. 2010). The presence of other grazer species also appeared to reduce the abundance of *Erichsonella*, likely because *Erichsonella* is a poor competitor, especially when grazer populations have reached carrying capacity in the mesocosms and competition for algal food resources is at a maximum. The apparent positive effects of the other two grazer species, *Elasmopus* and *Gammarus*, on each other in this SEM are likely an artifact of the substitutive design of this experiment: since initial numbers of all species were lowest in the three-species treatments, identical final densities of species across treatments would appear as higher population growth rates in the three-species treatment. Finally, *Gammarus* had a strong negative effect on macroalgal biomass through consumption. This effect is significant despite *Gammarus'* documented aversion to consuming red algae, which usually leads to overall higher macroalgal biomass (Duffy and Harvilicz 2001). However, *Gammarus* is a strong consumer of most other macro- and microalgae (Zimmerman et al. 1979), which could explain this effect.

This study showed that grazer presence and diversity can have comparable or larger effects on community structure than predicted warming and nutrient enrichment stressors. Diversity is important for maintaining critical ecosystem processes, such as grazing, in the face of multiple stressors (Elmqvist et al. 2003; Larson et al. 2005; Valentine and Duffy 2006; Blake and Duffy 2010), and grazing in seagrass systems has effects similar in magnitude to water-column nutrient enrichment (Neckles et al. 1993; Hughes et al. 2004). However, nutrients and warming favor ephemeral and epiphytic algae over stable eelgrass, potentially leading to a shift in basal primary producer species as coastal nutrient enrichment remains unabated, and the climate continues to warm. These results might suggest a bleak future for temperate estuarine seagrass systems, but also emphasize that these processes depend strongly on stressor identity and the composition of the grazer assemblage. Experimental grazer richness manipulations such as ours do not fully capture the range of species richness in the field,

Table 4 Results from structural equation model examining the experimental effects of warming, nutrients, and grazer species richness in 2007 (see Figure 4). Significant path coefficients (p = 0.05) are in bold. Whole-model chi-square = 22.404, degrees of freedom = 21, p = 0.377.

Model Path			**Standardized Estimate**	**Unstandardized Estimate**	**S.E.**	**C.R.**	**P**
Final *Elasmopus* abundance	<---	Warming	-0.022	24.45	71.07	-0.344	0.731
Final *Gammarus* abundance	<---	Warming	-0.032	-36.191	66.047	-0.548	0.584
Final *Erichsonella* abundance	<---	Warming	-0.124	-27.007	13.764	-1.962	**0.05**
Final *Elasmopus* abundance	<---	Initial *Erichsonella* abundance	-0.043	-4.008	21.306	-0.188	0.851
Final *Gammarus* abundance	<---	Initial *Erichsonella* abundance	0.116	10.796	19.8	0.545	0.586
Final *Erichsonella* abundance	<---	Initial *Erichsonella* abundance	0.256	4.63	3.536	-1.309	0.19
Final *Elasmopus* abundance	<---	Initial *Gammarus* abundance	0.797	73.965	21.282	3.476	**<.0001**
Final *Gammarus* abundance	<---	Initial *Gammarus* abundance	0.11	10.101	19.777	0.511	0.61
Final *Erichsonella* abundance	<---	Initial *Gammarus* abundance	-1.096	19.526	3.617	-5.399	**<.0001**
Final *Elasmopus* abundance	<---	Initial *Elasmopus* abundance	-0.039	-3.607	21.259	-0.17	0.865
Final *Gammarus* abundance	<---	Initial *Elasmopus* abundance	0.969	87.523	19.757	4.43	**<.0001**
Final *Erichsonella* abundance	<---	Initial *Elasmopus* abundance	-1.053	18.527	3.615	-5.125	**<.0001**
Epiphytic Algal biomass	<---	Final *Elasmopus* abundance	-0.231	0	0	-1.596	0.111
Macroalgal biomass	<---	Final *Elasmopus* abundance	0.147	0	0	1.02	0.308
Epiphytic Algal biomass	<---	Final *Gammarus* abundance	0.067	0	0	0.47	0.638
Macroalgal biomass	<---	Final *Gammarus* abundance	-0.304	0	0	-2.148	**0.032**
Epiphytic Algal biomass	<---	Final *Erichsonella* abundance	-0.059	0	0	-0.43	0.668
Macroalgal biomass	<---	Final *Erichsonella* abundance	0.056	0	0	0.407	0.684
Epiphytic Algal biomass	<---	Nutrients	0.287	0.012	0.004	2.812	**0.005**
Macroalgal biomass	<---	Nutrients	-0.223	-0.014	0.006	-2.186	**0.029**
Epiphytic Algal biomass	<---	Warming	0.237	0.076	0.033	2.274	**0.023**
Macroalgal biomass	<---	Warming	-0.016	0.007	0.048	0.152	0.879
Zostera biomass	<---	Final *Elasmopus* abundance	-0.217	0	0	-1.48	0.139
Zostera biomass	<---	Final *Gammarus* abundance	-0.128	0	0	-0.885	0.376
Zostera biomass	<---	Final *Erichsonella* abundance	0.177	0.001	0.001	0.993	0.321
Zostera biomass	<---	Macroalgal biomass	-0.041	-0.082	0.188	-0.438	0.662
Zostera biomass	<---	Epiphytic Algal biomass	-0.155	-0.447	0.269	-1.661	0.097
Zostera biomass	<---	Nutrients	-0.439	-0.054	0.011	-4.912	**<.0001**
Zostera biomass	<---	Warming	-0.112	-0.104	0.095	-1.088	0.277

and experimental manipulations of warming and nutrient additions can only simulate actual conditions for a short duration. Whether these patterns of diversity and stressor effects hold true for other larger experimental and natural systems still needs to be investigated. It will also be critical to evaluate the impacts of climate warming on individual species as well as the entire system, and to re-examine the effects of potential interactions between environmental stressors and species diversity as climate change continues.

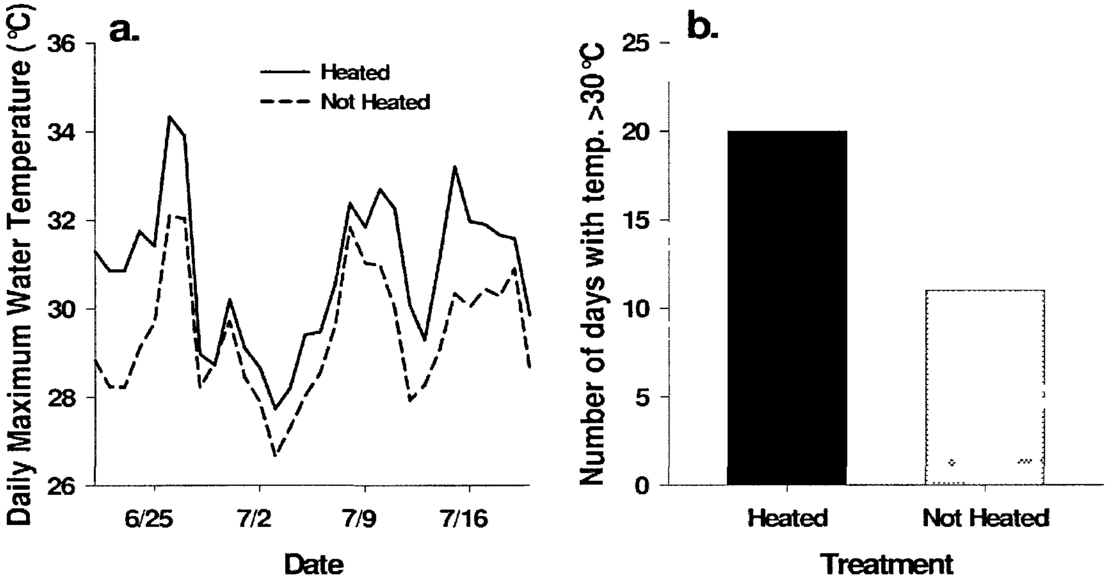

Figure 5 a) Average maximum daily water temperatures in heated (solid line) and unheated (dashed line) treatments over the duration of the experiment b) Number of days during the experiment with maximum water temperatures over 30°C in heated and unheated treatments.

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CHAPTER 3

Influence of environmental stressors and grazer immigration on ecosystem properties of an experimental eelgrass (*Zostera marina*) community.

Abstract

Anthropogenic stressors associated with climate change and shoreline development are increasingly changing conditions in coastal areas and impacting important habitats. But when multiple stressors act simultaneously, the results are often difficult to predict. Two anthropogenic stressors, warming temperatures and reduced light availability due to suspended materials, are expected to simultaneously affect estuarine communities, but the impacts of these stressors may be influenced by community processes such as immigration. Immigration of propagules can alter diversity and relative species abundances, rescue populations from extinction, and modify the capacity of communities to respond to environmental conditions. Effects of multiple stressors and their interactions with community processes are of particular concern in seagrass systems because these important estuarine habitats are vulnerable to anthropogenic impacts and provide valuable ecosystem services such as fisheries production and sediment stabilization. Using an experimental eelgrass (*Zostera marina*) system, we examined how warming, reduced light availability (shading), and periodic immigration of crustacean grazers influenced diversity, biomass, and structure of this community. Shading generally had large effects, and reduced biomass of all primary producers but did not affect grazer biomass. Warming reduced epiphytic algal biomass but only in the absence of grazers, while it increased sessile invertebrate biomass overall. Immigration of crustacean grazers did not interact significantly with stressors, and had little effect overall. Immigration did not influence diversity of grazers, relative abundance of grazer species, or biomass of primary producers and sessile invertebrates, however, immigration did increase biomass of grazers. Overall, reduced light availability by shading had strong effects on primary producers, whereas warming generally had weaker effects that differed with grazer presence. Thus, our results show that the impacts of reduced light availability and warming were independent and context specific, while crustacean grazer immigration effects were minimal in this model eelgrass system.

Introduction

Disturbance by natural environmental stressors can play an important role in structuring ecological systems (Paine et al. 1998), but anthropogenic stressors often exceed the range of natural environmental stressors. Thus, anthropogenic stressors can affect the functioning and persistence of ecosystems by reducing ecosystem resistance, resilience, and biodiversity (Folke et al. 2004; Darling and Cote 2008). These impacts become difficult to predict when multiple stressors simultaneously influence organisms and communities, but have effects that are non-additive (Breitburg et al. 1998; Paine et al. 1998; Vinebrooke et al. 2004). Thus, it is important to understand the impacts and consequences of multiple anthropogenic stressors, especially in the face of global climate change.

Many stressors are associated with climate change including climate warming, which is predicted to increase global temperature extremes. Air temperatures in the Chesapeake Bay region are predicted to warm by 2-6 °C by the end of the 21st century (Najjar et al. 2010), and because most of Chesapeake Bay is shallow, water temperatures will continue to closely follow increases in air temperatures (Najjar et al. 2000). This warming may impact nutrient and energy cycling, lifecycles and ranges of resident and transient organisms, fisheries production, and important habitats such as salt marshes, oyster reefs, and seagrass beds (Harley et al. 2006). These coastal habitats may also be simultaneously impacted by other climate warming-associated anthropogenic stressors.

Climate warming will also likely increase suspended sediment and nutrient inputs to coastal waters as a result of predicted increases in rainfall, storm frequency, and storm intensity (Najjar et al. 2000, 2008). Other human activity such as coastal development can also increase fine sediment inputs to coastal waters and contribute to shading effects (Jennings et al. 2003). Macrophytes such as seagrasses are effectively shaded when water column light levels are reduced due to suspended materials such as phytoplankton and sediments. Shading alone can effectively limit the extent of seagrasses in Chesapeake Bay (Orth and Moore 1983), but may also interact with warming temperatures to further limit seagrass extent by increasing the compensating light requirements at higher temperatures (Moore et al. 1997). Thus, warming temperatures and decreasing light availability, as a result of human activities, are expected to impact coastal areas generally, and Chesapeake Bay specifically.

A key question is whether multiple stressor impacts may be altered by community processes such as immigration, which can influence such ecosystem properties as diversity, abundance, and biomass (Loreau and Mouquet 1999; Mouquet et al. 2004). In unsaturated communities, ecological theory predicts that immigration will increase species diversity through resource partitioning and supply of novel or rare species (Loreau 2000; Chase 2003; Lee and Bruno 2009), while in saturated communities, immigration will maintain diversity by sustaining populations of inferior competitors that otherwise would be outcompeted (Loreau 2000). Thus, changes in immigration could alter diversity, abundance, and biomass, and result in communities that may be more or less able to cope with multiple stressors. Interactions between community processes and multiple stressors are of special interest in important coastal systems such as seagrass habitats, because anthropogenic stressors will likely have the most immediate impact in these systems.

Seagrass systems in Chesapeake Bay are vulnerable to climate change impacts, which is of concern because these important habitats provide refuge for juvenile fish and crabs, stabilize sediments and coastlines, and sequester carbon (Duarte 2000; Heck et al. 2003; Duarte et al. 2005; Polte et al. 2005; Canuel et al. 2007). Historically the dominant seagrass in Chesapeake Bay (Orth and Moore 1983, 1988; Orth et al. 2002), eelgrass (*Zostera marina*) has been declining at an accelerated pace in recent years due in part to light availability and record summer temperatures (Moore et al. 1996, 1997; Moore and Jarvis 2008). Elevated temperatures can lead to eelgrass (hereafter referred to as *Zostera*) die-offs (Bintz et al. 2003; Oviatt 2004) by fostering higher respiration than production, inducing internal carbon stress, and reducing growth rates (Short and Neckles 1999; Touchette and Burkholder 2000; Bintz et al. 2003; Touchette et al. 2003). Reductions in available light also cause eelgrass decline, especially in estuaries where turbidity is high due to suspended materials. *Zostera* cannot maintain a positive carbon balance when available light falls below compensating levels, leading to decreased growth and survival (Moore et al. 1997). However, in temperate systems grazing by amphipods and isopods on epiphytic algae can help maintain Zostera (Neckles et al. 1993; Duffy et al. 2003, 2005; Valentine and Duffy 2006). The effects of grazer immigration on maintaining this important function and on the community response to multiple stressors remain poorly understood. But, *Zostera* habitats in Chesapeake Bay and elsewhere are increasingly faced with multiple simultaneous stressors whose interactions are difficult to predict, so understanding these processes and responses is critical (Orth et al. 2006).

Impacts of multiple stressors and the role of diversity in altering those impacts have been tested in previous experiments, but those systems were closed (Blake and Duffy 2010). Here we used immigration to simulate an open system in which propagule supply from outside a community may have a rescue effect on a local stressed community (Gotelli 1991). We experimentally tested how multiple stressors simulating climate change effects and crustacean grazer immigration might influence the structure and functioning of eelgrass communities in a model system. We chose warming as a stressor because water temperatures in the Chesapeake Bay region are predicted to increase 2 - 6 °C in the next century (Najjar et al. 2010). Decreased light availability (shading), a result of suspended materials, will also likely increase in frequency and severity with coastal development and changing climate. Finally, we used crustacean grazers in our experimental system because they have strong impacts on primary producers, are relatively mobile, and reproduce quickly. The foci of our experiment were the following questions: 1) how do environmental stressors, warming and shading, interact and impact the structure and functioning of the eelgrass community, 2) does immigration alter the structure of the crustacean grazer community, 3) does immigration alter the effects of stressors.

Methods

Experimental Design

We manipulated grazer immigration, water temperature, and light in 84 outdoor mesocosms at the Virginia Institute of Marine Science, Gloucester Point, VA, USA (37.247653°N, 76.500809°W) during a six-week experiment in July and August 2009. Mesocosms (19 L buckets) were placed in large tanks in a 25 cm deep water bath, and supplied with through-flowing filtered estuarine water from the adjacent York River. York River water was initially filtered by a coarse sand filter, and secondarily filtered through 150-micron mesh to prevent unintentional introduction of mesograzers, but allow propagules of algae and sessile invertebrates to recruit. Approximately 2.5 L of sand and 0.5 L of defaunated organic mud were added to each mesocosm as substrate, and thirty shoots of defaunated *Zostera marina* were planted in each mesocosm.

Three grazer treatments were established using the six most common crustacean grazer species in York River seagrass beds in summer 2009: the isopod *Erichsonella attenuata*, and the amphipods *Elasmopus levis, Gammarus mucronatus, Dulichiella appendiculata, Cymadusa compta,* and *Ampithoe longimana* (grazer species hereafter referred to by genus). Treatments included: initial grazer introduction at the start of the experiment, initial and periodic grazer introductions during the experiment to simulate immigration events, and grazer-free control. 17 male-female grazer pairs were added to each mesocosm initially, in the same relative species abundances as grazer populations in York River eelgrass beds. Grazer additions during the experiment (at weeks two and four) were composed of between 20 – 24 individuals, in the same relative species abundances as found in the field. The experiment ran for six weeks, allowing the grazer species to have 1 - 2 generations and to reach carrying capacity in the mesocosms (Duffy and Harvilicz 2001). Two temperature treatments, warmed and unwarmed, were applied for the duration of the experiment. Treatments were warmed by one 200 watt aquarium heater per mesocosm, which increased temperatures approximately 3 - 4 °C (Blake and Duffy 2010). All mesocosms were covered by gray fiberglass window screen to reduce light levels, with un-shaded treatments covered by one layer of screen to approximate light levels in local eelgrass beds, and shaded treatments covered by four layers of screen to significantly reduce light levels (approximately 13% of insolation) (Moore et al. 1997).

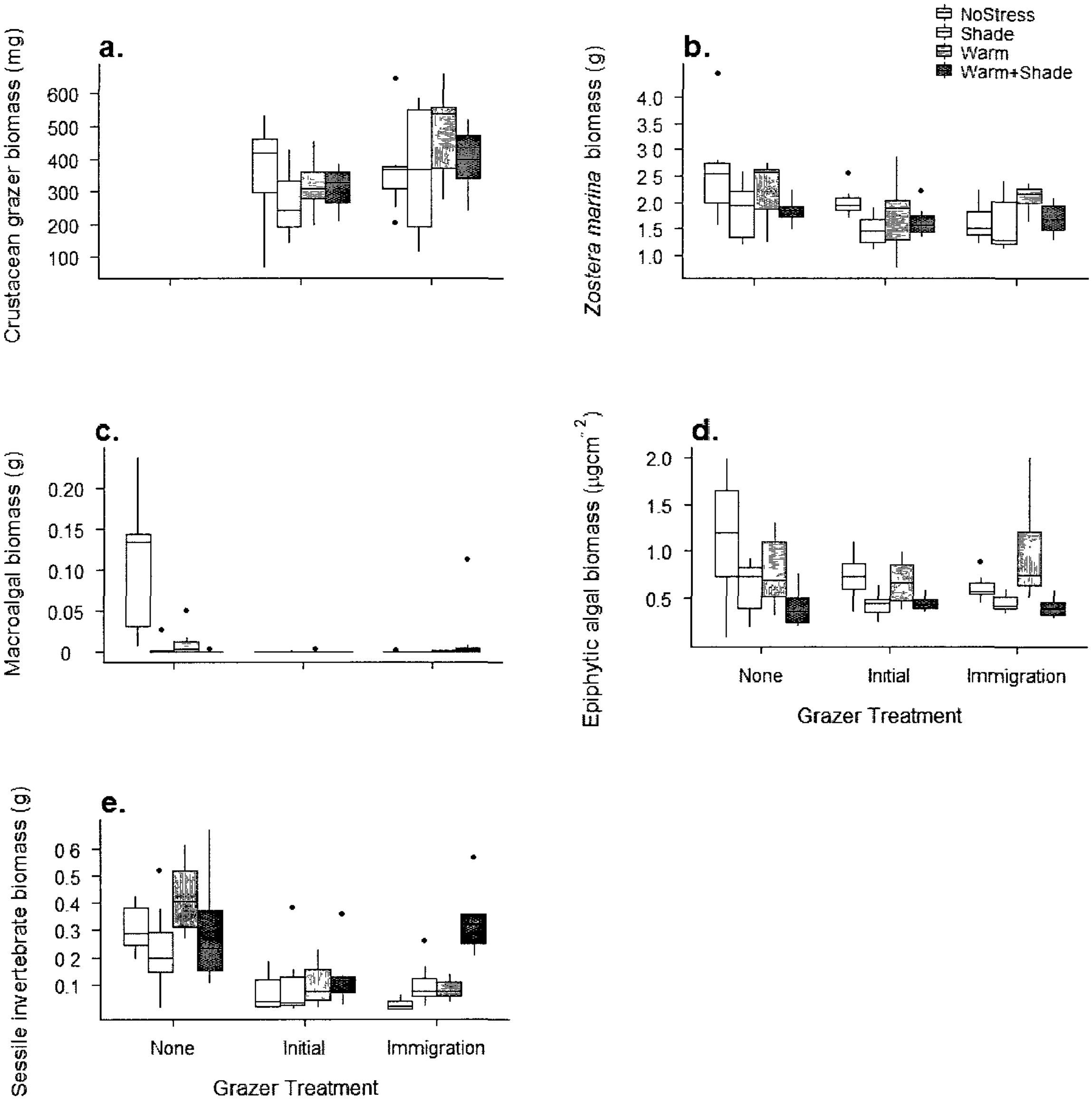

Figure 1. Boxplots of biomass for a) crustacean grazers, b) *Zostera marina*, c) macroalgae, d) epiphytic algae (as chlorophyll *a*), and e) sessile invertebrates. No-grazer controls were not significantly contaminated, and thus were considered to have zero grazers. Macroalgae included *Polysiphonia spp*, *Ulva spp*, *Cladophora spp*, and *Gracilaria spp*. Sessile invertebrates include the barnacle *Balanus improvisus*, and the tunicates *Molgula manhattensis* and *Botryllus schlosseri*.

Artificial seagrass (polypropylene curling ribbons) was placed in each mesocosm at the start of the experiment to provide a standardized substratum for accumulating and measuring epiphytic algal growth. Epiphytic chlorophyll *a* was sampled from the artificial seagrass at the end of the experiment as a proxy for epiphytic algal biomass (Parsons et al. 1984). Data are presented as mass of chlorophyll a normalized to area of eelgrass leaf blade (µg chl *a* cm^{-2}). At the end of the experiment, all plant, animal, and algal biomass was harvested from each mesocosm and frozen. All biomass retained on a 500 micron sieve was sorted, separated and identified to the lowest taxonomic level possible, dried at 60°C until mass was stable, and then combusted to obtain ash-free dry mass (AFDM). Grazers were sorted by size (using sieves of 8.0, 5.6, 4, 2.8, 2.0, 1.4, 1.0, 0.71, 0.05 mm), identified to species, and counted. Biomass was calculated using the empirical equation, $\log B = -1.01 + 2.64 \times \log S$, where B is AFDM (mg), and S is the sieve size (mm) on which the animal was retained (Edgar 1990).

Statistical Analysis

To evaluate the effects of water temperature, reduced light availability, and crustacean grazer immigration on the biomass of crustacean grazers, *Zostera*, epiphytic algae, macroalgae, and sessile invertebrates, we used factorial ANOVAs to test for main and interactive effects. Analysis was conducted in R 2.13.0 (R Development Core Team 2011). *Zostera* and epiphytic algal biomass were log transformed, and sessile invertebrate biomass was square-root transformed to improve homogeneity of variance. Two sets of orthogonal a-priori contrasts were conducted to test the effects of grazer absence (treatments with grazers vs. no-grazer controls) and grazer immigration (initial grazer introduction vs. periodic grazer introductions). To evaluate the effect size of each main and interactive effect in comparable units, we centered each variable (subtracted each value from the variable mean) and scaled it (divided each value by the variable standard deviation) to obtain standardized regression coefficients (Schielzeth 2010).

Results

Environmental stressor effects

Overall, shading had a much stronger effect on these model eelgrass communities than warming. Shading treatments reduced light to approximately 12% of ambient light levels, and significantly reduced the biomass of all primary producers, including *Zostera*, epiphytic algae, and macroalgae ($p = 0.002$, $p < 0.001$, $p = 0.011$ respectively; Figure 1, Table 1, Table 2). Shading also reduced sessile invertebrate biomass in the absence of grazers, but increased sessile invertebrate biomass in grazer immigration treatments (interaction $p = 0.0001$, Figure 1e., Table 1).

Warmed treatments were on average 2 °C warmer than ambient temperature treatments, with temperatures averaging 28.7 °C during this experiment. Warming significantly increased sessile invertebrate biomass ($p = 0.001$, Figure 1e., Table 1), but reduced macroalgal biomass ($p = 0.033$, Figure 1c., Table 1). However, in the absence of grazers, warming reduced epiphytic algal biomass ($p = 0.045$, Figure 1d., Table 1). Thus, warming effects were context specific, while shading effects on primary producers were strong and relatively consistent.

Table 1. Results from factorial ANOVA analysis, with a-priori contrasts for grazer presence and immigration. Significant values are in bold. Mean squares values were calculated using the type I sums of squares.

	Response														
	Crustacean grazer biomass			**Zostera biomass (log)**			**Macroalgal biomass**			**Epiphytic algal biomass (log)**			**Sessile invertebrate biomass (sqrt)**		
Factor	**Mean Sq**	**DF**	**p**	**Mean Sq**	**DF**	**p**	**Mean Sq**	**DF**	**p**	**Mean Sq**	**DF**	**p**	**Mean Sq**	**DF**	**p**
Grazers (Gzr)	107890	1	**0.017**	0.0266	2	**0.008**	0.0073	2	**0.001**	0.0078	2	0.291	0.5319	2	**<0.0001**
grazer absence	N/A			0.0521	1	**0.002**	0.0143	1	**<0.0001**	0.0147	1	0.127	1.0406	1	**<0.0001**
grazer immigration	107890	1	**0.017**	0.0011	1	0.646	0.0003	1	0.550	0.0008	1	0.714	0.0231	1	0.256
Warming (Warm)	17735	1	0.320	0.0008	1	0.692	0.0036	1	**0.033**	0.0025	1	0.525	0.2671	1	**0.0002**
Shading (Shade)	31248	1	0.188	0.0533	1	**0.002**	0.0052	1	**0.011**	0.1690	1	**<0.0001**	0.0467	1	0.108
Gzr*Warm	17100	1	0.329	0.0087	2	0.196	0.0067	2	**0.0004**	0.0154	2	0.090	0.0414	2	0.103
grazer absence	N/A			0.0054	1	0.313	0.0131	1	**<0.0001**	0.0257	1	**0.045**	0.0093	1	0.471
grazer immigration	17100	1	0.329	0.0119	1	0.134	0.0003	1	0.542	0.0050	1	0.370	0.0735	1	**0.045**
Gzr*Shade	476	1	0.870	0.0029	2	0.576	0.0083	2	**<0.0001**	0.0021	2	0.711	0.1949	2	**0.0001**
grazer absence	N/A			0.0053	1	0.317	0.0164	1	**<0.0001**	0.0024	1	0.538	0.2753	1	**0.0002**
grazer immigration	476	1	0.870	0.0005	1	0.757	0.0002	1	0.583	0.0019	1	0.583	0.1144	1	**0.013**
Warm*Shade	125	1	0.933	0.0037	1	0.404	0.0006	1	**0.005**	0.0030	1	0.485	0.0098	1	0.459
Gzr*Warm*Shade	22763	1	0.260	0.0086	2	0.198	0.0040	2	**0.008**	0.0072	2	0.318	0.0118	2	0.515
grazer absence	N/A			0.0012	1	0.630	0.0077	1	**0.002**	0.0022	1	0.554	0.0093	1	0.470
grazer immigration	22763	1	0.260	0.0159	1	0.084	0.0003	1	0.550	0.0122	1	0.164	0.0143	1	0.371
Residuals	17548	48		0.0052	72		0.0008	72		0.0062	72		0.0177	72	

Grazer immigration effects

Crustacean grazer presence also had a large effect in this experiment. Grazers virtually eliminated macroalgae from all mesocosms (Figure 1c.), and significantly reduced both *Zostera* above-ground biomass and sessile invertebrate biomass (the barnacle *Balanus improvisus*, and the tunicates *Molgula manhattensis* and *Botryllus schlosseri*) ($p = 0.002$, $p < 0.001$ respectively, Figure 1, Table 1). Grazer immigration, simulated via periodic addition of grazers during the experiment, increased grazer biomass at the end of the experiment ($p = 0.017$, Figure 1, Table 1), but had no other significant effect. Grazer immigration did not change grazer species richness (Figure 2) or relative species abundance (Figure 3). Thus, crustacean grazer presence had strong effects, while crustacean grazer immigration had minimal effects.

Discussion

With this experiment, we sought to understand how warming and shading impact eelgrass communities and whether immigration changes grazer community structure or alters stressor effects. These are critical issues to understand for seagrass systems that are increasingly faced with multiple simultaneous stressors from sources such as climate change and development in coastal areas. We found that generally, shading had strong negative impacts on primary producer biomass, warming shifted the fouling community from being dominated by macroalgae to being dominated by sessile invertebrates, and crustacean grazer immigration had little overall effect except to increase grazer biomass. Thus, we expect that projected warming temperatures and reduced light availability from suspended sediments will reduce primary producers but promote sessile invertebrates. In addition, based on the limited results of this mesocosm experiment, we think that crustacean grazer immigration may not alter the effects of stressors or the structure of the grazer community in seagrass beds.

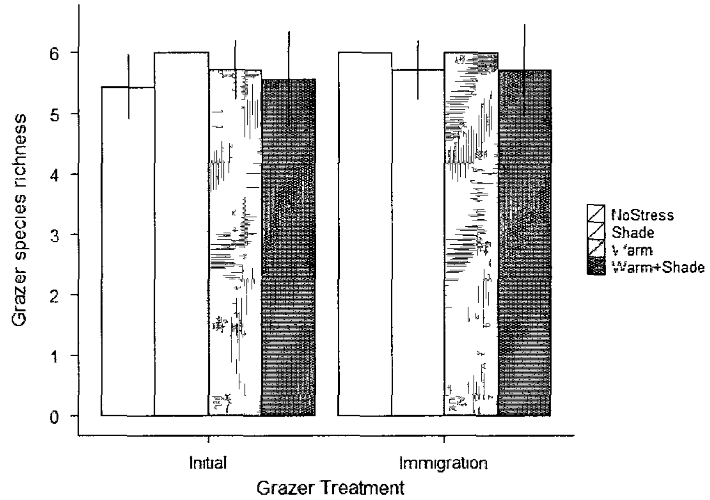

Figure 2
Mean grazer species richness in Initial and Immigration grazer treatments for each stressor treatment

Table 2. Results from linear models calculated using centered (subtracting each value from the variable mean) and scaled (dividing each value by the variable standard deviation) variables. The estimated coefficients therefore represent a measure of effect size relative to the intercept for each level of each factor.

	Response									
	Crustacean grazer biomass		Zostera biomass (log)		Macroalgal biomass		Epiphytic algal biomass (log)		Sessile invertebrate biomass (sqrt)	
Factor	**Coeff.**	**SE**	**Coeff.**	**SE**	**Coeff.**	**SE**	**Coeff.**	**SE**	**Coeff.**	**SE**
Intercept	<0.0001	0.1288	<0.0001	0.0989	<0.0001	0.0784	<0.0001	0.0944	<0.0001	0.0744
Grazer absence (GzrA)	N/A		0.3414	0.1148	0.3689	0.0911	0.1667	0.1097	0.6238	0.0864
Grazer immigration (GzrI)	0.3221	0.1299	0.0530	0.1148	0.0547	0.0911	0.0404	0.1097	0.0988	0.0864
Warming (Warm)	0.1306	0.1299	0.0395	0.0994	-0.1711	0.0789	-0.0607	0.0950	0.2910	0.0748
Shading (Shade)	-0.1734	0.1299	-0.3186	0.0994	-0.2054	0.0789	-0.4971	0.0950	0.1217	0.0748
GzrA*Warm	N/A		-0.0142	0.1155	-0.3000	0.0916	-0.1452	0.1104	0.0341	0.0869
GzrI*Warm	0.1294	0.1311	0.1752	0.1155	0.0562	0.0916	0.0996	0.1104	0.1773	0.0869
GzrA*Shade	N/A		-0.0828	0.1155	-0.3423	0.0916	-0.0895	0.1104	-0.1866	0.0869
GzrI*Shade	0.0216	0.1311	0.0359	0.1155	0.0505	0.0916	-0.0608	0.1104	0.2212	0.0869
Warm*Shade	0.0111	0.1311	0.0840	0.1000	0.2304	0.0794	-0.0670	0.0956	0.0561	0.0753
GzrA*Warm*Shade	N/A		-0.0531	0.1162	0.2814	0.0922	-0.0208	0.1110	-0.0156	0.0874
GzrI*Warm*Shade	-0.1507	0.1323	-0.2037	0.1162	0.0553	0.0922	-0.1561	0.1110	0.0787	0.0874
Model R^2	0.1898		0.2880		0.5519		0.3501		0.5971	
Model Residual SE	0.9635		0.9060		0.7187		0.8655		0.6815	

Warming had limited impacts in this experiment, but the effects are similar to previous experimental results. Sessile invertebrate biomass was increased by warming most likely due to faster growth and metabolic processes at increased temperatures. On the other hand, both macroalgal biomass and epiphytic algal biomass were reduced by warming in the absence of grazers, as demonstrated previously (Fong and Zedler 1993; Blake and Duffy 2010). However, caution is necessary in interpreting the macroalgal biomass results due to the very low overall biomass in this experiment.

Zostera biomass was not reduced by warming in this experiment as expected from previous experimental work (Blake, R.E. Chapter 2). One potential explanation for these different results is variation among years in summer air temperatures. In 2009, the year this experiment was conducted, Virginia experienced normal summer temperatures (compared to the 20th century average) (NOAA 2009). But, the previous experiment showing negative impacts of warming on *Zostera* was conducted in 2007, when summer temperatures in the U.S. were the sixth warmest since 1895, and Virginia temperatures were much above normal (NOAA 2007). Thus, even though our warming treatment effectively increased water temperatures in this experiment, those temperatures were likely not high enough in 2009 to reach the threshold above which *Zostera* is negatively impacted (Moore and Jarvis 2008).

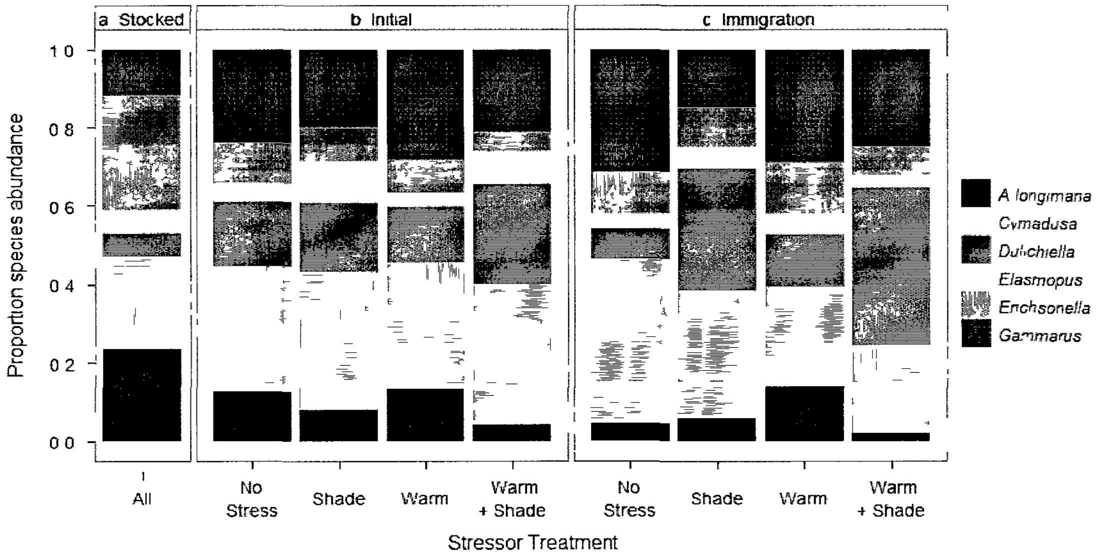

Figure 3. Relative species abundances of grazers a) that were stocked into all grazer treatments at the beginning of the experiment; b) at the end of the experiment in Initial grazer treatments; c) at the end of the experiment in Immigration grazer treatments.

Shading, our proxy for increased turbidity resulting from coastal development and storm events, reduced biomass of all primary producers as well as biomass of sessile invertebrates in the absence of grazers. This reduction in sessile invertebrates with shading makes sense if by limiting light availability we also limited phytoplankton, a food source for these filter-feeding animals. However, previous experiments have shown that the accumulation of phytoplankton is promoted at lower light levels, possibly due to release from competition with macroalgae for micronutrients (Fong and Zedler 1993). This may explain the increase in sessile invertebrate biomass with shading in the grazer immigration treatments: perhaps phytoplankton biomass was greater in shaded treatments, and thus supported higher biomass of barnacles and tunicates. Overall, shading had expected negative effects on macrophytes and epiphytic algae, but may have fostered phytoplankton production and thus filter feeding sessile invertebrates.

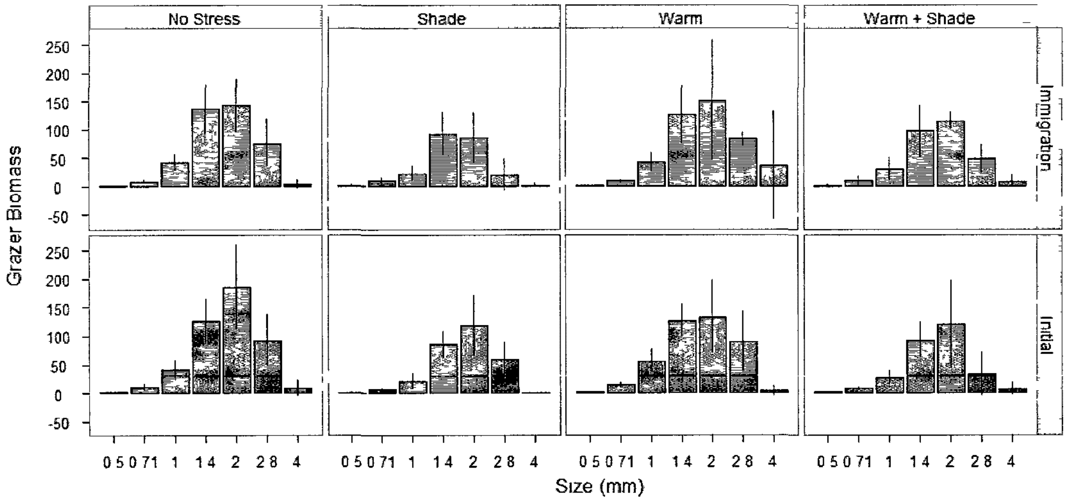

Figure 4. Total grazer biomass per sieve size (see Methods) for all treatment combinations.

We expected that periodically adding grazers to immigration treatments would mimic immigration events in the field, and thus increase grazer species richness via supply of rare species (Loreau 2000). A similar technique employed in previous experiments (Lee and Bruno 2009) yielded increases in grazer species richness with immigration treatments. Although we counted the grazers from the wild population that we added during each immigration pulse to insure uniform treatments, rather than taking blind random samples from the wild population as Lee and Bruno (2009) did, we still expected greater species richness in our immigration treatments. We expected that some of the rarer species (those stocked with only one male-female pair) would not persist and thus the species richness of initial grazer additions might decrease during the experiment. However, all grazer species were present at the end of the experiment and our periodic grazer additions did not change grazer species richness. Our immigration treatments also may not have changed grazer richness because our local crustacean grazer community is not very diverse, and thus the six species initially used in this experiment represented a majority of all possible species. Overall, it appears that our experimental grazer communities were stable, because immigration events did not change species richness and rarer species were able to persist in these communities.

Even though grazer immigration did not change species richness, relative abundance, or size distribution, it did increase grazer biomass (Figure 4, Table 1). Increased biomass with immigration has also been observed in terrestrial plant communities (Loreau 2000). We believe that in our experiment, this result does not indicate a transient effect of the final periodic grazer immigration treatment two weeks prior to the end of the experiment, because crustacean grazer generation time in the summer is approximately two weeks. Rather, we believe this indicates that grazer populations were neither at carrying capacity in the mesocosms, nor resource limited by epiphytic algae.

Grazing is a strong structuring process in seagrass communities, and the six grazer species used in this experiment represent the dominant summer grazer species in local seagrass beds. Though all grazers in this experiment consume micro- and macroalgae, the two ampithoid species, *Cymadusa compta* and *Ampithoe longimana*, may consume *Zostera* when their preferred algal food is in low supply (Nelson 1979). Ampithoids may also non-consumptively affect *Zostera* by using blades to help build their mucus tubes (personal observation), perhaps leading to the negative effect of grazer presence seen in this experiment (Table 1). Thus, it is possible that under warming temperatures and decreasing light availability, direct grazing on *Zostera* by crustacean grazers may increase. However, conditions under which this may happen will be highly dependent on the persistence of *Zostera* under low light conditions, the spatial and temporal consistency of stressor effects, the species composition of the grazer community, and the impact of predation on the grazer community, a scenario we did not test with this experiment.

In summary, shading and warming negatively impacted primary producers but positively impacted sessile invertebrates, while grazer immigration positively impacted grazer biomass but did not influence stressor effects or grazer community structure. This means that the stressors we examined favored sessile fouling animals and possibly phytoplankton over epiphytic micro- and macroalgae and habitat-forming eelgrass. However, care should be taken in extrapolating stressor effects because multiple stressor effects can be dependent on stressor identity. Our data also suggest that movement of crustacean grazers between habitat patches, here simulated via immigration treatments, may be unlikely to change stressor effects or grazer community composition in open seagrass systems. However, our experimental grazer communities were generally densely populated, well fed, and without predation. Immigration may be more important to population persistence and grazing under less favorable conditions. Results from our model eelgrass system indicate that crustacean grazers may be able to maintain grazing pressure and reduce overall algal biomass, an important ecosystem process, despite shifts in dominance of the fouling community and the effects of multiple simultaneous stressors.

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CHAPTER 4

Patterns of seagrass community response to local shoreline development

Abstract

Three quarters of the global human population will live in coastal areas in the next few decades and will continue to develop these areas as population density increases. Anthropogenic stressors, such as increased nutrient and sediment inputs, from coastal development may lead to changes in sediment characteristics, fragmented habitats, altered food webs, and eventual loss of near-shore vegetated habitats. Seagrass systems are important estuarine habitats that provide valuable ecosystem services, but are vulnerable to anthropogenic stressors. Understanding how coastal development impacts these habitats is key to maintaining important ecosystem services such as habitat for juvenile animals, water filtration, and sediment stabilization. To assess potential impacts of local shoreline development on these productive communities, we surveyed twenty seagrass beds in lower Chesapeake Bay, VA. We sampled primary producers, consumers, water quality, and sediment characteristics in seagrass beds, and we characterized the amount and type of development along the adjacent shoreline. We found that local coastal development significantly affected sediment organic matter and grain size, and that seagrass biomass tended to be related to sediment properties though not significantly affected by development. Epiphytic algal biomass and epibiont (epifauna and epiphyte) community composition differed significantly between Western and Eastern regions of the bay, and thus appear to be driven by regional rather than local conditions. We did not detect any effect of local coastal development on *Zostera* leaf nitrogen (a proxy for integrated nitrogen loading), crustacean grazer biomass, epifaunal predator abundance, or nekton abundance. Our data corroborate previous demonstrations of local coastal development impacts on sediment properties and benthic animals, but demonstrate that these patterns are different and more complex for mobile organisms in vegetated habitats. Thus, in seagrass communities, different factors appear to drive sediment and biological community properties on different spatial scales. This may be an important consideration for managing impacts of anthropogenic stressors, as management efforts will likely have the most impact when they match the specific drivers and spatial scale of a particular ecosystem process or service.

Introduction

Human populations in coastal areas continue to increase and by 2025, 75% of the global population is projected to live in coastal areas (Bulleri and Chapman 2010). Growing coastal populations will continue to develop coastal areas, converting vegetated pervious systems to unvegetated impervious areas by constructing buildings, paving roads, and armoring shorelines with riprap and bulkheads. Hundreds of miles of shoreline along Chesapeake Bay VA, USA, have been armored in the last several decades (Titus 1998; VIMS 2004), and in some sub-watersheds, 50% of shorelines are now hardened with armoring (Berman et al. 2000). This coastal development has thus changed a large portion of Chesapeake Bay shoreline from sandy, muddy soft sediments to rocks and concrete, and has the potential to impact important intertidal and subtidal communities by fragmenting habitats, changing movement of animals between habitats, altering wave energy and sediment composition, and increasing nutrient inputs to coastal systems (Irlandi and Crawford 1997; Bertness et al. 2002; Jennings et al. 2003; Groffman et al. 2004; Goforth and Carman 2005).

Many anthropogenic stressors result from of coastal development, including increased sediment and nutrient inputs. Coastal development frequently increases the amount of fine sediments input to adjacent waters (Jennings et al. 2003), and decreases the sediment stability adjacent to developed shorelines (Goforth and Carman 2005). These fine-grain, unstable sediments may support lower infaunal densities (Goforth and Carman 2005; Seitz et al. 2006), but their effects on submerged vegetated communities remain unclear.

Another important stressor in coastal systems is excessive nutrient loading. Changes in land-use during the last century have doubled the amount of nitrogen that is biologically available, leading to estuarine eutrophication (Vitousek et al. 1997). Eutrophication is a problem for estuaries with developed watersheds such as Chesapeake Bay, because nitrogen inputs to coastal waters are significantly higher when watersheds are dominated by development (Groffman et al. 2004). These nitrogen inputs can lead to simplified food webs, low dissolved oxygen, and losses in ecosystem functioning (Vitousek et al. 1997; Lerberg et al. 2000). Though eutrophication is widely known to negatively impact submerged vegetated habitats at watershed and basin-wide scales (Burkholder et al. 2007), local impacts in these habitats are less well understood but may be as important as in saltmarshes (Bertness et al. 2002).

Coastal development can fragment habitats, potentially leading to altered food web composition, structure, and functioning in submerged near-shore habitats (Irlandi and Crawford 1997; Eggleston et al. 1998; Reed and Hovel 2006). Immigration between habitat patches may be important to maintaining these ecosystem functions, because immigration can influence species diversity, abundance, and biomass (Loreau and Mouquet 1999; Mouquet et al. 2004). Species diversity is often positively related to biomass (Hooper et al. 2005; Cardinale et al. 2007), so we might expect development effects to be dampened by immigration between habitat fragments. However, previous studies have shown that nekton, benthic predators, and benthic prey occur in lower abundance in unvegetated habitats next to armored shorelines (Peterson et al. 2000; Rice 2006; Seitz et al. 2006). Whether similar patterns of reduced animal abundance may occur in vegetated habitats adjacent to developed shorelines remains unknown (but see Irlandi and Crawford 1997) and studies examining impacts on lower trophic levels such as epifauna are lacking. But, the effects of anthropogenic stressors and changes in diversity and biomass in vegetated habitats such as seagrass beds could be critical to the provision of valuable ecosystem services such as fisheries production and sediment stabilization.

Seagrasses are important foundation species that provide habitat for juvenile fish and crabs, stabilize sediments and coastlines, and sequester carbon (Duarte 2000; Heck et al. 2003; Duarte et al. 2005; Polte et al. 2005; Canuel et al. 2007). Eelgrass (*Zostera marina*),the dominant seagrass in Chesapeake Bay, has been declining due to multiple stressors such as continued eutrophication, suspended sediments, and record water temperatures (Moore, Neckles, and Orth 1996a; Moore et al. 1997; Moore and Wetzel 2000a; Moore and Jarvis 2008). Eutrophication can lead to reduced biodiversity, simplified food webs, and habitat loss (Deegan 2002; Tewfik et al. 2007). Excess nitrogen also stimulates fast-growing micro- and macro-algae which overgrow the eelgrass blocking light and reducing growth and shoot density (Neckles et al. 1993; Deegan 2002; Valentine and Duffy 2006). Increased inputs of fine sediments along developed shorelines can also reduce light, reducing eelgrass growth and survival (Moore et al. 1997). It is important to understand how changes to coastal habitats with shoreline development affect the community composition and ecosystem properties of these important habitats.

The goal of this study was to understand whether local shoreline development corresponds with altered ecosystem properties and community composition in adjacent seagrass beds, as has been demonstrated in unvegetated habitats (Peterson et al. 2000; Rice 2006; Seitz et al. 2006). To examine this, we surveyed twenty seagrass beds in the lower Chesapeake Bay, VA: we assessed shoreline development, and sampled sediment characteristics, water quality parameters, primary producers, and consumers. We asked the following questions: 1) Are there differences in seagrass-associated animal abundance and diversity with coastal development? 2) Are seagrass beds adjacent to developed shorelines nutrient enriched? 3) Does shoreline development correspond with an altered physical environment?

Methods

Field Sampling

Pilot sampling was conducted in 2005 and 2006 in eelgrass beds in the York River, VA (37° 15′ N, 76° 25′ W) to assess the statistical power of potential survey designs, and to determine the number of sites needed. We chose to sample 20 sites in the lower Chesapeake Bay, VA, 10 along the Eastern side and 10 along the Western side of the bay (see Figure 1). Potential sites were identified using seagrass coverage data available from the VIMS Seagrass Program website http://web.vims.edu/bio/sav/. Subsequent ground truthing verified that selected sites were dominated by eelgrass (*Zostera marina*), had at least 150 m of contiguous seagrass habitat, and were accessible by boat at low tide.

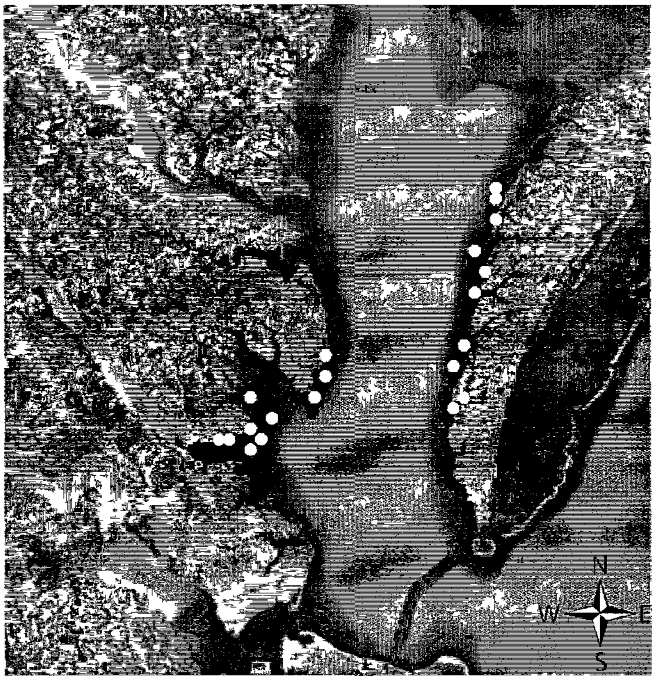

Figure 1
Map of lower Chesapeake Bay , VA, USA, showing location of sampling sites in white dots

In order to determine whether coastal development corresponded with variation in ecological characteristics of adjacent seagrass beds, we first assessed shoreline and riparian condition at each site. The shoreline type was measured along 150 m of linear shoreline, and the proportion of each shoreline type (riprap, bulkhead, beach, saltmarsh, etc.) was recorded (Bertness et al. 2002; King et al. 2005; Bilkovic et al. 2006). Likewise, a 150 m by 30 m area of riparian land was visually assessed, and the proportion of each land-use type was recorded (Bilkovic et al. 2006). Riparian and shoreline development assessments were later used to construct an index of development with three levels: U) undeveloped - neither shoreline nor riparian development of any sort; D1) either shoreline or riparian development but not both, for example - a pier, a bulkhead, a house, or a paved driveway; D2) both shoreline and riparian development, for example – a house and a pier, a bulkhead and a paved driveway, etc. In addition, wave exposure was evaluated at each site using the WEMo model developed by Fonseca and Malhotra (2010), which incorporates bathymetry, wind, and shoreline data to calculate wave energy in coastal waters.

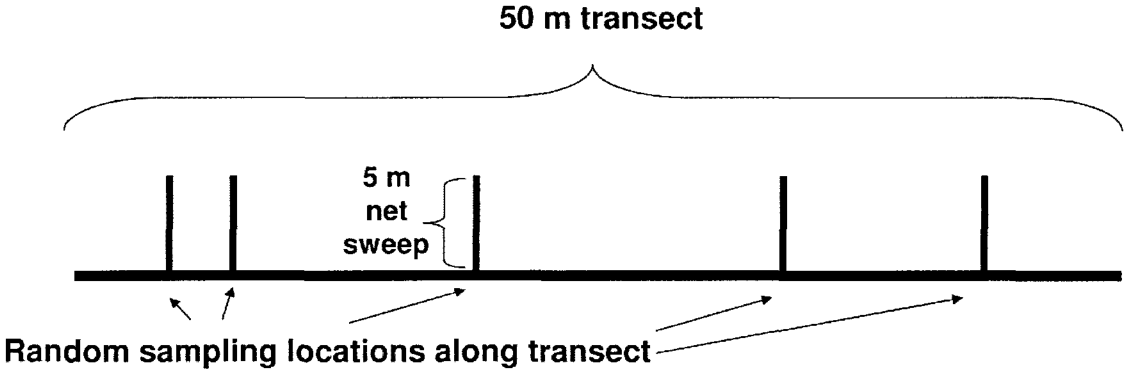

Figure 2. Illustration of transect used for sampling in seagrass beds. Dipnet sweeps and seagrass cover estimates were made along each 5m sub-transect. All other measures of primary and secondary production were sampled at each random location along the 50m transect (indicated in this case by the arrows).

We measured physical parameters at five randomly selected locations along a 50 m transect placed parallel to the shoreline in the middle of the seagrass bed at each site (Figure 2. Water temperature, conductivity, and dissolved oxygen were measured using a handheld data sonde (Yellow Springs Instruments, model 85), coincident with other sampling. To assess whether shoreline development affects the physical environment by altering the sediment composition, small sediment cores (approximately 24 cm3) were also taken at each random location along the transect, stored at -20°C until analysis, and analyzed for both sediment grain size (Plumb 1981) and organic matter content (loss on ignition).

We also sampled primary producers at each randomly selected location along the same 50 m transect. As a proxy for benthic microalgal biomass, small (approximately 1.77 cm3) surface sediment cores were analyzed for chlorophyll a using standard spectrophotometric methods (Lorenzen 1967; Strickland and Parsons 1972). Epiphytic microalgal biomass was estimated from algae scraped off a single *Zostera* shoot (both old and new blades) collected at each random transect location, and processed according to (Douglass et al. 2010). One additional *Zostera* shoot was collected for total nitrogen analysis, as a proxy for estimating integrated nitrogen levels at each site (Osgood and Zieman 1993; McClelland et al. 1997; Moore and Wetzel 2000b; Bertness et al. 2002; Burkholder et al. 2007). These shoots for nitrogen analysis were stored at -20°C until analysis, prepared using standard procedures, and analyzed on a CE Elantech, Inc. Flash 2000 elemental analyzer (Moore and Wetzel 2000b). To assess seagrass biomass, cores (approximately 10 cm deep and 15 cm in diameter) were taken at each random transect location, and sieved in the field to remove sediment. Above-ground material was separated from below-ground material, and all material was dried at 60°C until the mass was stable, and then combusted at 450°C to obtain the ash free dry mass (AFDM). We also assessed percent cover of seagrass at each random transect location, using presence/absence at 1 m intervals along a 5 m sub-transect (see Figure 2).

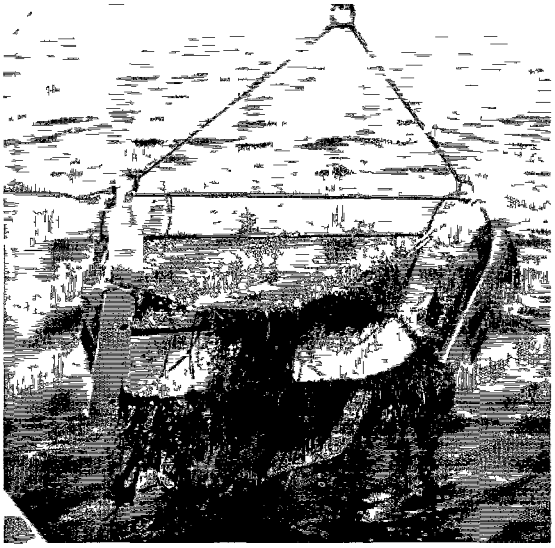

Figure 3
Scrape trawl used to sample nekton (fishes and crabs) in seagrass beds

We collected epifauna, specifically small mesograzers (amphipods and isopods), at each random transect location using a scissor-bucket collector (Virnstein and Howard 1987) that grabs a given quantity of seagrass along with the associated epifauna. Samples were stored at -20°C until analysis, at which time they were thawed, and material retained on a 500-micron sieve was separated and identified to the lowest possible taxonomic level. Eelgrass, algae, and other organisms were dried at 60°C until the mass was stable, and then combusted at 450°C to obtain the ash free dry mass (AFDM). Mesograzers were sieved into size classes (8.0, 5.6, 4, 2.8, 2.0, 1.4, 1.0, 0.71, 0.05 mm), identified to species, and counted. Biomass of grazers was calculated using the empirical equation, $\log B = -1.01 + 2.64 \times \log S$, where B is AFDM (mg), and S is the sieve size (mm) on which the animal was retained (Edgar 1990).

Finally, we measured epifaunal predators and nekton in the seagrass bed at each site. We collected epifaunal predators using a dipnet sweep (0.5 m wide) along the 5 m sub-transect at each random transect location (see Figure 2). We collected nekton predators in four 20 m2 trawls per site using a scrape trawl (see Figure 3). Trawl sampling took place at all sites approximately one week following the sampling for primary and secondary producers. For both dipnet sweep and scrape trawl samples, captured animals were identified to the lowest taxonomic level possible, counted, measured, and returned immediately to the seagrass bed.

Statistical analysis

After initial data exploration, we used Classification and Regression Trees (CART) to explore these data, because CART uses non-linear models to describe the complex patterns frequently found in ecological data (Olden and Jackson 2002), and is not only robust to non-linear relationships and high-order interactions, but produces an easily interpretable result (De'ath and Fabricius 2000; King et al. 2005). Regression and classification trees are built using several explanatory variables to describe the variation in a particular response variable, by repeatedly splitting the data into homogeneous groups to minimize sums of squares within groups (Breiman et al. 1982). This analysis was conducted using R (R Development Core Team 2011), and the package rpart (Therneau et al. 2011).

We also examined patterns in the community composition of epiphytes and epifauna (together referred to as epibiota) between regions and development levels using multivariate non-metric multi-dimensional scaling (NMDS). Data were square-root transformed prior to analysis to reduce the influence of abundant species. Analysis was conducted in R 2.13.0 (R Development Core Team 2011) using the metaMDS function in the vegan package (Oksanen et al. 2011), with a Bray-Curtis dissimilarity matrix. Results were plotted in two dimensions, with vectors showing macroalgae plotted using the envfit procedure in vegan (Oksanen et al. 2011). We also tested the statistical significance of the NMDS ordination using PERMANOVA (adonis function in vegan) (Oksanen et al. 2011), an analysis of variances using a dissimilarity matrix and a permutation test to determine significance.

Finally, because of our nested design we used linear mixed effects models to statistically test the effects of shoreline development on adjacent seagrass communities. Variables lacking homogeneity of variance were either log or square-root transformed. Interaction plots of the effects of a factor on the levels of another factor were generated and visually evaluated a-priori; plot lines that were approximately parallel indicated no or insignificant interactions and were thus excluded from the models. The optimal model was selected following the steps outlined in Zuur et al. (2009). First, we assembled the explanatory variables a-priori for each response variable, and included all the explanatory variables in the fixed part of the global model. Second, to determine the optimal random effects structure for our model, we fit several models with the global fixed effects structure but different random effects structures, including: a random intercept model, a random intercept and random slope model, an intercept-only model, and a model with a variance function to estimate variance within regions independently. We compared these models using Akaike's Information Criterion (AIC), and chose the model with the lowest score, thereby giving us the optimal structure for the random effects portion of the model (Burnham and Anderson 2010). Third, to determine the optimal fixed effects structure for our model, we outlined a-priori several fixed effects structures based on ecological principles, including: the global model, a biological model, a physical model, a development model, a region model, an intercept-only model, and in some cases top-down and bottom-up models (see Appendix A: Tables A1 – A8). Then, using the optimal random effects structure, we fit these different fixed effects structures, and compared the models using corrected AIC (AICc). We chose the model with the lowest AICc score as our optimal model (with the exception of the intercept-only model), or if several models had similar AICc scores (ΔAIC < 3), we chose the simplest among those similar models. The models we present were determined using this process, estimated using restricted maximum likelihood (REML), and evaluated using R 2.13.0 (R Development Core Team 2011), and the car, AICcmodavg, and ggplot2 packages (Wickham 2009; Fox and Weisberg 2011; Mazerolle 2011).

Results

Description of Sites

The amount of development varied among sites from no development to approximately 75% development, with half of all sites having some development along the local coastline. The relative wave exposure was also different among sites, but did not vary more than 0.048 between the lowest and highest exposure. Dissolved oxygen measured during sampling (daylight hours) ranged from 6 - 10 ppm, while water temperature ranged from 24 – 29°C, and salinity ranged from 15 - 20 among sites. There was no consistent difference in dissolved oxygen, temperature, or salinity between Eastern and Western regions. The sediment grain size at sites was generally course, with the proportion of sand ranging from 85 – 97%, and the sediment organic matter varying between 0.004 g and 0.122 g among sites (Appendix A: Table A9).

Sites were initially chosen for the presence of seagrass beds, but seagrass cover varied between 50 – 100%, and seagrass biomass varied from a low of 50 g m^{-2} to a high of 200 g m^{-2} among sites. The percent of nitrogen in *Zostera* leaves varied little among sites, and averaged 1.5%. Crustacean gazer biomass averaged 0.2 g, and predator species richness averaged 5 species across all sites (Appendix A: Table A9).

Trophic relationships and community composition

In general, classification and regression tree (CART) analysis shows that animals were more abundant or had greater biomass when resources were higher. For example, crustacean grazers (amphipods and isopods) had greater biomass when their food, epiphytic algae, had higher biomass (Table 1). Likewise, nekton abundance (e.g. silver perch) was greater when epifaunal predators were more abundant. However, epifaunal predators (e.g. grass shrimp) were more abundant when shelter, in the form of seagrass biomass, was greater. As expected, both nekton and epifaunal predator abundance were positively related to their respective species richness. Thus, animal abundance and biomass appear to follow expected biodiversity and trophic structure relationships.

On the other hand, primary producers appear to be influenced by substrate characteristics and location. Both seagrass biomass and benthic algal biomass were greatest when the percentage of fine sediments (clay and silt) was high, which usually occurs in low energy environments. These low energy conditions may also influence *Zostera* leaf nitrogen, which showed a similar relationship with sediment grain size (Table 4). Finally, epiphytic algal biomass was best described by differences between bay regions (Western and Eastern), and was greater in the West (tree R2 = 0.7, Table 1). Overall, primary producers in contact with the benthos appear most strongly related to sediment properties, while those growing as epiphytes and not in contact with the benthos appear to be related to larger regional factors.

Our examination of the community composition of epiphytes and epifauna (epibionts) between sites using non-metric multi-dimensional scaling (NMDS) indicates that epibiont communities differed significantly between regions (Figure 4a.; p = 0.003, Table 2, Appendix A: Table A10), but not with development (Figure 4b.; p = 0.090, Table 2). Vectors representing epiphytic macroalgal taxa show that *Cladophora*, *Ulva*, and *Ceramium* occurred in greater biomass at sites in the East, while *Gracilaria* and *Polysiphonia* were greater at sites in the West (Figure 4c.). Vectors representing crustacean grazer species indicate greater biomass of caprellid amphipods (*Paracaprella tenuis, Caprella penantis*), the isopod *Paracerceis caudata*, and the gammarid amphipod *Elasmopus levis* in the East (Figure 4d.), but greater biomass of the gammarid amphipod *Ampithoe valida* and the isopod *Erichsonella attenuata* in the West. Consistent with the CART analysis, crustacean grazer biomass was generally higher when epiphytic macroalgal biomass was greater (Figure 4c., d.). Overall, epibiont community composition differed not with development but primarily between the Western and Eastern sides of the bay.

Multivariate drivers of ecosystem properties

To explore in more depth the ecological patterns shown by our CART and NMDS analyses, we used a nested linear mixed effects approach. Our model selection process picked region as the best fixed explanatory variable for all biological responses. However, epiphytic algal biomass was the only response that differed significantly between regions; it was higher in the Western region, especially at several undeveloped sites (Figure 5b., p = 0.0003 Table 3). This corroborates the correlation between epiphytic algal biomass and region seen in the CART analysis (Table 1). At sites that had the highest epiphytic algal biomass, we also observed high grazer biomass, though this trend was not significant and there were no other significant effects on grazer biomass (Figure 5c.). We also found that the biomass of seagrass did not differ significantly either with region or with development, but did vary considerably both within and among sites (Figure 5a., Table 3). Neither did we see significant effects of region on predator and nekton abundance, nor on *Zostera* leaf nitrogen. Therefore, despite the domination of selected models by the explanatory variable region, we saw few significant effects (with the notable exception of epiphytic algal biomass) on biological responses.

Table 1. Summary of results from classification and regression tree (CART) analysis. Middle columns list conditions under which a given variable has the highest and the lowest values, respectively. R^2 values indicate the amount of variance in the variable explained by the model.

Variable	**Greatest when...**	**Least when...**	**R^2**
Seagrass biomass	Nekton abundance LOW % Clay HIGH	Predator abundance LOW % Clay LOW	0.25
Epiphytic algal biomass	Region is West % Silt is LOW Development is <10%	Region is East	0.7
Crustacean grazer biomass	Epiphytic algal biomass HIGH	Epiphytic algal biomass LOW Nekton abundance LOW	0.4
Predator abundance	Predator species richness HIGH Epiphytic algal biomass LOW	Predator species richness LOW Seagrass biomass LOW	0.4
Predator species richness	Predator abundance HIGH Epiphytic algal biomass HIGH	Predator abundance LOW	0.5
Nekton abundance	Nekton species richness HIGH Predator abundance HIGH	Nekton species richness LOW Epiphytic algal biomass HIGH	0.25
Nekton species richness	Nekton abundance HIGH % Clay HIGH	Nekton abundance LOW	0.65
Zostera leaf Nitrogen	Nekton species richness HIGH	Nekton species richness LOW % Silt LOW	0.45
Benthic algal biomass	% Silt is HIGH	% Silt is LOW	0.15

The best models for the sediment response variables contained a greater variety of explanatory variables. Sediment organic matter and sediment grain size varied inversely and significantly ($p < 0.0001$, Figure 5, Table 3). Sediment organic matter was also significantly affected by development, but not by region ($p = 0.002$, $p = 0.126$ respectively, Table 3). However, these development effects appear to vary between regions, with apparently higher sediment organic matter found at undeveloped sites in the West, but at sites with either shoreline or riparian development in the East (Figure 5). Sediment grain size was significantly different between regions ($p = 0.042$, Figure 5, Table 3) and development levels, however significant interactions make development main effects difficult to interpret ($p = 0.002$, $p = 0.005$ respectively, Table 3). Thus, in contrast to biological responses, sediment characteristics appear to be strongly affected by development as well as region. Therefore, biological and sediment properties of seagrass communities may be driven by different factors.

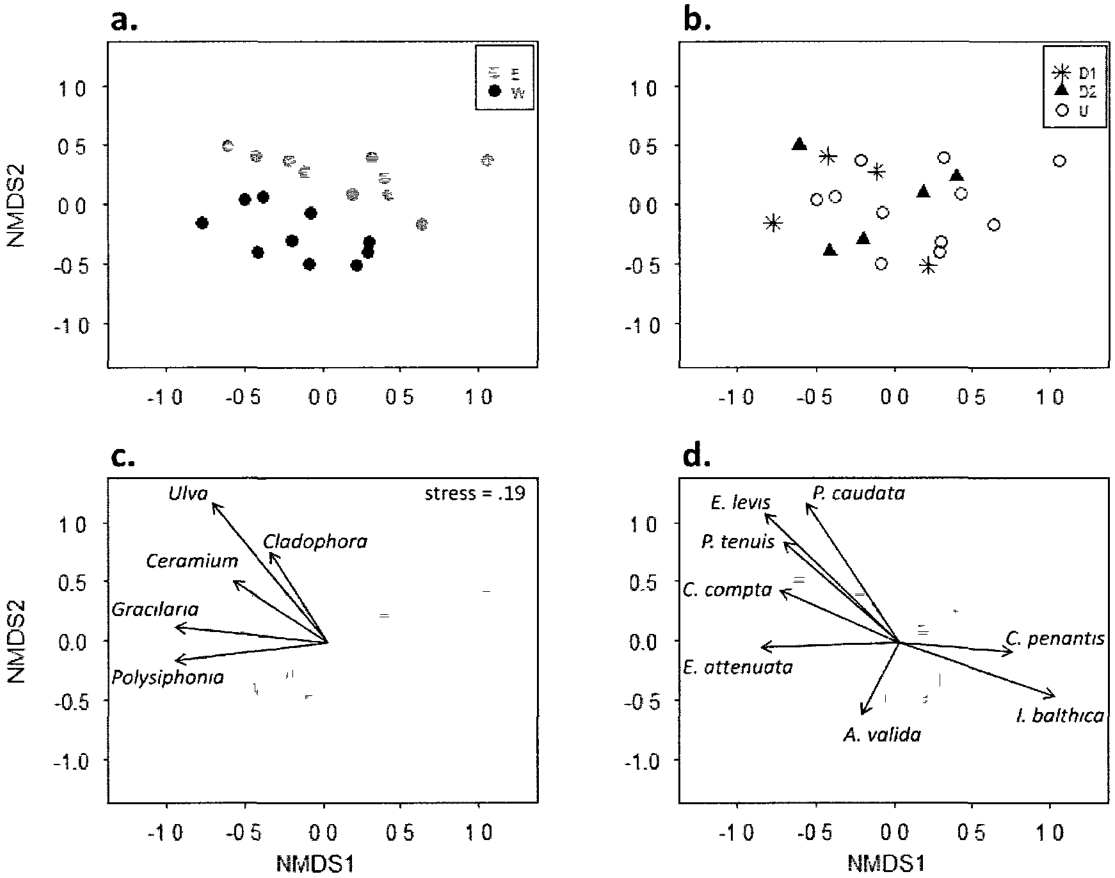

Figure 4. Plots from non-metric multi-dimensional scaling (NMDS) ordination of epibiota (normalized to seagrass biomass) collected using the scissor-bucket grab sampler. Each point represents the community at one site/seagrass bed. Stress = 0.19. a) plot of all sites grouped by region: gray = East, black = West; b) plot as in a, but grouped by development: U = undeveloped, D1 = either shoreline or riparian development, D2 = both shoreline and riparian development; c) plot as in a, but with vectors representing algal groups (*Polysiphonia spp., Gracilaria sp., Ceramium spp., Ulva spp., Cladophora sp.*); d) plot as in a, but with vectors representing crustacean grazer species (*Caprella penantis, Idotea balthica, Ampithoe valida, Erichsonella attenuata, Cymadusa compta, Paracaprella tenuis, Elasmopus levis, Paracerceis caudata*).

Discussion

With this survey, we aimed to determine whether coastal development was coincident with reduced biomass and abundance of seagrass-associated animals, increased nutrient inputs, and altered sediment characteristics. Generally, biological responses tended to be influenced by bay region (Western and Eastern) rather than development, but we saw few significant results with the notable exception of epiphytic algal biomass. *Zostera* leaf nitrogen, our indicator of integrated nitrogen inputs, likewise did not differ significantly with development or between regions. However, sediment characteristics were significantly affected by development and differed by region. Thus, our data suggest that different factors are influencing seagrass-associated animals, nutrient enrichment, and sediment composition.

Table 2. Results from PERMANOVA analysis, testing for differences in community composition with experimental treatment. This analysis used square-root transformed data and a Bray-Curtis dissimilarity matrix, as used for the NMDS ordination.

Factor	DF	SS	MS	F	p
Region (R)	1	0.538	0.538	2.985	**0.003**
Development (D)	2	0.518	0.259	1.436	0.090
R : D	2	0.435	0.217	1.206	0.250
Error	14	2.522	0.180		

Region had a large effect on several variables measured in this survey: the best-fitting mixed-effects models always included region, frequently as the only explanatory variable. However, abundance of nekton and small epifaunal predators did not differ significantly between regions, perhaps because these animals have patchy distributions, or because they are very mobile despite a high affinity for seagrass beds. Nekton and epifaunal predator abundances were positively related as expected, likely because there is some overlap in species represented in the two groups, but also because they both rely on the shelter provided by habitat-forming seagrasses. Previous studies focused primarily on infaunal organisms and their predators, which appear to respond to local coastal development, perhaps due to their comparative lack of mobility (King et al. 2005; Bilkovic et al. 2006; Seitz et al. 2006).

Epibiont (epifauna and epiphytes) community composition did differ between regions, perhaps driven by regional differences in macroalgal epiphyte biomass, as seen with microalgal epiphytes (measured as chlorophyll a). We expected that *Zostera* leaf nitrogen would be higher when epipihytic algal biomass was higher, indicating increased nitrogen inputs that could be driving epiphytic algal bimoass, but there was no difference in *Zostera* leaf nitrogen with either development or region. Therefore, we were not able to determine what mechanism may be causing these regional differences in epiphytic algae, nor were we able to demonstrate increased nutrient inputs along developed shorelines.

Crustacean grazer biomass appears unlikely to have influenced the observed differences in epibiont community composition between regions, as it did not differ significantly with region. Crustacean grazers, the primary grazers in temperate seagrass systems, play an important role in maintaining the dominance of seagrass over more ephemeral algae, but we saw no negative effect of grazers on epiphytic algae in this survey. In fact, grazer biomass was higher at several sites that also had high epiphytic

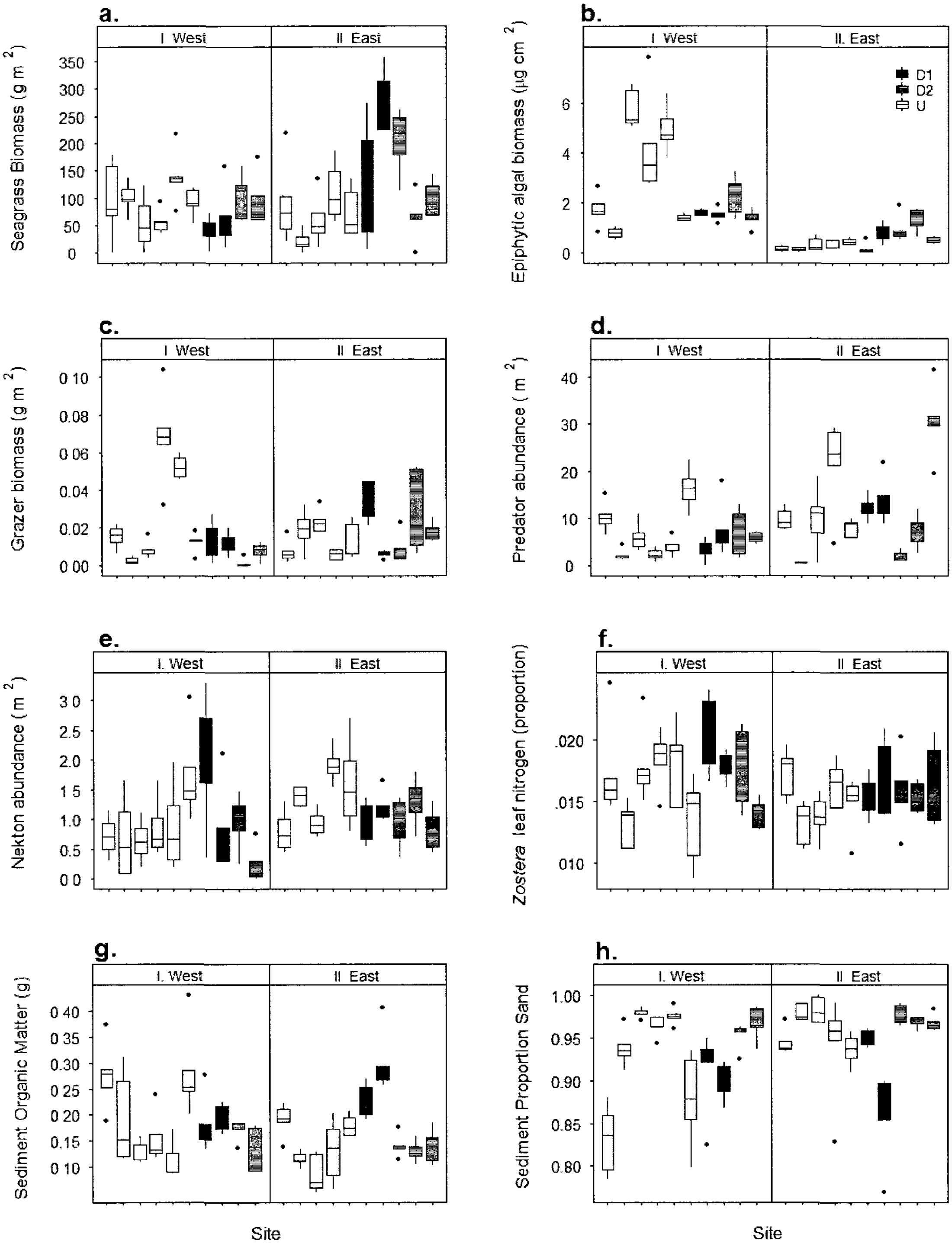

Figure 5. Boxplots of biomass, abundance, or proportion for each response variable: a) seagrass biomass, b) epiphytic algal biomass (as chl *a*), c) crustacean grazer biomass, d) epifauna predator abundance, e) nekton abundance, f) *Zostera* leaf nitrogen, g) sediment organic matter, h) sediment grain size. Colors represent level of development: U = undeveloped, D1 = either shoreline or riparian development, D2 = both shoreline and riparian development.

algal biomass, indicating that these grazer communities, which consume primarily epiphytic macroalgae and microalgae, may be controlled primarily by bottom-up factors such as food resources and shelter (Douglass et al. 2010). However, recent field experiments excluding grazers have shown strong crustacean grazer impacts on algal biomass in Chesapeake Bay seagrass beds (Whalen 2011). Thus, trophic controls may vary between species and across spatial and temporal scales (Douglass et al. 2010).

In this study, seagrass biomass also appears to have been influenced by bottom-up factors, primarily sediment grain size. Although sediment grain size was significantly affected by development, we saw no effect of development on seagrass biomass. It is likely that our study did not capture the effects of increased nutrient and sediment inputs, as the negative impacts of these stressors on seagrasses are widely known (Moore, Neckles, and Orth 1996b; Moore and Wetzel 2000a; Burkholder et al. 2007; Moore and Jarvis 2008). We did, however, observe some differences in spatial stability (lack of variability between sites within a region) between regions in seagrass biomass and *Zostera* leaf nitrogen (Garibaldi et al. 2011), but our survey design did not allow us to test this statistically. However, this raises an important question: do anthropogenic stressors associated with coastal development decrease the spatial stability of important ecosystem properties and processes in seagrass systems? For example, do increased nutrient inputs decrease the stability of seagrass biomass in developed watersheds? This is an important question that could be addressed with future research on both local and regional spatial scales.

Spatial scale is an important consideration in this survey. Not only is the scale of this study important, but also the scale at which anthropogenic stressors act, as well as the scale at which ecosystem functions and process occur. We expected anthropogenic stressors associated with coastal development to act on the local spatial scale at which we measured (hundreds of meters). However, our results suggest that drivers of animal abundance and biomass, epiphytic algal biomass, seagrass biomass, and sediment properties operate at different spatial scales. For example, sediment properties seem to vary at the local scale that we measured, which corroborates previous work (Jennings et al. 2003; Goforth and Carman 2005; King et al. 2005; Seitz et al. 2006). But, epiphytic algal biomass seemed to vary on a larger regional scale, between the West and East sides of the bay. This suggests that the driver of epiphytic algal biomass may act on the sub-watershed rather than the local scale (Bilkovic et al. 2006). If, as we hypothesized though our *Zostera* leaf nitrogen data did not show it, the driver was increased nutrient inputs, this pattern may be due to the well-mixed nature of Chesapeake Bay or the diffuse nature of non-point source nutrient inputs. Thus, our data highlight the need to better match the scale of sampling to the scale at which stressors may occur so that real patterns and influences can be more clearly detected.

Table 3. Results from nested linear mixed effects models. Results are from the best model (excluding the intercept-only model; based on AICc scores). Site was considered a random effect, so estimates of among-Site standard deviation (SD) and within-Site SD (residuals) are shown. Models for some response variables were estimated with independent variances by region (West and East), which represent the random "noise" at this level of nesting (Zuur et al. 2009).

		Seagrass Biomass				Epiphytic Algal Biomass			
		Value	DF	F	p	Value	DF	F	p
Fixed Effects:	**Intercept**	111.984	80	60.87	**<0.0001**	0.656	80	138.64	**0.0002**
	Region	-27.729	18	1.22	0.285	0.861	18	21.86	**0.0003**
Random Effects:	**among-Site SD**	50.910				0.401			
	within-Site SD (residuals)	53.446				0.223			
Variance (error)	**West**					0.223			
	East					0.202			

		Grazer Biomass				Predator Abundance			
		Value	DF	F	p	Value	DF	F	p
Fixed Effects:	**Intercept**	0.119	80	81.67	**<0.0001**	3.079	80	117.06	**<0.0001**
	Region	-0.002	18	0.01	0.935	0.742	18	2.21	0.155
Random Effects.	**among-Site SD**	0.056				1.071			
	within-Site SD (residuals)	0.035				0.675			
Variance (error):	**West**					0.675			
	East					0.756			

		Nekton Abundance				Zostera Leaf Nitrogen			
		Value	DF	F	p	Value	DF	F	p
Fixed Effects:	**Intercept**	1.062	59	525.25	**<0.0001**	0.016	80	1265.42	**<0.0001**
	Region	-0.159	18	2.95	0.103	0.001	18	2.29	0.148
Random Effects:	**among-Site SD**	0.139				0.002			
	within-Site SD (residuals)	0.380				0.003			
Variance (error):	**West**	0.380							
	East	0.201							

		Sediment Organic Matter				Sediment Grain Size			
		Value	DF	F	p	Value	DF	F	p
Fixed Effects:	**Intercept**	1.296	78	737.60	**<0.0001**	0.98901	72	40530	**<0.0001**
	Region (R)	-0.003	16	2.60	0.126	-0.014	16	4.87	**0.042**
	Development (D)	-0.024	16	9.12	**0.002**	0.072	16	9.01	**0.002**
	Sed. organic matter (OM)					0.028	72	152	**<0.0001**
	Seagrass Biomass (Sg)					0.000	72	1.89	0.174
	Sediment Size	-1.181	78	136.53	**<0.0001**				
	R : OM					-0.095	72	0.08	0.772
	R : Sg					0.000	72	2.84	0.096
	D : Sg					0.001	72	5.70	**0.005**
	Sg : OM					-0.001	72	7.74	**0.007**
Random Effects:	**among-Site SD**	0.025				0.019			
	within-Site SD (residuals)	0.032				0.018			

This is a particularly important consideration for the management of anthropogenic stressor impacts in coastal systems, because management efforts may have the most impact when they match the spatial scale at which drivers of ecosystem processes and services operate.

In summary, we found few clear effects of local coastal development on seagrass-associated consumers, but found substantial variation at regional scales in that epifauna and epiphytes differed between the Western and Eastern sides of Chesapeake Bay. Likewise, we could not detect nutrient enrichment in seagrass beds adjacent to development. In contrast, sediment characteristics appear to differ strongly with both development and region. Our results demonstrate that the factors driving biological and sediment properties in seagrass systems differ, and perhaps act on different scales. The scale of anthropogenic stressor impacts will also be key to the implementation of effective management of coastal development impacts on adjacent shallow-water habitats. Further research is necessary to tease apart the factors and mechanisms behind the patterns observed in this survey, and to understand how coastal development will affect coastal systems in the future.

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CONCLUSIONS

The overall goal of my research was to understand the ecological impacts and interactions of anthropogenic stressors stemming from climate change and coastal development on the persistence and functioning of seagrass systems in Chesapeake Bay. Specifically, my foci were to 1) assess the effects and interactions of multiple anthropogenic stressors and changes in animal diversity on the structure and functioning of eelgrass systems, and 2) examine the impacts of local coastal development on the composition, functioning, and structure of adjacent seagrass communities. My experimental results show that crustacean grazers maintained grazing pressure and that grazer diversity reduced the variation in epiphytic algal biomass in spite of multiple anthropogenic stressors. Stressor impacts were generally independent, without ecologically important interactions, and context specific, only occurring under certain conditions, while grazer diversity had an important but limited role in buffering certain ecosystem properties, such as epiphytic algal biomass, against stressors. My field survey results suggest that local development significantly altered sediment characteristics, that epifauna and epiphytes varied between Western and Eastern regions of the bay, and that grazer biomass and predator biomass did not vary with either local development or bay region. Thus, different factors appear to influence ecosystem properties at various spatial scales, an important consideration for predicting future impacts of multiple anthropogenic stressors in submerged vegetated systems.

One important factor in the predictability of ecosystem functions and services is stability. Ecologically stable ecosystems are able to maintain important ecosystem properties in the face of stressors. Stability should increase as species richness increases according to ecological theory (Yachi and Loreau 1999; Thebault and Loreau 2005), because increased functional redundancy provides biological insurance against the impacts of environmental stressors (Elmqvist et al. 2003; Hooper et al. 2005; Griffin et al. 2009). I chose to examine resistance stability (ability to withstand stressor impacts) in my experiments, and found some support for the importance of stability in this model seagrass system. Stability is of general importance in seagrass systems because these habitats may be able to provide more consistent ecosystem services, such as fisheries production, when they harbor more diverse communities and are thus able to better resist environmental changes. Spatial stability of ecosystem properties among seagrass beds may also be important to the maintenance of ecosystem services, but may be altered by changes in community composition (France and Duffy 2006). Though I did not test for spatial stability directly, it may also be an important consideration in the context of my field survey and will likely be tested in the next step of this research.

The stability of important ecosystem properties may be altered by anthropogenic stressors, especially when multiple stressors interact and produce unexpected results (Paine et al. 1998; Folt et al. 1999; Vinebrooke et al. 2004; Darling and Cote 2008). Anthropogenic stressors impact many coastal habitats, but are of particular concern in seagrass systems because these important habitats are vulnerable to many stressors and provide valuable ecosystem services such as fisheries production and sediment stabilization. Climate change and coastal development will likely continue to be important sources of anthropogenic stressors such as warming and increased freshwater, sediment and nutrient inputs to Chesapeake Bay seagrass systems (Najjar et al. 2000; Najjar et al. 2008; Najjar et al. 2010). The experimental data show that warming, shading (my proxy for the effects of suspended sediments), and nutrients negatively affected eelgrass, suggesting that these stressors continue to threaten the persistence of eelgrass in Chesapeake Bay. What these data do not show is strong interactions among stressors, suggesting that stressors likely act individually which may make their impacts in eelgrass systems more predictable in the future. The stressors used in these experiments were chosen because they can negatively impact eelgrass, but I found that warming and freshwater pulses both affected not only primary producers but also crustacean grazers and sessile invertebrates. This indicates that while the strongest anthropogenic stressor effects may be on the habitat-forming seagrass, epifaunal animals may not only be indirectly impacted by loss of habitat but also directly impacted as well.

Freshwater pulses had strong impacts on crustacean grazer species, but grazers were largely still able to maintain grazing pressure. However, it is important to note that my experimental systems did not include predators, which may affect the ability of the grazer community to maintain this important ecosystem function (Duffy et al. 2005). Results of the field survey, where predators were present, suggest that grazers were more abundant when epiphytic algal biomass is higher, a bottom-up rather than top-down effect as observed in my experiments ((Douglass et al. 2010), but see (Whalen 2011)). A fuller understanding of the trophic controls in these seagrass systems will require additional examination of the mechanisms controlling these populations.

The stability of ecosystem properties and services is predicted to increase as species richness increases. In my experiments, grazer diversity altered stressor effects in some cases, and buffered epiphytic algal biomass against stressor impacts. This means that epiphytic algal biomass was more stable in the presence of a greater number of grazer species. These results follow the predictions of ecological theory (Yachi and Loreau 1999; Thebault and Loreau 2005) in that resistance stability increased as species richness increased. This relationship occurs because the experimental grazer assemblages were functionally diverse in their response to environmental stressors, but largely functionally redundant in their grazing effects. For example, I found that some grazer species were negatively impacted by freshwater pulses while other species were not, but that all grazers generally reduced epiphytic micro- and macroalgal biomass. If diverse crustacean grazer assemblages are able to maintain grazing pressure and stabilize epiphytic algal biomass despite stressors under more realistic field conditions, perhaps stressor effects may be less than expected. This stabilizing effect of grazer diversity may be critical to predicting future impacts of increased nutrient inputs and climate warming on the dominance of habitat-forming seagrasses in Chesapeake Bay.

Stressor effects on ecosystem properties and faunal communities may occur on different spatial scales, making it important to consider not only the scale at which anthropogenic stressors act but also the scale at which ecosystem functions and processes occur. Data from this field survey suggests that drivers of sediment properties act on the local scale of this survey (hundreds of meters), while drivers of epiphytic algal biomass may act on the sub-watershed scale, and mobile animal abundances may potentially be driven by bay-wide factors. These data highlight the need for future studies to better match the scale of sampling to the scale at which stressors occur so that real patterns and influences can be more clearly detected. A related question is whether spatial stability (inverse of variation between sites within a region or watershed) of ecosystem properties is affected by anthropogenic stressors. Though I did not test this specifically, I think it is an important question that could be addressed with future research on both local and regional spatial scales.

Overall, my experiments and field survey indicate that anthropogenic stressors may negatively impact seagrass communities in Chesapeake Bay, especially as climate change and coastal development increase. Increased frequency and severity of storm events are predicted to increase freshwater inputs, which could shift salinity regimes towards the mouth of the bay. Distributions of animals may also shift as salinity regimes change and temperatures increase, and more southern species may take up residence in Chesapeake Bay. My experimental results suggest that pulsed changes in salinity may primarily impact animals, but larger shifts in salinity regimes may also affect macrophytes and other primary producers. In spite of these impacts, I have shown that crustacean grazers were generally able to maintain grazing, an important ecosystem process that promotes seagrasses over ephemeral algae, even in the face of multiple stressors. Diversity of these grazers may also play an important role in maintaining grazing such that epiphytic algal biomass is stabilized at consistently low levels. These results may be important to the persistence of vulnerable habitats in coastal areas, such as seagrass systems, as climate change continues.

Rising temperatures as well as reduced light levels may play a role in shifting the relative abundance of seagrass species in coastal systems, while coastal development may impact seagrass habitats not only through increased nutrient and sediment inputs, but also through fragmentation of existing seagrass beds. My results suggest that continued warming may negatively impact not only eelgrass but also epiphytic macro- and microalgae, while reduced light levels will likely negatively impact all primary producers. However, these stressors as well as others used in my experiments did not interact in ecologically important ways, indicating that predicting stressor impacts in these coastal systems may be more simple than previously assumed. My field survey results suggest that coastal development may alter sediment properties at the local scale, epiphytic macro- and microalgal biomass at the sub-watershed scale, and animal abundances at perhaps a larger estuary-wide scale. Thus, the impacts of development-associated habitat fragmentation could potentially have variable effects on different aspects of coastal seagrass communities, and emphasizes the need to implicitly incorporate the scale of anthropogenic stressor impacts in future research.

In order to be able to predict and manage the effects of climate change and development in coastal systems, future research should include identifying the mechanisms through which stressors act, and further identifying the scales at which they influence ecosystem processes. It will also be important to identify community-level responses to stressors and changes in community composition and diversity as a result of stressor impacts. Finally, management efforts in these systems may have the most impact when they are matched to the spatial scale of trophic controls and physical variables such as sediment properties, and thereby can promote the maintenance of these important ecosystem processes and services in the face of multiple anthropogenic stressors.

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APPENDIX

Table A1. Candidate fixed effects models for seagrass biomass specified a-priori, and evaluated using AICc

Seagrass Biomass	**Models**					
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Sediment Organic Matter (OM)	✓		✓			
Zostera Leaf Nitrogen (LN)	✓		✓			
Water Temperature (T)	✓		✓			
Grazer Biomass (Gz)	✓	✓				
Epiphytic Chlorophyll *a* (EChl)	✓	✓				
R : LN	✓		✓			
R : Gz	✓	✓				
R : OM	✓		✓			
R : EChl	✓	✓				
D : LN	✓		✓			
D : Gz	✓	✓				
D : OM	✓		✓			
LN : T	✓		✓			
LN : Gz	✓					
LN : OM	✓		✓			
LN : EChl	✓					
T : Gz	✓					
T : EChl	✓					
Gz : OM	✓					
Gz : EChl	✓	✓				
EChl : OM	✓					
AICc	1142.12	1129.32	1117.15	1122.29	1120.06	1119.2
ΔAICc	24.96	12.17	0	5.14	2.91	2.05
AICc weight	0	0	0.6	0.05	0.14	0.21

Table A2. Candidate fixed effects models for epiphytic algal biomass specified a-priori, and evaluated using AICc.

Epiphytic Chlorophyll *a*	Models							
Predictors	Global Model	Biological Model	Physical Model	Development Model	Region Model	Bottom-up Model	Top-down Model	Intercept Model
Region (R)	✓	✓	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		✓	✓	
Site	✓	✓	✓	✓	✓	✓	✓	✓
Zostera Leaf Nitrogen (LN)	✓		✓			✓		
Water Temperature (T)	✓		✓				✓	
Grazer Biomass (Gz)	✓	✓					✓	
Gz : T	✓						✓	
Gz : LN	✓							
LN : T	✓		✓					
Gz : D	✓							
LN : D	✓		✓			✓		
T : D	✓		✓					
AICc	67.45	46.72	57.65	44.43	40.87	50.15	51.15	54.54
ΔAICc	26.58	5.85	16.78	3.56	0	9.27	10.28	13.67
AICc weight	0	0.04	0	0.14	0.81	0.01	0	0

Table A3. Candidate fixed effects models for crustacean grazer biomass specified a-priori, and evaluated using AICc.

Grazer Biomass	Models					
Predictors	Global Model	Biological Model	Physical Model	Development Model	Region Model	Intercept Model
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Water Temperature (T)	✓		✓			
Seagrass Biomass (Sg)	✓	✓				
Epiphytic Chlorophyll *a* (EChl)	✓	✓				
Predator Biomass (Pd)	✓	✓				
R : Sg	✓	✓				
R : Pd	✓	✓				
R : EChl	✓	✓				
D : Sg	✓	✓				
D : Pd	✓	✓				
D : EChl	✓	✓				
T : Sg	✓					
T : Pd	✓					
T : EChl	✓					
Sg : Pd	✓	✓				
Sg : EChl	✓	✓				
EChl : Pd	✓	✓				
AICc	-292.4	-300.78	-321.5	-322.27	-325.59	-327.76
ΔAICc	35.35	26.98	6.26	5.49	2.16	0
AICc weight	0	0	0.03	0.04	0.23	0.69

Table A4. Candidate fixed effects models for predator abundance specified a-priori, and evaluated using AICc.

Predator Abundance	**Models**					
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Benthic Chlorophyll *a* (BChl)	✓	✓				
Water Temperature (T)	✓		✓			
Grazer Biomass (Gz)	✓	✓				
Seagrass Biomass (Sg)	✓	✓				
R : Gz	✓	✓				
D : BChl	✓	✓				
D : T	✓		✓			
D : Sg	✓	✓				
D : Gz	✓	✓				
BChl : T	✓					
BChl : Sg	✓	✓				
BChl : Gzr	✓	✓				
T : Sg	✓					
T : Gz	✓					
Sg : Gz	✓	✓				
AICc	308.29	297.73	284.83	279.5	275.1	275.2
ΔAICc	33.19	22.63	9.73	4.4	0	0.1
AICc weight	0	0	0	0.05	0.48	0.46

Table A5 *Candidate fixed effects models for nekton abundance specified a-priori, and evaluated using AICc*

Nekton Abundance	**Models**							
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Bottom-up Model**	**Top-down Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		✓	✓	
Site	✓	✓	✓	✓	✓	✓	✓	✓
Water Temperature (T)	✓		✓			✓		
Seagrass Biomass (Sg)	✓	✓				✓		
Grazer Biomass (Gz)	✓	✓					✓	
Predator Biomass (Pd)	✓	✓					✓	
R : T	✓		✓			✓		
R : Gz	✓	✓					✓	
R : Pd	✓	✓					✓	
D : T	✓		✓			✓		
D : Sg	✓	✓				✓		
D : Gz	✓	✓					✓	
D : Pd	✓	✓					✓	
Sg : Gz	✓	✓						
Sg : Pd	✓	✓						
T : Sg	✓					✓		
T : Gz	✓							
T : Pd	✓							
Gz : Pd	✓	✓					✓	
AICc	84 6	68 03	52 59	46 52	43 88	61 95	55.46	44 64
ΔAICc	40 72	24 15	8 71	2 64	0	18 07	11 58	0 76
AICc weight	0	0	0 01	0 14	0 51	0	0	0 35

Table A6. Candidate fixed effects models for *Zostera* leaf nitrogen specified a-priori, and evaluated using AICc.

Zostera Leaf Nitrogen	**Models**					
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Water Temperature (T)	✓		✓			
Sediment Organic Matter (OM)	✓		✓			
Epiphytic Chlorophyll *a* (EChl)	✓	✓				
R : OM	✓		✓			
D : OM	✓		✓			
D : T	✓		✓			
D : EChl	✓	✓				
OM : T	✓		✓			
OM : EChl	✓					
EChl : T	✓					
AICc	-853.69	-872.36	-861.51	-873.01	-873.4	-873.18
ΔAICc	19.71	1.04	11.89	0.39	0	0.22
AICc weight	0	0.18	0	0.25	0.3	0.27

Table A7. Candidate fixed effects models for sediment organic matter specified a-priori, and evaluated using AICc.

Sediment Organic Matter	**Models**					
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Sediment Grain Size (Ss)	✓		✓			
Benthic Chlorophyll *a* (BChl)	✓	✓				
Seagrass Biomass (Sg)	✓	✓				
R : BChl	✓	✓				
R : Sg	✓	✓				
D : Sg	✓	✓				
D : BChl	✓	✓				
Sg : BChl	✓	✓				
AICc	-345.94	-258.27	-361.86	-275.86	-275.02	-276.55
ΔAICc	15.92	103.59	0	86	86.83	85.31
AICc weight	0	0	1	0	0	0

Table A8. Candidate fixed effects models for sediment grain size specified a-priori, and evaluated using AICc.

Sediment Grain Size	**Models**					
Predictors	**Global Model**	**Biological Model**	**Physical Model**	**Development Model**	**Region Model**	**Intercept Model**
Region (R)	✓	✓	✓	✓	✓	
Development (D)	✓	✓	✓	✓		
Site	✓	✓	✓	✓	✓	✓
Sediment Organic Matter (OM)	✓		✓			
Seagrass Biomass (Sg)	✓	✓				
R : OM	✓		✓			
R : Sg	✓	✓				
D : Sg	✓	✓				
Sg : OM	✓					
AICc	-422.17	-372.11	-408.87	-365.79	-365.02	-366.02
ΔAICc	0	50.06	13.3	56.38	57.15	56.15
AICc weight	1	0	0	0	0	0

Table A9 Data used in all analyses Wave Exposure was calculated using the WEMo model (Fonseca and Malhotra 2010) Seagrass cover was measured as percent cover Sessile Invertebrate data is ash free dry mass (biomass) Seagrass biomass, Predator abundance, and Trawl abundance were normalized to m ^{2}, epiphytic chlorophyll *a* is normalized to cm ^{2}, grazer and sessile invertebrate biomass are normalized to biomass *of seagrass sampled Devel = Development, Temp = Temperature, OM = Organic Matter, Chl =* Chlorophyll, Invert = Invertebrate, Pred Abun = Predator Abundance, Sp = Species

	Western Region Sites										Western Region Mean
	Allen s East	Brown s Bay	Dyer Creek	Gaines Point	Goodwin Island	Goodwin Neck	Guinea Marsh	Jenkins Neck	New Pt Comfort	Potato Neck	
% Devel Shoreline	0	0	7	47	0	77	0	0	0	0	13 10
% Devel Riparian	0	0	0	0	0	48	0	0	0	0	4 80
Piers or Houses				1				1			
Devel Index	U	U	D1	D2	U	D2	U	D1	U	U	
Wave Exposure	0 047	0 023	0 029	0 015	0 015	0 029	0 014	0 026	0 011	0 044	0 025
DO	7 07	7 02	6 55	6 04	8 96	9 60	10 81	6 73	6 37	9 57	7 87
Salinity	19 50	18 60	17 50	19 90	19 70	19 60	18 30	19 70	20 96	16 64	19 04
Water Temp	28 84	27 68	27 92	24 40	28 86	28 06	27 52	24 60	25 04	29 38	27 23
Sediment % Sand	83 12	93 82	91 38	96 66	97 91	95 31	96 35	89 84	97 56	87 80	92 97
Sediment OM	*0 122*	*0 039*	*0 057*	*0 018*	*0 006*	*0 018*	*0 023*	*0 075*	*0 013*	*0 078*	*0 045*
Benthic Chl *a*	247 61	206 80	140 13	92 51	243 53	274 82	161 90	185 03	112 92	168 36	183 36
Seagrass Cover	100	100	88	80	64	56	72	96	92	100	84 80
Seagrass Biomass	97 20	100 71	41 72	93 13	55 14	103 80	56 74	62 33	139 34	92 46	84 26
Zostera Leaf % N	1 74	1 31	2 09	1 40	1 79	1 81	1 84	1 78	1 79	1 34	1 69
Epiphytic Chl *a*	1 74	0 79	1 62	1 37	5 76	2 34	4 27	1 53	4 96	1 41	2 58
Grazer Biomass	0 015	0 003	0 013	0 007	0 008	0 001	0 068	0 012	0 052	0 012	0 019
Sessile Invert	0 36	0 11	0 04	0 05	0 05	0 09	0 05	0 03	0 19	0 05	0 10
Pred Abun	10 24	2 24	3 28	5 84	6 08	6 00	2 32	8 00	3 84	16 32	6 42
Pred Sp Richness	5 00	2 00	2 40	4 00	4 40	3 20	2 20	4 00	4 40	3 80	3 54
Trawl Abun	0 71	0 70	2 05	0 24	0 64	0 96	0 86	0 79	0 88	1 75	0 96
Trawl Sp Richness	4 75	3 75	5 25	2 00	3 25	4 75	2 75	4 50	4 00	6 25	4 13

	Eastern Region Sites										Eastern Region Mean
	Cape Charles	Church Neck	Downings Beach	Hungars Creek	Hyslop Marsh	Mill Point	Occo Creek	Old Town Neck	Sandy Point	The Gulf	
% Devel Shoreline	33	0	0	73	0	0	0	0	13	47	16 60
% Devel Riparian	5	0	0	0	0	10	0	0	0	20	3 50
Piers or Houses						1					
Devel Index	D2	U	U	D1	U	D2	U	U	D1	D2	
Wave Exposure	0 020	0 027	0 018	0 025	0 013	0 025	0 025	0 033	0 059	0 018	0 026
DO	9 65	10 58	7 24	8 42	6 40	7 21	6 49	6 85	8 94	8 07	7 98
Salinity	19 14	16 28	15 04	17 80	15 10	18 80	14 92	18 00	15 32	18 60	16 90
Water Temp	28 14	26 86	28 08	26 30	23 40	28 18	24 52	24 54	29 02	27 36	26 64
Sediment % Sand	97 60	94 49	97 95	95 05	98 27	96 79	93 93	93 64	85 77	96 75	95 02
Sediment OM	0 004	0 028	0 002	0 024	0 004	0 007	0 036	0 030	0 083	0 014	0 023
Benthic Chl *a*	186 39	138 77	186 39	176 87	148 30	145 57	81 63	103 40	131 97	138 77	143 81
Seagrass Cover	96	100	80	100	92	80	92	72	100	100	91 20
Seagrass Biomass	203 44	91 66	21 06	126 36	60 04	64 07	112 25	74 00	270 46	96 50	111 98
Zostera Leaf % N	1 57	1 72	1 32	1 54	1 37	1 53	1 64	1 48	1 72	1 62	1 55
Epiphytic Chl *a*	0 96	0 16	0 14	0 16	0 36	1 37	0 39	0 45	0 85	0 51	0 53
Grazer Biomass	0 009	0 007	0 019	0 034	0 023	0 028	0 006	0 016	0 006	0 018	0 017
Sessile Invert	0 01	0 00	0 00	0 62	0 00	0 02	0 02	0 15	0 13	0 20	0 12
Pred Abun	2 24	9 84	0 56	12 16	9 92	7 20	21 44	8 00	14 08	30 72	11 62
Pred Sp Richness	2 60	4 40	1 00	4 60	3 60	2 60	4 00	2 80	3 00	3 60	3 22
Trawl Abun	0 94	0 80	1 39	0 95	0 94	1 30	1 91	1 60	1 20	0 81	1 18
Trawl Sp Richness	5 50	5 50	3 25	5 75	4 00	5 00	4 75	6 00	5 00	5 50	5 03

Table A10 Biomass of all epiphyte and epifauna taxa, normalized to seagrass biomass, that were collected in the scissor-bucket grab sampler

	West		East	
Taxon	Total Biomass (g)	% of Overall Biomass	Total Biomass (g)	% of Overall Biomass
Ampelisca sp	0.0002	0.003	0	0
Ampithoe longimana	0.1017	1.34	0.0503	0.65
Ampithoe valida	0.0063	0.08	0.0015	0.02
Anadara transversa	0	0	0.0009	0.01
Anemone	0.0003	0.004	0.0013	0.02
Balanus improvisus	0.2027	2.67	0.1076	1.38
Batea sp	0	0	0.0001	0.00
Bittium varium	0.4084	5.38	0.1607	2.06
Blenny	0.0275	0.36	0	0
Botryllus schlosseri	1.1516	15.18	2.4254	31.13
Bowerbankia sp	0.0735	0.97	0.0020	0.03
Bryozoan	0.6054	7.98	0.0923	1.18
Callinectes sapidus	2.1364	28.15	0	0
Caprella equilibria	0.0005	0.01	0.0011	0.01
Caprella penantis	0.3401	4.48	0.2574	3.30
Conopeum tenuissimum	1.1006	14.50	0.0652	0.84
Corophium spp	0.0003	0.004	0.0131	0.17
Crangon septemspinosa	0.0359	0.47	0.0174	0.22
Crepidula spp	0.0034	0.04	0.0237	0.30
Cymadusa compta	0.1044	1.38	0.1083	1.39
Doridella obscura	0.0046	0.06	0.0030	0.04
Dulichiella appendiculata	0.0004	0.01	0.0013	0.02
Edotea triloba	0.0043	0.06	0	0
Elasmopus levis	0.0115	0.15	0.0460	0.59
Erichsonella attenuata	0.2214	2.92	0.1270	1.63
Gammarus mucronatus	0.0731	0.96	0.1198	1.54
Haliplanella sp	0	0	0.0106	0.14
Hippolytes sp	0.0041	0.05	0.0271	0.35
Hydroids	0.0263	0.35	0.1853	2.38
Idotea balthica	0.0954	1.26	0.0840	1.08
Jassa falcata	0	0	0.0004	0.01
Larval fish	0.0007	0.01	0	0
Microprotopus raneyi	0.0003	0.003	0.0002	0.003
Mitrella lunata	0.0107	0.14	0.1127	1.45
Molgula manhattensis	0.1435	1.89	0	0
Mysids	0.0293	0.39	0.0053	0.07
Nassaria obsoletus	0	0	0.1729	2.22
Nassaria vibex	0.0270	0.36	0	0
Nereis spp	0.0406	0.54	0.1345	1.73
Nudibranch	0.0004	0.005	0	0
Pagurus annulipes	0	0	0.0109	0.14
Palaemonetes spp	0.0887	1.17	0.3904	5.01
Paracaprella tenuis	0.0005	0.01	0.0006	0.01
Paracerceis caudata	0	0	0.0071	0.09
Pennaria tiarella	0.0150	0.20	0.0221	0.28
Serpulid spp	0	0	0.1244	1.60
Sertularia argentea	0	0	0.0028	0.04
Spionid spp	0	0	0.0004	0.005
Spirorbis spp	0	0	0.2048	2.63
Sponge	0.0289	0.38	0	0
Stenothoe minuta	0	0	0.0009	0.01
Sygnathus spp	0.2766	3.64	2.6646	34.20
Tanaid	0.0208	0.27	0	0
Triphora sp	0	0	0.0002	0.002
Victorella pavida	0.1652	2.18	0.0028	0.04

Table A11. Abundance of all epifauna predators (normalized to m^{-2}) that were collected in dipnet sweeps in the West and East regions.

	West		East	
Taxon	Total Abundance	% of Overall Abundance	Total Abundance	% of Overall Abundance
Anguilla rostrata	0.8	0.25	0.4	0.07
Bairdiella chrysoura	2	0.62	0	0
Blenny	2.4	0.75	0	0
Callinectes sapidus	46.8	14.59	18	3.08
Crangon septemspinosa	17.6	5.49	45.2	7.75
Gobiesox strumosus	8	2.49	0.8	0.14
Gobiosoma spp.	1.2	0.37	0.4	0.07
Juvenile fish	0	0	5.6	0.96
Menidia menidia	0.4	0.12	0.4	0.07
Orthopristis chrysoptera	2.4	0.75	0	0
Palaemonetes spp.	154.4	48.13	452.8	77.59
Panopeus herbstii	1.2	0.37	4.8	0.82
Paralichthys dentatus	0.4	0.12	0	0
Penaeus sp.	0	0	0.4	0.07
Stickleback	0.4	0.12	1.2	0.21
Symphurus plagiusa	0	0	0.8	0.14
Syngnathus spp.	82.8	25.81	52.8	9.05

Table A12. Abundance of nekton species caught in all scrape trawls for both the West and East regions. Abundances were normalized to area (m^{-2}).

	West		East	
Taxon	Total Abundance	% of Overall Abundance	Total Abundance	% of Overall Abundance
Anguilla rostrata	1	0.13	0	0
Bairdiella chrysoura	21	2.69	21	2.15
Blenny	8	1.03	4	0.41
Callinectes sapidus	205	26.28	180	18.42
Centropristis striata	0	0	9	0.92
Cynoscion nebulosus	1	0.13	0	0
Fundulus heteroclitus	7	0.90	1	0.10
Gobiesox strumosus	29	3.72	18	1.84
Gobiosoma spp.	41	5.26	2	0.20
Hippocampus erectus	2	0.26	1	0.10
Lagodon rhomboides	1	0.13	14	1.43
Leiostomus xanthurus	85	10.90	6	0.61
Malaclemys terrapin	1	0.13	0	0
Menidia menidia	3	0.38	18	1.84
Opsanus tau	0	0	3	0.31
Orthopristis chrysoptera	22	2.82	122	12.49
Panopeus herbstii	11	1.41	38	3.89
Paralichthys dentatus	2	0.26	5	0.51
Stickleback	0	0	26	2.66
Strongylura marina	0	0	2	0.20
Symphurus plagiusa	2	0.26	9	0.92
Syngnathus spp.	335	42.95	495	50.67
Tautoga onitis	0	0	2	0.20
Trinectes maculates	3	0.38	1	0.10

VITA

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