Positive and Negative Feedbacks Within Zostera marina Beds Within the Chesapeake Bay, Virginia

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Positive and Negative Feedbacks Within *Zostera Marina* Beds Within the Chesapeake Bay, Virginia

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William & Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

by

Lance M. Gardner

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APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
The requirements for the degree of
Doctor of Philosophy

Lance M. Gardner

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................................................................... vii

LIST OF TABLES ............................................................................................................................... viii

LIST OF FIGURES ............................................................................................................................... x

ABSTRACT ........................................................................................................................................ xii

DISSERTATION INTRODUCTION ......................................................................................... 2

   Project Goals And Objectives .............................................................................................. 6

   Literature Cited ................................................................................................................... 10

CHAPTER 1: THE EFFECT OF WIND, TIDES, AND LOCATION ON WATER COLUMN
PARTICULATE LOADS WITHIN VEGETATED SHALLOWS OF THE YORK RIVER ESTUARY,
CHESAPEAKE BAY, VIRGINIA ............................................................................................. 15

   ABSTRACT .......................................................................................................................... 16

   INTRODUCTION ................................................................................................................. 18

   METHODS .......................................................................................................................... 24

      Continuous Monitoring and Vegetation Effects .................................................. 24

      Tidal Stage and Current Effects ............................................................................ 27

      Wind Effect ........................................................................................................... 28

      Statistical Analyses ............................................................................................... 29

RESULTS ............................................................................................................................ 31

   Vegetation Effects ......................................................................................................... 31

   Tidal Stage and Current Effects ..................................................................................... 31

   Wind Effects .................................................................................................................. 39

DISCUSSION ...................................................................................................................... 44

   Vegetation Effects ......................................................................................................... 45

   Tidal Effects .................................................................................................................. 46

   Wind Effects .................................................................................................................. 47

   Model Development .................................................................................................... 48

SUMMARY ......................................................................................................................... 55

REFERENCES CITED ......................................................................................................... 56
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LIST OF TABLES

CHAPTER 1
Table 1: Deployment dates, locations and shoreline orientation for the automated sensors ...... 25
Table 2: Table of cross correlation coefficient results, comparing chl a, NTU and Kd to tidal stage................................................................. 37
Table 3: Table of cross correlation coefficient results, comparing chl a, NTU and Kd to computed wind effect................................................................................. 43
Table 4: Linear regression coefficients and standard errors from selected sampling periods ...... 51

CHAPTER 2
Table 1: Mean zooplankton density by location and %SAV cover category, 2006 sampling season ......................................................................................... 74
Table 2: Results of 2-way ANOVA on total zooplankton density ............................................ 76
Table 3: Mean zooplankton density by location and inside vs. outside the SAV ...................... 79
Table 4: Results of 2-way ANOVA on total zooplankton daytime density ......................... 80
Table 5: Results of 3-way ANOVA on total zooplankton density .................................... 81

CHAPTER 3
Table 1: Regressions from ambient sampling of SAV shoot and root biomass, sediment organic content, and porewater sulfide concentrations ........................................................................... 119
Table 2: Grain size and initial Zostera biomass for the experimental sites ................................. 122
Table 3: p-values for shoot biomass by iron enrichment level .................................................. 129
Table 4: p-values for root biomass by iron enrichment level.................................................. 129

CHAPTER 4
Table 1: Model calibration and simulation analyses ................................................................. 177
Table 2: June – August mean, peak and final (day 365) Z. marina shoot biomass; June – August mean K_d and P_max for model runs as specified in Table 1 ........................................ 181
Table 3: June – August mean, peak and final (day 365) Z. marina root biomass for model runs as specified in Table 1 ................................................................. 182

APPENDIX 1
Table A1-1: Mean zooplankton density, standard deviation, and t-test results for three taxonomic groups and total zooplankton ........................................................................ 218

APPENDIX 3
Table A3-1: Results of 2-way ANOVA on log$_{10}$ transformed total zooplankton density, 2006 ............................................................ 225

Table A3-2: Results of 2-way ANOVA on log$_{10}$ transformed total zooplankton density, 2007 ............................................................ 225

Table A3-3: Results of 3-way ANOVA on log$_{10}$ transformed total zooplankton density, 2007 ............................................................ 226

Table A3-4: Results of 3-way ANOVA on TEE base difference values .................. 226

Table A3-5: Results of 2-way ANOVA on TEE early values ................................. 227

Table A3-6: Results of 2-way ANOVA on TEE late values ................................. 227
# LIST OF FIGURES

## INTRODUCTION

Figure 1: Adapted from Kemp et al. (2000) and Kemp et al. (2004), showing light transmission and attenuation, nutrient and grazer interactions and sulfide generation ........................................ 9

## CHAPTER 1

Figure 1: Basic diagram of the main theorized feedbacks affecting SAV growth ......................... 19

Figure 2: Fluorometer deployment sites in the lower York River and Mobjack Bay, Chesapeake Bay, Virginia .......................................................................................................................... 26

Figure 3: Box plot distributions of (A) chl a and (B) turbidity by location .................................. 32

Figure 4: Example time series plots ............................................................................................... 33

Figure 5: Power Spectral Density Analysis ..................................................................................... 35

Figure 6: Loess 2-D smoothed graphical representation ................................................................. 41

Figure 7: Plots of the best fitting regressions for chl a and turbidity inside of SAV beds ........... 52

Figure 8: Comparison of the difference between paired observations inside and outside of SAV beds for NTU and chl a ......................................................................................... 54

## CHAPTER 2

Figure 1: Zooplankton sampling sites from the summer of 2006 and 2007 in the vicinity of the lower York River and Mobjack Bay, Chesapeake Bay, Virginia, USA ........................................... 68

Figure 2: Shallow Water and Zooplankton Pumped Sampling device (SWaZooPS) .................... 69

Figure 3: Summer 2006 zooplankton densities ............................................................................. 75

Figure 4: Mean daytime zooplankton densities from 2007, grouped by identification category ................................................................. 82

Figure 5: Diel and tidal cycle zooplankton density results .......................................................... 83

Figure 6: Literature synthesis of zooplankton community dry weight as a function of salinity .... 93

## CHAPTER 3

Figure 1: Ambient sampling sites and iron enrichment experimental sites ................................. 111

Figure 2: Relationships between Zostera biomass, sulfide concentrations and sediment organic content .......................................................................................................................... 120

Figure 3: Total Eelgrass Estimator for the iron enrichment experiment ..................................... 123

Figure 4: Normalized Total Eelgrass Estimator mean and standard deviation across iron addition levels .................................................................................................................. 125

Figure 5: Porewater sulfide concentrations for the iron enrichment experiment ....................... 126

Figure 6: Sediment organic content for the iron enrichment experiment ................................. 128
Figure 7: Final shoot and root biomass for the iron enrichment experiment..............130
Figure 8: Molar ratio of carbon, nitrogen and iron to phosphorus for Zostera shoots........132
Figure 9: Molar ratio of carbon and nitrogen to phosphorus for Zostera roots..............133

CHAPTER 4

Figure 1: Basic diagram of the main theorized feedbacks affecting SAV growth........153
Figure 2: Measured chl a biomass from Harding et al. (2002) with seasonal and tidal models for chl a .................................................................161
Figure 3: Tidal and WE multipliers for TSS; modeled TSS.................................164
Figure 4: Benthic biomass as a function of SAV biomass based on the data of Orth and Van Montfrans (1982) and Bostroem and Bonsdorff (2000); temperature-dependence of benthic filtration rate.................................167
Figure 5: Measured in situ (Chapter 3) and modeled sulfide levels using various values for $S_M$; proportional reduction in $P_{max}$ due to modeled sulfide concentrations........174
Figure 6: Calibration of Z. marina shoot and root/rhizome biomass to field data from Buzzelli et al. (1999).................................................................180
Figure 7: Modeled light extinction coefficient ($K_d$) in a variety of model simulations; Modeled $K_d$ with all particulate input and loss processes included .................................................................183
Figure 8: Z. marina shoot response to increasing particulate inputs; Z. marina shoot response to particulate reduction due to physical and biotic removal ................................185
Figure 9: (a) Reduction in maximum photosynthetic rate ($P_{max}$) due to increased sulfide levels. (b) Modeled shoot biomass with the sulfide levels in (a) and all particulate inputs and reductions active in the model .................................................187
Figure 10: Modeled effects of increasing temperature in 1°C increments on Z. marina shoot biomass ....................................................................................190
Figure 11: Ten year simulated run.........................................................................192

APPENDIX 1

Figure A-1: Mean of zooplankton abundance from samples collected for comparison of net tows to SWaZooPS samples.........................................................217
Figure A-2: Three perspectives of the layout of the Shallow Water and Zooplankton Pumped Sampler (SWaZooPS).................................................219-220
DISSERTATION ABSTRACT

Particulate levels within marine, estuarine and freshwater vegetated shallows are often lower than in nearby open water, although most of the studies quantifying this trend are from non-tidal, freshwater systems. The potential positive feedbacks between vegetation, water clarity, and zooplankton clearance and the potential negative feedback from microbially-mediated sulfide production were investigated in several eelgrass (Zostera marina) beds in the lower York River and Mobjack Bay, Chesapeake Bay, Virginia and through the construction of a computer simulation model.

Paired automated chlorophyll a and turbidity sensors were deployed for eight one-week periods to compare particulate levels inside and outside of eelgrass beds. The vegetated estuarine shallows monitored appeared to behave differently than those in freshwater vegetated systems, in that they were not able to consistently maintain improved water clarity relative to adjacent, unvegetated areas. Predictive equations for particulate levels inside the eelgrass beds were developed by regressing chl a and turbidity against wind and tidal influences for use in a Zostera simulation model.

Zooplankton were sampled during two summer seasons to quantify their impact on water clarity. In 2006, zooplankton densities were significantly higher in vegetated than non-vegetated areas, but not in 2007. Zooplankton densities were significantly higher at night, both inside and outside of the vegetated beds. Overall, the zooplankton densities encountered within the SAV beds had the potential to filter approximately 2-6% of the water column per day, much less than typically encountered in freshwater.

Eelgrass density, sediment organic content and porewater sulfide levels were quantified in situ in several SAV beds throughout spring and summer. There was no significant difference in [S] between vegetated and unvegetated areas, [S] was not correlated with eelgrass cover or sediment organic levels, but field results demonstrated that porewater [S] above 900-1000 µM inhibited eelgrass growth within the study area. An iron enrichment experiment demonstrated some potential for iron to positively affect Z. marina growth and survival, but responses were site specific and highly variable.

Finally, a computer simulation model was constructed that incorporated positive and negative effects within Z. marina beds, including tidal- and wind-induced particulate loading, resulting attenuation of light, particulate removal due to biological and physical filtration, temperature stress and sulfide toxicity. Modeled Z. marina responded to reduced light with approximately proportional reductions in year-end shoot and root/rhizome biomass. The model was less sensitive to increased sulfides; increases of 1.5, 2.0 and 2.5 times background sulfide levels resulted in incremental reductions of year-end shoot biomass by 20-25% and root/rhizome biomass by 15-20%. The model was most sensitive to temperature; a 1°C increase reduced year-end shoot and root/rhizome biomass by 41%; sulfide and temperature stress combined reduced shoot and root/rhizome biomass by 64%. With eelgrass in the Chesapeake Bay growing near its southern limits, model results indicate that either sulfide or temperature stress may limit restoration efforts and induce continued losses of eelgrass. Internal feedbacks reduce some of the stress caused by light limitation, but do not compensate for a 1°C increase in temperature or increases in sulfide levels.
Positive and Negative Feedbacks Within *Zostera Marina* Beds Within the Chesapeake Bay, Virginia
It has been well established that submerged aquatic vegetation (SAV), either in marine, estuarine, or freshwater environments, can be depth limited by light availability (Wetzel and Penhale 1983; Duarte 1991; Dennison et al. 1993; Abal et al. 1994; Short et al. 1995; Livingston et al. 1998; Arnold et al. 2000; Moore et al. 2003; Kemp et al. 2004). In general, freshwater aquatic plants are able to tolerate lower light levels than marine angiosperms, while in clear water marine plants can grow to a greater depth (Duarte 1991; Kemp et al. 2000). In the Chesapeake Bay specifically, eelgrass requires a minimum of approximately 20% of surface irradiance to reach the leaf to support growth, and historically occupied depths up to 2 meters (Dennison et al. 1993; Orth et al. 2002; Kemp et al. 2004).

Over the past several decades, coverage of SAV beds in the Chesapeake Bay has declined (Alden 1997; Kemp et al. 2000; Cerco et al. 2002; Orth et al. 2002; Moore et al. 2003; Stankelis et al. 2003; Pomeroy et al. 2006). The precipitous decline in SAV coverage has been attributed to increased nutrient and sediment loading and its subsequent effect on water clarity (Figure 1) (Alden 1997; Cerco et al. 2002; Orth et al. 2002; Stankelis et al. 2003; Hagy et al. 2004; Kemp et al. 2005; Pomeroy et al. 2006). SAV coverage specifically has declined both in the depth at which it can survive, now occurring below 1 meter only rarely, and in its lateral extent, with many shorelines that previously had SAV beds now having little or no vegetation left (Dennison et al. 1993; Moore et al. 2000; Orth et al. 2002; Moore et al. 2003).
In the Chesapeake Bay, as in most coastal areas, SAV plays an important role in many estuarine functions, including enhancement of particulate removal, attenuation of waves and reduction of erosion, reduced sediment resuspension, and provision of habitat for many species including commercially important fisheries (Heck and Orth 1980; Orth and Heck 1980; Kemp et al. 1984; Ward et al. 1984; Olney and Boehlert 1988; Fredette et al. 1990; Ducnuigeeen et al. 1997; Buzzelli et al. 1999; Cerco and Moore 2001; Orth et al. 2002; Van Montfrans et al. 2003; Newell and Koch 2004). Because of the importance of SAV, the restoration of seagrass beds has been considered one of the major goals of Chesapeake Bay restoration (Orth et al. 2002; Stankelis et al. 2003). Direct seeding and transplantation of eelgrass, however, has met with inconsistent success (Moore et al. 1997; Kemp et al. 2000; Kemp et al. 2004; Moore et al. 2012; Orth et al. 2012).

Although increased nutrient and sediment loading, and subsequently reduced water clarity, has deleterious effects on seagrass growth (Fig. 1), work conducted almost entirely in freshwater systems has demonstrated the role of SAV in maintaining its own local water clarity and potentially water quality. In estuaries, this effect has received less attention. For example, the figure in Kemp et al. (2004) (Fig. 1) does not illustrate possible positive feedbacks, such as reduced particulate concentrations due to enhanced filtration by the benthic community resident within the beds and elevated rates of physical settling. Jones (1990) investigated water clarity in the upper tidal freshwater Potomac River in relation to SAV and found reduced chlorophyll a (chl a) in the beds, and Moore (2004) conducted a similar study in the York River that demonstrated reduced concentrations of total suspended solids (TSS) and chl a, but neither identified causative mechanisms.
Direct effects of vegetation on water clarity include possible allelopathic effects in which the SAV chemically inhibits phytoplankton growth (Jasser 1995; van Donk and van de Bund 2002; Erhard and Gross 2006), reduction of suspended sediments and adhered nutrients through reduced current velocity and subsequent settling (Lake and MacIntyre 1977; Short and Short 1984; Vermaat et al. 2000; Wigand et al. 2000; Madsen et al. 2001; Schulz et al. 2003; Takamura et al. 2003), particulate adherence to leaf structures (Pluntke and Kozerski 2003; Palmer et al. 2004), shading of phytoplankton as the SAV forms a canopy along the water surface (Buzzelli et al. 1998; Scheffer 1999), potential removal of nutrients directly from the water column (Short and Short 1984; Horppila and Nurminen 2003) and provision of habitat for a variety of fauna including filter feeding zooplankton and benthic infauna (Reusch and Reusch 1998; Bostrom and Mattila 1999; Bostroem and Bonsdorff 2000; Peterson et al. 2001; Hovel et al. 2002). Each of these effects alone may not be that large, but when considered as a whole, or several in combination, they may play a significant role in maintaining the water clarity necessary for continued SAV survival and growth.

Increased benthic infaunal biomass, production and filtration in brackish and marine SAV beds has been well established (Orth and Van Montfrans 1982; Fredette et al. 1990; Reusch and Reusch 1998; Bostroem and Bonsdorff 2000; Peterson et al. 2001). In freshwater systems, filter-feeding zooplankton (especially large-bodied cladocerans like Daphnia spp.) often occur in higher densities in the vegetated shallows compared to open water systems in the adjacent pelagic zone or in other aquatic systems with similar physical and nutrient characteristics but no vegetation (Scheffer 1999; van Donk and van de Bund 2002; Muylaert et al. 2003). In marine systems, however, the role of zooplankton in SAV beds has been little studied (Robertson et al. 1988; Jeppesen et al. 2007). Two studies comparing zooplankton densities in relation to marine
vegetated systems, using net tows through open areas adjacent to or within a bed or over the vegetation at high tide, produced conflicting results: Robertson et al. (1988) found higher densities of several types of zooplankton within the seagrass beds, while Meyer (1982) did not identify any differences.

Vegetated shallows may also demonstrate negative internal feedbacks. Some studies, such as those by Frederiksen (2004) and Morris and Virnstein (2004) have documented annual as well as spatial variability in the extent of seagrass beds, and Morris and Virnstein (2004) hypothesized that phytotoxin feedback within the sediments may be the cause of this variability. Azzoni (2001) studied the role of microbially-mediated feedbacks within the sediments, and found that in the eutrophic system studied, the accumulation of phytotoxins within the sediments exceeded the capacity of the SAV beds to ameliorate the negative effects, causing a rapid negative feedback on the survival of the vegetation. Increased organic matter deposition and subsequent microbial breakdown increased sulfide levels, which in turn increased root mortality. The loss of roots decreased oxygen concentrations in the sediments, which increased sulfide generation, leading to a sudden and catastrophic loss of the seagrass in the study area.

Little is known about sulfide levels and controls on these levels, such as organic matter accumulation and mineral sulfide binding, in Zostera beds within the Chesapeake Bay and few studies exist on sulfides in seagrass beds containing siliceous sediments (Goodman et al. 1995). Chambers et al. (2001) added iron oxide granules to a Thalassia bed in Florida, and found greater shoot growth in the iron addition treatments compared to the controls, due to binding of the sulfides by iron to form non-toxic precipitates in the calcareous sediments. Addition of iron to Zostera beds may similarly reduce sulfide stress within eelgrass beds in the Chesapeake Bay, but has yet to be tested with this temperate species and in the siliceous sediments typical
of the Bay. And if sulfide concentrations can be reduced in situ, the growth and survival of eelgrass may be enhanced.

The negative impacts of sulfides in SAV beds can be compounded by additional stresses due to high temperatures, variations in salinity and reduced light, and these stressors have the potential to interact in a non-linear fashion. Koch & Erskine (2001) conducted a laboratory study on the combined stressors of increased sulfides, temperature, and salinity and reduced light on the survival of *Thalassia*. The results clearly indicated that the combination of the different stressors had a greater negative effect than each stressor individually, such that when high sulfides were combined with either high temperature or salinity, significant mortality occurred. Further, when the plants were stressed with high temperature, salinity and sulfides, 100% mortality was induced. Goodman et al. (1995) concluded that the stressors of reduced light and increased sulfides on eelgrass growth were additive, and Holmer et al. (2005) recorded increased mortality and 75% lower growth rates in eelgrass subjected to low light and high sediment sulfides, while exposure to high sediment sulfides alone had no effect compared to the control.

**Project Goals and Objectives:**

Based on these previous studies and the amount of funding currently being allocated to SAV conservation and restoration, quantifying the relative roles and interactive effects of the positive and negative feedbacks within these beds is important for understanding the potential for long-term survival of existing and restored beds, and for verifying the cost effectiveness of these programs. The first goal of this project was therefore to gather background data to quantify possible positive and negative feedbacks described above for which limited data currently exist. These included (a) expanding previous studies of TSS and chl a concentrations
between vegetated and non-vegetated shallows across multiple seagrass beds and over an entire growing season; (b) quantification of zooplankton densities and potential rates of filtration within estuarine seagrass beds compared to open water; and c) quantification of \textit{in situ} sulfide concentrations within Chesapeake Bay eelgrass beds, potential controls on those concentrations, and the potential for iron enhancement to reduce sulfide levels and enhance eelgrass growth in siliceous estuarine sediments. The second goal was to construct a \textit{Z. marina} computer simulation model to examine the relative effects of multiple interacting stressors and the impact of the aforementioned positive and negative feedbacks on growth and survival of eelgrass beds in the lower Chesapeake Bay. Formulations based on the measured feedbacks described above were supplemented with information from the literature on benthic faunal densities in relation to eelgrass density, rates of benthic faunal filtration, rates of physical settling, and the impact of temperature and sulfides on eelgrass growth.

To address these goals the following objectives were undertaken:

\textbf{Objective 1 (Chapter 1):} To quantify total suspended solids (TSS) and phytoplankton (as chl \textit{a}) concentrations inside and outside of several vegetated sites in the lower York River and Mobjack Bay, and the effect of wind and tides on chl \textit{a} and TSS in these systems.

\textbf{Objective 2 (Chapter 2):} To quantify the density, biomass, and filtration potential of suspension feeding zooplankton in relation to eelgrass density and biomass. This objective includes construction of a novel, non-destructive, pumped sampler which is described in Appendix 1.

\textbf{Objective 3 (Chapter 3):} To quantify ambient sulfide concentrations and organic content in relation to eelgrass density and biomass in multiple SAV beds in the lower York River.
and Mobjack Bay.

**Objective 4 (Chapter 3):** To conduct an iron enrichment experiment to quantify the effect of particulate iron addition on sulfide concentrations and eelgrass growth.

**Objective 5 (Chapter 4):** To construct a *Zostera marina* computer simulation model that incorporates the results of Chapters 1-3 and several other identified feedbacks and inputs, including benthic faunal abundance and filtration, physical settling, and the effect of temperature and sulfides on eelgrass growth and survival. Eelgrass formulations are based on the model of Buzzelli et al. (1999), and the chapter documents the new formulations developed as part of this dissertation and modifications to Buzzelli et al.’s (1999) original model. Formulations for eelgrass and selected forcing functions that are taken directly from Buzzelli et al.’s (1999) model are provided in Appendix 2.
Figure 1: Adapted from Kemp et al. (2000) and Kemp et al. (2004), showing light transmission and attenuation, nutrient and grazer interactions and sulfide generation. PLW is percent light through the water, PLL is percent light at the leaf surface, SAV is submerged aquatic vegetation, DIN is dissolved inorganic nitrogen, DIP is dissolved inorganic phosphorus, P is phosphorus, N is nitrogen.
References cited:


Chapter 1:

The effect of wind, tides, and location on water column particulate loads within vegetated shallows of the York River Estuary, Chesapeake Bay, Virginia
Abstract:

Particulate levels within marine, estuarine and freshwater vegetated shallows are often lower than in nearby open water, although most of the studies quantifying this trend are from non-tidal, freshwater systems. Particulate levels can be affected by many factors, including tidal exchange and wind induced waves which may impact vegetated and non-vegetated shallows differently. To investigate the role which these factors play in particulate loads within beds of submerged aquatic vegetation (SAV) in a tidal estuarine system, paired automated chlorophyll \(a\) and turbidity sensors were deployed at multiple sites for a period of approximately 10 weeks to compare particulate levels inside and outside beds of *Zostera marina* (eelgrass). These recorders were deployed for eight approximately one-week periods across seven different locations in the lower York River and Mobjack Bay, Chesapeake Bay, Virginia. Median chlorophyll \(a\) concentrations were significantly lower inside the SAV beds relative to outside the beds early in the growing season, but generally higher inside the beds later in the season. Turbidity was generally significantly lower or unchanged inside the beds. Wind had a significant effect on turbidity (\(p<0.001\)) and in some cases chlorophyll \(a\) (\(p<0.01\)). Tides had a significant effect on both chlorophyll \(a\) (\(p<0.001\)) and turbidity (\(p<0.001\)) for some deployments. Chlorophyll \(a\) and turbidity were significantly positively correlated, suggesting that both types of particulates respond similarly to external influences. The vegetated estuarine shallows monitored in this study appear to behave differently than those in freshwater vegetated systems, in that they are not able to consistently maintain their water clarity advantage.
KEYWORDS: particulates, turbidity, chlorophyll, wind, tides, submerged aquatic vegetation, Zostera marina, eelgrass, estuary, Chesapeake Bay
Introduction:

Vegetated lakes, ponds, and shallows in eutrophic freshwater systems are known to have greater water clarity compared to adjacent open water or non-vegetated systems with similar environmental conditions, such as nutrient loading and turnover time (Hasler and Jones 1949; Hamilton et al. 1990; Jones 1990; Jasser 1995; Schriver et al. 1995; Ejsmont-Karabin et al. 1996; Perrow et al. 1999; Scheffer 1999; Biyu 2000; Jeppesen et al. 2002; van Donk and van de Bund 2002; Schulz et al. 2003). Beds of submerged aquatic vegetation (SAV) influence water clarity through reductions in total suspended solids and phytoplankton concentrations resulting from a series of biological, chemical and physical interactions (Fig. 1). These interactions include wave and current dampening, which reduces sediment resuspension and enhances settling of sediments and associated nutrients (Ward et al. 1984; Madsen et al. 2001; Horppila and Nurminen 2003; Takamura et al. 2003; Newell and Koch 2004), removal of nutrients directly from the water column (Short and Short 1984; Horppila and Nurminen 2003), possible allelopathic effects in which the SAV chemically inhibits phytoplankton growth (Jasser 1995; van Donk and van de Bund 2002; Erhard and Gross 2006), physical particle capture by the structure of the leaves (Pluntke and Kozerski 2003; Palmer et al. 2004; Hendriks et al. 2008), shading of phytoplankton as the SAV forms a canopy along the water surface (Buzzelli et al. 1998; Scheffer 1999), and attraction of organisms that actively filter particulates from the water column as a food source (Perrow et al. 1999; Blindow et al. 2002; Nurminen and Horppila 2002; van Donk and van de Bund 2002).
Figure 1: Basic diagram of the main theorized feedbacks affecting SAV growth. The inner and lower loops represent negative feedbacks, while the top outer loop represents positive feedbacks.
Limited work in vegetated shallows of estuarine systems has shown that SAV beds in these areas may also have reduced particulate levels and improved water clarity relative to adjacent unvegetated areas (Short and Short 1984; Ward et al. 1984; Jones 1990; Moore 2004; Newell and Koch 2004; Gruber and Kemp 2010). Moore (2004) found lower particulate levels within SAV beds above a threshold of 50-100 g dry mass m\(^{-2}\) or 25%-50% vegetative cover in the York River sub-estuary of Chesapeake Bay. Short and Short (1984) conducted a tank study which indicated that planted tanks reduced particulate and ammonia concentrations faster than unplanted tanks, while phosphorus removal was similar. Ward et al. (1984) reported significantly lower particulate levels within SAV beds compared to unvegetated areas in the estuarine Choptank River, MD; this difference persisted during wind events under normal water levels but not during spring tides or storm surges, indicating a combined role of vegetation, winds, tides, and water level in determining particulate levels within SAV beds. Newell & Koch (2004) also found that vegetation reduced resuspension in a cove in mid-Chesapeake Bay, but the active filtering of oysters in the bed played a larger role in particulate reduction than the vegetation itself. Finally, Paul et al. (2012) demonstrated the ability of vegetation to attenuate wind-induced waves as a function of stiffness and leaf area index, but these effects can be greatly reduced by the presence of tidal currents. These studies demonstrate the potential for reduced particulate loads within estuarine SAV beds, either due to physical or biological processes, but they also demonstrate the potential for these effects to be overcome by strong physical forcing in the form of winds, tidal currents, and high water levels. Additionally, most of these studies were conducted over a relatively
short period, typically 10 days or less, or in an enclosure, without consideration of seasonal or longer-term effects across multiple sites.

The reduction of suspended particulates within SAV beds has the potential to create a positive feedback, whereby SAV enhances its local water clarity through the mechanisms outlined above, which stimulates further SAV growth and increases the potential for further particulate removal (Fig. 1) (Jeppesen et al. 2007). There appears to be a threshold of turbidity beyond which these positive feedbacks become overwhelmed and a major shift in the local ecosystem may occur from a clear-water vegetated system to a turbid-water non-vegetated system (Scheffer et al. 2001; Jeppesen et al. 2007). Once this threshold is reached, hysteresis inhibits the system from returning to the vegetated state unless the forcing factors such as turbidity or nutrient loading are reduced beyond the point where the system shifted from vegetated to non-vegetated (Folke et al. 2004). On the other hand, increased particle settling within SAV beds and elevated productivity of the SAV community due to improved water clarity may also contribute to a negative feedback as increased organic matter deposition increases microbially mediated sulfide generation (Fig. 1) (Azzoni et al. 2001; Frederiksen et al. 2007).

In the vegetated shallows of the Chesapeake Bay, both the magnitude and timing of elevated suspended particulates may be important. Moore et al (1997) surmised that a springtime pulse of higher turbidity could reduce or eliminate *Zostera marina* stands for the rest of the growing season, or limit the colonization of an area, because spring is a critical growth and storage period for *Zostera* which in the Chesapeake is growing near its warm water southern limits. Episodic particulate loads can be caused by several
processes, including strong wind events that contribute to sediment resuspension and storm events which result in increased runoff (Ward et al. 1984; Caffrey and Day 1985; Short and Wyllie-Echeverria 1996; Koch 1999; Granata et al. 2001; Cerco et al. 2002; Morichon et al. 2008; Gruber and Kemp 2010). These episodic events can elevate particulate concentrations by various amounts for various durations, depending on the intensity, duration and timing of the events. Highly localized events can also contribute to locally elevated particulate levels, such as grazing by larger animals including the cow-nose ray (*Rhinoptera bonasus*), or boat traffic and other recreational activities (Orth 1975; Merriner and Smith 1979; Verney et al. 2007).

Previous studies of particulate loads within SAV beds in marine and estuarine systems have been fairly limited in their temporal and spatial scope, often lasting for a period of 10 days at 1 or 2 sites, or performed in enclosures or tanks, limiting their applicability to a variety of conditions and sites and across an entire growing season. Freshwater systems have been studied in more detail, but the periodic tidal influence is not a factor in most freshwater systems, nor do these systems typically have the long, open fetches characteristic of exposed estuarine systems. This study was designed to quantify the potential reduction of total suspended particulates, both in terms of phytoplankton chlorophyll *a* and suspended solids as reflected by turbidity, in estuarine SAV beds during an extended period of time and across a variety of locations. Since wind, tides, and location may influence particulate levels in these shallow areas (Ward et al. 1984; Gruber and Kemp 2010), wind and tidal data collected from other nearby monitoring stations were used to develop site-specific as well as global relationships.
between wind, tides, and particulate levels. Development of these relationships may also
be useful in predicting the effects of springtime wind and storm events on *Zostera*
survival (Moore et al. 1997).
Methods:

Continuous monitoring and vegetation effects:

One pair of WET Labs ECO fluorometers outfitted with nephelometric turbidity unit (NTU) and chlorophyll a (chl a) sensors was deployed simultaneously for periods of approximately one week at a time, with one fluorometer inside and one outside a seagrass bed at similar depths and in close proximity to each other (typically within 50 m), with a sampling frequency of 15 minutes. After the deployment period, the sensors were collected for data retrieval and maintenance and redeployed at a different site (Table 1). The total deployment period spanned from June 18 through August 22, 2007, for a total of eight deployments at seven different sites, with one site utilized both in mid-June and the beginning of August (Fig. 2).

Total suspended solids (TSS) and chl a grab samples were collected for calibration purposes concurrent with fluorometer deployment and retrieval. However, we were unable to obtain well-constrained calibration curves between sensor and laboratory measurements, which we attribute to high variability in the sensor data due primarily to wind, tides and disturbance of the area during deployment and sampling. We therefore had to rely on the factory calibration to laboratory standards which occurred immediately prior to deployment; while this may reduce the accuracy of the concentrations measured by the sensors, relative fluctuations between the sensors and throughout the season can nevertheless be quantitatively compared and analyzed. In addition, the sensors were checked in the laboratory prior to deployment to verify the sensors were recording the same values.
Table 1: Deployment dates, locations and shoreline orientation for the automated sensors.

<table>
<thead>
<tr>
<th>location</th>
<th>abbreviation</th>
<th>deployment period</th>
<th>shoreline orientation °N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins Neck</td>
<td>JN1</td>
<td>18 June – 25 June</td>
<td>90</td>
</tr>
<tr>
<td>Goodwin Islands</td>
<td>GI</td>
<td>25 June – 3 July</td>
<td>10</td>
</tr>
<tr>
<td>Guinea Marsh</td>
<td>GM1</td>
<td>3 July – 9 July</td>
<td>50</td>
</tr>
<tr>
<td>New Point Comfort</td>
<td>NPC</td>
<td>10 July – 17 July</td>
<td>140</td>
</tr>
<tr>
<td>Allens Island</td>
<td>AI</td>
<td>17 July – 25 July</td>
<td>90</td>
</tr>
<tr>
<td>Severn River</td>
<td>SR</td>
<td>25 July – 31 July</td>
<td>240</td>
</tr>
<tr>
<td>Jenkins Neck</td>
<td>JN2</td>
<td>31 July – 6 August</td>
<td>90</td>
</tr>
<tr>
<td>Guinea Marsh</td>
<td>GM2</td>
<td>7 August – 22 August</td>
<td>80</td>
</tr>
</tbody>
</table>
Figure 2. Fluorometer deployment sites in the lower York River and Mobjack Bay, Chesapeake Bay, Virginia. Black dots represent each deployment site; Jenkins Neck site was used twice.
As a means of analyzing the combined effects of chl $a$ and turbidity, the light extinction coefficient $K_d$ was calculated according to Xu et al. (2005), a light extinction model specifically developed for the Chesapeake Bay. This also enabled calculation of the effect of wind and tides on light attenuation. NTU was converted to TSS (mg L$^{-1}$) using a conversion of TSS=8.05*NTU based on the means of all calibration samples collected throughout the study. $K_d$ could then calculated as:

$$K_d = 1.17 + 0.024 \times \text{chl} + 0.006 \times \text{TSS} - 0.0225 \times \text{sal}$$

where $K_d$ is the attenuation coefficient in m$^{-1}$, chl is chlorophyll $a$ in $\mu$g L$^{-1}$, TSS is total suspended solids in mg L$^{-1}$, and sal is salinity = 20 PSU, the long-term mean in the lower York River based on monitoring data from the EPA Chesapeake Bay Program.

**Tidal stage and current effects:**

To analyze for the effect of tides and tidally generated currents, water level (i.e. tidal) data recorded every 15 minutes were obtained from the automated depth recorder maintained by the CBNERRVA (Chesapeake Bay National Estuarine Research Reserve – Virginia) at Goodwin Islands (Station #CHE019.38), which is located in a seagrass bed on the eastern side of the island; recorded water levels were used as a proxy for tidal stage. Assuming a standing wave relationship between tidal elevation and velocity, tidal currents were estimated as the absolute difference between successive water levels. A one hour running average of water depth was used in the calculations to limit the influence of spurious data points.
Comparing data from the Gloucester Point Continuous Monitoring Station (YRK005.40) maintained by the Virginia Estuarine and Coastal Observing System located approximately 10 km upstream from Goodwin Islands in the York River to the depth data at Goodwin Islands, a difference of 15-30 minutes was determined between tidal peaks for these two stations. The sampled sites in this study were located from 0 to 14 km upstream from the Goodwin Islands depth recorder, with the Severn River being the farthest site. Given the small difference in tidal stage across this distance, we assumed that the time of the tidal stage at each sampling site was equal to that at Goodwin Islands.

Wind effect:

Wind-induced wave action can vary based on several factors including orientation of the wind relative to the shoreline. Wave gauges were not deployed with the fluorometers, so the following formula was developed to quantify the effect of wind on a given shoreline:

\[ \text{WE} = \text{WS} \times \left( \frac{\left(\sin(\text{WD}-\text{SO})\right)}{2} + 1 \right) \]

where \( \text{WE} = \) wind effect; \( \text{SO} = \) shoreline orientation (°N); \( \text{WD} = \) wind direction (°N); \( \text{WS} = \) wind speed (m s\(^{-1}\)).

This formula reduces the wind speed by 0.5 when blowing perpendicular to the shoreline orientation from land to water, leaves wind speed unchanged when parallel to the shoreline and increases the wind speed by a maximum factor of 1.5 when blowing onshore perpendicular to the shoreline orientation.
The shoreline orientation should be drawn towards the east (0-180°) if the shoreline has most of the water towards the south and towards the west (180-360°) if the landmass is towards the south. Offshore shoals, shallow sand bars, islands, shoreline embayments or other wave breaks may attenuate wind induced wave action, sometimes without an obvious physical signature on a map. For the purposes of this study, the orientation utilized was estimated through a combination of personal site knowledge and mapped shoreline features with °N obtained from Garmin GPS mapping software (Table 1). Wind data (recorded every six minutes) were obtained from the NOAA National Ocean Service buoy at the mouth of the York River (Station YKRV2 – 8637611 – York River East Rear Range Light, VA). This station was chosen because it was located away from any land or other possible wind interference and was still within the vicinity of the sampling sites.

Statistical analyses:

Analyses were performed in SigmaPlot v. 11.0 and SPSS v. 18.0. The initial concept for the monitoring design was to compare mean values inside and outside the SAV bed from each deployment, through a t-test or similar paired analysis, to ascertain if there were significant differences in particulate levels (chl a or NTU) and to develop overall estimates of the effect of vegetation on particulate levels. Testing for normality indicated that all of the data sets were skewed. Transformations were not able to resolve the non-normal distributions, so the median based Mann-Whitney Rank Sum Test was used for the initial paired comparison of data from inside and outside the SAV beds for each location.
Power Spectral Density (PSD) analysis performed in SigmaPlot was used to identify tidal or diel periodicity in the data. A Loess 2-D smoothing algorithm was used to graphically enhance the interpretation of particulate levels as a function of wind effect. The smoothing period for chl $a$ and turbidity was set to one day with a second order polynomial to effectively remove the tidal signature. Wind was similarly smoothed but over a shorter 8-hour period to remove extremes but retain more detail.

The smoothing and PSD indicated that the data were often not stationary probably due to random events like wind, and perhaps boats, rays or other factors, which made the data collected too irregular for stationary time series analysis. An alternative acceptable filtering to limit periodic tidal signatures was not developed.

Cross correlation analysis (CCA) was used to test for time lags between wind effect, tidal stage and estimated tidal current with elevated particulate levels. CCA was performed on thirty minute discrete averages, the shortest common time interval of the datasets, with lag times of seven hours (both positive and negative) for a total window of 14 hours to encompass a full tidal cycle.
Results:

Vegetation effects:

Chl $a$ was statistically significantly different between paired fluorometer deployments for all time periods sampled ($p<0.05$) (Fig. 3a). One half of the deployment periods including the first three had lower median chl $a$ values inside the SAV, while the last three periods had higher median chl $a$ values inside the beds. Median chl $a$ values inside the SAV beds were higher as the summer season progressed while medians outside the beds were lower at the end of the season.

Five of the eight sampling periods had statistically significantly lower median turbidity inside the SAV beds, while only Guinea Marsh1 exhibited higher turbidity inside the SAV ($p=0.05$) (Fig. 3b). Three of the sampling periods, Guinea Marsh1, Allen’s Island, and Jenkins Neck2, had higher variability, while New Point Comfort and Severn River exhibited much lower variation than the other sites.

Tidal stage and current effects:

Periodic diel and tidal oscillations were evident in time-series plots of chl $a$ (Fig. 4a,c), with longer-term changes occurring on the order of a few days (e.g. multi-day increase and subsequent decline in chl $a$ values outside the bed at Jenkins Neck on June 20-22, Fig. 4a). NTU values displayed similar periodic oscillations although not as pronounced as for chl $a$ (Fig. 4b,d); visually Goodwin Islands NTU had the strongest response to tides. NTU time series displayed a greater occurrence of high values compared to chl $a$, with marked increases during certain wind events not related to tides (e.g. June 20 and 25 at Jenkins Neck, Fig. 4b, and July 1-3 at Goodwin Islands, Fig. 4d).
Figure 3: Box plot distributions of (A) chl \( a \) and (B) turbidity by location. Asterisks indicate significant differences between data inside and outside the SAV bed within a sampling period. Boxes show the 1st quartile, median, and 3rd quartile of the data, dashed lines show the mean, whiskers encompass the 10th and 90th percentiles, and solid circles show the 5th and 95th percentiles.
Fig. 4. Example time series plots from Jenkins Neck 1 (a, b) and Goodwin Islands (c, d) chl a (a, c) inside (●) and outside (○) the SAV bed, turbidity (b, d) inside (●) and outside (○) the SAV bed, wind speed (−) and water depth (---). Water depth is used as a proxy for tidal fluctuations. The chl a and turbidity scales were reduced to allow for better visualization of the data, eliminating the highest values; wind speed scale was adjusted to allow for better visualization.
Power Spectral Density (PSD) analysis provided strong evidence of tidal influence for some but not all sampling periods (Fig. 5). Several of the time series had a periodicity at once, twice, and four times per day, indicating both strong diurnal and tidal signatures, such as chl \( a \) at Goodwin Islands (Fig. 5c). Chl \( a \), NTU and wind effect all had peaks at approximately once and twice per day at Goodwin Islands (Fig. 5c, d), indicating that both wind and the effects of tides and diel cycles overlapped in their periodicity during some of the sampling periods.

PSD analyses displayed several peaks that were not related to any identifiable periodic events; e.g., chl \( a \) and turbidity inside the SAV bed at Jenkins Neck1 (Fig. 5a,b) and outside the SAV bed at Goodwin Islands (Fig. 5d). While the latter site had peaks at once and twice per day indicating diurnal and tidal influences, NTU outside the SAV bed displayed other stronger peaks (Fig. 5d). Guinea Marsh1, New Point Comfort, Severn River, Allen’s Island, and Jenkins Neck2 had relatively strong peaks at once and twice per day for chl \( a \) inside the SAV beds. New Point Comfort and Severn River had similar relatively strong peaks for chl \( a \) outside the SAV beds. Only Goodwin Islands had relatively strong tidal peaks for turbidity inside the SAV bed, although several of the sites (Jenkins Neck1, Guinea Marsh1, Severn River, Jenkins Neck2, Guinea Marsh2) had peaks at twice a day but these were mixed in with other stronger peaks. Turbidity outside of the SAV beds had strong diurnal and tidal peaks at Jenkins Neck1, Severn River, and Jenkins Neck2.
Fig. 5. Power Spectral Density analysis of Jenkins Neck1 (a, b) and Goodwin Islands (c, d) chl a (a, c), turbidity (b, d), and wind effect.
When the chl \( a \) and tidal data were offset according to the maximum correlation obtained from the cross correlation analysis, linear regression between the two variables was highly significant \((p<0.01)\) for all of the sites except Jenkins Neck1 \((p=0.032)\) and Guinea Marsh2 \((p=0.051)\) (Table 2). Even though the other regressions were highly significant, the correlation coefficient was less than 0.1 for all locations except Goodwin Islands, Guinea Marsh1 and New Point Comfort. Turbidity and tidal data had fewer statistically significant correlations even when offset: Jenkins Neck1 \((p=0.118)\), Jenkins Neck2 \((p=0.188)\), and Guinea Marsh2 \((p=0.381)\) had non-significant regressions, while regressions for the other sites were significant \((p<0.001)\). Goodwin Islands had the strongest correlation coefficient of \(r^2=0.23\), while all others had \(r^2\) less than 0.1.

Regressions between tidal stage and computed light extinction \((K_d)\) were similar to those for turbidity with only Goodwin Islands having \(r^2 > 0.1\).

The correlation coefficients from all of the tidal cross-correlation analyses changed gradually from maximum to minimum over approximately a six hour period (graphs not shown), even when the correlations were not strong. Only two out of the 32 particulate data sets had maximum and minimum \(r^2\) offset more than 7.5 hours (Table 2). The consistency of an approximately 6 hour separation between maximum and minimum correlations provides further evidence of the influence of tides.
Table 2: Table of cross correlation coefficient results, comparing chl $a$, NTU and $K_d$ to tidal stage. Both the highest and lowest correlations are listed, with the amount of offset given in the same order in the following rows. A positive offset means the response (chl $a$, NTU) comes after the cause (tide).

<table>
<thead>
<tr>
<th>Location</th>
<th>Cross correlation factors</th>
<th>Chl $a$</th>
<th>NTU</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inside bed</td>
<td>Outside bed</td>
<td>Inside bed</td>
</tr>
<tr>
<td>Jenkins</td>
<td>Maximum</td>
<td>0.12</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>Neck1</td>
<td>Minimum</td>
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<td>-0.03</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td>Amount of offset (hrs)</td>
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<td>7</td>
<td>-0.5</td>
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<tr>
<td>Goodwin</td>
<td>Maximum</td>
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<td>0.61</td>
<td>0.48</td>
</tr>
<tr>
<td>Islands</td>
<td>Minimum</td>
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<td>-0.37</td>
<td>-0.11</td>
</tr>
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<td></td>
<td>Amount of offset (hrs)</td>
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<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>Guine Marsh1</td>
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</tr>
<tr>
<td></td>
<td>Minimum</td>
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<td>-0.16</td>
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</tr>
<tr>
<td></td>
<td>Amount of offset (hrs)</td>
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<td>-3</td>
<td>0</td>
</tr>
<tr>
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<td>Comfort</td>
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<td>-0.11</td>
</tr>
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<td></td>
<td>Amount of offset (hrs)</td>
<td>4</td>
<td>-6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Allen's Island</td>
<td>Maximum</td>
<td>0.16</td>
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</tr>
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<td></td>
<td>Minimum</td>
<td>-0.05</td>
<td>-0.24</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>Amount of offset (hrs)</td>
<td>-6</td>
<td>-4</td>
<td>-6</td>
</tr>
<tr>
<td>Severn River</td>
<td>Maximum</td>
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<td>0.32</td>
<td>0.22</td>
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<tr>
<td></td>
<td>Minimum</td>
<td>-0.09</td>
<td>-0.13</td>
<td>-0.13</td>
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<tr>
<td></td>
<td>Amount of offset (hrs)</td>
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<td>0</td>
<td>0.5</td>
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<tr>
<td>Jenkins</td>
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<td>Neck2</td>
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<td>Amount of offset (hrs)</td>
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<td>Marsh2</td>
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</tr>
<tr>
<td></td>
<td>Amount of offset (hrs)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>-7</td>
<td>-6</td>
<td>-2</td>
</tr>
</tbody>
</table>
Maximum positive correlations with a minimal time lag (i.e. offset) suggests that the tides carried particulates into a site (greater water depth corresponds to higher particulate levels), while a six hour offset indicates higher particulate levels at low tide. The correlations developed from the cross-correlation analysis were not consistent across sampling periods in their strength or time lag, or even within a given site between NTU and chl a, even though the time difference between maximum and minimum correlations was consistently close to six hours. Goodwin Islands had the most consistently strong results; only NTU outside the SAV bed had a weaker regression at a longer offset, consistent with particulate levels increasing with the incoming tide and decreasing during ebb tide. Guinea Marsh1 and New Point Comfort also displayed relatively strong correlations between tidal stage and both chl a and turbidity within the SAV bed with a minimal offset.

New Point Comfort was the only location sampled that displayed a relatively strong correlation with a negative offset for chl a inside the SAV bed (elevated concentrations occur prior to the rising tide), and a similar offset for turbidity. Several locations displayed an approximately six hour positive offset between high tide and elevated particulates, including both of the sampling periods for Jenkins Neck, indicating higher particulate levels at low tide, but these correlations were not very strong (Table 2). Estimated tidal currents displayed weaker correlations with particulates than either tidal stage (data not shown) or wind effect (WE, see below).
Wind effects:

Particulates increased both inside and outside the SAV beds on June 20th and 25th (Fig. 4b) and July 2 (Fig. 4d), corresponding to peaks in wind speed during these times. Several other wind events were recorded during the sampling period with similar peak wind speeds but less response in chl $a$ and turbidity, possibly due to differences in wind direction, shoreline angle, and computed wind effect (Fig. 6). Chl $a$ inside the SAV bed increased rapidly in response to the wind event of June 20 (many of the chl $a$ values are above the scale); concentrations were initially lower than those outside the SAV bed but became greater during the event (Fig. 4a). The wind events of June 20, June 25, July 2 and July 3 corresponded to increases in turbidity with subsequent rapid decreases after the winds subsided (Fig. 4b, d).

Smoothed particulate levels inside the SAV beds reinforced the patterns above; i.e., concentrations were generally lower than those outside the beds except when spikes occurred due to wind events, which often reversed the trend (Fig. 6). This was not a consistent response as some peaks inside the SAV bed were offset from peaks outside the SAV bed or did not respond as strongly or even at all. While wind events generally resulted in elevated particulate levels, neither wind speed nor wind effect were consistently related to the strength of the response. For example, chl $a$ inside the SAV bed at Jenkins Neck 1 between June 19 and 22 displayed a large response to wind events characterized by a small computed wind effect, and a smaller response to a large computed wind effect (Fig. 6a). After June 23 chl $a$ did not appear to respond at all to wind, with low values occurring during two relatively large wind events. In contrast,
turbidity had a larger response to larger wind effects at Jenkins Neck1, both inside and outside the SAV beds (Fig. 6b). Goodwin Islands responded similarly, with chl $a$ responding to the smaller wind event on July 1, and turbidity responding to the larger wind event on July 2 (Fig. 6c, d).

Cross correlation analyses between computed wind effect and particulate levels demonstrated more consistent correlations for turbidity (Table 3), while chl $a$ was more consistently related to tides (Table 2). The WE cross correlation results did not display the same periodic oscillations as the tidal cross correlations (i.e. a consistent six hour offset), with variable offsets between the maximum and minimum correlations. Offsets also differed for chl $a$ and turbidity even though both appeared to respond to wind in a similar manner (Fig. 6). Often the maximum correlation with WE occurred by shifting the wind later in time, suggesting elevated particulate levels prior to elevated wind effects; an explanation for this was not readily apparent.
Fig. 6: Loess 2-D smoothed graphical representation of Jenkins Neck1 (a, b) and Goodwin Islands (c, d) chl $\alpha$ (a, c), turbidity (b, d), and wind effect.
Linear regressions between time-shifted turbidity inside the SAV beds and wind effect were all highly significant (p<0.001). Only two sites (Jenkins Neck1 and Severn River) had $r^2 < 0.1$, with Guinea Marsh1 having the highest correlation with $r^2 = 0.53$. Regressions between time-shifted chl $a$ inside the SAV beds and wind effect were highly significant (p<0.001) at all sites with the exception of Jenkins Neck1 and New Point Comfort, with $r^2 > 0.1$ at Goodwin Islands, Guinea Marsh1 and Guinea Marsh2.

Correlation results for wind effect were often different outside the SAV beds compared to those from inside the beds. Goodwin Islands, Guinea Marsh1, Allen’s Island, Jenkins Neck2 and Guinea Marsh2 all had stronger correlations inside the SAV than outside with very different offsets while only New Point Comfort had similar results. For example, NTU inside Jenkins Neck1 had a correlation of $r^2 = 0.05$ with an offset of -7 hours, while the correlation for NTU outside the bed was much stronger with $r^2 = 0.41$ at 7 hours offset. Regressions for chl $a$ inside and outside the SAV displayed similar differences in both the offset and strength of the association.
Table 3: Table of cross correlation coefficient results, comparing chl $a$, NTU and $K_d$ to computed wind effect. Both the highest and lowest correlations are listed, with the amount of offset given in the same order in the following rows.

<table>
<thead>
<tr>
<th>Location</th>
<th>Cross correlation factors</th>
<th>Chl $a$</th>
<th>NTU</th>
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<tr>
<td></td>
<td></td>
<td>Inside bed</td>
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<tr>
<td>Jenkins</td>
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Discussion:

The emphasis of this study was to quantify the extent to which vegetated estuarine shallows exhibit lower particulate levels and therefore improved water clarity relative to adjacent unvegetated areas. The potential for these effects has implications for restoration and management of SAV beds in the Chesapeake Bay and other coastal environments, as studies in freshwater systems have indicated a hysteresis effect in which vegetated shallows are able to improve water clarity within the bed which supports continued growth and survival (Jeppesen et al. 2007); however when vegetation is lost the absence of this positive feedback may make restoration difficult (Scheffer et al. 2001). If the same is true for estuarine SAV beds then preservation and expansion of existing beds might be more effective than restoration efforts which have met with mixed success (Orth et al. 2002).

This study was loosely modeled after one on vegetation density and water clarity by Moore (2004), in which periodic water samples were taken with an ISCO sampler over a ten day period during four different sampling times and at 2 locations in the lower York River estuary. Moore (2004) utilized 3-4 sampling sites over a range of SAV cover during each sampling period, and documented a positive relationship between water clarity and SAV cover.

The current study was different in several ways, including greater frequency of sampling with automated recorders, an increased number of sampling locations, and a longer time period over which sampling occurred. Results did not match those obtained by Moore (2004), possibly because of the differences in sampling protocol. Instead, the
results here support a much wider range of particulate levels and possible influences on water clarity, including SAV presence, tides, wind and landscape position. Factors affecting particulate levels, such as wind and tides, were unique at each site even when the locations were in the same general vicinity. There were some noticeable differences between particulate levels inside and outside the SAV beds at almost all sites, indicating an effect of the presence of structure or vegetation, but the inconsistency in these differences suggest variable effects of tides and wind even over relatively short distances.

**Vegetation effects:**

Chl a values were significantly lower inside the SAV beds (relative to outside the beds) for four of the eight sampling periods including the first three; the other four periods including the last three had significantly lower chl a levels outside the beds (Fig. 3a). This reversal throughout the sampling period may in part be due to the seasonal die-off of eelgrass in this area which occurs when summer water temperatures exceed 25°C, typically in the beginning of July (Orth and Moore 1986). Mortality and degradation of the eelgrass and associated epiphytes would have decreased the amount of structure available for slowing currents, led to elevated detrital concentrations within the beds, and released nutrients into the water column which could have stimulated phytoplankton growth. Phytoplankton biomass in this portion of the Chesapeake Bay display a bimodal peak, with the first peak around April and a second smaller peak around July (Harding et al. 2002), which may have contributed to the overall higher chl a levels in our July sampling.
Turbidity differences were more consistent in relation to vegetation as five out of the eight periods had significantly lower NTU levels inside the SAV beds even at the end of the sampling period, while only a single deployment (Guinea Marsh1) had higher levels inside the bed. NTU levels were also more variable than chl a, possibly due to the response of NTU to wind, discussed below.

_Tidal effects:_

CCA between tidal stage and both chl a and turbidity produced several maximum correlations with small temporal offsets from the maximum water depth, including Goodwin Islands and Allen’s Island, while other sampling periods had approximately six hour offsets between high water and high particulate levels, such as Jenkins Neck1 and Jenkins Neck2 (Table 2). A positive correlation between high tide and high particulate levels, as evidenced by a small time lag, suggests that flood tides carried particulates into the shallow areas. Although most of the particulate CCA results in Table 2 were not strong, the three sites with the strongest correlations to chl a and the one site with a stronger correlation to NTU also had short time lags and the strongest tidal signature in the PSD.

The time difference between the maximum and minimum correlation results was almost always approximately 6 hours and output from the CCA always had an approximately six hour cycle, indicating that tides played a role in almost every deployment. Even when the correlations were weak, the trend of a six hour separation was evident.
Wind effects:

Chl a inside the beds responded to wind events and tidal fluctuations to a similar degree, with a significant correlation (p<0.05) to both wind events and water depth for seven out of the eight sampling periods. However, these periods exhibited a low correlation coefficient ($r^2<0.1$) for four out of the seven significant sampling periods. Wind-induced elevations in chl a in shallow areas may not necessarily be attributable to phytoplankton but instead suspension of benthic and epiphytic algae, especially within SAV beds. This could explain why some wind events had higher chl a responses than others, as one wind event may result in loss of suspended material which would not be available for suspension on a subsequent wind event.

Turbidity displayed a stronger relationship to wind events than tidal fluctuations; five out of the eight sampling periods were significantly (p<0.001) correlated to water depth, while all eight sampling periods had significant (p<0.001) correlations to wind events. Similarly, the correlation coefficients for six of the sampling periods had $r^2>0.1$ in relation to wind, while only one correlation had $r^2>0.1$ in relation to tides. Landscape position may also have influenced the response to wind, as a larger fetch can induce larger waves, and therefore higher wave induced orbital velocities.

Sites with weaker correlations between tides and particulates tides did not necessarily display stronger correlations with wind, as some sites had the strongest correlations with both parameters, such as Goodwin Islands and Guinea Marsh, other sites had the weakest correlations with both parameters, including Jenkins Neck, Jenkins
Neck2 and Allen’s Island, and some sites had stronger correlations for one parameter than for the other, such as New Point Comfort (Tables 2, 3).

Particulate levels were expected to have an exponential or sigmoidal response to wind, whereby a threshold would be reached at which the wave and current dampening properties of the seagrass beds would be overcome, resulting in a more rapid increase in particulate levels until it reached a maximum. A comparison of fitted equations, as well as visual examination of the data, however, did not lend support to the presence of such an effect. A small improvement was observed for each sampling period, but always the correlation coefficient improved by less than 0.1. Therefore, linear regressions were used for all analyses.

Light attenuation more closely followed turbidity. In the development of the light attenuation model by Xu et al. (2005) the role of turbidity was found to be more important than chl \( a \). Combining chl \( a \) and turbidity into a single value did not greatly change the particulate relationship to either tides or wind because of the more pronounced role turbidity plays in light attenuation at the sampling sites.

**Model development:**

General predictive equations relating particulate levels within SAV beds to wind and tides were developed from the best fit correlations from the eight sampling periods. The criteria used to select the sampling periods included having a correlation coefficient greater than 0.1, for tides a relatively strong PSD signature at twice per day, and for wind a visual matching of the smoothed wind events and particulate concentrations from the Loess 2-D smoothing. Data from the selected sampling periods were offset based on
best-fitting values from the cross-correlation analysis, and then combined into a single paired data set to allow for development of six separate correlations—chl a, NTU, and $K_d$ inside the SAV beds as a function of both water depth ($D$, as a proxy for tidal stage) and computed wind effect (WE) (Table 4). Only one site met the criteria for NTU and $K_d$ as a function of tides but six sites were included for wind. Three sites met the criteria for chl a as a function of both tides and wind.

The combined correlations were not stronger than the individual site correlations but provided a more global predictive model (Table 4). The weakest relationship was between chl a and wind effect (Table 4, Fig. 7c), further supporting the finding above that chl a was more related to tides (Fig. 7a), while turbidity was influenced more by wind (Fig. 7d).

The data used to develop the equations had a large amount of variation, even after the criteria were met for selecting the sampling periods to combine (Fig. 7). These equations are meant to be used as a means to develop a general understanding between particulates and the external forcing factors of wind and tides. Landscape position of a given site will determine the relative strength of a given equation when wind and tides are combined to predict particulate levels. A site exposed to a larger fetch should have a stronger relationship to wind, while a site with stronger tidal currents may be more subject to tidal influences. Tides may carry either clearer water into a site, as in the case of the Chesapeake Bay mainstem potentially contributing to lower NTU at high tide at Jenkins Neck, or it may bring in more turbid riverine water, which seems to be the case for several of the other sites in this study (Table 3).
Because the combined datasets in Table 4 were not normally distributed and most did not have a constant variance, a series of regressions was performed on portions of the datasets to test how upper and lower values may affect the regression output. Use of the middle 80% of the data was selected as a means of eliminating values from both the high and low end of the dataset. Since there was greater scatter in the higher values (see Fig. 7), the lower 90% of the values were also used in the analyses. Regressions on other portions of the datasets did not improve and often degraded the fits.

The results of the series of regressions were mixed; regressions on the middle and full datasets were more similar for NTU and $K_d$, while regressions using the full and lower datasets were more similar for chl $a$ (Table 4). Chl $a$ as a function of wind effect was the only regression that had a correlation coefficients lower than 0.1, and the regression using the middle 80% of the data was not significant ($p = 0.07$). Regressions of NTU and tides displayed the most variation, most likely due to the smaller sample size.
Table 4: Linear regression coefficients and standard errors from selected sampling periods for regressions between chl $a$, NTU, and $K_d$ from either tidal fluctuations (water depth, D) or wind effect (WE). All regressions were significant ($p<0.001$) except when $r^2$ is denoted with an asterisk. "Full" indicates the regression was performed on the full dataset, "Middle" indicates the regressions were performed after eliminating the upper and lower 10% of values by count, to eliminate outlying values, and "Lower" indicates the regressions were performed after eliminating only the upper 10% of values.

<table>
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<th>Std. error</th>
<th>Slope</th>
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<td>0.97</td>
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Fig. 7: Plots of the best fitting regressions for chl \( a \) and turbidity inside of SAV beds based on water depth (a, b) and wind effect (c, d). Sampling periods used are listed in Table 4.
Although turbidity responded more strongly to wind and chl \(a\) was more related to tides, wind and tides had an effect on both particulate measures. The regressions developed indicated that what may be affecting chl \(a\) may also be affecting NTU; higher wind and higher tides increased both chl \(a\) and turbidity. Furthermore, there was a significant correlation between the differences in chl \(a\) and turbidity inside and outside the SAV beds across all deployments (Fig. 8).

1. All data –

\[ \text{NTU} = 2.048 + (1.332 \times \text{chl } a), \quad r^2 = 0.384, \quad p<0.001; \]

2. Data from inside the SAV beds only –

\[ \text{NTU} = 2.047 + (1.241 \times \text{chl } a), \quad r^2 = 0.382, \quad p<0.001; \]

3. Data from outside the SAV beds only –

\[ \text{NTU} = 1.939 + (1.460 \times \text{chl } a), \quad r^2 = 0.397, \quad p<0.001. \]

All three regressions were highly significant and similar regardless of location inside or outside the SAV beds. The regressions indicated that NTU values were typically higher and increased slightly faster than chl \(a\) values.
Fig. 8: Comparison of the difference between paired observations inside and outside of SAV beds for NTU and chl $a$. A positive difference indicates the value inside the bed was higher, while a negative difference indicates the value outside the bed was higher.
Summary:

Particulate levels were not consistently lower inside the SAV beds sampled in this study compared to values outside the beds; turbidity was generally lower within the beds while patterns in chl $a$ varied throughout the season. Chl $a$ levels within the studied vegetated beds were more influenced by tides than wind; turbidity was more strongly influenced by wind than tides. Effects of wind and tides varied among sites. Additional studies, specifically season long quantification of particulates at several sites, would be useful to develop stronger relationships between particulates, SAV abundance, and predictors including tides and wind.

General regression equations for predicting particulate levels inside the SAV beds in this study, and therefore the impact of particulates on water clarity and subsequent eelgrass growth, provide a means for analyzing major trends, such as the impact of wind and storm events on eelgrass growth at different times of the year. These regressions are considered only moderately predictive of particulate levels, however, due to their low correlation coefficients and the high variability in the data. Attempts to apply these regressions further should consider including stochastic variations around the mean predictions.
References cited:


56


Chapter 2:

*Zooplankton densities within the vegetated shallows of the York River Estuary, Virginia*
ABSTRACT

Zooplankton are known to concentrate within beds of submerged aquatic vegetation (SAV) in freshwater systems, resulting in elevated clearance rates within the vegetated shallows and serving to maintain water clarity and presumably enhance growth and survival of SAV. We investigated the potential for similar vegetation related differences in zooplankton density, as well as tidal and diurnal differences, in the York River Estuary, Virginia, a tributary of the Chesapeake Bay, and estimated their ability to enhance water clarity via filtration in these polyhaline SAV beds.

Zooplankton were sampled during the summers of 2006 and 2007 using a novel pumped sampling device designed specifically to sample the water column within shallow water structured environments. In 2006, zooplankton densities were significantly higher in vegetated areas than non-vegetated (p=0.05), but were not significantly different in 2007 (p=0.46). Zooplankton densities were significantly higher at night at all sites, both inside and outside of the vegetated beds (p<0.01). Zooplankton densities varied only slightly with tidal stage, with only one site exhibiting a significant relationship between zooplankton densities and water level as a proxy for tidal stage. There were significant differences in zooplankton density between sites in close proximity in both years (p<0.01), indicating patchy zooplankton distribution. Overall, the zooplankton densities typically encountered within the SAV had the potential to filter only 2-6% of the water column per day, providing a limited ability to improve water clarity in the studied systems.

KEYWORDS: zooplankton, submerged aquatic vegetation, Zostera marina, eelgrass, estuary, Chesapeake Bay
INTRODUCTION

Previous studies have shown that shallow vegetated areas, in both freshwater and marine environments, can have improved water clarity relative to nearby unvegetated areas (Hasler and Jones 1949; Jones 1990; Biyu 2000; Jeppesen et al. 2002; Moore 2004). There are a multitude of direct and indirect effects that submersed aquatic vegetation (SAV) can have on water clarity, including possible allelopathy in which the SAV chemically inhibits phytoplankton growth (Jasser 1995; van Donk and van de Bund 2002; Erhard and Gross 2006), reduction of suspended sediments and adhered nutrients through reduced current velocity and subsequent settling (Lake and MacIntyre 1977; Short and Short 1984; Vermaat et al. 2000; Wigand et al. 2000; Madsen et al. 2001; Schulz et al. 2003; Takamura et al. 2003; Li et al. 2008), the attraction of particulates to leaf structures (Palmer et al. 2004), shading of phytoplankton as the SAV forms a canopy along the water surface (Buzzelli et al. 1998; Wigand et al. 2000; Horppila and Nurminen 2003; Folke et al. 2004; Palmer et al. 2004), and removal of nutrients directly from the water column (Short and Short 1984; Horppila and Nurminen 2003). Each of these effects alone may not be large, but when considered in combination may play a significant role in maintaining the water clarity necessary for continued SAV survival and growth. The potential synergistic effects of combining several of these factors may also be larger than a simple additive effect (Folke et al. 2004).

Perhaps the most important indirect effect of SAV on water clarity is the physical structure of the SAV providing habitat for a variety of fauna, including suspension and filter feeding zooplankton and benthic invertebrates (Reusch and Reusch 1998; Bostrom
and Mattila 1999; Peterson et al. 2001; Hovel et al. 2002), as well as larval and juvenile fish (Orth and Heck 1980; Olney and Boehlert 1988; Jenkins et al. 1998; Baskin et al. 2003). Zooplankton (in freshwater) and macrobenthos (in marine systems) are frequently found in much higher densities within SAV beds than in the open water, and zooplankton may migrate horizontally into SAV on a diurnal basis to escape predation (Heck et al. 1995; Stansfield et al. 1995; Duffy 1997). In freshwater systems, zooplankton (especially large-bodied cladocerans like *Daphnia* spp.) often occur in higher densities in the vegetated shallows compared to open water systems in the adjacent pelagic zone or in other aquatic systems with similar physical and nutrient characteristics but no vegetation (Scheffer 1999; van Donk and van de Bund 2002; Muylaert et al. 2003). Since most zooplankton are suspension feeders, their increased density within vegetated areas compared to open water increases the removal of particulates, including phytoplankton, from the water column (Schriver et al. 1995; Ejsmont-Karabin et al. 1996; Biyu 2000; van Donk and van de Bund 2002). This allows for improved water clarity and a positive feedback between the zooplankton and vegetation, i.e., a refuge for the zooplankton improves the water clarity for the vegetation (Scheffer 2001; Jeppesen et al. 2007a).

In marine systems the role of zooplankton in SAV beds has been little studied (Robertson et al. 1988; Jeppesen et al. 2007b). Two studies compared zooplankton densities inside and outside of tidal marine vegetated systems by towing nets through open areas adjacent to or within a bed or over the vegetation at high tide, instead of sampling the water column within the structure (Meyer 1982; Robertson et al. 1988). These studies produced conflicting results: Robertson et al. (1988) found higher densities
of several types of zooplankton within the seagrass beds, while Meyer (1982) did not identify any differences. Monk (1988) reported increased abundance of multiple zooplankton groups within SAV beds of the tidal freshwater Potomac River, a tributary of the Chesapeake Bay, but the extent to which these patterns extend into the estuarine portion the bay has received little attention.

Given the potential of freshwater zooplankton to enhance the water clarity of vegetated freshwater shallows, the purpose of this study was to ascertain if the same potential exists within brackish estuaries. Three aspects regarding the possible effect of SAV on zooplankton are addressed: the role of SAV density, diel effects, and the role of tides. We predicted a positive relationship between vegetative density and daytime zooplankton density, although not necessarily in a linear fashion given the potential for a threshold SAV density beyond which its habitat value is enhanced. The possible benefit of vegetation for freshwater zooplankton has been hypothesized as providing protection from predation during the day and inducing diel horizontal migration into the vegetation during daylight (Perrow et al. 1999; Burks et al. 2002). A similar vertical migration is known for marine and estuarine zooplankton, even in shallow water systems, whereby zooplankton migrate deeper during the day for protection and towards the water surface during the night to feed (Roman et al. 1988; Cuker and Watson 2002). We also hypothesized that tides could play a role in the transport of zooplankton between the vegetated and unvegetated areas, as has been shown for estuarine zooplankton that migrate vertically to take advantage of currents and maintain their position within the estuary (Kimmerer and McKinnon 1987). It is possible that some zooplankton take
advantage of incoming tides to bring them into vegetated areas, and then migrate downwards as the tide goes out to maintain their position within the vegetation and avoid predation especially during the day, thereby increasing their density within the SAV.

To obtain samples directly within the SAV canopy and in adjacent unvegetated shallows, a shallow water sampling device was required to avoid disruption of the SAV caused by traditional net tows, and to allow sampling in waters too shallow for tows. A novel pumped sampling device was constructed and used to sample zooplankton densities inside and adjacent to multiple SAV beds in the lower York River Estuary, Virginia, a tributary of Chesapeake Bay, across a range of SAV densities, tide stages, and times of day. A description of the sampler used is located in Appendix 1.
MATERIALS AND METHODS

Sampling sites

Sampling was conducted in the lower Chesapeake Bay, in the general vicinity of the lower York River and Mobjack Bay, Virginia, USA (Fig. 1). Several sampling sites throughout the area were chosen based on the presence of densely vegetated shallows. The main vegetation in these sites is *Zostera marina*, with *Ruppia maritima* also present. During 2006, samples were collected in both *Z. marina* and *R. maritima* beds, while during 2007, samples were taken in areas dominated by *Z. marina*. Except for the diel and tidal cycle sampling, samples were taken around low to mid tide typically between 10:00 and 15:00, at depths of approximately 0.5 – 1.5 m, to avoid confounding factors of depth and time of day.

Pumped zooplankton sampler

All sampling was conducted with a Shallow Water and Zooplankton Pumped Sampler (SWaZooPS) (Fig. 2), loosely based on a similar proven design that included testing of the pumped samplers capability compared to traditional net tows (Dixon and Robertson 1986). Several important modifications were made to their design to ensure accurate measurement of the volume sampled, minimize disturbance to the area being sampled, avoid disrupting the zooplankton and water column, and allow for the simultaneous removal of water samples for additional analyses. Volumetric calibration verified this aspect of its accuracy; limited zooplankton calibration samples indicated it was comparable to net tows. In addition, since all samples in this study were collected with SWaZooPS, the samples were considered comparable to each other regardless of
this sampler's ability compared to other collection methods. A detailed description of the sampler is provided in Appendix 1.

SWaZooPS pumps water through a 200 μm plankton net at approximately 37 L per minute, allowing filtration of 350-400 L for each sample in about 10 minutes. While the specific parcel of water being sampled is difficult to determine due to ambient water currents, in the absence of currents we estimated the volume sampled around the intake to be a sphere 0.88 m in diameter based on a volume sampled of approximately 370 L. To obtain an integrated sample of the water column, the intake was initially positioned at mid-depth and slowly moved around a sphere of approximately 0.4 m radius while pumping, depending on water depth and SAV canopy structure at the time of sampling.
Figure 1. Zooplankton sampling sites from the summer of 2006 and 2007 in the vicinity of the lower York River and Mobjack Bay, Chesapeake Bay, Virginia, USA
Figure 2. Shallow Water and Zooplankton Pumped Sampling device (SWaZooPS). Water is pumped in through the pipe extending under the raft into the water, through the flow meter above the raft, and then into the collecting net. The smaller outlet located just prior to (left of) the flow meter is utilized for water samples and for rinsing, thereby not affecting the measurement of water volume for the zooplankton sample, and also allowing simultaneous sampling of the water and zooplankton.
Zooplankton collection and enumeration

In the summer of 2006, zooplankton samples were collected over a range of vegetative densities at Allen’s Island, Jenkins Neck, and Guinea Marsh (Fig. 1). Vegetative cover was visually estimated in 5% increments within a 1 m radius centered on the location of the pump inlet. The SAV densities were placed into 4 categories: no SAV (outside the SAV bed), 0-33% cover, 33-66% cover, and 66-100% cover. Three replicate samples were collected within each SAV cover category providing 12 samples during each sampling episode, with two complete sets of zooplankton samples collected at each location and a total of 72 samples during 2006.

To broaden the scope of sampling during the summer of 2007, samples were collected from more locations than 2006, but not over as many SAV densities. During the 2007 sampling period, samples were taken at all 5 sites shown on Figure 1 in a high (66-100% cover) SAV density and outside the SAV beds only, with 3 replicates (6 total samples) at each site for 30 samples. Also during 2007, three periods of extended sampling were performed to ascertain both tidal cycle and diel effects on zooplankton abundances at Allen’s Island, Jenkins Neck, and Guinea Marsh (Fig. 1). Tidal water depths were obtained online (www2.vims.edu/vecos) from the Chesapeake Bay National Estuarine Research Reserve System Goodwin Islands monitoring station near our Goodwin Islands sampling site (Fig. 1). Samples were collected during daylight at low tide, mid flood tide, high tide, and mid ebb tide, with an additional set of samples collected during that night. For each sampling period, 3 samples were collected inside and 3 samples outside the SAV beds, for a total of 30 samples (15 inside the SAV, 15
outside the SAV) and 90 samples combined for the diel and tidal cycle sampling. The total sampling effort for 2007 is therefore 120 samples.

A specific sampling point was chosen haphazardly while in the field that met the necessary cover criteria, the pump inlet was extended out approximately 1.5 m from the person holding the inlet, and the pump was turned on to begin sample retrieval. The initial several liters of pumped water to rinse out the pipes were discarded. During sample collection, care was taken to limit disturbance of the area to be sampled, including disturbance of the vegetation and sediments. As mentioned above, while the sample was being collected, the inlet was moved slowly around in a sphere of approximately 0.4 m diameter centered mid-depth to ensure an integrated sample from the water column.

After the collection of each zooplankton sample, the net was removed from its holder and thoroughly rinsed from the outside to wash all zooplankton into the cod end. The cod end was removed, the contents rinsed into pre-labeled glass jars which were placed on ice in a cooler and the samples preserved in buffered 4% Formaldehyde at the end of the day.

Zooplankton samples were enumerated to readily identifiable taxonomic groups. Since our main objective was to determine differences in overall zooplankton abundance and potential rates of particulate removal, we did not identify the zooplankton to genus and species levels, except for the copepod *Acartia tonsa*, the dominant species in most samples. Samples were enumerated with a zoom-stereo microscope utilizing a gridded or circular counting dish. Each sample was split using a Folsom plankton splitter until there were approximately 200 individuals of the most common taxa present. The number of
splits was recorded along with the counts, which were then used to calculate the number of each taxa per whole sample and divided by the volume of water filtered to obtain density (individuals L$^{-1}$).

**Statistical analyses**

Counts of various zooplankton taxa were combined into four general categories representing total zooplankton and the three consistently most numerous groups: *A. tonsa*, other copepods, and planktonic barnacles (barnacle nauplii and cyprids). Statistical analyses were performed with SigmaPlot version 11. Results for all analyses were first tested for normality, after which the data were necessarily log$_{10}$ transformed. Two-way ANOVAs on transformed data were utilized for most analyses for both years, with site and SAV density as factors and zooplankton density as the response variable. For 2007 data, two-way ANOVA was first conducted using zooplankton densities collected only during the day, with location and inside/outside the SAV beds as factors. To test for diel effects, samples collected at the same tidal stage during the day and night (approximately 12 hours apart) were compared with three-way ANOVA using day/night, location, and inside/outside the SAV beds as factors. Polynomial regressions ($1^{st}$ and $2^{nd}$ order to check both linear and non-linear fits) were fit to SAV cover and zooplankton density from 2006, and also used to test for tidal cycle effects on zooplankton density from 2007, using depth as a proxy for tidal cycle, using only the daytime samples.
RESULTS

2006 sampling

The results from the 2006 sampling indicated that denser zooplankton populations were generally found in denser (33-66% and 66-100% cover) SAV beds (Fig. 3, Table 1), although the high variability of the data resulted in limited statistically significant differences between cover classes (Table 2). When samples from all sites were combined, both planktonic barnacles and total zooplankton densities were significantly higher at sites with the densest SAV relative to sites with the least dense vegetation (Fig. 3, Table 2). Differences in A. tonsa and other copepods amongst different SAV coverage were not significant; however these groups did exhibit trends similar to barnacles and total zooplankton.

Total zooplankton densities in 2006 were significantly different between locations, with highest densities at Jenkins Neck, followed by Allen’s Island and then Guinea Marsh (p<0.01) (Table 2). There were no significant differences in zooplankton density with SAV cover at Allen’s Island, although sites with no vegetation were higher than the two middle vegetative densities and the densest vegetative cover had the highest densities (Table 2). Trends were similar at Guinea Marsh, with statistically significant higher densities of zooplankton in the absence of vegetation and in the highest cover class compared to the middle two cover classes. At Jenkins Neck significantly higher densities of zooplankton occurred in the two highest cover classes compared to no SAV and the lowest cover class.
Table 1. Mean zooplankton density (number L$^{-1}$) by location and %SAV cover category, 2006 sampling season. Values in parentheses are standard errors (SE), n=6 for each cell.

<table>
<thead>
<tr>
<th>Location</th>
<th>Allen's Island</th>
<th>Guinea Marsh</th>
<th>Jenkins Neck</th>
<th>combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>%SAV cover</td>
<td>No 0-33-66-100</td>
<td>No 0-33-66-100</td>
<td>No 0-33-66-100</td>
<td>No 0-33-66-100</td>
</tr>
<tr>
<td>Acartia tonsa</td>
<td>4.6 (1.8)</td>
<td>3.9 (1.8)</td>
<td>3.6 (2.5)</td>
<td>4.0 (1.2)</td>
</tr>
<tr>
<td></td>
<td>3.0 (1.8)</td>
<td>1.5 (2.2)</td>
<td>3.4 (2.5)</td>
<td>2.6 (1.2)</td>
</tr>
<tr>
<td></td>
<td>3.2 (2.2)</td>
<td>1.5 (1.9)</td>
<td>23.4 (2.5)</td>
<td>9.4 (1.3)</td>
</tr>
<tr>
<td></td>
<td>5.9 (1.8)</td>
<td>3.9 (1.8)</td>
<td>6.1 (2.5)</td>
<td>5.3 (1.2)</td>
</tr>
<tr>
<td>Other copepods</td>
<td>0.6 (0.7)</td>
<td>1.5 (0.7)</td>
<td>0.6 (1.0)</td>
<td>0.9 (0.5)</td>
</tr>
<tr>
<td></td>
<td>0.7 (0.7)</td>
<td>0.9 (0.9)</td>
<td>1.3 (1.0)</td>
<td>1.0 (0.5)</td>
</tr>
<tr>
<td></td>
<td>1.5 (0.7)</td>
<td>0.7 (0.8)</td>
<td>3.4 (1.0)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td></td>
<td>2.6 (0.7)</td>
<td>1.4 (0.7)</td>
<td>5.8 (1.0)</td>
<td>3.3 (0.5)</td>
</tr>
<tr>
<td>Barnacles</td>
<td>1.0 (0.6)</td>
<td>1.1 (0.6)</td>
<td>0.8 (0.9)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>1.3 (0.6)</td>
<td>0.5 (0.7)</td>
<td>1.2 (0.9)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>1.7 (0.7)</td>
<td>0.2 (0.7)</td>
<td>1.5 (0.9)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>4.3 (0.6)</td>
<td>0.5 (0.6)</td>
<td>6.5 (0.9)</td>
<td>3.8 (0.4)</td>
</tr>
<tr>
<td>total</td>
<td>7.2 (2.5)</td>
<td>7.4 (2.5)</td>
<td>5.3 (3.5)</td>
<td>6.9 (1.7)</td>
</tr>
<tr>
<td></td>
<td>5.4 (2.5)</td>
<td>3.2 (3.0)</td>
<td>6.2 (3.5)</td>
<td>4.9 (1.8)</td>
</tr>
<tr>
<td></td>
<td>6.8 (2.5)</td>
<td>2.7 (2.7)</td>
<td>28.9 (3.5)</td>
<td>12.8 (1.8)</td>
</tr>
<tr>
<td></td>
<td>13.4 (2.5)</td>
<td>6.3 (2.5)</td>
<td>18.9 (3.5)</td>
<td>12.9 (1.8)</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(2.5)</td>
<td>(2.5)</td>
<td>(1.7)</td>
</tr>
<tr>
<td></td>
<td>(3.0)</td>
<td>(2.7)</td>
<td>(3.5)</td>
<td>(1.8)</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(2.7)</td>
<td>(3.5)</td>
<td>(1.7)</td>
</tr>
</tbody>
</table>
Figure 3. Summer 2006 zooplankton densities, all locations combined. The error bars reflect one standard deviation. Significant differences between percent cover for a given taxa are indicated by a different letter within each zooplankton category. There were no significant differences between the different densities of SAV for either *A. tonsa* (p=0.26) or other copepods (p=0.07).
Table 2. Results of 2-way ANOVA on total zooplankton density, 2006 sampling season, with location and % cover as factors. Results for % cover are presented. Values in parentheses are standard errors (SE), bottom table values are p values from Holm-Sidak Multiple Comparison method. Statistically significant interactions between location and % cover were identified, see Appendix 3 for details.

<table>
<thead>
<tr>
<th>%SAV cover</th>
<th>Allen's Island</th>
<th>Guinea Marsh</th>
<th>Jenkins Neck</th>
<th>Locations combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%SAV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No  1- 33- 66- 100</td>
<td>No  1- 33- 66- 100</td>
<td>No  1- 33- 66- 100</td>
<td>No  1- 33- 66- 100</td>
</tr>
<tr>
<td>Mean number L^3±SE</td>
<td>7.2 (2.5)</td>
<td>5.4 (2.5)</td>
<td>6.8 (3.0)</td>
<td>13.4 (2.5)</td>
</tr>
<tr>
<td>1-33</td>
<td>0.51 - - -</td>
<td>0.06 - - -</td>
<td>0.59 - - -</td>
<td>0.16 - - -</td>
</tr>
<tr>
<td>33-66</td>
<td>0.62 0.63 - -</td>
<td>0.01 0.49 - -</td>
<td>0.00 0.01 - -</td>
<td>0.53 0.13 - -</td>
</tr>
<tr>
<td>66-100</td>
<td>0.32 0.16 0.35 -</td>
<td>0.63 0.07 0.02 -</td>
<td>0.01 0.01 0.52 -</td>
<td>0.05 0.00 0.09 -</td>
</tr>
</tbody>
</table>
Within the three zooplankton categories, Jenkins Neck had significantly higher *A. tonsa* (p=0.03) and nearly significantly higher copepod (p=0.053) densities than the other two locations and Guinea Marsh had significantly lower barnacle densities than the other sites (p<0.01). Results demonstrate the variability of zooplankton both across vegetative densities and location, and the potential for occasional dense patches of zooplankton even when the locations sampled are not physically far apart (Fig. 1).

Linear regression of total zooplankton density against % SAV cover as estimated in the field was statistically significant but only explained a small fraction of the variability (p=0.01, \( r^2=0.11 \)). Though significant, the increase in zooplankton density was small as indicated by the slope (\( Z = 5.5 + (0.072 \times \% \text{ cover}) \), where \( Z \) = total zooplankton density (number L\(^{-1} \)). A 2\(^{nd}\) order polynomial to account for a non-linear trend did not improve the fit (p=0.07, \( r^2=0.12 \); \( Z = 6.1 + (0.0079 \times \% \text{ cover}) + (0.00065 \times \% \text{ cover}^2) \)).

2007 sampling

Zooplankton densities in 2007 varied significantly with tidal stage (p<0.01), but the slope was so small the differences were considered inconsequential (slope = 0.02 outside the SAV bed and 0.08 inside the SAV bed), thus all daytime samples regardless of tidal cycle were included in the analysis of densities inside and outside the SAV beds, excluding only the nighttime samples. From this data zooplankton densities in 2007 were not significantly higher inside the SAV beds than outside, with total zooplankton density marginally higher outside the beds (p=0.46) (Fig. 4, Table 3, Table 4). There were also no significant differences between densities inside vs. outside the SAV for any of the
individual sampling sites, total zooplankton or the individual taxonomic groups, although Allen’s Island and New Point Comfort had slightly higher mean total zooplankton density inside the SAV beds (Fig. 4).

When diel sampling is included for the 3-way ANOVA analysis that utilized day/night, inside/outside of the SAV and location (Allen’s Island, Guinea Marsh, and Jenkins Neck) as factors, total zooplankton density in 2007 was significantly higher (p=0.02) outside the SAV bed than inside (Figure 6, Table 4). This result could have been influenced by three samples outside the SAV at night at Guinea Marsh, which had the highest zooplankton density in 2007, and three of the four highest zooplankton densities encountered for the entire study (Fig. 5b). *A. tonsa* occurred in significantly lower densities inside the SAV relative to densities outside during the day (p=0.03), but differences were not significant at night (p=0.08) and only significant at Guinea Marsh (p<0.01) for individual locations. In contrast, for other copepods, inside densities were significantly higher than outside overall (p=0.04) and at night (p<0.01), but higher outside (though not significant) during the day (p=0.08). Inside densities were lower than outside for planktonic barnacles for all locations combined during the day (p<0.01) and at Allen’s Island (p=0.03, outside higher), and for copepods at Guinea Marsh only (p=0.04).
Table 3. Mean zooplankton density (number L⁻¹) (±SE) by location and inside vs. outside the SAV (in/out), 2007 sampling season; n=15 for each cell under Allen’s Island, Guinea Marsh and Jenkins Neck, n=3 for each cell under Goodwin Islands and New Point Comfort, and n=51 for each cell under combined.

<table>
<thead>
<tr>
<th>Location</th>
<th>Allen's Island</th>
<th>Guinea Marsh</th>
<th>Jenkins Neck</th>
<th>Goodwin Islands</th>
<th>New Point Comfort</th>
<th>combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>Out</td>
</tr>
<tr>
<td>Acartia tonsa</td>
<td>0.4</td>
<td>(0.8)</td>
<td>4.3</td>
<td>(0.7)</td>
<td>0.5</td>
<td>(0.8)</td>
</tr>
<tr>
<td>Other copepod</td>
<td>0.3</td>
<td>(0.1)</td>
<td>0.7</td>
<td>(0.1)</td>
<td>0.5</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Barnacles</td>
<td>2.3</td>
<td>(0.9)</td>
<td>3.5</td>
<td>(0.9)</td>
<td>1.0</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Total</td>
<td>3.5</td>
<td>(1.2)</td>
<td>8.9</td>
<td>(1.1)</td>
<td>2.5</td>
<td>(1.2)</td>
</tr>
</tbody>
</table>
Table 4. Results of 2-way ANOVA on total zooplankton daytime density with location and inside vs. outside the SAV (in/out) as factors for 2007. Results for in/out are presented. No interactions were identified, see Appendix 3 for details.

<table>
<thead>
<tr>
<th>Location</th>
<th>In/out</th>
<th>mean number L$^1$ (±SE)</th>
<th>p-value, in vs. out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen's Island</td>
<td>In</td>
<td>3.5 (1.2)</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>3.2 (1.2)</td>
<td>-</td>
</tr>
<tr>
<td>Guinea Marsh</td>
<td>In</td>
<td>8.9 (1.1)</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>11.3 (1.1)</td>
<td>-</td>
</tr>
<tr>
<td>Jenkins Neck</td>
<td>In</td>
<td>2.5 (1.2)</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>2.8 (1.2)</td>
<td>-</td>
</tr>
<tr>
<td>Goodwin Islands</td>
<td>In</td>
<td>2.4 (2.7)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>4.7 (2.7)</td>
<td>-</td>
</tr>
<tr>
<td>New Point Comfort</td>
<td>In</td>
<td>1.5 (3.3)</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>1.3 (3.3)</td>
<td>-</td>
</tr>
<tr>
<td>Combined</td>
<td>In</td>
<td>3.8 (0.9)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>4.7 (0.9)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5. Results of 3-way ANOVA on total zooplankton density with location, inside vs. outside the SAV (in/out) and day/night as factors for 2007. Results for in/out and day/night are presented. Statistically significant interactions between in/out and both day/night and location were identified, but not between location and day/night; see Appendix 3 for details.

<table>
<thead>
<tr>
<th>Location</th>
<th>In/out</th>
<th>mean number L⁻¹ (±SE)</th>
<th>p-value in vs. out</th>
<th>Day/night</th>
<th>mean number L⁻¹ (±SE)</th>
<th>p-value day vs. night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen’s Island</td>
<td>In</td>
<td>1.7 (1.1)</td>
<td>0.11</td>
<td>day</td>
<td>1.2 (1.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>2.0 (1.1)</td>
<td></td>
<td>night</td>
<td>2.5 (1.1)</td>
<td>-</td>
</tr>
<tr>
<td>Guinea</td>
<td>In</td>
<td>11.3 (1.1)</td>
<td>&lt;0.01</td>
<td>day</td>
<td>11.0 (1.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Marsh</td>
<td>Out</td>
<td>28.6 (1.1)</td>
<td></td>
<td>night</td>
<td>28.9 (1.1)</td>
<td>-</td>
</tr>
<tr>
<td>Jenkins Neck</td>
<td>In</td>
<td>3.3 (1.2)</td>
<td>0.18</td>
<td>day</td>
<td>1.2 (1.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>1.8 (1.1)</td>
<td></td>
<td>night</td>
<td>3.9 (1.1)</td>
<td>-</td>
</tr>
<tr>
<td>Combined</td>
<td>In</td>
<td>5.5 (0.6)</td>
<td>0.02</td>
<td>day</td>
<td>4.5 (0.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>10.8 (0.6)</td>
<td></td>
<td>night</td>
<td>11.8 (0.6)</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 4. Mean daytime zooplankton densities from 2007, grouped by taxonomic category. Error bars represent one standard deviation. Differences between inside and outside the SAV bed were not statistically significant.
Figure 5. Diel and tidal cycle zooplankton density results for Allen's Island (a), Guinea Marsh (b), and Jenkins Neck (c). The last sample point for inside or outside the SAV bed was taken in the dark (shaded area). Water level data are from the Goodwin Islands CBNERRS monitoring station, which was used as a proxy for tide stage.
Significant differences were found for time of day (p<0.01), inside vs. outside the SAV beds (p<0.01), and location (p<0.01); see text for details.

The relationship between tide stage (using water level as a proxy) and total zooplankton density was analyzed by fitting 1st order (linear) and 2nd order (non-linear) polynomial regressions. Zooplankton densities responded inconsistently to tidal stage, with a mix of both very small positive and negative slopes depending on the site and location inside or outside of the SAV beds; most of the linear and polynomial regressions were not significant. With all of the daytime samples combined using both inside and outside the SAV beds, fits were not significant (1st order: p=0.09, r^2=0.04, slope=0.05; 2nd order: p=0.06, r^2=0.09), while regressions were significant for samples from inside the SAV beds for a 1st order equation but not for a 2nd order equation (1st order: p=0.04, r^2=0.12, slope=0.08; 2nd order: p=0.08, r^2=0.07). Fits for zooplankton density outside the SAV were a little better, but still not strong (1st order: p<0.00, r^2=0.27, slope=0.02; 2nd order: p<0.00, r^2=0.40). At the individual sites, regressions were strongest at Guinea Marsh, the only site to have significant fits, although a linear response was almost flat inside the SAV beds while a 2nd order equation had a much better fit (inside – 1st order: p=0.99, r^2=0.00, slope=0.00; 2nd order: p=0.01, r^2=0.67; outside – 1st order: p<0.00, r^2=0.64, slope=-0.31; 2nd order: p=0.02, r^2=0.65).

Zooplankton densities were significantly higher at night than during the day for all sites combined and each site separately (p<0.01 for each separately or sites combined, Fig. 5, Table 4), as well as for all combinations of location and position inside/outside of the SAV beds. Nighttime zooplankton densities were particularly high at Guinea Marsh,
with all three samples from outside the SAV bed having three of the four highest densities collected during both years of this study. *A. tonsa* and other copepods were significantly denser at night than during the day for almost all combinations of location and position inside/outside, similar to the total zooplankton densities. Barnacles, however, did not display significantly different diel trends overall or at any of the sites except for Guinea Marsh, where nighttime densities were significantly higher than day.
DISCUSSION

Overall, there were no consistent differences in zooplankton densities either in relation to SAV or tidal influences, although some individual locations had statistically significant differences between each other. There were consistent significant differences between night and day, with higher zooplankton density at night both inside and outside the SAV for all locations individually and combined. However, except for a few samples with very high zooplankton densities, estimates of the ability of zooplankton to improve water clarity through particulate removal was limited.

Zooplankton densities inside vs. outside of the SAV beds and across sites

When results from 2006 and 2007 are considered together, the variability of zooplankton densities and absence of consistent zooplankton habitat preference suggest that SAV beds do not act as refugia for zooplankton in this estuarine system as they do in some freshwater systems (Perrow et al. 1999; Muylaert et al. 2003). Hamilton et al. (1990) concluded that macrophyte beds in freshwater systems may actually reduce zooplankton densities, as lower densities of zooplankton were found down current of macrophyte mats compared to upstream from the mats. Burks et al. (2000) found that a combination of macrophyte and fish exudates inhibited Daphnia growth and reproduction, which indicates a possible detriment instead of benefit to these cladocerans.

Results from 2006 sampling indicated statistically significant higher zooplankton densities within SAV beds vs. outside, but the results were highly variable (Fig. 3, Table 2). In addition, 2006 zooplankton densities were also statistically significantly positively correlated with increasing SAV cover, but the slope of 0.07 is very small. This limited
slope essentially makes the trend meaningless in terms of variation in overall zooplankton density levels, as well as the particulate removal capacity of the zooplankton community, across varying SAV densities. In contrast, results of the daytime sampling in 2007 indicated no statistically significant differences between zooplankton densities inside and outside of the SAV beds; the trend was opposite from 2006 with mean densities slightly higher outside.

Zooplankton are well known to be patchily distributed, so variability in shallow systems is to be expected (Harding 2001). Zooplankton densities at all three locations in 2006 and most locations in 2007 were statistically significantly different from each other as well as having high variability within each sampling location. This variability could be the result of many factors not covered in this study, including localized food ability, predation avoidance, or aggregation or dispersal due to currents and eddies. The higher densities of zooplankton in the 33-66% cover category in 2006 were largely due to a single sample that was taken inside the SAV bed at Jenkins Neck adjacent to a stand of Ruppia maritima and had 40 A. tonsa and 48 total zooplankton L⁻¹, higher than any other single sample. A second sample nearby also had a relatively high zooplankton count of 28 zooplankton L⁻¹. Similarly, the 66-100% cover category had five samples with zooplankton densities over 15 zooplankton L⁻¹, again raising the mean of the 66-100% cover group, while the majority of the counts were similar to the rest of the zooplankton densities encountered. Without these seven samples it is likely that the 2006 results would not have displayed as many statistically significant differences and been similar to the results from 2007.
Not identifying consistent differences in zooplankton densities inside vs. outside the SAV beds in this study is in direct contrast to studies that have shown differences in freshwater systems (Beklioglu and Moss 1996; Perrow et al. 1999; Scheffer 1999; Burks et al. 2002; Lau and Lane 2002), but this is not entirely unexpected. SAV beds in marine and higher salinity estuarine systems are known for attracting benthos, both epibenthic and infaunal (Castel et al. 1989; Fredette et al. 1990; Heck et al. 1995; Mattila et al. 1999; Attrill et al. 2000; Bostroem and Bonsdorff 2000), while a recent study indicated an overall reduction in zooplankton density, especially large-bodied cladocerans, as salinity increases (Jeppesen et al. 2007b). This suggests that freshwater vegetated areas may be more prone to higher zooplankton densities while increases in salinity levels shifts the filter-feeding fauna to the benthos.

The amount of variability in this study is consistent with other studies, and reflects the overall patchiness of zooplankton distributions. Both freshwater and estuarine studies on zooplankton have documented this variability, with some questioning the degree to which zooplankton contribute to the improved water clarity within vegetated shallows because of this inherent patchiness (van Donk and van de Bund 2002), indicating other factors such as enhanced particulate settling may play a more important role (Blindow et al. 2000; Blindow et al. 2002).

Influence of tidal stage

Tidal stage also did not appear to appreciably affect zooplankton densities. Only one of the sampling locations, Guinea Marsh, exhibited significant trends with lower densities at higher water levels. Even then, only the area outside the Guinea Marsh bed
had a strong slope (-0.31 compared to 0.001 for inside the bed). The other locations sampled did not show any significant trends, with a mix of small positive and negative slopes for linear regressions. When all of the samples were combined, there was also no significant trend using 1st or 2nd order regressions. Given that the overall slope of the combined results was relatively flat, and that only one area exhibited a reasonably steep slope, zooplankton densities did not appear to differ in a predictable fashion with tidal stage which is in contrast to our predictions. Although more detailed studies may be necessary, our results indicate that zooplankton do not use the tides in this study area to migrate horizontally in and out of the SAV beds, or into shallow water areas in general.

**Diel differences**

Zooplankton densities were significantly higher at night than during the day both inside and outside the SAV beds, indicating that the zooplankton may be migrating up from the bottom (demersal) or towards the shore from the open water areas. Diel horizontal migrations by zooplankton to escape predation are known to occur in both directions – from open water to structured shallows either during the day or at night depending on the predation pressure encountered (Nurminen and Horppila 2002; Iglesias et al. 2007), and diel vertical migrations have also been reported previously in shallow estuarine systems (Roman et al. 1988; Cuker and Watson 2002). With higher zooplankton densities outside the beds than inside (but not statistically significant, p=0.08), it is also possible that zooplankton are either avoiding the beds or migrating out of them. Because the pump sampling was designed to integrate the water column, the samples are not depth stratified and it is not possible to quantitatively compare this study
to the previous ones, or definitively state whether the zooplankton were migrating up from the bottom or into the area from elsewhere. Therefore, the zooplankton encountered in this portion of the study could have been exhibiting predator avoidance in the open water areas by migrating into the shallows at night instead of during the day, or by migrating from the bottom up into the water column.

Zooplankton and water clarity

One motivation for this study was to ascertain if zooplankton could contribute to enhanced water clarity within vegetated brackish estuarine shallows, similar to that found in freshwater systems. Regardless that higher zooplankton density did not definitively occur in the vegetated shallows, an estimate of the filtering capacity of the zooplankton densities sampled in this study is useful for determining their potential impact on water clarity and how high these densities would need to be in order to appreciably affect particulate levels. The zooplankton used to estimate the filtering capacity of the zooplankton in this study included the three main groups – *A. tonsa*, other copepods, and barnacles – plus copepodites and polychaetes. Copepodites were separated from the other copepods because of their smaller size, and the rates for *A. tonsa* juveniles were used for the copepodites; polychaetes were included because they have a higher clearance rate per individual than the other groups. These five groups comprised over 90% of the total zooplankton densities encountered in this study. The filtration rates used were obtained from White & Roman (1992), who measured filtration rates of Chesapeake Bay zooplankton between March and October: 7.2 mL ind⁻¹ day⁻¹ for *A. tonsa*, 2.5 mL ind⁻¹ day⁻¹ for other copepods, 1.2 mL ind⁻¹ day⁻¹ for barnacles, 2.3 mL ind⁻¹ day⁻¹ for
copepodites and 5.6 mL ind^{-1} day^{-1} for polychaetes. These rates only provide a basis for estimating the filtering potential as variation in prey availability, temperature, predation and other factors can affect the filtration rates (Hansen et al. 1997).

The filtration capacity of the observed zooplankton densities was estimated within each SAV cover category in 2006 and 2007 using observed mean zooplankton densities and taxon-specific filtration rates from White & Roman (1992) as described above. The calculated volume of water filtered daily by each taxonomic group was summed within each SAV cover category (or inside vs. outside for 2007) and expressed as a proportion of the total water column (i.e., liters per liter of water per day). The zooplankton in the study area overall were estimated to filter between 2-6% of the water column per day on average based on these filtering rates; the lower value represents filtration in sites with 0-33% cover while the upper value represents filtration in sites with 66-100% cover, all other filtration estimates were in between the range of 2-6%. Even at the highest densities of zooplankton encountered, at Guinea Marsh in 2007 (Fig. 5b) and Jenkins Neck in 2006 (Table 2), the zooplankton were filtering about 30% of the water column on a daily basis. This upper estimate, combined with other particulate removal mechanisms, may produce a measurable impact on particulate levels within SAV beds, but these high densities of zooplankton were not common in this study and were not unique to the SAV beds. Based on the clearance rates above, it would take approximately 140 A. tonsa L^{-1} to filter the water column once per day, which is approximately 17 times higher than the overall average encountered in this study. These estimated filtering rates limit the ability of the zooplankton to play a large role in removing particulates from the water column,
thereby enhancing the water clarity within the SAV beds. As such, it seems that zooplankton play a limited role in particulate removal in the areas studied.

Our findings are in contrast to a range of freshwater studies that report enhanced zooplankton abundance and biomass within SAV beds (Scheffer 1999; van Donk and van de Bund 2002; Muylaert et al. 2003), including a study in a tidal fresh tributary to the Chesapeake (Monk 1988). This difference is likely due to an overall decrease in zooplankton biomass as a function of salinity that emerges from a synthesis of the literature and long-term monitoring data along the salinity gradient in Chesapeake Bay (Fig. 6). This pattern is also apparent from an enclosure experiment by Jeppesen et al (2007b), who demonstrated reduced zooplankton biomass when a shallow lagoon shifted from freshwater to brackish (Fig. 6). In that study, total zooplankton biomass was reduced, including copepods and cladocerans, and the zooplankton:chl a ratio decreased with the increase in salinity, indicating a decline in the zooplankton population in relation to available food resources. The large range in zooplankton biomass in freshwater systems highlights the differences between riverine systems and the riverine end-members of major estuaries including the Chesapeake, where zooplankton biomass tends to be low, and lakes where biomass tends to be higher (Fig. 6; Pace et al. 1992). While quantitative interpretation of Figure 6 is complicated by differing mesh sizes and averaging periods, and by differing filtration rates among the dominant zooplankton across this gradient, the data nevertheless suggest a steadily declining importance of zooplankton in regulating water clarity with increasing salinity from freshwater to estuarine environments.
This study...

Fig. 6 Literature synthesis of zooplankton community dry weight as a function of salinity, plotted with data from the present study, long-term (1985-2001) EPA Chesapeake Bay Program (CBP) zooplankton counts converted to biomass by Brush & Steinberg (unpublished data), and results from the enclosure experiment of Jeppesen et al. (2007b) in a brackish lagoon in Denmark. Freshwater values are from Quebec lakes (Pace 1986), the Hudson River pre- and post-zebra mussel invasion (Pace et al. 1998), four Danish lakes (Jeppesen et al. 1999), fluvial lakes of the St. Lawrence River (Basu et al. 2000), five Swedish and Latvian lakes (Blindow et al. 2000), a Lake Ontario marsh pre- and post-carp exclusion (Lougheed and Chow-Fraser 2001), Lake Hiidenvesi, Finland (Nurminen and Horppila 2002), Lake Blanca, Uruguay (Mazzeo et al. 2003), four Belgian lakes (Muylaert et al. 2003), and Lake Apopka, FL and Lago Trasimeno, Italy (Havens et al. 2009). Estuarine values are from Delaware Bay (Cronin et al. 1962), North...
Carolina estuaries (Williams et al. 1968), the Newport River estuary, NC (Thayer et al. 1974), North Inlet, SC (Lonsdale and Coull 1977), Narragansett Bay, RI (Durbin and Durbin 1981), Peconic Bay, NY (Turner 1982), the Neuse River estuary, NC (Mallin 1991), the Mpenjati estuary, South Africa (Kibirige and Perissinotto 2003), the Kasouga estuary, South Africa (Froneman 2004), Pensacola Bay, FL (Murrell and Lores 2004), the Senegal River estuary, Senegal and Mauritania (Champalbert et al. 2007), the Mondego estuary, Portugal (Marques et al. 2007), and Suisun Bay and the Sacramento-San Joaquin delta, CA (Winder and Jassby 2011). Densities from the present study were converted to biomass using conversion factors from White and Roman (1992). With the exception of data from the Chesapeake Bay, where we have detailed location and taxon specific biomass conversion information, studies that reported zooplankton counts were not used in this plot given conversion uncertainties. Reported biomass in carbon units was converted assuming 0.32 g C g⁻¹ dry weight; displacement volumes were converted to dry weight assuming a density of 1 g mL⁻¹ and 0.2 g dry weight (g wet weight)⁻¹. Two freshwater points lay off-scale at 2.2 and 4.6 mg L⁻¹. Most of the above-referenced studies, especially those done in more saline waterways, were done outside of SAV beds.
CONCLUSIONS

There were no consistent statistically significant differences in zooplankton densities either in relation to SAV or tidal influences, although some isolated sampling periods exhibited significant trends. Significant differences were found between sites in both years, highlighting the inherent patchiness of zooplankton distributions even in similar systems located in close proximity. There were consistent statistically significant differences between night and day with higher zooplankton densities at night both inside and outside the SAV for all locations. Based on calculations of clearance rates for the zooplankton densities encountered, it is not likely that zooplankton have a pronounced effect on particulate levels in these polyhaline vegetated systems.

ACKNOWLEDGEMENTS

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98


Chapter 3:

Sulfides, iron and *Zostera marina* in the lower Chesapeake Bay and the potential for iron addition to enhance restoration and management success
Abstract:

Submersed aquatic vegetation (SAV) in coastal and estuarine ecosystems is experiencing declines throughout the world in terms of both overall coverage and how deep it can grow. A variety of mechanisms have been identified as responsible for this decline, including disease, elevated temperatures, light limitation and increased concentrations of porewater sulfides. Elevated sulfides can result from microbially mediated anoxic degradation of increased organic matter deposition that is often associated with SAV beds and increasing coastal eutrophication. Previous studies have shown that addition of iron to coastal sediments has the potential to bind these toxic sulfides and enhance SAV growth and survival. Ambient sedimentary organic content, porewater sulfide levels and *Zostera marina* biomass and density were monitored throughout a growing season in the lower York River, Chesapeake Bay, Virginia to assess feedbacks among these parameters and the potential for sulfide limitation of eelgrass under *in situ* conditions. Additionally, an iron enrichment experiment was conducted at three sites to determine if particulate iron addition was a viable management technique for reducing porewater sulfide concentrations and enhancing eelgrass growth and survival. Significant linear trends could not be developed from the ambient sampling, although there was a significant 2nd order polynomial fit between *Zostera* shoot (p=0.01, $r^2=0.20$) and root (p<0.01, $r^2=0.28$) biomass and sediment organic content. Ambient sampling also indicated that porewater levels above 900-1000 μm [S] inhibited eelgrass growth within the study area. Sulfide levels were highly variable during the warmer summer months, with periodic higher levels encountered during the mid-summer
than the early spring or late summer sampling. The three experimental sites had different growth patterns, with one site developing higher biomass in relation to higher iron enrichment, while another site had a larger mean biomass at the moderate enrichment levels. These results indicate the potential for iron to positively affect *Z. marina* growth and survival, but responses tend to be very site specific and variable.

Keywords: *Zostera marina*, eelgrass, SAV, porewater sulfide, iron enrichment, sediment organic content, Chesapeake Bay
Introduction:

Eelgrass (Zostera marina) is a common marine aquatic plant in the lower Chesapeake Bay and western Atlantic coastal zone, extending from North Carolina north along the coastline (Orth et al. 2002; Moore et al. 2003). Eelgrass prefers cooler waters and is limited in its southern range by warmer summer water temperatures, with the Chesapeake Bay near its southern limits (Wetzel and Neckles 1986; Kemp et al. 2000; Kemp et al. 2004; Kemp et al. 2005). Near its warmer limits in Virginia and North Carolina, eelgrass biomass reaches a seasonal minimum during July-August because of thermally induced die-back, re-grows some during the early fall and overwinters in this condition, then has its most significant growth period from early spring through early summer (Buzzelli et al. 1998).

Submersed aquatic vegetation (SAV) coverage has decreased in the Chesapeake Bay both in how deep it can survive as well as in its lateral extent, with many shorelines previously containing SAV with little or no vegetation left (Dennison et al. 1993; Moore et al. 2000; Orth et al. 2002; Moore et al. 2003). This has made SAV the target of substantial restoration initiatives (Orth et al. 2002). Much of the recent decline in SAV coverage, as well as inability to recolonize, has been largely attributed to increased nutrient and sediment loading and its subsequent effect on water clarity (Alden 1997; Cerco et al. 2002; Orth et al. 2002; Stankelis et al. 2003; Hagy et al. 2004; Kemp et al. 2005). Global climate warming may also be affecting the southern range of Z. marina specifically because of its intolerance to warmer temperatures.
In addition to high temperature and light limitation, sulfide is a known phytotoxin to many plants including eelgrass (Goodman et al. 1995; Pedersen et al. 2004; Holmer et al. 2005b; Mascaro et al. 2009). Sulfide is a by-product of microbial breakdown of organic matter in anoxic sediments (especially in marine environments), with sulfate being reduced to sulfide (Raven and Scrimgeour 1997). Frederiksen et al. (2004) and Morris and Virnstein (2004) have documented the shifting spatial coverage of seagrass beds, and Morris and Virnstein (2004) hypothesized that phytotoxin feedback within the sediments may be the cause of such annual and spatial variability. Azzoni (2001) studied the role of microbially-mediated feedbacks within the sediments due to the breakdown of organic matter and found that in the eutrophic system studied, the accumulation of phytotoxins including sulfide within the sediments exceeded the capacity of the SAV beds to ameliorate the negative effects, causing a rapid negative feedback on the survival of the vegetation. Although Azzoni did not quantify the sources of organic matter, an increase in organic deposition can come from increased production of the vegetation itself including epiphytes (Penhale 1977), increased particulate removal including settling of phytoplankton (Lake and MacIntyre 1977; Short and Short 1984; Vermaat et al. 2000; Wigand et al. 2000; Madsen et al. 2001; Schulz et al. 2003; Takamura et al. 2003), and increased secondary production and its subsequent increase in fecal matter and faunal litter (Fredette et al. 1990; Reusch and Reusch 1998; Bostrom and Mattila 1999; Bostroem and Bonsdorff 2000; Peterson et al. 2001; Hovel et al. 2002).

The combined effect of stressors including high temperature, low light and elevated sulfides is cumulative, and can either be additive (Goodman et al. 1995) or

Goodman et al. (1995) concluded that the stressors of reduced light and increased sulfides on eelgrass growth were additive. Conversely, Holmer et al. (2005b) recorded increased mortality and 75% lower growth rates in eelgrass subjected to low light and high sediment sulfides, while exposure to high sediment sulfides alone had no effect compared to the control. Koch & Erskine (2001) conducted a laboratory study on the combined stressors of increased sulfides, temperature, salinity and reduced light on the survival of Thalassia. The results indicated that the combination of the stressors had a greater negative effect than each stressor individually, such that when high sulfides were combined with either high temperature or salinity, significant mortality occurred. Further, when the plants were stressed with high temperature, salinity and sulfides, 100% mortality was induced.

Sedimentary sulfide levels within seagrass beds have been quantified in many areas, primarily with calcareous sediments (Brueechert and Pratt 1996; Schaub and Van Gemerden 1996; Terrados et al. 1999; Erskine and Koch 2000; Azzoni et al. 2001; Chambers et al. 2001; de Wit et al. 2001; Borum et al. 2005; Bradley and Stolt 2006; Calleja et al. 2007; Frederiksen et al. 2007; Alongi et al. 2008). Conversely, information on sulfide levels in Zostera beds within the Chesapeake Bay are rare and few studies exist on sulfides in seagrass beds containing siliceous sediments (Goodman et al. 1995). Therefore one goal of this study was to quantify in situ sulfide levels as a function of organic content and Zostera density in siliceous sediment throughout the lower York River and Mobjack Bay in the lower Chesapeake Bay, Virginia.
Reduced iron has a strong affinity for reduced sulfur, so the addition of iron to sediments has the potential to directly bind sulfide to form non-toxic iron sulfides such as pyrite (de Wit et al. 2001). Sediment buffering of sulfide with iron has been documented in several locations, although most of the work has been done on calcareous sediments (Chambers et al. 2001; de Wit et al. 2001; Holmer et al. 2005a). Chambers et al. (2001) added iron oxide granules to a *Thalassia testudinum* bed in Florida and found greater shoot growth rates in the iron addition areas compared to the controls as the iron combined with sulfides to form non-toxic precipitates. Holmer (2005a) added an iron solution to organic enriched sediments and found *Z. marina* had greater leaf growth compared to non-iron amended controls. Even though the effect of sulfide on *Z. marina* has been documented, the studies identified have been done in laboratory and mesocosm manipulations (Goodman et al. 1995; Holmer and Bondgaard 2001; Pedersen et al. 2004).

If sulfide concentrations can be reduced *in situ* with a viable management option that is easily implemented, such as the addition of iron granules, the growth and survival of eelgrass may be enhanced similar to other seagrasses (Marba et al. 2007). However, iron can also bind phosphorus, limiting its availability to the vegetation (de Wit et al. 2001; Azzoni et al. 2005; Viaroli et al. 2008). Given the potential for iron enrichment to mitigate against sulfide stress, the second goal of this study was to test the ability of iron additions to reduce sulfide concentrations and enhance *Zostera* growth through a controlled field experiment in the lower York River.
Materials and Methods:

This study was carried out in the lower Chesapeake Bay, in the general vicinity of the lower York River and Mobjack Bay, Virginia, USA (Fig. 1). Guinea Marsh and Goodwin Islands have several islands and are not developed with Goodwin Islands being part of the Chesapeake Bay National Estuarine Research Reserve System; Jenkins Neck and Allen’s Island are both sites near shorelines with moderate housing development; the Severn River and New Point Comfort sites are near undeveloped shorelines. These sites have similar tidal fluctuations and are located within 20 km of each other, although they have different exposures to wind, riverine and tidal influences. Goodwin Islands, Guinea Marsh and New Point Comfort likely receive the most impact from wind due to extended fetch across the Chesapeake Bay, while Allen’s Island is more protected behind an island. Due to their proximity to the lower Chesapeake Bay, these sites were also expected to have similar water quality overall, although some site differences are possible due to the variable locations in relation to wind and riverine inputs, such as the Severn River site.

Ambient sampling and experimental sites were based on the presence of densely vegetated Zostera marina beds and the variety of areas were chosen to allow for a broad range of conditions. The main vegetation in these sites was Zostera marina, with Ruppia maritima also present. Samples were taken throughout the summer of 2008, starting with the initial samples during the experimental set-up in the beginning of March.

Monitoring of ambient conditions:

Quantification of ambient eelgrass conditions, sedimentary sulfide levels, and organic content was conducted at the sampling sites as indicated in Figure 1. After
locating a suitable general sampling location based on the presence or absence of *Zostera*, the exact location for each ambient sample was chosen haphazardly by tossing a marker while in the field. To limit disturbance of each successive measured parameter, the following order of measurements was utilized: non-destructive eelgrass measurements of % cover and canopy height, porewater sulfide samples, sediment organic matter samples and eelgrass biomass cores.

Canopy height was measured with a meter stick as outlined in Short and Coles (2001) and Short et al. (2006), whereby the top ~20% of the shoots are ignored, at 5 locations around the porewater sampling site, and percent cover was visually estimated within a 0.5 m radius of the porewater extraction location. Eelgrass root and shoot biomass was collected throughout the summer utilizing a 20 cm diameter PVC pipe coring device to remove the sediments and overlying eelgrass to a depth of 10 cm. The contents of the core were placed into a sieve and initially rinsed and sorted in the field. Eelgrass roots and shoots were placed into labeled bags, stored on ice in a cooler until returning back to the laboratory, then refrigerated until further sorting. Within a week, the samples were further cleaned, sorted, and rinsed in tap water. *Zostera* stem density was determined in the lab from the eelgrass biomass cores; *in situ* stem density was then calculated as the product of % cover and stem density from the core. Below ground biomass included the rhizomes and roots, if present, as recognized by lack of green in the stem. The samples were then placed into labeled pre-weighed aluminum foil pouches and dried at 50°-60°C until a constant mass was obtained.
Figure 1: Ambient sampling sites (circles•) and iron enrichment experimental sites (stars*) in the vicinity of the lower York River and Mobjack Bay, Chesapeake Bay, Virginia, USA.
Porewater samples were taken with a porewater sampling device constructed based on the design of Berg & McGlathery (2001). This apparatus consists of a 25 cm long, 2.4 mm (outside diameter) stainless steel collection tube inserted directly into the sediments through a stabilizing base plate, Tygon tubing inserted over the upper end of the stainless tubing with the other end connected to a 5 mL glass syringe used to create suction to withdraw and transfer the sample. All of the metal in the sampler was stainless steel to limit interactions with iron, and a glass syringe and Tygon tubing were used to avoid plastics potentially absorbing sulfide, oxygen or other contaminants. Several collection tubes were drilled with 0.5 – 1.5 mm holes near the sealed end to test the best size for porewater withdrawal in the sampling conditions encountered; preliminary sampling indicated that the 0.5 and 0.7 mm holes worked the best.

Porewater was collected at each location by extracting a 6 mL sample collected from 10 cm below the sediment surface. A depth of 10 cm was chosen as that is the typical maximum depth of core sampling for roots and rhizomes as well as root/rhizome penetration, while also deep enough to limit the potential for surface water to intrude into the porewater samples. The tubing was sized such that 5 mL of sample would be held in the stainless steel and Tygon tubing. The additional 1 mL of sample already in the syringe was used to rinse the syringe and discarded; an additional 4 mL of sample was then drawn into the syringe with the last 1 mL left in the tubing and subsequently also discarded, to limit exposure of the porewater to the atmosphere. A disposable Acrodisc 0.45 μm filter with a stainless steel discharge needle was placed on the end of the syringe and rinsed with at least 1 mL of sample from the syringe. Typically 1 mL of sample was
then discharged directly into 2 mL of 0.1M Zn acetate solution in a 20 mL scintillation vial to limit exposure to atmospheric oxygen and immediately fix the sulfide as ZnS for later analysis (Henrik Fossing, personal communication). After collection, the scintillation vial was tightly capped and swirled and all samples were stored in a closed cooler on ice for transport back to the laboratory where they were refrigerated until analysis. After each porewater sample was collected, the stainless steel and tygon tubing and syringe were all rinsed with at least 5 mL of dilute HCl acid and then at least 10 mL of distilled water.

Sulfide levels were determined in the laboratory based on the methods of Cline (1969). The samples were left in their collection vials, 0.75 mL of Cline’s reagent was added and the sample was diluted to 10 mL with deionized water. The samples were allowed to sit at room temperature for at least 30 minutes, and were then analyzed on a spectrophotometer for absorbance at 670 nm. A series of sulfide standards was prepared in the same manner during each analysis. Fixing the sulfides as ZnS precludes the need for purging oxygen from the dilution water, air, and rinse water as the formation of ZnS is fast. The binding of sulfide by the Cline’s reagent is also fast, with the sulfide being released from the Zn to the coloring reagent.

Samples for sediment organic content were collected with a 5 cm diameter PVC pipe to a depth of 10 cm. The sediments were placed into a plastic bucket, homogenized, and a portion removed for subsequent analysis. In the lab, samples were cleaned of large pieces of detritus and roots and approximately 25 mL sub-samples were placed into pre-
weighed aluminum pans to be dried. The sediments were dried at 50°-60° C, weighed, ashed at approximately 500°C, and weighed again for determination of organic content.

Iron enrichment experiment:

Experimental plots were set up in early to mid-March to test the potential for in situ iron enrichment to enhance Zostera growth and survival at three locations: Allen's Island, Jenkins Neck, and Guinea Marsh (Fig. 1). Experimental plots were set up in a 3X5 grid at each site, with 5 levels of iron addition and 3 replicates of each level, for a total of 15 plots at each site and 45 experimental plots overall. Plots were 1 m² with a 2 m buffer zone between each plot on all sides. Iron addition treatments were as follows: 0, 1.25, 2.50, 3.75, and 5.00 kg m⁻². Granular iron was pre-measured into plastic bags and slowly added to the surface of each plot. The iron pieces were large enough that they readily sank into place and provided an even coverage, which was visually checked. After the plots were established, six eelgrass biomass samples were collected adjacent to the experimental area to obtain initial biomass estimates.

Monitoring of the experimental sites, similar to the ambient sampling except as noted below, was performed eight times from March (during the initial set-up) to September. Since the experimental sites had to be monitored throughout the season, non-destructive eelgrass measurements of % cover, stem density, and canopy height were made until the end of the experiment. Canopy height was measured at each corner and at the center of each plot; values were averaged to give a single estimate. Percent cover was visually estimated within each m² plot. Stem density was counted in situ with a 100 cm²
grid placed near the center of each plot. Stem density for the entire plot was then
calculated as the product of % cover and stem density. Occasionally, there were so few
stems present that a total stem count was obtained for the entire square meter. Sediment
cores for organic content were collected within each plot in a space not occupied with
eelgrass as this was considered reasonable to adequately represent the sediments within a
given plot and limit disturbance to vegetation; a sub-sample from one set of these cores
was also used to determine sedimentary grain size. Eelgrass biomass cores were
collected during the last three sampling episodes at the end of the summer, taken from
one quarter of each square meter plot with a different quarter sampled at each successive
sampling, in the same manner as the ambient biomass sampling.

Because non-destructive Zostera measurements were utilized for the majority of
the experimental monitoring, a total eelgrass estimator (TEE) similar to that of Canfield
et al. (1984) was developed to estimate the total amount of eelgrass present for each
sampling episode within each plot. This factor was developed by multiplying % cover
times stem density to give an estimate of stem density within each square meter plot,
which was then multiplied by canopy height to provide an estimate of the amount of
Zostera present within the entire water column, better representing the total amount of
eelgrass present within each plot. The TEE, since it utilized more measurements and
could also compensate for anomalies of singular eelgrass parameters, was considered a
better estimate of the overall abundance of Zostera for the monitored experimental plots.
This estimator also provides a better estimate of the total amount of the water column
occupied by the eelgrass, which can be useful for analyzing the structural or habitat value of *Zostera*.

*Zostera* biomass varies seasonally and by location in the study area (Buzzelli et al. 1998; Orth et al. 2002). Since the main goal of the iron enrichment experiment was to assess the impact of iron additions on eelgrass growth, each TEE measurement was subtracted from the average of TEE in the control plots at a given site on a given date.

One set of experimental *Zostera* biomass samples was used for C, N, P and Fe tissue content analysis. Iron analysis was performed on shoots only as it was too difficult to separate external iron residues from the roots. Because of the limited amount of material at some sites, not all analyses could be performed on all samples. After drying and weighing the eelgrass samples, they were ground and homogenized. Iron samples were combusted at 500°C for 4 hours, then digested with 1M HCl. Iron content was determined spectrophotometrically at 526 nm based on the ferrozine method of Stookey (1970). Phosphorus samples were extracted using the method of Fourqurean et al. (1992). After extraction, the samples were analyzed for orthophosphate according to the standard procedures of Solorzano and Sharp (Solorzano and Sharp 1980). Carbon and nitrogen analysis was performed with an Exeter CHN Model 440 CE analyzer.

*Statistical analyses*:

Statistical analyses were performed in SigmaPlot version 11. Linear, polynomial and exponential regression was used on the ambient samples to analyze for relationships between the eelgrass measurements, sediment organic content and sulfide concentrations. The iron addition experiment was designed so that ANOVA or regression could be used
to analyze the data. Results for all analyses were first tested for normality; an initial attempt at running a 3-way ANOVA for the eelgrass biomass data, with site, date of collection and iron addition levels as factors, indicated that the data were not normally distributed but did have equal variance. For the eelgrass biomass cores obtained on the final sampling date, the lack of biomass in many of the plots was suspected as responsible for the non-normal distribution. Therefore, the biomass estimates from the three final sampling dates were combined by utilizing the total area sampled for all three collections to calculate biomass m$^{-2}$, which eliminated almost all of the zero values. A 2-way ANOVA was then performed using location and iron addition levels as factors, which satisfied the mathematical assumptions for the analysis. This was considered reasonable based on previous studies in this area that indicated a minimal difference in biomass during the time spanning our three final biomass collections (Orth and Moore 1986; Buzzelli et al. 1999). The Holm-Sidak Multiple Comparison Method was used to quantify differences identified from the ANOVA.
Results:

*Monitoring of ambient conditions:*

Field measurements of sediment organic content, porewater sulfide concentration, and *Zostera* biomass were extremely variable although some trends were apparent. No significant relationships were identified between sedimentary sulfide levels and eelgrass biomass roots or shoots (p=0.25 – 0.49) or sediment organic content (p=0.61 – 0.71), but there were statistically significant 2nd order polynomial regressions between *Zostera* shoot (p=0.01, r²=0.20) and root (p<0.01, r²=0.28) biomass and sediment organic content, as well as a significant exponential regression with the roots (p=0.03, r²=0.06) (Table 1, Fig. 2). The slope and correlation were small, however, and the trend was negative, suggesting the effects of organic content on *Zostera* colonization and survival are more important than the effects of *Zostera* on organic matter accumulation.

The relationships between both shoot and root biomass and sulfide, although non-significant, were negative suggesting that higher sulfide levels may have been inhibiting eelgrass growth in some areas. Highest *Zostera* biomass (>200 g m⁻²) occurred only when porewater sulfide concentrations were near zero, and intermediate biomass (100-200 g m⁻²) occurred when sulfide concentrations were below an apparent threshold of approximately 1,000 μM (Fig. 2a). However, *Zostera* biomass at the lower sulfide concentrations was also highly variable and reached as low as zero.
Table 1: Regressions from ambient sampling of SAV shoot and root biomass (shoot or root, g m\(^{-2}\)), sediment organic content (SOC, %), and porewater sulfide concentrations ([S], µM).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Equation order</th>
<th>Equation</th>
<th>( r^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV shoot biomass</td>
<td>1(^{st} )</td>
<td>( 92.552 - (0.0118*[S]) )</td>
<td>0.01</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>2(^{nd} )</td>
<td>( 94.483 - (0.0239*[S]) + (0.00000483*[S]^2) )</td>
<td>0.02</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>( 93.1<em>e^{-0.00016</em>[S]} )</td>
<td>0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>SAV root biomass</td>
<td>1(^{st} )</td>
<td>( 89.055 - (0.0179*[S]) )</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2(^{nd} )</td>
<td>( 87.306 - (0.00690*[S]) - (0.00000437*[S]^2) )</td>
<td>0.02</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>( 89.5<em>e^{-0.00025</em>[S]} )</td>
<td>0.02</td>
<td>0.39</td>
</tr>
<tr>
<td>sulfide</td>
<td>1(^{st} )</td>
<td>( 280.335 + (105.096*SOC) )</td>
<td>0</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>2(^{nd} )</td>
<td>( 302.456 + (57.091<em>SOC) + (22.016</em>SOC^2) )</td>
<td>0</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>( 1.0<em>e^{3.1</em>10^{-13}*SOC} )</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( 1.0<em>e^{3.69</em>10^{-5}} )</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>% organic</td>
<td>1(^{st} )</td>
<td>( 1.009 - (0.00106*shoot) )</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>2(^{nd} )</td>
<td>( 1.340 - (0.00852<em>shoot) + (0.0000274</em>shoot^2) )</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>( 0.9<em>e^{1.1</em>10^{-11}} )</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( 1.1<em>e^{-0.0017</em>shoot} )</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>1(^{st} )</td>
<td>( 1.002 - (0.00104*root) )</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>2(^{nd} )</td>
<td>( 1.251 - (0.00787<em>root) + (0.0000240</em>root^2) )</td>
<td>0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>( 0.9<em>e^{1.1</em>10^{-11}} )</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( 1.04<em>e^{-0.002</em>root} )</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Figure 2: Relationships between *Zostera* biomass, sulfide concentrations and sediment organic content. Only *Zostera* biomass and % SOC exhibited a statistically significant relationship which was negative. Secondary axis in (a) shows the proportional reduction in maximum photosynthetic production ($P/P_{max}$) due to sulfides based on Goodman et al (1995).
Iron enrichment experiment:

Initial sampling at the three study sites indicated similar grain size composition but markedly different biomass of *Zostera* shoots and roots (Table 2). Total eelgrass estimator (TEE) results reflected the typical seasonal growth patterns of *Zostera* in the Chesapeake Bay region (Buzzelli et al. 1999), with a peak in the early summer followed by a die-off due to the heat of the summer (Fig. 3). Allen’s Island (Fig. 3a) had the lowest values, with almost no eelgrass present in the beginning or end of the season, although there was some present at the end of sampling at all enrichment levels. Guinea Marsh had the highest TEE values (Fig. 3b), while TEE at Jenkins Neck (Fig. 3c) was intermediate but closer to the values at Guinea Marsh. No significant differences in TEE were identified as a function of iron enrichment. Allen’s Island had the highest mean TEE values in the plots that received the most iron, but the variability was too high for this pattern to be significant. The other plots did not show any consistent patterns among enrichment levels, although the intermediate enrichment plots at Guinea Marsh had higher mean TEE values than plots receiving either no iron or the highest enrichment level at the end of May and beginning of July, when plants were beginning to experience heat stress.
Table 2: Grain size and initial *Zostera* biomass for the experimental sites.

<table>
<thead>
<tr>
<th>Location</th>
<th>% sand</th>
<th>st. dev.</th>
<th>% silt</th>
<th>st. dev.</th>
<th>% clay</th>
<th>st. dev.</th>
<th>Shoot biomass (g m$^{-2}$)</th>
<th>st. dev.</th>
<th>Root biomass (g m$^{-2}$)</th>
<th>st. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen's Island</td>
<td>79</td>
<td>3.9</td>
<td>14</td>
<td>2.5</td>
<td>6</td>
<td>2.8</td>
<td>1.4</td>
<td>1.35</td>
<td>24.2</td>
<td>31.0</td>
</tr>
<tr>
<td>Guinea Marsh</td>
<td>84</td>
<td>2.7</td>
<td>12</td>
<td>2.0</td>
<td>4</td>
<td>1.0</td>
<td>52.7</td>
<td>11.7</td>
<td>101.2</td>
<td>45.5</td>
</tr>
<tr>
<td>Jenkins Neck</td>
<td>80</td>
<td>2.9</td>
<td>15</td>
<td>2.4</td>
<td>5</td>
<td>0.7</td>
<td>41.7</td>
<td>20.4</td>
<td>60.0</td>
<td>20.8</td>
</tr>
</tbody>
</table>
Figure 3: Total Eelgrass Estimator (TEE) for the iron enrichment experiment at Allen's Island (a), Guinea Marsh (b), and Jenkins Neck (c). Error bars are one standard deviation. There were no significant differences among the iron treatments.
When TEE values were normalized to the control plots, there were statistically significant differences among locations but still no significant differences among iron enrichment levels. We averaged the normalized TEE values from the second and third sampling events and from the last two sampling events to better reflect initial and final values (Fig. 4). Final values at Guinea Marsh showed several statistically significant differences among iron enrichment levels ($p < 0.001 – 0.01$), with the highest values under intermediate ($3.75 \text{ kg Fe m}^{-2}$) enrichment (Fig. 4), suggesting that moderate iron levels may enhance the recovery and re-growth of eelgrass at this site. Jenkins Neck, however, exhibited the opposite trend (not significant, $p = 0.8 – 0.9$) with lower TEE at higher enrichment levels, while Allen's Island had slightly higher TEE under the highest enrichment level, although again this difference was not significant ($p = 0.9 – 1.0$).

Porewater sulfide concentrations were not statistically significantly different among iron enrichment levels (Fig. 5). Variability and mean sulfide levels were higher during the warmer months of July and August and into September at Allen's Island (Fig. 5a) and Jenkins Neck (Fig. 5c). Variability in measured sulfide levels was high with the standard deviation often exceeding the mean, indicating very localized porewater differences as the entire experimental area at each given site was only $13 \times 9 \text{ m}$, with each individual plot separated by $2 \text{ m}$.
Figure 4: Normalized Total Eelgrass Estimator (TEE) mean and standard deviation across iron addition levels. Early and late values reflect averages from the 2rd and 3rd sampling periods and 7th and 8th sampling periods, respectively. To plot all values on a single axis, ‘AI late’ values were multiplied by 20 and ‘JN late’ values were multiplied by 10. Different letters for ‘GM late’ denote statistically significant differences. AI is Allen’s Island, GM is Guinea Marsh and JN is Jenkins Neck.
Figure 5: Porewater sulfide concentrations for the iron enrichment experiment at Allen’s Island (a), Guinea Marsh (b), and Jenkins Neck (c). Error bars are one standard deviation. There were no significant differences between the different levels of iron enrichment.
There were no significant differences in sediment organic content in the experimental plots in relation to iron enrichment levels (Fig. 6), TEE, or sulfide levels. There was also no significant seasonal difference in organic content. Occasionally high organic levels were encountered during the summer sampling as the eelgrass was going through its thermally-induced die-off, but the source of organic matter was not verified.

Eelgrass biomass harvested during the last three sampling periods did not exhibit the anticipated trends of increased biomass in relation to the iron enrichment (Table 3, Fig. 7). A two-way ANOVA indicated a significant difference among the sites, with Guinea Marsh having higher biomass than the other two sites (p<0.001), but no significant difference between Allen’s Island and Jenkins Neck (p=0.31 for roots, p=0.32 for shoots). Allen’s Island had the highest mean shoot and root biomass at the highest enrichment level of (5.00 kg Fe m\(^{-2}\)), while the other two sites had the highest mean biomass at the middle enrichment level (2.50 kg Fe m\(^{-2}\)). Allen’s Island had the lowest overall eelgrass biomass and TEE, indicating the plants at this location may have been more stressed than at the other two locations (Fig. 7). Because the vegetation was so sparse and patchy, the variability was too high to result in significant differences, but it is possible that the limited vegetation present was responding to the iron enrichment levels.
Figure 6: Sediment organic content for the iron enrichment experiment at Allen's Island (a), Guinea Marsh (b), and Jenkins Neck (c). Error bars are one standard deviation. There were no significant differences between the different levels of iron enrichment.
### Table 3: p-values for shoot biomass by iron enrichment level.

<table>
<thead>
<tr>
<th>Location</th>
<th>Allen's Island</th>
<th>Guana Marsh</th>
<th>Jenkins Neck</th>
<th>Locations combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (kg m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
</tr>
<tr>
<td>Mean shoot biomass (g m⁻² ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9 (2.3)</td>
<td>3.9 (2.6)</td>
<td>1.8 (2.3)</td>
<td>6.6 (8.4)</td>
<td>7.2 (5.4)</td>
</tr>
<tr>
<td>2.90 (10.7)</td>
<td>2.34 (9.7)</td>
<td>3.52 (7.6)</td>
<td>3.06 (6.6)</td>
<td>2.33 (4.7)</td>
</tr>
<tr>
<td>5.5 (5.1)</td>
<td>4.5 (3.9)</td>
<td>9.7 (10.7)</td>
<td>6.3 (2.2)</td>
<td>7.7 (3.5)</td>
</tr>
<tr>
<td>12.1 (14.1)</td>
<td>10.8 (11.1)</td>
<td>15.6 (16.6)</td>
<td>14.5 (13.3)</td>
<td>12.7 (8.9)</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.70</td>
<td>0.25 0.35</td>
<td>0.25 0.65</td>
<td>0.35 0.45</td>
<td>0.67 0.62</td>
</tr>
<tr>
<td>0.27</td>
<td>0.27 0.34</td>
<td>0.27 0.53</td>
<td>0.44 0.24</td>
<td>0.72 0.71</td>
</tr>
<tr>
<td>0.33</td>
<td>0.29 0.90</td>
<td>0.29 0.90</td>
<td>0.68 0.55</td>
<td>0.84 0.54</td>
</tr>
<tr>
<td>0.00</td>
<td>0.03 0.18</td>
<td>0.03 0.18</td>
<td>0.79 0.79</td>
<td>0.36 0.57</td>
</tr>
</tbody>
</table>

### Table 4: p-values for root biomass by iron enrichment level.

<table>
<thead>
<tr>
<th>Location</th>
<th>Allen's Island</th>
<th>Guana Marsh</th>
<th>Jenkins Neck</th>
<th>Locations combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (kg m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
<td>0.125 2.50 3.75 5.00</td>
</tr>
<tr>
<td>Mean root biomass (g m⁻² ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8 (0.3)</td>
<td>1.9 (2.3)</td>
<td>3.3 (5.1)</td>
<td>3.5 (2.1)</td>
<td>4.9 (3.1)</td>
</tr>
<tr>
<td>14.9 (6.9)</td>
<td>15.2 (3.6)</td>
<td>25.0 (7.1)</td>
<td>18.9 (8.5)</td>
<td>13.6 (4.9)</td>
</tr>
<tr>
<td>5.6 (4.1)</td>
<td>6.1 (3.6)</td>
<td>5.3 (6.2)</td>
<td>4.0 (2.5)</td>
<td>4.0 (0.8)</td>
</tr>
<tr>
<td>7.8 (6.8)</td>
<td>11.5 (11.5)</td>
<td>9.3 (8.0)</td>
<td>7.5 (5.5)</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.82</td>
<td>0.93</td>
<td>0.70</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>0.89</td>
<td>0.01</td>
<td>0.09</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>0.85</td>
<td>0.30</td>
<td>0.95</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>0.74</td>
<td>0.01</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>
Figure 7: Final shoot and root biomass for the iron enrichment experiment. Error bars are one standard deviation. No significant differences were detected among iron levels, but there were differences in biomass between Guinea Marsh and the other two locations (p<0.001). Location abbreviations are the same as in Fig. 4.
Iron addition did not significantly affect eelgrass C, N, P and Fe molar ratios among enrichment levels or among sites (Figs. 8 - 9). Iron content of shoots was significantly related to enrichment level at Guinea Marsh ($r^2=0.35$, $p=0.02$), which suggests that the iron was within the active uptake area of the eelgrass. Eelgrass at Allen’s Island exhibited a similar trend, but due to the lack of vegetation present within some plots, we lacked sufficient power to detect significance.

Although mean tissue P levels were lower in some of the iron addition treatments (Fig. 8), there were no significant trends across the iron enrichment levels for molar ratios, suggesting that iron addition did not cause P limitation. The difference was minimal at Guinea Marsh, the site which exhibited the largest difference in TEE and biomass across treatments, and most pronounced for Allen’s Island. Molar ratios and %C, %N and %P (data not shown) levels measured within the vegetation were all within values reported by other studies and consistently near the lower end of the range (Fourquelean et al. 1997; Moore and Wetzel 2000).
Figure 8: Molar ratio of carbon (C), nitrogen (N) and iron (Fe) to phosphorus for Zostera shoots. Error bars are one standard deviation; the absence of error bars indicates only one sample was available for analysis. There were no significant differences by site or treatment.
Figure 9: Molar ratio of carbon (C) and nitrogen (N) to phosphorus for *Zostera* roots. Error bars are one standard deviation. There were no significant differences by site or treatment.
Discussion:

Monitoring of ambient conditions:

We hypothesized that sites with higher eelgrass biomass would have higher sediment organic content, that sediment organic content would be proportional to sedimentary sulfide levels, and that higher sulfide concentrations could in turn potentially limit eelgrass biomass. Ambient sampling did not result in statistically significant relationships among eelgrass, porewater sulfides and sediment organic content. The only statistically significant trends were between sediment organic content and Zostera shoot and root biomass. However, these were near zero negative trends instead of the positive relationships as expected (Table 1). Lack of a strong positive correlation between eelgrass biomass and sediment organic content may be due to removal of organic detritus by wind- and tidally-induced currents prior to incorporation into the sediments; piles of eelgrass along shorelines and large floating mats were observed during several sampling events.

Ambient sampling indicated a threshold of porewater sulfide concentrations around 900-1000 μM above which Zostera was present only at relatively lower biomass. (Fig. 2a). Mean eelgrass root and shoot biomass were higher below 900 μM (roots = 90.5 ± 80.6 g m⁻², shoots = 95.1 ± 64.9 g m⁻²) than above (roots = 13.4 ± 7.3 g m⁻², shoots = 29.2 ± 20.0 g m⁻²). This threshold is higher than that found by Holmer and Bondgaard (2001), who reported reduced eelgrass growth from 50 – 100 μM S and mortality from 100 – 1000 μM sulfide. Goodman et al. (1995) however conducted a mesocosm study with environmental conditions similar to the lower Chesapeake Bay and documented a
reduction in maximum photosynthetic rate \( (P_{\text{max}}) \) as a function of porewater sulfide concentration. When Goodman et al.'s (1995) results for observed phytosynthetic rate \( (P) \) normalized to the maximum rate \( (P/P_{\text{max}}) \) as a function of sulfide concentrations are mapped over our ambient data and extrapolated across our observed range of porewater sulfides, photosynthetic rates are predicted to go to zero at sulfide concentrations around 1000 \( \mu \text{M} \) under conditions of low light and 1500 \( \mu \text{M} \) under conditions of high light (Fig. 2a). These experimental thresholds are similar to the values of 900 – 1000 \( \mu \text{M} \) observed in situ in the present study.

\( \text{Zostera} \) biomass spanned a wide range below sulfide concentrations of 900 \( \mu \text{M} \), likely due to other factors that influence the presence and growth of eelgrass including temperature and light (Marsh et al. 1987; Kemp et al. 2004); this variability made it impossible to fit a significant regression between porewater sulfide concentration and TEE or eelgrass biomass. Improved correlation might be obtained by conducting more intensive sampling during a shorter time frame, or by comparing values before and after the thermally induced die-off of eelgrass in this region. The latter was tested by analyzing the data separately before and after the seasonal die-back which occurred around July 1 in the current sampling year. This did not result in improved correlations, however, possibly because the sampling still spanned several months. Regardless, the observed difference in mean biomass above and below 900 \( \mu \text{M} \) porewater sulfide suggests that higher sulfide levels may be inhibiting eelgrass growth in the study area.

Strong correlations between eelgrass, porewater sulfide and sediment organic content were not identified due in part to a large amount of variability in the data. Some
of this variation may be explained by small-scale variations in sediment dynamics. For example, bio-turbation and bio-irrigation expose sediments to the higher oxygen content of the overlying water column, thus oxidizing sulfides to sulfates and reducing the localized porewater sulfide concentrations. Similarly, local patches of decaying organic matter may increase localized sulfide levels through higher rates of microbial sulfide production. The removal of larger pieces of organic matter from the samples prior to organic content analysis may have also contributed to a lack of correlation between Zostera abundance and sedimentary organic content. Larger pieces of detritus, such as dead eelgrass blades, could be one of the major sources of organic matter within SAV beds. These larger pieces were removed because it was difficult to consistently ascertain which types of organic matter was live or dead root matter, benthic faunal tunnels, or similar material.

*Iron enrichment experiment:*

The iron enrichment experiment resulted in only one statistically significant difference among response variables, excluding eelgrass iron content, although there were significant differences between locations. Guinea Marsh had statistically significantly higher TEE values with moderate iron enrichment at the end of the sampling period, in contrast with the other sites and Guinea Marsh immediately prior to the eelgrass die-off (Fig. 4). Both Allen’s Island and Guinea Marsh had higher TEE in some of the iron enrichment plots compared to the control immediately prior to and after the die-off (not statistically significant), suggesting that iron additions may enhance eelgrass
growth and re-growth in some areas. Jenkins Neck exhibited the opposite response (though not significant) of reduced Zostera TEE with iron additions at all levels except the highest enrichment before the mid-summer die-off. Since the response to iron enrichment was different at each site, it may be necessary to assess site-specific dosing requirements prior to widespread use of iron as a means of reducing sulfide with the intent to enhance SAV growth.

During the course of the experimental sampling, sediment samples were visually examined to ascertain mixing and reduction of the iron. It was anticipated that the iron would be adequately mixed into the sediments due to currents, sediment accretion, and bioturbation, which did appear to happen. The iron granules were always observed to be black, indicating it was reduced, and often mixed into the top several centimeters of sediment. Several times, clam burrows were observed to be lined with darkened iron, and crabs were observed in the field digging into the sediments.

Based on the visual evidence observed during the experimental biomass collection, it is also possible that the iron interfered with the roots and rhizomes of the eelgrass, which could be why some of the moderate iron addition treatments had the highest eelgrass TEE values. Iron concretions up to 5 cm across and blackened rhizomes were often observed in the root zone during harvesting. These iron concretions and plaques surrounding the roots and rhizomes could have interfered with exchange of solutes with the porewater (Mendelssohn and Postek 1982; Taylor et al. 1984; Batty et al. 2000; Batty et al. 2002; Povidisa et al. 2009). As such, elimination of wastes and uptake of nutrients within the sediments could have been limited or eliminated, and the iron
could also have had a direct toxic effect on the rhizomes if they were growing within an area of concentrated iron granules. These results further support development of site-specific iron enrichment levels based on small-scale field studies, as well as interspersing iron addition areas with areas that receive no additions to limit the potential for formation of iron pans and concretions.

Eelgrass collected during ambient surveys lacked the fine white root hairs typically responsible for solute exchange with the sediments during most of the summer, but were present during the initial and final sampling. This suggests that eelgrass in the study area may depend more on nutrient and waste exchanges with the water column than the sediments during the warmer months and also may limit the extent to which *Zostera* may be able to reduce toxic sulfides through active pumping of oxygen into the sediments. This may explain why phosphorus levels were not noticeably different between iron addition levels, as the plants may have been obtaining this nutrient from the water column instead of the sediments, and may also limit the degree to which sedimentary iron concretions impact solute exchange.

Porewater sulfide concentrations also did not respond to iron enrichment, although iron additions have been found to lower sulfide levels in previous studies (Chambers et al. 2001; Holmer et al. 2005a; Marba et al. 2007). The amount of variability encountered with porewater sulfides was similar for both the ambient and experimental sampling. As with the ambient samples, this variability could be indicative of localized sediment conditions such as bio-irrigation or accumulations of organic matter. Porewater was collected 10 cm below the sediment surface, and for a collection
volume of 6 mL with the water occupying a conservative 25% of the sediment volume, the radius of the collection sphere would be approximately 1.8 cm (Berg and McGlathery 2001). The depth of 10 cm was selected to limit the potential for surface water to be pulled into the sample, while still being within the root zone. However, the iron was added to the surface of the sediments, so porewater sampling may have been too deep to detect an effect of iron enrichment.

The porewater for the experimental plots was collected within 20 cm of the same location every time, allowing a comparison of sulfide levels across the summer for a specific location. When individual experimental plots were tracked through the entire sampling period, sulfide concentrations were still highly variable, indicating that the observed variability was not just due to spatial heterogeneity (data not shown). Because of the variation from one time to another, as well as during a single collection day even within the summer (Fig. 5), not all of the differences could be attributed to the time or location of sample collection. Since sulfide is so highly reactive, binding with oxygen and several minerals including iron, manganese, and magnesium, small scale variability is to be expected depending on the local sediment conditions at the time of sampling (de Wit et al. 2001).

Seasonality of sediment organic content and porewater sulfide levels was expected as increases in microbial, floral and faunal production occurs during the summer. Summer sampling documented the highest porewater sulfide concentrations and the greatest range in those concentrations. However, the anticipated seasonal increase in organic content was not observed. This further supports the supposition that
currents, storm events or other disturbances remove organic material from eelgrass beds in this area, limiting its build-up within the beds and reducing the build-up of high sulfide concentrations at the bed-scale.

Sediment organic content was expected to vary among levels of iron enrichment only if the eelgrass biomass was different. Organic content did demonstrate considerable variation, but mean values were similar across iron enrichment levels, time of sampling and location for both the experiment and the ambient sampling with the standard deviation occasionally exceeding the mean (Fig. 6). These results indicate that the patchiness of the sediment conditions within sites was about the same as the patchiness among sites, which is perhaps driving some of the variation in the measured sulfide levels.
Summary:

Ambient sampling of *Zostera* and porewater sulfides indicated a potential threshold concentration around 900-1000 μM, above which concentrations may have been inhibiting eelgrass growth. Ambient sulfide levels were highly variable, with particularly high concentrations during the summer months. No significant relationships were observed between eelgrass, porewater sulfide and sediment organic content, however, indicating that factors other than those sampled contributed to both sulfide and sediment organic levels within the study area.

The potential for utilizing particulate iron additions to enhance eelgrass growth and survival in the study area cannot be adequately addressed with the current results. The results suggest that higher iron enrichment levels may have provided some benefit at Allen's Island, moderate enrichment levels may have been beneficial at Guinea Marsh, and enrichment was not beneficial or slightly detrimental at Jenkins Neck, but only Guinea Marsh had statistically significant differences. It may be possible that small annual increases would equate to larger overall results in the long-term, and further studies are needed to determine the feasibility of iron enrichment to reduce sulfide phytoxicity and enhance the long term growth and survival of *Zostera marina*. Because each study site responded differently, performing small scale studies with variable iron enrichment levels would be useful to determine site specific optimum iron addition amounts.
References cited:


Chapter 4:

A computer simulation model incorporating biological and physical feedback effects
on Zostera marina survival and growth
Abstract

Vegetated freshwater shallows often have improved water clarity relative to adjacent non-vegetated regions through a series of positive feedbacks, but this effect is not well documented in vegetated estuarine and marine systems. If present, these feedbacks may offset the negative effects of stressors including light limitation, high summer temperatures, and porewater sulfides. A computer simulation model of eelgrass \((Zostera marina)\) growth and survival in the lower Chesapeake Bay was constructed that incorporated positive feedbacks of particulate removal due to biomass-dependent active biological filtration and passive particulate settling, as well as tidal- and wind-induced particulate loading. Reduction of photosynthetic capacity by sediment sulfides was also included as well as thermally-induced stress from elevated temperatures, allowing simulation of both positive and negative physical and biological processes known to affect estuarine vegetated shallows. Simulated incremental increases in particulates resulted in 1% - 5% increases in light attenuation that translated into 1% - 7% reductions in modeled year-end shoot and root/rhizome biomass. The model was relatively less sensitive to increased sulfides; stepwise increases of 1.5, 2, and 2.5 times background sulfide levels resulted in incremental reductions of year-end shoot biomass by 20-25% and root/rhizome biomass by 15-20%. The model was most sensitive to increased temperature; a 1°C increase reduced year-end shoot and root/rhizome biomass by 41%. A combination of sulfide and temperature stress reduced shoot and root/rhizome biomass by 64% and 61%; addition of elevated particulates reduced biomass by approximately 69% and 67%, respectively. The stressors exerted cumulative but not
multiplicative effects. Positive feedbacks from increased particulate removal were able
to compensate for some of the effects of elevated particulates but were not strong enough
to offset sulfide and temperature-induced reductions in biomass. With eelgrass in the
Chesapeake Bay growing near its southern limits, sulfide or temperature stress along with
the small but additional stress of increased particulate loads may be all that is necessary
to limit restoration efforts and induce continued losses of areal coverage and density.

KEYWORDS: turbidity, submerged aquatic vegetation, *Zostera marina*, eelgrass,
estuary, Chesapeake Bay, model
Introduction

It has been well established that submerged aquatic vegetation (SAV), either in marine, estuarine, or freshwater environments, can be depth limited by light availability (Wetzel and Penhale 1983; Duarte 1991; Dennison et al. 1993; Abal et al. 1994; Short et al. 1995; Livingston et al. 1998; Arnold et al. 2000; Moore et al. 2003; Kemp et al. 2004). In the Chesapeake Bay specifically, eelgrass requires a minimum of ~20% of surface irradiance to reach the leaf surface in order to survive, and historically occupied depths up to 2 meters although it currently occurs at depths over 1 meter only rarely (Dennison et al. 1993; Orth et al. 2002; Kemp et al. 2004). The precipitous decline in SAV coverage has been attributed to increased nutrient and sediment loading and its subsequent effect on water clarity (Alden 1997; Cerco et al. 2002; Orth et al. 2002; Stankelis et al. 2003; Kemp et al. 2005).

It has also been well documented that vegetated lakes, ponds, and shallows in eutrophic freshwater systems are known to have improved water clarity relative to non-vegetated systems with similar environmental conditions, such as nutrient loading and turnover time, or even compared to adjacent open water areas within the same system (Hasler and Jones 1949; Hamilton et al. 1990; Jones 1990; Jasser 1995; Schriver et al. 1995; Ejsmont-Karabin et al. 1996; Perrow et al. 1999; Scheffer 1999; Biyu 2000; Jeppesen et al. 2002; van Donk and van de Bund 2002; Schulz et al. 2003). These vegetated areas influence water clarity by reducing total suspended solids and phytoplankton levels through a series of biological, chemical and physical interactions (Fig. 1). Positive interactions that lead to improved water clarity can include wave and
current dampening, which discourages sediment resuspension and enhances particulate settling with its cohered nutrients (Ward et al. 1984; Madsen et al. 2001; Horppila and Nurminen 2003; Takamura et al. 2003; Newell and Koch 2004), removal of nutrients directly from the water column (Short and Short 1984; Horppila and Nurminen 2003), possible allelopathic effects in which the SAV chemically inhibits phytoplankton growth (Jasser 1995; van Donk and van de Bund 2002; Erhard and Gross 2006), physical particle capture by the structure of the leaves (Pluntke and Kozerski 2003; Palmer et al. 2004; Hendriks et al. 2008), shading of phytoplankton as the SAV forms a canopy along the water surface (Buzzelli et al. 1998; Scheffer 1999), and attraction of organisms that actively filter particulates from the water column as a food source (Perrow et al. 1999; Blindow et al. 2002; Nurminen and Horppila 2002; van Donk and van de Bund 2002).

In addition to these positive feedbacks, the potential for negative feedbacks exist primarily through accumulation of increased organic matter in the sediments of SAV beds due to enhanced particulate settling, mortality of eelgrass and epiphytes, and production of feces and pseudofeces by elevated densities of filtering fauna. This accumulation of organic matter may stimulate microbially-mediated sulfide production, which is toxic to most seagrasses including *Zostera marina* (Fredette et al. 1990; Azzoni et al. 2001; Koch and Erskine 2001). The combination of stressors from increased salinity, temperature, and sulfides can have synergistic effects, such that any one of these stressors may not greatly affect the growth of seagrass, but the combination of two or more stressors can have pronounced effects (Koch and Erskine 2001).
Figure 1: Basic diagram of the main theorized feedbacks affecting SAV growth. The inner and lower loops represent negative feedbacks, while the top outer loop represents positive feedbacks.
The potential for internal feedbacks within SAV beds to reduce particulate levels and enhance growth has not received as much attention in estuaries as in freshwater systems, although recent studies have begun to address these interactions (Newell and Koch 2004; Gruber and Kemp 2010). These studies have not, however, quantified the degree to which both positive and negative internal feedbacks may affect SAV (specifically *Z. marina* or eelgrass) survival and growth. The purpose of the present study was to develop a computer simulation model to quantify the role of these feedbacks within eelgrass beds of the lower Chesapeake Bay, Virginia. Several models have already been constructed to quantify eelgrass growth in multiple estuarine systems, with varying emphasis on internal and external factors that may affect the growth and survival of the eelgrass (Verhagen and Nienhuis 1983; Wetzel and Neckles 1986; Bach 1993; Buzzelli et al. 1999; Cerco and Moore 2001). Rather than construct a new eelgrass growth model, the goal of this study was to modify existing models (primarily that of Buzzelli et al., 1999) to examine the relative impact of selected physical, chemical, and biological factors on *Zostera* growth, with an emphasis on feedbacks mediated by *Zostera* abundance, especially factors affecting particulate levels and therefore light penetration. Formulations for quantifying these feedbacks were constructed using a combination of literature values and results from the first three chapters of this dissertation.

Positive feedbacks in the model include enhanced biological filtration from benthic fauna and physical particle reduction by the SAV canopy (biological and physical feedbacks via published data). The negative impact of increased sediment sulfide
concentrations was included based on forced seasonal sulfide concentrations typical of SAV beds in lower Chesapeake Bay (chemical feedback via published data and Chapter 3). Previous studies including Chapter 2 of this dissertation have shown little or no difference in zooplankton levels in brackish and marine vegetated systems compared to open water, in contrast to freshwater systems, so variable zooplankton filtration was not included (Jeppesen et al. 2007).

In addition to the internal factors listed above, several external factors affect the ability of eelgrass to survive, including temperature (Buzzelli et al. 1999), salinity, seasonal turbidity pulses, and increased particulate loads via tidal- and wind-induced resuspension and currents (Ward et al. 1984; Caffrey and Day 1985; Short and Wyllie-Echeverria 1996; Koch 1999; Granata et al. 2001; Morichon et al. 2008; Gruber and Kemp 2010). For example, Moore et al. (1997) suggested that spring time turbidity pulses in the lower Chesapeake Bay may limit or eliminate eelgrass in this area due to its reliance on increased light availability and lower temperatures in the spring to build up internal reserves. Further, Ward et al. (1984) found that particulates were reduced within a vegetated shallow of the Choptank River, MD during one storm event under normal water levels, but not a second one during spring tides, suggesting that both wind and tides play a role in these systems. The potential effects of wind and tides on particulate levels and light availability were included in the model based on results from Chapter 1. Water temperature is directly included as a variable in several of the Zostera formulations from Buzzelli et al. (1999). The effect of salinity on Z. marina has not been consistently documented, and no studies were identified combining sulfide and salinity stressors for
this species, so it was not included in the model (Hellblom and Bjoerk 1999; Kamermans et al. 1999; Van Katwijk et al. 1999).
Methods

Much of the model, including formulations for *Z. marina* growth and maintenance, tidal level fluctuations, incoming irradiance, water temperature and salinity, was based on the model of Buzzelli et al. (1999) for the Goodwin Islands area of the lower Chesapeake Bay, VA, with a mean depth of 1 m. Only functions that were modified from that study are reported below; remaining details can be located in that publication and Appendix 2. Formulations for internal and external feedbacks are described below. Information on biomass of filter feeders, biological filtration rates, physical settling rates, and their dependency on eelgrass density or biomass was highly variable or limited, so combined estimates based on the best information available was used for several of the formulations. Unlike many other models that transport particulates into shallow regions via coupling to a hydrodynamic model, particulate levels within this model were calculated at each iteration based on the inputs of season, tides, and wind, with subsequent particle reduction rates computed based on simulated benthic populations and physical settling rates. Negative feedbacks due to sulfide accumulation was specified using empirical measurements of sulfides in lower Chesapeake eelgrass beds and published data on the effect of sulfides on eelgrass growth. The model was set up to run for an annual cycle with a time step (DT) of one hour.

*Zostera growth*

Formulations for *Zostera marina* growth and maintenance were based on the carbon-based model of Buzzelli et al. (1999) for Goodwin Islands, VA in the lower Chesapeake Bay. Shoot growth is based on a temperature dependent maximum
photosynthetic rate ($P_{\text{max}}$), subsequently reduced by a Michaelis-Menten function to account for light limitation. Losses of eelgrass shoot production include shoot respiration, mortality and translocation to roots and rhizomes. Root biomass is lost due to respiration and mortality. This model included the formulations related to $Z. \text{marina}$ production and losses directly from Buzzelli et al.'s (1999) model with the following two modifications.

Buzzelli et al. (1999) did not include $Z. \text{marina}$ reproduction losses such as reproductive structures, pollen, seeds and reproductive shoot production. Orth and Moore (1986) reported that reproductive shoots comprised 10 – 42% of above ground standing crop with flowering shoot production between April and June and release of all seeds by mid-June. Reproductive shoots are inherently included in biomass estimates and the main reproductive losses while these shoots remain on the plants are releases including flowering parts, seeds and pollen. These losses were added to the model in spring and early summer up to a maximum value of 0.37% $d^{-1}$ which results in $15 \text{ g C m}^{-2}$ total annual loss or approximately 10% of annual peak biomass in the model calibration:

$$Z_{\text{rep}} = 0.0019 - (0.0019 \times \cos(2\pi \times (\text{Julian day}+70)/(365/3)))$$

(Eq. 1)

where $Z_{\text{rep}}$ is the biomass loss to reproduction, limited to the period between March 15 and June 29 within the model.

Reproductive shoot losses of 5% $d^{-1}$ continue after this period from June 30 to July 26, a period of rapid senescence (Orth and Moore 1986), which causes an additional loss of

158
82 g C m\(^{-2}\). These additional reproductive losses were calibrated to allow the final root
and shoot biomass at the end of the simulated annual cycle to be approximately equal to
the beginning biomass.

*Light attenuation*

Light attenuation was based on the optical model developed for the Chesapeake
Bay by Xu et al. (2005).

\[
\text{PAR}_z = \text{PAR}_0 \cdot e^{-K_d Z} \quad \text{(Eq. 2)}
\]

\[
K_d = 1.17 + 0.024 \cdot \text{chl} + 0.006 \cdot \text{TSS} - 0.0225 \cdot \text{sal} \quad \text{(Eq. 3)}
\]

Where \(\text{PAR}_z\) is light (\(\mu\text{E m}^{-2} \text{s}^{-1}\)) at depth \(Z\) (m), \(\text{PAR}_0\) is light at the water surface
(same units as \(\text{PAR}_z\)), \(K_d\) is the attenuation coefficient (m\(^{-1}\)), chl is chlorophyll \(a\) (\(\mu\text{g L}^{-1}\)),
TSS is total suspended solids (mg L\(^{-1}\)), and sal is salinity > 15 PSU. \(\text{PAR}_z\) was reduced
by an additional 10% to simulate epiphyte attenuation (Kemp et al. 2004).

*Chlorophyll \(a\) concentrations*

Chlorophyll \(a\) (chl \(a\)) levels were based on several formulations. Seasonal chl \(a\)
levels were based on the long-term data of (Harding et al. 2002) with the following
formulation:

\[
\text{Chl}_s = (3.5 - (2.5 \cdot \text{COS}(2 \cdot \pi \cdot (\text{Julian day} - 40)/(365/2))) + \\
(2 - (1.5 \cdot \text{COS}(2 \cdot \pi \cdot (\text{Julian day} + 20)/365))) \quad \text{(Eq. 4)}
\]

where \(\text{Chl}_s\) is the seasonal chl \(a\) level and Julian day is the numerical day of the year.
This replicated a biphasic pattern in annual phytoplankton biomass, with the first peak
around May and a second smaller peak around November (Fig. 2a). This seasonal cycle
in chl $a$ was modified based on wind, tides, biological filtration and physical settling as described below.

Tidal and wind effects on chl $a$ levels were formulated using results of continuous monitoring of several stations situated throughout the lower York River and vicinity (Chapter 1). Effects were included as dimensionless multipliers to the seasonal biomass cycle:

$$\text{Chl} = \text{Chl}_s \times \text{Chl}_t \times \text{Chl}_{WE} \quad \text{(Eq. 5)}$$

where Chl is the total chl $a$ level, Chl$_t$ is a proportional multiplier based on tides and Chl$_{WE}$ is a proportional multiplier due to wind effect. Tidal and wind effect formulations were derived using the regressions in Table 4 and Figure 7 of Chapter 1.
Fig. 2: (a) Measured chl $a$ biomass from Harding et al. (2002) with seasonal and tidal models for chl $a$. (b) Seasonally modeled chl $a$ from (a) and final model representation including both tidal and wind effects. Values for (a) seasonal + tidal and (b) seasonal + tidal + WE are estimated every hour.
\[
Chl_t = \frac{(1.98*Z+1.20)/3.30+0.04}{(Eq. 6)}
\]
\[
Chl_{WE} = \frac{(0.11*WE+3.57)/4.07}{(Eq. 7)}
\]

where \(Z\) (m) is the proxy for tidal influence based on water depth calculated in the model and \(WE\) is the wind effect. These regressions were normalized to the median observed values for chl \(a\) (3.30 and 4.07, respectively) to generate dimensionless multipliers to increase or decrease chl \(a\) around the seasonal trajectory (Fig. 2a, b). Median values were used rather than means because the data used to determine the regressions were not normally distributed (Chapter 1). The equation for \(Chl_t\) included an intercept of 0.04 to force the computed tidal influence to take a value of one at the mean depth of 1 m; without this intercept the multiplier at 1 m depth was 0.96, skewing computed chl \(a\) levels slightly below the seasonal chl \(a\) trajectory. \(Chl_{WE}\) was constrained to 1 or greater so wind did not decrease chl \(a\) levels.

Wind effect was modeled as a weekly periodic event that varied from 0 to 15 based on the minimum and maximum wind effect computed for SAV beds in the lower York River and Mobjack Bay in Chapter 1. Wind effect could be scaled to alter the maximum \(WE\) within the model:

\[
WE = (WE_m*7.5) - (WE_m*7.5*\cos(2*\pi*(Julian \text{ day} + 10)/(365/52))) \quad (Eq. 8)
\]

where \(WE\) is the wind effect and \(WE_m\) is the multiplier which ranged from 0 to 1 to reduce WE.
**Total suspended solids concentrations**

Total suspended solids (TSS) concentrations within SAV beds were also based on monitoring data from Chapter 1 by combining the effects of tides and wind as for chl $a$ but without a seasonal component. Long-term (1997-2011) monitoring data from the EPA Chesapeake Bay Program were analyzed at four stations near the mouth of the York River (LE 4.2, LE 4.3, WE 4.1, WE 4.2). These data did not demonstrate a seasonal trend, so the mean TSS concentration of 9.87 mg L$^{-1}$ at 1 m depth was used to predict baseline concentrations (constrained to not go below the minimum value of 3.00 mg L$^{-1}$ from the EPA dataset), subsequently modified by the effects of tides and wind:

$$TSS = 9.87 \cdot TSS_t \cdot TSS_{WE}$$  \hspace{1cm} (Eq. 9)

where TSS is total suspended solids, $TSS_{WE}$ is the effect of wind on turbidity, and $TSS_t$ is the effect of tide on turbidity:

$$TSS_t = \frac{(8.36 \cdot Z - 3.95) / 4.64}{4.64} + 0.05$$  \hspace{1cm} (Eq. 10)

$$TSS_{WE} = \frac{(1.10 \cdot WE + 1.95)}{6.15}$$  \hspace{1cm} (Eq. 11)

where WE is the wind effect, Z (m) is the calculated tidal water level, 4.64 and 6.15 are medians from their respective regression datasets in Chapter 1 and 0.05 adjusts the tidal formula in the same manner as it does for chl$_t$ (Fig. 3a, b). As for Chl$_{WE}$, $TSS_{WE}$ was constrained to 1 or greater so wind did not decrease TSS levels.
Fig 3: (a) Tidal and WE multipliers for TSS (Eq. 10-11). (b) Modeled TSS based on Eq. 9 ('total') and after reduction by filter feeding and settling (TSSfin, Eq. 21) with a biomass of 1 g SAV shoot C m\(^{-2}\) providing 7.97 g dry filter feeding benthos m\(^{-2}\), the model minimum. Graph values are end of day, not every DT or hour.
Biological effects on particulate levels

Once baseline TSS and chl $a$ concentrations are computed based on seasonal, background, tidal, and wind effects, the model applies reductions to these values to account for biological filtration and physical settling within the grass beds. As noted above, results from Chapter 2 indicated that zooplankton abundance was not significantly greater within the SAV beds of the lower York River relative to adjacent unvegetated areas, and calculations suggested that zooplankton within the beds typically filter only 2-6% of the water column each day. Therefore the effects of filtration by zooplankton were excluded from the model; however these can be easily tested by increasing benthic filtration through sensitivity analysis.

The biological reduction of TSS and chl $a$ due to high concentrations of benthic filter feeders within SAV beds was based on a literature review of the active pumping of the water column due to the benthic population and the relationship between benthic biomass and SAV. Pomeroy et al. (2006) performed an analysis of the filtering of benthic populations in the Chesapeake Bay specifically, and reported an overall filtration rate of 7.8 L (g dry weight$^{-1}$ h$^{-1}$ under optimal conditions, with a density of suspension feeding benthic fauna of 18 g dry weight m$^{-2}$. Filtration volume was modified from optimal levels by incorporating reported effects of temperature on the filtering activity of filter feeding benthos. Filtration efficiency is not always 100% and can vary based on many factors including particulate composition, size, and densities (Menon 1974; Fiala-Medioni 1978; Fiala-Medioni 1979; Hughes et al. 2005; Pomeroy et al. 2006). For the purposes of this model, filtration efficiency was assumed to be 100% but the volume of
water filtered was altered via sensitivity analysis which had the same effect as reducing filtration efficiency. To alter the filtering effect of the biota, a multiplier between 0.5 – 1.5 was used to modify the volume of water filtered and therefore particulate removal relative to the total water column.

The relationship between benthic biomass and SAV density or biomass has been documented but not extensively. Orth and Van Montfrans (1982) quantified benthic populations specifically within the Chesapeake Bay by population counts over a range of SAV densities within *Z. marina* and *Ruppia maritima* beds, with an emphasis on *Z. marina*. Bostroem and Bonsdorff (2000) also quantified benthos in relation to *Z. marina* in the Baltic sea by population counts. Other studies quantified benthic populations or biomass in relation to SAV, but these studies did not quantify the relationship between SAV and benthic abundance well enough for incorporation into this model, instead stressing the importance of other factors such as patch size and patchiness (Borg et al. 2010; Nohren and Odelgaard 2010), while others concentrated on benthic productivity within seagrass beds but did not quantify any relationship with SAV abundance (Fredette et al. 1990). The relationship developed here was based on data from Bostroem and Bonsdorff (2000) and Orth and Van Montfrans (1982) (Fig. 4a), with taxon specific counts of benthic populations converted to g dry weight m⁻² according to the conversions in Ricciardi and Bourget (1998):
Fig. 4: (a) Benthic biomass as a function of SAV biomass based on the data of Orth and Van Montfrans (1982) and Bostroem and Bonsdorff (2000); regression equation is given in Eq. 12. (b) Temperature-dependence of benthic filtration rate from Equation 13.
where SF is g dry weight of suspension feeders m$^{-2}$ and SAV$_{sh}$ is the mass of shoots in g C m$^{-2}$ ($r^2 = 0.55$). Eq. 12 is used in the model to compute SF from simulated eelgrass shoot biomass, which is then used to compute filtration rate (see below).

The effect of temperature on benthic suspension feeder filtration has been documented for several groups, including ascidians (Robbins 1983), polychaetes (Riisgaard and Ivarsson 1990; Riisgaard et al. 1992), bivalves (Newell and Koch 2004), and bryozoans (Lisbjerg and Petersen 2001). Since representatives of these groups are located within the eelgrass beds of the Chesapeake Bay (Orth and Van Montfrans 1982; Fredette et al. 1990), a mean $Q_{10}$ of 4.3 was computed from these studies and used to develop an exponential temperature function that reached the reported value from Pomeroy et al. (2006) of 7.8 L (g dry weight)$^{-1}$ h$^{-1}$ at a temperature of 22 °C:

$$FR = 7.395 \cdot e^{0.147 \cdot T} \quad (\text{Eq. 13})$$

where FR is the filtration rate in L (g dry suspension feeders)$^{-1}$ day$^{-1}$, and T is the water temperature in °C (Fig. 4b).

FR is then combined with computed filter feeder biomass to compute daily clearance rate of the water column:

$$CR = F_M \cdot (SF \cdot FR) \quad (\text{Eq. 14})$$

where CR is clearance rate in L m$^{-2}$ day$^{-1}$ and $F_M$ is a dimensionless multiplier to test the effect of changes in the volume of water filtered via sensitivity analysis; values were varied from 0.5 to 1.5. Equations 12-14 calculate a variable clearance rate from benthos.
due to both changes in SAV shoot density and water temperature, with 7.78 g suspension feeders m\(^{-2}\) with no SAV present, 57.5 g suspension feeders m\(^{-2}\) at 100 g SAV shoot C m\(^{-2}\), and the Chesapeake Bay mean from Pomeroy et al (2006) of 18 g suspension feeders m\(^{-2}\) at 42 g SAV shoot C m\(^{-2}\) reached in mid-April.

To reduce the particulates within the model, the volume of water filtered each day (CR) was divided by the total water volume in liters (per square meter) to obtain a proportional water volume filtered; this was used to compute the proportion of particles remaining each time step (DT, 1/24 d):

\[
\text{Red}_{\text{ben}} = (1-(\text{CR} \times \text{DT}/\text{WV}))
\]  \hspace{2cm} (Eq. 15)

where, Red_{\text{ben}} is the fraction of chl \(a\) and TSS remaining after removal by benthic filtration, WV is the water volume (L) and DT is the time step in the model (Fig. 3b). Values for Red_{\text{ben}} were constrained between 0.05 and 1, as the volume of water filtered sometimes exceeded the volume of water present.

**Physical effects on particulate levels**

Physical structures, including SAV leaves, can trap particles in addition to enhancement of settling due to current reduction (Palmer et al. 2004; Hendriks et al. 2008). Cerco & Moore (2001) used a variable settling rate for particles in their model of Chesapeake Bay SAV with an increase of 0.05 m d\(^{-1}\) for every 1 g shoot C m\(^{-2}\). The Chesapeake Bay Program Eutrophication Model uses a particulate settling rate in the absence of SAV of 1 - 4 m d\(^{-1}\) (0.04 - 0.17 m h\(^{-1}\)) for TSS and 0 - 0.25 m d\(^{-1}\) (0.01 m hr\(^{-1}\)) for phytoplankton (Cerco and Noel 2004). Other investigators have distinguished
between a rapidly settling component that is resuspended with each tide in the lower Chesapeake Bay, with settling rates ranging from 0.7 – 1.2 mm s\(^{-1}\) \((60.5 – 103.7 \text{ m d}^{-1}, 2.5 - 4.3 \text{ m hr}^{-1})\), and a second much slower settling component which largely stays in suspension throughout the entire tidal cycle, providing background TSS levels of 15-22 mg L\(^{-1}\) (Fugate and Friedrichs 2002; Fugate and Friedrichs 2003). The importance of currents, eddies, particle size, and location in affecting settling rates was also stressed (Fugate and Friedrichs 2003). Hendriks (2008) reported high particle loss rates within beds of *Posidonia oceanica*, with over 99% of particles lost within 20 minutes of particle loading under varying shoot densities and current velocities. The author’s own monitoring of particulate levels within vegetated shallows of the York River (Chapter 1) indicated that wind and tides both played important roles in particulate levels and can be used at least in part to predict concentrations, with NTU values rarely below 1.5 \((\sim 12 \text{ mg L}^{-1} \text{TSS})\), which is in close agreement with the minimum reported by Fugate and Friedrichs (2002).

Because reported physical settling rates even within the Chesapeake Bay are highly variable, the settling rate developed for this model was based on a formulation that allows variable particle settling from tidally induced currents as calculated within the model. This model has a maximum water depth of approximately 1.6 m and a time step of 1 hour, so a maximum TSS settling rate of 1.6 m hr\(^{-1}\) \((38.4 \text{ m d}^{-1})\) was used to allow full settling at slack tide (high or low), no physical settling at mid-flood or mid-ebb tide, with TSS values constrained to not fall below 3.0 mg L\(^{-1}\) based on the analysis of EPA data reported above. Wind induced currents can keep particles in suspension, so the tidal
settling rate was modified by \( WE \) to prevent settling at the maximum \( WE \) of 15, decreasing to no effect at a \( WE \) of zero; the settling rate due to SAV was left unchanged by winds as SAV shoots can reduce wind-induced currents (Ward et al. 1984; Gruber and Kemp 2010). The formulations are summarized below:

\[
\begin{align*}
\text{TSSSR}_{\text{SH}} &= 0.05 \times \text{SH} \times \text{SHM} \quad \text{(Eq. 16)} \\
\text{TSSSR}_{\text{t}} &= 38.4 - 142.2 \times \text{te} \quad \text{(Eq. 17)} \\
\text{TSSSR}_{\text{t,WE}} &= \text{TSSSR}_{\text{t}} \times (1 - 0.066 \times \text{WE}) \quad \text{(Eq. 18)} \\
\text{TSSSR}_{\text{tot}} &= 1 - ((\text{TSSSR}_{\text{SH}} + \text{TSSSR}_{\text{t,WE}}) \times \text{DT}) / \text{Z} \quad \text{(Eq. 19)}
\end{align*}
\]

where \( \text{TSSSR}_{\text{SH}} \) is the TSS settling rate due to SAV shoots (Cerco and Moore 2001), \( \text{SH} \) is g dry shoot C m\(^{-2}\), \( \text{SHM} \) is a multiplier to test the model response to increased or decreased shoot-dependent settling, \( \text{TSSSR}_{\text{t}} \) is the physical settling rate (m d\(^{-1}\)) as a function of tidal currents, \( \text{te} \) is the tidal exchange (see below), \( \text{TSSSR}_{\text{t,WE}} \) is the tidal settling rate modified by wind, and \( \text{TSSSR}_{\text{tot}} \) is the fraction of TSS remaining after physical settling, constrained from 0.05 to 1. Assuming a standing wave relationship between tidal elevation and velocity, tidal exchange (\( \text{te} \)) is calculated as the absolute difference between the previous water level and the current water level (m h\(^{-1}\)), and is used as a proxy for tidally generated currents.

Physical settling of chl \( a \) was based on the range of 0 - 0.25 m d\(^{-1}\) (0.01 m hr\(^{-1}\)) used for phytoplankton in the Chesapeake Bay Eutrophication Model (Cerco and Noel 2004) and modified similar to that for TSS:

\[
\text{ChlSR}_{\text{SH}} = 0.05 \times \text{SH} \times \text{SHM} \quad \text{(Eq. 20)}
\]
\[ \text{ChlSR}_t = 0.25-0.9259*_{te} \]  
(Eq. 21)

\[ \text{ChlSR}_{t\text{WE}} = \text{ChlSR}_t * (1-0.066*\text{WE}) \]  
(Eq. 22)

\[ \text{ChlSR}_{\text{tot}} = 1-(((\text{ChlSR}_{\text{SH}} + \text{ChlSR}_{t\text{WE}})*\text{DT})/\text{Z}) \]  
(Eq. 23)

where \( \text{ChlSR}_{\text{SH}} \) is the chl \( a \) settling rate due to SAV shoots based on Cerco and Moore (2001), \( \text{ChlSR}_t \) is the settling rate due to tidal currents in m d\(^{-1}\), \( \text{ChlSR}_{t\text{WE}} \) is the tidal settling rate modified by WE, and \( \text{ChlSR}_{\text{tot}} \) is the fraction of chl \( a \) remaining after physical settling, constrained between 0.05 and 1.

The final concentrations of Chl and TSS each time step are computed by reducing the values resulting from seasonal, background, tidal, and wind effects (Eq. 5 and 9 above) by the fraction remaining after benthic filtration and physical settling (Eq. 19 and 23):

\[ \text{TSS}_{\text{fin}} = \text{TSS} * \text{Redben} * \text{TSS}_{\text{SR tot}} \]  
(Eq. 24)

\[ \text{Chl}_{\text{fin}} = \text{Chl} * \text{Redben} * \text{Chl}_{\text{SR tot}} \]  
(Eq. 25)

These final values for Chl and TSS are used in the calculation of light attenuation \( (K_d) \) in Equation 2 above.

**Sulfide effects on Zostera marina**

Enhanced organic levels are often associated with seagrass beds due to increased internal production, particle settling, and deposition of feces and pseudofeces from filter feeders, inducing microbially-enhanced sulfide levels which are toxic to most seagrasses including *Zostera marina* (Fredette et al. 1990; Azzoni et al. 2001; Koch and Erskine 2001). Monitoring of sulfide levels within SAV beds in Chapter 3 did not indicate...
increased organic loading or sulfide levels compared to adjacent unvegetated areas, but
did indicate seasonality to the sulfide levels with the potential for much higher levels
during the warmer summer months. The summer months had a range of values from
almost 0 $\mu$M to over 1000 $\mu$M. A seasonal $[S]$ curve was developed (Fig. 5a) based on
the increase in sulfide levels from Chapter 2:

$$[S] = S_M \cdot 260 - (S_M \cdot 250 \cdot \cos(2\pi \cdot (\text{Julian day} + 95)/(365/3))) \quad (\text{Eq. 26})$$

where $[S]$ is the sulfide concentration in $\mu$M, $S_M$ is a multiplying factor to change
the maximum and mean sulfide concentrations while leaving the minimum little changed.
Sulfide concentration is set to a constant low value that will not affect $P_{\text{max}}$ before June 1
or after September 12 ($152 > \text{Julian day} > 256$).

Goodman et al. (1995) quantified the reduction in photosynthetic capacity of $Z.\
marina$ in a mesocosm study on the Eastern Shore of Virginia which is in close proximity
to the York River. Utilizing the reduction in maximum photosynthetic rate ($P_{\text{max}}$) as
reported by Goodman et al (1995), the effect of sulfides on eelgrass production was
formulated as:
Fig. 5: (a) Measured *in situ* (Chapter 3) and modeled sulfide levels using various values for $S_M$. (b) Proportional reduction in $P_{\text{max}}$ due to modeled sulfide concentrations in (a).
\[ P_{\text{maxr}} = P_{\text{max}} \cdot P_{\text{red}} \]  
\[ P_{\text{red}} = 1 - (0.749 \cdot \ln([S]) - 4.468) \]  
(Eq. 28)  
(Eq. 29)

where \( P_{\text{maxr}} \) is the reduced value of \( P_{\text{max}} \) after accounting for sulfides, \( P_{\text{max}} \) is the temperature-dependent maximum rate (Eq. 3), \( P_{\text{red}} \) is the proportional reduction in \( P_{\text{max}} \) values, and \( S \) is the sulfide concentration in \( \mu \text{M} \) (Fig. 5b). \( P_{\text{red}} \) is constrained within a range of 0.2 – 1.0, corresponding to sulfide concentrations of 1,130 and 400 \( \mu \text{M} \), respectively, allowing \( P_{\text{max}} \) to remain minimally changed at low sulfide levels, and reducing the value significantly as sulfide levels approach 1,130 \( \mu \text{M} \) to a maximum reduction of 0.2 or 80%. This range corresponds to the range of sulfides observed in Goodman et al.’s (1995) study; values were not extrapolated outside this range.

**Calibration and simulation analysis**

The model was run over an annual cycle with an hourly time step and calibrated to the above- and below-ground biomass data of Buzzelli et al. (1999) from the Goodwin Islands, Chesapeake Bay, Virginia. Calibration was conducted with initial eelgrass shoot and root/rhizome biomass of 25 and 10 g C m\(^{-2}\), respectively, and chl \( a \) and TSS concentrations of 4.7 \( \mu \text{g L}^{-1} \) and 9.87 mg L\(^{-1}\), respectively (Table 1, Run 1). The Chl \( a \) concentration used in calibration was the seasonal mean value measured inside the vegetated shallows from high frequency field sampling across seven SAV beds in lower Chesapeake Bay (Chapter 1), while the TSS concentration was a 15 year mean from the above referenced EPA Chesapeake Bay Program long term monitoring data. The EPA data set was used for TSS because of its extended monitoring over several years even
though values were lower than some published values (Fugate and Friedrichs 2002; Fugate and Friedrichs 2003). The value for chl $\alpha$ was based on field sampling rather than the EPA dataset because it was in better agreement with the reported seasonal values from Harding et al. (2002) used above.

After calibration, a series of model scenarios were run to evaluate the effects of individual and combined particulate processes (both inputs and reductions), feedbacks and sulfide concentrations (Table 1). The first scenario was run with minimum particulate concentrations of 3.0 mg L$^{-1}$ TSS and 1.0 $\mu$g L$^{-1}$ chl $\alpha$, based on minimums from the same datasets used for the calibration, to quantify eelgrass response to the most favorable conditions possible within the model (Table 1, Run 2). Subsequent runs incrementally activated particulate inputs to gauge model response to the particulate formulations (Runs 3-7), followed by a series of runs to quantify the effect of increasing porewater sulfide concentrations (Runs 8-12) with values based on data from Chapter 3. Particulate removals were then included to gauge their potential to offset the negative impacts of sulfides (Runs 13-15). Moderate sulfide levels of 2X the base level were chosen for these runs. Lastly, the effect of increased temperature was included by incrementally increasing the modeled temperature in 1°C increments to a maximum increase of 3°C; these runs are summarized below but are not included in Tables 1-3.
Table 1: Model calibration and simulation analyses. "X" denotes values or processes that were included in each run; numbers represent the multiplier used for sulfide concentrations.

<table>
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<tr>
<th>Conditions</th>
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177
Results

Calibration:

The model reproduced the annual cycle of *Z. marina* shoot and root growth and biomass at Goodwin Islands with an early summer peak followed by a thermally induced die-off and minor regrowth in the fall (Fig. 6). This baseline calibration resulted in a peak shoot biomass of 139 g C m\(^{-2}\), summer mean biomass of 67.9 g C m\(^{-2}\), and ending biomass of 24.5 g C m\(^{-2}\) (Table 2, Run 1), and peak root/rhizome biomass of 49.1 g C m\(^{-2}\), summer mean biomass of 30.0 g C m\(^{-2}\), and ending biomass of 8.8 g C m\(^{-2}\) (Table 3, Run 1). It is important for year-ending biomass to be similar to the beginning biomass as eelgrass is perennial in this region and the following year’s growth is dependent on the biomass present at the end of the current year’s run. Regressions of model results against the biomass data from Buzzelli et al. (1999) were sufficiently well constrained to proceed with simulation analysis (Fig. 6c, d). Shoot biomass had a stronger agreement (\(r^2=0.87\)) than root biomass (\(r^2=0.38\)) most likely because summer peak root/rhizome modeled biomass was off-set from Buzzelli et al.’s (1999) data by about 2-3 weeks even though the magnitudes were similar (Fig. 6d).

Light attenuation:

Modeled light attenuation in the calibration run followed a seasonal cycle even though the particulate inputs were held constant because the light extinction coefficient follows seasonal salinity as well as TSS and chl \(a\) (Eq. 4, Fig. 7a). Computed \(K_d\) was 14% lower in the run with minimum chl \(a\) and TSS compared to the calibration run (Fig. 7a, Table 2 – Runs 1 and 2). When seasonally-variable chl \(a\) was included, \(K_d\) tracked...
the seasonal chl \( a \) curve with a mean approximately equal to that in the calibration run. When all processes that lead to elevated chl \( a \) and TSS were included, the combination of wind and tidal influences led to widely fluctuating \( K_d \) with minimum values approximately equal to those predicted from seasonal chl \( a \). When particulate removal processes were included, modeled summertime \( K_d \) decreased by 10 to 20\% (Fig. 7b, Table 2 – Runs 7, 13-15).
Fig. 6: Calibration of *Z. marina* shoot and root/rhizome biomass to field data from Buzzelli et al. (1999). (a, b) Daily model output and monthly field data; (c, d) Regressions between monthly mean model output and field data.
Table 2: June – August mean, peak and final (day 365) *Z. marina* shoot biomass (g C m$^{-2}$); June – August mean $K_d$ and $P_{\text{max}}$ for model runs as specified in Table 1. The starting biomass for *Zostera* shoots in all runs was 25 g C m$^{-2}$.

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181
Table 3: June – August mean, peak and final (day 365) *Z. marina* root biomass (g C m\(^{-2}\)) for model runs as specified in Table 1. The starting biomass for *Zostera* roots in all runs was 10 g C m\(^{-2}\).

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Fig. 7: (a) Modeled light extinction coefficient ($K_d$) in a variety of model simulations; (b) Modeled $K_d$ with all particulate input and loss processes included (no sulfides).
Zostera response to particulates:

Relative to the calibration run, simulation with minimum concentrations of chl \(a\) and TSS (Run 2, Table 1) resulted in 14% greater year-end shoot and 13% greater root biomass due to a 14% decrease in summer mean light attenuation (Tables 2-3). Addition of seasonally- and tidally-modulated chl \(a\) (Runs 3-4) produced results similar to those from the calibration run (Tables 2-3, Run 1), since the tidal chl \(a\) formulation varies around the seasonal chl \(a\) concentrations. Including the influence of wind on chl \(a\) (Run 5) reduced final biomass by only 0.5 g C m\(^{-2}\) or 2%.

Incrementally activating the various sources of elevated particulates (both chl \(a\) and TSS) steadily decreased all measures of eelgrass biomass (Fig. 8a, Tables 2-3, Runs 3 – 7). Increased chl \(a\) concentrations had less of an effect on eelgrass growth compared to increased TSS concentrations, which is to be expected since light attenuation in this region of the Chesapeake Bay is more strongly affected by TSS than phytoplankton chlorophyll (Xu et al 2005). Particulates had a greater effect on % reduction in year-end shoot biomass than on summer biomass, although absolute year-end losses were lower. Zostera shoots and roots responded similarly because growth of root biomass was formulated based on shoot biomass and production. Overall, losses of summer mean shoot biomass due to elevated particulates ranged from 0.4 to 2.0 g C m\(^{-2}\) (0.6% - 3%), root biomass losses ranged from 0.1 to 0.8 g C m\(^{-2}\) (0.7% - 3%), year-end shoot losses ranged from 0.3 to 1.6 g C m\(^{-2}\) (1% - 7%) and root losses ranged from 0.1 to 0.6 g C m\(^{-2}\) (1% - 7%). The percent reductions in year-end biomass were similar to the percent change in summer mean \(K_d\) (1% - 5%) across these runs.
Fig 8: (a) *Z. marina* shoot response to increasing particulate inputs. (b) *Z. marina* shoot response to particulate reduction due to physical and biotic removal; sulfide at 2x. Values are end of day.
**Zostera response to sulfides:**

Buzzelli et al.'s (1999) maximum photosynthetic rate formulation was based on temperature and therefore did not respond to variations in light fields due to changing particulate loads (Table 2). Seasonal sulfide inputs reduced summer mean $P_{\text{max}}$ in the model by 10% - 17% which coincided with the annual temperature-induced die-back in the Chesapeake region (Fig. 9a). The timing of sulfide-induced $P_{\text{max}}$ reductions on top of thermal reductions may have limited the modeled response to sulfide even though up to 80% reductions in $P_{\text{max}}$ occurred during the summer, with the largest percent reduction in shoot biomass between 1 and 1.5 times the base sulfide level (Table 2, Runs 8-9, Fig. 9b). Since modeled sulfide continued to suppress $P_{\text{max}}$ into the early fall regrowth period, year-end biomass had the largest response with approximately 20% reductions of shoot biomass with every 50% increase in [S]. Continuing the annual decline over several years could result in a large decline or loss of SAV beds.
Fig. 9: (a) Reduction in maximum photosynthetic rate ($P_{\text{max}}$) due to increased sulfide levels. (b) Modeled shoot biomass with the sulfide levels in (a) and all particulate inputs and reductions active in the model.
Zostera response to particulate removal processes

When physical and biotic particulate removals were both included in the model runs, year-end shoot biomass increased by approximately 10% relative to runs that included only particulate loading. When these removals were individually included along with particulate input processes, eelgrass responded with 5% - 7% increases in biomass, which were not enough to compensate for losses due to increased attenuation of light (Fig. 8b, Table 2, Runs 10–11, 13–15). Benthic suspension feeders were predicted to filter the entire water column in summer (Pomeroy et al. 2006), resulting in low and limited variations in $K_d$ during mid-summer (Fig. 7b). With fewer particulates in the water column light attenuation decreased and growth increased, although the increase in growth was more evident during the summer (peak biomass) than for ending biomass due to sulfide induced reductions in these runs (Figs. 6b, 7b, Tables 2-3).

Zostera response to increased temperature in combination with other stressors:

_Z. marina_ is considered a cool weather plant and starts to experience thermal stress at about 25°C, although regional and local vegetative strains can respond differently (Orth and Moore 1986; Van Katwijk et al. 1999). Increased water temperatures resulted in earlier and greater peak biomass in the model but an earlier die-back and decreased year-end values (Fig. 10). Since the biomass at the end of the year is important for determining growth during the following year, this decrease has important implications for long-term survival of eelgrass beds in the region. The initial 1°C increase resulted in a 41% decline in year-ending biomass with incremental 1°C increases reducing year-ending shoot biomass incrementally by 40-50%, which highlights that
eelgrass in lower Chesapeake Bay is near its southern limits (Fig. 10a) (Moore and Jarvis 2008).

Combining temperature increases with particulate inputs had less of an impact on final biomass than did combining temperature with increased sulfides (Fig. 10b,c). Peak shoot biomass had the opposite trend with greater reductions from particulates than from sulfides (Fig. 10a, c). Since both increased sulfides and temperature above 25°C reduce $P_{\text{max}}$, combining these two stressors produced a larger response than either stressor alone with a 1°C increase, with less of an impact on subsequent temperature increases (Fig. 10a-c). Combining temperature increases, sulfide increases and particulate inputs did not appear to reduce ending or peak biomass more than that observed with increased temperature and sulfides (Fig. 10c, d).
Fig. 10: Modeled effects of increasing temperature in 1°C increments on $L$. marina shoot biomass under scenarios with (a) minimum particulates and no sulfides; (b) minimum particulates and 2X sulfides; (c) all particulate inputs and removals active and no sulfides; (d) all particulate inputs and removals active and 2X sulfides.
Discussion

Eelgrass has gone through a series of both sudden and less pronounced long-term variations in its annual coverage in the lower Chesapeake Bay (Moore et al. 2012; Orth et al. 2012). Buzzelli et al.’s (1999) model predicted a gradual decline due to increased particulate levels with a final 40% loss of shoot biomass over a 10 year simulation period. Long term monitoring of Chesapeake Bay-wide eelgrass coverage supports similar long-term trends with gradual declines except in the case of catastrophic events (Orth et al. 2010). Small annual differences in eelgrass biomass over extended periods of time can lead to long-term changes in biomass.

The model presented here produced similar results, with limited differences between modeled eelgrass biomass when internal particulate loading or reductions were incorporated one at a time (Tables 2-3). Similarly, if internal positive feedbacks were increased the response of eelgrass was small. For example, if all particulate inputs and removals were active, sulfides were set at 1X and shoot feedbacks were reduced by ½, final eelgrass shoot biomass was 21.0 g C m⁻²; if shoot feedbacks were increased by 1.5, final shoot biomass was 22.5 g C m⁻², a difference of only 7%. However, if these small differences continued over several years large changes in eelgrass biomass similar to the documented trajectories in Chesapeake Bay could be realized (Fig. 11). If larger annual losses in biomass occur then almost complete loss of the SAV bed can occur in just a few years, limiting the potential for any recovery, whereas smaller annual losses require longer for complete losses and provide a standing stock to aid recovery of the SAV bed (Fig. 11).

191
Fig. 11: Ten year simulated run incorporating all the inputs and removals, including either twice the base sulfide levels for the second run or a 1°C increase in ambient water temperature for the third run and both increased sulfides and temperature for the fourth run.
Internal feedbacks did not produce as large a response as that observed in some freshwater systems (Scheffer et al. 2005), perhaps because the continuous particulate loading caused by tides in marine systems limits the effect of filtration and particle settling. Shoot-related feedbacks associated with filtration by benthic fauna were small but greater than the effect of physical settling (Table 2, Runs 13-14). Pomeroy et al. (2006) predicted that suspension feeding benthic fauna are able to remove all particulates within the lower Chesapeake Bay during the summer months which was supported by this model; computed summer benthic filtration rates exceeded the volume of water available to filter, resulting in complete particulate removal over long periods of time at elevated eelgrass densities. However, the limited ability of benthic fauna to access the upper portion of the water column limits the depth to which the water column can be cleared in estuarine and marine systems (Pomeroy et al., 2006), thereby contributing to the depth limitation of the vegetation. This is in contrast to freshwater systems, where the primary drivers of biological water filtration are zooplankton and semi-planktonic fauna which are able to access the full water column (Scheffer 1999).

Another reason for limited response to internal feedbacks may be due to the relationship of eelgrass photosynthesis to water temperature. The maximum photosynthetic rate is a bi-phasic formulation that declines at warm summer temperatures, exactly when the positive feedbacks of increased particulate removal are increasing. The declining $P_{\text{max}}$ may also limit the impact of negative feedbacks including decreased light. For example, for Runs 13-14, $K_d$ decreased by approximately 10% but year-end biomass increased by only 1.5%. Other runs exhibited a closer correspondence
between differences in $K_d$ and biomass; for example, Runs 4-5 resulted in a difference in $K_d$ of about 7% and a difference in biomass of about 6%.

The model responded strongly to increased sulfide levels which reduced eelgrass biomass. The modeled base curve for sulfide was formulated to reduce $P_{\text{max}}$ for approximately 3.5 months with the biggest reduction during July and August. Increasing this base curve increased the time as well as extent to which sulfide could reduce $P_{\text{max}}$ and affect eelgrass growth (Fig. 9). Increasing sulfide levels in increments of 0.5X caused a 20-25% reduction in year-end shoot and root biomass (Table 2, Runs 8, 9, 11, and 12). Including particulate removal allowed approximately 10% of this loss to be regained (Table 2, Runs 11 and 15), but was not enough to fully offset the negative impact of sulfides.

The model responded the strongest to temperature increases. At minimum particulate loads, a $1^\circ C$ increase in temperature reduced the year-end shoot and root biomass by 41% and each incremental $1^\circ C$ increase in water temperature resulted in incremental 40-50% declines in year-ending biomass. Combining a $1^\circ C$ temperature increase with a 2X increase in sulfide levels reduced year-end shoot biomass by 64% and root biomass by 61%, compared to a 43% reduction in shoots and 40% reduction in roots when increasing sulfides only. Including all of the particulate functions (4% increase in $K_d$) resulted in a decline of 69% for shoots and 67% for roots (Fig. 10); including particulates only resulted in a 14% decline in final root and shoot biomass with a 4% increase in $K_d$. 

194
The combined stressors of temperature, sulfides and particulates were cumulative and resulted in a larger negative effect than each stressor alone. Since eelgrass in the lower Chesapeake is near its southern limits, even small biomass losses can contribute to a long-term decline, further highlighting the combined role of temperature, sulfide and particulate stressors in reducing eelgrass biomass in the lower Chesapeake Bay beyond the capacity of internal feedbacks to compensate for those stressors.

Simulated benthic filtration improved eelgrass survival by approximately 1 g shoot C m\(^{-2}\) or 5-10% incrementally, depending on which other factors were active (Table 2, Runs 11, 13 – 15). This limits the effect that benthic filtration may have on eelgrass survival due to increased sulfides or temperature. However, increases in turbidity due to increased wind, including spring wind events (Moore et al. 1997), resulted in a decline in eelgrass biomass of the same magnitude (1-1.6 g shoot C m\(^{-2}\)), suggesting that benthic removal of particulates can play an important role in offsetting negative impacts of elevated particulates.

The annual eelgrass biomass cycle in the Chesapeake Bay and nearby regions such as the coastal bays of the Delmarva Peninsula may limit the extent to which internal positive feedbacks are able to enhance eelgrass survival beyond limited external stresses. Because a large portion of annual \textit{Z. marina} growth occurs during the early spring when water temperatures are cooler, benthic filtration is lower than during the warmer summer months. The limited shoot biomass present at the beginning of the growth cycle also limits the extent to which it can influence physical settling (Hansen and Reidenbach 2012). Recent seeding restoration successes in the coastal bays of the Delmarva
Peninsula indicates that conditions were amenable to eelgrass growth without internal positive feedbacks (Orth et al. 2012). Positive feedbacks may allow the eelgrass to survive should conditions begin to deteriorate, but long-term losses may still occur should temperature, sulfide levels or particulates increase even minimally, and catastrophic losses may occur if these three stressors increase simultaneously (Fig. 11). The lack of restoration success in the Chesapeake Bay indicates that conditions are not presently sufficient for sustaining increases in eelgrass in these areas, and the continued variation in areal coverage may also indicate that internal feedbacks are not strong enough to overcome the stressors inherent in the lower Chesapeake Bay.
Summary and Conclusions

The model developed in this study was able to reproduce annual growth and biomass cycles typical of *Z. marina* in the lower Chesapeake Bay, with an early summer peak in biomass followed by a thermally-induced decline and subsequent re-growth in fall. This model expanded upon that of Buzzelli et al. (1999) as it incorporated physical and biological influences on particulate removal and the potential effects of elevated sulfides. Additional formulations included seasonal eelgrass reproductive losses, and wind- and tidally-influenced loading of particulates. Internal feedbacks that cause particulate reduction had a positive but small effect on eelgrass growth, increasing year-end shoot biomass approximately 10% relative to including all of the particulate inputs without any reductions. This positive feedback was able to offset the modeled wind effect, but was not able to counter reductions in biomass due to all of the simulated particulate inputs.

Increased sulfide concentrations had a large impact on year-end eelgrass biomass, with a 20-25% reduction resulting from each 0.5X increase in sulfide levels, suggesting sulfides may have a larger impact on short-term eelgrass survival than particulates. Increased temperature had the largest negative impact, with an initial 1°C increase resulting in a 41% reduction in year-end shoot biomass with declines of 40-50% for each additional 1°C increase in water temperature. Combined sulfide and temperature stress increased the loss to 64%, while addition of particulates increased the loss slightly to 69%. Modeled stressors to eelgrass were cumulative in that the % reduction in year-ending biomass was greater when multiple stressors were included in the simulations.
relative to runs with each stressor acting individually. Some stressor combinations were additive in that the reduction in year-ending biomass was equal to the sum of the percent reductions for each stressor individually; other combinations resulted in enhanced losses which were less than the sum of the individual percent reductions.

With eelgrass in the Chesapeake Bay growing near its southern limits, model results indicate that either sulfide or temperature stress may be all that is necessary to limit restoration efforts and induce rapid losses of areal coverage and density, while chronic elevated light attenuation increases these losses, contributing to long-term gradual declines and resistance to recovery. Internal feedbacks are able to reduce some of the stress caused by light limitation due to elevated particulate levels, but do not appear to compensate for even a 1°C increase in temperature or increases in sulfide levels. With predicted increases in temperature due to global climate warming, continued management to increase eelgrass survival and restoration may not be a cost-effective measure in the Chesapeake Bay, and instead an alternative to eelgrass or breeding to increase eelgrass resistance to thermal stress may be worth pursuing.
References cited:


DISSERTATION SUMMARY AND CONCLUSIONS

Extended particulate monitoring (NTU and chl a) of several sites in the general vicinity of the lower York River did not demonstrate consistently improved water clarity within vegetated shallows compared to adjacent unvegetated areas. Turbidity was generally lower within the beds while patterns in chlorophyll a varied throughout the season. Chlorophyll a levels within the SAV beds were more influenced by tides than wind; turbidity was more strongly influenced by wind than tides. Effects of wind and tides varied among sites. Additional studies, specifically season-long quantification of particulates at several sites, would be useful to develop stronger relationships between particulates, SAV abundance, and predictors including tides and wind.

General regression equations were developed for predicting particulate levels inside the SAV beds in this study. The formulations provide a means for analyzing major trends and their impact on subsequent eelgrass growth, such as the impact of wind and storm events at different times of the year. These regressions are considered only moderately predictive of particulate levels, however, due to their low correlation coefficients and the high variability in the data. Attempts to apply these regressions further should consider including stochastic variations around the mean predictions.

There were no consistent statistically significant differences in zooplankton densities either in relation to SAV or tidal influences, although some isolated sampling periods exhibited significant trends. Significant differences were found between sites in both years, highlighting the inherent patchiness of zooplankton distributions even in similar systems located in close
proximity. There were consistent statistically significant differences between night and day with higher zooplankton densities at night both inside and outside of SAV beds for all locations. Based on calculations of clearance rates for the zooplankton densities encountered, it is not likely that zooplankton have a pronounced effect on particulate levels in these polyhaline vegetated systems with an overall clearance rate of approximately 2-6% of the water column. These results are in stark contrast to those from freshwater systems where zooplankton reach much higher abundance and exert a strong effect on water clarity.

Ambient sampling of *Zostera* and porewater sulfides indicated a potential threshold concentration around 900-1000 μM, above which concentrations appeared to inhibit eelgrass growth and abundance. Ambient sulfide levels were highly variable, with particularly high concentrations during the summer months. No significant relationships were observed between eelgrass, porewater sulfides and sediment organic content, however, indicating that factors other than those sampled contributed to both sulfide and sediment organic levels within the study area.

The potential for utilizing particulate iron additions to enhance eelgrass growth and survival in the study area cannot be adequately addressed with the current results. The results suggest that higher iron enrichment levels may have provided some benefit at Allen's Island, moderate enrichment levels may have been beneficial at Guinea Marsh, and enrichment was not beneficial or slightly detrimental at Jenkins Neck, but only Guinea Marsh had statistically significant differences. It may be possible that small annual increases would equate to larger overall results in the long-term, and further studies are needed to determine the feasibility of iron enrichment to reduce sulfide phyto-toxicity and enhance the long term growth and survival of *Zostera marina*. Because each study site responded differently, performing small scale
studies with variable iron enrichment levels would be useful to determine site specific optimum iron addition amounts.

The *Z. marina* simulation model was able to reproduce annual growth and biomass cycles typical of lower Chesapeake Bay. An annual early summer biomass peak followed by a thermally-induced reduction in biomass and subsequent fall re-growth was replicated with approximately equal values of initial and final biomass. Internal particulate reduction feedbacks had a positive but small effect on eelgrass growth, increasing year-end shoot biomass approximately 10%. This positive feedback was able to offset the modeled wind effect, but was not able to counter reductions in biomass due to all of the modeled particulate inputs.

Increased sulfide concentrations had a larger impact on eelgrass year-end biomass, with 20-25% reductions resulting from every 0.5X increase in sulfide levels, suggesting that sulfides may have a larger impact on short-term eelgrass survival than particulates. Increased temperature had the largest negative impact, with a 1°C increase in causing a 41% reduction in year-end shoot biomass. Combined sulfide and temperature stresses increased the loss to 64%, and inclusion of particulates resulted in a 68% loss. Modeled stressors to eelgrass were cumulative rather than multiplicative.

With eelgrass in the Chesapeake Bay growing near its southern limits, model results indicate that either sulfide or temperature stress may be all that is necessary to limit restoration efforts and induce rapid losses of areal coverage and density, while chronic elevated light attenuation increases these losses, contributing to long-term gradual declines and resistance to recovery. Internal feedbacks are able to reduce some of the stress caused by light limitation due to elevated particulate levels, but do not appear to compensate for even a 1°C increase in temperature or increases in sulfide levels.
Global climate warming is adding thermal stress on top of the other anthropogenic stressors already encountered by eelgrass in the lower Chesapeake Bay such as nutrient enrichment, sediment loading, and reduced water clarity. The anticipated increase in water temperature could exacerbate the stress of elevated particulates and sulfides, as well as directly impact eelgrass by stimulating an earlier die-back, increased mortality and delayed fall regrowth. Microbially-mediated porewater sulfides were shown in this study to increase in the warmer summer months; porewater sulfide concentrations could increase earlier and reach higher values with an increase in water temperature. Particulate levels due to increased run-off and elevated winds could also increase as more intense storms are predicted as the result of climate change in the region, further reducing light levels within eelgrass beds. The combination of increases in negative stressors could have devastating effects on Zostera, such that it may be eliminated as a major SAV species in the polyhaline areas of the Bay. From this perspective, it may be more important to study a possible alternative to eelgrass in this region as opposed to continueing management options for a plant that is likely not to return to pre-colonial aerial coverage.
APPENDIX 1

Calibration, use and construction of the zooplankton sampler SWaZooPS

Overview

The sampling performed for this study was conducted with a Shallow Water and Zooplankton Pumped Sampler (SWaZooPS), based loosely on the design of Dixon and Robertson (1986). An initial version that more closely followed their published design was limited by inherent inaccuracies in measuring the volume of water sampled due to turbulence caused by internal pipe hydraulics. Most hydraulic flow meters are designed to measure laminar instead of turbulent flow. Several important modifications were made to their design to ensure accurate measurement of the volume pumped, minimize disturbance to the area being sampled, avoid disrupting the zooplankton and water column, and allow for the simultaneous removal of water samples for additional analyses.

The re-designed sampler is easy to assemble, disassemble and transport, with most of the pieces fitting within the floats. In addition, the sampler was designed to be free-floating and self-contained, with a marine battery utilized for the power source and operated while wading as opposed to deployment over the side of or behind a boat. It is ideal for sampling in a variety of shallow and structured aquatic habitats that would not be suitable for towing a net behind a boat. Additional features include operation of the sampler by only two people, small sample and equipment holders held in place on top of
the platform with hook and loop fasteners, and ability of the entire sampler to withstand temporary submersion.

**Calibration, water flow and sampling**

A calibration check on the flow meter indicated it was correctly measuring the volume of water filtered ($r^2=0.99$, $p<0.01$). Tests with rhodamine red dye and field experience confirmed that the sampler generated no discernible currents while sampling, while any ambient currents appeared unaffected by the pumping. Given a sampling duration of about 10 minutes and the small sphere of water sampled, even in the presence of tidal currents SWaZooPS is collecting a local water sample. SWaZooPS pumps water through a 200 μm plankton net at approximately 37 L per minute, allowing filtration of 350-400 L for each sample in about 10 minutes, depending on battery charge. While the specific parcel of water being sampled is difficult to determine due to ambient water currents, in the absence of currents we estimated the volume sampled around the intake to be a sphere 0.88 m in diameter based on a volume sampled of approximately 370 L. To obtain an integrated sample of the water column, the intake was initially positioned at mid-depth and slowly moved around a sphere of approximately 0.4 m radius while pumping, depending on water depth and SAV canopy structure at the time of sampling.

SWaZooPS was based on a similar proven design that included testing of the pumped samplers capability compared to traditional net tows (Dixon and Robertson 1986). Since this testing was already performed and several other pumped samplers have been tested with comparable results, limited testing of this design was performed to verify its consistency (Miller and Judkins 1981; Taggart and Leggett 1984; Omori and Jo
1989; Mallin 1991; Nayar et al. 2002). In addition, since all samples in this study were collected with SWaZooPS, the samples were considered comparable to each other regardless of this sampler’s ability compared to net tows. Therefore three samples were collected with SWaZooPS and three samples were collected with a traditional net tow, one pair each at Allen’s Island, Jenkins Neck, and Guinea Marsh to provide a limited comparison of this sampler’s collection ability to a net tow. These samples were collected in open shallow water deep enough for the boat to operate without clogging the plankton net, with the pumped sampler deployed over the side of the boat in the same general location.

Student’s t-test was used for comparing zooplankton densities determined from the towed plankton net vs. pump. Comparison of the test samples from SWaZooPS and traditional net tows indicates no significant difference in zooplankton density for the three main taxonomic groups or for total zooplankton (Fig. A-1, Table A-1), although “other copepods” were noticeably higher in samples collected by SWaZooPS. We also found no significant differences between sampling methods for each individual taxonomic group prior to combining them into the three main groups used for analysis. These results indicate that SWaZooPS was performing similar to other pumped samplers and traditional net tows.

Construction

To build the sampler, 2 cm (1 inch) thick, 28.5 cm (12 inch) wide foam core PVC lumber was used for the raft or platform, battery box, support braces under the platform and around the sides of the battery box, and supports for the flow meter and pipes. PVC
lumber is resistant to splitting, allowing screws to be driven in close to the edges or in thin pieces without pre-drilling the holes. This lumber also floats, while solid PVC does not. It is waterproof and resistant to degradation in the water, and although the specific type used here was not UV resistant, will last for a long time if stored out of direct sunlight. It is also lighter to transport and easier to clean than wood, with none of the metals and other possible contaminants found in treated lumber. The pipe pieces, except for the floats, are schedule 40 PVC, and the hoses are braided reinforced clear flexible tubing, as unreinforced tubing tended to kink and restrict flow. All of the hardware is stainless steel to resist corrosion.

The platform is 88 cm x 81.5 cm, using 4 pieces of the PVC lumber, with a space in the center for the battery box. The platform is held together with 4 support braces running underneath, screwed and glued into place. The battery box is sized to fit a 12V marine battery, but the dimensions need to fit the size of battery available, including posts and handles, as they vary. The whole in the center of the raft needs to be sized to fit the battery box inside, with the supports around the edge of the battery box resting on the platform (Fig. A-2).

The floats for the sampler are constructed of thin walled, or sewer grade, 6 inch PVC pipe approximately 1.5 m long. One end has a cap permanently attached, while the other end has a black rubber cap with a hose clamp that can be removed to facilitate storage of the other pieces. Each float is attached to the underside of the platform with two lengths of 2.5 cm webbing with buckles so they can be easily installed and removed. Small slots are drilled through the platform to allow the straps to pass through the
platform and around the floats, with the floats pushed up tight against the outermost support brace.

A 12V 2200 GPH (gallons per hour) bilge pump was used for the pump, secured to the platform with bolts and wing nuts. The intake of the bilge pump is outfitted with a 4 inch to 3 inch ID (inside diameter) flexible black rubber reducer. A 3 inch rigid PVC elbow redirects the inflow towards the front, connected to a short piece of 3 inch PVC pipe, followed by a black rubber 3 inch to 2 inch reducer, and then by a short length of flexible 2 inch ID braided hose to allow for increased maneuvering of the intake. This is further reduced to 1 ¼ inch ID PVC pipe, with an elbow at the end to allow the inflow to be positioned from above the sampling point. The elbow has additional 2 inch ID braided hose connected to it to reach the desired depth, in this case about 0.4 m.

The outtake from the pump is one inch flexible braided hose connected to rigid 1 inch ID PVC pipe. The length of straight pipe prior to the flow meter should typically be a minimum of 10x the width of flow meter pipe, in this case at least 25 cm, to avoid internal turbulence interfering with the accuracy of the meter. Likewise, straight pipe length at least 5x the pipe width needs to come after the flow meter. A t-connection with ½ inch ID PVC pipe, a valve, and flexible hose is connected to the inflow approximately 30 cm prior to the flow meter. This allows water samples to be taken simultaneously with the zooplankton samples, without affecting measurements of volume filtered for zooplankton. After filtering the zooplankton sample, this hose can also be used to rinse the plankton net from the outside to ensure the zooplankton are in the cod end of the net.
The flow meter is a paddlewheel type from Blue-White Industries rated 5 – 50 gallons per minute (20 – 200 L min⁻¹), and is easy to take apart to remove debris that may interfere with its functioning. A calibration check on the flow meter indicated it was correctly measuring the volume sampled ($r^2=0.99$, $p<0.01$). The flow meter, being threaded on each end, fits in between its supports and is screwed into place with the PVC pipe connectors. After the flow meter, water flow is directed through a valve, then through a 1 inch ID flexible braided hose into the zooplankton net. A small length of webbing with hook and loop fasteners can be used to secure the flexible hose to the pipe above the net to ensure the hose stays in position. The valve is used to shut off the water to this hose for rinsing and cleaning after the sample is filtered. Most of the PVC pipe connectors are threaded to allow them to be easily disconnected for transport and storage. These are made watertight with Teflon tape, which also allows for easier threading and removal.

The upper reinforced rim of a 12 inch, 200 μm mesh plankton net is supported on top of a 25 cm length of 12 inch ID PVC pipe. Three or four small pieces of the PVC lumber can be glued to the inside of the 12 inch pipe to provide more support for the net. These should be rounded off and sanded to ensure they do not snag or tear the net. After the sample is filtered, the hose can be removed from the net, and the net can be lifted out of the support for rinsing and removal of the sample from the cod end.

The net and flow meter supports are attached to the platform with 3 small blocks glued and screwed into place on the top of the platform. The supports are then attached to these blocks and each other with wing nuts and bolts so they can be readily tightened.
or removed in the field without tools. The plankton net hangs through a 29 cm diameter hole in the platform inside the pipe support, and the filtered water is returned directly back to the water column below the sampler.

The battery for the power supply is fully enclosed within a watertight box made of the PVC lumber, utilizing marine grade sealant along the inside edges of the box, and foam strips inside the lid. It is held closed with a hasp and hinges across the back. Braces are attached to the outside of the box near the top to act as supports, holding the box in place in the middle of the platform. This allows the weight of the box to steady the platform and prevent it from tipping over.

Power is routed through a rubber encased switch on the top of the box which allows the pump to be easily turned on and off. The wires run through a hole in the side of the box sealed with marine grade sealant, connected to a basic 2 prong trailer plug which can be easily connected or disconnected for assembly or disassembly. A hole through the platform allows the power supply wires to connect to the bilge pump underneath. All connections, except the trailer plug, are made waterproof through the use of heat shrink tubing, marine grade sealant, and self-adhesive silicone tape. The trailer plug is rated for outdoor use and is water resistant.

A cooler for sample storage can be either tied in between the floats behind the sampler, or placed on top of the platform in the back. Several containers were constructed from PVC pipe with thin (1/8 inch) plastic glued to the bottom. Hook and loop fasteners were attached to their bottoms, with the opposing fastener piece glued to the top of the platform and battery box (not shown in Fig. A-2). These containers were

215
used to hold sample jars, markers, a cod end holder, water bottles, and other necessary equipment, securing and allowing easy access on top of the platform during sampling. Additional views of SWaZooPS are provided below.

**Summation**

Our novel, shallow water pumped zooplankton sampler, SWaZooPS, successfully collected both water and zooplankton samples in the shallow, structured SAV habitats of the lower York River and Mobjack Bay. The advantages of using this sampler in structured shallow water habitats across the salinity gradient from freshwater to marine make SWaZooPS ideal for quantifying zooplankton densities where conventional net tows will not work. The sampler enabled us to address our three main objectives, namely to determine the potential for increased zooplankton densities within SAV beds relative to adjacent unvegetated areas and the influence of SAV cover on zooplankton densities, the potential for zooplankton to occur at greater densities during low tides as opposed to high tides, and the presence of diel effects on zooplankton densities within SAV beds (i.e. prevalence of horizontal migration into the SAV over vertical migration). Comparisons to traditional net tows indicated the sampler produced comparable estimates of zooplankton abundance.
Figure A-1. Mean of zooplankton abundance from samples collected for comparison of net tows to SWaZooPS samples. Error bars represent one standard deviation for 3 replicate samples (6 total). Differences were not statistically significant (see Table A-1).
Table A1-1. Mean zooplankton density (number L$^{-1}$), standard deviation, and t-test results for three taxonomic groups and total zooplankton, 3 samples collected by SWaZooPS and 3 samples collected by traditional net tows.

<table>
<thead>
<tr>
<th>Zooplankton category</th>
<th>SWaZooPS</th>
<th>Net Tow</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. tonsa</em></td>
<td>3.2 (± 1.4)</td>
<td>4.2 (± 3.0)</td>
<td>0.62</td>
</tr>
<tr>
<td>Other copepods</td>
<td>1.7 (± 1.2)</td>
<td>0.4 (± 0.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>Barnacles</td>
<td>0.2 (± 0.2)</td>
<td>0.5 (± 0.5)</td>
<td>0.46</td>
</tr>
<tr>
<td>Total zooplankton</td>
<td>5.4 (± 2.7)</td>
<td>5.2 (± 3.6)</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Figure A-2: Three perspectives of the layout of the Shallow Water and Zooplankton Pumped Sampler (SWaZooPS)
References cited:


APPENDIX 2

Additional formulations in the computer simulation model

Novel and modified formulations included in the Zostera marina computer simulation model were presented in Chapter 4. Below are the formulations that were used directly from Buzzelli et al. (1999) describing eelgrass growth and losses and selected forcing functions.

Physical forcing functions:

(1) Water depth

\[ Z = MSL + (0.356 \times \cos(0.5059 \times t - 1.583)) + (0.067 \times \cos(0.5236 \times t - 5.039)) + (0.074 \times \cos(0.4964 \times t - 1.264)) + (0.047 \times \cos(0.2625 \times t + 0.332)) + (0.037 \times \cos(0.2434 \times t + 0.332)) \]

where \( Z \) is water depth in m, MSL is mean sea level which was set at 1 m in the model, and \( t \) is time in hours.

(2) Water temperature

\[ (16.5 - (14.5 \times \cos(2 \times \pi \times (Julian \text{ day} - 31)/365))) + T_i \]

where Julian day is the numerical day of the year and \( T_i \) is the °C increase in water temperature which varied from 0-3; water temperature varied from 2°C to 31°C under normal conditions.

(3) Salinity

\[ 19.85 - (3.13 \times \cos(2 \times \pi \times (Julian \text{ day} - 110)/365)) \]

where salinity is in PSU and varied from 17 to 23.

(4) Photosynthetically active radiation

\[ PAR = ((\text{Solar PAR}/\text{photoperiod}) \times 277.78) \]

\[ \text{Photoperiod} = 11.75 - (2.25 \times \cos((2 \times \pi \times (Julian \text{ day} - 354))/365)) \]

\[ \text{Solar PAR} = 28.25 - (16.75 \times \cos((2 \times \pi \times (Julian \text{ day}-354))/365)) \]
where PAR is photosynthetically active radiation at the water surface in \( \mu E \ m^{-2} \ s^{-1} \), Solar PAR is the daily PAR in \( E \ m^{-2} \ d^{-1} \), and Photoperiod is daylength in hours.

**Zostera growth and loss functions:**

1. Photosynthesis

\[
P_{\text{max}} = ((0.0025 \times T) + 0.0049) \times (1 - (\text{MAX}(T - 25,0)/10))
\]

where \( P_{\text{max}} \) is the temperature (T)-dependent maximum photosynthetic rate (d\(^{-1}\)) of *Z. marina* and the MAX function limits temperature to 25°C or less. This function was modified to include the MAX function based on Buzzelli (pers. comm.).

\[
P_{\text{gross}} = P_{\text{max}} \times (\text{PAR}_z/(\text{PAR}_z+I_k))
\]

where \( P_{\text{gross}} \) is the gross photosynthetic rate (d\(^{-1}\)), \( \text{PAR}_z \) is the available light at depth \( Z \) (based on Eq. 3 in Chapter 4), and \( I_k \) is the half saturation constant in \( \mu E \ m^{-2} \ s^{-1} \) for *Z. marina* photosynthesis, set at 57.5.

2. Shoot growth and biomass

\[
Z_{\text{sh}} = P_{\text{gross}} \times Z_{\text{sh}}
\]

where \( Z_{\text{sh}} \) is the biomass of *Zostera* shoots in g C m\(^{-2}\) and \( Z_{\text{sh}} \) is the biomass of *Zostera* shoots from the previous time step.

3. Shoot respiration

\[
Z_{\text{shresp}} = Z_{\text{shrespR}} \times Z_{\text{sh}}
\]

\[
Z_{\text{shrespR}} = 1.5 \times (P_{\text{gross}} \times (0.00317 \times T + 0.105) + e^{(0.137 \times T - 10.1)})
\]

where \( Z_{\text{shresp}} \) is the loss of shoots due to respiration in g C m\(^{-2} \) d\(^{-1} \) and \( Z_{\text{shrespR}} \) is the respiration rate (d\(^{-1} \)).

4. Shoot mortality

\[
Z_{\text{shm}} = Z_{\text{sh}} \times Z_{\text{shmR}}
\]

\[
Z_{\text{shmR}} = Z_{\text{shm1}} + Z_{\text{shm2}}
\]

\[
Z_{\text{shm1}} = 0.0135 \times e^{(-0.0005 \times (333 - \text{Julian day}))^2}
\]

\[
Z_{\text{shm2}} = (0.0175 - 0.0125 \times \text{COS}(2 \times \pi \times \text{Julian day}/365)) \times \text{MAX}(T - 20,0)/(10)
\]

223
(5) Translocation from shoots to roots

For $\text{Zsh} < 200 \text{ g C m}^{-2}$, $\text{Zt}_{\text{trans}} = 0.25 \times \text{NPP}$

For $\text{Zsh} \geq 200 \text{ g C m}^{-2}$, $\text{Zt}_{\text{trans}} = \text{NPP}$

$\text{NPP} = (\text{Zsh} \times \text{P}_{\text{green}}) - \text{Zshresp}$

where $\text{Zt}_{\text{trans}}$ is the amount of production in $\text{g C m}^{-2} \text{ d}^{-1}$ that is translocated to the roots/rhizomes, $\text{Zrt}$ is amount of roots/rhizomes in $\text{g C m}^{-2}$, NPP is the net shoot primary production in $\text{g C m}^{-2} \text{ d}^{-1}$. This formulation allows 25% of production to be transferred to the roots/rhizomes when the total shoot biomass is less than the maximum density of 200 g C m$^{-2}$, and all of the net production goes to the roots/rhizomes when shoot biomass is at its maximum limit.

(6) Root respiration

$\text{Zrtresp} = \text{ZrtrespR} \times \text{Zrt}$

$\text{ZrtrespR} = (0.0005 \times 1.25^{(T-20})}$

where $\text{Zrtresp}$ is total root respiration ($\text{g C m}^{-2} \text{ d}^{-1}$) and $\text{ZrtrespR}$ is the rate of root respiration ($\text{d}^{-1}$).

(7) Root mortality

$\text{Zrtm} = \text{Zrt} \times \text{ZrtmR}$

where $\text{Zrtm}$ is the root/rhizome mortality ($\text{g C m}^{-2} \text{ d}^{-1}$) and $\text{ZrtmR}$ is the mortality rate which is identical to $\text{ZshmR}$ above.

Reference cited:

APPENDIX 3

ANOVA results – addendum to values reported in main text

Chapter 2 - Zooplankton densities within the vegetated shallows of the York River Estuary, Virginia

Table A3-1: Results of 2-way ANOVA on log$_{10}$ transformed total zooplankton density, 2006 sampling season, with location and % cover as factors.

<table>
<thead>
<tr>
<th>variable/interaction</th>
<th>degrees of freedom</th>
<th>sum of squares</th>
<th>mean squares</th>
<th>F ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cover category</td>
<td>3</td>
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<td>0.3</td>
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<td>0.005</td>
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<tr>
<td>location</td>
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<td>1.2</td>
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<tr>
<td>cover category X location</td>
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<td>0.2</td>
<td>4.2</td>
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<tr>
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<tr>
<td>total</td>
<td>54</td>
<td>5.6</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A3-2: Results of 2-way ANOVA on log$_{10}$ transformed total zooplankton density, 2007 sampling season, with location and % cover as factors.

<table>
<thead>
<tr>
<th>variable/interaction</th>
<th>degrees of freedom</th>
<th>sum of squares</th>
<th>mean squares</th>
<th>F ratio</th>
<th>P-value</th>
</tr>
</thead>
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<td>cover category</td>
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<tr>
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<td>0.2</td>
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<td></td>
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</table>
Table A3-3: Results of 3-way ANOVA on $\log_{10}$ transformed total zooplankton density, 2007 sampling season, with day/night, location and in/out as factors.

<table>
<thead>
<tr>
<th>variable/ interaction</th>
<th>degrees of freedom</th>
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<th>mean squares</th>
<th>F ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>day/night</td>
<td>1</td>
<td>1.8</td>
<td>1.8</td>
<td>54.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>location</td>
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<td>7.1</td>
<td>3.6</td>
<td>106.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>in/out</td>
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<td>0.2</td>
<td>0.2</td>
<td>6.3</td>
<td>0.020</td>
</tr>
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<td>day/night x location</td>
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<td>0.621</td>
</tr>
<tr>
<td>date x location</td>
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<td>13554</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>plot x location</td>
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<td>31545</td>
<td>3943</td>
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<td>0.516</td>
</tr>
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<td>date x plot x location</td>
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<td>0.3</td>
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</table>

Chapter 3 - Sulfides, iron and *Zostera marina* in the lower Chesapeake Bay and the potential for iron addition to enhance restoration and management success

Table A3-4: Results of 3-way ANOVA on TEE base difference values included in Fig. 3.

<table>
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<th>mean squares</th>
<th>F ratio</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>date</td>
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<td>58624</td>
<td>8375</td>
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<td>plot</td>
<td>4</td>
<td>24673</td>
<td>6168</td>
<td>1.4</td>
<td>0.231</td>
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<tr>
<td>location</td>
<td>2</td>
<td>96324</td>
<td>48162</td>
<td>11.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>date x plot</td>
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<td>109799</td>
<td>3921</td>
<td>0.9</td>
<td>0.621</td>
</tr>
<tr>
<td>date x location</td>
<td>14</td>
<td>189760</td>
<td>13554</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>plot x location</td>
<td>8</td>
<td>31545</td>
<td>3943</td>
<td>.09</td>
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</tr>
<tr>
<td>date x plot x location</td>
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Table A3-5: Results of 2-way ANOVA on TEE early values included in Fig. 4.

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<th>P-value</th>
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</thead>
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<tr>
<td>iron enrichment level</td>
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<td>74630</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>total</td>
<td>89</td>
<td>1452318</td>
<td>16318</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A3-6: Results of 2-way ANOVA on TEE late values included in Fig. 4.

<table>
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<th>variable/interaction</th>
<th>degrees of freedom</th>
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<th>F ratio</th>
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</tr>
</thead>
<tbody>
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<tr>
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<td>&lt;0.001</td>
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<tr>
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</tr>
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<td>89</td>
<td>15245</td>
<td>171.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
VITA

LANCE M. GARDNER

Lance was born in Wilmington, Delaware, on February 23, 1965. He graduated from Caesar Rodney High School in 1983, obtained a Bachelor of Arts degree in Biology and Chemistry from Skidmore College in 1987, and a Master of Science degree in Limnology and Oceanography from the Center for Limnology at the University of Wisconsin – Madison in 1992. He moved to Virginia to accept a position with the Commonwealth as a Clean Water Act §401 Water Quality Certification and Virginia Water Protection Permit writer and compliance inspector. He left government employment and accepted a position as an environmental consultant specializing in wetland delineations, soil surveys, and aquatic ecology studies before returning to school. He will graduate from the Virginia Institute of Marine Science Biology Department, College of William & Mary, with a Doctorate of Philosophy in 2012.