Sustainable seagrass restoration in the Virginia coastal bays.

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Abstract

Seagrass depth limits are important to consider when thinking about the future of coastal ecosystems through climate change and nutrient loading scenarios. Seagrasses provide many ecosystem services to coastal areas worldwide, including providing habitat for many economically important species, acting as a significant carbon sink, and improving water quality. Because seagrasses are declining globally, it is important to be able to identify areas for restoration where seagrasses could be successful in order to maximize use of time and money. Current models for the Virginia Coast Reserve (VCR) only consider effects of light and point measurements of temperature on the maximum depth limit for eelgrass. However, it has been shown that multiple factors can affect light requirements in plants such as sediment characteristics and pore-water chemistry. Sediment characteristics, such as grain size and organic matter content increase light requirements in plants by physically blocking light and decreasing oxygen concentrations in the sediments allowing for the intrusion of phytotoxins such as pore-water sulfide and ammonium. With climate change causing a rise in global temperatures, seagrasses will become even more sensitive to changes in their light environment, such as those caused by coastal eutrophication, and will need to increase light requirements further to maintain a positive carbon balance. This may affect depth limits in seagrasses by limiting their range for growth at the minimum depth limit due to increases in temperature and at the maximum depth limit due to declining light conditions with depth. Because the persistence of restoration projects is dependent on the feedbacks between hydrodynamics, light attenuation, and temperature at the meadow scale, it is important to consider the effects of light and temperature measurements over time in terms of other stressors such as pore-water chemistry and sediment characteristics to accurately find the maximum and minimum depths for eelgrass growth.

This thesis addresses how maximum and minimum depth limits change over an environmental gradient of sediment grain size and organic matter content in the Virginia coastal bays. The impact of changes in light attenuation in terms of water quality and temperature on maximum and minimum depth limits was investigated through spatial analysis of field and bathymetry data. The predicted depth ranges were compared to ranges of transplanted plants along a depth gradient from 0.4 m to 2.0 m MSL (mean sea level) bracketing the known range for eelgrass growth in Hog Island Bay, 0.8 m to 1.6 m MSL. I found that the maximum depth limit for eelgrass growth can be predicted by light levels in areas with low pore-water sulfide concentrations; however, in areas with high sediment pore-water sulfide concentrations there may be a more complex interaction occurring where light requirements increase due to sulfide intrusion. Predicting the minimum depth limit involves considering a more complex interaction between light and temperature. In a mesocosm study, I attempted to separate the effects of light and sediment characteristics, specifically pore-water sulfide concentrations, by measuring optimal quantum yield using a pulse amplitude modulated fluorometer and plant productivity. There was no significant relationship between sediment treatment and photosynthesis; however, I did find a significant effect of light on photosynthesis. This indicates that in this controlled environment, sediment grain size and organic matter content do not have an effect on the efficiency of photosystem II or productivity of eelgrass. However, results may have been skewed due to poor mesocosm set-up and a drop in sediment sulfide concentrations between the field and mesocosm experiments. With this knowledge, these effects can be separated in future

experiments and the information from this thesis can be used to constrain eelgrass growth and distribution models in the VCR.

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Introduction

Seagrasses are submerged aquatic vegetation (SAV) found in coastal areas worldwide (Orth et al. 2006b, Waycott et al. 2009). They provide many valuable ecosystem services to coastal bays and estuaries including habitat and food for marine organisms, improved water quality and clarity, sediment stabilization, carbon sequestration and, provision of organic matter and nutrients to the ocean (Orth et al. 2006b, Waycott et al. 2009, Short et al. 2011). These ecosystem services have been valued at over US$34,000 per hectare (Costanza et al. 1997, Short et al. 2011). However, seagrass ecosystems are rapidly declining worldwide due to coastal development, increasing human population, increasing sediment and nutrient loading (Orth et al. 2006b), and the effects of climate change (Orth et al. 2006b, Waycott et al. 2009). Because seagrass ecosystems are so valuable, mitigating the loss of seagrasses and ensuring current restoration projects are sustainable through future climate change and nutrient loading scenarios is of particular concern to scientists and policy makers alike.

History and restoration of seagrasses on the Delmarva Peninsula

Eelgrass (*Zostera marina* L.) is found in coastal areas along the North Atlantic and North Pacific basins (Orth et al. 2010). Historically, eelgrass was found in the Chesapeake Bay and in the coastal bays of the Delmarva Peninsula (Orth et al. 2010). However, in the Virginia Coastal bays the wasting disease *Labyrinthula zosterae* followed by a hurricane extirpated the eelgrass population in 1933 (Orth et al. 2006a). With the collapse of the eelgrass ecosystem came the collapse of the commercially important scallop and brant industries (Orth et al. 2006a). Discovery of small patches of eelgrass in the 1990s prompted a restoration project in the coastal bays of the Virginia Coast Reserve Long Term Ecological Research site (VCR-LTER) Bays by seeding in South Bay in 1999 and 2000 (Orth et al. 2003, Orth et al. 2006a) Cobb Bay in 2001, Spider Crab Bay in 2003 and Hog Island Bay in 2006 (Orth et al. 2012). In the first ten years of the restoration, more than 1700 hectares of eelgrass were restored to the Virginia Coastal Bays, and meadows continue to expand (Orth et al. 2012; http://web.vims.edu/bio/sav/index.html).

These restored seagrass beds have been shown to engineer their landscape by increasing nitrogen fixation (Cole and McGlathery 2012), restoring carbon and nutrient sequestration (Greiner et al. 2013), and increasing water clarity through sediment deposition (McGlathery et al. 2012, Hansen and Reidenbach 2012, Hansen and Reidenbach 2013). Cole and McGlathery (2012) found that biological nitrogen fixation increases as meadow density and sediment organic content increase. This creates a positive feedback loop between *Z. marina* meadows and nitrogen fixing bacteria and epiphytes where eelgrass provides carbon exudates and the nitrogen fixing bacteria and epiphytes provide essential nitrogen to the eelgrass (Cole and McGlathery 2012). As *Z. marina* density increases, wave energy and near-bottom currents are attenuated (Hansen and Reidenbach 2012). This causes sediment in the water column to settle to the bottom decreasing turbidity and increasing the amount of light reaching eelgrass meadows (Orth et al. 2012). As light attenuation through the water column increases, eelgrass growth ceases at the maximum depth limit, 1.6 m MSL (mean sea level) (McGlathery et al. 2012). Because the Virginia coastal bays are at the southern range for eelgrass (*Z. marina*) growth, increasing water temperatures may also negatively affect eelgrass growth in these coastal bays in the future as it has in the Chesapeake Bay with extensive die-offs seen after summer heat waves (Moore et al. 2012, Carr et al. 2010).

Controls on Seagrass Distribution

Light

Seagrasses require from 4% to 30% incident light at the sediment surface for carbon fixation by photosynthesis to compensate for losses from respiration; *Zostera marina* (L.) requires about 20% incident light at the sediment surface (Dennison et al. 1993). The maximum depth limit for seagrasses is constrained by this minimum light requirement due to increased light attenuation with depth (Duarte 1991). Several factors influence water column light attenuation, including water quality parameters such as total suspended solids, water column chlorophyll, and water column nutrient concentrations, (McGlathery et al. 2007), macro-algal growth, (McGlathery 2001) and sediment suspension from wave action or storminess which contributes to the concentration of total suspended solids in the water column (Biber et al. 2009). These parameters cause light to decline exponentially with depth through the water column, changing the maximum depth limit for seagrass growth as the light attenuation coefficient (K_d) changes (Duarte 1991, Dennison et al. 1993).

Water Quality

High water quality (low total suspended solids, low water column chlorophyll, low nutrient concentrations) is essential for the persistence of seagrass beds (Waycott et al. 2009, Biber et al. 2008, Dennison et al. 1993). However, many coastal areas world-wide are being negatively affected by increasing human populations along the coastlines which can increase sediment and nutrient loading and decrease water quality (Waycott et al. 2009). Areas where there is increased nutrient loading may have excess algal growth which can decrease the amount of light reaching the sediment surface (Duarte 2002, Bryars et al. 2011). Even with the stabilizing effects of seagrasses, sediment suspension can increase the amount of total suspended solids in the water column (Duarte 2002, Lawson et al. 2007, Pedersen et al. 2012). In the Virginia coastal bays, water quality is high with respect to water column nutrient concentrations and water column chlorophyll (McGlathery et al. 2012, www1.vcrlter.virginia.edu/home1/?q=data_wq). However, there is high wind-driven sediment suspension in the coastal bays which can cause spikes in total suspended solids in the water column and increase light attenuation (Lawson et al. 2007). It has been shown that wave attenuation by seagrass meadows increases water clarity, therefore, increasing eelgrass growth (Hansen and Reidenbach 2012, Carr et al. 2010).

Sediment Characteristics

Sediment grain size is important when considering the amount of light attenuation caused by these sediment suspension events in seagrass beds (Krause-Jensen et al. 2011, Pedersen et al. 2012). Seagrass meadows are usually areas with high sediment deposition and stability (Hansen and Reidenbach 2012, Lawson et al. 2012), which contributes to accumulation of finer sediment in seagrass beds compared to bare sediments (McGlathery et al. 2012). However, muddy sediment in seagrass beds can be less stable compared to sandy sediment (Widdows et al. 2008) and suspended particles have been found to be a greater percentage clay and silt than sand (Pedersen et al. 2012). Sediment suspension can also increase in seagrass beds depending on the height of the water column: if seagrasses occupy the entire water column, sediment deposition increases because current velocities are reduced more than in areas where seagrass meadow height does not occupy the entire water column (Ward et al. 1984). Increasing seagrass density in a meadow has been shown to help reduce flow within the canopy and therefore bed shear stress and sediment resuspension (Widdows et al. 2008). Lawson et al. (2012) found that at low densities seagrasses can increase sediment suspension to values higher than seen in bare sediment during similar wind and wave events.

The Lambert – Beer equation is used to find the percentage of incident light at the maximum depth limit for submerged aquatic vegetation:

Equation 1: $$I_z = I_o \cdot e^{-k_d \cdot Z}$$

where I_z is photosynthetically active radiation (PAR) at water depth z, I_o is PAR just below the water surface, and K_d is the light attenuation coefficient (Dennison et al. 1993). Differences in water quality have a direct effect on the light attenuation coefficient which can explain world-wide differences in the maximum depth limits of seagrass species

Equation 2: $$\log Z_c(m) = 0.26 - 1.07 \log K_d(m^{-1})$$

where Z_c is the maximum depth limit in meters (Duarte 1991). Many models have tried to explain the relationship between the light attenuation coefficient and water quality (Biber et al. 2008, Carr et al. 2012, Dennison et al. 1993, Kemp et al. 2004, Wall et al. 2011, Christian and Sheng 2003). Dennison et al. (1993) found the minimum water quality characteristics using water column chlorophyll a (15 μg/L), total suspended solids (15 mg/L), dissolved inorganic nitrogen (DIN) (10 μM) and dissolved inorganic phosphorous (DIP) (0.67 μM) to sustain submerged aquatic vegetation (SAV) in the Chesapeake Bay and its tributaries. Biber et al. (2008) modified an optically active constituent model (Gallegos et al. 1990) to find the levels of optically active constituents, non-algal particulates and phytoplankton, needed for seagrass growth in order to set goals for ecosystem managers. They verified their model at the maximum depth limits for seagrasses in the Albemarle-Pamlico Sound off the coast of North Carolina, USA. However, there are few studies that evaluate the minimum depth limit for seagrass growth (Reynolds et al. 2012), which may be related to high temperature stress or wave-disturbance.

Temperature

Water temperature is strongly correlated with seasonal seagrass productivity (Lee et al. 2005). Seagrass metabolism is sensitive to changes in temperature, specifically temperature increases (Collier et al. 2011, Winters et al. 2011). When temperatures exceed thresholds for seagrass species, 28°C for *Z. marina* (Moore and Jarvis 2008, Moore et al. 2012), respiration exceeds photosynthesis causing a carbon deficit within the plant, decreasing growth and above-ground biomass (Bulthuis 1987, Collier et al. 2011, Olsen et al. 2012). With the predicted average increase of 1.3°C of coastal water temperatures before 2100 (Najjar et al. 2000), many seagrasses at the warm latitudinal limit for their growth may experience decline (Collier et al. 2011, Massa et al. 2009).

Seagrasses at these lower latitudes, including the VCR, may experience a bimodal form of growth where optimal temperatures for growth occur in the spring and fall and reductions in growth occur in mid-summer due to high temperature stress, as seen with *Zostera marina* (Kenworthy et al. 1982, Dennison 1987, Lee et al. 2005, Lee et al. 2007). With increased light attenuation through the water column, the optimal temperature for seagrass photosynthesis declines because of the high respiratory demands (Bulthuis 1987, Lee et al. 2007). During the summer at shallow water depths, temperatures are higher than at deeper depths which increases the amount of light needed for growth (Lee et al. 2005); however, because these plants are at shallower depths they may have enough light for growth even with high temperatures (Collier et al. 2011). Reductions in light during the summer months can further increase the amount of stress on seagrasses, reducing growth (Lee et al. 2007, Lee et al. 2005).

Chemical Stressors

Chemical stressors have a great impact on production with increasing summer water temperatures (Lee et al. 2007). As temperatures increase, microbial activity in the rhizosphere also increases and in sediments with high organic matter content, oxygen levels decline quickly (Goodman et al. 1995). Sulfide concentrations also increase with high summer temperatures due to anoxic conditions (Moore and Jarvis 2008). At temperatures greater than 30°C, eelgrass meristematic tissue becomes anoxic (Greve et al. 2003). Low oxygen content in these tissues is correlated to increases in sulfide in roots and rhizomes in many seagrass species (Borum et al. 2005, Pedersen et al. 2004). The phytotoxin sulfide has been found to negatively impact photosynthesis, aerobic metabolism, and growth and nutrient uptake of plants (Borum et al. 2005) by inhibiting cytochrome oxidase from functioning, causing the cell to die and breakdown (Pederson et al. 2004). This increases light requirements for seagrasses so photosynthesis can make-up for carbon lost through respiration caused by sulfide toxicity (Greve et al. 2003).

Excessive ammonium in the pore-water and water column can also increase light requirements for seagrass (van Katwijk et al. 1997). In anoxic areas, nitrification does not occur to decrease ammonium concentrations and decomposition aided by anoxic conditions increases ammonium concentrations (van Katwijk et al. 1997). Ammonium in the pore-water or water column can be taken up by roots and leaves passively (Villazan et al. 2013). In a low ammonium environment, the ammonium would be converted to amino acids immediately when entering the cell (Villazan et al. 2013); however, when concentrations are high, ammonium can enter the plant cell and uncouple photosynthetic electron transport inhibiting photosynthesis from occurring (van Katwijk et al. 1997, Villazan et al. 2013). When this occurs, seagrasses must increase light requirements in order to photosynthesize enough to oxygenate the rhizosphere to allow for nitrification to occur and to maintain a positive carbon balance when carbon demands increase from respiration (van Katwijk et al. 1997). However, in eutrophied areas it may be impossible to photosynthesize enough to maintain an oxygenated rhizosphere and balance carbon demands (Marba et al. 2006). Coastal eutrophication may be a cause of increased sulfide and ammonium concentrations in the pore-water (Marba et al. 2006). However, coastal eutrophication is not an issue in the VCR (Orth et al. 2012, www1.vcrlter.virginia.edu/home1/?q=data_wq). In temperate regions, the minimum depth for seagrass growth is often bound by temperature (McGlathery et al. 2012). In shallow areas, the water column is heated by incoming solar radiation creating an environment that may have increased temperatures compared to areas at deeper depths (Duarte 2002). Because of this, effects of nutrient stressors, such as the phytotoxins sulfide and ammonium, and increasing respiration may severely decrease production in these areas.

Motivation

In the Virginia coastal bays, the loss of eelgrass and the state change to a benthic algae dominated system (Orth & McGlathery 2012) resulted in the loss of many ecosystem services that seagrasses provide, such as habitat for many organisms including scallops, sediment deposition, and carbon and nitrogen sequestration (McGlathery et al. 2012). In the mid-1990s a natural patch of eelgrass was found in the coastal bays of the VCR-LTER site, which prompted a large restoration effort starting in the early 2000s (Orth et al. 2006a). Since 2001, restoration of *Z. marina* has resulted in over 17 km^2 of restored habitat (Orth et al. 2012) where water quality (total suspended solids, chlorophyll, dissolved nitrogen) is high and sediments are sandy. Long-term monitoring of restored sites and modeling of sediment suspension, light attenuation, temperature, and plant growth has shown that the maximum depth for *Z. marina* survival in these bays is 1.6 m MSL (mean sea level) and the minimum depth limit is 0.8 m MSL (Carr et al. 2010, McGlathery et al. 2012, Reynolds et al. 2012). However, variations in sediment characteristics (organic matter (OM), grain size, carbon, nitrogen, hydrogen sulfide, exchangeable ammonium) and water quality (dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorous (DIP), dissolved oxygen (DO), total suspended solids (TSS), chlorophyll a), as well as increasing summer sea surface temperatures may influence *Z. marina* light requirements and depth limits for survival and thus potential suitable habitat.

Research Questions

Chapter 1: How does variation in water quality, temperature, and sediment characteristics affect the maximum and minimum depths for Zostera marina L. growth?

In this chapter, I investigate the growth and productivity response of *Zostera marina* L. (eelgrass) to various sediment, water quality, and temperature conditions by 1) using known light and temperature thresholds and field data to find the maximum and minimum depths for *Z. marina* growth in two unrestored bays, Gargathy Bay and Magothy Bay, and one bay in the process of being restored, Hog Island Bay, in the VCR through spatial analysis, and 2) by transplanting *Z. marina* across a depth gradient from 0.4 m to 2.0 m mean sea level (MSL) to test the predictions from spatial analysis. Past models have attempted to find the maximum depth limit using point light and temperature data (Carr et al. 2010), but have not accounted for variations in sediment characteristics (grain size, organic matter content) and pore-water chemistry (sulfide, ammonium) which can affect the light requirements of *Z. marina* (Borum et al. 2005, van Katwijk et al. 1997). Because of this, it is important to understand the compounding effects these multiple stressors have on *Z. marina* light requirements and the maximum and minimum depth limits for eelgrass growth. Through a transplant experiment and spatial analysis, I show that the maximum depth limit of *Z. marina* in the Virginia coastal bays is dependent on the amount of fine sediment in a bay while the minimum depth limit is dependent on the interaction between light and temperature. The results of this study are to be submitted to Marine Ecology Progress Series.

Chapter 2: How do sediment characteristics and light attenuation affect Zostera marina L. productivity and optimal photosynthetic yield?

In this chapter, I attempt to separate the effects of sediment characteristics and light attenuation on *Zostera marina* L. (eelgrass) productivity and optimal photosynthetic yield in a controlled mesocosm experiment. In the previous chapter, I found the maximum and minimum depth limits for eelgrass growth over a sediment grain size and organic matter content gradient in the Virginia Coast Reserve. However, in the field it is difficult to separate the effects of light, temperature, and sediment characteristics on eelgrass growth. Because of this, I planted eelgrass in sediments from four different coastal bays in the VCR in mesocosms in order to control the light environment and separate effects of sediment characteristics and pore-water chemistry on eelgrass productivity and optimal quantum yield from the effects of light attenuation. In terms of the effect of sediment characteristics and pore-water chemistry on photosynthetic yield and productivity, the experiment was inconclusive because pore-water sulfide concentrations in sediments in the mesocosms were lower than expected. However, it did indicate that in a controlled environment where pore-water sulfides are generally low, sediment grain size and organic matter content have no impact on the efficiency of photosystem II or productivity of *Z. marina*. This result may have been different if pore-water chemistry gradient from the coastal bays could have been maintained. Our light treatment indicated that all plants in the dark treatment were light stressed in terms of optimal quantum yield, but there were no significant differences between sites. Because pore-water sulfide concentrations in sediments in the mesocosms differed from concentrations in the field, the experiment needs to be modified in order to separate the effects of sediment characteristics and light attenuation on seagrass photosynthesis and productivity. Modifications would include using a paired-flume mesocosm system and pairing the laboratory experiment with a field experiment. This would allow me to quantify transplant stress within the mesocosm system, control flushing of the sediments, and control for temperature. The results of this study are to be submitted to Marine Ecology Progress Series.

References

Biber, P. D., C. L. Gallegos, and W. J. Kenworthy. 2008. Calibration of a Bio-optical Model in the North River, North Carolina (Albemarle–Pamlico Sound): A Tool to Evaluate Water Quality Impacts on Seagrasses. *Estuaries and Coasts* **31**: 177 – 191.

Biber, P. D., W. J. Kenworthy, H. W. Paerl. 2009. Experimental analysis of the response and recovery of *Zostera marina* (L.) and *Halodule wrightii* (Ascher.) to repeated light-limitation stress. *Journal of Experimental Marine Biology and Ecology* **369**: 110 – 117.

Borum, J., O. Pedersen, T. M. Greve, T. A. Frankovich, J. C. Zieman, J. W. Fourqurean, C. J. Madden. 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology* **93**: 148 – 158.

Bryars, S., G. Collings, D. Miller. 2011. Nutrient exposure causes epiphytic changes and coincident declines in two temperate Australian seagrasses. *Marine Ecology Progress Series* **441**: 89 – 103.

Bulthuis, D. A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany* **27**: 27 – 40.

Carr, J., P. D'Odorico, K. McGlathery, P. Wiberg. 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. *Journal of Geophysical Research* **115**: doi:10.1029/2009JG001103.

Carr, J. A., P. D'Odorico, K. J. McGlathery, P. L. Wiberg. 2012. Modeling the effects of climate change on eelgrass stability and resilience: future scenarios and leading indicators of collapse. *Marine Ecology Progress Series* **448**: 289 – 301.

Christian, D., Y. P. Sheng. 2003. Relative influence of various water quality parameters on light attenuation in Indian River Lagoon. *Estuarine, Coastal, and Shelf Science* **57**: 961 – 971.

Cole, L. W., K. J. McGlathery. 2012. Nitrogen fixation in restored eelgrass meadows. *Marine Ecology Progress Series* **448**: 235 – 246.

Collier, C. J. S. Uthicke, M. Waycott. 2011. Thermal tolerance of two seagrass species at contrasting light levels: Implications for future distribution in the Great Barrier Reef. *Limnology and Oceanography* 56: 2200 – 2210.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'neill, J. Paruelo, R. G. Raskin, P. Sutton, M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. *Nature* **387**: 253 – 260.

Dennison, W. C. 1987. Effects of light on seagrass photosynthesis, growth, and depth distribution. *Aquatic Botany* **27**: 15 – 26.

Dennison, W. C., R. J. Orth, K. A. Moore, J. C. Stevenson, V. Carter, S. Kollar, P. W. Bergstrom, R. A. Batiuk. 1993. Assessing Water Quality with Submersed Aquatic Vegetation: Habitat requirements as barometers of Chesapeake Bay Health. *BioScience* **43**: 86 – 94.

Duarte, C. M. 1991. Seagrass depth limits. *Aquatic Botany* **40**: 363 – 377.

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

Gallegos, C. L., D. L. Correll, J. W. Pierce. 1990. Modeling spectral diffuse attenuation, absorption, and scattering coefficients in a turbid estuary. *Limnology and Oceanography* **35**: 1486 – 1502.

Greiner, J. T., K. J. McGlathery, J. Gunnell, B. A. McKee. 2013. Seagrass restoration enhances "Blue Carbon" sequestration in coastal waters. *PLoS ONE* **8**: e72469. doi:10.1371/journal.pone.0072469.

Greve, T. M., J. Borum, O. Pederson. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnology and Oceanography* **48**: 210 – 216.

Hansen, J. C. R. and M. A. Reidenbach. 2012. Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension. *Marine Ecology Progress Series* **448**: 271 – 287.

Hansen, J. C. R., M. and M. A. Reidenbach. 2013. Seasonal growth and senescence of a *Zostera marina* seagrass meadow alters wave-dominated flow and sediment suspension within a coastal bay. *Estuaries and Coasts* **36**: 1099 – 1114.

Kemp, M. W., R. Batiuk, R. Bartleson, P. Bergstrom, V. Carter, C. L. Gallegos, W. Hunley, L. Karrh, E. W. Koch, J. M. Landwehr, K. A. Moore, L. Murray, M. Naylor, N. B. Rybickr, J. C. Stevenson, D. L. Wilcox. 2004. Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: water quality, light regime, and physical – chemical factors. *Estuaries* **27**: 363 – 377.

Kenworthy, W. J., J. C. Zieman, G. W. Thayer. 1982. Evidence of the influence of seagrasses on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA). *Oecologia* **54**: 152 – 158.

Krause-Jenson, D., J. Cartensen, L. Nielsen, T. Dalsgaard, P. B. Christensen, H. Fossing, M. B. Rasmussen. 2011. Sea bottom characteristics affect depth limits of eelgrass *Zostera marina*. *Marine Ecology Progress Series* **425**: 91 – 102.

Lawson, S. E., P. L. Wiberg, K. J. McGlathery, D. C. Fugate. 2007. Wind-driven sediment suspension controls light availability in a shallow coastal lagoon. *Estuaries and Coasts* **30**: 102 – 112.

Lawson, S. E., K. J. McGlathery, P. L. Wiberg. 2012. Enhancement of sediment suspension and nutrient flux by benthic macrophytes at low biomass. *Marine Ecology Progress Series* **448**: 259 – 270.

Lee, K. S., S. R. Park, J. B. Kim. 2005. Production dynamics of the eelgrass, *Zostera marina* in two bay systems on the south coast of the Korean peninsula. *Marine Biology* **147**: 1091 – 1108.

Lee, K. S., S. R. Park, Y. K. Kim. 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology* **350**: 144 – 175.

Massa, S. I., S. Arnaud-Haond, G. A. Pearson, E. A. Serrao. 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal). *Hydrobiologia* **619**: 192 – 201.

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient – enriched coastal waters. *Journal of Phycology* **37**: 453 – 456.

McGlathery K. J., K. Sundback, I. C. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Marine Ecology Progress Series* **348**: 1 – 18.

McGlathery, K. J., L. K.Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Marine Ecology Progress Series* **448**: 209 – 221.

Moore, K. A., J. C. Jarvis. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: Implications for long-term persistence. *Journal of Coastal Research* **55**: 135 – 147.

Moore, K. A., E. C. Shields, D. B. Parrish, R. J. Orth. 2012. Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures. *Marine Ecology Progress Series* **448**: 247 – 258.

Najjar, R. G., H. A. Walker, P. J. Anderson, E. J. Barron, R. J. Bord, J. R. Gibson, V. S. Kennedy, C. G. Knight, J. P. Megonigal, R. E. O'Connor, C. D. Polsky, N. P. Psuty, B. A. Richards, L. G. Sorenson, E. M. Steele, R. S. Swanson. 2000. The potential impacts of climate change on the mid-Atlantic coastal region. *Climate Research* **14**: 219 – 233.

Olsen, Y. S., M. Sanchez-Camacho, N. Marba, C. M. Duarte. 2012. Mediterraean seagrass growth and demography responses to experimental warming. *Estuaries and Coasts* **35**: 1205 – 1213.

Orth R. J., J. R. Fishman, M. C. Harwell, S. R. Marion. 2003. Seed-density effects on germination and initial seedling establishment in eelgrass *Zostera marina* in the Chesapeake Bay region. *Marine Ecology Progress Series* **250**: 71 – 79.

Orth R.J., M. L. Luckenbach, S. R. Marion, K. A. Moore, D. J. Wilcox. 2006a. Seagrass recovery in the Delmarva coastal bays, USA. *Aquatic Botany* **84**: 26−36.

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. Williams. 2006b. A Global Crisis for Seagrass Ecosystems. *BioScience* **56**: 987 – 996.

Orth, R. J., S. R. Marion, K. A. Moore, D. J. Wilcox. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of the Mid-Atlantic coast of the USA: Challenges in conservation and restoration. *Estuaries and Coasts* **33**: 139 – 150.

Orth R. J., K. J. McGlathery. 2012. Eelgrass recovery in the coastal bays of the Virginia Coast Reserve, USA. *Marine Ecology Progress Series* **448**: 173 – 176.

Orth R. J., K. A. Moore, S. R. Marion, D. L. Wilcox. 2012. Seed addition facilitates eelgrass recovery in a coastal bay system. *Marine Ecology Progress Series* **448**: 177 – 195.

Pederson, O., T. Binzer, J. Borum. 2004. Sulfide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell and Environment* **27**: 595 – 602.

Pedersen, T. M., C. L. Gallegos, S. L. Nielsen. 2012. Influence of near-bottom re-suspended sediment on benthic light availability. *Estuarine, Coastal and Shelf Science* **106**: 93 – 101.

Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. In: McRoy CP, Helfferich C (eds) Seagrass ecosystems. Marcel Dekker, New York, NY, p 1−51.

Reynolds, L. K., K. J. McGlathery, M. Waycott. 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. *PLoSONE* **7**: e38397. doi:10.1371/journal.pone.0038397.

Short, F. T., B. Polidoro, S. R. Livingstone, K. E. Carpenter, S. Bandeira, J. S. Bujang, H. P. Calumpong, T. J. B. Carruthers, R. G. Coles, W. C. Dennison, P. L. A. Erftemeijer, M. D. Fortes, A. S. Freeman, T. G. Jagtap, A. H. M. Kamal, G. A. Kendrick, W. J. Kenworthy, Y. A. La Nafie, I. M. Nasution, R. J. Orth, A. Prathep, J. C. Sanciangco, B. van Tussenbroek, S. C. Vergara, M. Waycott, J. C. Zieman. 2011. Extinction risk assessment of the world’s seagrass species. *Biological Conservation* **144**: 1961 – 1971.

van Katwijk, M. M., L. H. T. Vergeer, G. H. W. Schmitz, J. G. M. Roelofs. 1997. Ammonium toxicity in eelgrass *Zostera marina*. *Marine Ecology Progress Series* **157**: 159 – 173.

Villazan, B. M. F. Pederson, F. G. Brun, J. J. Vergara. 2013. Elevated ammonium concentrations and low light form a dangerous synergy for eelgrass *Zostera marina*. *Marine Ecology Progress Series* **493**: 141 – 154.

Wall, C. C., B. J. Peterson, C. J. Gobler. 2011. The growth of estuarine resources (*Zostera marina, Mercenaria mercenaria, Crassostrea virginica, Argopecten irradians, Cyprinodon variegatus*) in response to nutrient loading and enhanced suspension feeding by adult shellfish. *Estuaries and Coasts* **34**: 1262 – 1277.

Ward, L. G., W. M. Kemp, W. R. Boynton. 1984. The influence of waves and seagrass communities on suspended particulates in an estuarine embayment. *Marine Geology* **59**: 85 – 103.

Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, Jr., A R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Publications of the National Academy of Sciences* **106**: 12377 – 12381.

Widdows, J. N. D. Pope, M. D. Brinsley, H. Asmus, R. M. Asmus. 2008. Effects of seagrass beds (*Zostera noltii* an d *Z. marina*) on near-bed hydrodynamics and sediment resuspension. *Marine Ecology Progress Series* **358**: 125 – 236.

Winters, G., P. Nelle, B. Fricke, G. Rauch, T. B. H. Reusch. 2011. Effects of a simulated heat wave on photophysiology and gene expression of high and low-latitude populations of *Zostera marina*. *Marine Ecology Progress Series* **435**: 83 – 95.

Chapter 1: Changes in the maximum and minimum depth limits for *Zostera marina L.* (eelgrass) growth across an environmental gradient in the Virginia Coastal Bays

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Abstract

Restoration of eelgrass (*Zostera marina*) has been successful in the Virginia coastal bays, where >17 km^2 of eelgrass has been restored since 2001. In the regions of the bays where restoration efforts have been focused, water quality (as quantified by total suspended solids, chlorophyll, dissolved nitrogen) is high and sediments are sandy. Long-term monitoring of restored sites and modeling of sediment suspension and light attenuation have shown that the minimum depth for *Z. marina* survival in these bays is 0.8 m mean sea level (MSL) and the maximum depth is 1.6 m MSL. Understanding the effects of varying environmental conditions, such as sediment, water quality, and temperature on minimum and maximum depth limits for seagrass is important for extending restoration efforts to other regions in the Virginia coastal bays. High temperatures in shallow areas, increased light attenuation at deeper depths, high sulfide concentrations, and high organic matter content in fine-grained sediments act as stressors in eelgrass meadows and may reduce potential areal coverage. I quantified changes in light attenuation and temperature from depths of 0.4 m to 2.0 m MSL (1.8 m MSL in Gargathy Bay) in two unrestored bays, one with fine-grained sediment (Gargathy Bay) and the other with coarser sediment, (Magothy Bay), and compared these with sites in a centrally located bay in the process of being restored (Hog Island Bay) in order to model the potential distribution of *Z. marina* in each of these bays. I then tested the model by comparing it to transplanted eelgrass survival in the unrestored bays. I found significant differences in the potential distribution for *Z. marina* growth in each bay, with Gargathy Bay being uninhabitable due to poor conditions that may increase the light compensation point and Magothy Bay having a slightly shallower minimum depth limit than Hog Island Bay due to interactions between light and temperature, but

sharing the same maximum depth limit. With this information, better more informed models for potential seagrass distribution in the Virginia Coastal Bays can be built.

Introduction

Seagrass meadows are declining worldwide due to coastal development, increasing human population, increased sediment and nutrient loading (Orth et al. 2006b), and the effects of climate change (Orth et al. 2006b, Waycott et al. 2009). With this loss, comes the loss of ecosystem services seagrasses provide to coastal bays and estuaries, such as habitat and food for marine organisms, improved water quality and clarity, sediment stabilization, carbon sequestration, and provision of organic matter and nutrients to the ocean (Orth et al. 2006b, Waycott et al. 2009, Short et al. 2011). It has been found that restoration of seagrass meadows can bring back lost ecosystem services to coastal bays and estuaries (McGlathery et al. 2012). However, there has been uncertainty over what environmental conditions seagrass restoration will be most successful as there are many feedbacks between light availability, water quality, sediment characteristics, and temperature (Benson et al. 2013, Carr et al. 2010, van der Heide et al. 2009).

Seagrasses require from 4% to 30% incident light at the sediment surface for carbon fixation by photosynthesis to compensate for losses from respiration (Dennison et al. 1993). The maximum depth limit for seagrasses is constrained by this minimum light requirement due to increased light attenuation with depth (Duarte 1991). At the minimum depth limit, high temperatures increase respiration in seagrasses; therefore, light requirements also increase to maintain a positive carbon balance (Lee et al. 2007). Other stressors related to pore-water, such as high sulfide and low oxygen concentrations caused by high organic matter content, as well as high percent carbon and percent nitrogen which are related to organic content, and high exchangeable ammonium concentrations, can also increase light requirements (Gustafsson and Bostrum 2013, Holmer and Bondgaard 2001, Goodman et al. 1995). Microbes in the sediment decompose organic matter, reducing the amount of oxygen in the rhizosphere and increasing sulfide concentrations (Goodman et al. 1995). When oxygen concentrations in the rhizosphere and water column are low the internal partial pressure of O_2 (pO_2) in seagrasses declines allowing for sulfide intrusion into the plant inhibiting re-oxidation of sulfides to sulfate by bacteria present in the sediment (Pederson et al. 2004). Sulfide within the plant then inhibits cytochrome oxidase from functioning causing the cell to die and breakdown (Pederson et al. 2004). Higher light requirements would ensure enough photosynthesis is occurring to keep internal pO_2 in the seagrass high enough to prevent sulfide intrusion (Greve et al. 2003). When oxygen concentration in the rhizosphere is low, nitrification does not occur to decrease ammonium concentrations (van Katwijk et al. 1997). Ammonium in the pore-water can be taken up by roots passively (Villazan et al. 2013). Usually this ammonium is converted to amino acids as soon as it enters the cell (Villazan et al. 2013); however, when ammonium enters plant cells and is not immediately converted to amino acids due to high concentrations, it uncouples photosynthetic electron transport inhibiting photosynthesis from occurring (van Katwijk et al. 1997, Villazan et al. 2013). Several factors influence water column light attenuation, including water quality parameters such as total suspended solids, water column chlorophyll and nutrient concentrations, (McGlathery et al. 2007), macro-algal growth, (McGlathery 2001) and sediment suspension from wave action or storminess which contributes to the concentration of total suspended solids in the water column (Biber et al. 2009). These parameters cause light to decline with depth through the water column decreasing the maximum depth limit for seagrass growth as the light attenuation coefficient (K_d) increases (Duarte 1991, Dennison et al. 1993).

Sediment suspension can have a large effect on the light environment in seagrass beds and sediment grain size is an important factor when considering the amount of light attenuation caused by these sediment suspension events paired with wind and current conditions (Krause-Jensen et al. 2011, Pedersen et al. 2012). Seagrass meadows are usually areas with high sediment deposition and stability (Hansen and Reidenbach 2012, Lawson et al. 2012) which contributes to accumulation of finer sediment in seagrass beds compared to bare sediments (McGlathery et al. 2012). However, muddy sediment in seagrass beds can be less stable compared to sandy sediment (Widdows et al. 2008) and suspended particles have been found to comprise a greater percentage clay and silt than sand (Pedersen et al. 2012). Sediment suspension can also increase in seagrass beds depending on the height of the water column: if seagrasses occupy the entire water column, sediment deposition increases because current velocities are reduced more than in areas where seagrass meadow height does not occupy the entire water column (Ward et al. 1984). Increasing seagrass density in a meadow has been shown to help reduce flow within the canopy and therefore bed shear stress and sediment resuspension (Widdows et al. 2008). This has also been shown to occur in the restored *Zostera marina* (L.) meadows of the Virginia coastal bays (Lawson et al. 2012, Hansen and Reidenbach 2013).

Most previous studies related to the light environment of seagrass beds have considered established meadows with high seagrass densities. It remains unclear what effects sediment properties combined with other stressors such as water quality, pore-water properties, and temperature have on the depth limits for newly established seagrasses. This is an important consideration in the likely success of seagrass restoration efforts. The objective of this study was to develop a better understanding of minimum light requirements and maximum temperature requirements of newly established seagrass to help identify locations where restored seagrass meadows are likely to be successful and sustainable in the Virginia Coastal Bays. To do this I determined how differences in sediment and water quality characteristics affected light attenuation across an environmental gradient of grain size and organic matter content, mapped the predicted range of seagrass growth based on these environmental parameters, and transplanted eelgrass across this depth range to test the modeled predictions. In this study, I looked at three bays: unrestored Gargathy Bay with fine sediment grain sizes, unrestored Magothy Bay with coarser sediment grain sizes, and Hog Island Bay, in the process of being restored, with intermediate sediment grain sizes. Because of these differences in grain size, I expected differences in the maximum depth for eelgrass growth due to increased light attenuation in areas with fine sediment because of increased sediment suspension with fine sediments and decreased light attenuation in areas with coarser sediment.

Methods

Study Sites

This study was carried out in the shallow coastal bays of the Virginia Coast Reserve - Long Term Ecological Research Site (VCR-LTER) on the Eastern Shore of Virginia (Figure 1.1). The coastal bays are bounded by a barrier island system to the east and the mainland portion of the Delmarva Peninsula to the west with narrow inlets for exchange with the Atlantic Ocean. These bays are shallow with 50% of the area <1 m relative to mean low water and a tidal range of 1.2 to 1.3 m (McGlathery et al. 2012). Most of the bay bottom in the area is dominated by micro-and macro-algae. However, in four of the southern bays, South, Hog Island, Spider Crab, and Cobb Bays, >1700 hectares of eelgrass (*Zostera marina*) have been restored since 2001 (Orth et al. 2012).

This study focused on two unrestored bays within the VCR, Gargathy Bay (WGS 84: 450992.3 easting, 4179866 northing) and Magothy Bay (WGS 84: 418923 easting, 4113632 northing), and a region in the process of being restored with seagrass within Hog Island Bay

(WGS 84: 435467 easting, 4140227 northing) (Figure 1.1). In Hog Island Bay, the minimum depth limit for eelgrass survival under current conditions was 0.8 m mean sea level (MSL) and the maximum depth limit was 1.6 m MSL (McGlathery et al. 2012, Reynolds et al. 2012). The restoration sites, Hog Island Bay and South Bay had similar bathymetry, sediment and water quality, and hydrologic characteristics such as current speeds (McGlathery et al. 2012). However, Gargathy Bay and Magothy Bay, because of differing bathymetry from Hog Island Bay and South Bay, were expected to have differing water and sediment quality characteristics.

Three sampling transects were established in Gargathy Bay (WGS 84: GA: 4140227 easting, 4180109 northing; GB: 451155 easting, 4179905 northing; GC: 450992 easting, 4179866 northing), Magothy Bay (MA: 419060 easting, 4112585 northing; MB: 418923 easting, 4113632 northing; MC: 419238 easting, 4115681 northing) and Hog Island Bay (Table 1.1) (Figure 1.2). In Gargathy Bay, all transects were located near the eastern side of the bay. Transects were placed from the shallow area near the center of the bay toward a channel closer to the eastern edge of the bay. The depth gradient for each of the transects bracketed the minimum (0.6 m MSL) and maximum (1.6 m MSL) depth limits in Hog Island Bay and ranged from 0.4 m to 1.8 m MSL and were each about 30 m in length to test how these limits vary. Because Gargathy Bay is very small and shallow with >50% of the bay at depths <1 m MSL, transects were located in relatively close proximity with about 150 m between transects. In Magothy Bay, all transects were located on the eastern edge of the bay along the western edge of Mockhorn Island. Transects ran over a depth gradient of 0.4 m MSL closest to Mockhorn Island to 2.0 m MSL approaching the channel closer to the center of the bay to test how the depth limit varies from Hog Island Bay. Distance between transects in this bay varied from 800 m between transects MA and MB to 3.5 km between MB and MC. In Hog Island Bay, all transects were located on the eastern side of the bay near Hog Island and ran from 0.4 m MSL near the shallow spit on the north end of the bay to 2.0 m MSL to the south (Figure 1.2).

Water Quality

Three 2L water samples were collected randomly at the midpoint of each transect monthly from May to August 2012 and August 2013 and were used to determine concentrations of water column chlorophyll a, nutrients, and total suspended solids. Dissolved oxygen concentration was measured during August 2012 at the midpoint of each transect. Three 1200 cm^3 (15 cm depth, 10 cm diameter) sediment cores were collected from the midpoint of each transect with a minimum distance of 1 m between cores and used for pore-water sulfide and exchangeable ammonium analysis.

Water column chlorophyll a

Water column chlorophyll a concentrations were determined using the standard 90% acetone spectrophotometric technique (Holm-Hansen et al. 1965). 400 mL of sample was filtered through a 25 µm glass fiber filter (Whatmann GF/C) and 1 mL of $MgCO_3$ slurry was added to the filter at the completion of water filtration. The filter was then placed in an aluminum foil envelope and was kept in the freezer until further analysis. The filters were ground in 8 mL of 90% acetone, placed in a foil-covered Nalgene test tube and placed in the freezer overnight for extraction (Holm-Hansen et al. 1965). Chlorophyll samples were shaken for re-suspension and centrifuged for 5 minutes before absorbance was read at 665 nm and 750 nm. HCl was used to acidify each sample and the absorbance was read at 665 nm and 750 nm.

Water column chlorophyll a concentrations were calculated using the following equation:

$$Chl\ a = \frac{26.7(665_o - 665_a) \times v}{V \times l}$$

Where 665_o = 665 – (750 – blank value) before acidification, 665_a = 665 – (750 – blank value) after acidification, v is volume of extract in mL, V is the volume of water filtered in liters, and l is the path-length of the cuvette.

Water column nutrients

Water column nutrient concentrations were determined by filtering two 30 mL sub-samples of water from each sample through a Whatman puradisc 25 µm syringe filter into separate whirl-pak sample bags. Samples were frozen for later chemical analysis on a Lachat Quik Chem Auto-analyzer using standard methods for total dissolved nitrogen and phosphate, nitrate and nitrite, and ammonium (Environmental Protection Agency 1983).

Total suspended solids (TSS)

Total suspended solid concentrations were determined using a 100 mL subsample from each 2L Nalgene bottle using standard filtration, drying, and combustion methods (Standard Methods 1993).

Water column oxygen

Water column oxygen concentrations were measured using Precision Measurement Engineering miniDOT dissolved oxygen temperature loggers. Sample interval was set to 15 minutes for one week monthly at the most central transect in each bay, MB and GB, near the midpoint of each transect, at 1 m MSL, with the loggers set 20 cm above the sediment surface.

Pore-water sulfides

Pore-water was extracted at a depth of 8 cm from the sediments in the lab using an anoxic pore-water probe and methods from Berg and McGlathery (2001). After 1.5 mL of pore-water was extracted, pore-water was extruded into a vacutainer with 1.5 mL of Zinc Acetate solution and a subsample was diluted depending on the amount of precipitate in the sample. The diluted sample was vortexed before dye was added. Sulfide concentrations were determined after incubation for 30 minutes using the spectrophotometric Cline method (Cline 1969). If concentrations exceeded the linear range of absorbance, a subsample of the original pore-water sample was diluted further.

Exchangeable ammonium

Exchangeable ammonium concentrations were determined using standard methods on a Lachat Quik Chem Auto-analyzer (Environmental Protection Agency 1983). A 60cc syringe was used to collect sediment to a depth of 5cm from the large sediment cores. Samples were homogenized, placed into 20 mL of KCl, and shaken on a shaker table for extraction.

Light and temperature profiles

Temperature and relative light profiles were obtained using HOBO® light and temperature loggers for two weeks every month from May to August 2012 and from June 13 to August 7 2013. HOBO®s were placed every 0.2 m depth change from 0.4 m to 2.0 m MSL, 1.8 m MSL in Gargathy, along each transect at 20 cm above the sediment surface. Light was measured at every 0.2 m depth interval from 0.4 m to 2.0 m MSL (1.8 m MSL in Gargathy), along each transect 20 cm above the sediment surface using Odyssey Photosynthetic Active Radiation Loggers and was compared to a sensor above the water surface in order to calculate % incident light at the sediment surface. Instantaneous light profiles were measured in photosynthetically active radiation (PAR) which was obtained using a Li-Cor Spherical Quantum Sensor. In order to compare relative light and PAR measurements, the HOBO®s and Spherical Quantum Sensor and Odyssey Sensor and Spherical Quantum Sensor were calibrated using methods from Long et al. (2012). The light attenuation coefficient (K_d) was calculated using the Lambert-Beer Equation:

$I_z = I_o * e^{-Kd * Z}$

where I_z is light at depth of measurement, I_o is light at water surface, and Z is depth (Lee et al. 2007, Dennison et al. 1993). Incident light at the sediment surface was calculated using the formula for sediment surface irradiance (SI):

$SI = ((I_z)/I_o) * 100$

where I_z is PAR at the sediment surface and I_o is PAR at the water surface (Dennison et al. 1993).

Mapping potential eelgrass distribution

Light attenuation and temperature data were combined to create maps of potential eelgrass distribution using ArcMap 10 in Gargathy Bay, Magothy Bay, and Hog Island Bay. Bathymetry data for Gargathy Bay, Magothy Bay, and Hog Island Bay were converted from raster to point maps and mean high water was converted to mean sea level using a calculator tool. Temperature and light data were joined to the bathymetry point data and specific temperature and light parameters were selected. For temperature, areas were separated based on percent of time over 28°C. Areas selected for the light layer had greater than 20% IL at the sediment surface during summers 2012 and 2013. Once selected, the data was exported to new layers and point maps were converted to raster in order to create spatial distribution maps.

The resulting maps were compared to the distribution found during studies of the eelgrass beds in the process of being restored in Hog Island Bay (McGlathery et al. 2012, Reynolds et al. 2012). Percent of time over 28°C from summer 2012 and 2013 was compared to transplant data from Gargathy and Magothy and distribution data in Hog Island Bay in order to find the maximum temperature threshold for eelgrass in the Virginia Coastal Bays. The maximum depth limit was based on the percentage of incident light at the sediment surface, with areas receiving greater than 20% incident light considered suitable for eelgrass growth. The distribution map in Hog Island Bay created using these methods was also compared to a map created mapping the distribution of eelgrass based on an equation for light compensation point in Hog Island Bay (Rheuban et al. in press).

Sediment Characteristics

Particle size analysis

Bed sediment size distribution in each bay was measured using a Beckman Coulter LS(TM) 13320 Laser Diffraction Particle Size Analyzer (PSA) which can detect particle sizes between 0.4 µm and 2000 µm. A 60cc syringe was used to collect sediment to a depth of 5 cm every 0.2 m change in depth along each transect from 0.4 m to 2.0 m MSL. Each sample was extruded from the syringe and sieved to remove any particles larger than 2000 µm. Bleach was added to each sample to remove any organic matter before standard analysis with the PSA (Beckman Coulter 2011).

Organic matter

Sediment organic matter in bottom sediment in each bay was analyzed using the loss on ignition method for organic content analysis (McGlathery et al. 2012). A 60cc syringe was used to collect sediment to a depth of 5 cm at every 0.2 m change in depth along each transect. Sediments were dried at 60°C for 48 hours and were combusted in a 500°C muffle furnace for 6 hours. Between collection and analysis, each sample was extruded into a whirl-pak and frozen.

Porosity

Porosity was analyzed using standard methods for porosity analysis (Krause-Jenson et al. 2011). The same sediment samples used for organic content were used for porosity. Porosity was calculated by finding the volume of water lost after drying each sediment core. In order to ensure that water did not drain out of the samples during sampling, samples were covered after being taken out of the sediment and immediately placed into bags.

Sediment Carbon and Nitrogen content

Sediment Carbon (C) and Nitrogen (N) content was analyzed using standard methods on a Carlo Erba elemental analyzer (McGlathery et al. 2012). A 60cc syringe was used to collect sediment to a depth of 5 cm every 0.2 m change in depth along each transect. Each sample was dried for a minimum of 48 hours at 60°C or until completely dry, ground, and placed in aluminum tins for analysis on the Carlo Erba elemental analyzer.

Transplant experiment

Eelgrass was collected from South Bay and transplanted along the depth gradient from 0.4 m to 2.0 m MSL in Magothy Bay and 0.4 m to 1.8 m MSL (maximum depth) in Gargathy Bay using the staple method (Davis and Short 1997) during fall 2012. This depth range was used to extend the known depth range in Hog Island Bay (0.8 m - 1.6 m MSL) in order to capture variability among sites. Plants were processed to check for reproductive shoots and standardized so each plant had 3 nodes on the rhizome, a rhizome at least 2 cm long, three leaves, and one shoot. The plants were then bundled into groups of three and groups of two with a paper coated twist tie to create each planting unit. A quadrat, 0.2 m by 1.0 m with string used to mark off ten spaces equal in size, was used as a planting frame. The quadrat was laid down at each depth interval along each transect in Gargathy Bay and Magothy Bay. One three plant and one two plant planting unit were planted in each space in the quadrat with a total of 50 plants and 20 planting units per depth (Figure 1.3). Two separate planting unit sizes were used in order to prevent overcrowding in each section of the quadrat while still maintaining five plants per space.

Three control plots were planted in South Bay using the same methods used in Gargathy Bay and Magothy Bay to insure the staple method worked properly.

Shoots were counted once monthly during June, July, and August 2013. Productivity was measured in August 2013 at the termination of the experiment using methods from Zieman 1974.

Statistical Analyses

For all sediment and water quality analyses, differences between sites were found using an ANOVA using Statistical Analysis System (SAS) version 9.2 software. Data that were not normally distributed were log transformed or square root transformed to meet the normality assumption. A Tukey post-hoc comparison test was used to determine which sites differed from each other. Linear regression analysis was used to test the relationship between depth and survival as well as grain size and survival. A principal components analysis was performed to test the relationship between transplant survival and depth, grain size, temperature, and light attenuation.

Results

Site Characteristics: sediment characteristics, pore-water quality, and water quality

Gargathy Bay had significantly lower water, sediment, and pore-water quality than Hog Island Bay and Magothy Bay with respect to water column chlorophyll a concentration, total suspended solids, water column ammonium concentration, orthophosphate concentration, nitrate and nitrite concentration, organic matter content, sediment grain size, pore-water sulfide concentration, and exchangeable ammonium concentration. Magothy Bay was similar to Hog Island Bay in terms of water, sediment, and pore-water parameters (organic matter, exchangeable ammonium concentration, nitrate and nitrite concentration, total dissolved nitrogen concentration) except pore-water sulfide concentration, water column chlorophyll a concentration, and water column ammonium concentration.

Sediment analyses confirmed that Gargathy Bay had a higher percentage of clay (diameter ≤ 2 μm) and silt (diameter between 2 μm and 63 μm) in areas sampled than areas sampled in Hog Island Bay and Magothy Bay (Figure 1.4) ($p < 0.05$) with an average of 7.0 +/- 0.4 % clay, 3.8 +/- 0.7 % clay, and 3.5 +/- 0.5 % clay respectively and 45.5 +/- 2.1 % silt, 16.1 +/- 3.4 % silt, and 15.1 +/- 2.7 % silt respectively. All sites differed significantly in fine sand (diameter between 63 μm and 200 μm) content (Figure 1.4) ($p < 0.05$) with an average of 37.1 +/- 2.5 % for sites sampled in Gargathy Bay, 78.8 +/- 4.0 % for sites sampled in Hog Island Bay, and 16.9 +/- 2.4 % for sites sampled in Magothy Bay. Sites in Magothy Bay had a higher percentage of medium sand (diameter between 200 μm and 630 μm) than sites in Hog Island Bay and Gargathy Bay (Figure 1.4) ($p < 0.05$) with an average of 62.8 +/- 4.5 %, 1.3 +/- 0.2 %, and 7.7 +/- 0.9 % respectively. Coarse sand (> 630 μm) content did not differ significantly among bays ($p > 0.05$). Sites sampled in Gargathy Bay contained significantly higher percent organic matter than sites sampled in Hog Island Bay and Magothy Bay ($p < 0.05$) (Figure 1.5a) (Table 1.2). As expected for sediments of fine particle size and high organic matter, bottom sediments in Gargathy Bay had significantly higher porosity than sediments in Hog Island Bay or Magothy Bay ($p < 0.05$) (Figure 1.5b) (Table 1.2). Similarly, the coarser sediments of Magothy Bay had a significantly lower porosity than in Hog Island Bay ($p < 0.05$) (Figure 1.5b). C and N contents in the sediment were significantly higher in Gargathy Bay and Magothy Bay than in Hog Island Bay ($p < 0.05$) (Table 1.2) (Figure 1.5c and 1.5d). C and N contents in Gargathy Bay, where sediment organic content was also found to be high, were also significantly higher than in Magothy Bay ($p < 0.05$) (Table 1.2) (Figure 1.5c and 1.5d).

Pore-water parameters in Gargathy Bay reflected what would be expected with fine sediment grain size and high percent organic matter, with pore-water sulfide and exchangeable ammonium concentrations significantly higher than those seen in both Hog Island Bay and Magothy Bay ($p < 0.05$) (Figure 1.6) (Table 1.2). Pore-water sulfide concentrations in Magothy Bay were also significantly higher than in Hog Island Bay ($p < 0.05$) (Figure 1.6a) (Table 1.2), which was not expected due to the coarser sediment. Exchangeable ammonium concentrations in Magothy Bay were not significantly different from concentrations in Hog Island Bay (Figure 1.6b) (Table 1.2).

Water quality followed similar patterns to pore-water concentrations in the coastal bays confirming that Gargathy Bay had lower water quality than that of Hog Island Bay and Magothy Bay. However, as seen with pore-water quality, Magothy Bay did not have higher water quality than Hog Island Bay. During the summer of 2012, Gargathy Bay had significantly higher water column chlorophyll a concentrations than both Hog Island Bay and Magothy Bay ($p < 0.05$) (Figure 1.7a) (Table 1.3). Magothy Bay had significantly higher water column chlorophyll a concentrations than Hog Island Bay ($p < 0.05$) (Figure 1.7a) (Table 1.3). Water column nutrient concentrations varied depending on the type of nutrient analyzed. Gargathy Bay had significantly higher concentrations of water column ammonium, ortho-phosphate, and total dissolved nitrogen (TDN) than Hog Island Bay and Magothy Bay ($p < 0.05$) (Table 1.3) (Figure 1.7). However, there was no statistical difference between any of the bays for nitrate and nitrite concentrations (Table 1.3) (Figure 1.7e). Magothy Bay had a significantly lower concentration of water column ammonium than Hog Island Bay ($p < 0.05$) (Table 1.3) (Figure 1.7b). However, there was no statistical difference between Magothy Bay and Hog Island Bay for ortho-phosphate and TDN (Table 1.3) (Figure 1.7c and 1.7d). Gargathy Bay had significantly higher total suspended solid

(TSS) concentrations than Hog Island Bay ($p < 0.05$) (Table 1.3) (Figure 1.7f). However, Magothy Bay did not differ significantly from Hog Island Bay in terms of TSS concentration (Table 1.3) (Figure 1.7f). Oxygen concentrations in Gargathy Bay, Hog Island Bay, and Magothy Bay were not significantly different (Table 1.2).

Even though there were very clear patterns for water quality during the summer of 2012, these patterns were not always observed during summer 2013 with water column chlorophyll a, total dissolved nitrogen (TDN), and total suspended solids (TSS) differing between years in Gargathy Bay, water column chlorophyll a, ammonium, and ortho-phosphate differing between years in Hog Island Bay, and water column chlorophyll a, ortho-phosphate, and TDN differing between years in Magothy Bay (Table 1.4). During this time period, both Gargathy and Magothy Bays had significantly higher water column chlorophyll a concentrations than Hog Island Bay ($p < 0.05$) but did not differ from one another (Table 1.5) (Figure 1.8). Concentrations of nutrients (ammonium, ortho-phosphate, nitrate+nitrite, TDN) and TSS did not differ among bays (Table 1.5).

Light and Temperature Profiles

Both light and temperature were attenuated with depth in each bay (Gargathy: light $p = 0.0014$, $R^2 = 0.8134$; temperature $p = 0.0079$, $R^2 = 0.6705$; Magothy: light $p = 0.0007$, $R^2 = 0.7981$; temperature $p = 0.0035$, $R^2 = 0.6875$; Hog Island: light $p = 0.0077$, $R^2 = 0.6130$; temperature $p < 0.0001$, $R^2 = 0.9067$). During summer 2012 and 2013, temperatures in Gargathy Bay were significantly cooler than temperatures in Hog Island and Magothy Bays ($p < 0.05$) and temperatures in Magothy Bay were significantly warmer than those in Hog Island Bay ($p < 0.05$). The maximum temperature found in each bay did not differ significantly, however the amount of time over 28°C was different among bays. In the shallow areas of Gargathy Bay, where high temperature limitation would be expected to occur, temperatures exceeded 28°C for only 11.5% of the summer. In Hog Island Bay, temperatures in the shallow areas (< 0.8 m MSL) exceeded 28°C for 29% of the summer. Time over 28°C was even higher in the shallow areas of Magothy Bay with high temperatures for 33% of the summer at the shallowest site, 0.4 m MSL.

Even though light was attenuated with depth in each bay, the light extinction coefficient was different among bays and followed patterns in grain size as expected. In Gargathy Bay, the area with the finest sediment, the light extinction coefficient was significantly greater than that seen in both Hog Island Bay and Magothy Bay ($K_d = 3.468\ m^{-1}$, $K_d = 1.685\ m^{-1}$, $K_d = 2.163\ m^{-1}$ respectively) ($p < 0.05$). However, the light extinction coefficients in Hog Island Bay and Magothy Bay were not significantly different ($p > 0.05$). This indicates that light was attenuated more quickly in Gargathy Bay than in Hog Island and Magothy Bays. This pattern was repeated when looking at % incident light at the sediment surface. In Gargathy Bay, the amount of light at the sediment surface fell below the threshold for eelgrass growth, 20% incident light, at 0.8 m MSL. In Hog Island Bay and Magothy Bay, this threshold was much deeper at 1.6 m MSL (Figure 1.9).

Environmental Factors Affecting Density

The principal component analysis followed by a multiple regression revealed that principal component 1 explained the most variance for density ($p > 0.0002$) (Table 1.6). This component had high loadings for clay, silt, medium sand, and temperature (Table 1.7) meaning that fine grain size and temperature had the most effect on density.

Maximum and Minimum Depths for Eelgrass Growth

Spatial Analysis

Potential distribution maps of the maximum and minimum depths for eelgrass survival were created using the light and temperature data above. In Gargathy, only a very shallow and small range for eelgrass growth is possible based on light (Figure 1.10**)** and temperature data (Figure 1.11). Because areas in Gargathy Bay did not exceed 28°C for long periods during the summer, there should not be a limit on the minimum depth other than that defined by dessication. However, because Gargathy Bay had a high light attenuation coefficient, % incident light at the sediment surface only exceeded 20% in the shallowest areas with the maximum depth limit at 0.8 m MSL (Figure 1.12). In Hog Island Bay, I would expect the minimum depth limit to be deeper than seen in Gargathy Bay due to increased temperature stress (Figure 1.11). The maximum depth limit in Hog Island Bay was also much deeper than that seen in Gargathy Bay, 1.6 m MSL (Figure 1.10). In Magothy Bay, the minimum depth limit was expected to be deeper than that seen in Hog Island Bay due to increased time over 28°C in the shallow areas (Figure 1.11, Figure 1.12). However, the light and temperature spatial analysis indicated that the maximum depth limit is predicted to be the same as in Hog Island Bay, 1.6 m MSL (Figure 1.10).

Transplant Experiment

Transplant survival followed patterns similar to predictions from spatial analysis based on light and temperature profiles. In Gargathy Bay, there was some transplant survival across the transects during the winter months. However, density declined drastically from June to July 2013 with no survival by July 2013 (Figure 1.13). In Magothy Bay, survival declined throughout the summer, but plants did not completely disappear from the area (Figure 1.13). The range for eelgrass growth for Magothy Bay found during the transplant experiment was 0.6 m MSL to 1.6 m MSL (Figure 1.15). This was similar to the range for eelgrass growth in Hog Island Bay 0.8 m MSL to 1.6 m MSL (Figure 1.15). In Magothy Bay, transplant density decreased with depth but not at a significant level (Figure 1.15) ($y = -39.81x + 87.22$; $F = 3.54$, $p = 0.0717$) and there was a significant negative relationship between transplant productivity and depth ($y = -2.32x + 3.92$; $R^2 = 0.8629$; $F = 19.89$, $p = 0.0468$).

Discussion

Water Quality

This study represents the first attempt to quantify the effect of differences in sediment characteristics, water quality, and temperature on the range of *Z. marina* in the Virginia Coastal Bays. Gargathy Bay had significantly higher concentrations of water column chlorophyll a, ammonium, ortho-phosphate, total dissolved nitrogen (TDN), and total suspended solids (TSS) than Magothy Bay and Hog Island Bay in 2012 (Figure 1.7, Table 1.3) with no differences between Gargathy and Magothy in 2013 (Table 1.5). Gargathy Bay had high water column chlorophyll a concentrations (33.0 +/- 2.4 μg/L) compared to literature values for healthy eelgrass beds in estuaries in Massachusetts, New York, Maryland, and the Chesapeake Bay (5.1 μg/L, 1 – 4 μg/L, < 15 μg/L and, < 15 μg/L respectively) (Benson et al. 2013, Carrol et al. 2008, Wazniak et al. 2003, Kemp et al. 2004) while concentrations in Magothy Bay (12.5 +/- 0.4 μg/L) and Hog Island Bay (6.8 +/- 0.6 μg/L) fell within the range listed in the literature.

Healthy eelgrass beds in Denmark and the Chesapeake Bay had concentrations of water column ammonium less than 1.2 μM and 0.7 μM respectively (Villazan et al. 2013, Moore and Jarvis 2008). Villazan et al. (2013) found that when levels of ammonium are elevated above the natural range found in healthy eelgrass beds (< 1.2 μM), eelgrass is subject to ammonium toxicity when in low light conditions. Concentrations of water column ammonium in Gargathy Bay and Hog Island Bay exceeded this threshold (Figure 1.7); however, with sufficient light, ammonium toxicity does not occur in concentrations up to 25 μM (Villazan et al. 2013) indicating that plants in Gargathy Bay would be more susceptible to decline from ammonium toxicity than plants in Hog Island Bay.

Ortho-phosphate, total dissolved nitrogen, and nitrate + nitrite concentrations were also found to be variable in healthy eelgrass beds with the water column in healthy eelgrass beds in Mumford Cove, Connecticut containing <1 µM ortho- phosphate and <0.2 µM nitrate + nitrite (Vaudrey et al. 2010), healthy eelgrass beds near Goodwin Island in the Chesapeake Bay containing < 0.1 µM of ortho-phosphate and < 1.2 µM nitrate + nitrite (Moore and Jarvis 2008), and healthy eelgrass beds in Barnegat Bay-Little Egg Harbor Estuary, New Jersey containing < 40 µM of total dissolved nitrogen (Fertig et al. 2013). For ortho-phosphate concentrations, Gargathy, Magothy, and Hog Island Bay exceeded 1 µM during the 2012 – 2013 time period (Table 1.4). None of the areas studied exceeded the amount of total dissolved nitrogen (TDN) found in healthy seagrass beds in New Jersey and all sites studied remained below the threshold value of 1.2 µM for the Chesapeake Bay area (Table 1.4). Total suspended solids (TSS) concentrations in Gargathy, Magothy, and Hog Island bays (Table 1.4) exceeded the threshold value of 15 mg/L set for eelgrass in the Chesapeake Bay and Maryland Coastal Bays (Wazniak et al. 2003, Kemp et al. 2004, Moore and Jarvis 2008).

Even though water quality nutrient and TSS concentrations in the VCR sometimes exceeded threshold values for healthy eelgrass beds, it may not necessarily mean these areas are not fit for restoration. Water quality was highly variable in each bay between years with water column chlorophyll having significantly lower concentrations in each bay in 2013 compared to 2012, water column ammonium concentrations staying the same in Gargathy and Magothy, but decreasing in Hog Island, ortho-phosphate concentrations rising in Hog Island and Magothy, TDN concentrations increasing in Gargathy and Magothy, and TSS concentrations falling in

Gargathy (Table 1.4). There is often interannual variability of water quality parameters in estuaries due to changes in nutrient loading from the terrestrial environment (Moore and Jarvis 2008, Vaudrey et al. 2010, Fertig et al. 2013). Nutrient loading can be affected by changes in public policy (Vaudrey et al. 2010) and can also be affected by yearly variability in rainfall (Mallin et al. 2003). It has been shown that there is spatial variability in water quality as well as sediment characteristics across coastal bays (Son and Wang 2012, Cloern 1996); however, this study only addresses variability across the eastern edge of the study bays. When considering thresholds for water quality characteristics used to predict eelgrass success, it is important to consider the compounding effects water quality, sediment characteristics, and temperature have on the plants across the seascape.

Light and Temperature

Light is often directly affected by sediment characteristics in estuaries (Yang et al. 2013). Areas with fine sediment grain sizes and high organic matter had a smaller and shallower range for eelgrass growth than areas with coarser sediment grain sizes and low organic matter (Figure 1.14). However, the percentage of fine sediment was a better predictor of the maximum depth limit than the percentage of coarse sediment (Figure 1.4, Table 1.7) as seen in Magothy Bay (Figure 1.14).

The maximum depth limit in Gargathy Bay was not predicted as accurately as Magothy and Hog Island bays by this approach (Figure 1.4, Figure 1.14). One reason for this may be the presence of large clumps of the macroalgae *Gracilaria vermiculophylla* that were found covering *Z. marina* transplant plots in Gargathy Bay. Presence of such mats can cause declines in the amount of light reaching the sediment surface and, therefore, cause declines in eelgrass growth (Holmquist 1997, Martinez-Luscher and Holmer 2010, Canal-Verges et al. 2014). Peckol and

Rivers (1996) found a 100% reduction in irradiance through 16 cm mats of *Gracilaria tikvahiae* which is morphologically similar to *G. vermiculophylla* (Thomsen et al. 2005). During summer 2013 in Gargathy Bay, *G. vermiculophylla* mat depth declined from an average of 22.8 +/- 3.6 cm in June to 9.9 +/- 2.3 cm in July over a 1 m by 0.2 m quadrat (Figure 1.17). Studies indicate that *G. vermiculophylla* is a drift algae but can become attached to mollusc shells and stones (Nyberg et al. 2009), *Diopatra cuprea* tube caps (Thomsen and McGlathery 2005), and can become tangled around seagrass blades (Thomsen et al. 2010). Including light attenuation through the mat with water column light attenuation, *Z. marina* would not receive enough light to survive at any depth in Gargathy Bay.

Along with differing light distributions, bays differed in temperature distribution with Magothy Bay having a higher percentage of time over 28°C than both Hog Island and Gargathy bays (Figure 1.11). Differences in temperature distribution in Magothy Bay compared to Hog Island Bay can be explained by differences in residence times with Magothy Bay having higher water residence times than Hog Island Bay (Safak et al. *in prep*). However, in Gargathy Bay, residence times were high (Safak et al. *in prep*) while temperatures were cooler than found in Hog Island Bay. These lower temperatures may suggest groundwater input because groundwater is usually cooler than ocean water during the summer months (Mulligan and Charette 2006).

Differences in temperature among the bays did not explain changes in the minimum depth limit. This is especially true for Magothy Bay where the predicted minimum depth limit was deeper than the actual minimum depth limit found through the transplant experiment (Figure 1.11 and Figure 1.14). When looking at transplant density across the depth range in Magothy Bay, transplant density declined linearly with depth (Figure 1.15). Transplant productivity also declined linearly over the depth range. This indicates that there is no temperature stress occurring at the minimum depth limit. If temperature stress was occurring as predicted in the model, density and transplant productivity in Magothy Bay would have followed a parabolic pattern with lower density and productivity at the minimum and maximum depth limits and high density and productivity in the center of each transect.

Temperature stress can often be a problem in shallow areas because of interactions between temperature and light requirements of seagrasses. When temperatures exceed 28°C, light requirements for *Z. marina* increase (Bulthuis 1987, Lee et al. 2005). Because of this, it is important to consider changes in light requirements with increases in temperature. In bays with small percentages of fine sediment and low light attenuation in shallow areas, temperature may not be the factor limiting the minimum depth for *Z. marina* growth because plants receive enough light to maintain a positive carbon balance. This means that the minimum depth limit would be shallower than predicted by temperature alone. Because light compensation point increases with temperature (Olesen and Sand-Jensen 1993, Abe et al. 2003), light compensation point may be a better factor for predicting the minimum depth limit than temperature alone. However, finding the light compensation point for *Z. marina* in each bay requires more complicated measurements than used in this study and cannot be done for Magothy Bay or Gargathy Bay at this point. However, maximum and minimum depth limits can be modeled for Hog Island Bay using the light compensation point found in Rheuban et al. (*in press*).

Rheuban et al. (*in press*) used seasonal oxygen metabolism measurements paired with light data to find the equation for light compensation point in Hog Island Bay. When using this equation, the minimum depth limit for *Z. marina* growth in Hog Island Bay can be calculated by calculating hours of saturation and applying 10 hours as the cut off for *Z. marina* (Carr et al. 2010). This causes the predicted minimum depth limit in Hog Island Bay to shift to a shallower depth, 0.4 m MSL (Figure 1.16). However, this does not match the actual minimum depth limit in Hog Island Bay (Figure 1.14) which may be attributed to other factors.

The potential distribution maps based off of light, transplant survival, and hours of saturation as well as the temperature distribution map are extrapolated to the entire bay from the transects measured (Figure 1.2). Because light, temperature, and sediment characteristics were not measured on the western side of each bay, it cannot be assumed that the western side of each bay is suitable for eelgrass growth. These characteristics can vary from the eastern side of the bay where measurements were taken. It has been shown that water quality and sediment characteristics change as measurements are taken moving away from the mainland in both the Chesapeake Bay (Son and Wang 2012) and San Francisco Bay (Cloern 1996). This is not unusual for estuaries because there is often a build-up of fine sediment along the marsh edge in the form of a mudflat (Allen and Duffy 1998, Cooper et al. 2001, Warrick 2012) and a higher input of nutrients from terrestrial sources than from marine sources causing higher nutrient concentrations on the landward side of the bay (Cloern 1996). Residence time has also been shown to change from the western to eastern side of the bays with a higher residence time along the western side of Magothy Bay than the eastern side (Safak et al. *in prep*). A higher residence time is associated with fine sediment grain sizes and build-up of organic matter (Houwing 1999, Molinaroli et al. 2009) possibly leading to conditions closer to those seen in Gargathy.

Chemical Stressors

Chemical stressors, such as organic content and pore-water sulfide concentrations, can also combine with light and temperature stressors to limit the depth range for *Z. marina*. In Magothy Bay and Hog Island Bay, organic content and pore-water sulfide concentrations were low (Figure 1.5 and Figure 1.6). Yang et al. (2013) found that organic-rich sediment is a better indicator for intertidal seagrass seedling recruitment than both desiccation and nutrient availability. This could be the reason for the difference between the minimum depth limit found in Hog Island Bay and the minimum depth limit predicted by the light compensation point and hours of saturating light in Hog Island Bay. In Hog Island Bay and Magothy Bay, organic content is inversely proportional to grain size (Sherman et al. *unpublished data*) and grain size declines with depth (Figure 1.18); therefore, sediments in shallow areas have less organic matter than deeper areas. This may make it more difficult for seagrasses to spread to shallow areas in Hog Island Bay and Magothy Bay naturally because seed germination is associated with anaerobic environments that can result from high organic content (Marba et al. 2006). Anaerobic areas are often associated with high sulfide concentrations (Holmer and Nielson 2007). Korhonen et al. (2012) found that *Z. marina* survival declined at sulfide concentrations greater than 1000 μM. Low pore-water sulfide concentrations in Magothy Bay and Hog Island Bay indicate an aerobic rhizophere suggesting these areas are more suitable for eelgrass restoration than Gargathy Bay.

In Gargathy Bay, both sediment organic content and pore-water sulfide concentrations were higher than in Magothy Bay and Hog Island Bay (Figure 1.5 and Figure 1.6). Dense filamentous macroalgal mats can also increase sulfide intrusion into *Z. marina* by creating an anaerobic microenvironment around the plants and preventing enough light from reaching the plants to counter the effects of sulfide intrusion (Holmer and Nielson 2007). However, I cannot conclude whether the reason for transplant loss is caused by the direct effects of light attenuation through the *G. vermiculophylla* mats or due to effects of chemical stressors. Al-Haj et al. (*in prep*) attempt to separate these effect by looking at changes in photosynthetic efficiency of *Z. marina* transplants planted into different sediment types in mesocosms. Another way to separate effects of direct light stress due to increased light attenuation from chemical stress due to the anaerobic environment is to perform an analysis of sulfur isotopes in the sediment and roots, shoots, and leaves of the plants (Kilminster et al. 2014); however, this is beyond the scope of the present study.

Conclusions

Differences in sediment characteristics, water quality, and temperature need to be considered when identifying areas for *Z. marina* restoration in order to maximize chances of restoration success. As summer temperatures increase chances of sulfide stress, light stress can also increase (Garcia et al. 2013). Previous models, such as Carr et al.'s (2010) hydrodynamic-growth model for eelgrass in the Virginia Coastal Bays, Biber et al.'s (2008) bio-optical model for Pamlico Sound and Kemp et al.'s (2004) submerged aquatic vegetation (SAV) distribution model for the Chesapeake Bay, focus on light attenuation through the water column without identifying how light requirements of seagrass may change when exposed to temperature stress and chemical stressors. However, there have been attempts to quantify these factors with other seagrass species (Kenov et al. 2013) and in locations where temperature stress is not an issue for *Z. marina* (Kenworthy et al. 2013).

In this study, I found that the maximum and minimum depths for eelgrass growth vary across the seascape in the Virginia Coastal Bays with temperature, light attenuation, and chemical stressors. By using spatial analysis to compare the effects of light and temperature on the maximum and minimum depth limits and transplanting *Z. marina* across a depth limit that bracketed and extended the minimum and maximum depth limit in Hog Island Bay in the unrestored bays, the effects of compounding stressors could be compared. I found that the maximum depth limit is dependent on light attenuation, that the minimum depth limit is dependent on a much more complex interaction between light and temperature that can be affected by chemical stressors, and that fine sediment grain sizes and temperature are important factors for density across the depth range. However, more work needs to be done in order to separate the effects of chemical stressors on the minimum depth limit.

The results of this study will allow researchers to build more accurate models for predicting potential *Z. marina* coverage in the Virginia Coastal Bays and other areas world-wide in order to help mitigate the global net loss of seagrasses. In order to more accurately characterize the Virginia Coastal Bays for *Z. marina* restoration, modelers need a full-scale survey of environmental conditions including the western boundaries of each bay. Without this information, it is difficult to characterize entire bays because water quality may change as you move away from the mainland as shown in the Chesapeake Bay (Son and Wang 2012) and San Francisco Bay (Cloern 1996).

Literature Cited

Abe, M., N. Hashimoto, A. Kurashima, M. Maegawa. 2003. Estimation of light requirement for the growth of *Zostera marina* in central Japan. *Fisheries Science* **69:** 890 – 895.

Al-Haj, A. N., K. J. McGlathery, P. L. Wiberg. A. C. Schwarzschild. Quantifying changes in Zostera marina L. productivity and photosynthetic capacity with changes in sediment characteristics and light attenuation. *In preparation*.

Allen, J. R. L., M. J. Duffy. 1998. Medium-term sedimentation on high intertidal mudflats and salt marshes in the Severn Estuary, SW Britain: the role of wind and tide. *Marine Geology* **150**: 1 – 27.

Beckman Coulter. 2011. Instructions for use: LS 13 320 laser diffraction particle size analyzer, PN B05577AB, revision 10/11, Brea, California.

Benson, J. L., D. Schlezinger, B. L. Howes. 2013. Relationship between nitrogen concentration, light, and *Zostera marina* habitat quality and survival in southeastern Massachusetts estuaries. *Journal of Environmental Management* **131**: 129 – 137.

Berg, P. and K. J. McGlathery. 2001. A High-Resolution Pore Water Sampler for Sandy Sediments. *Limnology and Oceanography* **46**: 203 – 210.

Biber, P. D., C. L. Gallegos, and W. J. Kenworthy. 2008. Calibration of a Bio-optical Model in the North River, North Carolina (Albemarle–Pamlico Sound): A Tool to Evaluate Water Quality Impacts on Seagrasses. *Estuaries and Coasts* **31**: 177 – 191.

Biber, P. D., W. J. Kenworthy, H. W. Paerl. 2009. Experimental analysis of the response and recovery of *Zostera marina* (L.) and *Halodule wrightii* (Ascher.) to repeated light-limitation stress. *Journal of Experimental Marine Biology and Ecology* **369**: 110 – 117.

Boese, B. L., B. D. Robbins. 2008. Effects of erosion and macroalgae on intertidal eelgrass (*Zostera marina*) in a northeastern Pacific estuary (USA). *Botanica Marina* **61**: 247 – 257.

Bulthuis, D. A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany* **27**: 27 – 40.

Canal – Verges, P., M. Potthoff, F. T. Hansen, N. Holmboe, E. K. Rasmussen. M. R. Flindt. 2014. Eelgrass re-establishment in shallow estuaries is affects by drifting macroalgae – Evaluated by agent-based modeling. *Ecological Modelling* **272**: 116 – 128.

Carr, J., P. D'Odorico, K. McGlathery, P. Wiberg. 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. *Journal of Geophysical Research* **115**: doi:10.1029/2009JG001103.

Carrol, J., C. J. Gobler, B. J. Peterson. 2008. Resource-restricted growth of eelgrass in New York estuaries: light limitation, and alleviation of nutrient stress by hard clams. *Marine Ecology Progress Series* **369**: 51 – 62.

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* **14**: 454 – 458.

Cloern, J. E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. *Reviews of Geophysics* **34**: 127 – 168.

Cooper, N. J., T. Cooper, F. Burd. 2001. 25 years of salt marsh erosion in Essex: Implications for coastal defense and nature conservation. *Journal of Coastal Conservation* **7**: 31 – 40.

Davis R. C. and F. T. Short. 1997. Restoring eelgrass, *Zostera marina* L., habitat using a new transplanting technique: The horizontal rhizome method. *Aquatic Botany* **59**: 1 – 15.

Dennison, W. C., R. J. Orth, K. A. Moore, J. C. Stevenson, V. Carter, S. Kollar, P. W. Bergstrom, R. A. Batiuk. 1993. Assessing Water Quality with Submersed Aquatic Vegetation: Habitat requirements as barometers of Chesapeake Bay Health. *BioScience* **43**: 86 – 94.

Duarte, C. M. 1991. Seagrass depth limits. *Aquatic Botany* **40**: 363 – 377.

Environmental Protection Agency. 1983. Methods for chemical analysis of water and waste, EPA-600/4-79-020, revision 03/83, Washington, DC.

Fertig, B., M. J. Kennish, G. P. Sakowicz. 2013. Changing eelgrass (*Zostera marina* L.) characteristics in a highly eutrophic temperate coastal lagoon. *Aquatic Botany* **104**: 70 – 79.

Garcia, R., M. Holmer, C. Duarte, N. Marba. 2013. Global warming enhances sulphide stress in a key seagrass species (NW Mediterranean). *Global Change Biology* **19**: 3629 – 3639.

Goodman, J. L., K. A. Moore, W. C. Dennison. 1995. Photosynthetic responses of eelgrass (*Zostera marina*) to light and sediment sulfide in a shallow barrier lagoon. *Aquatic Botany* **50**: 37-48.

Gustafsson, C. and C. Bostrom. 2013. Influence of neighboring plants on shading stress resistance and recovery of eelgrass, *Zostera marina* L. *PLoSONE* **8**: e64064. doi:10.1371/journal.pone.0064064.

Greve, T. M., J. Borum, O. Pederson. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnology and Oceanography* **48**: 210 – 216.

Hansen, J. C. R. and M. A. Reidenbach. 2012. Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension. *Marine Ecology Progress Series* **448**: 271 – 287.

Hansen, J. C. R., M. and M. A. Reidenbach. 2013. Seasonal growth and senescence of a *Zostera marina* seagrass meadow alters wave-dominated flow and sediment suspension within a coastal bay. *Estuaries and Coasts* **36**: 1099 – 1114.

Holmer, M., E. J. Bondgaard. 2001. Photosynthesis and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. *Aquatic Botany* **70**: 29 – 38.

Holmer, M., R. M. Nielson. 2007. Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology* **353**: 245 – 252.

Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, J. D. H. Strickland. 1965. Fluorometric determination of chlorophyll. *Journal du Conseil Permanent International pour l'Exploration de la Mer* **30**: 3 – 15.

Holmquist, J. G. 1997. Disturbance and gap formation in a marine benthic mosaic: influence of shifting macroalgal patches on seagrass structure and mobile invertebrates. *Marine Ecology Progress Series* **158**: 121 – 130.

Houwing, E. J. 1999. Determination of the critical erosion threshold of cohesive sediments on intertidal mudflats along the Dutch Wadden Sea coast. *Estuarine, Coastal and Shelf Science* **49**: 545 – 555.

Kemp, M. W., R. Batiuk, R. Bartleson, P. Bergstrom, V. Carter, C. L. Gallegos, W. Hunley, L. Karrh, E. W. Koch, J. M. Landwehr, K. A. Moore, L. Murray, M. Naylor, N. B. Rybickr, J. C. Stevenson, D. L. Wilcox. 2004. Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: water quality, light regime, and physical – chemical factors. *Estuaries* **27**: 363 – 377.

Kenov, I. A., R. Deus, C. N. Alves, R. Neves. 2013. Modelling seagrass biomass and relative nutrient content. *Journal of Coastal Research* **29**: 1470 – 1476.

Kenworthy, W. J., C. L. Gallegos, C. Costello, D. Field, G. di Carlo. *In press*. Dependence of eelgrass (*Zostera marina*) light requirements on sediment organic matter in Massachusetts coastal bays: Implications for remediation and restoration. *Marine Pollution Bulletin* http://dx.doi.org/10.1016/j.marpolbul.2013.11.006.

Kilminster, K., V. Forbes, M. Holmer. 2014. Development of a 'sediment-stress' functional – level indicator for the seagrass *Halophila ovalis*. *Ecological Indicators* **36**: 280 – 289.

Koch, E. 2001. Beyond Light: Physical, Geological, and Geochemical Parameters as Possible Submersed Aquatic Vegetation Habitat Requirements. *Estuaries* **24**: 1 – 17.

Korhonen, L. K., V. Macias – Carranza, R. Abdala, F. L. Figueroa. A. Cabello – Pasini. 2012. Effects of sulfide concentration, pH, and anoxia, on photosynthesis and respiration of *Zostera marina*. *Ciencias Marinas* **38**: 625 – 633.

Krause-Jenson, D., J. Cartensen, L. Nielsen, T. Dalsgaard, P. B. Christensen, H. Fossing, M. B. Rasmussen. 2011. Sea bottom characteristics affect depth limits of eelgrass *Zostera marina*. *Marine Ecology Progress Series* **425**: 91 – 102.

Lawson, S. E., K. J. McGlathery, P. L. Wiberg. 2012. Enhancement of sediment suspension and nutrient flux by benthic macrophytes at low biomass. *Marine Ecology Progress Series* **448**: 259 – 270.

Lee, K. S., S. R. Park, J. B. Kim. 2005. Production dynamics of the eelgrass, *Zostera marina* in two bay systems on the south coast of the Korean peninsula. *Marine Biology* **147**: 1091 – 1108.

Lee, K. S., S. R. Park, Y. K. Kim. 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology* **350**: 144 – 175.

Li, W. T., S. H. Kim, J. W. Kim, J. H. Kim, K. S. Lee. 2013. An examination of photoacclimatory responses of Zostera marina transplants along a depth gradient for transplant-site selection in a disturbed estuary. *Estuarine, Coastal and Shelf Science* **118**: 72 – 79.

Long, M. H., J. E. Rheuban, P. Berg. J. C. Zieman. 2012. A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. Limnology and Oceanography – Methods **10**: 416 – 424.

Mallin, M. A., H. W. Paerl, J. Rudek, P. W. Bates. 1993. Regulation of estuarine primary production by watershed rainfall and river flow. *Marine Ecology Progress Series* **93**: 199 – 203.

Marbà, N., M. Holmer, E. Gacia, C. Barròn. 2006. Seagrass beds and coastal biogeochemistry. In Seagrasses: Biology, ecology and conservation, ed. A.W.D. Larkum, R.J. Orth, and C.M. Duarte, 135–157. Dordrecht: Springer.

Martinez-Luscher, J., M. Holmer. 2010. Potential effects of the invasive species *Gracilaria vermiculophylla* on *Zostera marina* metabolism and survival. *Marine Environmental Research* **69**: 345 – 349.

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient – enriched coastal waters. *Journal of Phycology* **37**: 453 – 456.

McGlathery K. J., K. Sundback, I. C. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Marine Ecology Progress Series* **348**: 1 – 18.

McGlathery, K. J., L. K.Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Marine Ecology Progress Series* **448**: 209 – 221.

Molinaroli, E., S. Guerzoni, G. De Falco, A. Sarretta, A. Cucco, S. Como, S. Simeone, A. Perilli, P. Magni. 2009. Relationships between hydrodynamic parameters and grain size in two contrasting transitional environments: The Lagoons of Venice and Cabras, Italy. *Sedimentary Geology* **219**: 196 – 207.

Moore, K. A., J. C. Jarvis. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: Implications for long-term persistence. *Journal of Coastal Research* **55**: 135 – 147.

Mulligan, A. E., M. A. Charette. 2006. Intercomparison of submarine groundwater discharge estimates from a sandy unconfined aquifer. *Journal of Hydrology* **327**: 411 – 425.

Nyberg, C. D., M. S. Thomsen, I. Wallentinus. 2009. Flora and fauna associated with the introduced red alga *Gracilaria vermiculophylla*. *European Journal of Phycology* **44**: 395 – 403.

Olesen, B., K. Sand-Jensen. 1993. Seasonal acclimatization of eelgrass *Zostera marina* growth to light. *Marine Ecology Progress Series* **94**: 91 – 99.

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. Williams. 2006b. A Global Crisis for Seagrass Ecosystems. *BioScience* **56**: 987 – 996.

Orth R. J., K. A. Moore, S. R. Marion, D. L. Wilcox. 2012. Seed addition facilitates eelgrass recovery in a coastal bay system. *Marine Ecology Progress Series* **448**: 177 – 195.

Peckol, P., J. S. Rivers. 1996. Contribution of macroalgal mats to primary production of a shallow embayment under high and low nitrogen-loading rates.

Pederson, O., T. Binzer, J. Borum. 2004. Sulfide intrusion in eelgrass (*Zostera marina* L.). *Plant, Cell and Environment* **27**: 595 – 602.

Pedersen, T. M., C. L. Gallegos, S. L. Nielsen. 2012. Influence of near-bottom re-suspended sediment on benthic light availability. *Estuarine, Coastal and Shelf Science* **106**: 93 – 101.

Reynolds, L. K., K. J. McGlathery, M. Waycott. 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. *PLoSONE* **7**: e38397. doi:10.1371/journal.pone.0038397.

Rheuban J. E., P. Berg, K. J. McGlathery. *In Press*. Seasonal oxygen metabolism in a restored *Zostera marina L.* (eelgrass) meadow at the Virginia Coast Reserve measured by eddy correlation.

Safak, I, P.L. Wiberg and M.O. Kurum. Controls on residence time and exchange in shallow coastal bay systems. *In preparation*.

Sherman, A. E., A. N. Al-Haj, K. J. McGlathery. Covariance of sulfide scncentration and percent organic matter with grain size in the Virginia Coastal Bays. *Unpublished data*.

Short, F. T., B. Polidoro, S. R. Livingstone, K. E. Carpenter, S. Bandeira, J. S. Bujang, H. P. Calumpong, T. J. B. Carruthers, R. G. Coles, W. C. Dennison, P. L. A. Erftemeijer, M. D. Fortes, A. S. Freeman, T. G. Jagtap, A. H. M. Kamal, G. A. Kendrick, W. J. Kenworthy, Y. A. La Nafie, I. M. Nasution, R. J. Orth, A. Prathep, J. C. Sanciangco, B. van

Tussenbroek, S. C. Vergara, M. Waycott, J. C. Zieman. 2011. Extinction risk assessment of the world's seagrass species. *Biological Conservation* **144**: 1961 – 1971.

Skinner, M. A., S. C. Courtenay, C. W. McKindsey. 2013. Reductions in distribution, photosynthesis, and productivity of eelgrass *Zostera marina* associated with oyster *Crassostrea virginica* aquaculture. *Marine Ecology Progress Series* **486**: 105 – 119.

Son, S., M. Wang. 2012. Water properties in Chesapeake Bay from MODIS-Aqua measurements. *Remote Sensing of Environment* **123**: 163 – 174.

Standard Methods for the Examination of Water and Wastewater. 1993. 18th edition, Washington, D.C., prepared and published by the American Public Health Association, American Water Works Association, and Water Environmental Federation.

Thomsen, M. S., C. F. D. Gurgel, S. Fredericq, K. J. McGlathery. 2005. *Gracilaria vermiculophylla* (Rhodophyta, Gracilariales) in Hog Island Bay, Virginia: A cryptic alien and invasive macroalga and taxonomic correction. *Journal of Phycology* **42**: 139 – 141.

Thomsen, M. S., K. J. McGlathery. 2005. Facilitation of macroalgae by the sedimentary tube forming polychaete *Diopatra cuprea*. *Estuarine, Coastal and Shelf Science* **62**: 63 – 73.

Thomsen, M. S., T. Wernberg, A. Altieri, F. Tuya, D. Gulbransen, K. J. McGlathery, M. Holmer, B. R. Silliman. 2010. Habitat cascades: The conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and Comparative Biology* **50**: 158 – 175.

van der Heide, T., E. T. H. M. Peeters, D. C. R. Hermus, M. M. van Katwijk, J. G. M. Roelofs, A. J. P. Smolders. 2009. Predicting habitat suitability in temperate seagrass ecosystems. *Limnology and Oceanography* **54**: 2018 – 2024.

van Katwijk, M. M., L. H. T. Vergeer, G. H. W. Schmitz, J. G. M. Roelofs. 1997. Ammonium toxicity in eelgrass *Zostera marina*. *Marine Ecology Progress Series* **157**: 159 – 173.

Vaudrey, J. M. P., J. N. Kremer, B. F. Branco, F. T. Short. 2010. Eelgrass recovery after nutrient enrichment reversal. *Aquatic Botany* **93**: 237 – 243.

Villazan, B. M. F. Pederson, F. G. Brun, J. J. Vergara. 2013. Elevated ammonium concentrations and low light form a dangerous synergy for eelgrass *Zostera marina*. *Marine Ecology Progress Series* **493**: 141 – 154.

Wagner, E., B. R. Dumbauld, S. D. Hacker, A. C. Trimble, L. M. Wisehart, J. L. Ruesink. 2012. Density-dependent effects of an introduced oyster, *Crassostrea gigas*, on a native intertidal seagrass, *Zostera marina*. *Marine Ecology Progress Series* **468**: 149 – 160.

Ward, L. G., W. M. Kemp, W. R. Boynton. 1984. The influence of waves and seagrass communities on suspended particulates in an estuarine embayment. *Marine Geology* **59**: 85 – 103.

Warrick, J. A. 2012. Dispersal of fine sediment in nearshore coastal waters. *Journal of Coastal Research* **29**: 579 – 596.

Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, Jr., A R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Publications of the National Academy of Sciences* **106**: 12377 – 12381.

Wazniak, C., L. Karrh, T. Parham, M. Naylor, M. Hall, T. Carruthers, R. J. Orth. 2003. Maryland’s Coastal Bays: Ecosystem Health Assessment. 6-2 – 6-17.

Widdows, J. N. D. Pope, M. D. Brinsley, H. Asmus, R. M. Asmus. 2008. Effects of seagrass beds (*Zostera noltii* an d *Z. marina*) on near-bed hydrodynamics and sediment resuspension. *Marine Ecology Progress Series* **358**: 125 – 236.

Yang, S., E. E. Wheat, M. J. Horwith, J. L. Ruesink. 2013. Relative impacts of natural stressora on life history traits underlying resilience of intertidal eelgrass (*Zostera marina* L.). *Estuaries and Coasts* **36**: 1006 – 1013.

Zieman, J. C. 1974. Methods for the study of growth and production of Turtle Grass *Thalassia testudinum*. *Aquaculture* **4**: 139 – 143.

Figures

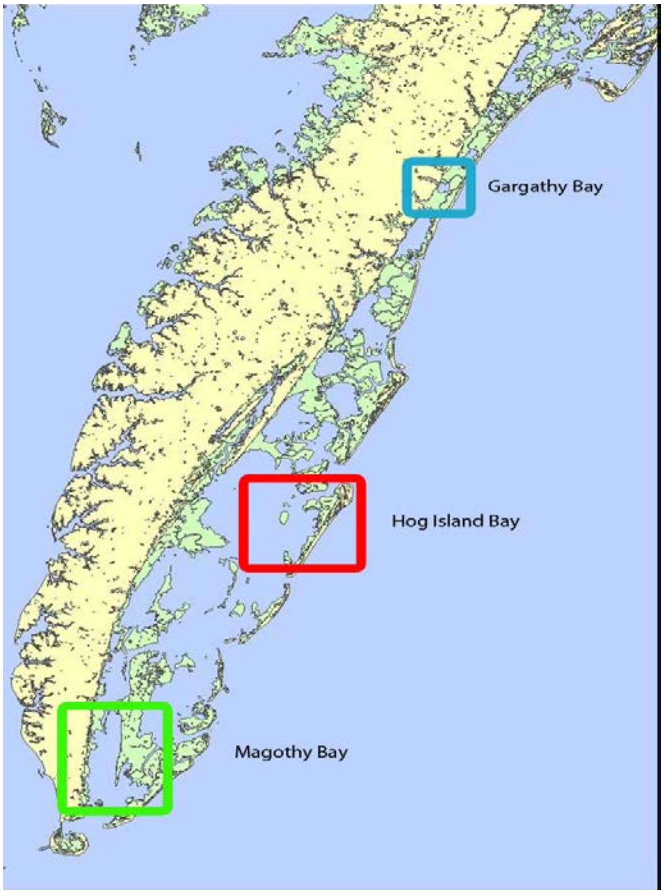


Figure 1.1 Image of the Delmarva Peninsula with study sites outlined; unrestored Gargathy Bay in blue, Hog Island Bay in the process of being restored in red, and unrestored Magothy Bay in green.

Figure 1.2 Site locations in Magothy Bay (green), Gargathy Bay (blue), and Hog Island Bay (red).

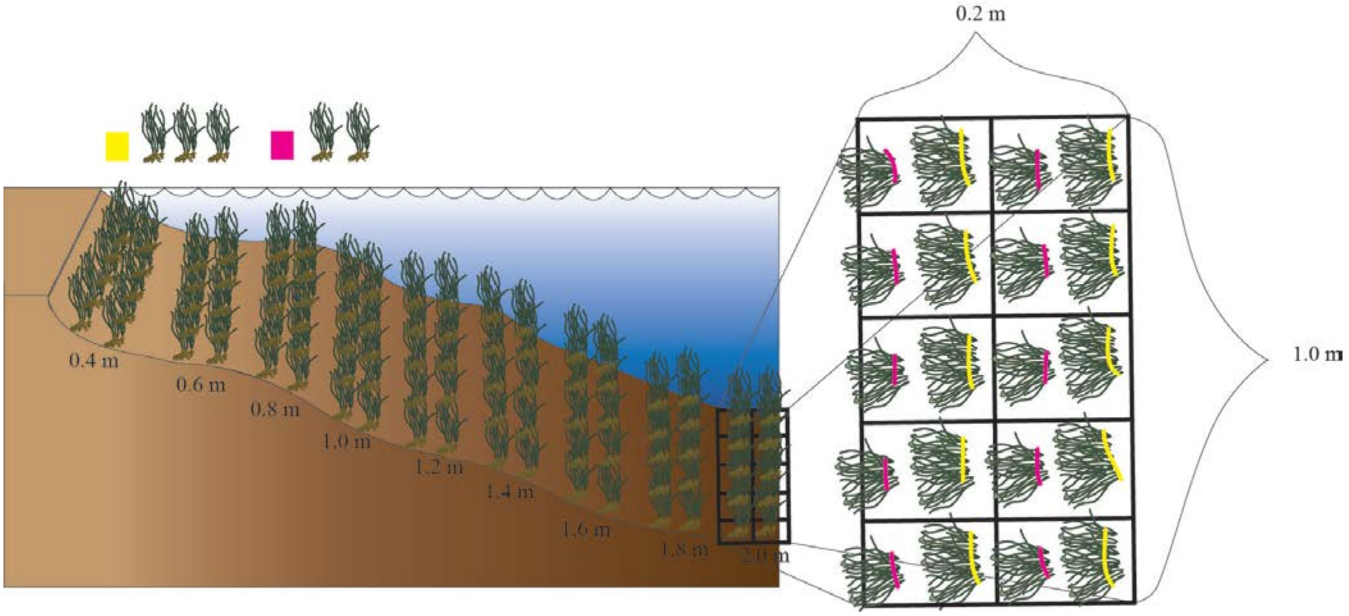


Figure 1.3 Diagram of transect orientation. When transplanting, a 0.2 m by 1.0 m quadrat will be set at each 0.2 m change in depth along a depth gradient from 0.4 m to 2.0 m MSL. A three plant planting (yellow) unit and a two plant planting unit (pink) were planted in each section of the quadrat. Eelgrass image: Tracey Saxby, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

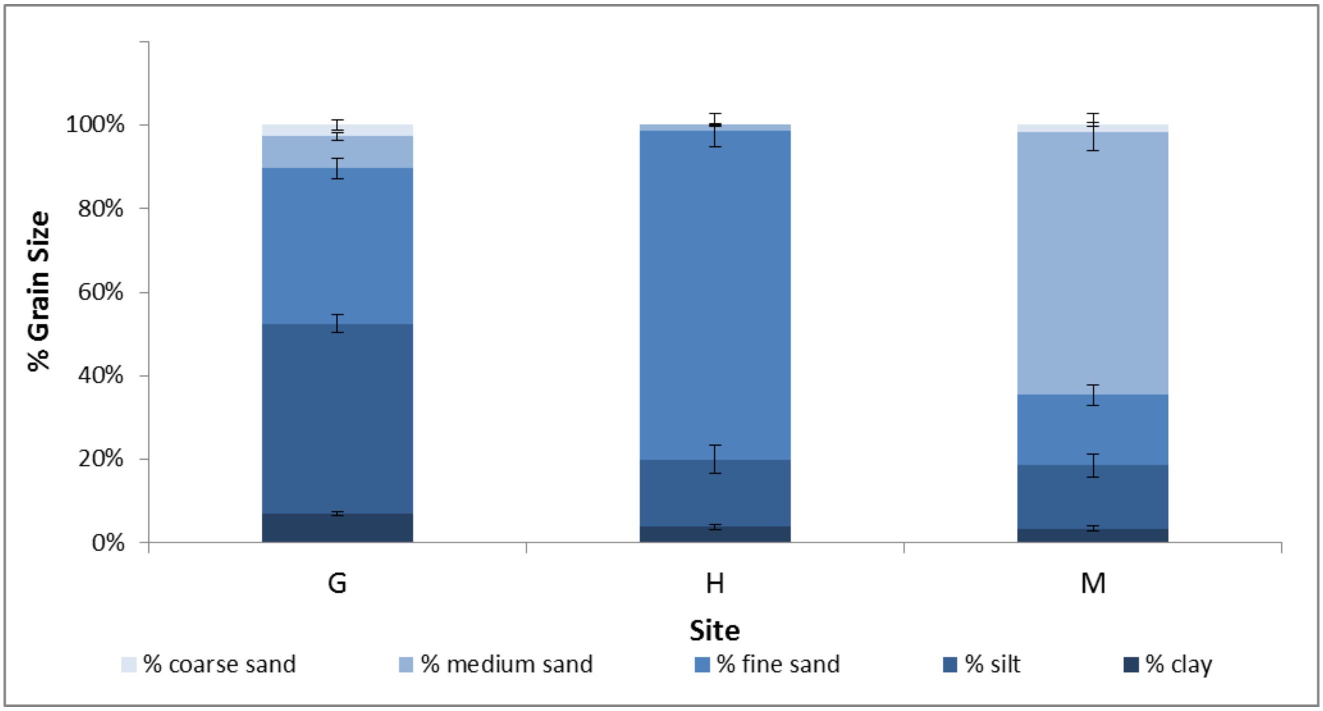


Figure 1.4. Distribution of clay (<2 µm), silt (2 – 63 µm), fine sand (63 – 200 µm), medium sand (200 – 630 µm) and coarse sand (>630 µm) at sites in Gargathy Bay, Magothy Bay, and Hog Island Bay with error bars representing standard error (n = 27).

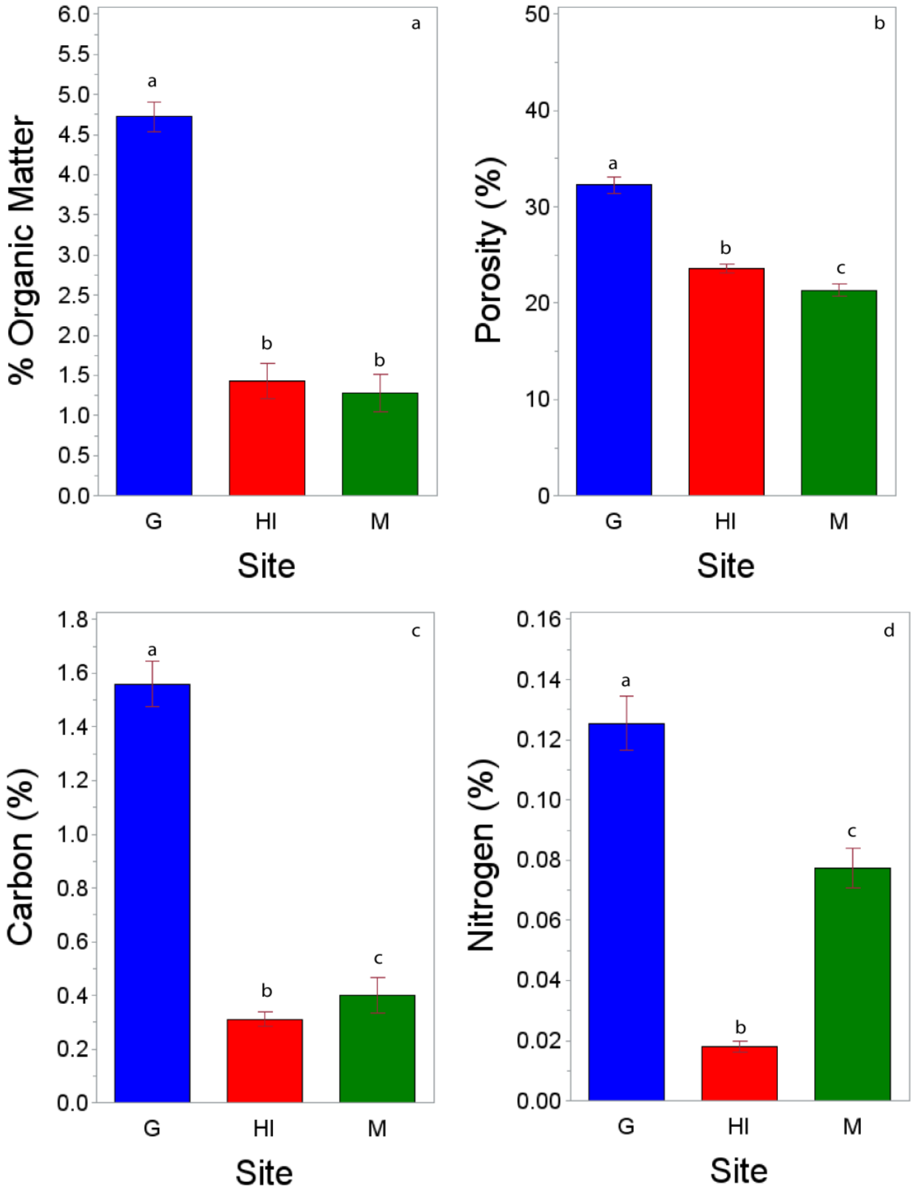


Figure 1.5. Sediment characteristics of sites in unrestored bays, Gargathy (G) and Magothy (M) compared to bare sites in a bay in the process of being restored, Hog Island Bay (HI). Sediment characteristics include (a) % organic matter (b) % porosity (c) %C and (d) %N. Error bars represent standard error (n = 27). Letters represent bars statistically the same.

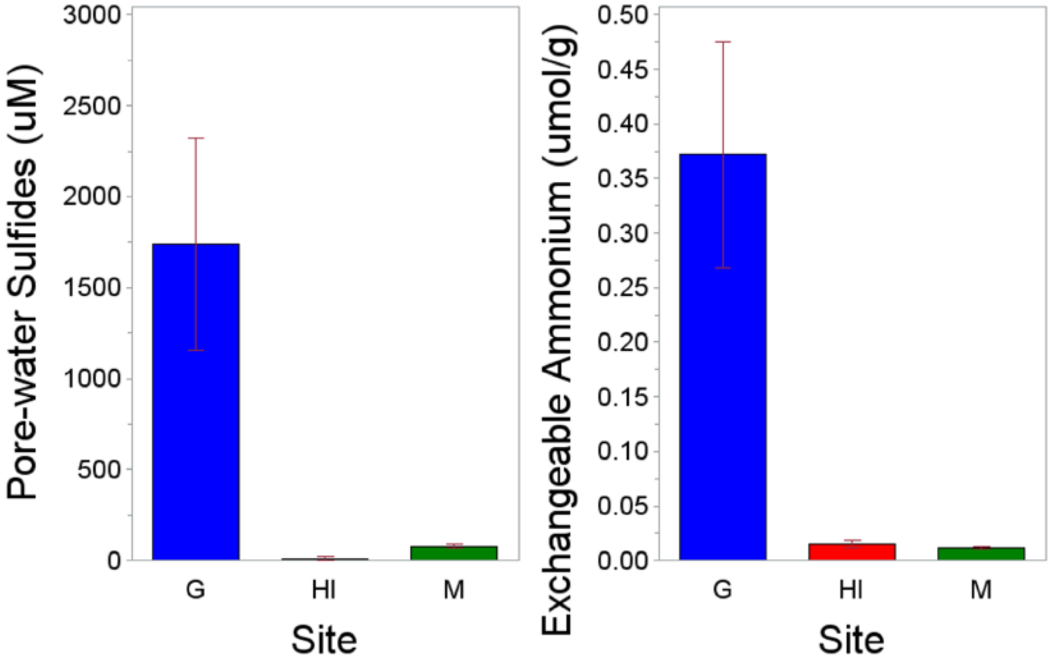


Figure 1.6. Sediment pore-water characteristics of sites in unrestored bays, Gargathy (G) and Magothy (M), compared to bare sites in a bay in the process of being restored, Hog Island Bay (HI). Sediment pore-water parameters include (a) sulfide concentration and (b) exchangeable ammonium concentration. Error bars represent standard error (n = 9). Letters represent bars statistically the same.

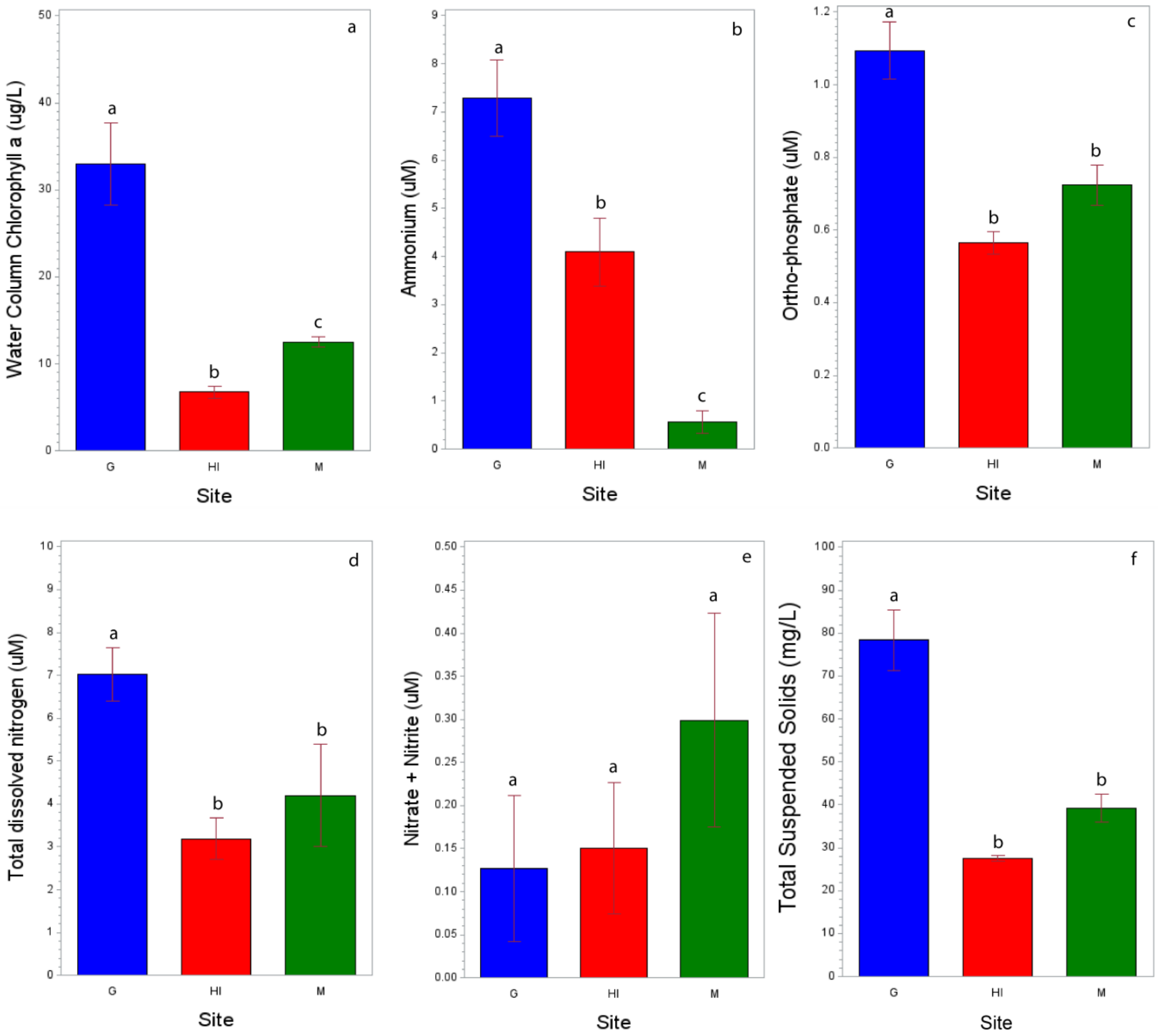


Figure 1.7. Water quality characteristics from summer 2012 of sites in unrestored bays, Gargathy (G) and Magothy (M), and a bay in the process of being restored, Hog Island Bay (HI). Water quality parameters include (a) water column chlorophyll a concentration, (b) ammonium concentration, (c) ortho-phosphate concentration, (d) total dissolved nitrogen concentration, (e) nitrate + nitrite concentration, and (f) total suspended solids. Error bars represent standard error (n = 9). Letters represent bars statistically the same.

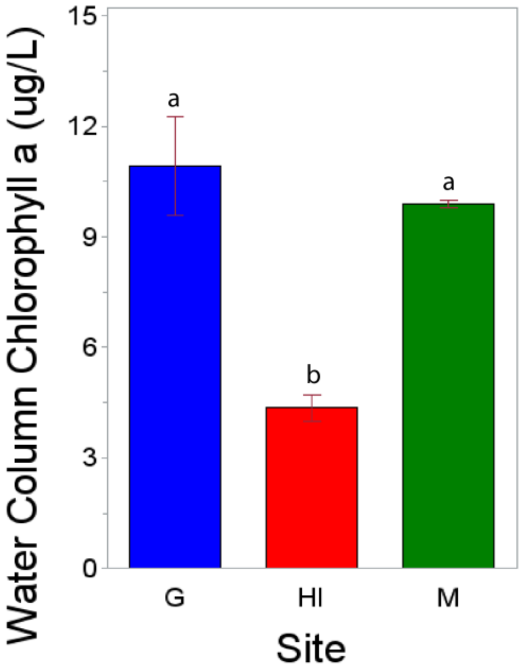


Figure 1.8. Water column chlorophyll a from summer 2013 of sites in unrestored bays, Gargathy (G) and Magothy (M), and a bay in the process of being restored, Hog Island Bay (HI). Error bars represent standard error (n = 9). Letters represent bars statistically the same.

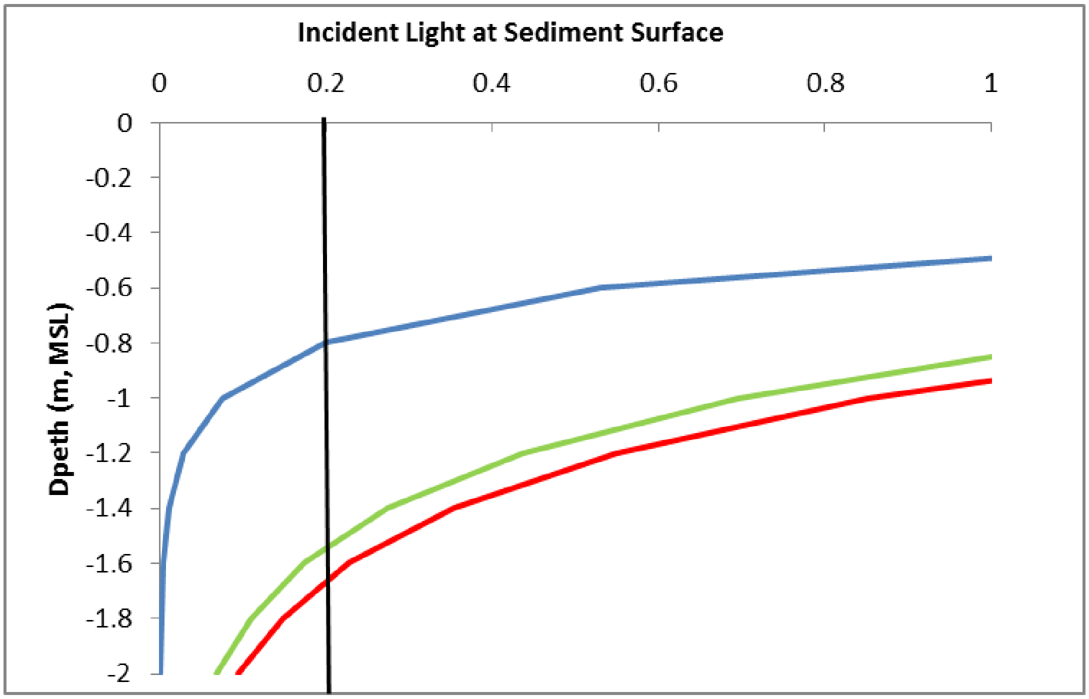


Figure 1.9. Percent incident light at the sediment surface with depth for Gargathy Bay (blue), Magothy Bay (green), and Hog Island Bay (red). The black line represents 20% incident light at the sediment surface, the threshold for eelgrass growth.

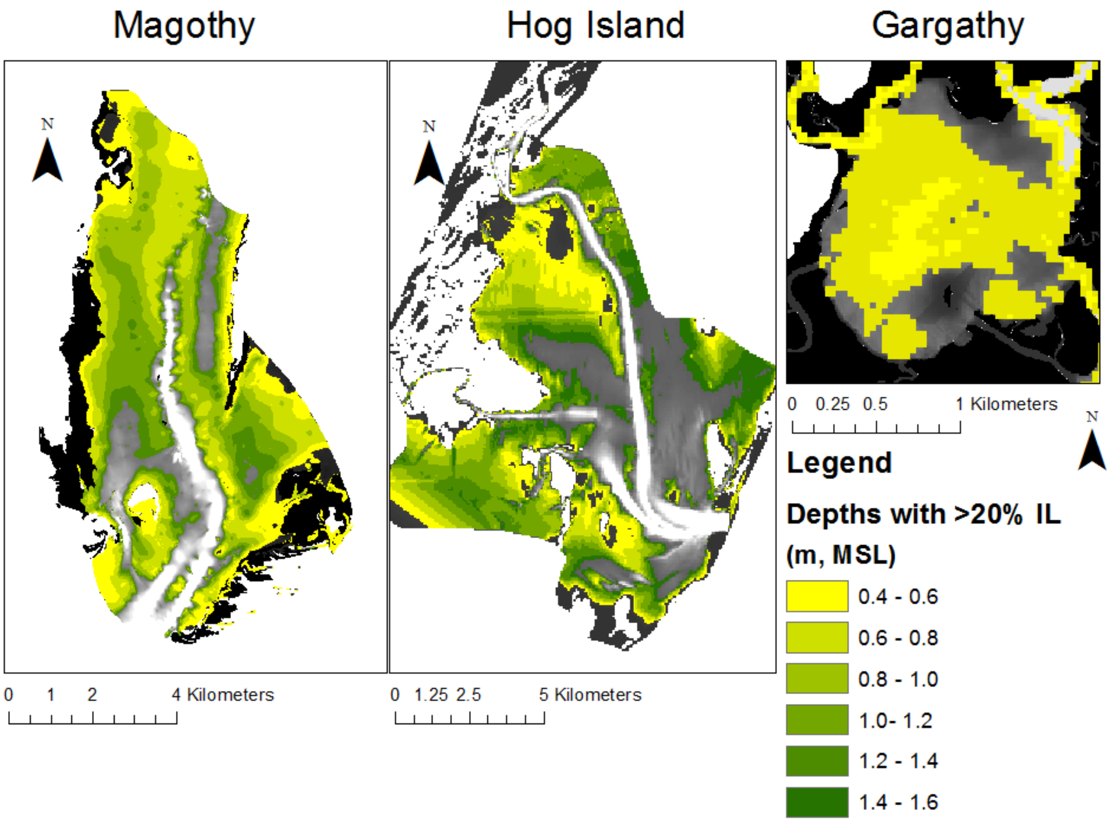


Figure 1.10. Modeled area suitable for eelgrass growth in Magothy Bay, Hog Island Bay, and Gargathy Bay when considering depths where average % incident light at the sediment surface was > 20% during summer 2012 and 2013. Areas shaded in yellow to green are suitable for eelgrass growth with greener areas in each map representing the maximum depth limit in each bay.

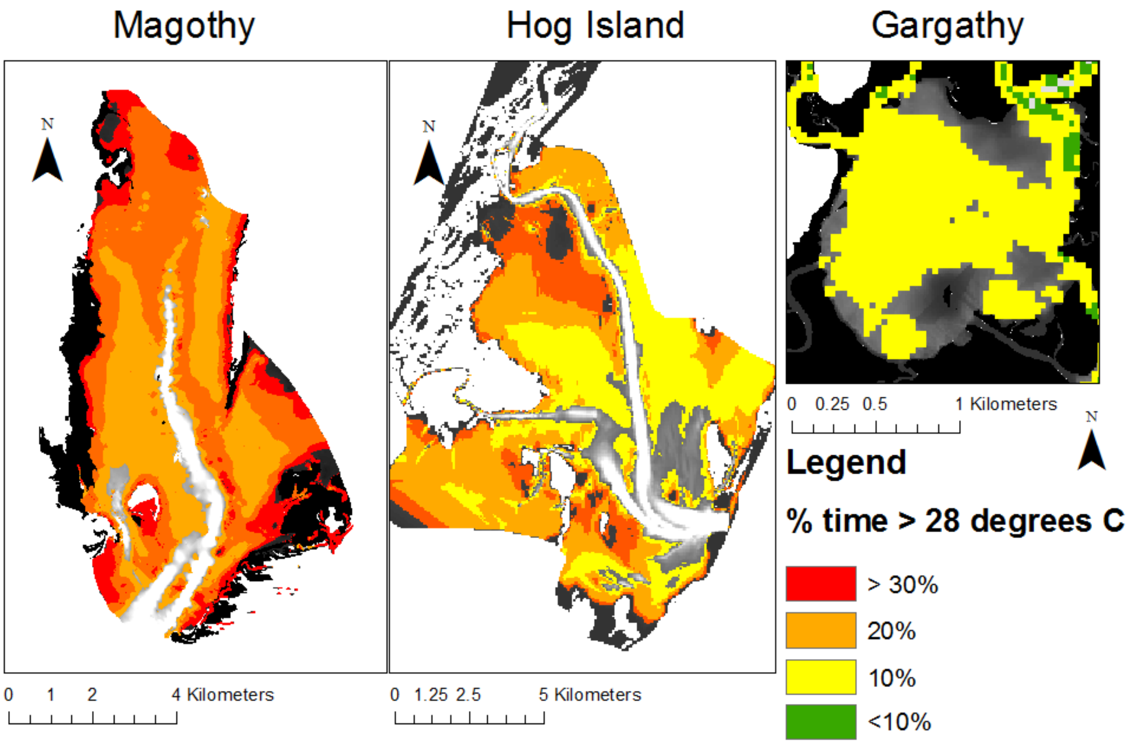


Figure 1.11. Percentage of time depths exceeded 28°C during summers 2012 and 2013 in Magothy Bay, Hog Island Bay, and Gargathy Bay. Areas shaded in green represent areas with <10% of the summer over 28°C and areas shaded in red represent areas with >30% of the summer over 28°C. Redder areas in each map generally represent shallower depths than greener areas on a map by map basis with greener areas deemed more suitable for eelgrass growth than redder areas.

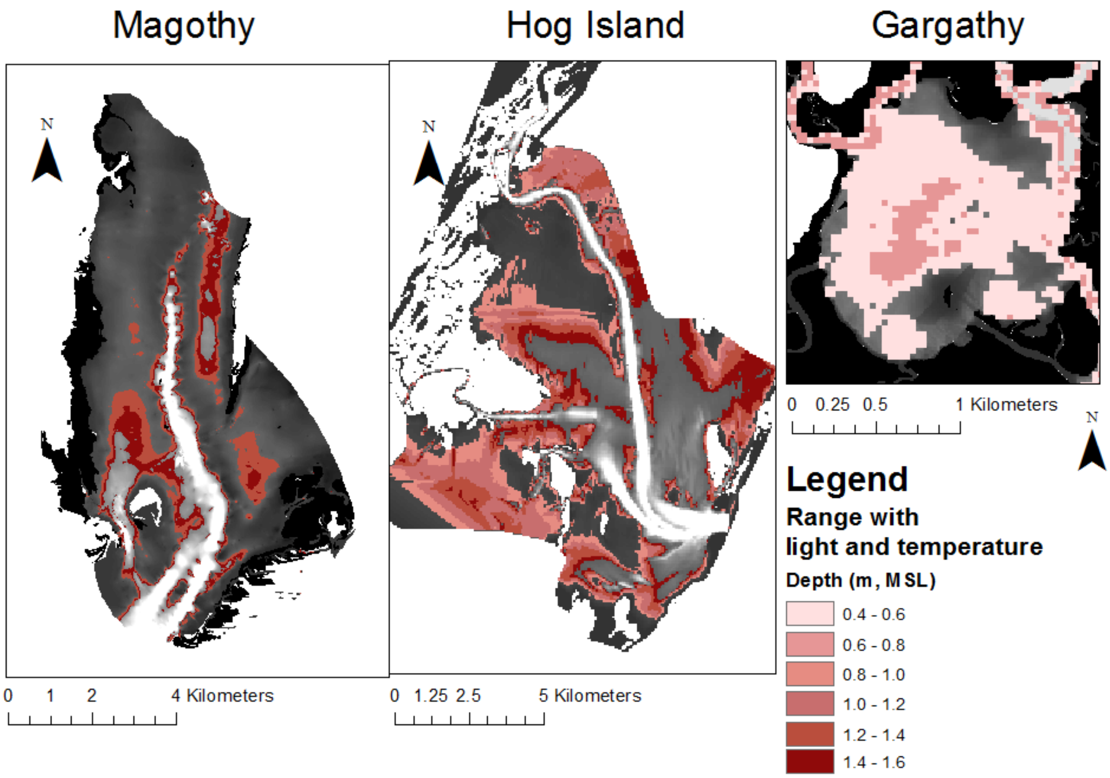


Figure 1.12. Predicted range based on light and temperature parameters with % incident light at the sediment surface dictating the maximum depth limit and % time > 28°C dictating the minimum depth limit. Areas shaded in pink to red are suitable for eelgrass growth with shallow areas depicted in light pink and deeper areas depicted in red.

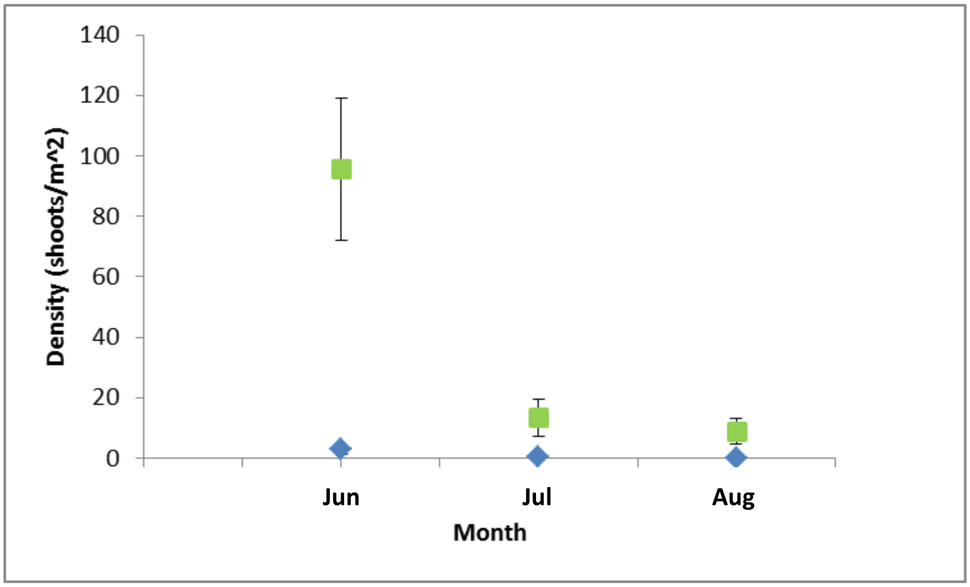


Figure 1.13. Density over summer 2013 of transplanted eelgrass in Gargathy Bay (blue) and Magothy Bay (green). Error bars represent standard error.

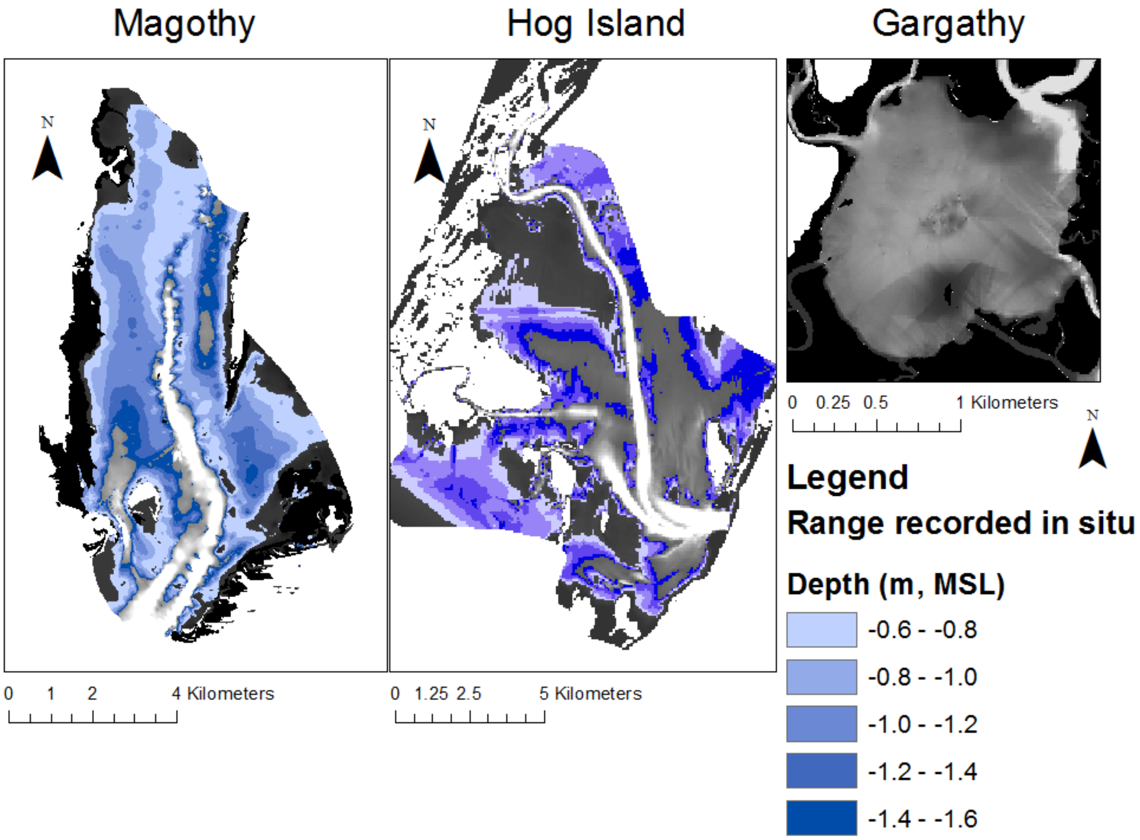


Figure 1.14. Area suitable for eelgrass growth in each bay based on transplant survival in unrestored Gargathy and Magothy Bays during summer 2013 and meadow depth in Hog Island Bay from McGlathery et al. 2012 and Reynolds et al. 2013. Areas shaded in blue are suitable for eelgrass growth.

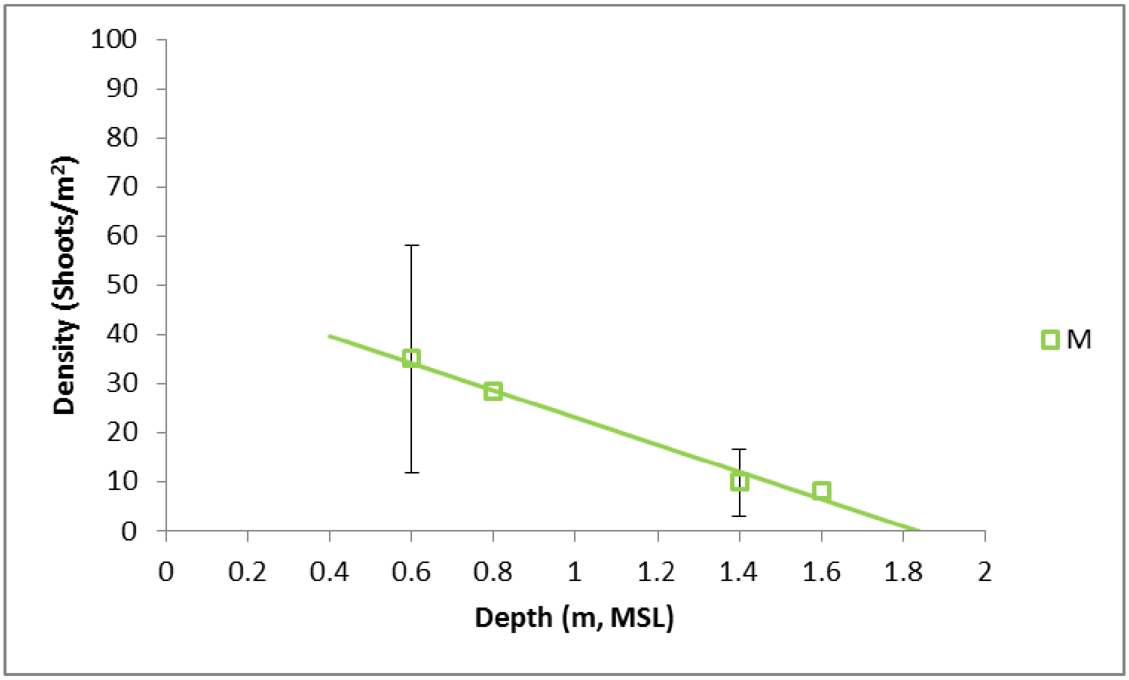


Figure 1.15. During August 2013, density decreases with depth from 0.6 m MSL to 1.6 m MSL in Magothy Bay (y = -39.81x + 87.22; F = 3.54, p = 0.0717). Error bars represent standard error.

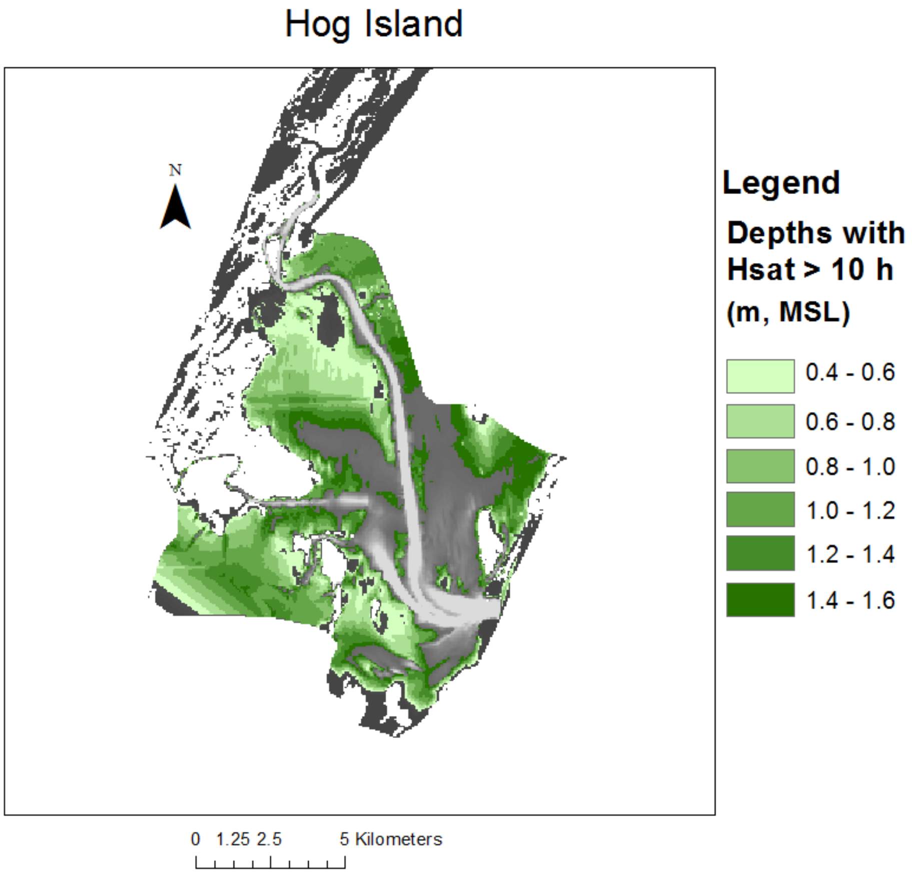


Figure 1.16. Areas with greater than 10 hours of saturation based on the equation for light compensation point in Hog Island Bay from Rheuban et al. *in press*.

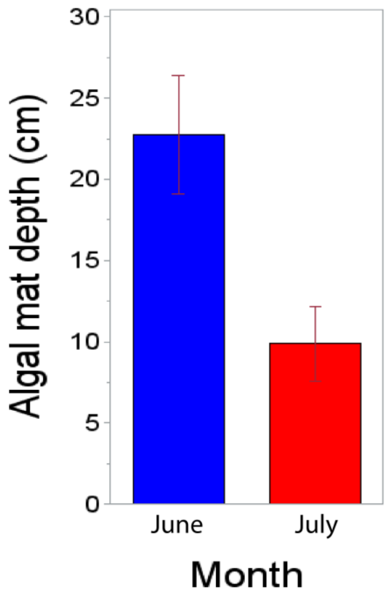


Figure 1.17. Average depth of *Gracilaria vermiculophylla* mats in Gargathy Bay during June and July 2013. Error bars represent standard error.

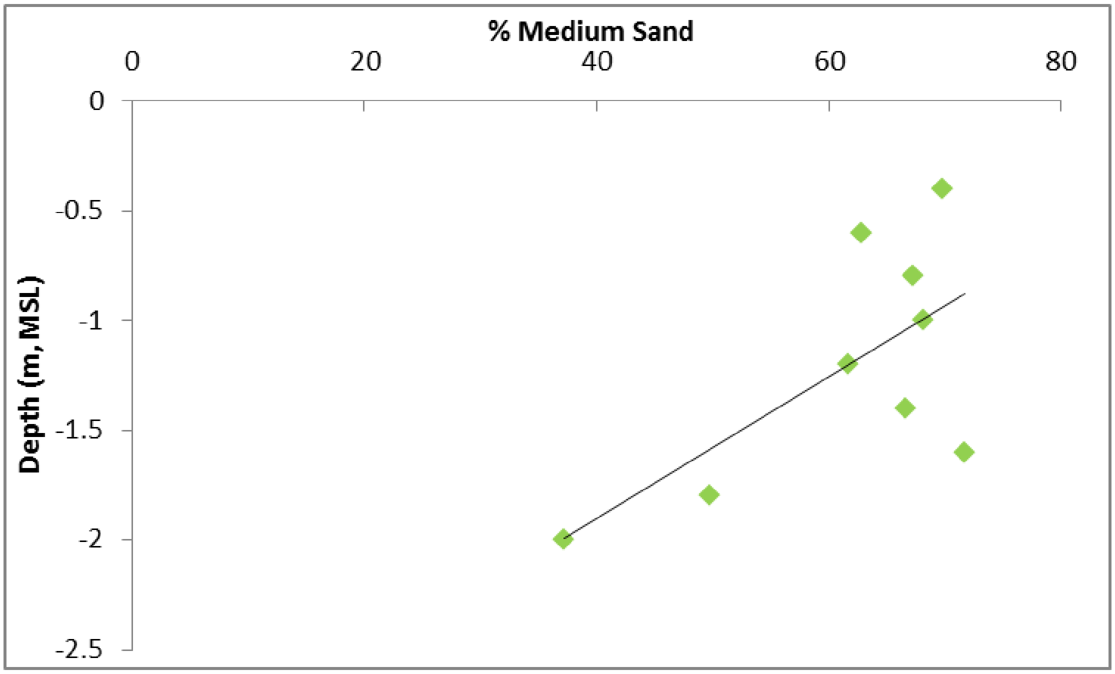


Figure 1.18. Percent medium sand declined with depth from 0.4 m to 2.0 m MSL in Magothy Bay ($y = 0.0323x - 3.1925$, $R^2 = 0.4384$).

Tables

Table 1.1. Locations of sites along each transect in Hog Island Bay.

Site	easting	northing
HA 0.4	435430	4141654
HA 0.6	435393	4141574
HA 0.8	435302	4141541
HA 1.0	435575	4140650
HA 1.2	435425	4140650
HA 1.4	435950	4140520
HA 1.6	435579	4140266
HA 1.8	435898	4140464
HA 2.0	434985	4139336
HB 0.4	435575	4141659
HB 0.6	435650	4141559
HB 0.8	435800	4141559
HB 1.0	435800	4141299
HB 1.2	435800	4141039
HB 1.4	436025	4140650
HB 1.6	436700	4140520
HB 1.8	436131	4140444
HB 2.0	436199	4140335
HC 0.4	435801	4141997
HC 0.6	435868	4141740
HC 0.8	435875	4141675
HC 1.0	435950	4141559
HC 1.2	436250	4141559
HC 1.4	436550	4141299
HC 1.6	437189	4141272
HC 1.8	437252	4141307
HC 2.0	437188	4141006

Table 1.2. Table of water and sediment quality results including standard error. Water quality results combine summers 2012 and 2013. Parameters that are not statistically different are shaded in grey.

	Gargathy Bay	**Hog Island Bay**	**Magothy Bay**
Sediment Quality (October 2011, n = 27)	*Mean +/- SE*		
Sediment Grain Size: Clay (% < 2 µm)	6.97 +/- 0.43	3.82 +/- 0.66	3.49 +/- 0.53
Sediment Grain Size: Silt (% 2 - 63 µm)	45.53 +/- 2.05	16.09 +/- 3.36	15.06 +/- 2.67
Sediment Grain Size: Fine Sand (% 63 - 200 µm)	37.10 +/- 2.47	78.76 +/- 3.98	16.89 +/- 2.44
Sediment Grain Size: Medium Sand (% 200 - 630 µm)	7.74 +/- 0.90	1.34 +/- 0.22	62.77 +/- 4.46
Sediment Grain Size: Course Sand (% > 630 µm)	2.66 +/- 1.10	0.0 +/- 0.0	1.81 +/- 0.45
Sediment Organic Matter (%)	4.72 +/- 0.24	1.43 +/- 0.20	1.28 +/- 0.16
Porosity (%)	32.28 +/- 0.85	23.63 +/- 0.72	21.35 +/- 0.59
Carbon Content (%)	1.56 +/- 0.09	0.31 +/- 0.03	0.40 +/- 0.07
Nitrogen Content (%)	0.130 +/- 0.010	0.018 +/- 0.002	0.077 +/- 0.007
Pore-Water Quality (2012, n = 9)			
Pore-water Sulfide (µM)	1244.1 +/- 310.6	12.6 +/- 3.7	494.0 +/- 185.9
Exchangeable Ammonium (µmol/g)	0.37 +/- 0.10	0.02 +/- 0/0	0.01 +/- 0.00
Water Quality (2012-2013, n = 12)			
Water Column Chlorophyll a (µg/L)	26.43 +/- 3.37	5.56 +/- 0.59	11.86 +/- 0.42
Ammonium (µM)	2.630 +/- 0.756	2.831 +/- 0.523	0.876 +/- 0.195
Ortho-phosphate (µM)	0.718 +/- 0.139	0.821 +/- 0.108	0.551 +/- 0.111
Total Dissolved Nitrogen (µM)	9.109 +/- 1.119	3.189 +/- 0.451	7.564 +/- 1.844
Nitrate + Nitrite (µM)	0.008 +/- 0.008	0.212 +/- 0.045	0.184 +/- 0.103
Total Suspended Solids (mg/L)	54.04 +/- 3.46	29.50 +/- 1.68	38.60 +/- 2.92
Dissolved Oxygen (mg/L) **(n = 3)**	7.00 +/- 0.13	7.09 +/- 0.10	7.06 +/- 0.06

Table 1.3. Table of water quality results from summer 2012 including standard error. Parameters that are not statistically different are shaded in grey.

	Gargathy Bay	**Hog Island Bay**	**Magothy Bay**
Water Quality 2012 (n = 9)	*Mean +/- SE*		
Water Column Chlorophyll a (µg/L)	33.0 +/- 2.4	6.8 +/- 0.6	12.5 +/- 0.4
Ammonium (µM)	7.296 +/- 0.746	4.097 +/- 0.667	0.56 +/- 0.221
Ortho-phosphate (µM)	1.094 +/- 0.074	0.564 +/- 0.030	0.724 +/- 0.052
Total Dissolved Nitrogen (µM)	7.025 +/- 0.578	3.189 +/- 0.451	4.2 +/- 1.113
Nitrate + Nitrite (µM)	0.127 +/- 0.079	0.151 +/- 0.072	0.299 +/- 0.117
Total Suspended Solids (mg/L)	78.33 +/- 6.69	27.56 +/- 0.69	39.22 +/- 3.10

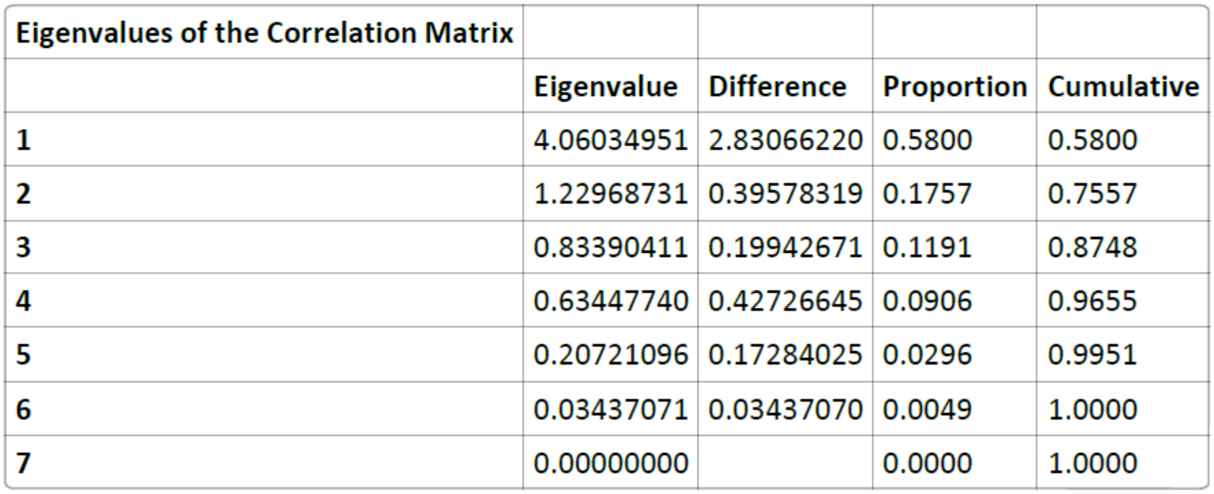
Table 1.4. Table comparing water quality results from 2012 and 2013 in each bay. Results include average for each parameter and standard error. Parameters that are not statistically different between each year are shaded in gray.

	Gargathy		**Hog Island**		**Magothy**	
Water Quality (n = 9)	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
Water Column Chlorophyll a (µg/L)	33.0 +/- 2.4	10.93 +/- 1.09	6.8 +/- 0.6	4.36 +/- 0.51	12.5 +/- 0.4	9.90 +/- 0.13
Ammonium (µM)	7.296 +/- 0.746	1.973 +/- 0.432	4.097 +/- 0.667	1.567 +/- 0.110	0.56 +/- 0.221	1.509 +/- 0.354
Ortho-phosphate (µM)	1.094 +/- 0.074	0.871 +/- 0.090	0.564 +/- 0.030	1.078 +/- 0.052	0.724 +/- 0.052	1.182 +/- 0.114
Total Dissolved Nitrogen (µM)	7.025 +/- 0.578	14.667 +/- 0.562	3.189 +/- 0.451	N/A	4.2 +/- 1.113	16.533 +/- 0.054
Nitrate + Nitrite (µM)	0.127 +/- 0.079	0.034 +/- 0.027	0.151 +/- 0.072	0.273 +/- 0.019	0.299 +/- 0.117	0.735 +/- 0.188
Total Suspended Solids (mg/L)	78.33 +/- 6.69	43.17 +/- 8.70	27.56 +/- 0.69	35.33 +/- 5.08	39.22 +/- 3.10	32.67 +/- 6.08

Table 1.5. Table of water quality results from summer 2013 including standard error. Parameters that are not statistically different are shaded in grey.

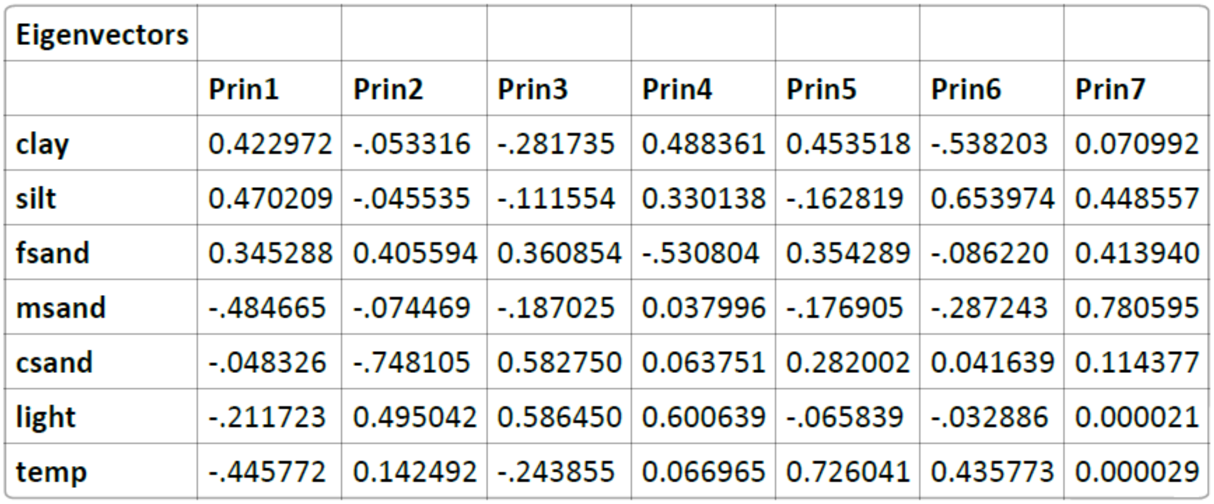
	Gargathy Bay	**Hog Island Bay**	**Magothy Bay**
Water Quality 2013 (n = 9)	*Mean +/- SE*		
Water Column Chlorophyll a (µg/L)	10.93 +/- 1.09	4.36 +/- 0.51	9.90 +/- 0.13
Ammonium (µM)	1.973 +/- 0.432	1.567 +/- 0.110	1.509 +/- 0.354
Ortho-phosphate (µM)	0.871 +/- 0.090	1.078 +/- 0.052	1.182 +/- 0.114
Total Dissolved Nitrogen (µM)	14.667 +/- 0.562	N/A	16.533 +/- 0.054
Nitrate + Nitrite (µM)	0.034 +/- 0.027	0.273 +/- 0.019	0.735 +/- 0.188
Total Suspended Solids (mg/L)	43.17 +/- 8.70	35.33 +/- 5.08	32.67 +/- 6.08

Table 1.6. Eigenvalues of the Correlation Matrix from the principal components analysis with density as the dependent variable and clay, silt, fine sand, medium sand, course sand, light, and temperature as the independent variables. The numbers in the first column represent the principal component number.



Eigenvalues of the Correlation Matrix				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	4.06034951	2.83066220	0.5800	0.5800
2	1.22968731	0.39578319	0.1757	0.7557
3	0.83390411	0.19942671	0.1191	0.8748
4	0.63447740	0.42726645	0.0906	0.9655
5	0.20721096	0.17284025	0.0296	0.9951
6	0.03437071	0.03437070	0.0049	1.0000
7	0.00000000		0.0000	1.0000

Table 1.7. Loading factors of the independent variables from the principal components analysis. Principal component 1 explains the most variance. Loading factors greater than +/- 0.4 are significant.



Eigenvectors							
	Prin1	**Prin2**	**Prin3**	**Prin4**	**Prin5**	**Prin6**	**Prin7**
clay	0.422972	-.053316	-.281735	0.488361	0.453518	-.538203	0.070992
silt	0.470209	-.045535	-.111554	0.330138	-.162819	0.653974	0.448557
fsand	0.345288	0.405594	0.360854	-.530804	0.354289	-.086220	0.413940
msand	-.484665	-.074469	-.187025	0.037996	-.176905	-.287243	0.780595
csand	-.048326	-.748105	0.582750	0.063751	0.282002	0.041639	0.114377
light	-.211723	0.495042	0.586450	0.600639	-.065839	-.032886	0.000021
temp	-.445772	0.142492	-.243855	0.066965	0.726041	0.435773	0.000029

Chapter 2: Quantifying changes in *Zostera marina* L. (eelgrass) productivity and photosynthetic capacity with changes in sediment characteristics and light attenuation

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Abstract

The effects of sediment characteristics and light attenuation on photosynthesis are important to consider when pinpointing areas for seagrass restoration. The maximum and minimum depths for eelgrass growth in the Virginia Coastal Bays were found over an environmental gradient in Al-Haj et al. (*in preparation*). This study uses a mesocosm experiment to separate the effects of light limitation and sediment characteristics on *Zostera marina* photosynthesis in the Virginia Coastal Bays using pulse amplitude modulated (PAM) fluorometry and measures of productivity. I took sediment from four bays: unrestored Gargathy Bay with high sulfide concentrations, high organic matter content, and a high percentage of fine sediment; unrestored Magothy Bay with low sulfide concentrations, low organic matter content, and a low percentage of fine sediment; Hog Island Bay in the process of being restored with low sulfide concentrations, low organic matter content, and a medium percentage of fine sediment; and South Bay the control where eelgrass was harvested. High organic content is associated with pore-water anoxia which can lead to increased sulfide concentrations in the pore-water and sulfide intrusion into plants. This can lead to declines in photosynthesis due to the toxic nature of these compounds. I found that based on the conditions used these sediment characteristics did not have an effect on optimal quantum yield or productivity, but that there was a strong light effect on optimal quantum yield indicating stress in shaded plants. Due to the lack of sediment effects, I could not identify a sediment-light effect on photosynthesis. This could be due to lack of flow in the mesocosms causing a difference between sulfide concentrations in the field and mesocosms. Future work could use a flume study paired with a mesocosm experiment to replicate a more natural flow environment and other parameters of stress such as non-

photochemical quenching, or heat dissipation, could be used to identify the effect of sediment characteristics and light on photosynthesis.

Introduction

Seagrasses provide many important ecosystem services to coastal areas such as habitat and food for marine organisms, improved water quality and clarity, sediment stabilization, carbon sequestration, and provision of organic matter and nutrients to the ocean (Orth et al. 2006b, Waycott et al. 2009, Short et al. 2011). However, seagrasses are declining globally due to environmental changes from anthropogenic impacts (Waycott et al. 2009, Orth et al. 2006b). Researchers have found that restoring seagrasses to coastal environments can also restore the ecosystem services they provide (McGlathery et al. 2012). In order to restore areas efficiently, it is important to be able to identify the types of environments that are most suitable for seagrasses by focusing on feedbacks between light availability, water quality, sediment characteristics and temperature (Benson et al. 2013, Carr et al. 2010, van der Heide et al. 2009). Finding the optimum habitat for seagrass growth has been the focus of many studies to date (Dennison et al. 1993, Biber et al. 2008, Carr et al. 2012), with a majority of these studies focusing on changes in the light environment as the driver for seagrass survival (Duarte 1991, Pederson et al. 2012, Gustaffson and Bostrom 2013). However, it is important to be able to identify how sediment characteristics affect seagrass growth separately from the light environment to enhance the success of restoration projects.

Seagrasses need 4% to 30% incident light at the sediment surface to survive (Dennison et al. 1993). Light attenuation through the water column constrains the maximum depth limit for seagrass survival (Duarte 1991, Li et al. 2013, Manuel et al. 2013). Many factors that affect water quality, such as sediment suspension, water column nutrient concentrations, and temperature, can change light attenuation through the water column as well as impact seagrass light requirements (Christian and Sheng 2003, Biber et al. 2008). Pederson et al. (2012) found that sediment suspension was inversely related to both particle size and organic matter content. Areas with fine sediment, high organic content, and high light attenuation, like those in the Pederson et al. (2012) study, often have low oxygen availability and high pore-water sulfide concentrations which can restrict seagrass growth by increasing light requirements (Goodman et al. 1995). Increasing temperature in high nutrient environments is linked to increasing algal productivity (Flanagan et al. 2003) as well as seagrass decline caused by increasing light requirements due to increases in seagrass respiration (Bulthuis 1987, Lee et al. 2007, Massa et al. 2009).

There are many methods used to identify if environmental conditions are causing seagrasses to be stressed. Survival and abundance are often used as measures of habitat quality over long periods of time (Moore and Jarvis 2008, Vaudrey et al. 2010, Benson et al. 2013). Benson et al. (2013) used survival of transplanted eelgrass as an indicator of habitat quality in Massachusetts estuaries. Moore and Jarvis (2008) used measures of eelgrass abundance as an indicator of habitat quality during summers in the Chesapeake Bay. Others use plant morphometrics such as leaf area, productivity, and above and below-ground biomass to indicate stress (Fertig et al. 2013, Villazan et al. 2013, Borum et al. 2014). Measures of carbohydrate reserves after environmental manipulation are used to study the effects of stressors on plant chemistry (Villazan et al. 2013). However, methods used to measure plant morphometrics and carbohydrate reserves are often destructive and may involve the removal of plants from the experiment (Fertig et al. 2013, Villazan et al. 2013, Borum et al. 2014).

Quantum yield and electron transport rate measurements can be used as a proxy for plant stress and are a non-invasive alternative to plant morphometric and carbohydrate reserve measurements often used in short-term, a few month-long, experiments (Campbell et al. 2007, Villazan et al. 2013). These measurements are an indicator of the “health” of photosystem II, or the amount of light utilized by photosystem II, in plants (Howarth and Durako 2013). Photosystem II is measured because it is considered the most sensitive to stress in electron transport (Howarth and Durako 2013). To measure maximum, or optimal, quantum yield (F_v/F_m) a saturating light pulse is applied to a plant leaf to close photosystem II receptors and the difference between minimum and maximum fluorescence is measured (Howarth and Durako 2013). Electron transport rate uses maximal fluorescence yield (F_m) and maximal variable fluorescence (F_v) to calculate the rate of electron movement across photosystem II during photosynthesis (Howarth and Durako 2013) where F_m is the fluorescence reached without heat dissipation, photochemical quenching, and F_v is maximal fluorescence minus minimal fluorescence (Walz 1998). Lideman et al. (2013) used a diving – pulse amplitude modulated (PAM) fluorometer to measure the effect of changes of temperature and light on photosynthetic activity in algae in order to find the exact range of temperatures to maintain high rates of photosynthetic activity in commercially grown algae. PAM fluorometry has been used to evaluate plant stress in a variety of experiments. Rapid light curves (RLC) from PAM fluorometry can be used to look at acclimation to an environment after transplanting (Sharon et al. 2009). Sharon et al. (2009) used reciprocal transplant experiments between shallow and deep depths to measure the acclimation potential of the seagrass. This method can also be used for long-term measurements of seagrass health (Durako 2012).

In the previous chapter, I identified areas in the Virginia coastal bays that are suitable for *Zostera marina* (eelgrass) restoration based on light, temperature, and sediment characteristics. However, it is difficult to separate the effects of light and temperature stress from sediment stressors in order to apply the information gained from the previous study to restoration projects in other regions. In order to do this, I planted eelgrass in sites with different sediment characteristics in a low light and control light environment and measured optimal quantum yield using a pulse amplitude modulated (PAM) fluorometer over a 28-day period during July 2013. Based on high pore-water sulfide concentrations, high organic matter, and fine sediment grain sizes in Gargathy Bay, plants in sediment from Gargathy Bay should have a lower photosynthetic efficiency and therefore show signs of more stress than plants in Hog Island, Magothy, and South Bay sediments at all light levels. When compounded with a low light environment, differences between photosynthetic efficiency of plants in Hog Island, Magothy, and South Bay should be identifiable.

Methods

Study Sites

This study was carried out in a salt-water flow-through mesocosm system on the dock of the Anheuser-Busch Coastal Research Center in Oyster Harbor on the eastern side of the Delmarva Peninsula facing the Virginia Coastal Bays. Six mesocosm tanks were used with light and temperature constantly monitored in each tank. Sediment cores were collected from two sites where seagrass has not been restored, Gargathy Bay (37°45'53.30"N, 75°33'23.10"W) and Magothy Bay (37° 9'56.12"N, 75°54'47.12"W), and two sites where seagrass restoration has been successful, Hog Island Bay (37°24'23.70"N, 75°43'45.05"W) and South Bay (37°15'53.60"N, 75°49'7.20"W) (Figure 2.1). Sediments from South Bay acted as control sediment because this is where seagrass was harvested. Sediments from Gargathy Bay, Magothy Bay, and Hog Island Bay represent a grain size (Table 2.1) (Figure 2.2), organic matter (4.72 +/- 0.24 %,1.28 +/- 0.16 %, 1.43 +/- 0.20 % respectively**)** (Figure 2.3), and pore-water sulfide concentration (1244.1 +/- 310.6, 494.0 +/- 185.9, 12.6 +/- 3.7) (Figure 2.4) gradient.

Water was pumped from the harbor and filtered through a 125 µm bag filter above each tank to prevent fouling. Each tank was cleaned daily through the seven-day acclimation period and the twenty-eight-day experimental period. When plants were acclimating to the environment in the mesocosms, the tanks remained uncovered. During the experimental period, three tanks were covered with 80% shade cloth (shaded treatment) and three tanks were covered with 10% shade cloth (unshaded treatment) (Figure 2.5).

Plant and Sediment Collection

Seven sediment cores were collected from each of Gargathy Bay, Magothy Bay, Hog Island Bay, and South Bay (control) and put into separate pots. Eelgrass was collected from South Bay, processed to check for reproductive shoots and standardized so each plant had three nodes on the rhizome, a rhizome at least 2 cm long, three leaves, and one shoot. Three plants were bundled together using a paper twist tie into a planting unit and were transplanted into each pot of sediment using a metal staple to hold the planting units in place (Davis and Short 1997) (Figure 2.5). Four pots, containing each type of sediment, were placed into each of the six running water mesocosms.

Pore-water sulfide concentrations were measured in each pot of sediment using the Cline method (Cline 1969) at the beginning and end of the experimental period. Organic matter content and sediment grain size averages for each sediment type were obtained from Al-Haj et al. (*in prep*) and McGlathery et al. (*unpublished data*). Optimum quantum yield (F_v/F_m) and electron transport rate (ETR) were measured using a Diving-Pulse Amplitude Modulated Flourometer (DIVING-PAM) (Walz, Germany) on each plant every day through the acclimation period and three days into the experimental period at which point frequency of measurements declined to every three to ten days. At the end of the acclimation period and every three days after in the experimental period, rapid light curves (RLCs) were taken on one plant from each sediment and light treatment. After the seven days for acclimation and fourteen days into the experimental period, each plant was pricked for productivity and collected two weeks later (Zieman 1974). Shoot-specific productivity was quantified by using mass (dry weight) and leaf area by measuring leaf length and width.

Statistics

Differences between sites were found using an ANOVA using Statistical Analysis System (SAS) version 9.3 software. Data that were not normally distributed were log transformed or square root transformed to meet the normality assumption. A Tukey post-hoc comparison test was used to determine which sites differ from each other. A repeated measures ANOVA was used to look at differences between treatments for optimal photosynthetic efficiency and electron transport rate.

Results

Mesocosm light and temperature

During the pre-experimental period, light and temperature did not vary significantly between mesocosms (Table 2.2). During the experimental period, light and temperature changed significantly between treatments (Figure 2.6 and Figure 2.7). Average temperature for the light treatments was significantly higher than average temperature for the dark treatments ($p < 0.0001$) (Table 2.3). Temperature in both treatments exceeded the temperature threshold, 28°C, for greater than 62% of the experimental period (Figure 2.7). As expected, average light intensity was significantly higher for the light treatments than the dark treatments ($p < 0.0001$) (Table 2.3) and % incident light at the sediment surface declined significantly from light to dark treatments ($p < 0.0001$) (Table 2.3).

Pore-water sulfide, organic matter content, and sediment grain size analysis

Sediment from Gargathy Bay contained higher concentrations of pore-water sulfides than sediment from Magothy Bay, Hog Island Bay, and South Bay (103.5 +/- 19.6 µM, 64.7 +/- 7.9 µM, 43.6 +/- 11.5 µM, and 62.3 +/- 9.8 µM respectively) (Figure 2.8); however, differences were not significant ($p = 0.087$). By the end of the experimental period, pore-water sulfide concentrations dropped in the Gargathy Bay treatments (Table 2.4) and South Bay treatments. However, concentrations did not differ significantly between Gargathy Bay, Magothy Bay, Hog Island Bay, or the South Bay dark treatment and the pre-experiment values ($p = 0.052$) (Table 2.4) (Figure 2.8 and Figure 2.9), but did differ between the South Bay light treatment and the pre-experiment values and the Hog Island Bay light treatment and South Bay and Magothy Bay pre-experiment concentrations ($p < 0.0001$). For the dark treatment, the Gargathy Bay treatment had higher pore-water sulfide concentrations than Magothy Bay, Hog Island Bay, and South Bay treatments (316.4 +/- 203.9 µM, 29.4 +/- 21.6 µM, 20.7 +/- 14.1 µM, and 28.5 +/- 19.1 µM respectively) (Figure 2.9a), but differences were not significant ($p = 0.212$) (Table 2.4). For the light treatment, there were no significant differences among bays ($p = 0.414$) (Table 2.4) (Figure 2.9b). Only South Bay light and dark treatments were significantly different (Table 2.4) (Figure 2.9).

Sediment organic matter content and grain size varied among bays. Sediment from Gargathy Bay had significantly higher sediment organic matter content than sediment from Hog Island Bay, Magothy Bay, and South Bay (4.72 +/- 0.18, 1.43 +/- 0.22, 1.28 +/- 0.23, 2.10 +/- 0.15 respectively) (Figure 2.10). Grain size varies based on clay, silt, fine sand, medium sand, and coarse sand percentages (Figure 2.11). Gargathy Bay had a higher clay (< 2 µm in diameter) content than Magothy Bay ($p < 0.0001$), but was not statistically different from Hog Island and

South bays (6.96 +/- 0.43 %, 3.47 +/- 0.53 %, 3.82 +/- 0.66 %, 4.21 +/- 0.28 % respectively). Sediment from Gargathy Bay also had higher clay (2 µm – 63 µm in diameter) content than sediment from both Hog Island Bay and Magothy Bay ($p < 0.0001$), but did not differ significantly from sediment from South Bay (45.53 +/- 2.05 %, 16.09 +/- 0.66 %, 15.06 +/- 2.67 %, 24.66 +/- 1.97 % respectively). Sediment from Magothy Bay had significantly less fine sand (63 µm – 200 µm in diameter) than sediments from Gargathy, Hog Island, and South bays (16.89 +/- 2.44 %, 37.10 +/- 2.47 %, 78.76 +/- 3.98%, 69.93 +/- 2.57%) ($p < 0.0001$). Sediment from Magothy had significantly more medium sand (200 µm – 630 µm) than Gargathy, Hog Island, and South bays (62.77 +/- 4.46 %, 7.74 +/- 0.90 %, 1.34 +/- 0.22 %, 1.20 +/- 0.66 %) and sediments from Gargathy had significantly more medium sand than sediment from Hog Island Bay and South Bay ($p < 0.0001$) which did not differ significantly. All sediment types were statistically the same for course sand (630 µm – 2000 µm) ($p = 0.7620$).

Stress measurements

The electron transport rate (ETR) declined throughout the experimental period in both light and dark treatments for each sediment type (Figure 2.12). The change in ETR over the experimental period was dependent on the light treatment ($F = 39.83$, $p < 0.\ 0001$), sediment treatment ($F = 1.96$, $p = 0.0376$), and the interaction between light treatment and sediment type ($F = 2.03$, $p = 0.0307$). Only the light treatment had an effect on the overall mean ETR ($F = 5.02$, $p = 0.0379$) with the least squares mean for the light treatment higher than the dark treatment for the first four measurement days and the least squares mean for the dark treatment higher than the light treatment for the last four measurement days (Figure 2.13). Sediment treatment did not have an effect on the overall mean ETR ($F = 0.34$, $p = 0.7974$).

Optimum quantum yield measurements taken at the same time as the ETR measurements did not appear to change over time (Figure 2.14); however, repeated measures ANOVA indicated that optimum quantum yield did depend on the time of measurement during the experimental period (F = 31.78, $p < 0.0001$). The change in optimum quantum yield over the experimental period was dependent on the light treatment (F = 40.28, $p < 0.0001$), sediment treatment (F = 2.08, p = 0.0265), and the interaction between the light treatment and sediment type (F = 2.36, p = 0.0120). A statistically significant difference exists between average optimum quantum yield for the light treatments (F = 14.94, p = 0.0011) with the pattern for the least squares mean of yield following the same pattern as the least squares mean of ETR (Figure 2.15). Even though there is an interaction effect between sediment treatment and light treatment, sediment treatment did not have an effect on the overall mean optimum quantum yield (F = 0.47, p = 0.7048) and light treatment and sediment treatment did not combine to influence overall average optimum quantum yield (F = 0.36, p = 0.7804).

Data from the rapid light curves is included in Appendix 4.

Productivity (Leaf area produced per square meter) and aboveground biomass did not vary significantly between light treatments (p = 0.7584, p = 0.3252 respectively) or among sediment types (Dark: p = 0.9407, Light: p = 0.3958; Dark: p = 0.9992, Light: p = 0.9407 respectively) (Figure 2.16, Figure 2.17).

Discussion

This study attempts to separate the effects of sediment characteristics and light attenuation on photosystem II in *Zostera marina* (eelgrass). Even though sediment grain size, organic matter, and sulfide concentrations varied significantly between bays (Figure 2.11, Figure 2.10, Figure 2.4), sulfide concentrations in the sediment collected did not maintain this pattern during the mesocosm experiment (Figure 2.8) and did not vary significantly between sediment or light treatments by the end of the experimental period (Figure 2.9). The lack of differences in sulfide concentration was reflected in both the electron transport rate and optimum quantum yield where the only differences evident were caused by differences in the light environment between shaded and unshaded mesocosms (Figure 2.12 and Figure 2.13).

In previous experiments, sulfide concentrations over 25 μM have been found to cause a decline in optimum quantum yield (F_v/F_m) over short (1.5 hour) incubation periods with an upper threshold for *Z. marina* survival at 1000 μM (Korhonen et al. 2012). Dooley et al. (2013) found that *Z. marina* seedlings were killed at concentrations of H_2S above 680 μM. These high concentrations also caused a decline in photosynthetic output and a complete shutdown of photosystem II (Dooley et al. 2013). This indicates that in this experiment sediment sulfide concentrations did not vary enough to show visible signs of stress in photosystem II because all concentrations varied from 25 – 125 μM. This would mean all plants were equally stressed by pore-water sulfide conditions within the mesocosms making it difficult to separate sediment effects. However, measurements in the field indicate that seagrasses cannot survive in sediments in Gargathy Bay because sulfide concentrations exceed the 1000 μM upper threshold (Al-Haj et al. *in preparation*, Korhonen et al. 2012). Field measurements and mesocosm measurements of pore-water sulfides varied greatly with sediments from Gargathy Bay containing 1244.1 +/- 310.6 μM in the field and an average of 103.5 +/- 19.6 μM in the mesocosms. Sediments from Magothy Bay also showed a decline in sulfide measurements between the field and mesocosm experiments (field: 494.0 +/- 185.9 μM, mesocosm: 64.7 +/- 7.9 μM). Measurements from sediments from Hog Island Bay did not change greatly between the field and mesocosm experiments (field: 12.6 +/- 3.7, mesocosm: 43.6 +/- 11.5 μM) (Al-Haj et al. *in preparation*).

However, all measurements, except for the Hog Island Bay field average, are indicative of a stressful environment for eelgrass.

Many studies use plant productivity as a way to quantify photosynthetic stress (Peterson and Heck 2001, Carroll et al. 2008, Wall et al. 2011, Skinner et al. 2013). Peterson and Heck (2001) used productivity to quantify positive interactions between *Thalassia testudinum* and bivalves and found that the presence of mussels at densities greater than 500 m^{-2} reduced epiphytic loads on leaves and resulted in increased productivity. In other studies, productivity was used in conjunction with measures of optimal quantum yield in order to more accurately differentiate between stressors (Flores et al. 2013, Howarth and Durako 2013). Flores et al. (2013) used leaf elongation measurements paired with optimal quantum yield to look at the effects of pesticides on photosystem II and productivity in four seagrass species found in the Great Barrier Reef lagoon. Howarth and Durako (2013) compared optimal quantum yield and leaf growth in seedlings in high and low light environments with different salt treatments. They found that plants in low-light environments had high optimal quantum yield indicating acclimation and plants in hypersaline solutions had low optimal quantum yield indicating stress (Howarth and Durako 2013). In our study, there were no significant differences between light treatments or sediment treatments in terms of productivity or aboveground biomass. Many studies have found that plants in muddy areas with high organic matter content, such as plants in sediment from Gargathy Bay, show increases in aboveground biomass (Wicks et al. 2009, Lee and Dunton 2000). This indicates the experimental period may have not been long enough for plants to alter their morphology or growth patterns. However, there were significant differences in both ETR and optimal quantum yield over all treatments with time indicating that the plants were not doing well in the mesocosms and were not differently affected by sediment treatment.

Stress due to mesocosm environment can be detected by looking at the ETR curves. For each light treatment and sediment type, ETR declines exponentially at the same point in time and remains low. One reason for this can be explained by high temperatures in the mesocosms with temperature exceeding 28°C for over 62% of the experimental period for all treatments (Figure 2.7). Studies have shown that long durations of high temperatures can cause the decline of *Z. marina* (Lee et al. 2005, Moore and Jarvis 2008). In the Virginia coastal bays and Chesapeake Bay, *Z. marina* experiences a bimodal form of growth where they lose leaves due to high temperature stress in the summer and have low biomass in the winter due to low temperature and lack of light (Olesen and Sand-Jenson 1994, Moore and Jarvis 2008).

Light limitation is only one of many factors influencing seagrass survival (Dennison 1987, Dennison et al. 1993, Biber et al. 2009). Other variables that can affect growth include hydrodynamics (Koch 2001), geologic factors (Koch 2001), nutrient availability (McGlathery et al. 2007), and concentration of toxic compounds (Flores et al. 2013). In mesocosm experiments, it is difficult to replicate physical conditions, such as hydrodynamics, which can have an effect on pore-water nutrient concentrations (Koch 1999). Koch (1999) found that very low and high flow velocities can lower the biomass of *Thalassia testudinum* by increasing pore-water sulfide concentrations and reducing nutrient concentrations within the pore-water. In the mesocosms used for this experiment, water entered the tanks from a pipe above and exited from a pipe about 7 cm below the water surface. This configuration did not provide for complete flow to the bottom of the tank, about 0.8 m, where seagrasses were placed. Theoretically this should cause an increase in sulfide concentrations over time because factors controlling microbial activity, such as sediment organic matter, and porosity, clay content and grain size, did not change. However, there was no statistical difference between pore-water sulfide concentrations between the beginning and end of the experiment (Figure 2.8 and Figure 2.9) indicating that the sediments were being flushed. Because there was a difference between sulfide concentrations within the bays and sulfide concentrations in the sediments used in the mesocosm experiment, there could have been advection occurring in the cores due to their shallow depth and the flow of the water through the tanks into and out of the top. This can be controlled by increasing core diameter and depth and altering flow patterns through the tanks by having water come down one side of the tank through a long pipe and leave through the other side.

Another reason for the lack of differences among sediment types could be due to the variable concentrations of sulfides in the sediment in these areas based on microclimate (Sherman et al. unpublished data, Borum et al. 2014). Because sulfide concentration is very variable across transects in Hog Island Bay (Sherman et al. unpublished data) and is also shown to be variable over the seascape in eelgrass beds on the coast of Denmark (Borum et al. 2014), the small cores taken for this experiment may not have represented the range of variability within each bay that was represented during the field experiment. This indicates that a higher sample size may be needed to represent the full range of sulfide concentrations in sediment types studied.

Another way to control for sulfide concentrations would be to pump an inert gas such as N_2 or Ar into the sediments in the mesocosms to slow down sediment aeration and to control for iron concentrations within the sediment to prevent sulfide oxidation from occurring. Moscaro et al. (2009) controlled organic matter concentrations and aeration of the sediments in order to test the conditions for sulfide intrusion into *Z. marina*. However, they did not change the aeration of the sediment by adding N_2 or Ar; instead they turned off the water supply to the low aeration treatment at night (Moscaro et al. 2009). This would not work with our mesocosm system due to the high temperatures within the tanks. If water were allowed to stagnate, temperatures could rise even higher. Even though there is not a clear method in the literature for creating anoxic sediments using N_2 and Ar, N_2 and Ar are often used to create an anoxic environment for sulfide analysis (Cline 1969). It may have also been beneficial to check iron concentrations within the sediment throughout the experimental period because a metal staple was used to hold the seagrasses in place after transplanting. This artificial increase in iron within the sediment can inhibit the reduction of sulfide because sulfate reducing bacteria may use iron as an electron acceptor instead of sulfate (Moeslund et al. 1994, Moscaro et al. 2009).

The motivation for this study was to be able to distinguish between sediment and light stressors limiting the range for eelgrass growth in Al-Haj et al. (*in* preparation). Due to differences between sulfide concentrations in the mesocosms and in the field, this study does not represent an accurate representation of sediment characteristics within the Virginia Coastal Bays. However, it does indicate a need to modify the experiment to control for more factors such as increased flushing of the sediments in the mesocosms. This supports the findings of hydrodynamic studies that found that the wave, current, and tidal environment interact with sediment characteristics, nutrients, and chemical stressors in order to predict seagrass survival (Koch 1999, Koch 2001, Wicks et al. 2009). Wicks et al. (2009) found that *Z. marina* in Chincoteague Bay could only survive in sediments with < 4% sediment organic content, but could survive in mesocosms in sediments with up to 6% sediment organic content. In high organic content sediments, plants put more resources into building aboveground biomass than belowground biomass because they are not as nutrient limited in the soil (Lee and Dunn 2000). Wicks et al. (2009) suggested that increases in *Z. marina* leaf width and shoot length paired with less root growth in sediments with high organic content introduced more shear stress on the plants in the hydrologic conditions present in Chincoteague Bay which may have caused the plants with more aboveground biomass to be ripped out of the sediment; however, they did not replicate these conditions within mesocosms (Wicks et al. 2009). The increase in aboveground to belowground biomass ratios increases respiratory demand which increases light requirements (Collier et al. 2007). In the future, it would be beneficial to perform mesocosm experiments in flumes or to pair mesocosm experiments with field experiments as in Wicks et al. (2009) in order to account for the effect of hydrodynamic conditions on sediment organic content, grain size, and sulfide concentrations in coastal lagoons.

Other modifications can be made to this experiment in order to help distinguish between sediment and light stressors. Measuring photochemical quenching, the amount of open photosystem II reaction centers, and non-photochemical quenching, a measure of the amount of heat dissipation occurring, may be a better way of determining differences in stress among treatments. Non-photochemical quenching is a photoprotective process that occurs when a plant has more light coming in than it can use (Marin-Guirao et al. 2013). When photosynthetic capacity declines due to increases in stress, photoprotective processes take over (Marin-Guirao et al. 2013). Collier et al. (2009) compared the non-photochemical quenching of shaded plants to control plants during a recovery period after shading and found that the plants that had been shaded had much higher non-photochemical quenching than control plants indicating stress in the shaded plant treatment. Marin-Guirao et al. (2013) compared non-photochemical quenching of plants at different salinity levels and found that high salinity did not have an effect on the plants, but light conditions did. Allowing for a recovery period where all tanks are subjected to normal light conditions after the experimental period, would allow for measurement of continued stress due to non-photochemical quenching and may allow for a better separation of sediment stressors. Since temperatures in the light and dark treatments exceeded 28°C for greater than 62% of the experimental period (Figure 2.7), it would be beneficial to control for temperature as well.

Conclusions

Effects of sediment characteristics and light attenuation on *Z. marina* photosystem II and productivity need to be quantified in order to help identify areas where eelgrass can persist. This is particularly important when considering the warming that is predicted in the Virginia Coastal Bays due to climate change in the next century. Increased warming can lead to increased microbial activity in areas with high organic matter content, such as mature seagrass beds (Goodman et al. 1995, Holmer and Bondgaard 2001). This can cause anoxia in the rhizosphere; therefore, increasing sulfide concentrations in the pore-water (Goodman et al. 1995, Holmer and Bondgaard 2001, Raun and Borum 2013). Based on this study, being able to pinpoint areas for restoration only based on sediment characteristics and light attenuation is not realistic because hydrodynamics play an important role in dictating morphological characteristics for plant survival based on shear stress and flushing of the pore-water (Koch 1999, Koch 2001, Wicks et al. 2009).

In the Virginia Coastal Bays, a hydrodynamic seagrass growth model exists (Carr et al. 2010, Carr et al. 2012), but more field studies on the impact of sediment characteristics on eelgrass photosystem II and productivity need to be performed in order to accurately apply the model to other areas of the Virginia Coast Reserve. Mesocosm experiments were not sufficient to separate the impacts of light, organic content, grain size, and sulfide concentrations on eelgrass growth due to small sample size and lack of flow through the mesocosms. Using a paired mesocosm-flume system as well as having an accompanying field study would alleviate some of the issues found during this study for separating the effects of light and sediment characteristics to feed back in to models. Having more accurate models of coastal areas, will help managers pinpoint areas for seagrass restoration world-wide returning many of the ecosystem services lost due to decline (Waycott et al. 2009).

Literature Cited

Al-Haj, A. N., K. J. McGlathery, P. L. Wiberg, A. C. Schwarzschild. Variations in the maximum and minimum depth limits for Zostera marina L. (eelgrass) growth across an environmental gradient in the Virginia Coastal Bays. *In preparation*.

Benson, J. L., D. Schlezinger, B. L. Howes. 2013. Relationship between nitrogen concentration, light, and *Zostera marina* habitat quality and survival in southeastern Massachusetts estuaries. *Journal of Environmental Management* **131**: 129 – 137.

Biber, P. D., C. L. Gallegos, and W. J. Kenworthy. 2008. Calibration of a Bio-optical Model in the North River, North Carolina (Albemarle–Pamlico Sound): A Tool to Evaluate Water Quality Impacts on Seagrasses. *Estuaries and Coasts* **31**: 177 – 191.

Biber, Patrick D., W. Judson Kenworthy, and Hans W. Paerl. 2009. Experimental analysis of the response and recovery of Zostera marina (L.) and Halodule wrightii (Ascher.) to repeated light-limitation stress. *Journal of Experimental Marine Biology and Ecology* **369**: 110 – 117.

Borum, J., A. L. Raun, H. Hasler – Sheetal, M. O. Pederson, O. Pederson, M. Holmer. 2014. Eelgrass fairy rings: sulfide as inhibiting agent. *Marine Biology* **161**: 351 – 358.

Bulthuis, D. A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany* **27**: 27 – 40.

Campbell, S. J., L. J. McKenzie, S. P. Kerville, J. S. Bite. 2007. Patterns in tropical seagrass photosynthesis in relation to light, depth and habitat. *Estuarine, Coastal and Shelf Science* **73**: 551 – 562.

Carr, J., P. D'Odorico, K. McGlathery, P. Wiberg. 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. *Journal of Geophysical Research* **115**: doi:10.1029/2009JG001103.

Carr, Joel A., Paolo D'Odorico, Karen J. McGlathery, Patricia L. Wiberg. 2012. Stability and resilience of seagrass meadows to seasonal and interannual dynamics and environmental stress. *Journal of Geophysical Research* **117**: doi:10.1029/2011JG001744.

Carroll, J., C. J. Gobler, B. J. Peterson. 2008. Resource-restricted growth of eelgrass in New York estuaries: light limitation, and alleviation of nutrient stress by hard clams. *Marine Ecology Progress Series* **369**: 51 – 62.

Christian, D., Y. P. Sheng. 2003. Relative influence of various water quality parameters on light attenuation in Indian River Lagoon*. Estuarine, Coastal, and Shelf Science* **57**: 961 – 971.

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* **14**: 454 – 458.

Collier, C. J., P. S. Lavery, R. J. Masini, P. J. Ralph. 2007. Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Marine Ecology Progress Series* **103**: 103 – 115.

Collier, C. J., P. S. Lavery, P. J. Ralph, R. J. Masini. 2009. Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. *Journal of experimental marine biology and ecology* **370**: 89 – 103.

Davis R. C. and F. T. Short. 1997. Restoring eelgrass, *Zostera marina* L., habitat using a new transplanting technique: The horizontal rhizome method. *Aquatic Botany* **59**: 1 – 15.

Dennison, W. C. 1987. Effects of light on seagrass photosynthesis, growth, and depth distribution. *Aquatic Botany* **27**: 15 – 26.

Dennison, W. C., R. J. Orth, K. A. Moore, J. C. Stevenson, V. Carter, S. Kollar, P. W. Bergstrom, R. A. Batiuk. 1993. Assessing Water Quality with Submersed Aquatic Vegetation: Habitat requirements as barometers of Chesapeake Bay Health. *BioScience* **43**: 86 – 94.

Dooley, F. D., S. Wyllie – Echeverria. M. B. Roth. P. D. Ward. 2013. Tolerance and response of *Zostera marina* seedlings to hydrogen sulfide. *Aquatic Botany* **105**: 7 – 10.

Duarte, C. M. 1991. Seagrass depth limits. *Aquatic Botany* **40**: 363 – 377.

Durako, M. J. 2012. Using PAM fluorometry for landscape-level assessment of *Thalassia testudinum*: can diurnal variation in photochemical efficiency be used as an ecoindicator of seagrass health?. *Ecological Indicators* **18**: 243 – 251.

Fertig, B., M. J. Kennish, G. P. Sakowicz. 2013. Changing eelgrass (*Zostera marina* L.) characteristics in a highly eutrophic temperate coastal lagoon. *Aquatic Botany* **104**: 70 – 79.

Flanagan, K. M., E. McCauley, F. Wrona, T. Prowse. 2003. Climate change: The potential for latitudinal effects on algal biomass in aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* **60**: 635 – 639.

Flores, F.. C. J. Collier, P. Mercurio, A. P. Negri. 2013. Phytotoxicity of four photosystem II herbicides to tropical seagrasses. *PLOSone* **8**: e75798, doi:10.1371/journal.pone.0075798.

Goodman, J. L., K. A. Moore, W. C. Dennison. 1995. Photosynthetic responses of eelgrass (*Zostera marina*) to light and sediment sulfide in a shallow barrier lagoon. *Aquatic Botany* **50**: 37-48.

Gustafsson, C. and C. Bostrom. 2013. Influence of neighboring plants on shading stress resistance and recovery of eelgrass, *Zostera marina* L. *PLoSONE* **8**: e64064. doi:10.1371/journal.pone.0064064.

Holmer, M., E. J. Bondgaard. 2001. Photosynthesis and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. *Aquatic Botany* **70**: 29 – 38.

Howarth, J. F., M. J. Durako. 2013. Diurnal variation in chlorophyll fluorescence of *Thalassia testudinum* seedlings in response to controlled salinity and light conditions. *Marine Biology* **160**: 591 – 605.

Koch. E. 1999. Preliminary evidence on the interdependent effect of currents and porewater geochemistry on *Thalassia testudinum* Banks ex Konig seedlings. *Aquatic Botany* **63**: 95 – 102.

Koch, E. 2001. Beyond Light: Physical, Geological, and Geochemical Parameters as Possible Submersed Aquatic Vegetation Habitat Requirements. *Estuaries* **24**: 1 – 17.

Korhonen, L. K., V. Macias – Carranza, R. Abdala, F. L. Figueroa. A. Cabello – Pasini. 2012. Effects of sulfide concentration, pH, and anoxia, on photosynthesis and respiration of *Zostera marina*. *Ciencias Marinas* **38**: 625 – 633.

Lee, K. S., K. H. Dunton. 2000. Effects of nitrogen enrichment on biomass allocation, growth, and leaf morphology of the seagrass *Thalassia testudinum*. *Marine Ecology Progress Series* **196**: 39 – 48.

Lee, K. S., S. R. Park, J. B. Kim. 2005. Production dynamics of the eelgrass, Zostera marina in two bay systems on the south coast of the Korean peninsula. *Marine Biology* **147**: 1091 – 1108.

Lee, K. S., S. R. Park, Y. K. Kim. 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology* **350**: 144 – 175.

Li, W. T., S. H. Kim, J. W. Kim, J. H. Kim, K. S. Lee. 2013. An examination of photoacclimatory responses of Zostera marina transplants along a depth gradient for transplant-site selection in a disturbed estuary. *Estuarine, Coastal and Shelf Science* **118**: 72 – 79.

Manuel, S. A., K. A. Coates, W. J. Kenworthy, J. W. Fourqurean. 2013. Tropical species at the northern limit of their range: Composition and distribution in Bermuda's benthic habitats in relation to depth and light availability. *Marine Environmental Research* **89**: 63 – 75.

Marin-Guirao, L., J. M. Ruiz, J. M. Sandoval-Gil, J. Berbardeau-Esteller, C. M. Stinco, A. Melendez-Martinez. 2013. Xanthophyll cycle-related photoprotective mechanism in the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* under normal and stressful hypersaline conditions. *Aquatic Botany* **109**: 14 – 24.

Massa, S. I., S. Arnaud-Haond, G. A. Pearson, E. A. Serrao. 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal). *Hydrobiologia* **619**: 192 – 201.

McGlathery K. J., K. Sundback, I. C. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Marine Ecology Progress Series* **348**: 1 – 18.

McGlathery, K. J., L. K. Reynolds, L. W. Cole, R. J. Orth, S. R. Marion, A. Schwarzschild. 2012. Recovery trajectories during state change from bare sediment to eelgrass dominance. *Marine Ecology Progress Series* **448**: 209 – 221.

Moeslund, L., B. Thamdrup, B. B. Jorgensen. 1994. Sulfur and iron cycling in a coastal sediment: Radiotracer studies and seasonal dynamics. *Biogeochemistry* **27**: 129 – 152.

Moore, K. A., J. C. Jarvis. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: Implications for long-term persistence. *Journal of Coastal Research* **55**: 135 – 147.

Moscaro, O., T. Valdemarsen, M. Holmer, M. Perez, J. Romero. 2009. Experimental manipulation of sediment organic content and water column aeration reduces *Zostera marina* (eelgrass) growth and survival. *Journal of Experimental Marine Biology and Ecology* **373**: 26 – 34.

Olesen, B., K. Sand-Jensen. 1994. Demography of shallow eelgrass (*Zostera marina*) populations – shoot dynamics and biomass development. *Journal of Ecology* **82**: 379 – 390.

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. Williams. 2006b. A Global Crisis for Seagrass Ecosystems. *BioScience* **56**: 987 – 996.

Pederson, T. M., C. L. Gallegos, S. L. Nielsen. 2012. Influence of near-bottom re-suspended sediment on benthic light availability. *Estuarine, Coastal, and Shelf Science* **106**: 93 – 101.

Peterson, B. J., K. L. Heck Jr. 2001. Positive interactions between suspension-feeding bivalves and seagrass – a facultative mutualism. *Marine Ecology Progress Series* **213**: 143 – 155.

Raun, A. L., J. Borum. 2013. Combined impact of water column oxygen and temperature on internal oxygen status and growth of *Zostera marina* seedlings and adult shoots. *Journal of experimental marine biology and ecology* **441**: 16 – 22.

Sharon, Y., J. Silva, R. Santos, J. W. Runcie, M. Chernihovsky, S. Beer. 2009. Photosynthetic responses of *Halophils stipulacea* to a light gradient. II. Acclimations following transplantation. *Aquatic Biology* **7**: 153 – 157.

Sherman, A. E., A. N. Al-Haj, K. J. McGlathery. Covariance of sulfide scncentration and percent organic matter with grain size in the Virginia Coastal Bays. *Unpublished data*.

Short, F. T., B. Polidoro, S. R. Livingstone, K. E. Carpenter, S. Bandeira, J. S. Bujang, H. P. Calumpong, T. J. B. Carruthers, R. G. Coles, W. C. Dennison, P. L. A. Erftemeijer, M. D. Fortes, A. S. Freeman, T. G. Jagtap, A. H. M. Kamal, G. A. Kendrick, W. J. Kenworthy, Y. A. La Nafie, I. M. Nasution, R. J. Orth, A. Prathep, J. C. Sanciangco, B. van Tussenbroek, S. C. Vergara, M. Waycott, J. C. Zieman. 2011. Extinction risk assessment of the world's seagrass species. *Biological Conservation* **144**: 1961 – 1971.

Skinner, M. A., S. C. Courtenay, C. W. McKindsey. 2013. Reductions in distribution, photosynthesis, and productivity of eelgrass *Zostera marina* associated with oyster *Crassostrea virginica* aquaculture. *Marine Ecology Progress Series* **486**: 105 – 119.

van der Heide, T., E. T. H. M. Peeters, D. C. R. Hermus, M. M. van Katwijk, J. G. M. Roelofs, A. J. P. Smolders. 2009. Predicting habitat suitability in temperate seagrass ecosystems. *Limnology and Oceanography* **54**: 2018 – 2024.

Vaudrey, J. M. P., J. N. Kremer, B. F. Branco, F. T. Short. 2010. Eelgrass recovery after nutrient enrichment reversal. *Aquatic Botany* **93**: 237 – 243.

Villazan, B. M. F. Pederson, F. G. Brun, J. J. Vergara. 2013. Elevated ammonium concentrations and low light form a dangerous synergy for eelgrass *Zostera marina*. *Marine Ecology Progress Series* **493**: 141 – 154.

Walz. 1998. Underwater fluorometer DIVING-PAM submersible photosynthesis yield analtzer: Handbook of operation, 2.128 / 07.98, 1st Edition, Effeltrich, Germany.

Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, Jr., A R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Publications of the National Academy of Sciences* **106**: 12377 – 12381.

Wicks, E. C., E. W. Koch, J. M. O'Neil, K. Elliston. 2009. Effects of sediment organic content and hydrodynamic conditions on the growth and distribution of *Zostera marina*. *Marine Ecology Progress Series* **378**: 71 – 80.

Zieman, J. C. 1974. Methods for the study of growth and production of Turtle Grass Thalassia testudinum. *Aquaculture* **4**: 139 – 143.

Figures

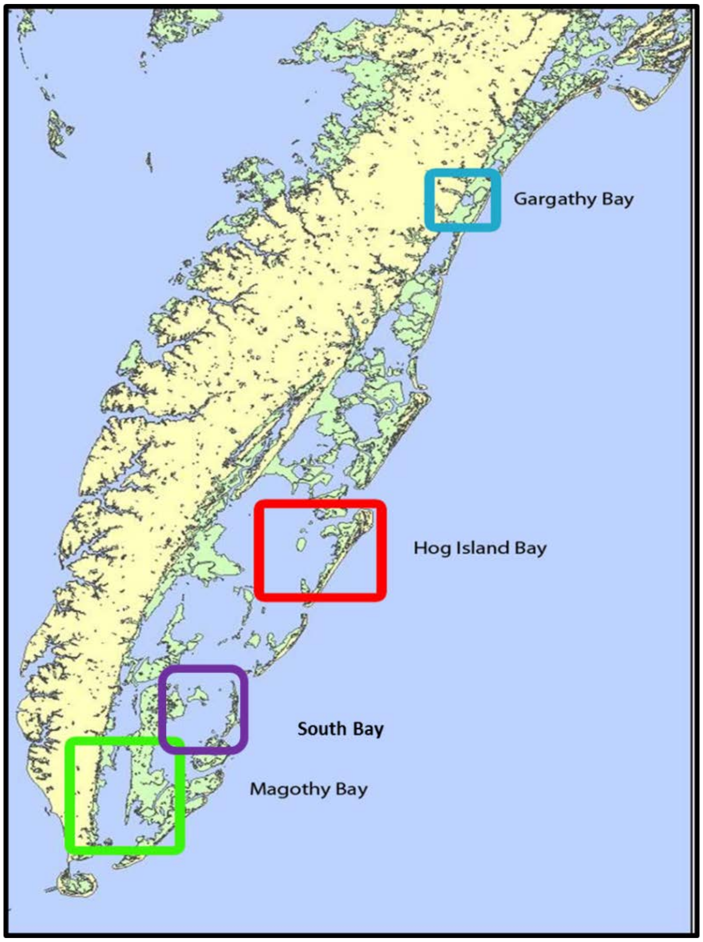


Figure 2.1. Image of the Delmarva Peninsula with study sites outlined; unrestored Gargathy Bay in blue, Hog Island Bay in the process of being restored in red, restored South Bay in purple, and unrestored Magothy Bay in green.

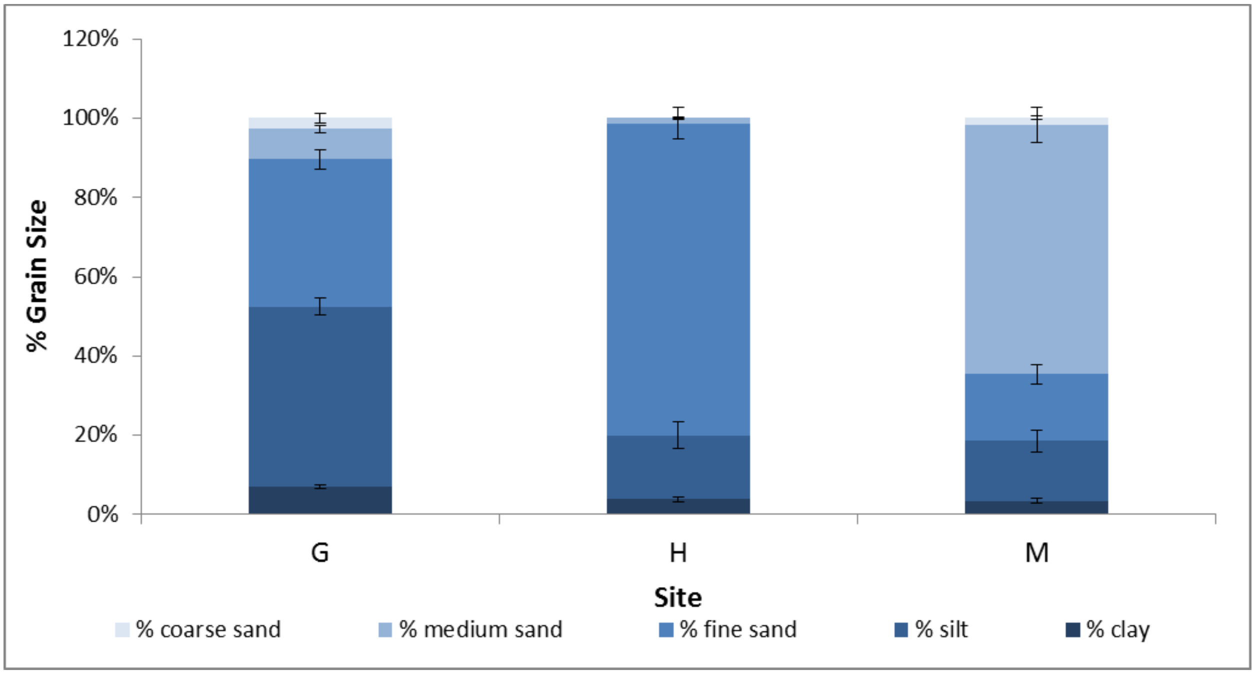


Figure 2.2. Distribution of clay (<2 µm), silt (2 – 63 µm), fine sand (63 – 200 µm), medium sand (200 – 630 µm) and coarse sand (>630 µm) at sites in Gargathy Bay, Magothy Bay, and Hog Island Bay with error bars representing standard error (n = 27).

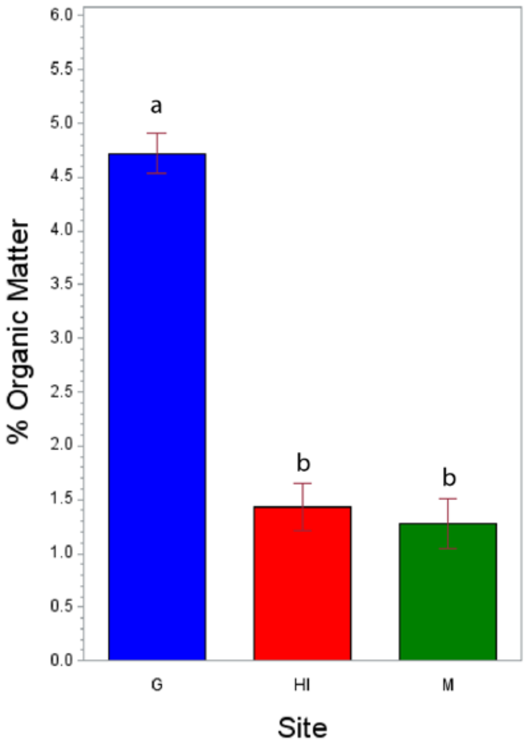


Figure 2.3. Percent organic matter in sediment of sites in unrestored bays, Gargathy (G) and Magothy (M) compared to bare sites in a bay in the process of being restored, Hog Island Bay (HI). Error bars represent standard error (n = 27). Letters represent bars statistically the same.

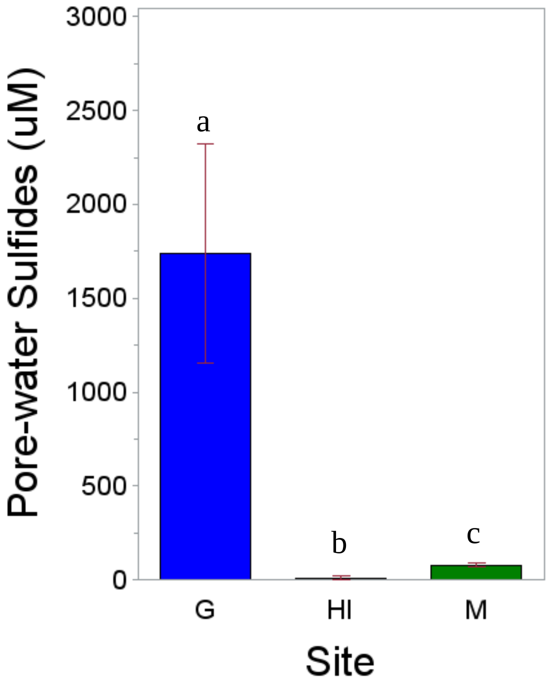


Figure 2.4. Sediment pore-water sulfide concentrations of sites in unrestored bays, Gargathy (G) and Magothy (M), compared to bare sites in a bay in the process of being restored, Hog Island Bay (HI). Error bars represent standard error (n = 9). Letters represent bars statistically the same.

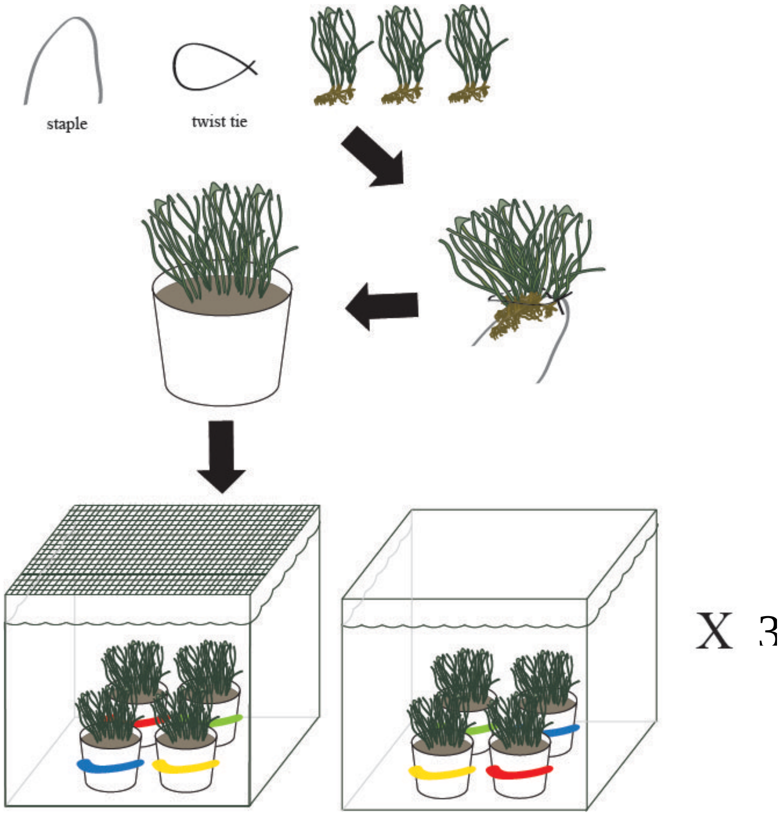


Figure 2.5. Conceptual diagram of the mesocosm planting method. To create a planting unit, a twist tie was wrapped around a three-plant bundle of eelgrass and a metal staple was attached. Each planting unit was planted in sediment from Magothy Bay (green), Gargathy Bay (blue), Hog Island Bay (red), or the control, South Bay, (yellow) and one pot of each sediment type was randomly placed in each mesocosm. Half of the mesocosms were shaded with 80% shade cloth in order to understand how sediment parameters and light interact to affect eelgrass productivity. Pot and eelgrass image: Tracey Saxby, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

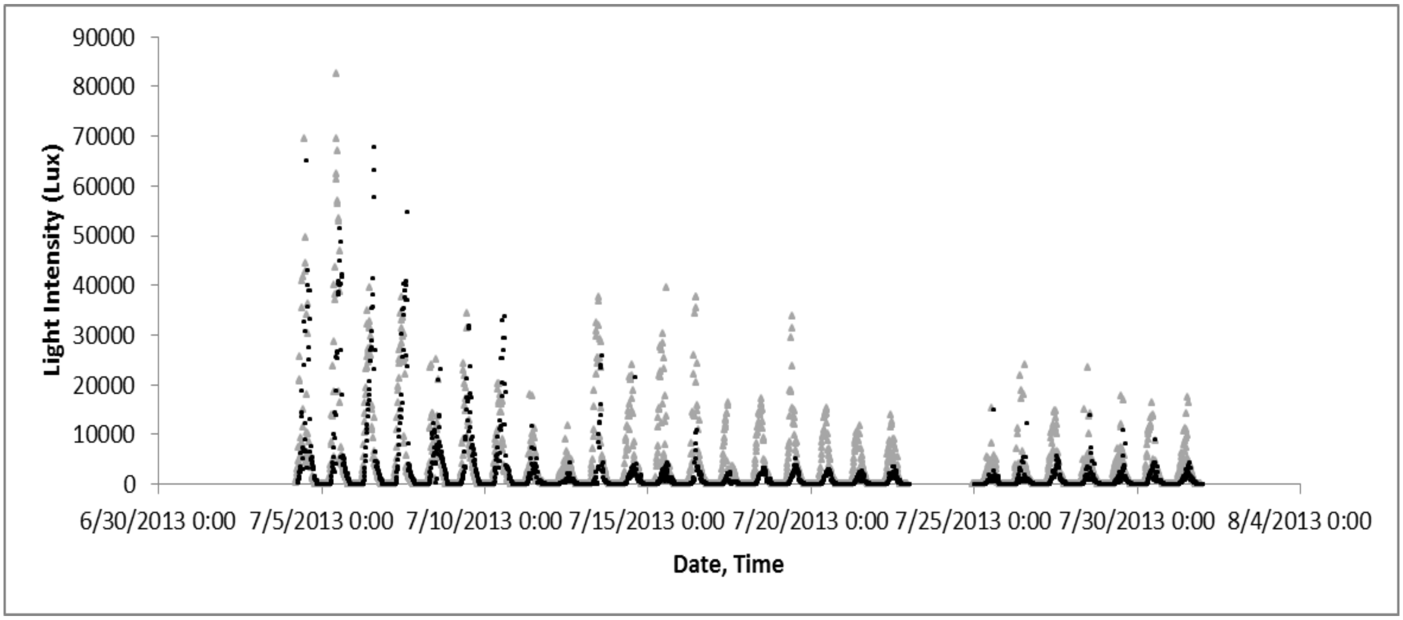


Figure 2.6. Light intensity for the light (grey) and dark (black) treatments during the pre-experimental period (July 4 – 9) and experimental period (July 10 – August 2). No data was collected July 23 and 24.

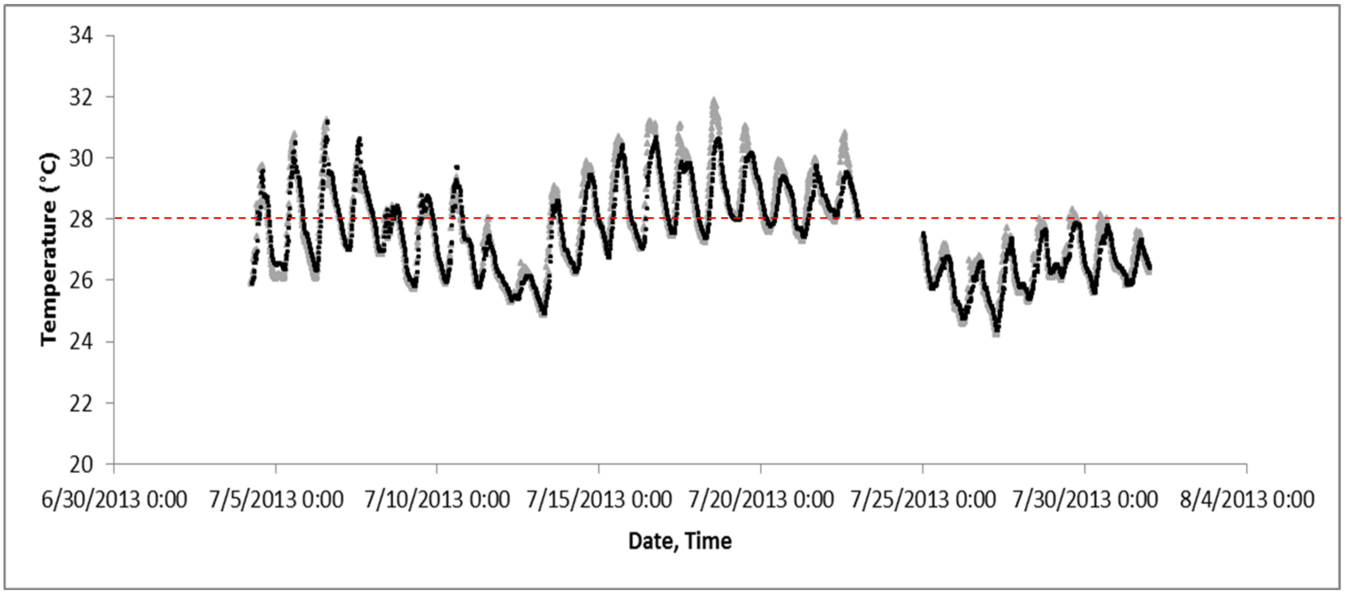


Figure 2.7. Temperature in degrees Celsius for the light (grey) and dark (black) treatments during the pre-experimental period (July 4 – 9) and experimental period (July 10 – August 2). No data was collected July 23 and 24. The red dotted line separates time below the temperature threshold (28°C) from time above the threshold.

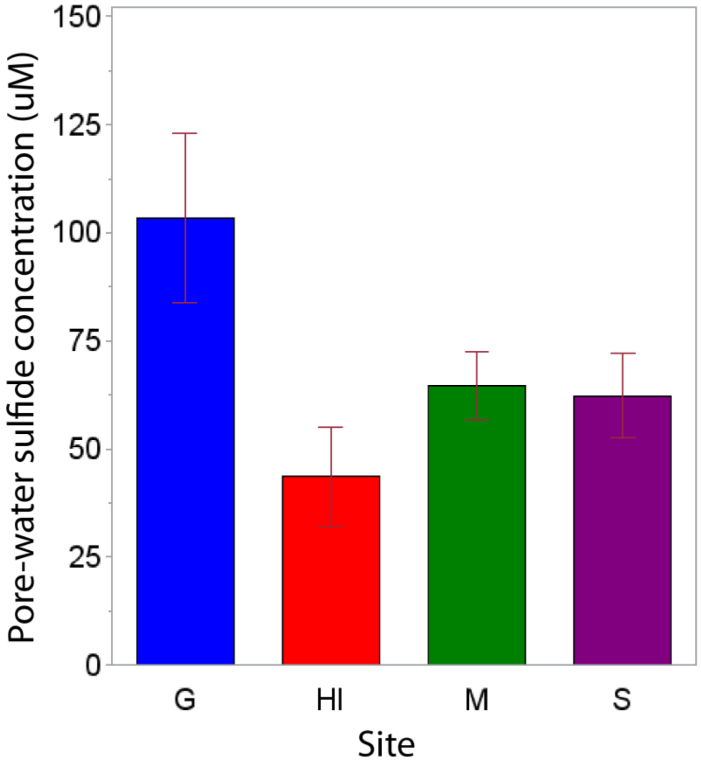


Figure 2.8. Pre-experiment pore-water sulfide concentrations from sediment cores collected in Gargathy Bay (blue), Hog Island Bay (red), Magothy Bay (green), and South Bay (purple). Error bars represent +/- standard error. Sites not statistically different.

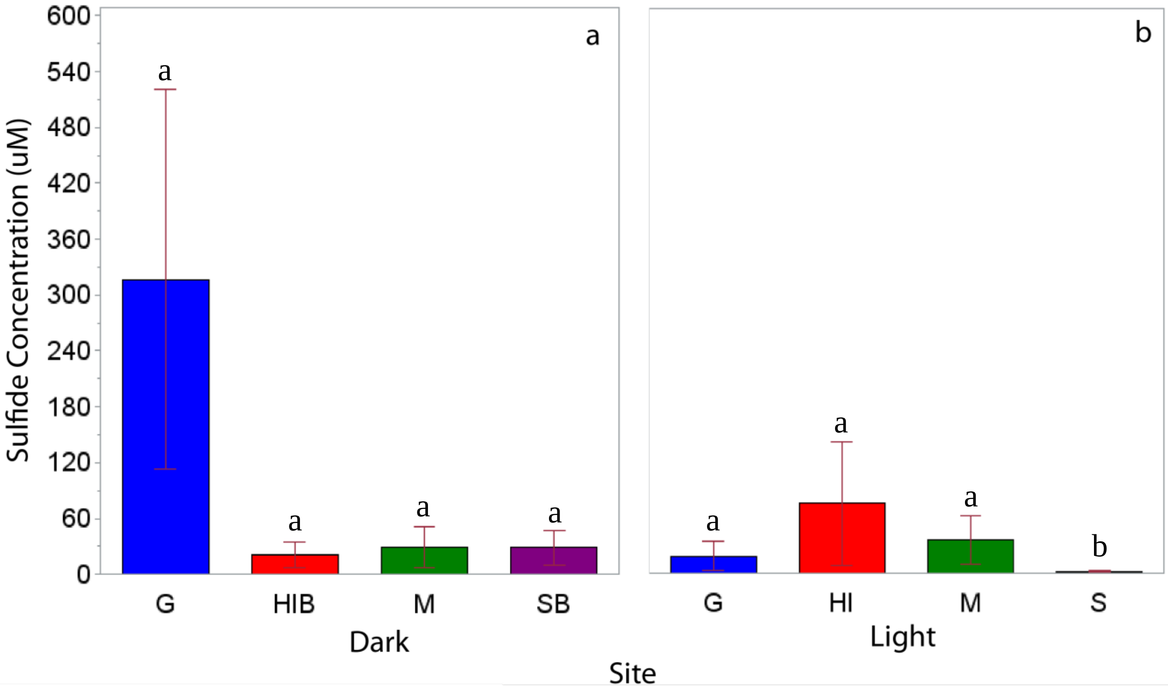


Figure 2.9. Post-experiment pore-water sulfide concentrations from dark (a) and light (b) treatments with sediment cores collected in Gargathy Bay (blue), Hog Island Bay (red), Magothy Bay (green), and South Bay (purple). Error bars represent +/- standard error. Letters indicate statistical difference.

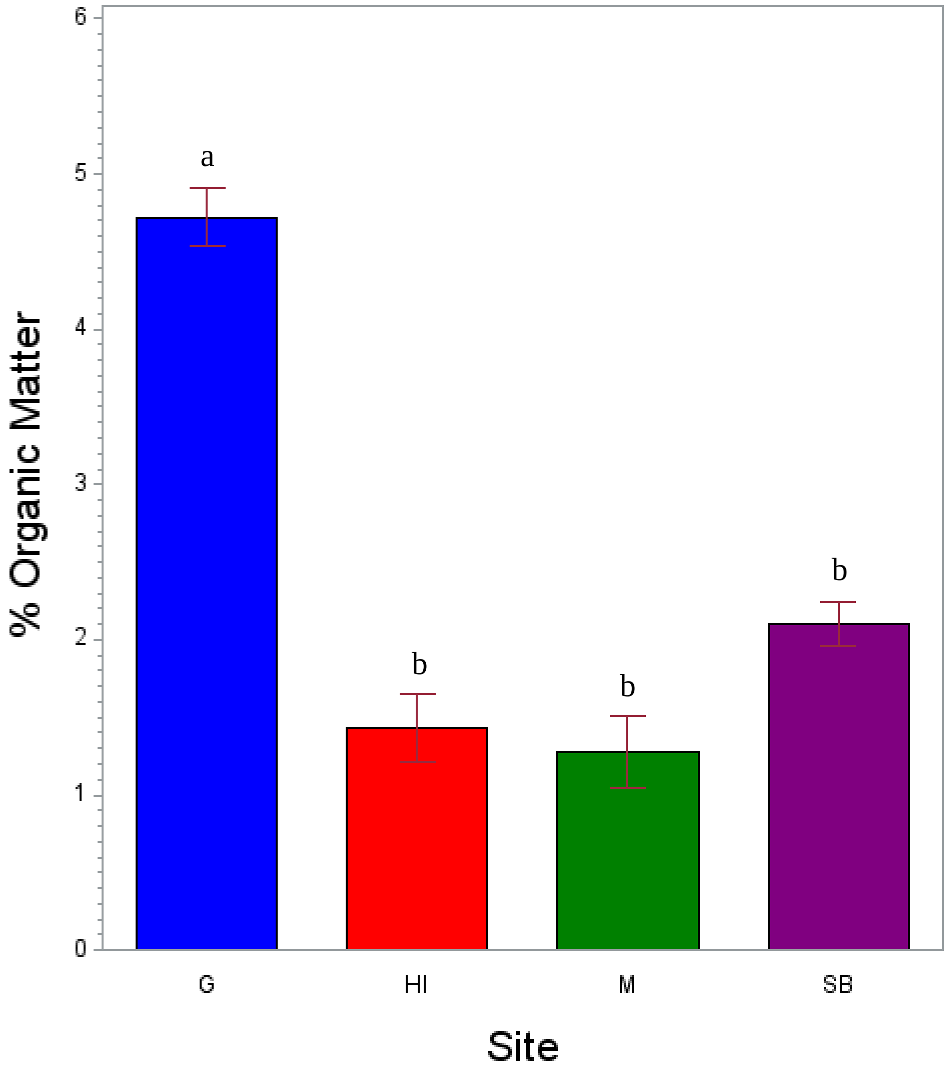


Figure 2.10. Percent organic matter in sediment of sites in unrestored bays, Gargathy (G) and Magothy (M) compared to sediment from a bay in the process of being restored, Hog Island Bay (HI) and South Bay (SB). Error bars represent standard error (n = 27). Letters represent bars statistically the same.

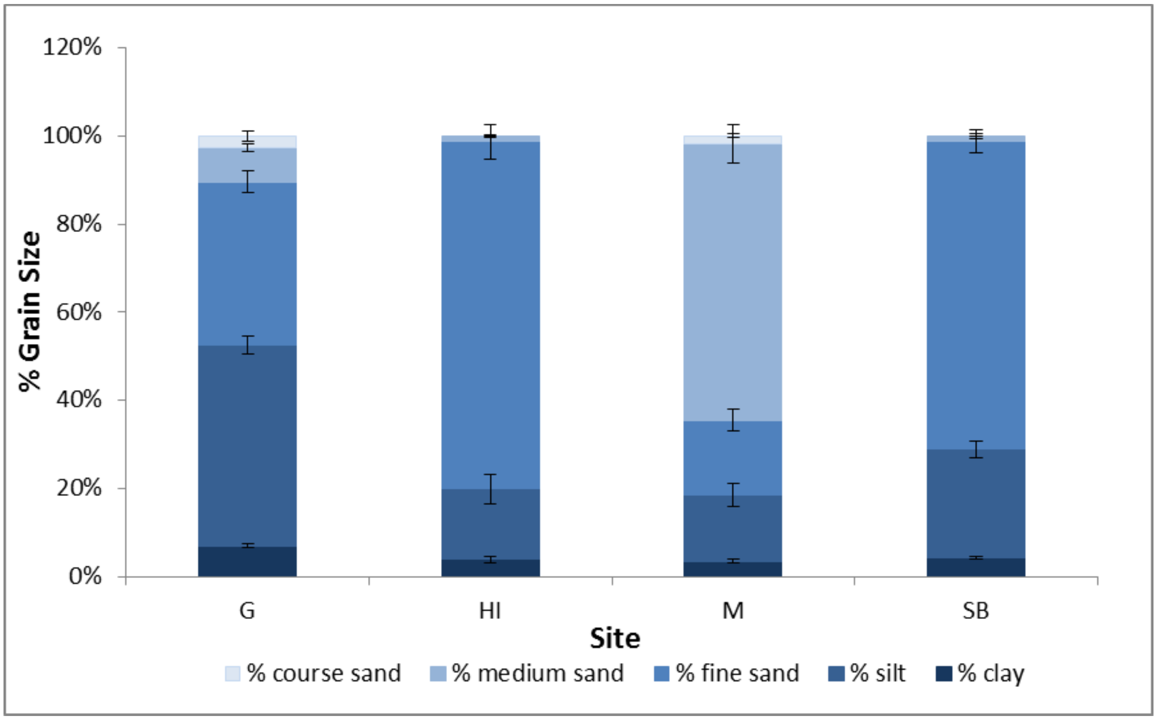


Figure 2.11. Distribution of clay (<2 µm), silt (2 – 63 µm), fine sand (63 – 200 µm), medium sand (200 – 630 µm) and coarse sand (>630 µm) at sites in Gargathy Bay, Magothy Bay, Hog Island Bay, and South Bay with error bars representing standard error (n = 27 for all but SB n = 4).

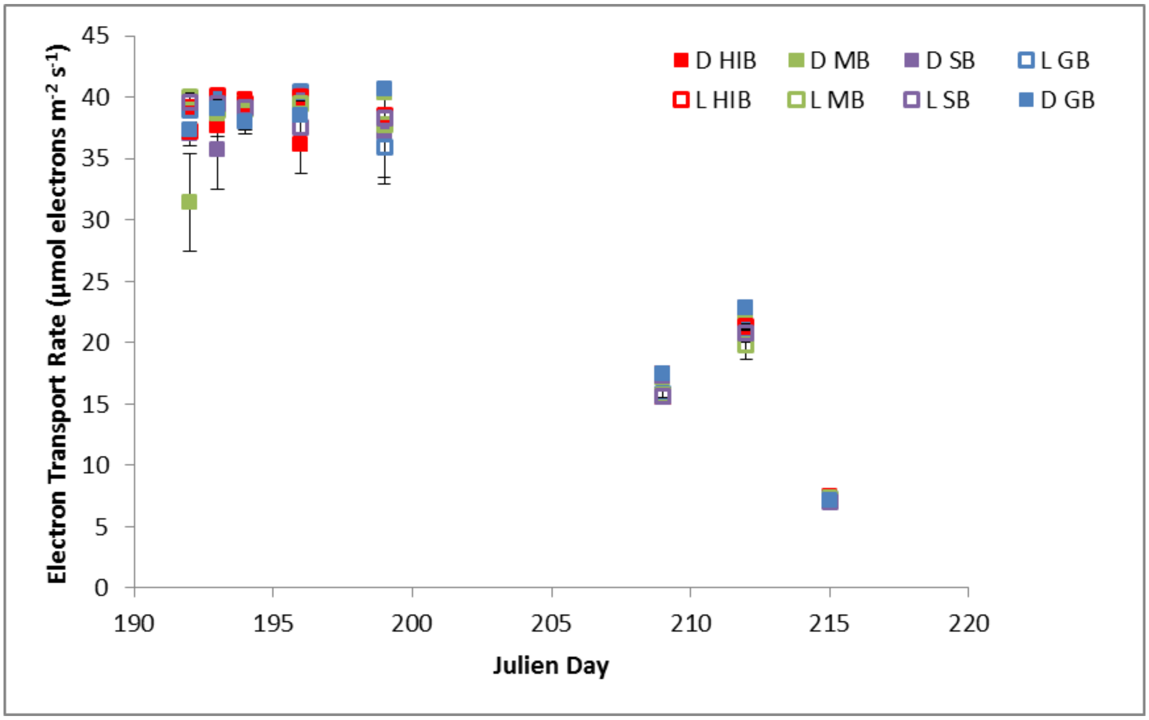


Figure 2.12. Electron transport rate during the experimental period for light (hollow) and dark (filled) treatments by sediment treatment: Hog Island Bay (HIB, red), Magothy Bay (MB, green), South Bay (SB, purple), and Gargathy Bay (GB, blue). Error bars represent standard error.

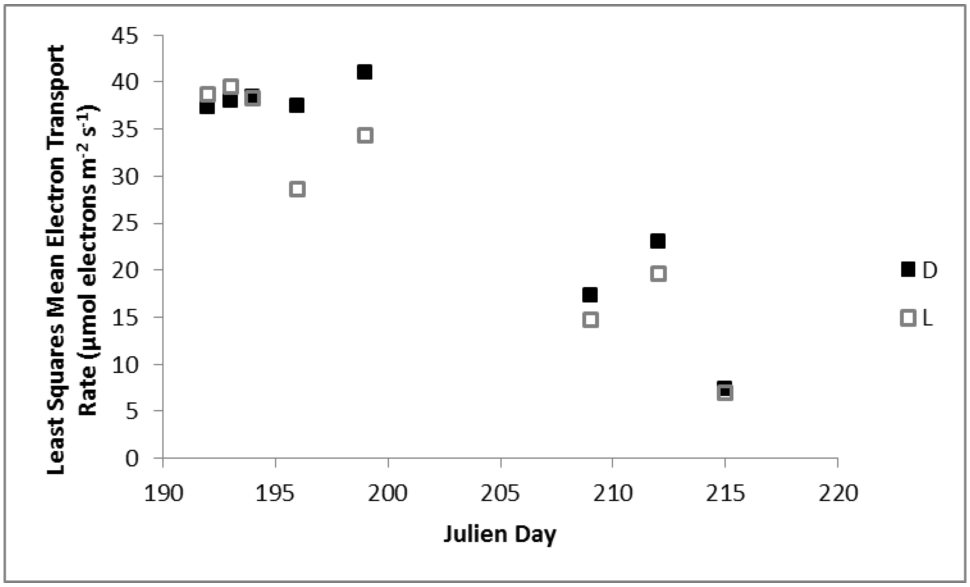


Figure 2.13. Least squares mean electron transport rate over time during the experimental period for light (grey, unfilled) and dark (black, filled) treatments.

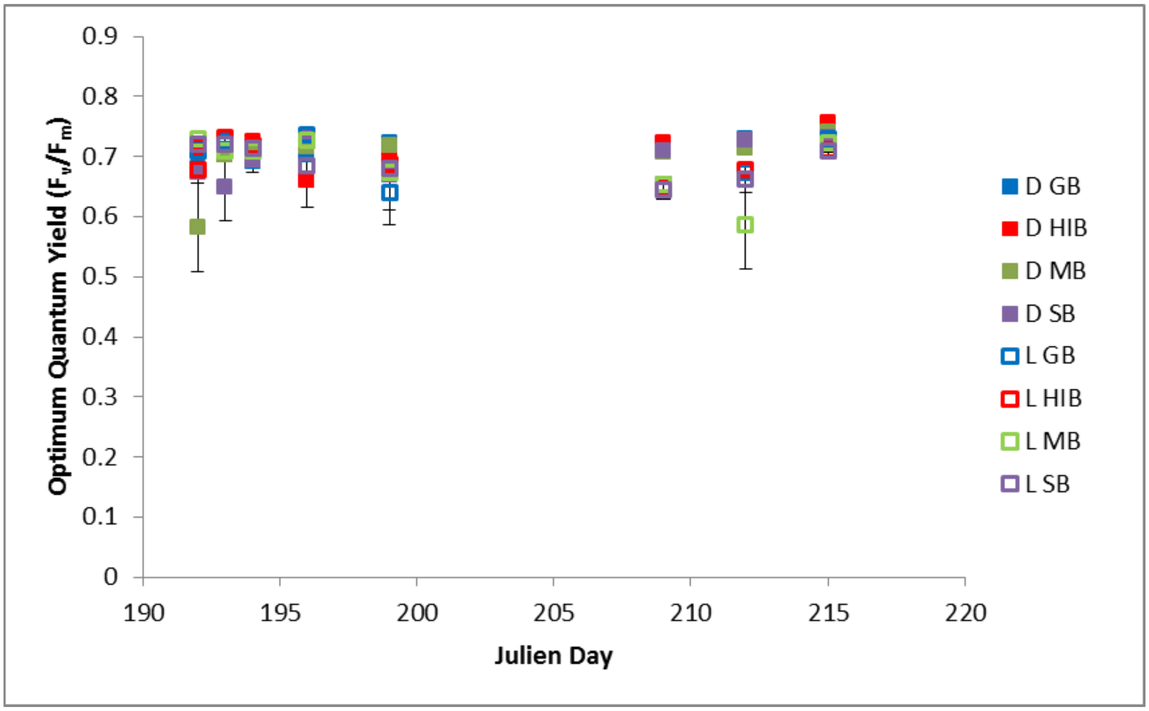


Figure 2.14. Optimum quantum yield during the experimental period for light (hollow) and dark (filled) treatments by sediment treatment: Hog Island Bay (HIB, red), Magothy Bay (MB, green), South Bay (SB, purple), and Gargathy Bay (GB, blue). Error bars represent standard error.

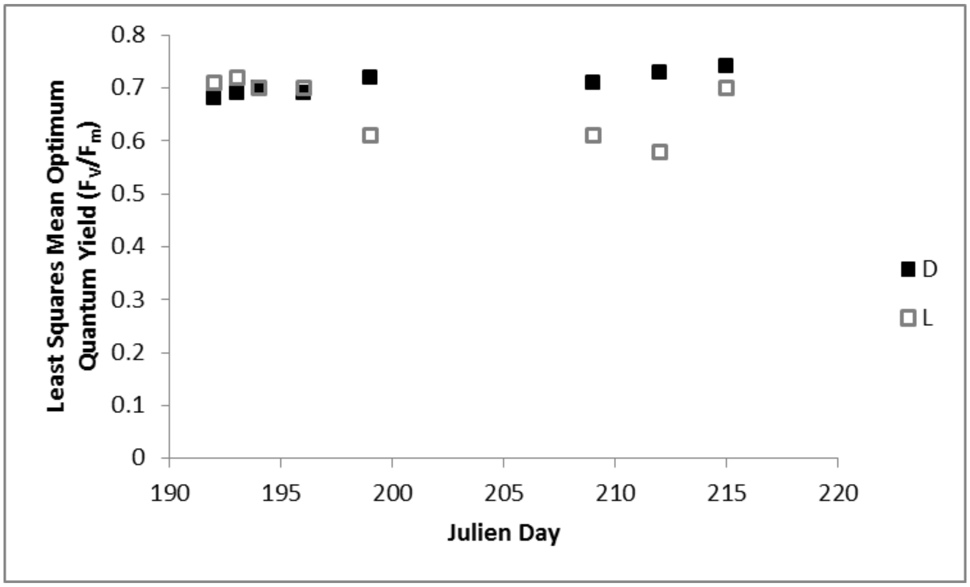


Figure 2.15. Least squares mean optimum quantum yield over time during the experimental period for light (grey, unfilled) and dark (black, filled) treatments.

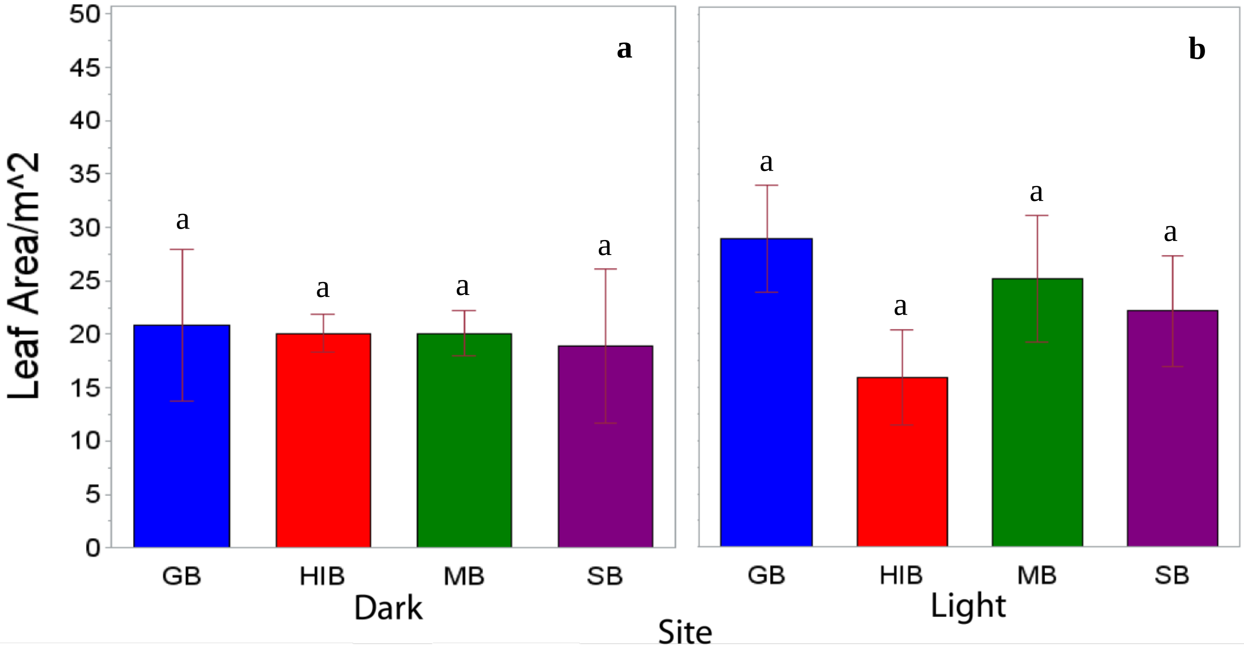


Figure 2.16. Post-experiment average productivity in leaf area per square meter from dark (a) and light (b) treatments with sediment cores collected in Gargathy Bay (blue), Hog Island Bay (red), Magothy Bay (green), and South Bay (purple). Error bars represent +/- standard error. Letters indicate statistical difference.

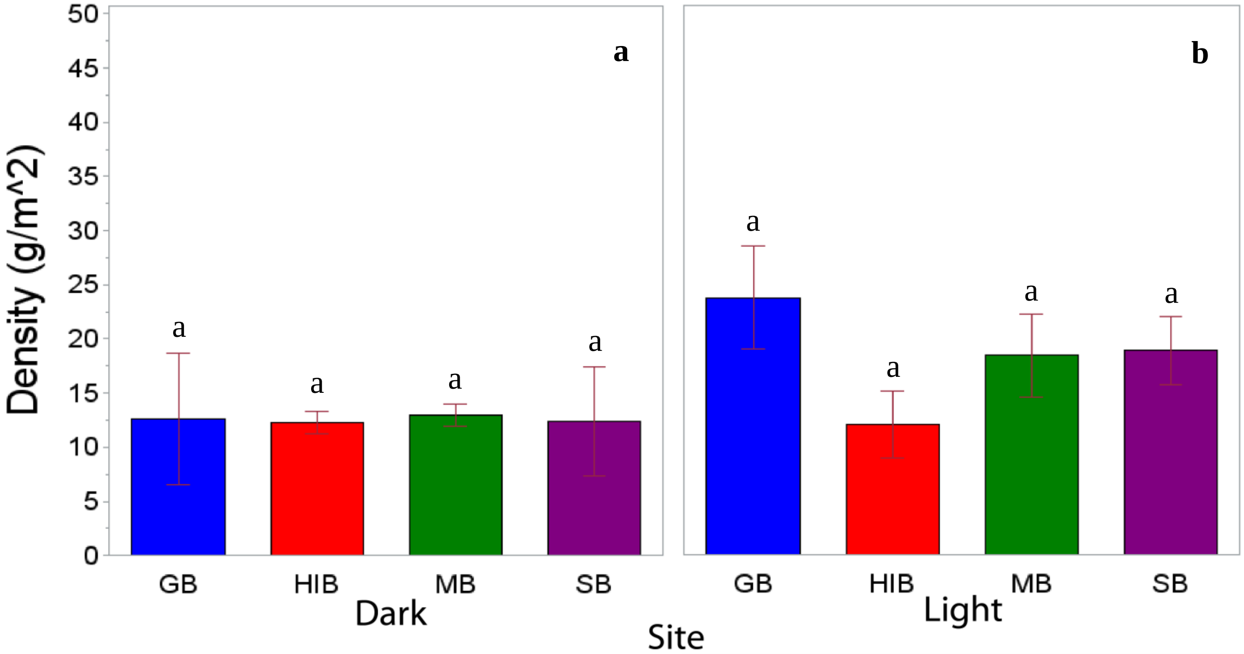
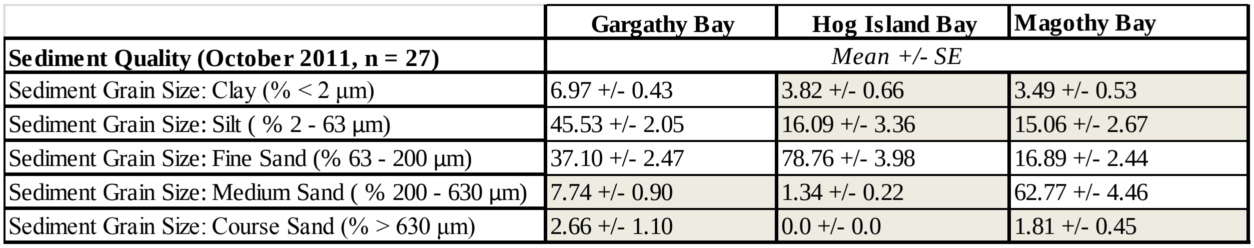


Figure 2.17. Post-experiment plant aboveground biomass in grams of leaf per square meter from dark (a) and light (b) treatments with sediment cores collected in Gargathy Bay (blue), Hog Island Bay (red), Magothy Bay (green), and South Bay (purple). Error bars represent +/- standard error. Letters indicate statistical difference.

Tables

Table 2.1. Table of sediment grain size results including standard error from October 2011.

Parameters that are not statistically different are shaded in grey.



	Gargathy Bay	**Hog Island Bay**	**Magothy Bay**
Sediment Quality (October 2011, n = 27)	*Mean +/- SE*		
Sediment Grain Size: Clay (% < 2 µm)	6.97 +/- 0.43	3.82 +/- 0.66	3.49 +/- 0.53
Sediment Grain Size: Silt (% 2 - 63 µm)	45.53 +/- 2.05	16.09 +/- 3.36	15.06 +/- 2.67
Sediment Grain Size: Fine Sand (% 63 - 200 µm)	37.10 +/- 2.47	78.76 +/- 3.98	16.89 +/- 2.44
Sediment Grain Size: Medium Sand (% 200 - 630 µm)	7.74 +/- 0.90	1.34 +/- 0.22	62.77 +/- 4.46
Sediment Grain Size: Course Sand (% > 630 µm)	2.66 +/- 1.10	0.0 +/- 0.0	1.81 +/- 0.45

Table 2.2. Pre-experimental mesocosm temperature and light intensity means +/- standard error. Tanks marked with “L#” were light tanks during the experimental period and tanks marked with “D#” were dark tanks during the experimental period. Parameters that are not statistically different are shaded in grey.

	Tank					
	L1	***L3***	***L5***	***D2***	***D4***	***D6***
Temperature (°C)	27.97 +/- 1.50	27.76 +/- 1.52	27.79 +/- 1.55	27.96 +/- 1.33	27.87 +/- 1.46	27.93 +/- 1.42
Light Intensity (Lux)	5622.8 +/- 408.3	6958.5 +/- 562.0	8910.2 +/- 701.9	7195.9 +/- 540.2	6129.4 +/- 480.8	7251.7 +/- 576.0

Table 2.3. Mesocosm mean +/- standard error of temperature, light intensity, and, % incident light at the sediment surface for light and dark treatments during the experimental period in July 2013. All parameters are statistically different.

	Treatment	
	Light	***Dark***
Temperature (°C)	27.55 +/- 0.02	27.41 +/- 0.02
Light Intensity (Lux)	3168.3 +/- 89.7	1026.6 +/- 46.6
% Incident Light	15.7 +/- 0.4	3.6 +/- 0.1

Table 2.4. Table of sediment sulfide concentrations including standard error from the mesocosm experiment during summer 2013. Parameters in the same shade of gray are values in columns that are not statistically different. Parameters that are mottled are not statistically different within the sediment treatment.

	Pre-experiment	**Post-experiment**	
Sediment treatment		***Light***	***Dark***
Gargathy	103.5 +/- 19.6	18.7 +/- 15.4	316.4 +/- 203.9
Magothy	64.7 +/- 7.9	36.4 +/- 26.1	29.4 +/- 21.6
Hog Island	43.6 +/- 11.5	75.6 +/- 66.6	20.7 +/- 14.1
South	62.3 +/- 9.8	2.1 +/- 0.3	28.5 +/- 19.1

Significance

The goal of this thesis was to develop a better understanding of how variable minimum light requirements of *Zostera marina* in response to water quality, temperature, and sediment characteristics influence habitat suitability in the Virginia coastal bays in order to identify locations where restored seagrass is likely to successfully propagate and persist. Even though many studies have tried to identify and model optimal environmental conditions for *Zostera marina* (eelgrass), their focus was mainly on the light environment in established seagrass meadows and how it affected the maximum depth limit for eelgrass growth. These studies do not address effects of temperature or sediment characteristics on changes in the maximum depth limit and do not discuss the drivers of the minimum depth limit. None of the previous field studies of light requirements for *Zostera marina* were carried out in the restored area of the Virginia coastal bays.

I used water quality, light, temperature, and sediment characteristic surveys to identify areas where eelgrass could grow based on literature values for light and temperature thresholds and compared this distribution to the natural distribution of *Z. marina* in a restored bay. I showed that the maximum depth limit is bound by light, that the minimum depth limit is bound by a combination of light and temperature, and that fine sediment grain sizes and temperature are important factors for density across the depth range. The results of this study will be used to modify models of the range for eelgrass growth to include changes in sediment characteristics in order to more accurately predict the areas suitable for eelgrass restoration in the Virginia Coast Reserve.

I used a mesocosm experiment to attempt to separate effects of light and sediment characteristics, specifically pore-water sulfide concentration, on eelgrass productivity and optimal quantum yield. My mesocosm results were inconclusive, due to differences between field and mesocosm pore-water sulfide concentrations likely caused by advection. Finally, I found that plants in both the dark treatment and light treatment were photosynthetically stressed indicating mesocosm or transplant stress. The results of this study indicate that hydrodynamics play a major role in seagrass growth and call for a need for more paired flume and chemical studies in order to accurately quantify the effects of chemical stressors on natural populations of seagrass.

This thesis addresses questions related to the effects of multiple stressors on the maximum and minimum depth limits of *Z. marina* in the Virginia coastal bays. I found that the maximum depth limit in the Virginia coastal bays is bound by light in sandy, low organic matter areas and bound by a combination of light and temperature effects at the minimum depth limit. However, I was unable to separate the effects of light and chemical stressors within the pore-water in order to quantify the effect chemical stressors have on plant productivity and photosynthesis separately from light. This work would be improved by this information, which could be obtained by pairing a flume experiment, where I would use a similar set-up as in the mesocosms except control for flow and temperature in order to maintain field pore-water sulfide concentrations within the mesocosms, with a field experiment, where photosynthetic stress would be measured in a healthy meadow to provide baseline measurements for the mesocosm experiment.

According to our results, seagrasses can still propagate to or be restored to areas that were not previously restored in the VCR, such as Magothy Bay. Eelgrass in the VCR can grow in sandy sediments with low organic matter content and low pore-water sulfide concentrations due to feedbacks between light, temperature, and sediment characteristics across depth gradients. Data from this thesis can be used to parameterize and validate existing models of maximum depth limits in the Virginia coastal bays and parameterize future models of seagrass propagation. These results will help increase the understanding of seagrass depth limits across the seascape in the VCR and will help managers identify areas where restoration is likely to be successful. In order to most accurately predict seagrass depth limits future work should focus on the following questions: How do hydrodynamics affect concentrations of chemical stressors of eelgrass in the sediments? What are the effects of light and sediment characteristics on eelgrass photosynthesis? Are there factors other than light and temperature that can help predict the minimum depth limit for eelgrass growth? Answering these questions will further our understanding of the drivers of eelgrass distribution and help managers worldwide identify possible areas for restoration.

Appendices

Appendix 1 – Site Characteristics

Table A.1.1 Site Characteristics

Table of water and sediment quality results including variance and n. Water quality results combine summers 2012 and 2013.

	Gargathy Bay			Hog Island Bay			Magothy Bay		
Sediment Characteristics (2012)	mean	variance	n	mean	variance	n	mean	variance	n
Sediment Grain Size: Clay (% < 2 µm)	6.97	5.57	27	3.82	3.10	6	3.49	8.77	27
Sediment Grain Size: Silt (% 2 - 63 µm)	45.53	125.98	27	16.09	81.48	6	15.06	220.83	27
Sediment Grain Size: Fine Sand (% 63 - 200 µm)	37.10	183.72	27	78.76	114.00	6	16.89	184.11	27
Sediment Grain Size: Medium Sand (% 200 - 630 µm)	7.74	24.06	27	1.34	0.34	6	62.77	618.62	27
Sediment Grain Size: Course Sand (% > 630 µm)	2.66	36.38	27	0.00	0.00	6	1.81	2.29	27
Sediment Organic Matter (%)	4.72	1.85	27	1.43	0.29	6	1.28	0.79	27
Porosity (%)	32.28	22.44	27	23.63	3.73	6	21.35	10.95	27
Carbon Content (%)	1.56	0.22	27	0.31	0.02	26	0.4	0.13	27
Nitrogen Content (%)	0.130	0.002	27	0.018	0.00	26	0.077	0.001	27
Pore-Water Quality (2012)									
Pore-water Sulfide (µM)	1244.1	976559	9	12.6	261.3	18	494	349877	9
Exchangeable Ammonium (µmol/g)	0.37	0.097	9	0.02	0.00	5	0.01	0.00	4
Water Quality (2012-2013)									
Water Column Chlorophyll a (µg/L)	26.43	137.29	11	5.56	2.50	6	11.86	2.35	12
Ammonium (µM)	2.630	7.484	12	2.831	1.972	6	0.876	0.498	12
Ortho-phosphate (µM)	0.718	0.252	12	0.821	0.084	6	0.551	0.162	12
Total Dissolved Nitrogen (µM)	9.109	15.162	11	3.189	1.563	9	7.564	41.125	11
Nitrate + Nitrite (µM)	0.008	0.001	12	0.212	0.014	6	0.184	0.140	12
Total Suspended Solids (mg/L)	54.04	157.11	12	29.50	36.95	12	38.60	94.77	10
Dissolved Oxygen (mg/L)	7.00	1.67	3	7.09	0.3	3	7.06	1.67	3

Appendix 2 – Light and Temperature

Table A.2.1 Light data by site

Average light in lux measured by HOBOs© from each depth in Gargathy Bay, Magothy Bay, and Hog Island Bay during summers 2012 and 2013. There was no data from Hog Island Bay in 2012. SE represents standard error.

		Light (Lux)					
Site	Depth	2012			2013		
		average	SE	n	average	SE	n
Gargathy	0.4	10110.14	4209.843	3	11968.09	1361.711	3
Gargathy	0.6	6725.501	2776.724	3	10128	2078.495	3
Gargathy	0.8	5376.671	2218.093	3	7641.972	1760.62	3
Gargathy	1.0	3623.678	1008.9	3	2698.922	520.6511	3
Gargathy	1.2	1820.926	1039.011	3	2516.692	18.73247	3
Gargathy	1.4	1960.946	1028.625	3	1829.809	52.59177	3
Gargathy	1.6	1034.129	195.2904	3	2245.511	72.47177	3
Gargathy	1.8	1078.366	177.6779	3	986.8173	264.965	3
Magothy	0.4	14286.83	4160.299	3	13280.21	1113.605	3
Magothy	0.6	10618.54	2698.852	3	7163.071	1461.177	3
Magothy	0.8	7373.431	2059.581	3	4747.615	1811.84	3
Magothy	1.0	4123.68	224.2167	3	4070.75	1057.451	3
Magothy	1.2	2558.752	945.5756	3	3549.348	1074.532	3
Magothy	1.4	1925.719	944.9594	3	1452.626	165.4995	3
Magothy	1.6	1848.129	131.4963	3	519.7447	580.1552	3
Magothy	1.8	1625.372	173.3352	3	266.6377	121.1904	3
Magothy	2.0	1414.212	4376.557	3	86.80052	5980.974	3
Hog Island	0.4			0	13471.77	205.8481	3
Hog Island	0.6			0	11684.7	135.8799	3
Hog Island	0.8			0	2880.603	164.5827	3
Hog Island	1.0			0	2490.95	827.8339	3
Hog Island	1.2			0	2257.86	283.9131	3
Hog Island	1.4			0	1134.066	10.9139	3
Hog Island	1.6			0	2839.883	94.77837	3
Hog Island	1.8			0	360.0273	48.47751	3
Hog Island	2.0			0	688.3623	3348.176	2

Table A.2.2 Temperature data by site

Average temperature in °C measured by HOBOs© from each depth in Gargathy Bay, Magothy Bay, and Hog Island Bay during summers 2012 and 2013. There was not data from all sites in Hog Island Bay in 2012. SE represents standard error.

		Temperature (°C)					
Site	Depth	2012			2013		
		average	SE	n	average	SE	n
Gargathy	0.4	26.5804	0.997172	3	23.76677	0.521345	3
Gargathy	0.6	26.47492	1.01471	3	23.89076	0.538292	3
Gargathy	0.8	26.47993	1.003738	3	23.94873	0.545241	3
Gargathy	1.0	26.34259	1.007299	3	23.83792	0.585762	3
Gargathy	1.2	25.88036	1.313702	3	23.67126	0.581918	3
Gargathy	1.4	26.19698	1.002425	3	23.66029	0.584436	3
Gargathy	1.6	26.16421	1.002796	3	23.52501	0.584625	3
Gargathy	1.8	26.4034	0.857755	3	23.4391	0.604446	3
Magothy	0.4	28.25171	0.707268	3	27.00276	1.034797	3
Magothy	0.6	28.31078	0.711903	3	26.79092	0.928859	3
Magothy	0.8	28.20037	0.712998	3	26.74082	0.899334	3
Magothy	1.0	28.12479	0.749048	3	26.75305	0.944116	3
Magothy	1.2	28.06417	0.752516	3	26.72196	0.980662	3
Magothy	1.4	27.98022	0.796859	3	26.6435	0.962154	3
Magothy	1.6	27.95541	0.789778	3	26.61683	0.977892	3
Magothy	1.8	27.92674	0.798194	3	26.6645	1.02248	3
Magothy	2.0	27.89643	0.77053	3	26.63215	0.16058	3
Hog Island	0.4			0	26.39659	0.398669	3
Hog Island	0.6			0	26.42067	0.371245	3
Hog Island	0.8			0	26.25775	0.366052	3
Hog Island	1.0	28.61616		1	26.14882	0.390224	3
Hog Island	1.2	28.14777		1	26.04954	0.404846	3
Hog Island	1.4	28.49002		1	25.93088	0.405888	3
Hog Island	1.6	28.26518		1	25.583	0.404027	3
Hog Island	1.8	28.18532		1	25.60135	0.433798	3
Hog Island	2.0			0	25.67193	0.053391	2

Appendix 3 – Transplant Data

Table A.3.1 Transplant Density and Productivity

Average density and productivity of transplants in Gargathy Bay, Hog Island Bay, and South Bay (control) during summer 2013. n = 3 for all sites. South Bay data was not used due to concerns over meadow encroachment into transplant control plots.

		Density (Shoots/m2)						Productivity (LA/m^2)	
Site	Depth	June	SE	July	SE	August	SE	August	SE
Gargathy	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	1.0	1.7	1.4	3.3	2.7	0.0	0.0	0.0	0.0
Gargathy	1.2	6.7	5.4	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	1.4	13.3	10.9	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gargathy	1.8	1.7	1.4	0.0	0.0	0.0	0.0	0.0	0.0
Magothy	0.4	73.3	44.6	0.0	0.0	0.0	0.0	0.0	0.0
Magothy	0.6	235.0	75.6	46.7	38.1	35.0	28.6	2.3	0.0
Magothy	0.8	113.3	66.1	46.7	38.1	28.3	23.1	2.5	0.0
Magothy	1.0	165.0	51.4	15.0	12.2	0.0	0.0	0.0	0.0
Magothy	1.2	61.7	32.2	0.0	0.0	0.0	0.0	0.0	0.0
Magothy	1.4	105.0	43.7	8.3	6.8	10.0	8.2	0.3	0.0
Magothy	1.6	108.3	47.6	5.0	4.1	8.3	6.8	0.4	0.0
Magothy	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Magothy	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
South Bay		550.0	8.2	1046.7	31.4	1318.3	81.0	97.2	15.1

Appendix 4 – PAM Data

Table A.4.1 Electron Transport Rate

Average electron transport rate (ETR) in μmol e^{-} m^{-2} s^{-1} and standard error (SE) for light treatments and sediment treatments during the mesocosm experiment of summer 2013.

		Date															
		7-Jul		8-Jul		9-Jul		11-Jul		14-Jul		24-Jul		27-Jul		1-Aug	
Light Treatment	Sediment Treatment	ETR	SE	ETR	SE	ETR	SE	ETR	SE	ETR	SE	ETR	SE	ETR	SE	ETR	SE
Dark	Gargathy	37.3	0.6	39.0	0.6	37.9	1.0	38.6	0.4	40.6	0.5	17.4	0.1	22.9	0.3	7.2	0.1
Dark	Hog Island	39.2	0.6	37.7	0.9	39.8	0.4	36.2	2.4	40.5	0.5	17.5	0.2	22.5	0.3	7.5	0.1
Dark	Magothy	31.4	4.0	38.6	0.7	39.5	0.4	38.6	0.5	40.3	0.2	17.1	0.2	22.2	0.5	7.4	0.0
Dark	South Bay	37.0	0.9	35.7	3.2	38.1	0.8	40.3	0.4	37.2	3.7	17.2	0.1	22.8	0.2	7.3	0.1
Light	Gargathy	38.9	0.1	39.8	0.3	39.3	0.6	40.5	0.3	35.9	3.0	15.8	0.3	21.2	0.2	7.3	0.0
Light	Hog Island	37.2	0.4	40.2	0.4	39.5	0.4	40.0	0.3	38.5	0.7	15.6	0.4	21.3	0.3	7.1	0.1
Light	Magothy	40.1	0.3	38.9	0.4	39.0	0.2	39.5	0.3	37.8	1.1	15.8	0.4	19.9	1.2	7.2	0.0
Light	South Bay	39.6	0.4	39.5	0.6	39.1	0.6	37.5	2.0	38.3	0.4	15.7	0.4	20.8	0.7	7.1	0.1

Table A.4.2 Optimal Quantum Yield

Optimal quantum yield represented by F_v/F_m in and standard error (SE) for light treatments and sediment treatments during the mesocosm experiment of summer 2013.

	Date															
	7-Jul		8-Jul		9-Jul		11-Jul		14-Jul		24-Jul		27-Jul		1-Aug	
Sediment Treatment	YIELD	SE	YIELD	SE	YIELD	SE	YIELD	SE	YIELD	SE	YIELD	SE	YIELD	SE	YIELD	SE
Gargathy	0.680	0.012	0.711	0.011	0.691	0.018	0.702	0.007	0.724	0.008	0.719	0.005	0.729	0.009	0.721	0.014
Hog Island	0.714	0.011	0.703	0.007	0.725	0.008	0.660	0.044	0.712	0.015	0.722	0.008	0.718	0.009	0.756	0.013
Magothy	0.582	0.074	0.703	0.012	0.718	0.008	0.716	0.009	0.718	0.003	0.707	0.008	0.715	0.010	0.740	0.005
South Bay	0.673	0.017	0.650	0.057	0.695	0.015	0.735	0.007	0.669	0.059	0.710	0.006	0.726	0.006	0.731	0.010
Gargathy	0.709	0.003	0.725	0.006	0.716	0.011	0.737	0.006	0.639	0.053	0.653	0.014	0.674	0.008	0.730	0.003
Hog Island	0.678	0.007	0.732	0.007	0.719	0.007	0.728	0.006	0.686	0.012	0.646	0.016	0.678	0.009	0.711	0.009
Magothy	0.729	0.005	0.709	0.007	0.710	0.004	0.726	0.008	0.673	0.020	0.653	0.015	0.588	0.075	0.723	0.005
South Bay	0.721	0.008	0.720	0.011	0.713	0.010	0.684	0.035	0.682	0.007	0.646	0.017	0.662	0.022	0.710	0.007

Table A.4.3 Light Treatment Rapid Light Curves

Response and recovery yield (F_v/F_m) from rapid light curves taken in light treatment mesocosms during summer 2013. Plants were dark adapted for 15 minutes before measurements were taken with a DIVING-PAM. Measurements were taken 5 cm from the base of each leaf.

		Response Pulse Number									Recovery Pulse Number					
Time	Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Day 1	GB	0.741	0.243	0.168	0.123	0.088	0.061	0.043	0.02	0.016	0.484	0.627	0.686	0.7	0.701	0.703
Day 2	GB	0.747	0.23	0.182	0.122	0.093	0.068	0.042	0.032	0.013	0.484	0.618	0.687	0.701	0.705	0.708
Day 3	GB	0.649	0.3	0.244	0.209	0.186	0.141	0.118	0.076	0.03	0.384	0.518	0.6	0.602	0.613	0.615
Day 4	GB	0.66	0.251	0.192	0.114	0.062	0.056	0.066	0.017	0.009	0.398	0.527	0.586	0.619	0.623	0.631
Day 5	GB	0.701	0.236	0.177	0.126	0.098	0.054	0.025	0.032	0.026	0.454	0.594	0.637	0.656	0.666	0.671
Day 6	GB	0.7	0.226	0.166	0.123	0.088	0.064	0.033	0.028	0.071	0.398	0.519	0.63	0.656	0.667	0.661
Day 7	GB	0.721	0.287	0.191	0.14	0.096	0.061	0.047	0.012	0.032	0.473	0.601	0.665	0.684	0.684	0.684
Day 8	GB	0.739	0.309	0.251	0.183	0.141	0.108	0.08	0.023	0.024	0.486	0.623	0.678	0.697	0.703	0.702
Day 1	HIB	0.733	0.281	0.197	0.14	0.11	0.063	0.052	0.033	0.023	0.494	0.64	0.683	0.69	0.698	0.643
Day 2	HIB	0.698	0.224	0.17	0.141	0.101	0.063	0.064	0.05	0.022	0.35	0.502	0.63	0.663	0.664	0.67
Day 3	HIB	0.558	0.25	0.222	0.194	0.164	0.138	0.104	0.081	0.05	0.313	0.451	0.523	0.548	0.552	0.567
Day 4	HIB	0.671	0.316	0.246	0.19	0.152	0.112	0.085	0.044	0.045	0.465	0.595	0.623	0.631	0.636	0.652
Day 5	HIB	0.675	0.474	0.377	0.289	0.222	0.153	0.104	0.065	0.032	0.487	0.559	0.587	0.6	0.613	0.619
Day 6	HIB	0.677	0.25	0.184	0.139	0.099	0.08	0.038	0.015	0.008	0.361	0.512	0.608	0.635	0.637	0.642
Day 7	HIB	0.64	0.28	0.214	0.156	0.118	0.097	0.055	0.038	0.059	0.422	0.553	0.588	0.592	0.597	0.609
Day 8	HIB	0.734	0.201	0.161	0.109	0.084	0.058	0.031	0.039	0.014	0.482	0.646	0.678	0.69	0.688	0.687
Day 1	MB	0.71	0.215	0.157	0.112	0.083	0.051	0.039	0.023	0.006	0.474	0.628	0.675	0.682	0.682	0.675
Day 2	MB	0.743	0.281	0.223	0.163	0.127	0.08	0.056	0.038	0.028	0.53	0.652	0.685	0.693	0.707	0.709
Day 3	MB	0.638	0.238	0.2	0.151	0.136	0.095	0.058	0.04	0.01	0.339	0.446	0.55	0.6	0.605	0.61
Day 4	MB	0.614	0.224	0.185	0.138	0.097	0.083	0.035	0.045	0.054	0.341	0.497	0.542	0.565	0.579	0.583
Day 5	MB	0.723	0.278	0.194	0.132	0.102	0.076	0.048	0.04	0.021	0.497	0.631	0.656	0.669	0.672	0.678
Day 6	MB	0.686	0.231	0.171	0.124	0.085	0.048	0.031	0.012	0.019	0.417	0.558	0.623	0.641	0.653	0.657
Day 7	MB	0.711	0.228	0.155	0.108	0.08	0.051	0.043	0.034	0.006	0.484	0.618	0.67	0.682	0.68	0.679
Day 8	MB	0.734	0.231	0.167	0.124	0.083	0.051	0.038	0.04	0.012	0.478	0.62	0.676	0.688	0.694	0.701
Day 1	SB	0.719	0.321	0.22	0.16	0.12	0.075	0.051	0.027	0.023	0.545	0.662	0.679	0.64	0.693	0.69
Day 2	SB	0.735	0.3	0.231	0.175	0.138	0.094	0.071	0.049	0.026	0.449	0.621	0.675	0.688	0.69	0.692
Day 3	SB	0.613	0.302	0.238	0.192	0.181	0.148	0.125	0.059	0.03	0.319	0.418	0.534	0.573	0.563	0.565
Day 4	SB	0.64	0.292	0.239	0.188	0.142	0.088	0.05	0.038	0.039	0.408	0.511	0.582	0.602	0.609	0.603
Day 5	SB	0.661	0.282	0.1	0.14	0.106	0.069	0.055	0.045	0.03	0.414	0.529	0.578	0.585	0.596	0.604
Day 6	SB	0.657	0.175	0.128	0.083	0.063	0.033	0.046	0.024	0.012	0.38	0.527	0.608	0.613	0.616	0.62
Day 7	SB	0.662	0.271	0.199	0.14	0.102	0.071	0.063	0.03	0.012	0.423	0.564	0.621	0.636	0.631	0.639
Day 8	SB	0.729	0.212	0.149	0.11	0.079	0.048	0.045	0.018	0.006	0.481	0.61	0.664	0.681	0.686	0.684

Table A.4.4 Dark Treatment Rapid Light Curves

Response and recovery yield (F_v/F_m) from rapid light curves taken in dark treatment mesocosms during summer 2013. Plants were dark adapted for 15 minutes before measurements were taken with a DIVING-PAM. Measurements were taken 5 cm from the base of each leaf.

		Response Pulse Number									Recovery Pulse Number					
Time	Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Day 1	GB	0.644	0.245	0.192	0.131	0.104	0.059	0.035	0.045	0.028	0.395	0.544	0.61	0.616	0.617	0.618
Day 2	GB	0.673	0.225	0.183	0.141	0.109	0.072	0.065	0.057	0.025	0.333	0.506	0.586	0.613	0.636	0.643
Day 3	GB	0.724	0.298	0.244	0.188	0.162	0.112	0.071	0.043	0.039	0.551	0.644	0.676	0.684	0.685	0.682
Day 4	GB	0.713	0.334	0.277	0.206	0.164	0.103	0.061	0.038	0.04	0.448	0.581	0.661	0.662	0.679	0.683
Day 5	GB	0.724	0.239	0.184	0.133	0.093	0.056	0.039	0.02	0.005	0.47	0.625	0.669	0.679	0.684	0.695
Day 6	GB	0.685	0.228	0.189	0.121	0.095	0.071	0.03	0.015	0.008	0.389	0.519	0.613	0.647	0.654	0.665
Day 7	GB	0.741	0.285	0.206	0.145	0.107	0.071	0.052	0.032	0.019	0.498	0.625	0.686	0.7	0.71	0.713
Day 8	GB	0.747	0.185	0.135	0.098	0.07	0.042	0.033	0.013	0.014	0.584	0.683	0.704	0.712	0.717	0.72
Day 1	HIB	0.732	0.256	0.193	0.13	0.093	0.073	0.042	0.025	0.027	0.472	0.646	0.688	0.695		
Day 2	HIB	0.74	0.222	0.179	0.125	0.102	0.044	0.04	0.036	0.007	0.482	0.63	0.673	0.688	0.689	0.694
Day 3	HIB	0.712	0.3	0.229	0.17	0.132	0.1	0.064	0.029	0.03	0.524	0.638	0.661	0.669	0.681	0.684
Day 4	HIB	0.734	0.318	0.259	0.191	0.155	0.097	0.086	0.038	0	0.5	0.634	0.684	0.694	0.696	0.697
Day 5	HIB	0.726	0.286	0.204	0.136	0.104	0.073	0.051	0.036	0.018	0.43	0.581	0.65	0.67	0.681	0.687
Day 6	HIB	0.725	0.223	0.167	0.125	0.078	0.056	0.038	0.017	0.023	0.469	0.605	0.675	0.685	0.696	0.689
Day 7	HIB	0.739	0.275	0.194	0.136	0.094	0.057	0.041	0.021	0.011	0.516	0.629	0.686	0.698	0.706	0.706
Day 8	HIB	0.74	0.14	0.112	0.076	0.058	0.046	0.025	0.021	0.011	0.535	0.663	0.69	0.702	0.703	0.709
Day 1	MB	0.68	0.198	0.161	0.129	0.116	0.108	0.083	0.048	0.01	0.396	0.54	0.616	0.637	0.643	0.636
Day 2	MB	0.663	0.286	0.21	0.18	0.125	0.117	0.088	0.089	0.048	0.41	0.589	0.628	0.634	0.638	0.645
Day 3	MB	0.732	0.329	0.276	0.212	0.168	0.121	0.086	0.054	0.019	0.461	0.609	0.665	0.68	0.693	0.689
Day 4	MB	0.711	0.344	0.277	0.208	0.161	0.119	0.092	0.068	0.03	0.481	0.607	0.665	0.663	0.672	0.682
Day 5	MB	0.71	0.318	0.232	0.167	0.121	0.081	0.058	0.037	0.009	0.445	0.548	0.602	0.628	0.654	0.665
Day 6	MB	0.741	0.183	0.14	0.096	0.078	0.044	0.041	0.024	0.006	0.46	0.601	0.679	0.701	0.708	0.709
Day 7	MB	0.715	0.225	0.177	0.135	0.109	0.074	0.047	0.025	0.006	0.468	0.62	0.662	0.676	0.688	0.689
Day 8	MB	0.749	0.156	0.126	0.093	0.07	0.045	0.03	0.015	0.003	0.604	0.68	0.703	0.711	0.717	0.72
Day 1	SB	0.666	0.215	0.16	0.121	0.099	0.064	0.038	0.008	0.008	0.432	0.586	0.62	0.639	0.704	0.702
Day 2	SB	0.67	0.196	0.138	0.12	0.093	0.07	0.063	0.037	0.038	0.346	0.534	0.603	0.628	0.634	0.646
Day 3	SB	0.752	0.291	0.232	0.174	0.125	0.084	0.061	0.038	0.018	0.575	0.689	0.698	0.706	0.709	0.713
Day 4	SB	0.72	0.313	0.248	0.189	0.15	0.1	0.061	0.037	0.032	0.419	0.558	0.656	0.673	0.682	0.677
Day 5	SB	0.688	0.248	0.192	0.141	0.1	0.062	0.046	0.033	0.009	0.509	0.609	0.63	0.639	0.649	0.642
Day 6	SB	0.733	0.258	0.188	0.125	0.098	0.068	0.044	0.02	0.021	0.5	0.643	0.7	0.701	0.71	0.704
Day 7	SB	0.733	0.244	0.183	0.118	0.093	0.072	0.035	0.022	0.007	0.503	0.659	0.683	0.695	0.695	0.7
Day 8	SB	0.634	0.284	0.217	0.175	0.132	0.092	0.068	0.033	0.015	0.504	0.563	0.582	0.593	0.593	0.601

Appendix 5 – Mesocosm Environmental Data

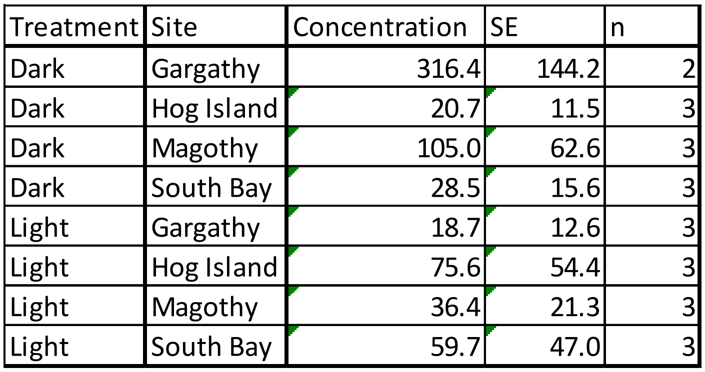
Table A.5.1 Pre-experiment Mesocosm Sulfide Concentrations

Average sulfide concentration (µM) for sediment treatments during the pre-experimental period for the mesocosm experiment summer 2013. SE represents standard error.

Site	Concentration	SE	n
Gargathy	103.5	17.5	5
Hog Island	43.6	10.3	5
Magothy	64.7	7.1	5
South Bay	62.3	9.0	6

Table A.5.2 Post-experiment Mesocosm Sulfide Concentrations

Average sulfide concentration (μM) for sediment treatments taken after the experimental period for the mesocosm experiment summer 2013. SE represents standard error.



Treatment	Site	Concentration	SE	n
Dark	Gargathy	316.4	144.2	2
Dark	Hog Island	20.7	11.5	3
Dark	Magothy	105.0	62.6	3
Dark	South Bay	28.5	15.6	3
Light	Gargathy	18.7	12.6	3
Light	Hog Island	75.6	54.4	3
Light	Magothy	36.4	21.3	3
Light	South Bay	59.7	47.0	3

Appendix 6 – Mesocosm Plant Measurements

Table A.6.1 Mesocosm Productivity and Aboveground Biomass

Productivity (leaf area*m^{-2}) and aboveground biomass (g*m^{-2}) of plants in mesocosm experiment. The measurements were taken at the end of the experimental period. SE indicates standard error.

Light Treatment	Sediment Treatment	aboveground biomass	SE	Productivity	SE
Light	Gargathy	23.7	3.9	29.0	4.1
Light	Magothy	18.4	3.2	25.2	4.8
Light	Hog Island	12.0	2.6	16.0	3.6
Light	South Bay	18.9	2.6	22.2	4.3
Dark	Gargathy	12.6	4.9	20.9	5.8
Dark	Magothy	13.0	0.8	20.1	1.7
Dark	Hog Island	12.3	0.8	20.1	1.5
Dark	South Bay	12.4	4.1	18.9	5.9