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The role of porewater sulfide toxicity among other multiple stressors in *Zostera marina* populations in Long Island South Shore Estuaries

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Abstract of the Thesis

The role of porewater sulfide toxicity among other multiple stressors in *Zostera marina* populations in Long Island South Shore Estuaries

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Eelgrass (*Zostera marina*) is the dominant seagrass in Long Island's South Shore Estuaries (LISSE). Eelgrass meadows in LISSE are heavily influenced by anthropogenic impacts and have been declining over the last three decades. A combination of stressors, including increased porewater sulfide concentrations, eutrophic shading, increased temperature and habitat modification have been implicated as causes for extensive seagrass loss here and world-wide. Porewater sulfide surveys performed in this study evaluated eelgrass meadows and unvegetated substrates across Great South Bay (GSB), New York, and confirmed that toxic porewater sulfide concentrations currently exist in the bay. Survey results revealed that patchy eelgrass meadows had significantly higher porewater sulfide concentrations than dense meadows. This suggests that toxic porewater sulfide concentrations are currently present in some stressed seagrass beds in GSB.

Through manipulative laboratory and field experiments the role of porewater sulfide toxicity and other stressors (shading and habitat modification) was investigated. Laboratory experiments using aquaria found no negative effects in eelgrass productivity and survival when eelgrass was subjected to sub-toxic porewater sulfide concentrations and a mild shade reduction in light intensity. Specific productivity in high sulfide treatments with hard clams was greater than treatments without clams. This difference

was found regardless of high shading (80% reduction in light intensity) that was used in the experiment. This increase in specific productivity however was much greater amongst shaded treatments with clams. This suggests that hard clam presence stimulates eelgrass productivity, particularly when facing shading stress. A field experiment was conducted to investigate the effects of increased porewater sulfide and shading in a dense grass bed in GSB. Glucose perfusers were used in the field experiment and successfully increased the porewater sulfide pool. Statistically significant differences were found in percent survival and standing crop (g/m^2) among control and glucoses mediated sediments. A non-significant trend was also seen in eelgrass productivity (mg/SS/day), with Control replicates exhibiting higher productivity than high glucose enriched sediments. Control replicates experienced toxic porewater sulfide concentrations and at termination had a 55% survival rate. Medium and High glucose mediated sulfide treatments experienced very high porewater sulfide concentrations and had lower survival rates (20% and 15% respectively). Results suggest that the effects of porewater sulfide toxicity, shading, and habitat modification (presence/absence of hard clams) are relevant components of eelgrass decline in GSB. It is important that future research be conducted to elucidate these interactions of multiple stressors now that fundamental procedures and methods have been established.

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Background

Seagrasses serve several important functions in coastal ecosystems: such as shelters and nurseries for juvenile fish and shellfish, substrate for larval attachment, and food for a wide variety of organisms (Kikuchi 1980). Seagrass ecosystems also improve water quality by preventing sediment resuspension (Garcia & Duarte 2001, Garcia et al. 2002). As fully submerged photosynthetic organisms, seagrasses have physically adapted to the underwater environment. The most important of these adaptations are the development of aerenchyma tissue (spongy tissue that allows for internally produced O_2 to be transported throughout the seagrass), the development of a photosynthetic epidermal layer, and the loss of stomata (Larkum et al. 2006). These adaptations optimize seagrass photosynthesis in the marine environment and allow for oxygen to be transported to non-photosynthetic root and rhizome biomass found in sediments. Although they are marine plants, seagrasses are true angiosperms with adventitious roots. Since migrating into aquatic systems, they have radiated to marine and estuarine systems world-wide, with the exception of Antarctica (Spalding et al. 2003, Short et al. 2006). *Zostera marina* (eelgrass) is a common seagrass distributed on the western and eastern coasts of the North Pacific and North Atlantic oceans, as well as in the Mediterranean Sea (Kuo & Den Hartog 2001). Eelgrass is the dominant seagrass on the western Atlantic coast, particularly in estuaries on the eastern U.S. coast, including New York State's Long Island south shore estuaries (LISSE). This estuary system is a series of connected shallow bar built estuaries (Bokuniewicz & Schubel 1991), with the largest being Great South Bay.

Great South Bay (GSB) is a shallow water estuary (average depth ~2 m) spanning approximately 40 km in length and possessing a maximum width of 11 km (Schubel 1991). Tides, wind events, and ocean exchange through the Fire Island and Moriches Inlets are the dominant factors responsible for water circulation and mixing that occurs in the Bay (Wilson et al. 1991). Great South Bay has and continues to support large commercial, industrial, residential, and recreational activities (Carpenter et al. 1991). In the past, Great South Bay shellfish fisheries dominated the U.S. domestic market, producing Eastern oysters (*Crassostrea virginica*) in the early 1900's and hard clams (*Mercenaria mercenaria*) in the following decades (COSMA 1985, McHugh 1991). Since then, these fisheries have respectively crashed in the 1930's and 1980's, largely due to overharvesting, deteriorating water quality, and ineffective management policies (COSMA 1985, McHugh 1991). Concurrently with the oyster fishery crash in the 1930's, there was a significant reduction in eelgrass meadow coverage in the Bay (Carpenter et al. 1991), from the regional eelgrass "wasting disease" (*Labyrinthula zosterae*) (Short et al. 1987, Vergeer et al. 1995). Eelgrass was seen as a public nuisance in GSB, which made seagrass restoration activities virtually non-existent during this time (Dennison et al. 1991). Eelgrass populations subsequently recovered after the oyster fishery crash and wasting disease events, but endured a second significant decline in close proximity to the GSB hard clam industry crash in the 1980s (Dennison et al. 1989). Reduced biogenic filtration and diminished water quality resulted in recurrent blooms of "brown tide" phytoplankton (*Aureococcus anophagefferens* (Gobler et al. 2005)). These

intense blooms were responsible for this second eelgrass decline in Great South Bay (Cosper et al. 1987, Dennison et al. 1991, Gobler et al. 2005).

Coastal eutrophication fueled mostly from land run-off and ground water discharge stimulates these harmful phytoplankton blooms (Dennison et al. 1991) and is a growing concern as GSB becomes more heavily populated. At peak hard clam abundances, the entire volume in GSB was filtered thorough the clam community once every 2.6 days, in contrast, today the system filtration rate is approximately once every 100 days (Cerrato et al. 2004). The loss of hard clams and subsequent increased system filtration time of GSB could encourage the persistence of intense phytoplankton blooms that were previously controlled through biotic filtration. Currently, hard clam populations are at historic lows (NYDEC 1999) and eelgrass populations have continued to decline for the last three decades. This loss of critical habitat has lead to increased interest in restoration activities (Short & Neckles 1999) as suspension feeding bivalves and seagrasses have strong positive relationships with each other (Peterson & Heck 2001). Bivalves remove phytoplankton that can shade seagrasses as well as fertilize the sediments with fecal and pseudo-fecal deposits (Peterson & Heck 2001, Carroll et al. *in press*). Seagrasses provide a refuge from predation for juvenile shellfish (Kikuchi 1980) and reduce water column turbidity by preventing sediment resuspension (Gacia & Duarte 2001, Gacia et al. 2002) and slow flow, enhancing particle concentrations at the benthic boundary layer, both of which increases suspension feeding rates of hard clams (Malouf 1991). This strong positive relationship was recently supported by (Wall et al. 2008), who found that eelgrass productivity increased in the presence of hard clams in eutrophic

mesocosm environments as a result of bivalve filtration of the water column. The increase in turnover rate in GSB is particularly important for eelgrass survival, as phytoplankton blooms can significantly shade eelgrass blades, decreasing photosynthesis and causing seagrass stress (Dennison et al. 1989, Goodman et al. 1995, Holmer & Laursen 2002). The combination of lower photosynthetic rates through increased shading and impacts from other stresses, such as increased porewater sulfide due to higher organic loading of sediments (Terrados et al. 1999, Holmer & Laursen 2002, Holmer et al. 2005), increased salinity and temperature, (Koch & Erskine 2001) or habitat modification have been implicated as causes for extensive seagrass loss world-wide.

The potential stressors of eelgrass populations in coastal New York, particularly sulfide toxicity, are similar to those implicated in causing seagrass mortality in *Thalassia testudinum* in Florida Bay (Carlson et al. 1994, Koch & Erskine 2001). Eelgrass populations in Chincoteague Bay, Maryland (Goodman et al. 1995), the French Mediterranean Sea (Plus et al. 2003), Denmark (Holmer & Laursen 2002, Holmer et al. 2005) and the Long Island Sound (Koch & Beer 1996) have also experienced mortality due to interactions of multiple stressors.

Introduction

Sediment toxicity in marine and coastally flooded terrestrial sediments are strongly influenced by sulfides. Sulfides (ΣH_2S i.e H_2S, HS^-, S^{2-} but primarily H_2S) are produced by heterotrophic bacteria carrying out dissimilatory sulfate reduction [$2CH_2O + SO_4^{2-} \rightarrow HCO_3^- + HS^- + CO_2 + H_2O$]. This bacterial metabolic process remineralizes organic matter in the absence of oxygen (Wang & Chapman 1999). Sulfides influence sediment toxicity in four major ways: 1) Sulfides are strong metabolic toxins to many aerobic organisms (Wang & Chapman 1999); 2) Sulfides can act as both a source and sink for the exposure of other metal and metalloid toxicants (i.e. Cd and As, respectively) by binding toxic metal ions into insoluble complexes not bioavailable to organisms (Burgess & Scott 1992) and releasing toxic metal ions when metal sulfide complexes dissociate (Peters et al. 1997); 3) Sulfides can change sediment dynamics and overall sediment toxicity by altering the behavior of benthic organisms (i.e. burrowing depth, irrigation, feeding rates) (Wang & Chapman 1999); and 4) Sulfides can work in conjunction with other toxicants and environmental stressors, such as increased temperature, eutrophication and habitat modification to enhance overall sediment toxicity (Goodman et al. 1995, Peters et al. 1997, Koch & Erskine 2001). A diagram of some abiotic and biotic factors affecting sediment toxicity is represented in Figure 1.

Dissolved sulfides (ΣH_2S) are toxic to a wide range of organisms, from single celled cyanobacteria (Miller & Bebout 2004) to multicellular fauna (Ip et al. 2004, Blackstone et al. 2005) and flora (Koch & Mendelssohn 1989, Goodman et al. 1995, Koch & Erskine 2001, Armstrong & Armstrong 2005). Sulfides act as metabolic poisons

to fauna by inhibiting cytochrome c oxidase, preventing ATP production from oxidative phosphorylation in the electron transport chain in mitochondria (Ip et al. 2004). In flora, sulfides act as cytotoxins, binding to proteins and inhibiting oxygenic photosynthesis and respiration (Bagarinao 1992, Miller & Bebout 2004). Although sulfides are potent metabolic toxicants, many organisms that live within or in contact with waterlogged anoxic and sulfidic sediments have evolved adaptive mechanisms to reduce sulfide toxicity (Goodman et al. 1995, Grieshaber & Volkel 1998, Ip et al. 2004, Armstrong & Armstrong 2005, Choi et al. 2006).

Macrophytes, such as marsh grasses, rice plants, mangroves and seagrasses, prevent sulfide toxicity by transporting oxygen derived from photosynthetic processes or in the overlying water and air, to root and rhizome systems located within anoxic sediments (Goodman et al. 1995, Choi et al. 2006). The oxidation of sediments around root and rhizome structures is critical in reducing the toxicity of sulfides to submerged aquatic vegetation (Goodman et al. 1995, Eldridge & Morse 2000, Armstrong & Armstrong 2005, Choi et al. 2006). In *Zostera marina* oxygen flux to below-ground biomass is mostly derived from photosynthesis and this leakage rate becomes saturated when the eelgrass experiences light irradiance values around 500 μmol photons/m^2/s (Frederiksen & Glud 2006) or ~30000 lux using conversion equations from (Inada 1984). Eelgrass photosynthesis has been found to become saturated at ~200 μmol photons/m^2/s (Dennison & Alberte 1982) or ~12000 lux using equations from (Inada 1984). Light irradiance values close to these are only naturally possible during daylight hours in this region, as eelgrass begin to experience negative effects when receiving < 15% of surface

irradiance (Moore & Short 2006). Minimal oxygen has been shown to be transported to below-ground biomass when light is not present, if overlying water oxygen concentrations are greater than 450 μmol/L, based on laboratory experiments (Frederiksen & Glud 2006). Oxygen transported to below-ground biomass leaks from roots and forms an oxygenated buffer around the rhizosphere (Frederiksen & Glud 2006, Marba et al. 2006). This buffer oxidizes sulfides in and around the roots into non-toxic sulfur species, reducing sulfide toxicity (Holmer et al. 2005, Marba et al. 2006). Therefore, if the transport of oxygen to below-ground biomass decreases, because of eutrophic shading, sulfide penetration and toxicity to below ground biomass is enhanced. Sulfide intrusion is a major seagrass stressor and in addition with other stressors has been implicated in causing the loss of critical seagrass habitats (Carlson et al. 1994, Goodman et al. 1995, Terrados et al. 1999, Koch & Erskine 2001). This research attempts to evaluate and further understand the possible role and significance of sulfide toxicity on eelgrass health and survival in eutrophic Great South Bay thru manipulative field and laboratory experiments.

Objectives

The primary objectives of this study were: 1) to evaluate current porewater sulfide concentrations within Great South Bay in *Z. marina* grass beds and in adjacent unvegetated sediments; 2) to experimentally examine the effect of shading and increased porewater sulfide concentration on the growth and survivorship of eelgrass in a laboratory setting; and 3) to experimentally examine the effect of shading and increased porewater sulfide concentrations on eelgrass growth and survivorship in the field.

Methods

I. GSB porewater sulfide survey

To determine the range of current porewater sulfide concentrations within GSB sediments, 20 sites and over 100 samples were taken between April and August 2007 (see map, Figure 2). At each site, several porewater samples were taken at a depth of 7 cm. Sites were chosen based on the presence of eelgrass with the intent to survey the breadth of the bay. Weather conditions (clear / overcast) were noted on sampling days so that data could be grouped into two general light intensity categories, clear day and overcast day. Porewater samples were taken on April 5th, May 18th, and June 21st, 2007 all during daylight hours. On both April 5th and June 21st the sky was sunny with some clouds. The remaining sampling day was overcast with light showers. Porewater samples were categorized into the following groups: dense seagrass, patchy seagrass and unvegetated sediment.

Porewater was collected from 7 cm sediment depth by divers using a constructed porewater "sipper" device. Sipper devices consisted of a 60 ml plastic syringe, a 3 way Luer-lok plastic stopcock, Tygon tubing, a 1 ml plastic syringe, and a Porex© filter tip (pore size 15-35 μm) (Figure 3). Porewater was collected by first inserting the sipper into the sediment to the desired depth. The syringe piston was slowly pulled and shut off after approximately 10 ml of porewater was collected by turning the 3 way stopcock. This isolated the collected porewater sample. The 1 ml syringe used acts as a stopper for the perpendicular port of the 3 way stopcock, preventing ambient water from diluting the

porewater sample (Fig. 3). Collected porewater sulfide samples were immediately fixed by pipetting 250 µl of the sample into labeled vials containing 500 µl of 0.05M zinc acetate solution. Sample vials were sealed and stored in a cooler with ice until returning to the laboratory. Porewater sulfide analysis occurred within 24 hours of collection and followed the methods described in Cline 1969.

II. Laboratory sulfide and shading experiments

A laboratory experiment was conducted at the Marine Sciences Research Center's Aquaculture facility on Great South Bay to assess what role increased sulfide and shading would have on eelgrass survival and productivity. Experiments were conducted in both the summer and the fall of 2007. Experimental units consisted of 38L (10 gal) glass aquaria with a plastic seagrass planter (LxWxH = 33x19x11.5cm) inside. Seagrass planters were wrapped with high-density black plastic sheets to prevent light penetration on all sides. Porewater injection and sampling devices (Figure 4) were placed in each planter and buried until they were ~5 cm below the sediment water interface. Devices consisted of 3 equal lengths (~20cm) of perfuser tubing attached to two PVC tees. Tubing with 3 way stopcocks were attached to each perfuser, allowing for porewater sulfide injections and porewater sampling to be administered without physically disturbing the sediment. The benefits of sampling the sediment porewater in this manner is that it allows for horizontally integrated porewater samples to be easily obtained from a constant depth, while also providing a direct method for sulfide injection into the sediment. All experimental aquaria received continuous unfiltered GSB seawater for the

duration of both experiments. Both light and temperature were measured in shaded and un-shaded replicates throughout the experiment using HOBO (Onset Technologies) light and temperature loggers.

Summer Laboratory Experiment

A 3 x 2 randomized block design was used in this experiment. Forty experimental aquaria were set up for a 28 day experiment. The following treatments were randomly assigned to experimental microcosms: (Control, un-shaded (CA)); (Medium Sulfide, un-shaded (MA)); (High Sulfide, un-shaded (HA)); (Control, shaded (CS)); (Medium Sulfide, shaded (MS); and (High Sulfide, shaded (HS)) (Figure 5). Shaded treatments received a sleeve of 22% light reduction greenhouse shade cloth which covered all sides and the top of the aquaria, blocking out ambient light from all directions. This light reduction percentage is equivalent to reductions in GSB from minor algal blooms (Gobler et al. 2005). Light intensity (lux) and water temperature (˚C) were measured throughout the experiment using HOBO light/temperature probes in two replicates in both shaded and un-shaded treatments. Hourly light intensity data (Figure 6) and temperature data (Figure 7) throughout the experiment were analyzed. Temperature differences between shaded and un-shaded replicates were greatest in the afternoon (~13:00), with an average temperature difference in un-shaded replicates of +2.25 ˚C from shaded replicates (Figure 8). Temperature values ranged from 15˚C to 30˚C throughout the experiment, and were similar to values from GSB during that time (Rountos this study). Temperatures below 5˚C and above 30˚C have been found to

adversely affect eelgrass (Nejrup & Pedersen 2008). Light intensity data loggers showed that shaded replicates received about 44% less average light intensity than un-shaded replicates during daylight hours, using the 22% shade cloth (Figure 9). This calculation excludes light intensity averages for 16:00 and 17:00, as shaded replicates for these times recorded higher light intensities than un-shaded replicates. Light intensity values for shaded (~65000 lux) and un-shaded (~80000 lux) replicates were about an order of magnitude higher than light intensity values in GSB at a depth of 1m (~8000 lux) (Rountos this study). Saturating light intensity values for eelgrass photosynthesis and rhizosphere oxygen leakage have been found to be ~200 μmol m^{-2} s^{-1} (~12000 lux, (Inada 1984)) (Dennison & Alberte 1982) and 500 μmol m^{-2} s^{-1} (~30000 lux, (Inada 1984))(Frederiksen & Glud 2006) respectively, suggesting that both light treatments experienced saturating light levels for photosynthesis and oxygen leakage.

Porewater sulfide addition treatments consisted of: control (ambient filtered seawater), medium sulfide (~800 μM NaS-H_2S solution), and high sulfide (~1600 μM NaS-H_2S solution). In summation, control porewater addition treatments consisted of 6 replicates for each light treatment, while medium and high H_2S porewater addition treatments both had 7 replicates for each light treatment respectively.

Eelgrass was collected from a dense meadow in Great South Bay. Healthy terminal shoots were separated and care was taken to make sure that each of the selected shoots had an adequate and consistent amount of root and rhizome material (rhizome ~2.5 cm length) for successful transplantation. Shoots were planted at a consistent depth at least 3 cm from the next shoot and allowed to acclimate and heal for 10 days, which is

consistent with previous experiments using *Z. marina* (1 – 2 weeks) (Holmer & Bondgaard 2001).

Four-hundred of the healthiest looking shoots (shoots that visually resembled healthy grass in the field) were selected and ten shoots were planted in each seagrass planter as seen in Figure 10, making sure that the roots and rhizomes penetrated the diffusive matrix created by the injection device. Shoots with discolored blades and or appeared infirm, were not selected for this experiment. The following porewater parameters were measured during this experiment: porewater sulfide, ammonium (NH_4), and pH. Porewater sulfide was measured in order to verify that the respective sulfide treatments were in fact experiencing different porewater sulfide concentrations. Porewater ammonia was measured to ensure that seagrasses were not exposed to toxic porewater ammonium concentrations and to determine if concentrations between experimental microcosms were similar. While an exact toxic concentration for porewater ammonium for eelgrass is not currently available (Tennant 2006), porewater concentrations less than 500 μM have been found to not be toxic to eelgrass (Zimmerman et al. 1987). Finally, porewater pH was measured to ensure that all microcosms exhibited field porewater pH levels and to verify that all microcosms exhibited similar porewater pH, as sulfide species are sensitive to pH fluctuations (Bagarinao 1992).

Porewater samples were collected prior to the initiation of the experiment and analyzed for sulfide, ammonium and pH during the acclimation process. This was done to ensure that eelgrass shoots were not experiencing porewater stress, based on parameter from previous research (Dennison et al. 1987, Goodman et al. 1995). Upon experimental

initiation, porewater sulfide and pH samples were analyzed from all microcosms on Days 0, 7, 11, 13, 17, 21, 25, and 28 of the experiment, while porewater ammonium samples were analyzed less frequently (Day 2, 4, 5, 13, 21, and 28). Porewater samples were consistently collected between 15:30 – 18:30, with the last aquarium being sampled at least one hour before sunset. Porewater was collected by applying gentle pressure to a syringe piston until ~20 ml of porewater was collected. Porewater sulfide samples were immediately fixed by pipetting 250 µl of sample into vials containing 500µl of zinc acetate solution for further analysis as described in Cline 1969. Sample vials were capped and placed on ice until analysis. Porewater pH was measured by filling a 1 ml syringe and attaching it to a piece of plastic tubing coupled to a pH electrode (Figure 11). Porewater ammonium samples were frozen for future NH_4 analysis in the lab.

Experimental porewater injections for all treatments were conducted for the first five days of the experiment and then administered every 3-4 days thereafter until termination. Porewater injections were done by filling three 60 ml plastic syringes each with 25ml of stock solution (control, medium sulfide (~800 µM), or high sulfide (~1600 µM) respectively). Control and sulfide solutions were prepared using filtered de-aerated ocean water buffered to porewater pH (6.8-7.2) in the lab using additions of 1N HCl solution. Injections always followed sampling and were consistently administered approximately 1hr before sunset to try and mimic natural diel root exposure to sulfide in the field.

Eelgrass shoots were marked for productivity on Day 14 using a modified blade puncturing technique (Zieman 1974). At the conclusion of the experiment, all eelgrass

shoots were collected and sediment cores were taken from each microcosm for loss on ignition (LOI) analysis.

Fall Laboratory Experiment

A second laboratory experiment was conducted in the fall (Oct. 11, 2007 – Nov. 7, 2007) using 24 aquaria, consisting of 8 independent treatments of 3 replicates each (see Figure 12). In this experiment shaded treatments were covered with an 80% light reduction sleeves. Temperature and light intensity were continuously monitored hourly throughout this experiment. Light intensity data throughout the experiment is plotted in Figure 13. Un-shaded replicates experienced average daylight light intensity levels (6,740 lux) similar to levels in GSB at a depth of 1m (8000 lux)(Rountos this study) and under photosynthetic saturating levels (~12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982)). Un-shaded light intensity values during daylight hours ranged from 28 lux to 15686 lux. Shaded replicates received an average ~80% reduction in light intensity with an average daylight light intensity value of 1429 lux (Figure 14), which is comparable to natural reductions in light intensity by dense algal blooms in GSB (Gobler et al. 2005). Temperature plots between un-shaded and shaded replicates were also analyzed (Figure 15). Temperature plots showed the largest temperature differences between shaded and un-shaded treatments (~2.75 ˚C) at around 13:00 (Figure 16). Aquaria temperatures ranged between 6.5 ˚C to 23˚C, and were not under or above temperatures known to adversely affect eelgrass health and productivity (Nejrup & Pedersen 2008).

Porewater injection solutions remained the same as the previous experiment, with an additional two new Hard Clam treatments at the High sulfide level both shaded and un-shaded. In these additional treatments 3 Hard Clams (maximum shell length ~25mm) were added into the respective seagrass planters. Sampling and injecting procedures followed protocols discussed in the previous experiment, with the exception that NH_4 samples were not analyzed.

III. Field sulfide and shading experiment

To examine the effects of sulfide toxicity and shading on eelgrass growth and survivorship in the field, experiments were conducted in the summer and fall in separate seagrass meadows in two LISSEs, Great South Bay and Shinnecock Bay. Black 19 L buckets (Height = 34cm, Diameter = 30cm) with the bottoms removed were inserted into a continuous eelgrass bed and served as experimental units. Buckets physically isolated eelgrass biomass inside the buckets from the surrounding seagrass meadow preventing any chemical or physical exchanges. Porewater sulfide concentrations were elevated in sulfide treatment buckets by burying three cylinder shaped glucose filled perfusers (Ave L=11cm, I.D. = 2.5cm) inside the respective buckets. Control (no glucose) buckets received three filled perfusers with filtered ocean water. Previous laboratory experiments using LISSE sediments found that glucose enrichment of the sediment greatly stimulated porewater sulfide pools (Rountos unpublished). Experimental shading was achieved by installing PVC pipe frames (LxWxH = 55x55x110cm) covered with a 22% density screen

over shaded replicates (Figure 17). This light reduction is similar to reductions by minor algal blooms in GSB (Gobler et al. 2005).

Summer Field Experiment

Experiments were conducted for 4 weeks in a natural eelgrass meadow (depth ~1m) within Great South Bay in August 2007. Eighteen black buckets were inserted into the eelgrass meadow, making sure that they were at least 1 m away from each other and the edge of the grass bed. Eelgrass patches isolated by the buckets were allowed to recover for about 3 weeks prior to experimental initiation. Experimental treatments consisted of three levels of glucose addition (no glucose, 5%, and 20%) and two light treatments, ambient and shaded (22% light reduction screen added, similar to light reduction by minor algal blooms in GSB (Gobler et al. 2005)). Replicates for the respective treatments were randomly assigned to buckets. Perfusers filled with glucose solutions passively leak glucose into the surrounding sub-oxic sediments fueling bacterial sulfate reduction and leading to enhanced sulfide production. Sulfides in turn accumulate in these sediments and increase the overall sulfide concentrations inside the bucket.

Light intensity and temperature were measured in a shaded and un-shaded replicate continuously using HOBO light/temperature data loggers (Onset Technologies). Light intensity throughout the experiment is plotted in Figure 18. Shaded treatments experienced an average 38% decrease in light intensity compared to un-shaded treatments during daylight hours (Figure 19). This corresponds to a decrease in average light

intensity from ~8600 lux to ~3300 lux respectively. Both averages were under light saturating intensity values for photosynthesis (~12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982)), but shaded treatments were about 4 times less. Water temperature was measured hourly in both shaded and un-shaded replicates and trends are plotted in Figure 20. Average water temperatures in shaded and un-shaded replicates never varied more than 0.053 ˚C throughout the 30 day experiment.

At experimental initiation, perfusers were buried approximately 5 cm below the sediment surface in an equidistant triangle formation. The initial numbers of short shoots were recorded and porewater samples were taken from each bucket using sipper devices. Porewater sulfide, ammonium, and pH were fixed and analyzed as described earlier on Day 0, 16 and 30. Eelgrass shoots in every bucket were marked for productivity following techniques described in Zieman (1974) on Day 21. At the conclusion of the experiment all eelgrass biomass within the bucket was harvested for processing.

A fall experiment was attempted and initiated before being destroyed two weeks into the experiment, most likely by fishing gear. The data from this experiment could not be salvaged and this experiment was abandoned.

IV. Seagrass analysis

In all laboratory and field experiments, eelgrass growth, productivity, and survival were assessed as the dependant variables. Eelgrass survival was determined by the loss of short shoots over time. Growth rate and total new biomass were quantified using a modified seagrass blade puncturing procedure described in Zieman (1974). In this

procedure, an 18 gauge needle was used to punch a hole through the base of eelgrass shoots. Puncturing just above the basal meristem (region of actively dividing cells in seagrass plants) creates a hole that will be present in all germinated blades of the shoot. As seagrasses grow from the basal meristem found at the base of the shoot, the oldest parts of eelgrass blades are the blade tips and the newest tissue is closer to the shoot base. The movement of the punctured hole from the shoot base is used as an indicator of both growth rate and total new production. Since the distance the pin hole has moved from the shoot base indicates the amount of new growth, a growth rate (cm^2 new eelgrass / time) can be calculated, because the amount of time since the hole has been at the shoot base is known. Growth rates and total new eelgrass biomass were assessed at the conclusion of all experiments. At the end of all experiments, all seagrass material was harvested. Blades of each shoot were separated and epiphytes were removed by gently scraping with a razor blade. Leaf lengths and widths were measured to the nearest mm and recorded. Once the epiphytes were removed, the blades were separated into new and old growth. All eelgrass biomass from the shoot base to the pin hole and newly germinated seagrass blades with no pin hole were considered as new biomass. Separated new and old growth plant tissue were dried at 70 ^{o}C for 48 hr in a convection oven then weighed and recorded. Epiphyte biomass was not quantified, as there was very little biomass on all blades.

V. Porewater analysis

Porewater sulfide samples were analyzed in the lab using a modified colorometric method following Cline 1969. Porewater samples were fixed with 500 µl of 0.05M zinc acetate immediately after samples were obtained. Within 24 hours these samples were analyzed colorometrically using a Diamine reagent to develop color in the samples following this protocol. To ensure complete mixing and development of the samples, samples were gently shaken. Once samples and reference standards fully developed absorbances were read at 670nm using a manual spectrophotometer. All measurements were recorded and porewater sulfide concentrations were then calculated based on reference standard linear regression. Porewater sulfide concentrations for the fall laboratory aquarium experiment were measured using a plate reader spectrophotometer and not the manual spectrophotometer. Although preliminary quality control experiments conducted to compare the data output from the plate reading spectrophotometer and the manual spectrophotometer found encouraging correlations, time did not permit for all samples to be run also with the manual spectrophotometer for this second laboratory experiment.

Porewater ammonium samples were analyzed using a plate reading spectrophotometer using modified methods from Parsons et al. 1984. In this colorimetric determination ammonium samples are mixed with a phenolic / sodium nitroprusside solution. This forms a blue indophenolic dye where ammonium is present, with darker colors suggesting higher ammonium concentrations. To analyze porewater ammonium samples all stored samples were completely thawed. Once thawed porewater samples

and freshly prepared ammonium standards were pipetted into the wells of multiple 96 well plates. Since only 280µl of sample or standard were pipetted in each well, three wells were used for each standard solution and 2 for each sample. This allowed for a more robust reading of both the ammonium standards and samples to be obtained. All plate wells were treated with the necessary reagents for color development and after an hour the plates were read. All well plates for ammonium analysis were read at a wavelength of 640nm. All measurements from the plate reading spectrophotometer were recorded and porewater ammonium concentrations were calculated using the linear regression found from the reference standards.

VI. Statistical analysis

All collected data were organized using Microsoft Excel 2003 and statistical analysis was performed using SigmaStat (SYSTAT©). Differences between treatments were considered to be significant when $P < 0.05$. Treatments in all experiments were compared using One-way and Two-way analysis of varience tests (ANOVAs), and t-tests (where noted within) to determine levels of significance between treatments for all experiments. Multiple comparison procedures included the Holm-Sidak method, Student-Newman-Keuls method and Tukey Test method were suggested by statistical software. If any comparisons did not pass normality tests data was square root transformed and re-analyzed.

Results

I. GSB Porewater Sulfide Survey

A total of 20 sites were sampled over three non-consecutive days (4/5/07, 5/18/07, and 6/21/07) across southern Great South Bay (see map, Figure 2). At each site a minimum of two porewater sulfide samples were taken for analysis. The sky was clear and sunny on two of the sampling days (4/5/07, 6/21/07) and overcast for the remaining sampling day (5/18/07). Each site sampled had a combination of the following substrate categories: dense eelgrass (DG), patchy eelgrass (PG), or unvegetated sediment (S). Substrates were separated into categories by visual qualitative measurements of eelgrass density by divers. Actual estimates of eelgrass density were not taken from all substrates during the survey, so no quantitative categorization could be used based on eelgrass density. However, eelgrass density estimates in dense grass substrates in GSB in a non-sampled meadow ranged from 240 shoots/m^2 to >400 shoots/m^2 and unvegetated substrates exhibited no eelgrass densities (0 shoots/m^2). Although eelgrass density estimates were not taken for Patchy grass substrates, divers could confirm that this substrate always appeared significantly less dense than dense grass substrates. Data from all survey sites and sampling days were organized into substrate and light (clear/overcast) categories for graphical and statistical analyses (Figure 21). Data for clear sampling days consisted of 6 samples from dense grass, 8 samples from patchy grass, 6 from unvegetated sediment, and 4 from an unvegetated Nature Conservatory hard clam restoration site (HC)(samples taken with permission). On the overcast sampling day, 21 samples were obtained from dense grass beds, 10 from unvegetated substrate, and 2

samples from a patchy grass bed. Mean porewater sulfide concentrations on clear sampling days were 44μM ± 21 S.E., 329μM ± 106 S.E., 178μM ± 68 S.E., and 26μM ± 9 S.E.) for dense grass, patchy grass, unvegetated sediment, and unvegetated Hard Clam restoration site respectively (Figure 22). Porewater sulfide concentrations for substrates sampled on the clear day exhibited the following ranges: dense grass (0:117 μM), patchy grass (~50: ~950μM), unvegetated sediment (~5: ~400μM) and hard clam restoration site (0-~50μM). Hard Clam restoration site samples were only taken once (4/5/2007) and had no overcast porewater sulfide data for comparison. On the overcast sampling day mean porewater sulfide values in dense grass, patchy grass, and unvegetated sediment were 609μM ± 71 S.E., 980μM ± 242 S.E., and 636μM ± 166 S.E.) respectively. These porewater sulfide concentrations are about 14 times greater for dense grass, ~3x greater for patchy grass, and ~3.4x greater for unvegetated sediments on the clear sampling days. Mean porewater sulfide concentrations for all treatments on the overcast sampling day were above 600μM, with the largest average porewater sulfide concentration being around 1000μM for patchy grass. Overcast porewater sulfide concentrations for sampled substrates ranged as follows: dense grass (~110: ~1300μM), patchy grass (~750: ~1200μM), and unvegetated sediment (~70: ~1650μM). Porewater sulfide concentrations in excess of 600μM are classified as toxic to eelgrass, based on EC50 laboratory experiments for eelgrass photosynthesis (Goodman 1995).

Two-Way ANOVAs were used to analyze the porewater sulfide survey data. The main factors were light (clear and overcast) and bottom type (dense grass, patchy grass and unvegetated sediment). All data was square root transformed to meet the

assumptions of normality (p=0.738) for statistical analysis. A highly significant difference was found in porewater sulfide concentration between clear (184μM ± 53 S.E.) and overcast (742μM ± 70 S.E.) sampling days, for all substrates combined (p<0.001), excluding the hard clam site data. In addition, there was a statistically significant difference in average porewater sulfide concentrations between the substrates when combining clear and overcast sampling days (p=0.025) (DG = 327μM ± 73 S.E., PG = 654μM ± 126 S.E., S= 407μM ± 119 S.E.). The porewater sulfide average concentration for dense grass substrate was significantly less than the patchy grass concentration (unadjusted p=0.007, Holm-Sidak method), this significant difference was only seen on the clear sampling days (p=0.002, One-Way ANOVA (SQRT TRANSFORM) (Figure 22)). The average Hard Clam restoration site porewater sulfide concentration (26μM ± 9 S.E.) was found to only be significantly different from the patchy grass concentrations (329μM ±106 S.E.) during clear sampling days (p=0.005, (SQRT TRANSFORM)). Overcast porewater sulfide average concentrations among substrates did not differ significantly from each other (p=0.442, One-Way ANOVA (SQRT TRANSFORM).

II. Laboratory Sulfide & Shading Experiment

Summer Experiment

The summer laboratory experiment was conducted for 28 days. By day 7 of the experiment significantly different porewater sulfide treatments were established regardless of shading treatment (p<0.001, Two-Way ANOVA, Control (0μM ± 10.2 S.E.), Medium (59μM ± 9 S.E.), and High sulfide treatments (161μM ± 9 S.E.)).

Significantly different sulfide treatments were maintained until experimental termination (Figure 23). Average porewater sulfide concentrations in treatments never exceeded a maximum of ~500μM for the High sulfide treatment, ~200μM for the Medium treatment, and ~100μM for the Control treatment. No significant differences in average porewater sulfide concentrations were found when comparing sulfide treatments between ambient and 22% shaded treatments ($p=0.990$, Two-Way ANOVA)(Figure 23). Average sulfide concentrations of stock solution administered for porewater injection were significantly different from each other (Control = 0μM ± 0 S.E., Medium = 851μM ± 55 S.E., High = 1574μM ± 94 S.E.) ($p<0.001$, Kruskal-Wallis One-Way ANOVA on Ranks w/ Tukey Test) (Figure 24). Average values for High and Medium sulfide stock solutions were within 10% of the desired 1600μM and 800μM target treatment concentrations. Average porewater ammonium (NH_4) concentrations remained below 200 μM and were well below the toxic range for eelgrass in all replicates throughout the experiment (Zimmerman et al. 1987). Porewater ammonium concentrations usually ranged inside non-toxic growth saturation concentrations (10-100μM) (Dennison et al. 1987, Zimmerman et al. 1987). A statistically significant difference in porewater ammonium concentrations were found among sulfide treatments on Day 28 (Control = 38μM ± 5 S.E., Medium = 49μM ± 8 S.E., and High = 57μM ± 11 S.E.) ($p=0.049$, Two-Way ANOVA). A statistically significant difference in porewater ammonium concentrations was also found when comparing light treatments irrespective of sulfide treatments (Shade= 25μM ± 3 S.E. and Ambient= 71μM ± 5 S.E.) ($p<0.001$, Two-Way ANOVA) (Figure 25). Porewater pH values monitored throughout this experiment were seldom

outside typical porewater pH ranges (7.5-6.8) for L.I. sediment (Berner 1980)(pers. comm. R. Aller). There were no significant differences in average porewater pH values on Day 28 when comparing sulfide treatments (Control= 7.06 ±0.02 S.E., Medium= 7.07 ± 0.03 S.E., High= 7.12 ± 0.26 S.E.) (p=0.158, Two-Way ANOVA), however shaded treatments were statistically higher than ambient treatments (Shade= 7.12 ± 0.1 S.E., Ambient= 7.05 ± 0.02 S.E.) (p=0.010, Two-Way ANOVA), but all within typical porewater pH ranges for L.I. sediment (Berner 1980). No differences were found comparing organic matter in treatment sediments by loss on ignition (LOI) analysis (CA= 0.11% ± 0.007 S.E., MA= 0.1% ± 0.005 S.E., HA= 0.1% ± 0.004 S.E., CS= 0.11% ± 0.007 S.E., MS= 0.1% ± 0.007 S.E, HS= 0.11% ± 0.004 S.E.). Organic matter values were comparable to values found in natural eelgrass beds in GSB (Peterson unpublished).

No statistically significant differences or trends were found in eelgrass productivity averages in this experiment comparing all treatments. Eelgrass survival was between 90 to 100% in all replicates. The productivity parameters analyzed in this experiment included specific productivity (mg/g/day)(Figure 26), short shoot (SS) productivity (mg/SS/day)(Figure 27), and leaf area productivity (cm^2/m^2/day)(Figure 28). All productivity parameters were compared using either Two-Way ANOVA or One-Way ANOVA statistical tests values were similar to levels found in healthy eelgrass beds in GSB (Peterson unpublished).

Fall Experiment

Significantly different sulfide treatments were not established throughout the entire 28 days of the fall laboratory experiment. Porewater sulfide averages for Control (1000µM H2S ± 193 S.E), Medium (344µM ± 79 S.E), and High (701µM ± 111 S.E) no-clam replicates ranged an order of magnitude from hundreds to thousands of µM sulfide on all sampling days (Figure 29), irrespective of light treatment. Average concentrations for sulfide stock solutions for porewater injections were significantly different from each other (C=0µM H_2S ± 0 S.E., M= 606µM H_2S ± 48 S.E., H= 1491µM H_2S ± 223 S.E.) (p=<0.001, Kurskal-Wallis One-Way ANOVA on Ranks, SQRT Transform data). Medium and High sulfide stock solutions were within 25% and 7% respectively of the target treatment concentrations of 800µM and 1600µM. High sulfide treatments with and without clams did not have significantly different average porewater sulfide concentrations (High sulfide, un-shaded, no-clam (HANC)= 570µM ± 144 S.E., High sulfide, shade, no-clam (HSNC)= 832µM ± 161 S.E, High sulfide, un-shaded, clam (HAC)= 669µM ± 182 S.E, and High sulfide, shaded, clam (HSC)= 575µM ± 127 S.E.) (Two-Way ANOVA, Light: p=0.596, Sulfide: p=0.618) (Figure 30). Porewater pH and ammonium (NH_4) were not analyzed in this experiment and sediment organic matter (loss on ignition) averages across treatment aquaria were not statistically different (Light: p=0.408, Sulfide: p=0.894 Two-Way ANOVA), ranging from 0.16% to 0.20% OM.

Porewater sulfide treatments were not established between Control, Medium, and High sulfide treatments in this experiment. Eelgrass growth, productivity, and survival could not be compared between sulfide treatments with the exception of the High sulfide

treatments. High sulfide treatments experienced similar average porewater sulfide concentrations, allowing for eelgrass productivity comparisons to be made to examine the possible effects of shading and hard clam presence. Increases in specific productivity (mg/g/d) were found when hard clams were present in both un-shaded ((HANC)= 15.5 ± 4.5 S.E., (HAC)= 19.1 ± 4.5 S.E.) and shaded ((HSNC)= 11.6 ± 4.5 S.E., (HSC)= 28.1 ± 4.5 S.E.) treatments. A small increase in specific productivity was seen in un-shaded treatments with clams, and a nearly 3 fold increase is seen in the shaded treatment with clams. This increase in productivity between shaded treatments with and without clams was marginally statistically different (p=0.059, t-test) (Figure 31).

III. Field sulfide and shading experiment

Summer Experiment

Glucose additions from glucose filled perfusers successfully increased porewater sulfide concentrations in both shaded and un-shaded treatments by Day 16 and through experimental termination (Figure 32). On Day 16 average porewater sulfide concentrations for the 5% glucose addition treatment (5A) (5818μM ± 846 S.E.) were significantly greater than the Control (CA) (1000μM ± 229 S.E., (p=0.003) and 20% glucose treatments (20A) (2161μM ± 1006 S.E.) (p=0.007), when comparing only un-shaded treatments (Two-Way ANOVA with Student-Newman-Keuls Method for multiple comparison). Amongst shaded replicates on Day 16 the average porewater sulfide concentration for 20% glucose replicates (20S) (5484μM ± 490 S.E.) were significantly larger than the control treatment (CS) (904μM ± 237 S.E.) (p=0.004, Student-Newman-

Keuls Method). A marginally significant increase in average porewater sulfide concentration was also found between 5% glucose shaded replicates (5S) (3313µM ± 1285 S.E.) and the Control shaded treatment (p=0.052). No significant differences in porewater sulfide concentrations were found amongst shaded and un-shaded control replicates (p=0.933, Two-Way ANOVA, Student-Newman-Keuls method). Averaged porewater sulfide concentrations in the 5A treatment were significantly larger than the 5S treatment (p=0.044). Porewater sulfide average concentrations in the 20A treatment were significantly less than the 20S treatment (p=0.012). On Day 30 no significant differences in average porewater sulfide concentration amongst treatments were found when comparing light or glucose addition treatments (Light p=0.750, Glucose p=0.078, Two-Way ANOVA). Initial experimental porewater sulfide averages were mostly below toxic levels (600µM, (Goodman et al. 1995)): CA= 496µM ± 133 S.E., CS= 924µM ± 449 S.E., 5A= 338µM ± 217 S.E., 5S= 635µM ± 248 S.E., 20A= 386µM ± 160 S.E., and 20S= 1424µM ± 423 S.E., with the exceptions of the shaded 5% and 20% glucose treatments. The majority of porewater sulfide samples had concentrations below 600µM, however few sample concentrations did range from 1000µM to 2000µM in all shaded treatment means. Maximum porewater sulfide concentrations were recorded on Day 16 in the 5A and 20S treatments, both above ~5500µM H_2S.

Porewater ammonium concentrations were all well below toxic concentrations for eelgrass, ranging from 17µM to 110µM NH_4. No significant differences were found in porewater NH_4 concentrations for each sampling day when comparing the effects of light or glucose addition (Day 0: Light p=0.392, Glucose p=0.320; Day 16: Light p=0.777,

Glucose p=0.708; Day 30: Light p=0.277, Glucose p=0.230). Porewater pH was sampled throughout the experiment. A decreasing trend in porewater pH values was found after initiation of the experiment, with the only significant decrease occurring on Day 16 (p=0.039 Two-Way ANOVA), comparing control (7.33 ± 0.07 S.E.) and 20% glucose addition (7.04 ± 0.07 S.E.) treatments (p=0.036, Two-Way ANOVA Student-Newman-Keuls Multiple Comparison Method). Sediment organic matter was evaluated in all replicates using loss of ignition methods. Treatment averages ranged from 0.4% to 0.5% at experimental termination and there were no significant differences in sediment organic matter and when comparing Light (p=0.072) or Glucose addition (p=0.423).

Analysis of eelgrass productivity for this experiment found significant differences when comparing eelgrass standing crop (g/m^2) (Figure 33). Light had a non-statistically significant effect on decreasing standing crop (p=0.293) while glucose amended treatments had a significant effect (p=0.042, Two-Way ANOVA). Standing crop means for un-shaded treatments decreased from Control (20.343 ± 4.35 S.E) to 5% (16.463 ± 4.35 S.E.) and 20% (15.023 ± 5.33 S.E.) treatments. Standing crop means decreased more sharply in the shaded treatments following the same respective order (CS= 25.8 ± 5.3 S.E., 5S= 10.6 ± 4.35 S.E., and 20S= 2.73 ± 4.35 S.E.). Eelgrass short shoot productivity (mg/SS/day) means were not significantly different when comparing treatments for Light (p=0.196) and Glucose (p=0.200, Two-Way ANOVA) (Figure 34), however averages in shaded treatments decreased sharply with increasing glucose addition (CS= 2.34 ± 0.38 S.E., 5S= 1.88 ± 0.46 S.E., and 20S= 0.80 ± 0.46 S.E.). Eelgrass survival at experimental termination decreased in both un-shaded (CA= 55% ± 9

S.E., 5A= 38% ± 7 S.E., 20A= 25% ± 9 S.E.) and shaded treatments (CS= 41% ± 7 S.E., 5S= 13% ± 9 S.E., 20S= 17% ± 9 S.E.) with respect to glucose addition (Figure 35). Shading had a marginally significant effect on decreasing eelgrass survival ($p=0.051$), while glucose addition had a statistically significant effect ($p=0.025$, Two-Way ANOVA). A Q-test was used to remove outlying survival data from 1 bucket in each CA and 20S treatments. Percent survival was reduced by 17% and 30% in un-shaded glucose treatments (5A and 20A respectively) and reduced 28% and 24% in shaded glucose treatments (5S and 20S respectively) when compared to respective control treatments.

Discussion

I. GSB Porewater Sulfide Survey

Porewater sulfide surveys extended from West Fire Island to Bellport Bay in southern Great South Bay, NY. Survey data confirmed that toxic porewater sulfide concentrations (≥600μM, (Goodman et al. 1995)) are currently present in eelgrass beds and surrounding unvegetated sediments in Great South Bay. The influence of shading due to cloud cover (i.e. clear/overcast) had a significant effect on increasing porewater sulfide concentrations in both dense and patchy grass beds during daylight hours. This indicates that a reduction in ambient light for above ground eelgrass biomass may correspond to increases in the porewater sulfide pool. Toxic porewater sulfide concentrations (i.e. ≥600μM, (Goodman et al. 1995)) were not found in any samples from dense grass beds, unvegetated sediment, or Nature Conservancy Hard Clam restoration sites on clear days. Average porewater sulfide concentrations for patchy grass sampled

on clear days were also below toxic concentrations, however one sample did exceed the toxic concentration threshold by about 300μM (~900μM). Average porewater sulfide concentrations for patchy grass on clear days were about ~285μM greater than dense grass concentrations. Seagrasses naturally experience various porewater sulfide concentrations corresponding with natural diel photosynthetic cycles and overlying O_2 concentrations (Frederiksen & Glud 2006, Marba et al. 2006). Porewater sulfide concentrations in seagrass meadows are generally lower during the daylight hours when receiving saturating light levels, due to direct oxidation of the rhizoshere by photosynthetically derived oxygen (Lee & Dunton 2000). When light saturation concentrations are not available because of a reduction in light (overcast skies or shading from algal blooms) than production and transport of photosynthetically derived oxygen is reduced to the rhizosphere (Marba et al. 2006). Eelgrass meadows that have not adequately oxidized the sediments during daylight hours may face increased risks of sulfide intrusion and toxicity (Dennison et al. 1989, Goodman et al. 1995, Holmer & Laursen 2002). Maximum concentrations of porewater sulfide in seagrass beds are found right before sun-rise, which exhibits maximum respiration for the sediments. Dense seagrass beds (which have high above-ground photosynthetic biomass) are able to oxygenate the rhizosphere much faster and more completely than patchy seagrass beds, which have less above ground photosynthetic biomass. As a result, dense eelgrass beds can possibly oxygenate the rhizosphere quicker, reducing or eliminating root and rhizome exposure to toxic sulfide concentrations (Dennison et al. 1989, Goodman et al. 1995, Holmer & Laursen 2002). In contrast, the rhizosphere of patchy eelgrass beds could face

increased exposure time to toxic levels of sulfide, as oxygenation of sediments is reduced, due to reduced photosynthetic above-ground biomass. The effects of sulfide toxicity in patchy grass beds in particular would be expected to increase during phytoplankton blooms or overcast skies. Porewater sulfide concentrations amongst dense grass (609μM ± 73 S.E.), patchy grass (980μM ± 242 S.E.) and unvegetated (636μM ± 166 S.E.) substrates in this study were not significantly different on the overcast sampling day. Although substrates were not significantly different from each other and were all above toxic levels (600 μM, (Goodman et al. 1995)), it is important to recognize that porewater sulfide concentrations in patchy grass replicates did have noticeably higher average porewater sulfide concentrations than dense grass replicates of about 370μM (Figure 21). Increased porewater sulfide concentrations in patchy grass substrates may be due to a decreased photosynthetic capacity and ultimately ineffective rhizosphere oxidation. Patchy grass substrates may face longer exposures to toxic porewater sulfide concentrations than dense grass substrates simply because dense grasses are capable of oxygenating the sediments quicker and more efficiently due to increased above-ground photosynthetic biomass. This study reveals that porewater sulfide concentrations in natural eelgrass beds in GSB are significantly increased during daylight shading and qualitative reductions in above-ground photosynthetic biomass.

II. Laboratory sulfide and shading experiments

The summer laboratory experiment found that eelgrass productivity and survival was not adversely affected by a ~44% decrease in ambient light intensity or a

manipulated increase in porewater sulfide concentrations. Average light intensity measurements for both shaded and un-shaded treatments were an order of magnitude greater than natural light intensity values in GSB. Light intensity data for both light treatments were also 5 to 7 times greater than photosynthetic light intensity saturation values (~12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982)). Administered sulfide stock solutions for the high (1600μM NaS-H_2S) and medium (800μM NaS-H_2S) treatments were within 10% from target concentrations. It can not be confirmed however that eelgrass roots and rhizomes were effectively exposed to these target concentrations. Porewater sulfide samples taken ~22hr following injections revealed that original stock solution concentrations were reduced up to 5 times while in the sediment, as average sulfide stock concentrations in Medium (851μM ± 55 S.E.) and High (1574μM ± 94 S.E.) treatments were reduced to 126μM ± 11 S.E. and 318μM ± 28 S.E. respectively by experimental termination. This indicates that actual eelgrass exposure concentrations must have been between these two porewater sulfide concentrations for a 24hr period. The drastic decrease in porewater sulfide concentrations from initial injections to sampling is most likely do to sediment characteristics, particularly from using low porosity iron rich sediment in this experiment. Iron rich sediments serve as a sulfide buffer, removing sulfide species and forming pyrite (FeS_2) (Chambers et al. 2001, Marba et al. 2006). Porewater sulfide treatments (C, M and H) were found to be significantly different from each other throughout the experiment, although no significant differences were found between sulfide concentrations in shaded and un-shaded treatments. This suggests that the sediment

characteristics may be the most important factor affecting the porewater sulfide pool. Shaded treatments experienced lower average temperatures of as much as 2.25 ˚C during mid-day hours compared to un-shaded replicates. Water temperatures in experimental aquaria ranged from ~15 to ~30˚C throughout experimental duration. These temperatures were similar to those measured during the same time period in GSB at a depth of 1m. Temperatures at and above 30˚C have been found to adversely affect eelgrass survival and photosynthesis (Nejrup & Pedersen 2008) and were only reached one day during the experiment by un-shaded replicates (Figure 7).

All experimental replicates had porewater pH and ammonium levels that were consistent with values in natural eelgrass beds in GSB (Rountos this study). Porewater ammonium was significantly greater in shaded treatments compared to un-shaded treatments. Since eelgrass in both shaded and un-shaded treatments were exposed to super-saturating light intensity values it is unlikely that increases in ammonium are do to denitrification in the sediments, as the rhizosphere should be fully oxygenated. It is likely that this difference in porewater ammonium may be the response of the benthic microbial community. Loss on ignition analysis of organic matter in sediments revealed no significant differences among all treatments.

Eelgrass productivity and shoot survival analyses showed no significant differences between treatments in this experiment. Seagrasses were exposed to sub-toxic porewater sulfide concentrations (below 600μM), while also experiencing light intensity values almost 40000 lux greater than photosynthetic saturating values (~12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982). Under these conditions effects

from sulfide and shading would not be expected to adversely affect eelgrass survival and productivity.

In the fall laboratory experiment shading was increased to reduce ambient light intensity levels by ~80% while administered sulfide injection solutions remained at the same concentrations. The increase in shading was meant to mimic the shading effect due to attenuation by a natural dense phytoplankton bloom (Gobler et al. 2005). This experiment examined the interactions between this increased shading and similar porewater sulfide concentrations on eelgrass productivity and survival. Light intensity values for shaded replicates (~4000 lux) were 3 time less than photosynthetic saturating levels (12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982)) and 50% less than ambient values in GSB (~8000 lux). This experiment was unsuccessful at creating distinguishable porewater sulfide treatments regardless of light treatment (Figure 29). Eelgrass productivity and survival therefore was not able to be analyzed based on sulfide treatments. This experiment did find valuable information when analyzing the effects of hard clam presence in both shaded and ambient light treatments. Since porewater sulfide concentrations in all High treatments were similar and not significantly different from each other, the effects of shading and clam presence on eelgrass productivity could be assessed. Comparing specific productivity (mg/g/day) (Figure 31), hard clam present treatments had higher average productivity rates compared to no clam treatments. This difference was particularly seen when comparing shaded treatments, as specific productivity increased by 16.5 (mg/g/day) when clams were present.

Porewater pH and ammonium were not measured in this experiment and may have provided clues as to the variability in porewater sulfide concentrations in treatments. No significant differences in organic matter were found amongst all treatments from loss on ignition analysis, which eliminates it as a possible contributor to experimental porewater sulfide variability. Water temperature in experimental aquaria ranged from ~9 to ~23 ˚C throughout the duration of the experiment, which are similar to optimum laboratory growing temperatures for eelgrass (~10 to ~20˚C) (Nejrup & Pedersen 2008).

III. Field sulfide and shading experiment

In the summer field experiment significant differences in eelgrass standing crop (g/m^2) and survival were found when eelgrass was exposed to glucose mediated sediments and a 38% reduction in average ambient light intensity. Light intensity levels in shaded treatments were about 4 times lower than levels at which photosynthetic saturation occurs (~12000 lux, (Inada 1984) converted from (Dennison & Alberte 1982)), while ambient treatments were just below saturation levels (8600 lux). Glucose additions successfully increased the porewater sulfide pool an order of magnitude greater than toxic concentrations (600µM, (Goodman et al. 1995)), to about 6000µM. These concentrations were not found in eelgrass beds during the porewater sulfide survey in GSB, but would be possible in organically enriched sediments in the bay. Porewater sulfide concentration averages for Control treatments were frequently around 1000µM and were statistically lower than glucose addition treatments. Porewater sulfide concentrations of 1000µM are natural in eelgrass beds in GSB and were found during the sulfide survey. This confirms

that eelgrass shoots in all experimental treatments were exposed to different degrees of toxic porewater sulfide concentrations.

Standing crop (g/m^2) decreased in both ambient and shaded treatments as glucose treatment increased (C to 5% to 20%). The trend was gradual amongst ambient treatments from (20.343 g/m^2 ± 4.35 S.E) to 5% (16.463 ± 4.35 S.E.) and 20% (15.023 ± 5.33 S.E.). A sharper and more significant decrease in standing crop was found in the shaded treatment, from Control (25.8g/m^2 ± 5.3 S.E.) to 5% (10.6 ± 4.35 S.E.) and finally 20% (2.73 ± 4.35 S.E.). Standing crop was reduced by >50% compared to the Control treatment in 5% glucose treatments, and reduced by ~90% in 20% glucose treatments. This decrease in standing crop correlates well with the average porewater sulfide concentration in both 5% (~2500μM) and 20% (~4250μM) glucose treatments. These concentrations were achieved by averaging Day 16 and Day 30 porewater sulfide averages, which is what eelgrass plants were exposed to inside the buckets. It is also worth noting that the increased standing crop value in the Control shaded treatment compared to the Control ambient treatment may be a result of lower average porewater sulfide concentrations at the end of the experiment (557μM ± 187 S.E. and 1099μM ± 400 S.E., respectively).

To examine the effects of shading and glucose addition on eelgrass survival a Q-test was first used to remove 2 different outlying replicates (Figure 35). This revealed a statistically significant decrease in survival from control to glucose amended treatments ($p=0.025$). This decrease was step-wise in the ambient treatments and more abrupt in shaded treatments. Since control replicates often exhibited toxic porewater sulfide

concentrations (>600μM, (Goodman et al. 1995)), it is reasonable that the average survival rate found in control treatments (55%) might have been reduced due to sulfide toxicity. As porewater sulfide concentrations were greater in both 5% and 20% glucose treatments reductions in survival would be expected, particularly when all replicates were experiencing sub-saturating light intensity concentrations described earlier. No significant differences were found amongst treatments when looking at porewater pH, ammonium, and organic matter, which suggest that glucose addition and increased porewater sulfide may have been the only geochemical variables affecting eelgrass in experimental treatments.

Conclusion

Eelgrass ecosystems in Great South Bay have been declining over the last several decades and continue to face new challenges in the decades to come. Unlike historical die-off events, that mostly occurred from singular causes, like the regional eelgrass "wasting disease" (*Labyrinthula zosterae*) (Short et al. 1987, Vergeer et al. 1995), eelgrass meadows are currently facing a combination of multiple-stressors. Among these multiple stressors, eutrophic related shading, porewater sulfide toxicity, increased water temperature, and habitat modification (the significant removal of *Mercenaria mercenaria* (hard clams) by overharvesting) seem to be the most important parameters affecting eelgrass health in GSB. Sulfide toxicity is a major seagrass stressor and in addition with other stressors has been implicated in causing the loss of critical seagrass habitat in temperate regions.

The GSB survey study confirmed that toxic porewater sulfide concentrations are currently found in *Zostera marina* meadows and unvegetated sediments throughout southern Great South Bay. Toxic concentrations were found mostly on overcast sampling days and primarily in patchy grass substrates. During daylight hours on clear days, porewater sulfide concentrations in sediments were significantly reduced, probably from photosynthetically derived oxygenation of the rhizosphere by the plants. Eelgrass plants prevent sulfide toxicity by transporting oxygen derived from photosynthesis to root and rhizome systems located within anoxic sediments (Goodman et al. 1995, Eldridge & Morse 2000). If light decreases, transport of photosynthetically derived oxygen to below-ground biomass will also decrease, leading to rhizosphere anoxia and possibly sulfide

toxicity to below ground biomass (Goodman et al. 1995, Frederiksen & Glud 2006, Marba et al. 2006). This oxygenation of the rhizosphere is especially apparent in dense grass substrate samples, whose average porewater concentrations decreased ~550μM to very low concentrations when adequate light was available. Dense grass meadows in GSB effectively reduced porewater sulfide concentrations to non-toxic concentrations on clear days. A reduction in average porewater sulfide concentrations was also found in patchy and unvegetated sediments sampled during clear days; however some samples remained at toxic porewater sulfide concentrations. Porewater sulfide average concentrations for patchy grass substrates were generally greater than concentrations in dense grass substrates. This suggests that dense eelgrass meadows are better than patchy grass substrates at oxidizing their rhizospheres, and ultimately reducing the porewater sulfide pool. The survey shows that patchy grass meadows are less able to reduce porewater sulfide concentrations when similar amounts of light were available. Porewater sulfide averages for patchy grass substrates were always between 285μM and 370μM higher than dense grass averages on both clear and overcast days respectively. This suggests that patchy grass substrates may always experience porewater sulfide concentrations ~300μM greater than dense grass substrates in GSB under different light conditions. These findings suggest that dense eelgrass meadows are better able to reduce porewater sulfide toxicity in GSB than patchy or infirm eelgrass meadows. This information may help managers in selecting restoration sites and in classifying unhealthy eelgrass meadows in Great South Bay.

Laboratory and field experiments were designed to elucidate the effects and interactions of shading and porewater sulfide toxicity on *Zostera marina* populations in GSB. Eelgrass productivity and survival was not adversely affected by saturating light intensity values in the shaded treatments, and sub-toxic porewater sulfide concentrations (<350μM) in the summer laboratory experiment. Differences in productivity were also not found when light intensity was reduced to sub-saturating dense phytoplankton bloom light levels (~80% light intensity reduction,(Gobler et al. 2005)) in the fall laboratory experiment. In this experiment, the presence of hard clams was found to increase the specific productivity (mg/g/d) of eelgrass in the High sulfide treatments (both shaded and un-shaded). In the shaded treatments specific productivity increased by over 50% with hard clams present, suggesting that hard clam presence can increase eelgrass productivity particularly under shade stress. In field manipulations, glucose additions to GSB sediment successfully increased the porewater sulfide pool for a 30 day period. A PVC density screen canopy was also successful at simulating shading similar to dense phytoplankton blooms (Gobler et al. 2005). Significant differences were found in eelgrass standing crop (g/m^2) and percent survival amongst the glucose mediated sediment treatments and light treatments. Eelgrass standing crop in ambient and shaded treatments decreased as average porewater sulfide concentrations increased in glucose amended treatments. Standing crop decreased almost exponentially in the shaded treatment, and an almost 90% decrease in standing crop in the highest glucose treatment was found compared to the control value. Glucose addition (increased porewater sulfide) had a statistically significant effect ($p=0.042$) on reducing eelgrass standing crop. After a

month long field experiment eelgrass survival rates in glucose amended (high sulfide) sediment were ~50% less than control treatment values. The effects of shading reduced survival percentages in the Control, 5%, and 20% treatments, however increases in porewater sulfide in glucose mediated treatments significantly reduced eelgrass survival ($p=0.025$). This experiment demonstrates that a reduction in light intensity, similar to those observed by a dense phytoplankton bloom, and increased porewater sulfide concentrations (through glucose additions) have a dramatic effect on eelgrass survival and density in GSB. It is important to note that the direct effect of glucose additions on eelgrass was not examined in this thesis. Glucose additions, like any addition of labile organic matter to anoxic sediment, would enhance anaerobic diagenic processes. In GSB, sulfate reduction is the major remineralizing process in anoxic sediments, thus glucose addition would stimulate sulfate reduction primarily.

The decline of *Zostera marina* meadows in Great South Bay and other Long Island's estuaries has huge ecological consequences for our coastal marine environment. It is important for all coastal researchers and managers to understand interactions between multiple stressors for *Zostera marina*. Understanding the interactions of multiple stressors will provide vital information for restoring eelgrass biomass and preventing future declines. This thesis research has developed and tested new research methods and approaches in which the effects of multiple stressors on eelgrass can be investigated in both the field and laboratory settings. It is important that future research on this topic be conducted in order to develop a clearer understanding of the role of multiple stressors in eelgrass ecosystems in Long Island South Shore Estuaries. Future

research should include a comprehensive porewater sulfide survey for autumn, spring and summer seasons. In addition to this survey diurnal porewater sulfide sampling should be conducted in dense and patchy eelgrass meadows as well as unvegetated sediments. This may provide important information into porewater sulfide dynamics in eelgrass meadows in Great South Bay. Finally, it is important that laboratory and field experiments be conducted in order to verify data from this study.

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Appendix

Figure 1. Some abiotic and biotic factors affecting sediment toxicity.

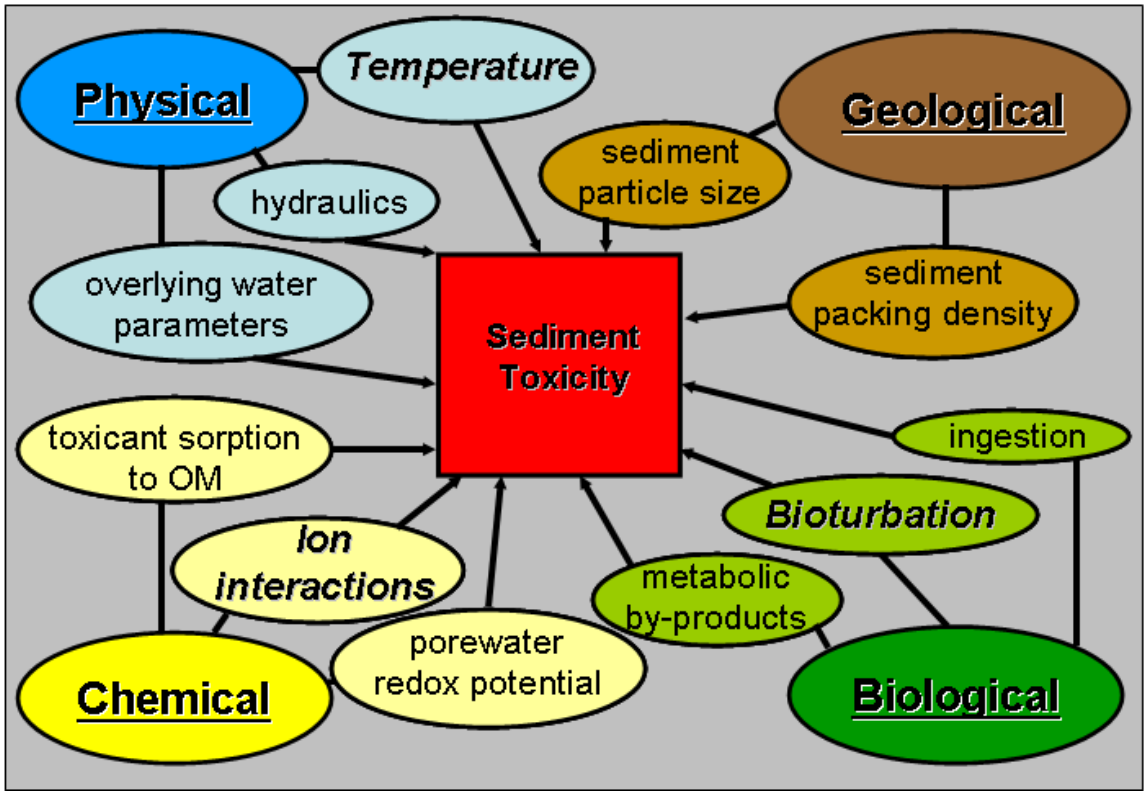

Figure 2. Map of porewater sulfide sampling sites in Great South Bay, New York. Black circles= sampling sites on overcast days, White circles= sampling sites on clear days.

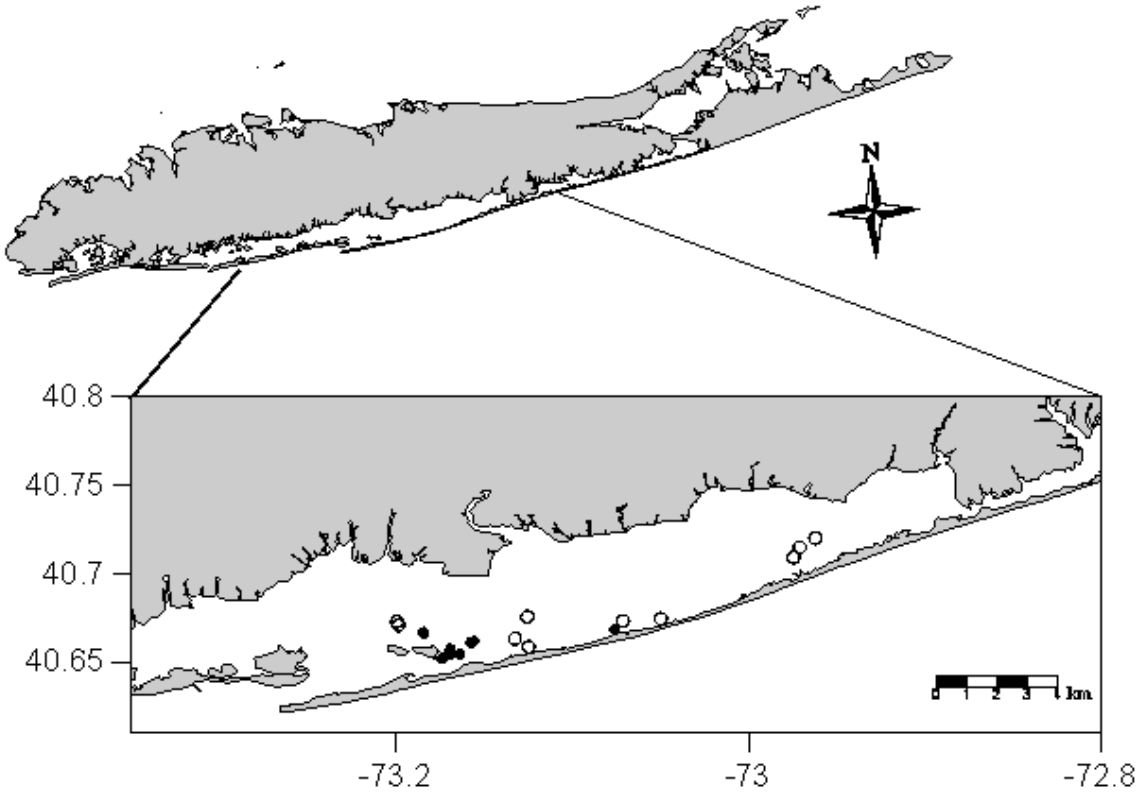

Figure 3. Porewater sipper device.

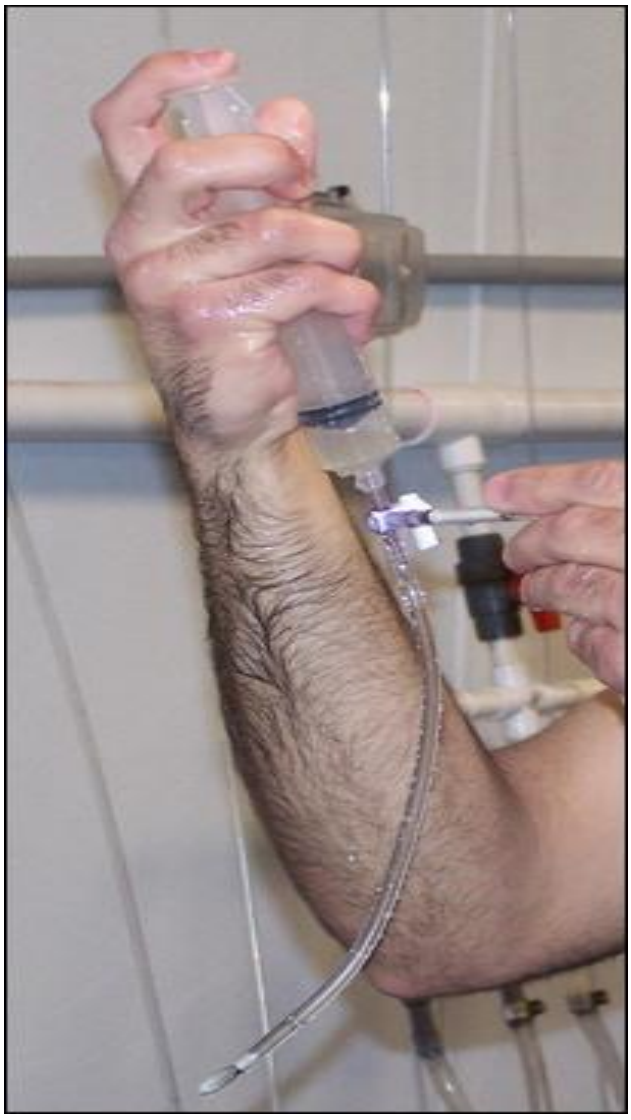

Figure 4. Porewater injection and sampling device.

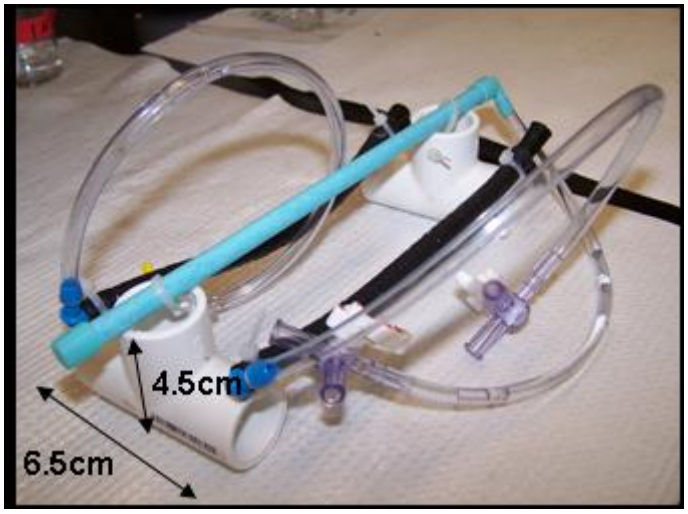

Figure 5. Summer laboratory experimental aquaria layout. C= Control, M= Medium sulfide, H= High sulfide, A= Un-shaded, S= Shaded

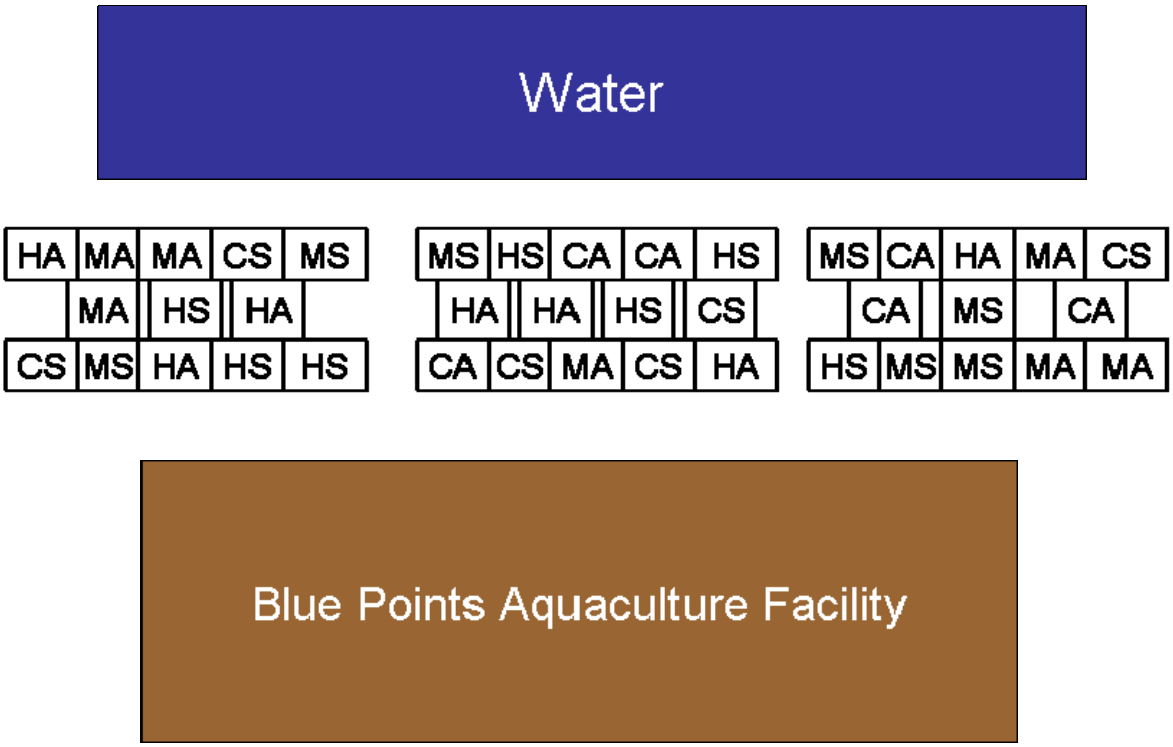

Figure 6. Light intensity plot for the summer laboratory experiment.

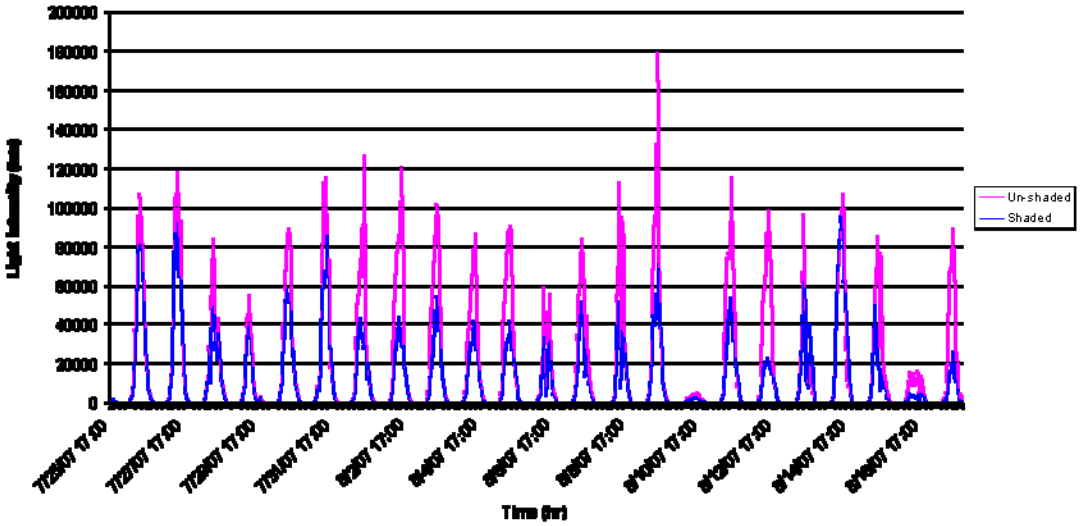

Figure 7. Water temperature plot for the summer laboratory experiment.

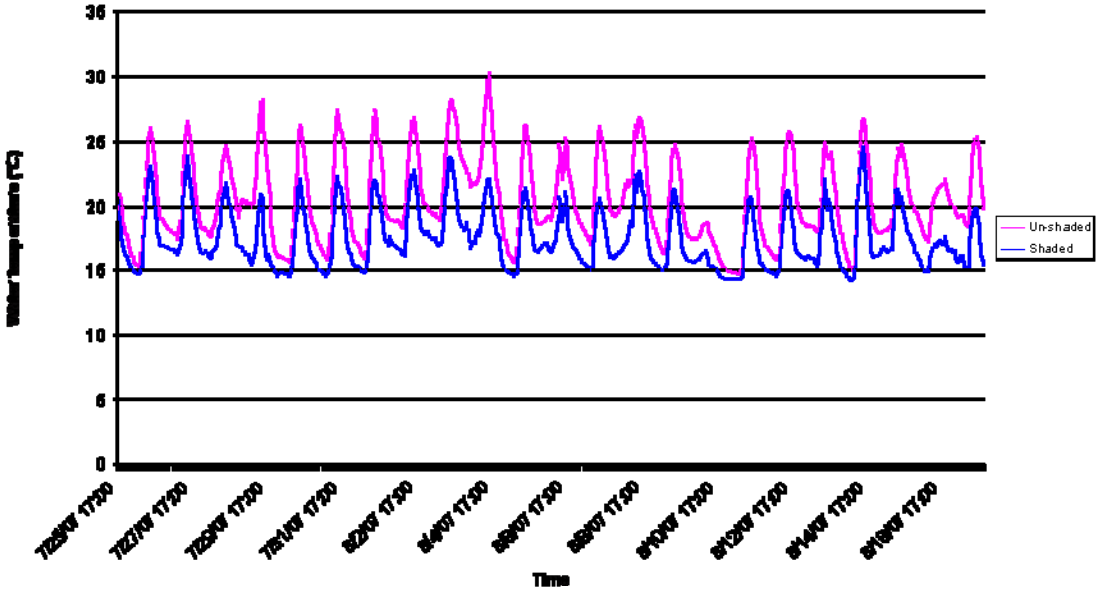

Figure 8. Average temperature differences during daylight hours in summer laboratory experiment.

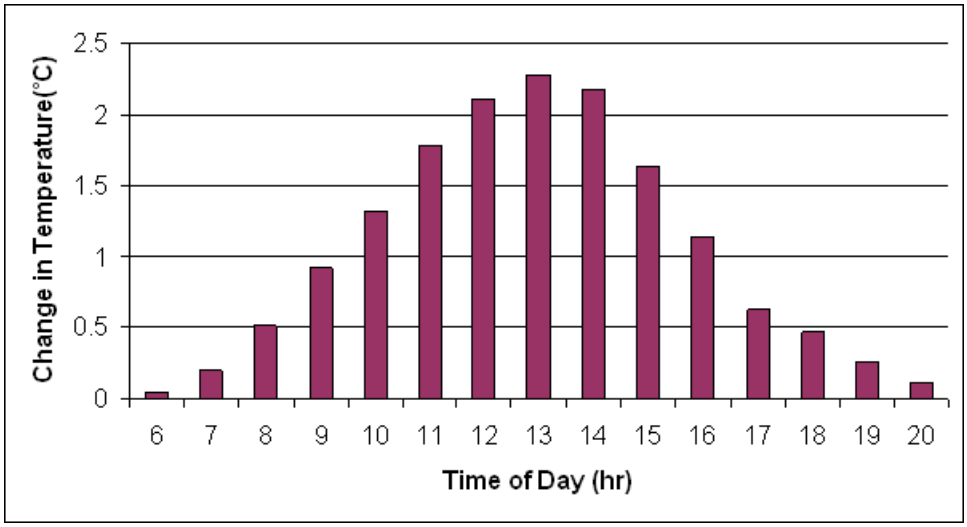

Figure 9. Average change in light intensity (lux) during daylight hours in the summer laboratory experiment.

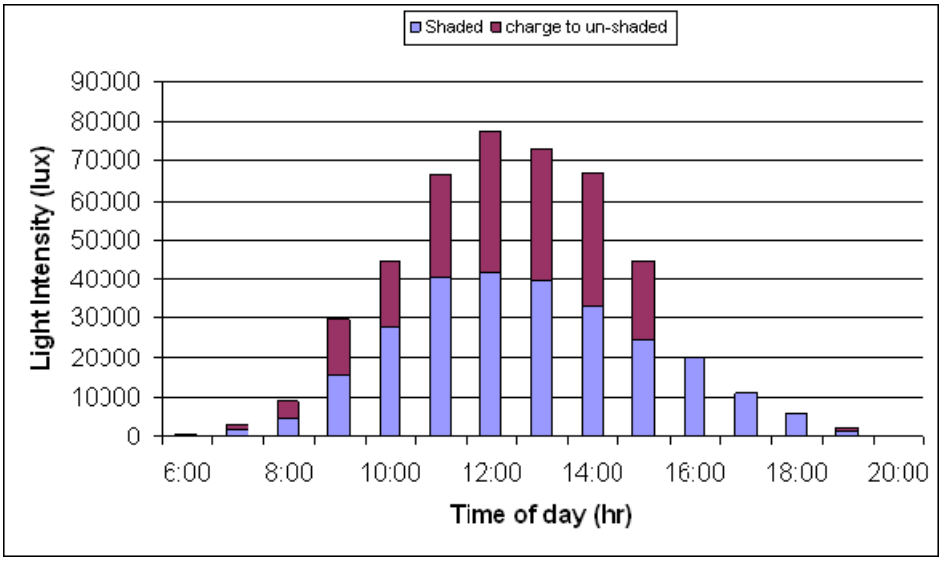

Figure 10. Seagrass planter set-up for laboratory experiments. X denotes eelgrass shoots.

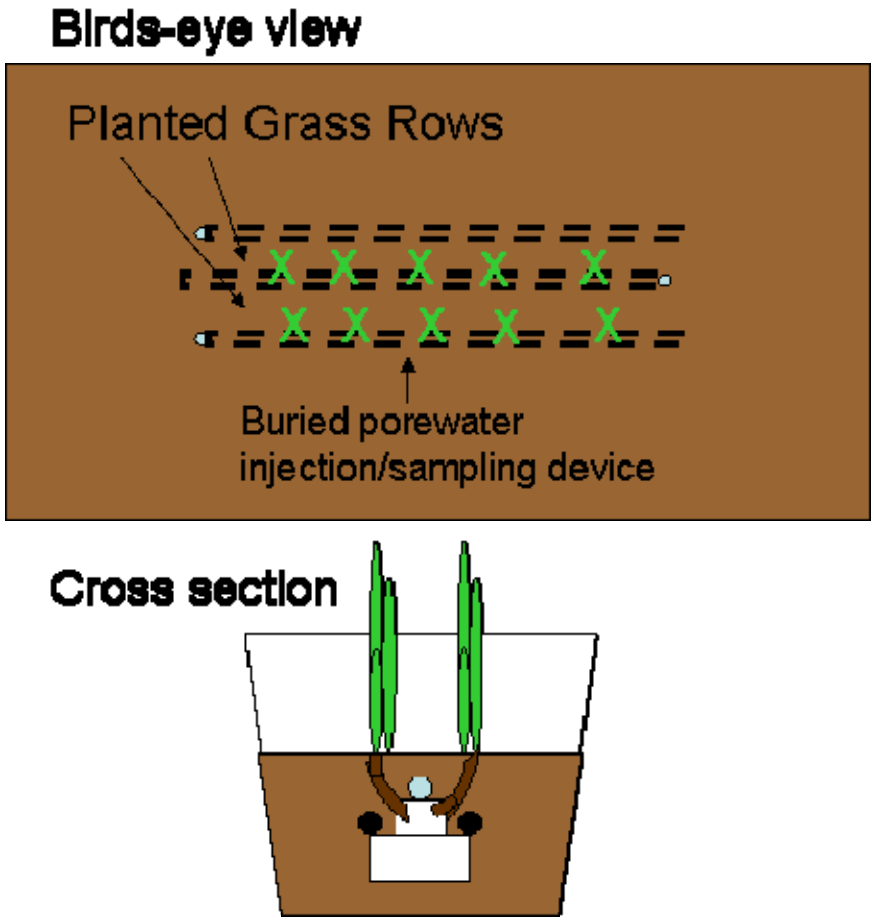

Figure 11. Coupling device for pH meter.

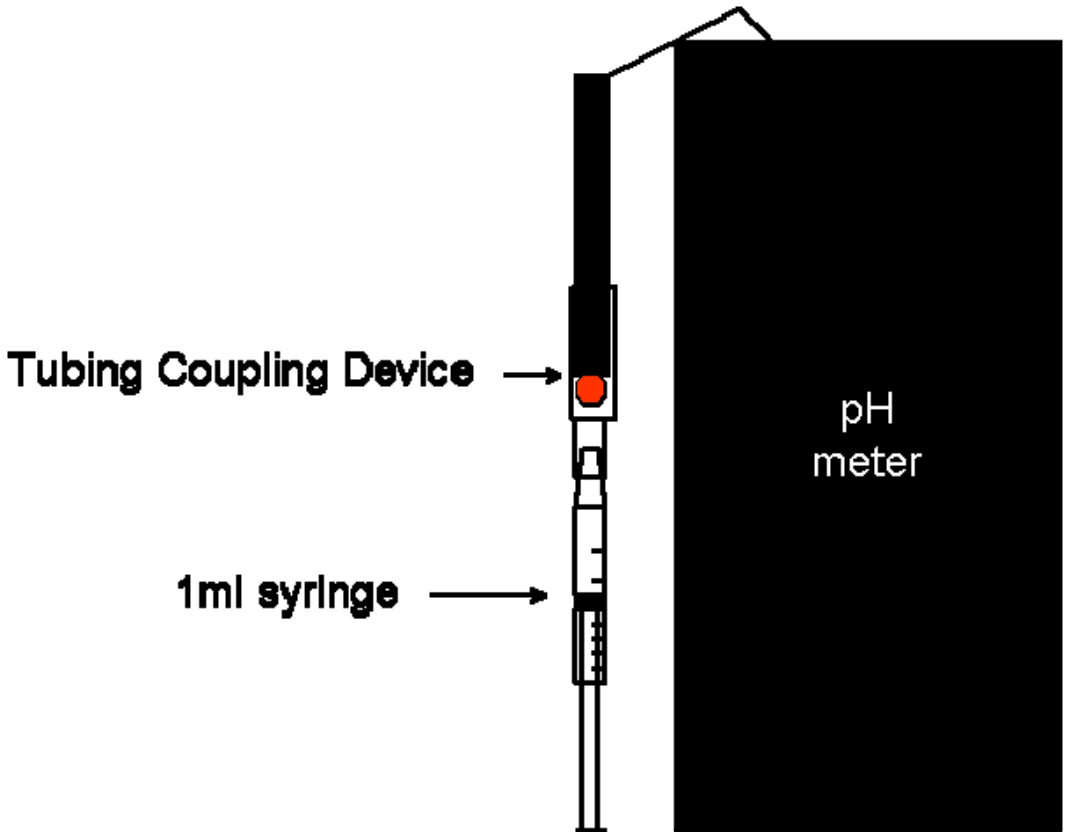

Figure 12. Fall laboratory experiment layout. C= Control, M=Medium sulfide, H= High sulfide, A= Un-shaded, S= Shaded, HAC= High un-shaded clam, HSC= High un-shaded clam.

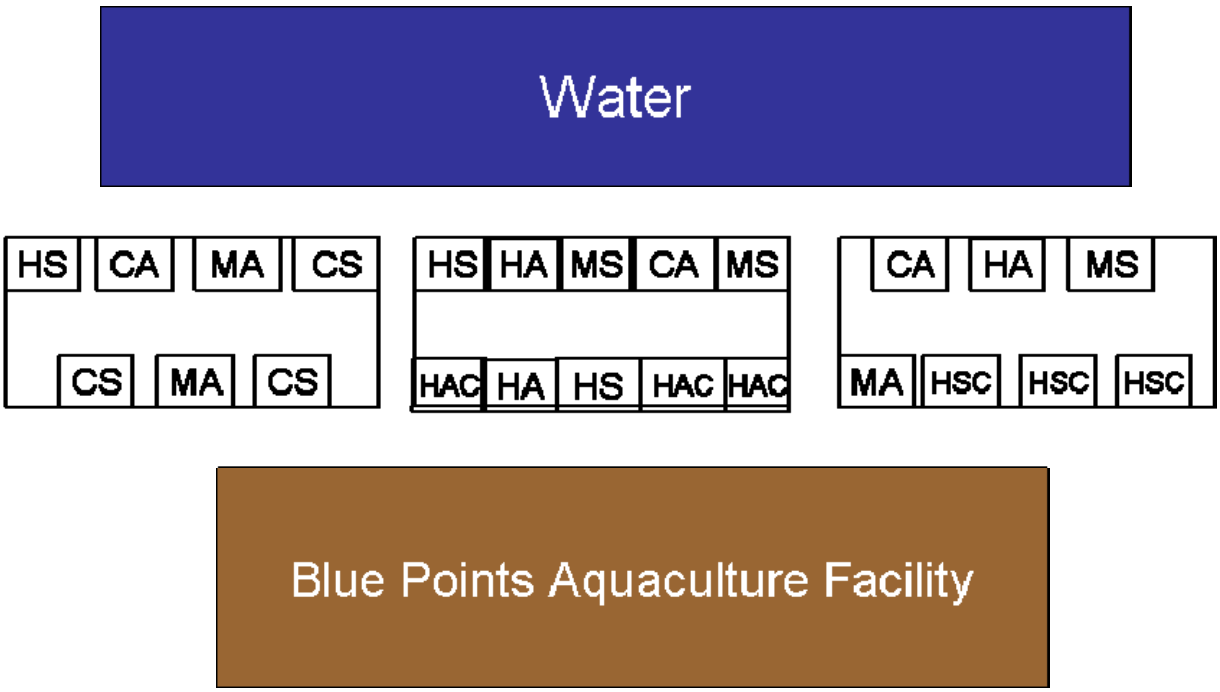

Figure 13 Light intensity plot in fall laboratory experiment.

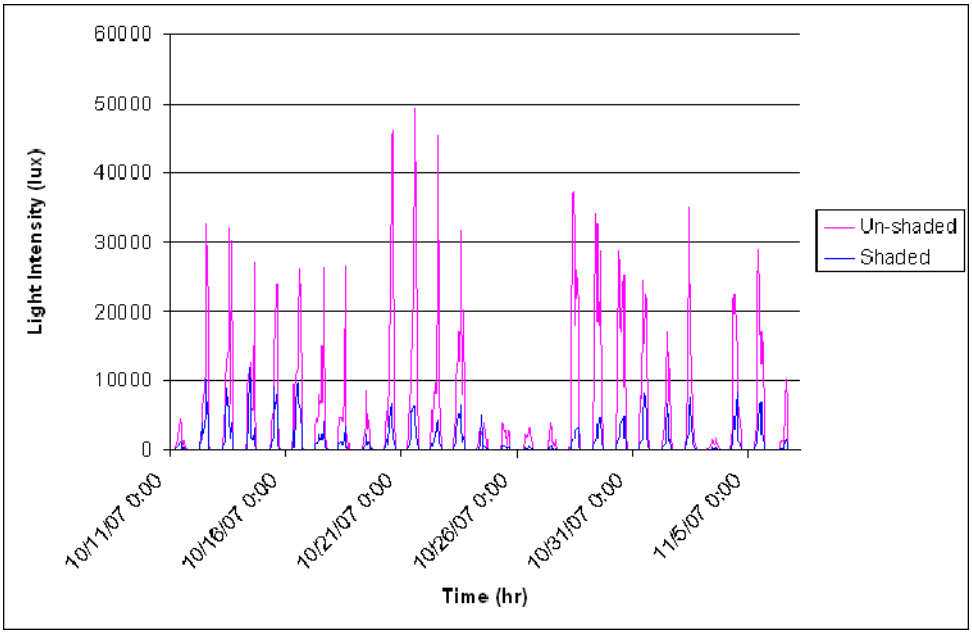

Figure 14. Average change in light intensity (lux) during daylight hours in the fall laboratory experiment.

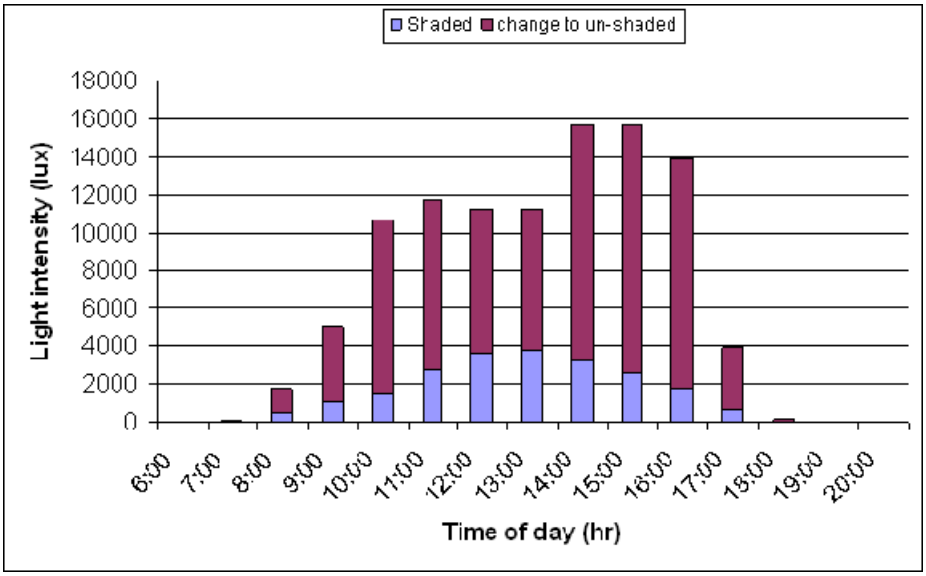

Figure 15. Water temperature plot for the fall laboratory experiment.

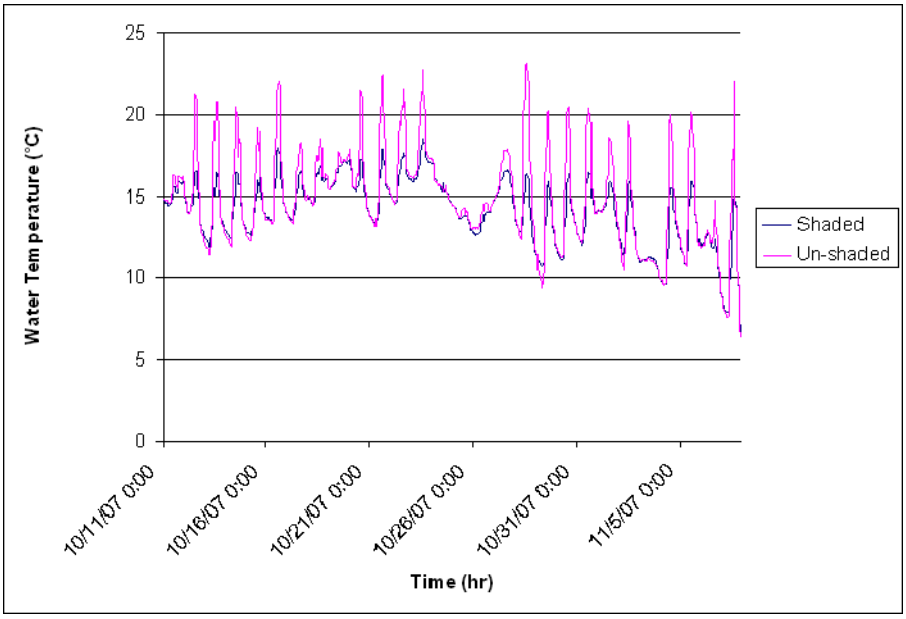

Figure 16. Average temperature differences during daylight hours in fall laboratory experiment.

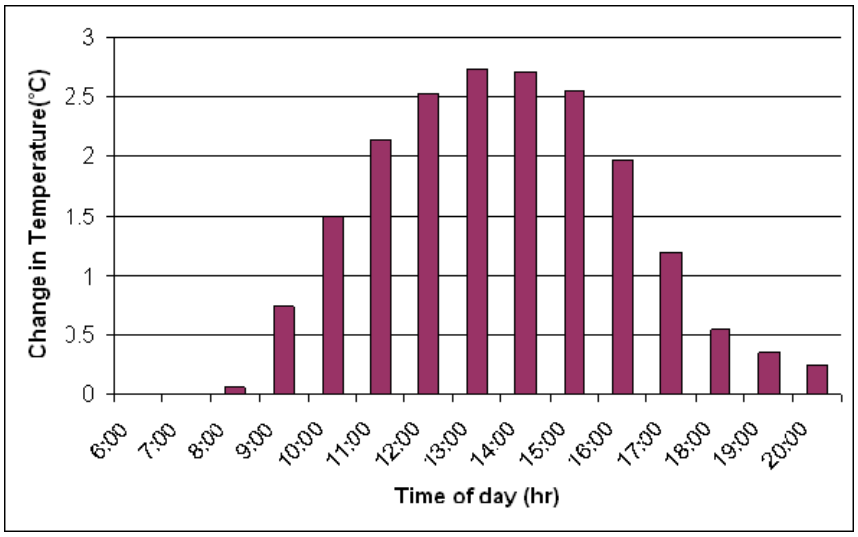

Figure 17. Experimental set-up for summer field experiment. Courtesy of B. Rodgers.

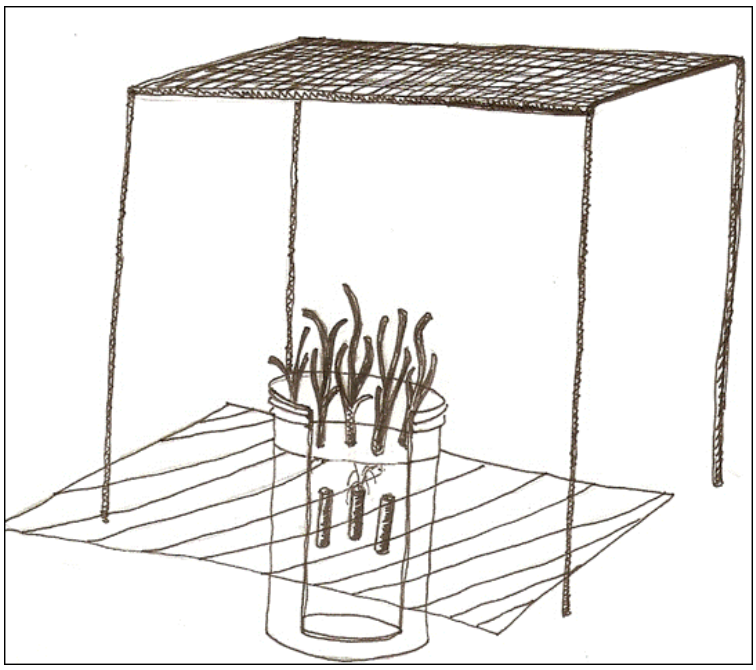

Figure 18. Light intensity plot in summer field experiment.

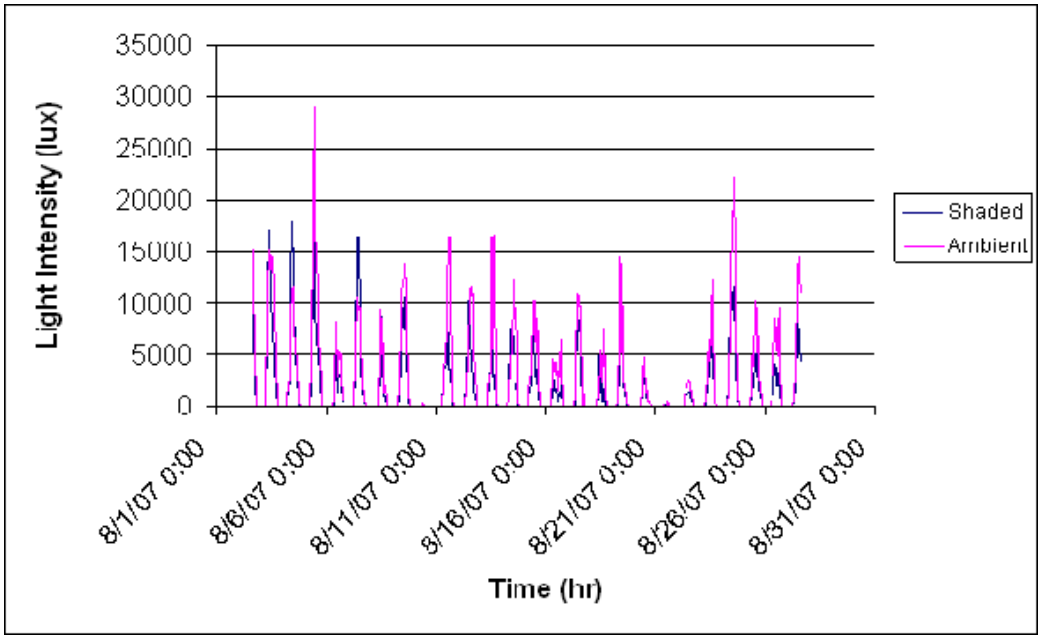

Figure 19. Average change in light intensity (lux) during daylight hours in the summer field experiment.

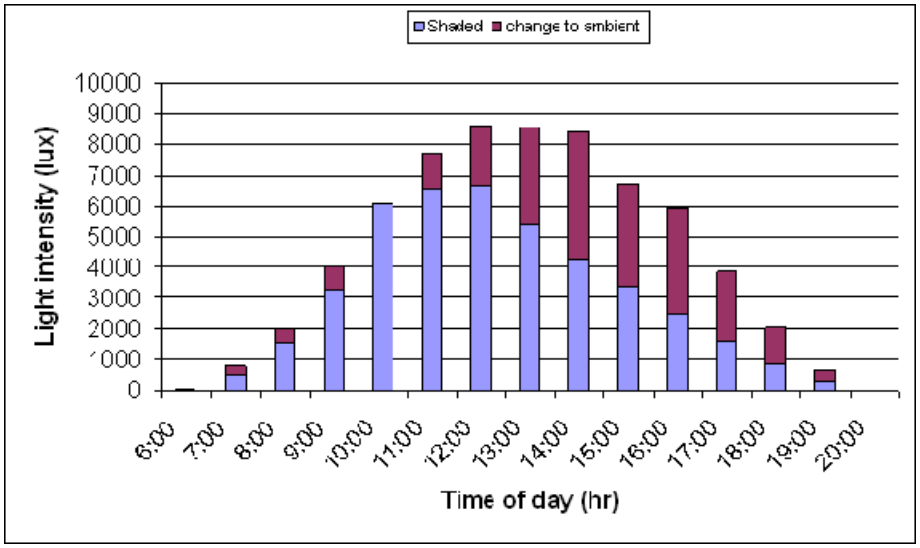

Figure 20. Water temperature plot in summer field experiment.

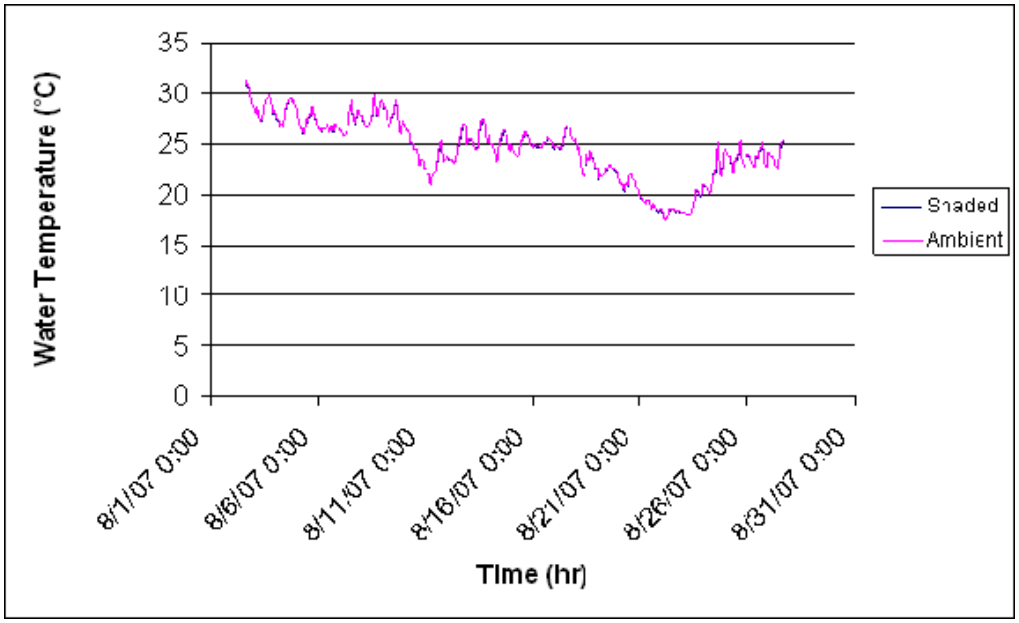

Figure 21. Porewater sulfide concentrations in Great South Bay, NY. Clear days= yellow bars, Overcast day= grey bars). Bars show standard error.

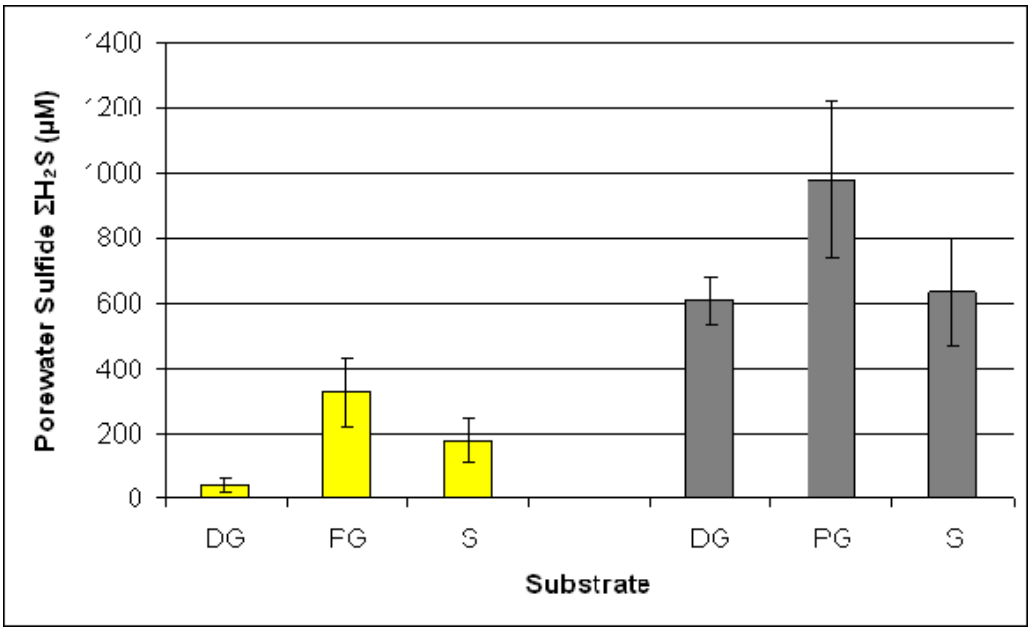

Figure 22. Porewater sulfide concentrations for clear sampling days in Great South Bay, NY. Bars show standard error.

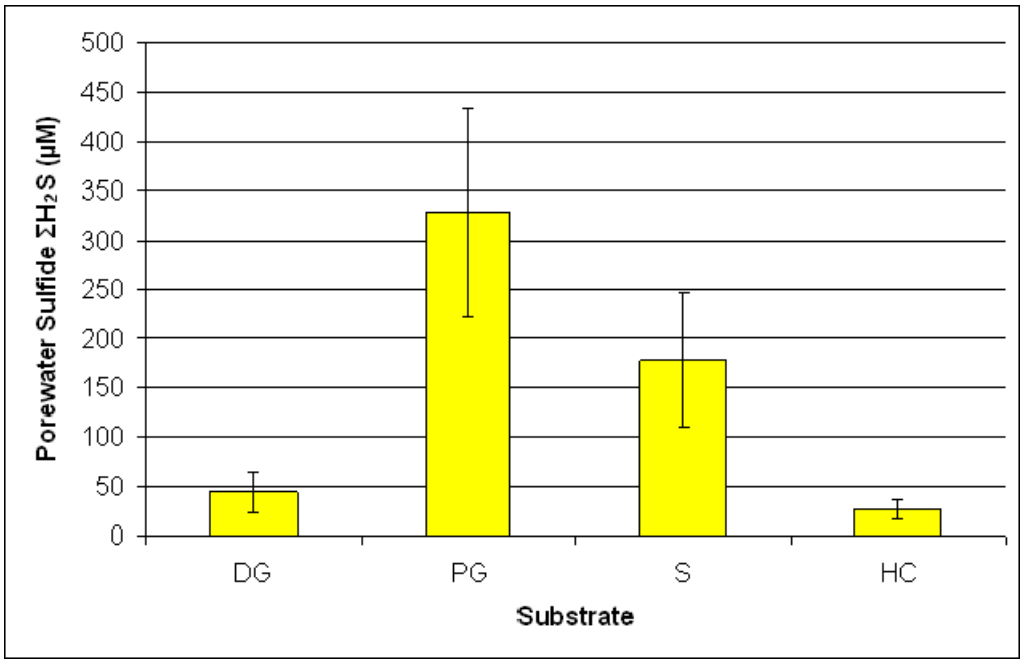

Figure 23. Porewater sulfide concentrations throughout the summer laboratory experiment. CA= Control un-shaded, MSA= Medium sulfide un-shaded, HSA= High sulfide un-shaded, CS= Control shaded, MSS= Medium sulfide shaded, HSS= High sulfide shaded. Bars show standard error.

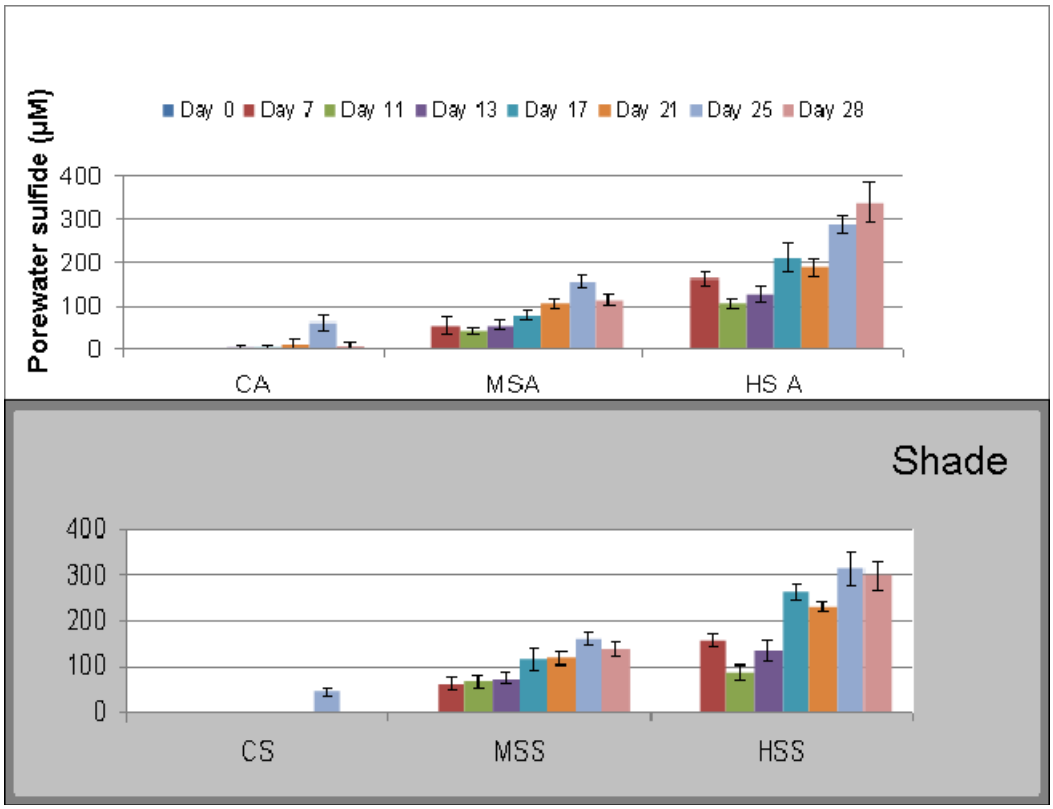

Figure 24. Sulfide concentrations for administered stock solutions for summer laboratory experiment. Average concentration shown in bars. Bars show standard error

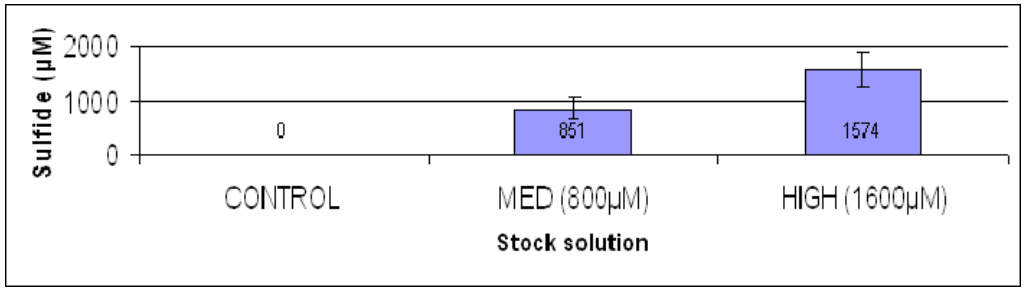

Figure 25. Porewater ammonium concentrations in the summer laboratory experiment. Un-shaded treatments shown in yellow, Shaded treatments shown in grey. C= Control, M= Medium sulfide, H= High sulfide. Bars show standard error

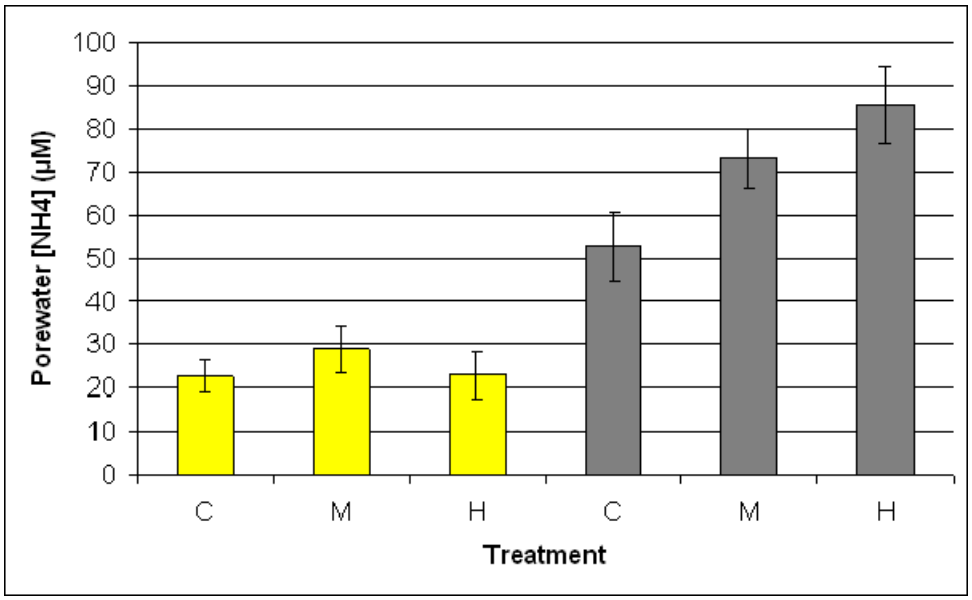

Figure 26. Specific productivity (mg/g/day) of eelgrass in the summer laboratory experiment. Un-shaded (A)= yellow, Shaded (S)= grey. C= Control, M= Medium sulfide, H= High sulfide. Bars show standard error

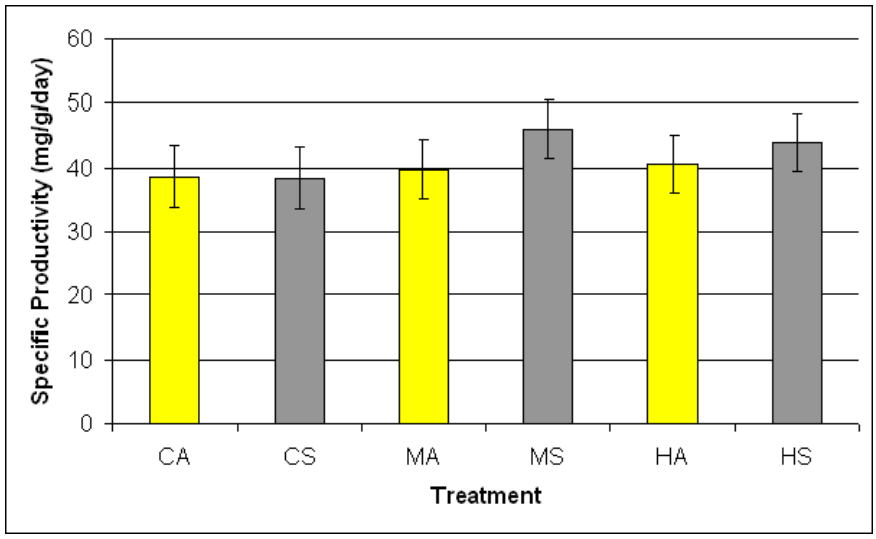

Figure 27. Short shoot (SS) productivity (mg/SS/day) of eelgrass in the summer laboratory experiment. Un-shaded (A)= yellow, Shaded (S)= grey. C= Control, M= Medium sulfide, H= High sulfide. Bars show standard error

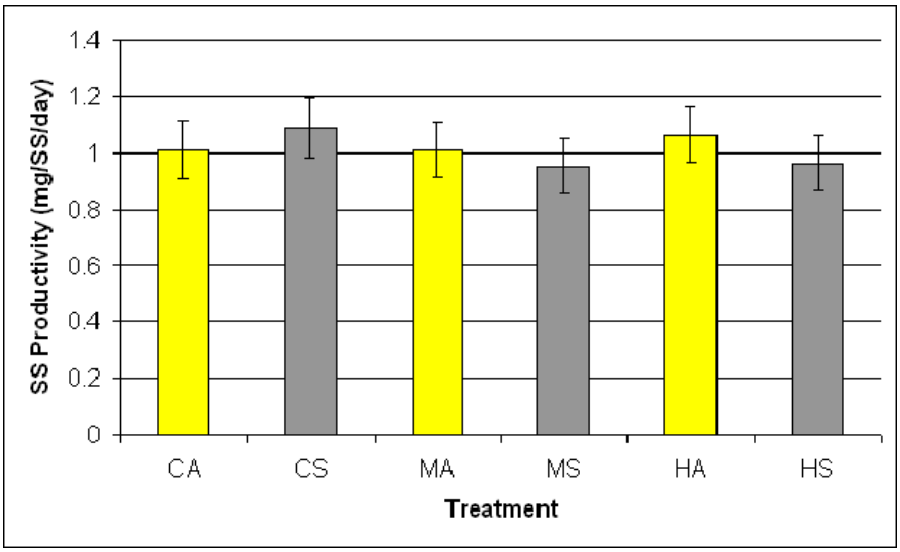

Figure 28. Leaf area productivity ($cm^2/m^2/day$) of eelgrass in the summer laboratory experiment. Un-shaded (A)= yellow, Shaded (S)= grey. C= Control, M= Medium sulfide, H= High sulfide. Bars show standard error

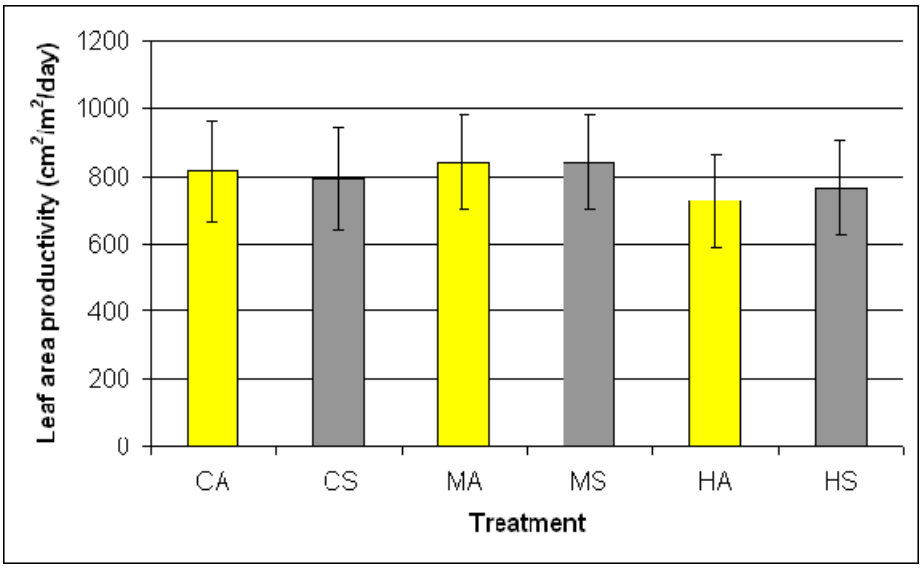

Figure 29. Porewater sulfide concentrations for the fall laboratory experiment. Un-shaded (A)= yellow, Shaded (S)= grey. C= Control, M= Medium sulfide, H= High sulfide, HANC= High sulfide un-shaded no clam, HSNC= High sulfide shaded no clam, HAC= High sulfide un-shaded clam, HSC= High sulfide shaded clam. Bars show standard error

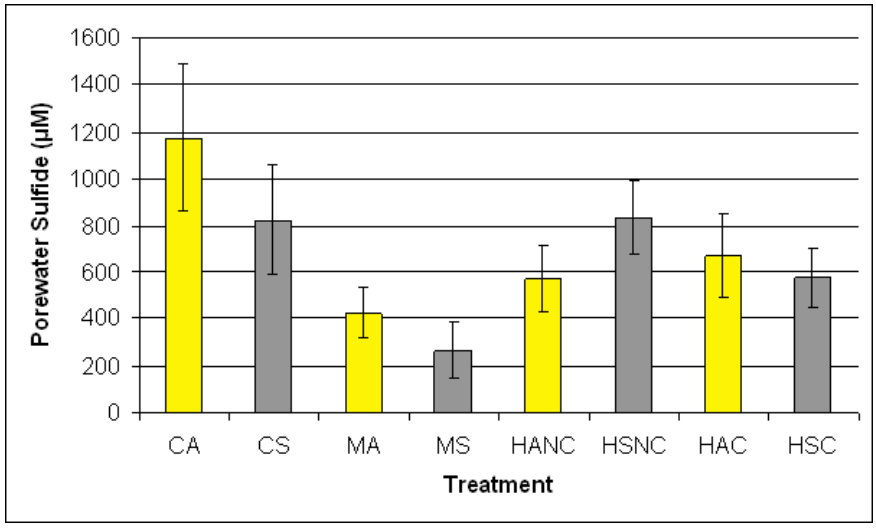

Figure 30. Porewater sulfide concentrations for the fall laboratory experiment (High sulfide treatments only). Un-shaded= yellow bars, Shaded= grey bars. HANC= High sulfide un-shaded no clam, HSNC= High sulfide shaded no clam, HAC= High sulfide un-shaded clam, HSC= High sulfide shaded clam. Averages displayed in bars. Bars show standard error

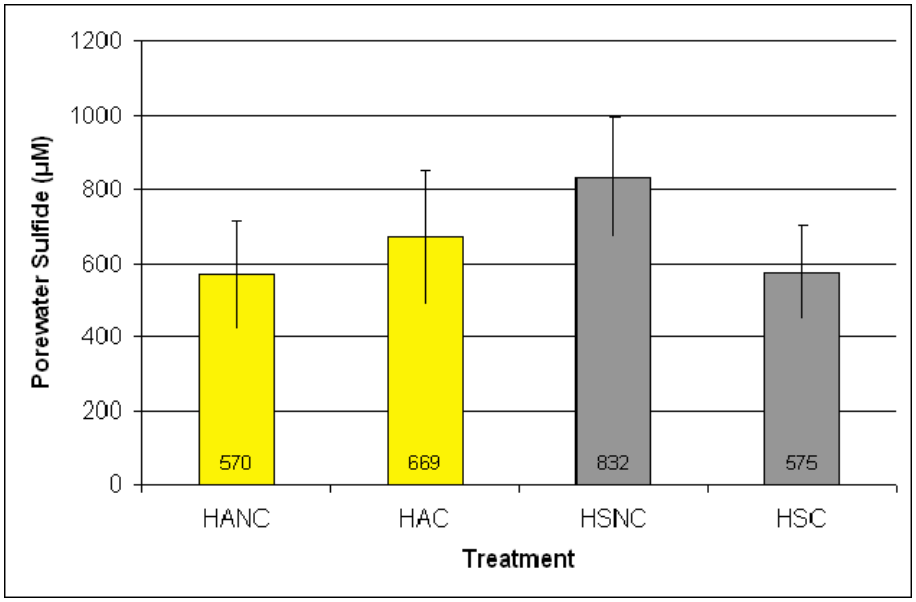

Figure 31. Specific productivity (mg/g/day) in High sulfide treatments in the fall laboratory experiment. Un-shaded= yellow, Shaded= grey. HNC= High sulfide no clam, HC= High sulfide clam. Bars show standard error

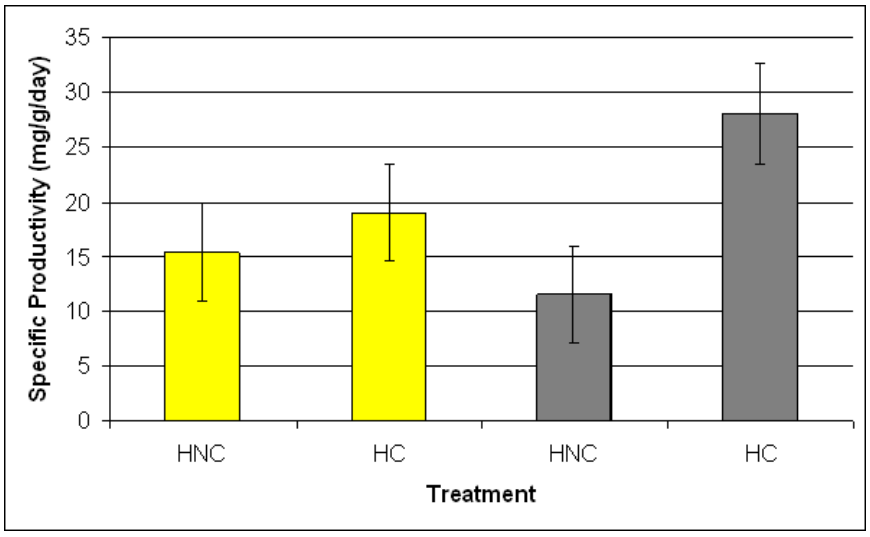

Figure 32. Porewater sulfide concentrations for summer field experiment. C= Control, 5= 5% glucose addition, 20= 20% glucose addition. A= un-shaded, S= shaded. Bars show standard error.

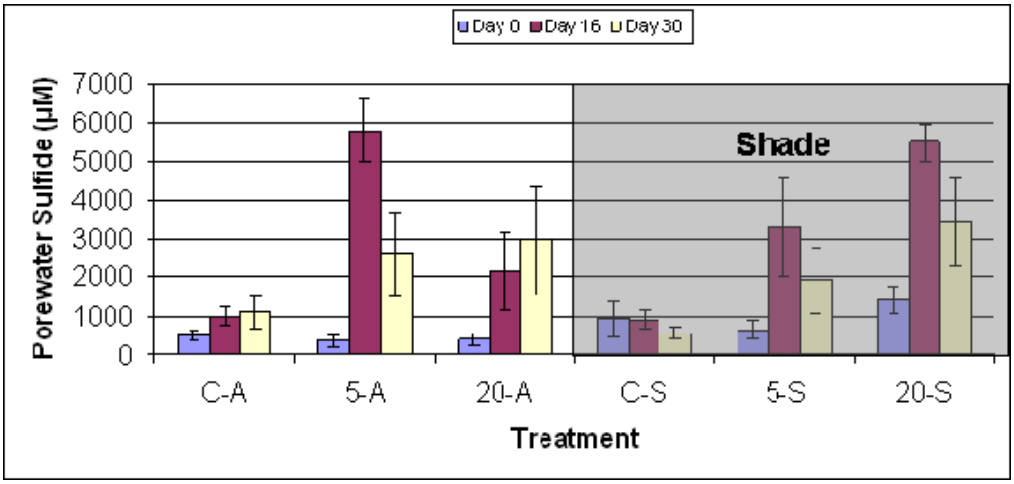

Figure 33. Standing crop (g/m^2) values for eelgrass in the summer field experiment. Un-shaded treatments= yellow, Shaded treatments= grey. C= Control, 5= 5% glucose addition, 20= 20% glucose addition. Bars show standard error

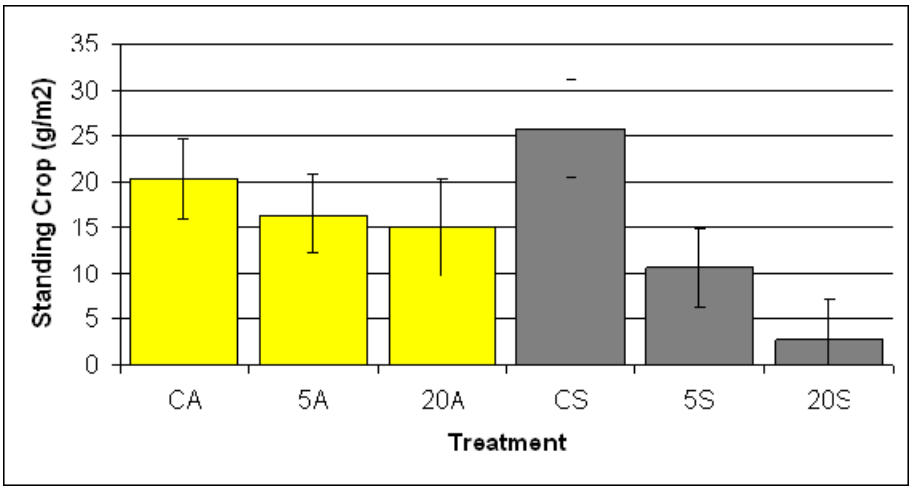

Figure 34. Short shoot (SS) productivity (mg/SS/day) of eelgrass in the summer field experiment. Un-shaded treatments= yellow, Shaded treatments= grey. C= Control, 5= 5% glucose addition, 20= 20% glucose addition. Bars show standard error

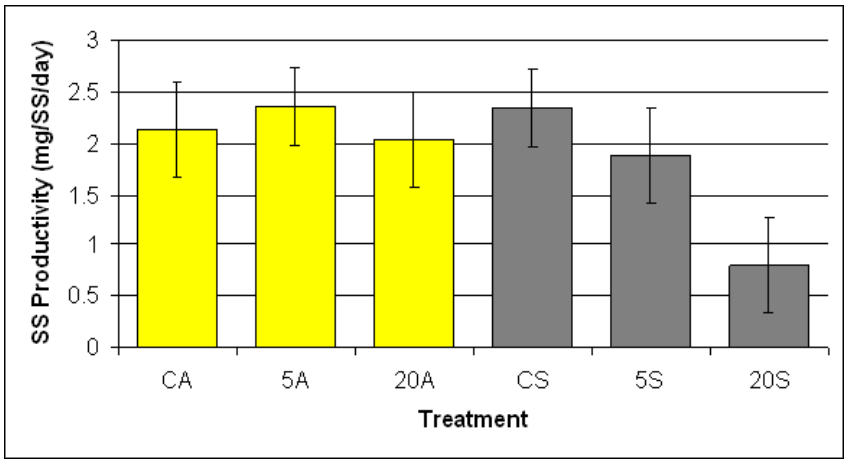

Figure 35. Percent survival of eelgrass in the summer field experiment. Un-shaded treatments= yellow, Shaded treatments= grey. C= Control, 5= 5% glucose addition, 20= 20% glucose addition. n= number of replicates per treatment. Bars show standard error