How does groundwater impact eelgrass in Long Island? The role of nitrogen and herbicide in reducing eelgrass growth, survival and photosynthetic efficiency.

A Thesis presented

By

Brooke Shannon Rodgers

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

Marine and Atmospheric Science

Stony Brook University

August 2010

Stony Brook University

The Graduate School

Brooke Shannon Rodgers

We, the thesis committee for the above candidate for the

Master of Science degree, herby recommend acceptance of this thesis.

Bradley Peterson-Thesis Advisor
Assistant Professor
School of Marine and Atmospheric Science

Christopher Gobler-Chairperson of Defense
Associate Professor
School of Marine and Atmospheric Science

Glenn Lopez
Professor School of Marine and Atmospheric Science

This thesis is accepted by the graduate school

Lawrence Martin
Dean of the Graduate School

Abstract of the Thesis

How does groundwater impact eelgrass in Long Island? The role of nitrogen and herbicide in reducing eelgrass growth, survival and photosynthetic efficiency.

by

Brooke Shannon Rodgers

Master of Science

in

Marine and Atmospheric Science

Stony Brook University

2010

Eelgrass communities in the Peconic estuary of Long Island NY have been in decline for several decades prompting resource managers to reduce nitrogen loads into the estuary. Despite improved water quality in recent years, eelgrass continues to decline. This lack of response has caused the management agencies to suggest that seepage of groundwater contaminated with herbicides such as Diuron and high nitrate from regions with a long history of agriculture may be to blame. We performed two types of manipulative experiments during the summer of 2009 to assess the possible impacts of groundwater on eelgrass growth and survival. We exposed the plants to multiple stressors of decreased light availability and increased water temperatures as well as Diuron exposure to the root and rhizome exclusively. In addition, we conducted a mesocosm experiment that delivered high nitrogen groundwater to the sediment of eelgrass planters via peristaltic pumps to assess the relative stimulation of grass growth versus phytoplankton biomass. In addition to measuring eelgrass survival and productivity, a PAM fluorometer was used to assess the impact that the herbicide had on the photosynthetic efficiency of the plants. Diuron in concentrations 80-200μg/l was found to decrease growth and productivity of eelgrass in the 1st root exposure experiment. Plants subjected to elevated temperature, reduced light and Diuron had the lowest mass and productivity.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to thank the following people who made the completion of this thesis possible: My advisor, Dr Bradley Peterson and my other readers Dr Chris Gober and Dr Glenn Lopez. Many friends and fellow graduate students helped and taught me a great deal especially: Bengt Allen, Jamie Brisbin, John Carroll, Tara Duffy, Adam Ehmer, Carolyn Hall, J.Matt Hoch, Florian Koch, Jesse Moore, Neilie Meyer, Amber Stubler, Chuck Wall and Brandon Wheeler. I would like to thank the Department of Environmental Conservation of New York for funding. Finally, I would like to thank my family and friends for all the support and love.

INTRODUCTION

Seagrass ecosystems are important for a variety of ecological and economic reasons. They grow in shallow coastal waters subjecting them to many impacts of nearby human populations. This anthropogenic impact has led to substantial loss of seagrass coverage which is an increasing cause of concern. Among the possible causes of these declines are declining water quality related to agricultural practices that contaminate groundwater with nutrients and herbicides. It is the goal of this work to examine how submarine groundwater discharge of agriculturally derived nitrate and the herbicide Diuron into the Peconic estuary, NY could be impacting the growth, survival and restoration of eelgrass *Zostera marina*.

Seagrasses are an important group of marine angiosperms that are distributed worldwide, existing throughout tropic and temperate ocean regions. They live submerged in marine and estuarine environments, anchored into the sediment and reproduce both asexually and sexually producing flowers and seeds. When reproducing asexually via root propagation, the plant grows laterally from the apical meristem and a large clonal bed can be formed. Materials produced or absorbed in one portion of the clone can be transported between these connected ramats. Seagrass unlike algae have true adventitious roots and rhizomes which anchor it into the substrate and absorb nutrients within the sediments. Spatial distribution is primarily controlled by light levels reaching the seabed which must be 15-25% of surface irradiance for the eelgrass to survive, whereas algae can survive with as little is 1% of surface irradiance. In some areas seagrass can grow deeper than 40 meters but is often found in less than 2 meters. Both algae and seagrass use inorganic carbon in the form of carbon dioxide (CO_2) and bicarbonate ions (HCO_3^-) as a carbon source for photosynthesis but algae does so much more efficiently. Eelgrass must maintain non-photosynthetic root and rhizome material, which can be equal to or exceed above ground tissues. Thus seagrass, unlike algae can become carbon limited. Finally, temperature and salinity are also important drivers of seagrass distributions (Larkum 2006).

Seagrass meadows are among the most productive and diverse ecosystems on earth (Duarte 2002, Waycott et al. 2009). They provide structural complexity with above ground leaves and below ground rhizome matrix that functions as a predation refuge supporting much greater abundance and biomass of organisms within the seagrass meadows than in adjacent un-vegetated areas. Roots and rhizomes stabilize and oxygenate the sediment and provide structure below ground which prevents erosion while above ground biomass slows water movement. The attenuated water motion allows fine particles to settle out of the water column reducing turbidity and increasing organic matter under the canopy. It also encourages settlement of planktonic larvae while the trapped organic material enhances juvenile and adult bivalve growth rates (Peterson & Heck 2001, Peterson et al. 2009). The higher O_2 level in the sediments converts sulfide to sulfate reducing its toxic effect making the associated sediment habitable for more organisms. Dissolved organic carbon exuded from the roots supports high microbial activity and efficient recycling of organic material. High primary productivity in the form of eelgrass and epiphytes growing on leaf surfaces support a rich food web including many commercially important finfish and shellfish (Larkum 2006). These services have lead to seagrass ecosystems being considered more valuable than salt marshes or coral reefs (Orth et al. 2006).

Unfortunately, seagrass communities are in decline worldwide (Duarte 2002, Orth et al. 2006, Waycott et al. 2009). Over a billion people live within 50 km of coastal regions where seagrass ecosystems occur. These huge human population densities have many damaging impacts on seagrass such as coastal development, dredging and nutrient inputs that all lead to declining water quality. A recent analysis of existing data found a 29% loss of seagrass cover worldwide over the last 127 years. Both natural and anthropogenic causes are responsible for this decline. In addition to damage from storms and tsunamis, boat propellers, coastal engineering, destructive fishing practices, aquaculture and the introduction of invasive species all have negative impacts.

Not only has the area of seagrass loss increased, but the rate of decline of these ecosystems has accelerated in the last thirty years from <1% $year^{-1}$ before 1940 to 5% $year^{-1}$ after 1950. The major driver of these declines is thought to be increased anthropogenic eutrophication of coastal waters leading to planktonic, epiphytic and macro algal blooms (Waycott et al. 2009). These negative impacts affect seagrass ecosystems simultaneously requiring the consideration of multiple stressors and nonlinear responses of seagrass to these cumulative impacts (Larkum 2006, Orth et al. 2006).

Eelgrass (*Zostera marina)* is the most widespread and ecologically important of two seagrass species in Long Island, New York waters. It reproduces both sexually and asexually. Eelgrass is a protogynous hermaphrodite having both male and female flowers on the same plant. Pollen is released into the water column and seeds develop within a spadix and sheath. The seeds can disperse from the spadix or it can separate from the plant and drift with the tide some distance before being released. Optimal temperature ranges are 10-20˚C and optimal salinity is 20-31ppt. *Zostera* is found on both coasts of the US as well as throughout Europe and Eastern Asia. New York eelgrass populations have recently been impacted by two major disturbances. An epidemic of the normally endemic slime mold *Labyrinthula zostera* destroyed ~90% of eelgrass cover on the Atlantic coasts of North America and Europe in the 1930's (Short & Wyllie-Echeverria 1996) . In the 1980's, blooms of *Aureococcus anophagefferens,* commonly know as brown tide, caused large scale die-offs of eelgrass by attenuating light reaching the plants in Great South Bay, NY and the Peconic Estuary, NY (Cosper et al. 1987). These blooms have reoccurred since then. New York eelgrass is also negatively impacted by warming temperatures, shoreline hardening, dredging, boating, destructive fishing techniques and blooms of macro algae that smother eelgrass (Gobler & Sanudo-Wilhelmy 2001, Gobler & Boneillo 2003, Larkum 2006).

Eelgrass cover in the estuaries of Long Island is limited and in decline. In Long Island Sound (LIS), eelgrass is limited to shallow near shore regions. On the New York side of the Sound, only 236 acres of seagrass coverage remain, which is less than 1% of historic acreage remains (2006 LIS report). The South Shore Estuary Reserve (SSER) covers approximately 108,000 acres, of which only 20,015 acres contained eelgrass during a 2002 survey. Approximately all (99%) of that eelgrass is found at depths less than 2m. In the Peconic estuary, seagrass was historically found in shallow waters from Flanders Bay to Gardiners Bay. However, eelgrass is now limited to the eastern part of the Peconic Estuary System, east of Shelter Island. While estimated seagrass coverage in the 1930's was approximately 8,720 acres, current aerial surveys reveal only 1,552 acres remain (Figure 1). In the last twenty years water quality in the Peconic estuary seems to be improving (Figure 2), but eelgrass coverage continues to decline (Figure 3). Eelgrass remaining in Long Island as in estuaries world wide is subjected to multiple stressors of low light availability and increasing concerns of rising water temperatures from global climate change (Short & Wyllie-Echeverria 1996, Lotze et al. 2006, Orth et al. 2006).

Submarine groundwater discharge (SGD) is very common along coastal regions of Long Island (Bokuniewicz 1980). As groundwater is the sole source of drinking water on Long Island, most research has been targeted toward its protection, although there are three aquifers, the upper glacial near the surface and the Lloyd and Magothy below

(Figure 4), the water of the deeper aquifers is older and relatively clean. Less than 40% of groundwater recharges the Lloyd and Magothy aquifers while the remaining 60% flows no deeper than the upper glacial aquifer (Buxton & Modica 1992). This aquifer extends to from 5 to 75 meters, is greatly impacted by human land use, such as agriculture, industry and sewage treatment (Eckhardt & Stackelberg 1995), and has the greatest impact on surface waters (Bokuniewicz 1980).

Long Island's North fork has a long history of agriculture including corn, potatoes and viticulture. Thus, large amounts of fertilizer, herbicides and pesticides have been and continue to be used (Buxton & Modica 1992) resulting in some of the highest recorded levels of nitrate in groundwater worldwide (Figure 5; (Gobler & Boneillo 2003). Long Island's sandy soil allows greater mobility of groundwater constituents, such as herbicides and pesticides which have been found in the aquifer (Suffolk County Department of Health). Unpredictably high mobility led to the banning of the pesticide Aldicarb in 1979 when it was found to have contaminated the aquifer and endangered public health (Jones & Marquardt 1987).

Much of the research on the effects of herbicides and pesticides in marine systems have been focused on relatively large doses delivered via runoff from heavy rain shortly after application (Haynes et al. 2000a, Macinnis-Ng & Ralph 2004). When contamination of an aquifer occurs, the delivery would differ from such singular pulses. Instead of an overland pulse to the estuary, contamination of an aquifer would lead to a chronic seepage to the estuary via groundwater of agriculturally derived chemicals and nutrients (Gardner & Vogel 2005). Such steady delivery would expose the roots and rhizomes of seagrass to higher concentrations than the leaves. Seagrasses in this region are not nutrient limited so it is unlikely they derive a benefit from the fertilizer in groundwater, the presence of which could contribute to phytoplankton blooms and cause light limitation at the seabed (Short & Wyllie-Echeverria 1996, Gobler & Sanudo-Wilhelmy 2001, Gobler & Boneillo 2003).

Regarding the impacts of agriculturally contaminated groundwater discharging into estuaries, Diuron is an herbicide of concern which has been found in Long Island's upper glacial aquifer (Paulsen, R. personal communication). Diuron N-(3,4-dichlorophenyl)-N,N-dimethyl urea is a substituted urea herbicide produced by Du Pont which is known to be moderately toxic to fish and highly toxic to invertebrates (Extoxnet 1993). . The herbicide works by disabling photo-system II preventing the plant from fixing carbon and can also subject the plant to damage from UV radiation (Extoxnet 1993(Haynes et al. 2000b).

Growing concern about the impact of pesticides on seagrasses has led to a variety of research techniques. Limited research is available on both the species *Zostera marina* and the herbicide Diuron but work done using one or the other is informative. Some researchers looked for delivery of pesticides in river flow or presence in sediments (Bester 2000, Haynes et al. 2000a, McMahon et al. 2005). Correll and Wu (1982) found significant inhibition of eelgrass leaf area by Atrazine at high concentrations and stimulation at low concentrations. Schwarzschild, Moore and Libelo (1994) used a peristaltic pump to simulate root exposure of *Zostera marina* via groundwater flow with the herbicide Atrazine and found no significant mortality or growth effects. To verify this, they used a split chamber setup to maintain root exposure to the herbicide in the absence of sediment and diffusion into the water column as well as a static whole plant exposure (Schwarzschild et al. 1994). Haynes, Muller and Carter (2002) found Diuron levels as high as 10.1µg/Kg in sediments near the Great Barrier Reef (Haynes et al. 2000a). In the same year Haynes, Ralph, Pranges and Dennison used a Diving PAM fluorometer to determine maximum quantum yield (F_v/F_m) and showed inhibition of *Cymodocea serrulata, Halophila ovalis* and *Zostera capricorni* at concentrations as low as 0.1 µg/l (Haynes et al. 2000b). The PAM remains a favored technique for determining sublethal impacts of herbicides on seagrasses in part because it can measure the efficiency of photosystem II the site of action of many herbicides of concern (Ralph et al. 1998). Macinnis-Ng and Ralph (2003) used the same technique to show inhibition of *Zostera capricorni* by leaf exposure to 10-100 µg/l Diuron for a 10 hour exposure and following a four day recovery period (Macinnis-Ng & Ralph 2003). Similarly, Chesworth, Donkin and Brown (2003) showed inhibition of maximum quantum yield (F_v/F_m) and leaf specific biomass of *Zostera marina* via whole plant exposure to Diuron at concentrations as low as 2.5 and 5 µg/l respectively during a 10 day exposure.

The primary objectives of this research project were to address the following questions. (1) Does Diuron exposure via submarine groundwater discharge impact the growth, photosynthetic efficiency or survival of eelgrass? (2) Do the multiple stressors (heat and reduced light) affect the impact of Diuron on eelgrass? (3) Does nitrate present in submarine groundwater discharge impact the growth, photosynthetic efficiency or survival of eelgrass or chlorophyll a in the water column? (4) Does Diuron impact water column chlorophyll a?

METHODS

This study consisted of a series of root exclusive exposure and water column mesocosm experiments that were conducted between July and September 2009 to assess the impact of the herbicide Diuron and nitrate on eelgrass. The root exclusive exposure experiments isolated the roots in a flask with Diuron and subjected the whole plant to the multiple stressors of reduced light availability and elevated temperatures.. Herbicide exposure via submarine groundwater discharge would expose the roots of the plant to the highest concentration of herbicide present in groundwater which would subsequently be diluted into the water column. It is difficult to determine how much herbicide adsorbs onto sediments in an experimental setup versus how much reaches the plant roots. The evenness of diffusion and dilution of herbicides in discharging groundwater is also difficult to control. The split chamber setup with plant roots isolated in a flask greatly simplifies this system. It is a worst case scenario with the eelgrass being exposed to the full concentration of the herbicide without the buffering effects of adsorbtion and diffusion. If no effect of herbicide can be seen in a split chamber setup it can be assumed that there will be none in the more realistic circumstance with sediment adsorbtion and diffusion and dilution into the water column.

In the peristaltic pump Submarine Groundwater Discharge (SGD) experiments the complexity of sediment and diffusion of the herbicide into the water column were addressed. This setup simulates groundwater flow through sediment containing eelgrass root material which is then diluted into the mesocosm chamber. Seeing no impact of herbicide in this experiment would not rule out an impact of the herbicide on the grass in this much more complex and realistic setup. Any impact of the herbicide seen in would be in spite of the buffering effects of sediment adsorbtion and diffusion into the simulated water column.

Diuron (N-(3,4-dichlorophenyl)-N,N-dimethyl urea) stock solutions for both experiments were made by dissolving Diuron in 5ml of acetone then diluting into de-ionized freshwater to desired concentration (150 µg/L or 200 µg/L) (Haynes et al. 2000a, Haynes et al. 2000b).

<u>Diuron Root Exclusive Exposure with Multiple Stressors Experiment</u>:

Direct exposure of eelgrass roots to a low concentration of Diuron was examined by growing eelgrass in a split chamber consisting of a 125ml Erlenmeyer flask fitted with a holed rubber stopper (Figure 6). .The flasks were filled with Diuron in de-ionized freshwater (150 µg/L or 200 µg/L) or de-ionized freshwater without Diuron for the control (Schwarzschild et al. 1994, Haynes et al. 2000b). *Zostera marina* shoots, 20-30 cm long, were harvested from eastern Shinnecock Bay on the day that each experiment commenced. Eelgrass was sorted to remove reproductive shoots, rinsed in seawater, separated into individual shoots with a segment of the attached rhizome, and marked with a small pinhole at the top of the sheath using an 18 gauge needle, according to the method of Zieman (1974). Individual shoots were fitted into the holed stoppers taking care not to damage the root and sealed with silicone grease then fitted into the filled flask, according to the method of Schwarzschild (1994). Sixteen weighted wood racks of six flasks each were grown in an outdoor flow through system of eight 1100 liter black plastic mesocosms at the Southampton marine station. Four of the mesocosm tanks were maintained at a temperature 2-4˚C above ambient using four Finnex 800 watt titanium heaters and a thermistor/thermostat system to detect the temperature in the ambient tanks and turn the heaters on and off as needed to maintain the elevated temperature in the heated tanks. Shade cloth covered two heated and two unheated mesocosms to achieve an 80% reduction of light.

Water samples were collected at the end of each experiment and were analyzed by the Suffolk County Department of Health for Diuron concentration.

Productivity was measured by puncturing each grass shoot in the leaf sheath region with a hypodermic needle. After two weeks of growth, plants were harvested and new growth (below the puncture) separated from old (above the puncture). Length and width of leafs were measured to calculate a total leaf area. Leaf tissue dry weights were measured for the old and new production.

Chlorophyll fluorescence parameters and stress to photosystem II were measured with a portable underwater Pulse Amplitude Modulated (PAM) fluorometer (Walz, Germany) (Ralph et al. 1998). Plastic Waltz clips were used to dark adapt the leaves and assure a constant distance between the plant surface and the fiber optics (10mm). Maximum quantum yield measurements were taken on the adaxial or upper surface of the second youngest leaf at the midpoint. Quantum yield measurements and Fv/Fm measurements were taken on the same leaf in the same position, with Fv/Fm measurements occurring after a 10 minute dark adaptation period determined adequate for relaxation of the chlorophyll a reaction center based on preliminary work (Rodgers, unpublished data).

At the end of the experiment, the first five leaves utilized for fluorescence measurements in each treatment were removed at the base of the leaf, placed in a dark plastic bag and returned to the laboratory for chlorophyll analysis. Immediately upon collection, leaves were scraped free of epiphytes, cut with a razor blade to a standard length of 1 cm, and frozen. Chlorophyll from each leaf segment was then extracted using N,N-dimethylformamide DMF as described by Inskeep and Bloom (1985). After 24 hours, the extracts were analyzed using Turner Trilogy fluorometer (Parsons et al., 1984).

A three factorial experimental design was used for this study with herbicide presence/absence, temperature ambient/+2 or 4˚C and light ambient/80% shade as the main factors. A three way analysis of variance was used to detect differences between treatments. All data was log transformed when necessary to meet assumptions of normality and equal variance. When a significant difference was observed between treatments Students Tukey multiple comparisons analysis was conducted. Differences were considered significant when $P < 0.05$.

Peristaltic Pump SGD Simulation Experiment:

Mesocosm experiments were carried out at the Stony Brook - Southampton Marine Science Center on Old Fort Pond in Southampton, New York between July and September 2009. Old Fort Pond exchanges tidally with Shinnecock Bay, one of the major Long Island south shore estuaries. The experiments were carried out in a series of 300 L polyethylene tanks (Nalgene©; depth 122 cm, inside diameter 60 cm), which have been used successfully in the past to examine the impacts of filer-feeding bivalves on pelagic algal communities (Cerrato et al. 2004, Wall et al. 2008). Prior to each experiment, all tanks were scrubbed, rinsed with fresh water, and then filled with seawater from Old Fort Pond. The mesocosms were ~90% immersed in Old Fort Pond to maintain a uniform ambient temperature. Small aquarium pumps (Rio® 180

Mini, pumping rate: 456 L h^{-1}) were added to mix the water column of each mesocosm, but were suspended only a few centimeters below the surface to minimize re-suspension of sediments or biodeposits. Measurements taken at the start of each experiment and every 1 -2 days during experiments included temperature, salinity, dissolved oxygen, chlorophyll *a*, and light attenuation. Surface and bottom readings of temperature and salinity during experiments confirmed that aquarium pumps kept the mesocoms well-mixed during experiments. Chlorophyll *a* (chl *a*) was measured by filtering mesocosm samples onto replicated GF/F filters, freezing and extracting in acetone, and measuring fluorescence with a Turner Trilogy fluorometer (Parsons et al., 1984). Each mesocosm contained a weighted plastic planter with clean sand and 10 shoots of the seagrass *Zostera marina*. *Zostera* shoots, 20-30 cm long, were harvested from eastern Shinnecock Bay on the day that each experiment commenced. Eelgrass was sorted to remove reproductive shoots, rinsed in seawater, separated into individual shoots with a segment of the attached rhizome, and marked with a small pinhole at the top of the sheath using an 18 gauge needle, according to the method of Zieman (1974). Twelve marked shoots were randomly assigned to each mesocosm, gently buried in the planter, making sure the roots were intact and covered with sand, and the planters were carefully lowered to the bottom of the mesocosm.

Nitrogen-contaminated groundwater for these experiments was collected from the eastern shore of the Forge River, NY, USA. This region is surrounded by dense housing and agriculture, features which can commonly lead to N-enriched groundwater on Long Island (Gobler and Boneillo 2003, Gobler and Sañudo-Wilhelmy 2001). A PVC-well was drilled to 40m, and 5 m of Teflon tubing connected to a parastaltic pump was used to pump groundwater to the surface. High groundwater flow rates within seeps typically ensured this well contained with fresh groundwater (salinity < 0.1 PSU; practical salinity units). Groundwater were sampled using a low flow (< 100 mL min^{-1}), peristaltic pump equipped with acid-washed Teflon tubing. Dissolved oxygen, temperature, and conductivity levels of pumped groundwater were measured with submersible electrodes (YSI 85). To ensure representative groundwater was obtained, samples were not collected until the dissolved oxygen, temperature, and conductivity of the pumped groundwater stabilized (Puls & Powell 1992, Puls & Paul 1995). Previous research on Long Island has demonstrated that groundwater collected from coastal well with such methods is representative of the groundwater which enters surface waters (Gobler & Sañudo-Wilhemy 2001). Nutrient samples were filtered with pre-combusted GF/F glass fiber filters in the field, and immediately stored on ice, and analyzed for ammonium, nitrate, and phosphate according to Parsons et al (1984).

The effect of Submarine Groundwater Discharge (SGD) and herbicides on seagrass was assessed by simulating ground water seepage using a peristaltic pump with 24 separate lines delivering a groundwater to the bottom of the seagrass planters within the mesocosms. The pure groundwater contained 700µM and in the first SGD experiment was mixed with de-ionized water to create a gradient of N levels (0, 231, 462 or 700 µM nitrate). Groundwater was delivered at a rate of 2ml/minute, mimicking rates observed within Long Island estuaries (Gobler and Boneillo 2003). This resulted in N fluxes rates of 0, 665.28, 1330.56, and 2016.0 M nitrate/day. The groundwater was delivered to the eelgrass for two weeks after which measurements of survival, growth, productivity and photosynthetic efficiency were made as described above. One Way ANOVAs were used to analyze this experiment.

In the second SGD experiment setup was the same but the groundwater treatments differed, the four treatments were de-ionized fresh water with and without Diuron (200µg/L) and 700µM nitrate groundwater with and without Diuron (200µg/L). This experiment was run for two weeks after which the above data was collected. Two way ANOVAs were used to analyze this experiment.

RESULTS

Root Exclusive Exposure with Multiple Stressors Experiments

Two root exclusive exposure experiments were conducted for 14 days in July 2009. The experiment had three treatments that consisted of Herbicide (with and without 200µg/l Diuron), Shade (with and without 80% shade cloth) and Heat (either ambient water temp or + 2˚C). The heat and shade treatments were significantly different (p=0.001 for both). The response variables of the plants to the treatments varied significantly over the course of the experiment. Leaf number was significantly reduced in the Heat and Shade treatments (p=0.002 and 0.01 respectively, 3-Way ANOVA). Leaf width and length were significantly reduced in the Herbicide treatment (p=0.01 and 0.04 respectively). While leaf area (cm^2) was significantly reduced in both Herbicide and Shade treatments (p=0.001 and 0.004 respectively). In addition, leaf mass (mg short $shoot^{-1}$) was significantly reduced in both Herbicide and Shade treatments (p=0.008 and 0.001 respectively; Figure 7), but standing crop was only significantly reduced in the Shade treatment (p=0.001).

Productivity measurements based on either changes in leaf area or leaf mass were significantly affected by the experimental treatments. Leaf area productivity (cm^2 Short $Shoot^{-1}$ day^{-1}) was significantly reduced in herbicide, heat and shade treatments (p=0.01, 0.01, 0.02; Figure 8), as was leaf mass productivity (mg Short $Shoot^{-1}$ day^{-1}; p=0.03, 0.01 and 0.001 respectively). PAM measurements of photosynthetic efficiency (dark yield) were significantly reduced in the Shade treatment (p=0.001; Figure 9), but no significant difference was found in the amount of chlorophyll a per leaf tissue area for herbicide, shade or heat (P=0.11, 0.17, 0.52 respectively).

The second root exclusive exposure experiment had the same three treatments as the first, but the level of Diruon was reduced to 150µg/l in the herbicide treatment and the heat treatment was +4˚C. The treatments were again significantly different for both heat and shade (p=0.001 for both; Table 2). Leaf number was again significantly reduced in the heat treatment (p=0.001, 3-Way ANOVA), but not in the herbicide treatment as before (p=0.68). Neither did the Diuron treatment significantly reduced leaf widths or lengths (p=0.63 and 0.73 respectively). Similarly, leaf area was only significantly reduced in the heat treatment (p=0.04). While leaf mass was significantly reduced only in the heat treatment (p=0.005; Figure 10), standing crop was significantly reduced in both the heat and shade treatments (p=0.001 and 0.032 respectively). All measures of eelgrass productivity either based on increases in mass or area were significantly reduced only in the heat treatment (mass short shoot^{-1} d^{-1}, specific productivity mg g^{-1} d^{-1} and leaf area productivity cm^{2} d^{-1}; p=0.001 for all three; Figure 11). PAM measurements of photosynthetic efficiency (dark yield) were significantly different for heat and shade treatments (p=0.001 for both; Figure 12). There was also a significant interaction between heat and shade (p=0.001).

Peristaltic Pump SGD Simulation Experiments

For this mesocosm experiment, there were no significant differences between treatments for any of the eelgrass growth morphometrics (leaf number, length, width or area). There were also no significant differences between treatments in leaf mass, standing crop or any productivity measure (Figure 13 & 14). Water column chlorophyll a was significantly different for both the water treatments and by day ($p=0.001$ for both; Figure 16) being higher in the high N loading treatments. These increases in algal biomass significantly reduced light availability within the mesocosms with a significant difference between all treatments ($p<0.001$ for all). These changes in light resulted in significant differences in photosynthetic efficiency (dark yield) of the eelgrass. The treatment with the highest levels of natural groundwater had significantly higher photosynthetic efficiency (100% groundwater versus 0% ground water and 100% groundwater versus 33% groundwater; $p<0.05$ for both, 1-Way ANOVA; Figure 15). There was no significant difference between treatments for epiphytes ($p=0.33$) but the trend of the data was similar to that seen in the photosynthetic efficiency data above.

In the second peristaltic pump SGD experiment initiated August 11th, the treatment solutions were de-ionized water with and without 200µg/l Diuron and groundwater with and without 200µg/l Diuron. These treatments allowed the impacts of herbicide to be decoupled from that of high nitrogen. Diuron significantly reduced eelgrass leaf mass and standing crop ($p=0.005$ for both; 2-Way ANOVA; Figure 17). In addition, leaf number and leaf area were significantly reduced by Diuron ($p=0.01$ and

0.018 respectively). However, there were no significant differences between treatments for leaf length and width. Both leaf mass productivity (mg Short Shoot^{-1} day^{-1}) and leaf area productivity (cm^2 Short Shoot^{-1} day^{-1}; Figure 18) were significantly reduced by the presence of herbicide (p= 0.01 and 0.04 respectively). Phytoplankton biomass showed a significant increase in the groundwater treatment and decrease in the herbicide treatment (p=0.001 and 0.009 respectively; Figure 19). The impact of these changes on light resulted in a significant reduction in photosynthetic efficiency for herbicide and an increase in photosynthetic efficiency for groundwater treatments (p=0.001 for both; Figure 20).

DISCUSSION

New York eelgrass ecosystems like many around the world are far from pristine. By looking at the multiple stressors (+4˚C and an 80% reduction in light availability) the impact of herbicide exposure on eelgrass being impacted by a warming climate and poor water quality was examined. Many people have used the diving PAM fluorometer to access impacts from herbicide exposure but few have collected morphometeric data as well. By using morphometrics and maximum quantum yield (F_v/F_m) more comparisons can be made between this work and that of researchers using either method. Much prior work has examined the impact of leaf tissue and whole plant exposure to herbicides. When overland transport is the source of herbicide contamination concern, these methods are appropriate whereas submarine groundwater discharge would expose almost exclusively root and rhizome tissue having potentially very different effects. Schwarzschild, Moore and Libelo (1994) are the only group to examine closely the impact of root exposure but they were concerned with the herbicide Atrazine. In the water column mesocosm experiments, we looked from herbicide impacts to the phytoplankton community (via chlorophyll a) as well as the plants. In this series, we simulated groundwater flow into planters with sediment. This brought the factors of sediment adsorption and diffusion into the water column into the setup. We collected fluorescence and morphometric data as well as water column chlorophyll a. We included a treatment with groundwater collected from a region with high nitrate (700µm) and added Diuron as areas with herbicide contamination from agriculture often have very high nitrate from fertilizer. This assessment of nitrate with and without herbicide using fluorescence and morphometric data as well as water column chlorophyll a has not been done before.

This research project focused on several specific questions. *Does Diuron exposure via submarine groundwater discharge impact the growth, photosynthetic efficiency or survival of eelgrass?* Diuron exposure reduced leaf mass, and leaf area productivity in the first split chamber experiment. In the second root exclusive exposure experiment, the heat treatment dominated the results and no differences were found for the Diuron treatment in mass, productivity or maximum quantum yield (F_v/F_m). In the second peristaltic pump SGD experiment Diuron negatively impacted leaf mass, productivity and maximum quantum yield (F_v/F_m) of the eelgrass as well as chlorophyll a in the water column. These experiments both with and without a sediment matrix demonstrated that Diuron between 80 and 200 μg/L had significant effects on *Zostera marina* and phytoplankton.

Diuron is only one of many agricultural chemicals to have been detected in groundwater and it is still being used as a weed control and in some areas as an antifoulant (Extoxnet 1993). Herbicide exposure via submarine groundwater discharge is a concern in part because it is a chronic long term source of stress. If *Zostera marina* is living in suboptimal conditions any additional stressor could affect the survival of the plant. Excessive heat and shade are always stressful to eelgrass but in the presence of Diuron that stress is intensified (Haynes et al. 2000a, Haynes et al. 2000b). We don't yet know the distribution and concentrations of the various herbicides in the groundwater of long island that may be affecting Zostera. This knowledge may be vital to understanding how water quality and the changing climate will impact eelgrass in the years to come.

We know that groundwater contaminated by agriculture often has not one but many chemicals present. Over the last 30 years more than a dozen herbicides and pesticides have been restricted or banned due to contamination concern. The pesticide Aldicarb was banned in 1980 but is still present in groundwater (Extoxnet 1993). By the time contamination is discovered and use of the chemical is banned we may have 30 years or more of contaminated groundwater that will be discharged into our estuaries (R. Paulsen *personal communication*).

Do multiple stressors (heat and reduced light) affect the impact of Diuron on eelgrass? In the first root exclusive exposure experiment the shade treatment negatively impacted leaf mass, but no differences were seen from the heat treatment. Leaf area productivity was significantly reduced by heat and shade. Reduced light significantly increased maximum quantum yield (F_v/F_m). The cumulative effect of these stressors causes statistically and ecologically significant declines in eelgrass mass and productivity. The increase in maximum quantum yield (F_v/F_m) is evidence of the plants costly adaptation to stressful low light conditions. In the second root exclusive exposure experiment the heat treatment overwhelmed all other treatment effects and was the only significant difference between treatments for leaf mass, leaf area productivity and maximum quantum yield (F_v/F_m). In both experiments, one or many stressors negatively impacted the condition of the plant. As we saw in the second root exclusive exposure experiment a major stressor can mask the presence of other factors harmful to the plant. It is clear that each of these factors have a cost and in the presence of other stressful conditions such as high heat and low light the added effect of herbicide and pesticide exposure could lead to a failure of the individual or the population (Short & Wyllie-Echeverria 1996, Haynes et al. 2000b, Ralph 2000). Without careful investigation it would be difficult to pinpoint that additional stressor from the background established stressful conditions.

Eelgrass in the Peconic estuary is regularly exposed to episodes of high temperatures, low light and poor water quality that are thought to affect biomass and productivity. The impact of Diuron and other herbicides must be considered in the light of these underlying conditions. Major efforts are being made to improve water quality and to restore eelgrass. If these costly efforts are made in a region that will be steeped in an herbicide and pesticide cocktail for decades to come during an era of increasing temperatures it may mean the difference between success and failure. The stressors of eelgrass must be studied in concert as they occur if we hope to determine thresholds for stressors affecting the viability of these ecosystems.

Does nitrate present in submarine groundwater discharge impact the growth, photosynthetic efficiency or survival of eelgrass or chlorophyll a in the water column? The first peristaltic pump SGD experiment that compared different levels of nitrate in groundwater and the corresponding concentration of nitrate showed a trend of declining leaf mass with increasing nitrate concentration, but had no impact on productivity. Although it was not statistically significant, it is likely a result of light limitation. Water column chlorophyll a and maximum quantum yield (F_v/F_m) increased with increasing nitrate concentration. In the second water column experiment, the groundwater treatment high in nitrate significantly increased the water column phytoplankton biomass and decreased light levels. Ensuing reduction in light may have driven the increase of maximum quantum yield (F_v/F_m) for the eelgrass in response to the shading from phytoplankton growth stimulated by the groundwater (Williams & Ruckelshaus 1993, Short et al. 1995).

Anthropogenic eutrophication from point sources such as sewage effluent is a subject of concern (Orth et al. 2006, Waycott et al. 2009). This work suggests that the nitrate present in groundwater has the potential to stimulate phytoplankton biomass. If this is true, the costly efforts being made to reduce point source inputs into our estuaries could fail to improve water quality and reduce Harmful Algal Blooms (HABs) as hoped (Long Island Sound Study 1998). The long residence time of groundwater creates a lag time of decades before any regulatory or behavioral changes have an impact on contamination levels found in submarine groundwater discharge.

Does Diuron impact water column chlorophyll a? In the second peristaltic pump SGD experiment that introduced the impact of a herbicide, there was significantly lower chlorophyll a in the presence of Diuron. This was an unexpected finding as the Diuron concentration after dilution into the mesocosm tank was below detection limits. This reduction in phytoplankton biomass was greater for the treatments without elevated nitrate.

Alteration of phytoplankton communities by Diuron or other herbicides could have far reaching consequences. Changes in the magnitude or composition of the plankton community could have cascading impacts to upper trophic levels and potential reductions in larval recruitment. If this occurred on a large scale it could change the distribution of commercially important species and alter food webs regionally. Although this project was focused on the impact of groundwater on seagrass, the implications of the effect of herbicides delivered via groundwater on phytoplankton biomass need to be considered. Efforts to restore scallop and clam populations rely on the quality of phytoplankton available as food to the larvae and adults. If the phytoplankton community in a region is shifted toward less palatable species or those of a different size it could affect the success of the restoration (SPAT Cornell Cooperative Extention, Peconic Estuary Program).

This study clearly demonstrated that herbicide delivered to the root matrix had a negative impact on *Zostera*. These impacts were intensified when other stressors were present. Nitrate in groundwater stimulated phytoplankton biomass that could lead to intensified light limitation for the benthic plant community. Surprisingly, there was a statistically significant reduction of phytoplankton biomass when Diuron was present in the groundwater. These results must lead us to consider what impact Diuron or other herbicides and pesticides have in our coastal waters. In 1998 there were 24 pesticides detected in Long Island groundwater by the Suffolk County Department of Health Services, by 2002 there were 52. More than half of private wells tested were contaminated by agricultural chemicals and 15% contained five or more pesticides. In 2005 a dozen herbicides and pesticides were detected in two creeks discharging in to the

Peconic estuary (Paulsen, R. personal communication). It is clear that agricultural chemicals are reaching our estuaries. What is not yet well known is how this cocktail of herbicides combined with increasing temperatures and poor water quality impact seagrass ecosystems. From this work it is clear that low concentrations of herbicides such as Diuron can negatively impact eelgrass. Herbicides delivered via groundwater flow are a chronic stressor and if paired with a period of high temperatures or light limitation from chronic algae blooms the survival of eelgrass could be affected. Extensive testing is needed to determine the types and concentrations of agricultural chemicals discharging our estuaries. When considering the concentration at which harm is done to eelgrass or other species, other stressors and "chemical cocktails" need to be considered.

The new paradigm of ecosystem based management has brought attention to the importance of eelgrass habitat. Substantial funds are being spent on restoration in the Peconic estuary (Peconic Estuary Program 2000). If substances in groundwater have a negative effect on eelgrass, it would have important management implications. The presence or absence of groundwater flow could guide choices of restoration site selection. However if these substances are not found to harm grass, the nutrients and cooler temperatures of high groundwater flow sites could prove optimal for restoration activities.

References

Bester K (2000) Effects of pesticides on seagrass beds. Helgoland Mar Res 54:95-98

Bokuniewicz H (1980) Groundwater seepage into Great South Bay, New-York. Estuarine and Coastal Marine Science 10:437-444

Buxton HT, Modica E (1992) Patterns and rates of groundwater-flow on Long-Island, New-York. Ground Water 30:857-866

Cerrato RM, Caron DA, Lonsdale DJ, Rose JM, Schaffner RA (2004) Effect of the northern quahog Mercenaria mercenaria on the development of blooms of the brown tide alga Aureococcus anophagefferens. Marine Ecology-Progress Series 281:93-108

Cosper EM, Dennison WC, Carpenter EJ, Bricelj VM, Mitchell JG, Kuenstner SH, Colflesh D, Dewey M (1987) Recurrent and persistent brown tide blooms perturb coastal marine ecosystem. Estuaries 10:284-290

Duarte CM (2002) The future of seagrass meadows. Environmental Conservation 29:192-206

Eckhardt DAV, Stackelberg PE (1995) Relation of groundwater quality to land-use on Long-Island, New-York. Ground Water 33:1019-1033

Gardner KK, Vogel RM (2005) Predicting ground water nitrate concentration from land use. Ground Water 43:343-352

Gobler CJ, Boneillo GE (2003) Impacts of anthropogenically influenced groundwater seepage on water chemistry and phytoplankton dynamics within a coastal marine system. Mar Ecol-Prog Ser 255:101-114

Gobler CJ, Sanudo-Wilhelmy SA (2001) Temporal variability of groundwater seepage and brown tide blooms in a Long Island embayment. Marine Ecology-Progress Series 217:299-309

Haynes D, Müller J, Carter S (2000a) Pesticide and Herbicide Residues in Sediments and Seagrasses from the Great Barrier Reef World Heritage Area and Queensland Coast. Marine Pollution Bulletin 41:279-287

Haynes D, Ralph P, Prange J, Dennison B (2000b) The Impact of the Herbicide Diuron on Photosynthesis in Three Species of Tropical Seagrass. Marine Pollution Bulletin 41:288-293

Jones RL, Marquardt TE (1987) Monitoring of Aldicarb residues in Long-Island, New-York potable wells. Arch Environ Contam Toxicol 16:643-647

Larkum AWD, Orth, R.J., Duarte, C.M. (2006) Seagrasses: Biology, Ecology and Conservation, Vol. Springer, Dordrecht

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806-1809

Macinnis-Ng CMO, Ralph PJ (2003) Short-term response and recovery of Zostera capricorni photosynthesis after herbicide exposure. Aquatic Botany 76:1-15

Macinnis-Ng CMO, Ralph PJ (2004) In situ impact of multiple pulses of metal and herbicide on the seagrass, Zostera capricorni. Aquatic Toxicology 67:227-237

McMahon K, Nash SB, Eaglesham G, Muller JF, Duke NC, Winderlich S (2005) Herbicide contamination and the potential impact to seagrass meadows in Hervey Bay, Queensland, Australia. Pergamon-Elsevier Science Ltd, p 325-334

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. Bioscience 56:987-996

Peterson BJ, Carroll J, Wall CC, Gobler CJ (2009) The facilitation of seagrass (Zostera marina) growth and productivity by mercenaria mercenaria via light and nutrient stress alevition. Journal of Shellfish Research 28:720-720

Peterson BJ, Heck KL (2001) Positive interactions between suspension-feeding bivalves and seagrass - a facultative mutualism. Marine Ecology-Progress Series 213:143-155

Puls, RW, Powell, RM,. 1992 Acquisition of representative groundwater samples for metals. Ground Water Monitoring Review, 12(3), 167-176Ralph PJ (2000) Herbicide toxicity of Halophila ovalis assessed by chlorophyll a fluorescence. Aquatic Botany 66:141-152

Ralph PJ, Gademann R, Dennison WC (1998) In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. Marine Biology 132:367-373

Schwarzschild AC, Macintyre WG, Moore KA, Libelo EL (1994) Zostera-marina growth-response to Atrazine in root-rhizome and whole plant exposure experiments. Journal of Experimental Marine Biology and Ecology 183:77-89

Short FT, Burdick DM, Kaldy JE (1995) Mesocosm experiments quantify the effects of eutrophication on eelgrass, Zostera-marina. Limnol Oceanogr 40:740-749

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. Environmental Conservation 23:17-27

Wall CC, Peterson BJ, Gobler CJ (2008) Facilitation of seagrass Zostera marina productivity by suspension-feeding bivalves. Marine Ecology-Progress Series 357:165-174

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci U S A 106:12377-12381

Williams SL, Ruckelshaus MH (1993) Effects of nitrogen availability and herbivory on eelgrass (Zostera-marina) and epiphytes. Ecology 74:904-918

Zieman JC (1974) Methods for study of growth and production of turtle grass, Thalassia-testudinum konig. Aquaculture 4:139-143

Table 1. First Root Exposure Experiment Light and Temperatures

1st Root Exposure Experiment	Light Lumins/ft^2	St Dev	Temp ˚C	St Dev
No Shade	5013.37	2742.49	24.14	0.36
Shade	1149.29	1592.60	26.23	0.84

Table 2. Second Root Exposure Experiment Light and Temperature

2nd Root Exposure Experiment	Light Lumins/ft^2	St Dev	Temp ˚C	St Dev
No Shade	40805.9	27572.4	24.75	0.37
Shade	4432.9	3459.019	28.09	0.60

Table 3. First Peristaltic Pump SGD Experiment Light and Epiphytes

1st Peristaltic Pump	Light Lumins/ft^2	St Dev	Epiphytes (mg/treatment)	St Dev
0 μM nitrate	7474.5	8509.0	15.9	13.8
231 μM nitrate	3313.0	4327.2	24.7	4.4
462 μM nitrate	4649.3	5005.8	30.3	11.6
700 μM nitrate	2615.1	3312.7	29.2	4.9

Table 4 First Root Exposure Experiment p-values

Diuron Root Exposure Multiple Stressors # 1	Leaf Mass	Productivity	Maximum Quantum Yield
Diuron	p=0.008	p=0.009	p=NS
Heat	p=NS	p=0.009	p=NS
Shade	p=0.001	p=0.02	p=0.001

Table 5 Second Root Exposure Experiment p-values

Diuron Root Exposure Multiple Stressors # 2	Leaf Mass	Productivity	Maximum Quantum Yield
Diuron	p=NS	p=NS	p=NS
Heat	p=0.005	p=0.001	p=0.001
Shade	p=NS	p=NS	p=0.001

Table 6 First Peristaltic Pump SGD Simulation Experiment p-values

Peristaltic Pump SGD nitrate	Leaf Mass	Productivity	Max Quantum Yield	Water Chl a
700 µM vs 0 µM	p=NS	p=NS	p<0.05	p<0.05
700 µM vs 231 µM	p=NS	p=NS	p<0.05	p<0.05
700 µM vs 462 µM	p=NS	p=NS	p=NS	p=NS

Table 7 Second Peristaltic Pump SGD Simulation Experiment p-values

Peristaltic Pump SGD Experiment #2	Leaf Mass	Productivity	Max Quantum Yield	Water Chl a
Diuron	p=0.005	p=0.036	p=0.001	p=0.009
Groundwater	p=NS	p=NS	p=0.001	p=0.001

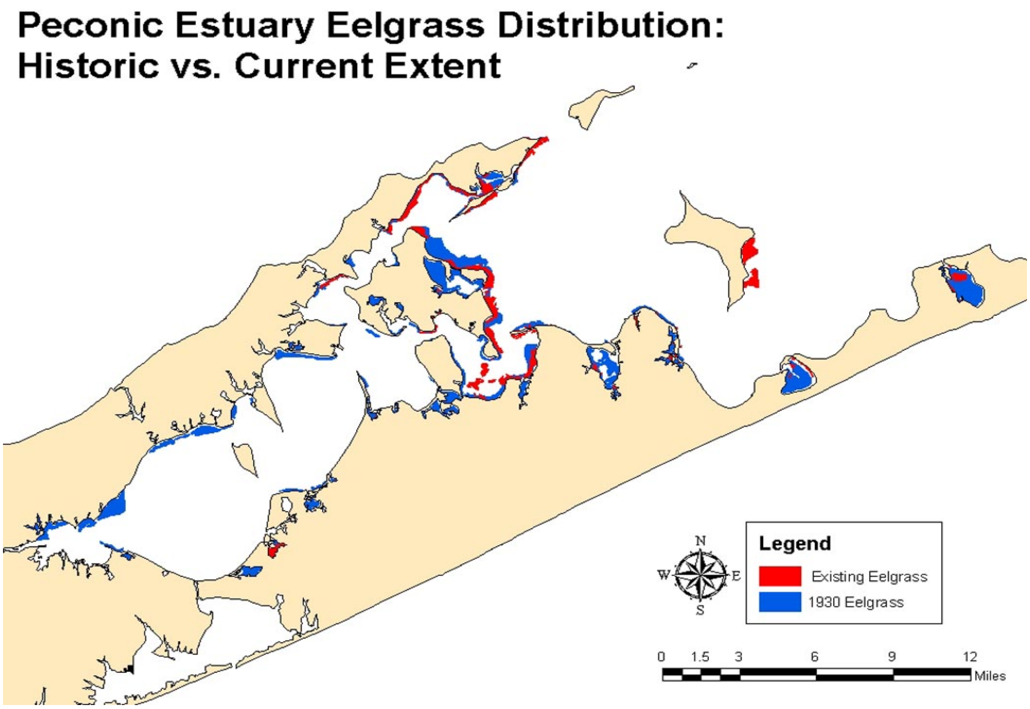

Figure 1. Change in spatial coverage of eelgrass (*Zostera marina*) between 1930 and 2007 (created by Cornell Marine Extention)

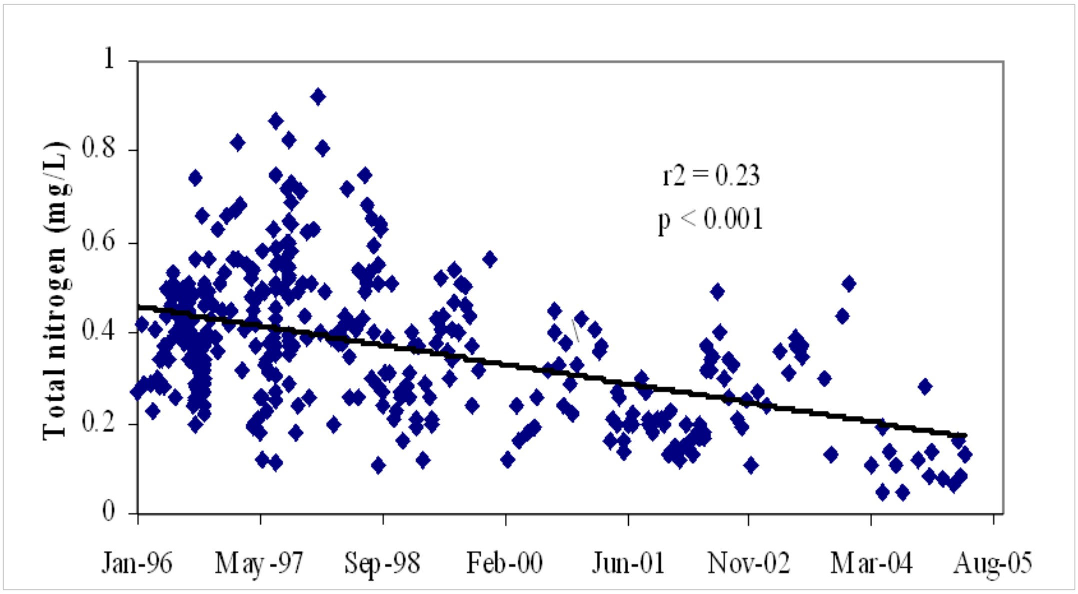

Figure 2. Trend of decreasing nitrogen in Great Peconic Bay (Suffolk County Health Department water quality monitoring program)

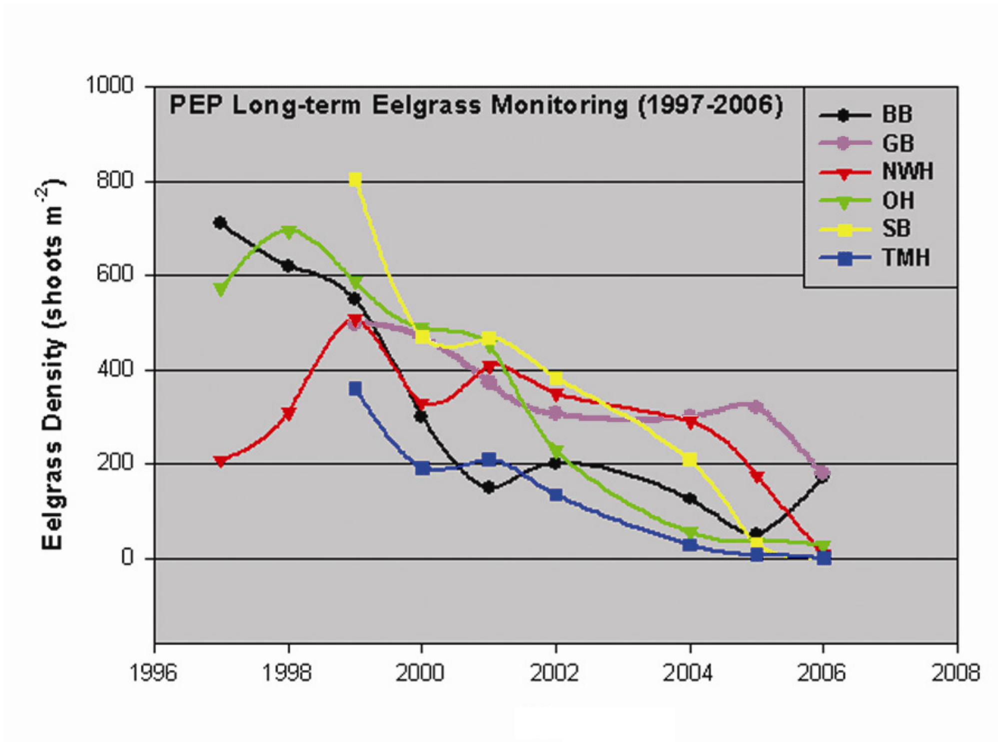

Figure 3. Eelgrass decline at several sites long term (Peconic Estuary Program)

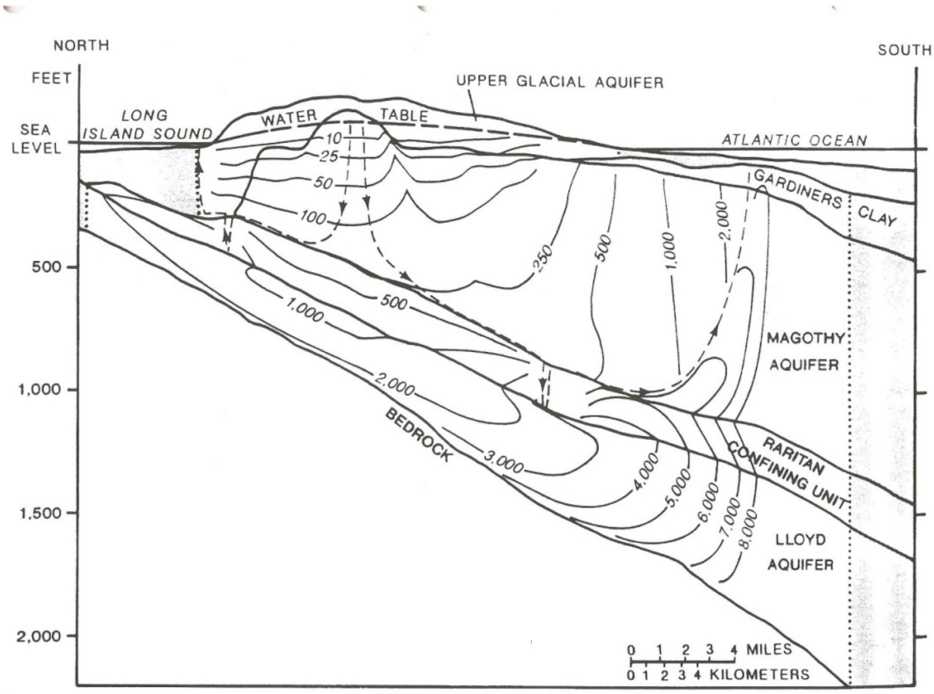

Figure 4. Long Island Aquifers with lines of equal groundwater travel time in years

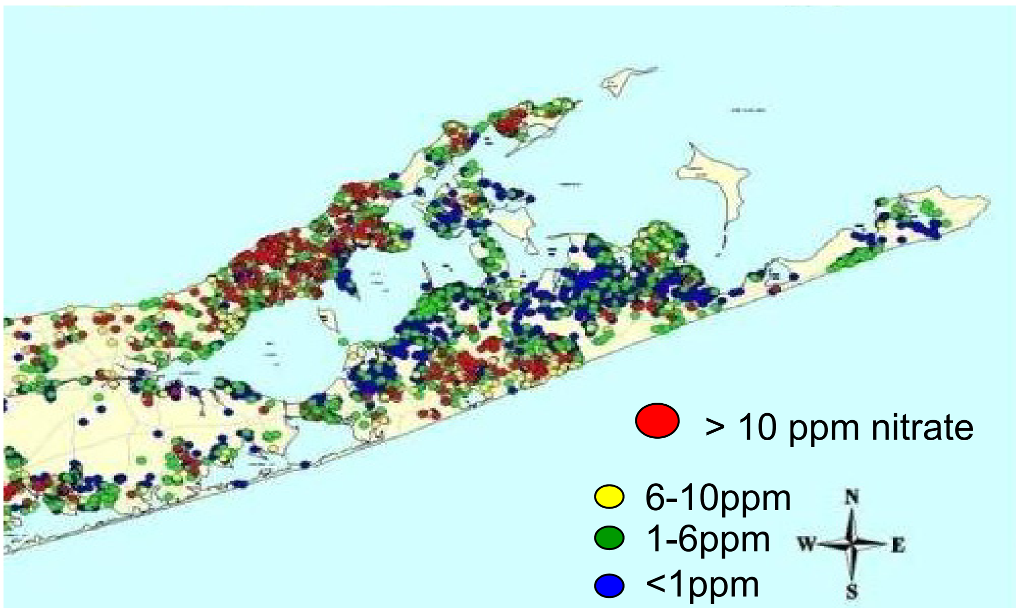

Figure 5. Nitrate concentrations in private wells 1997-2006 (Suffolk County Department of Health Services)

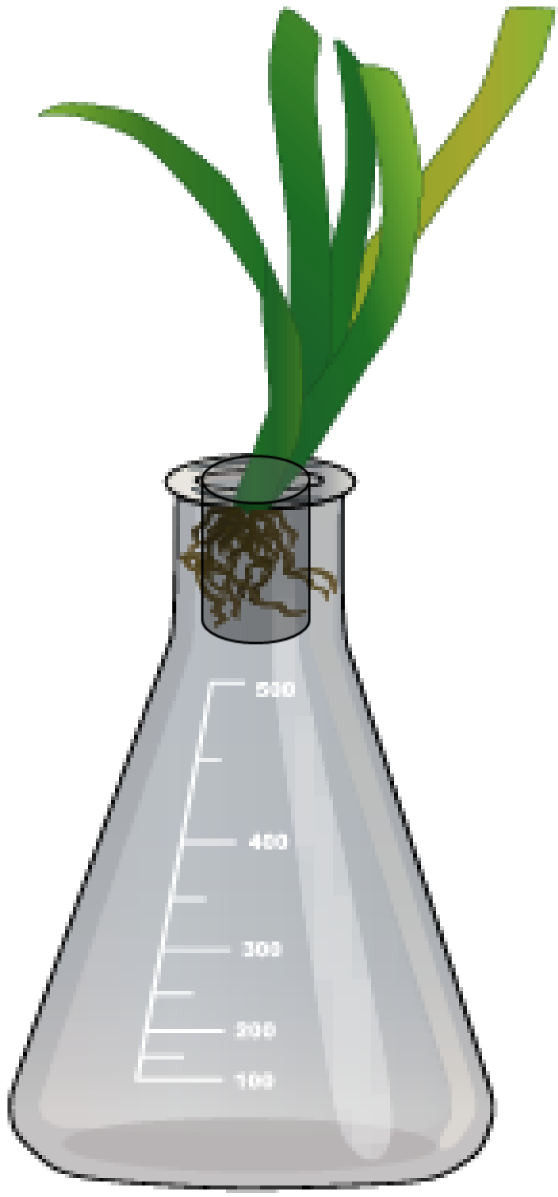

Figure 6. Split Chamber

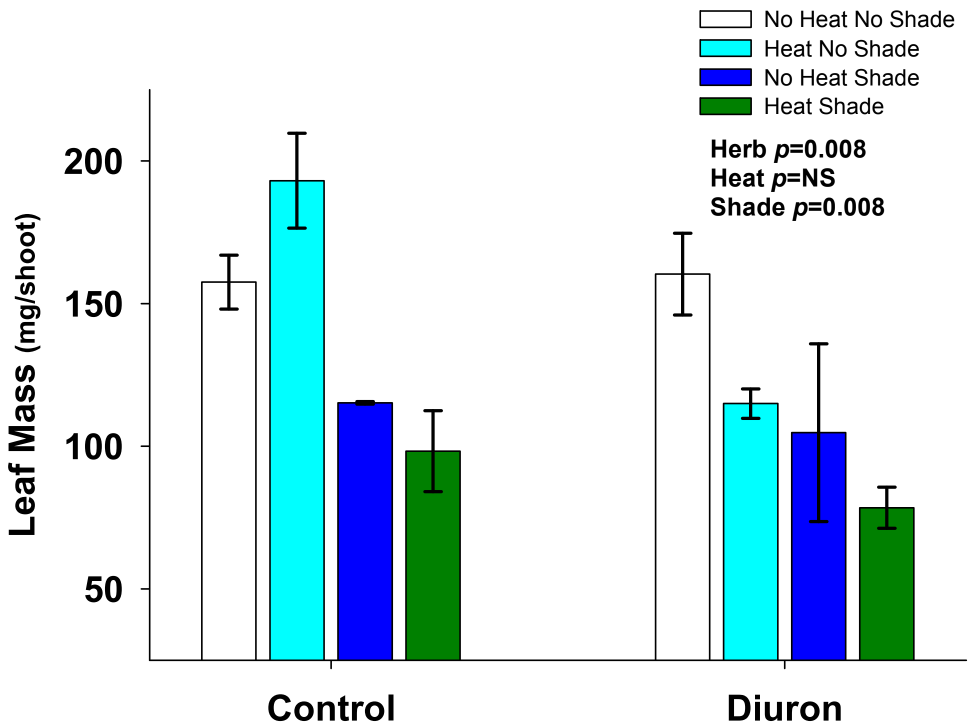

Figure 7 First Root Exposure Experiment

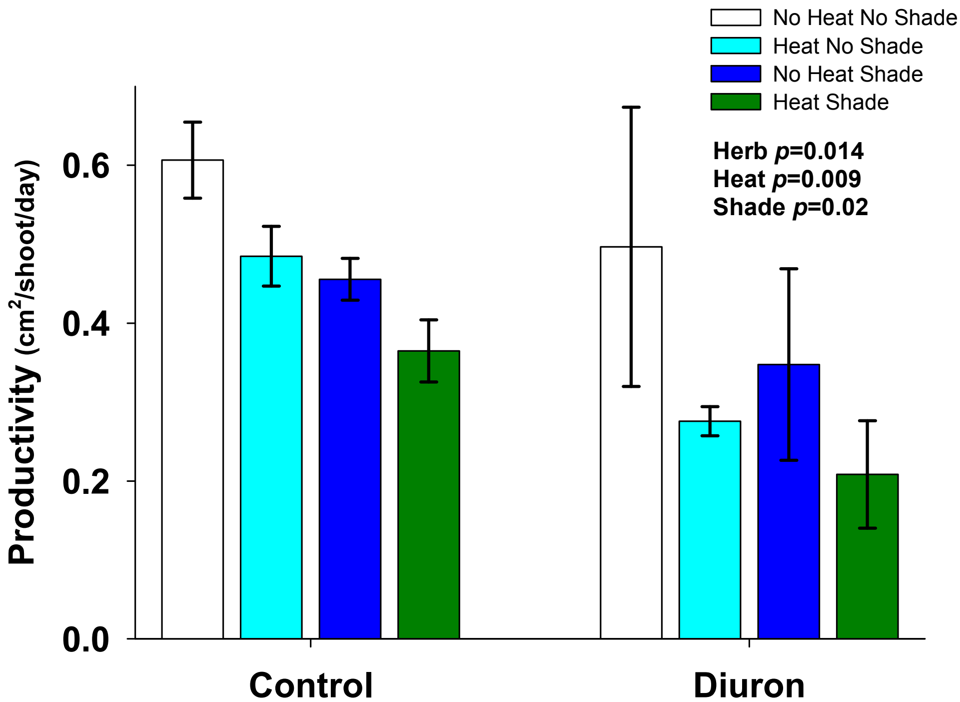

Figure 8 First Root Exposure Experiment

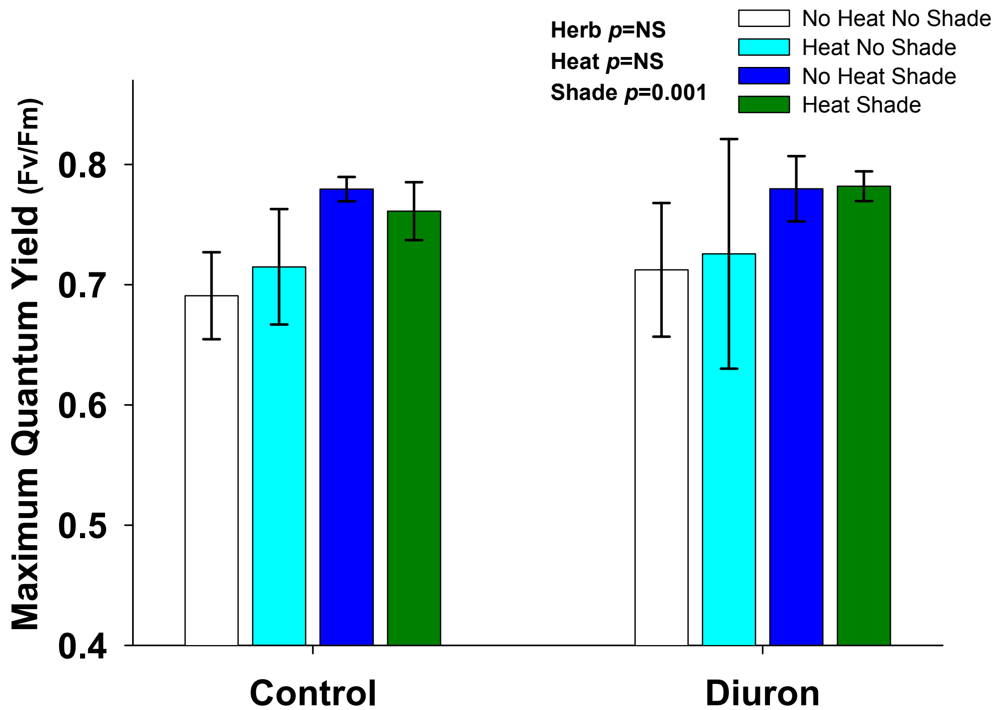

Figure 9 First Root Exposure Experiment

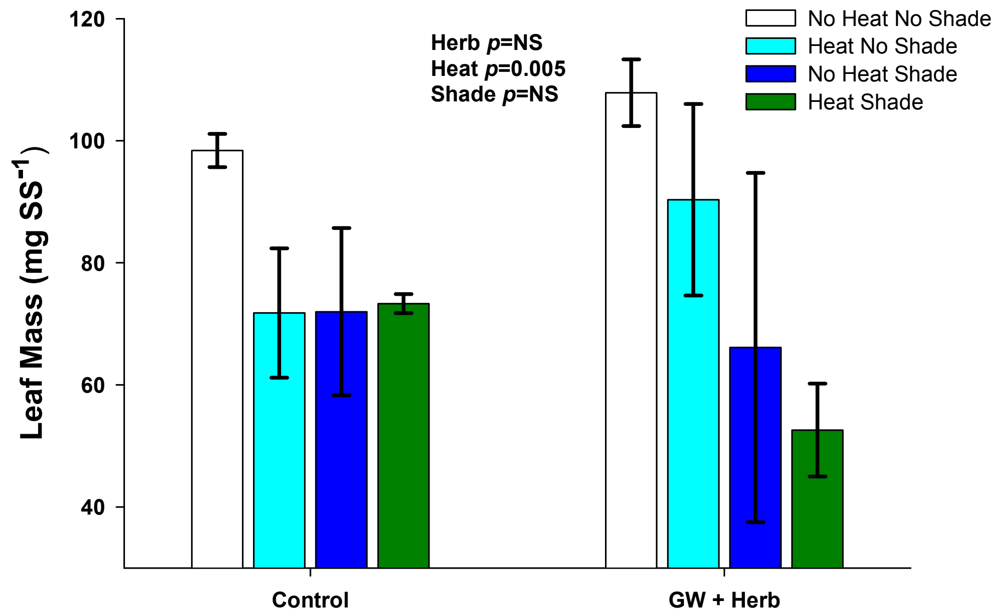

Figure 10 Second Root Exposure Experiment

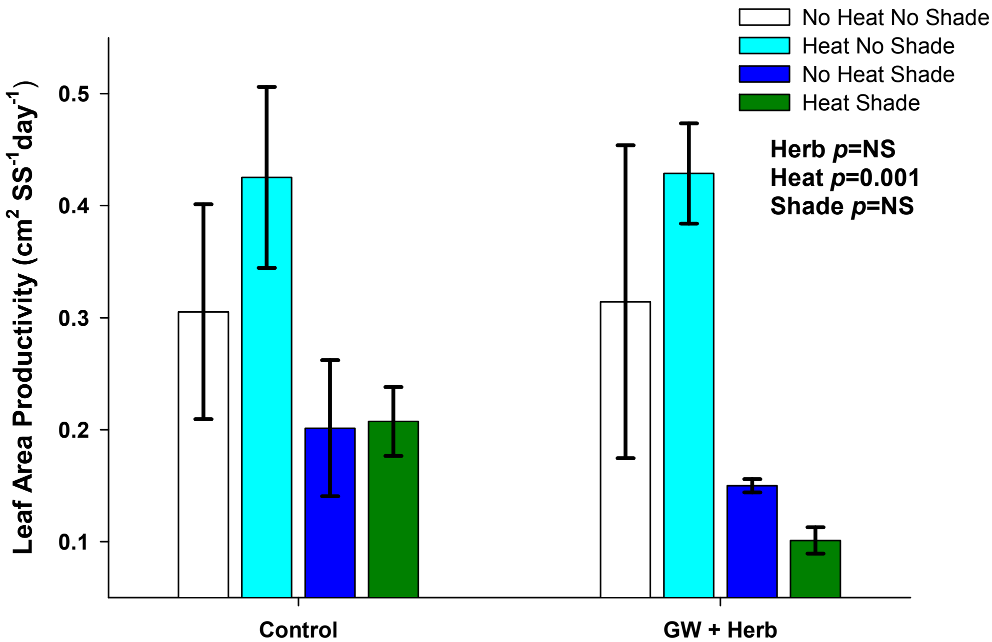

Figure 11 Second Root Exposure Experiment

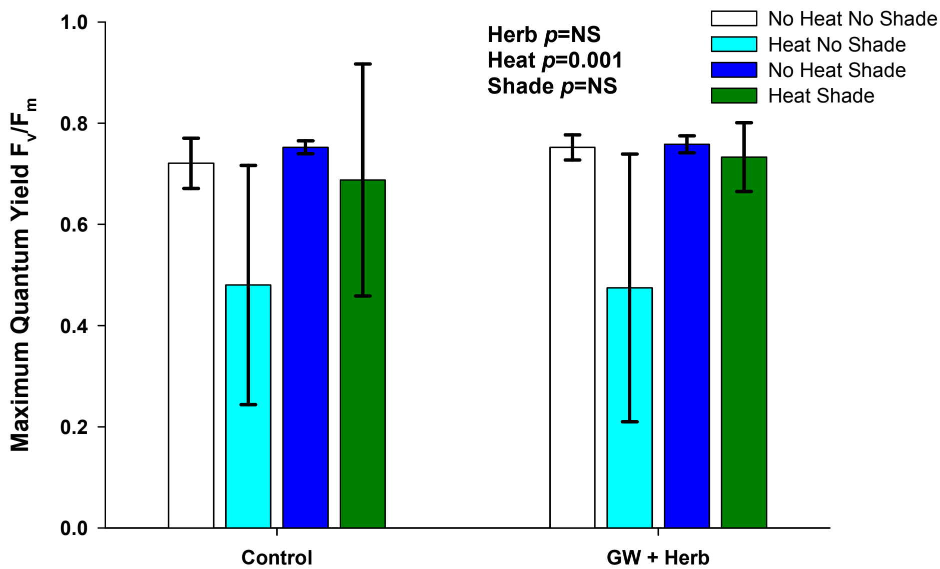

Figure 12 Second Root Exposure Experiment

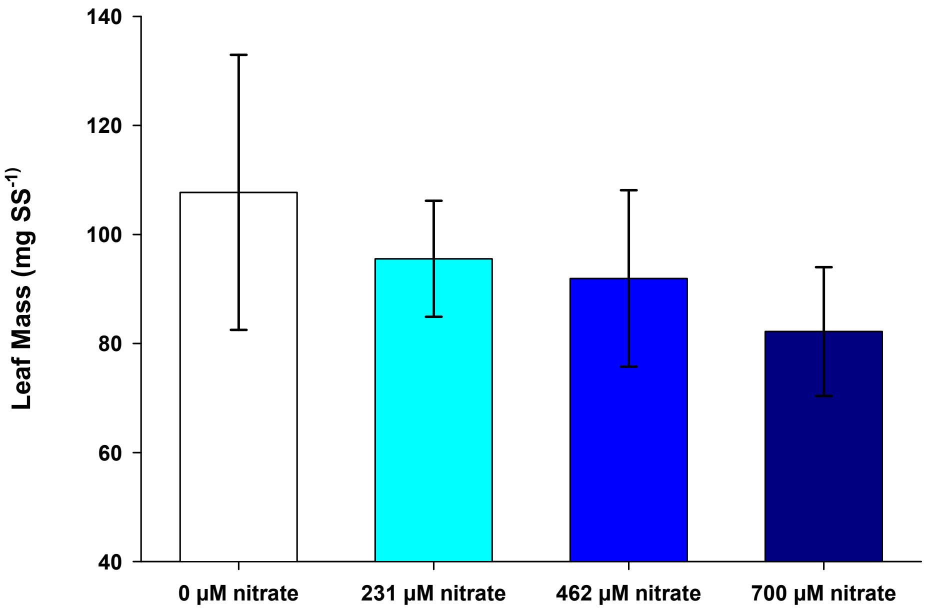

Figure 13 First Peristaltic Pump Experiment

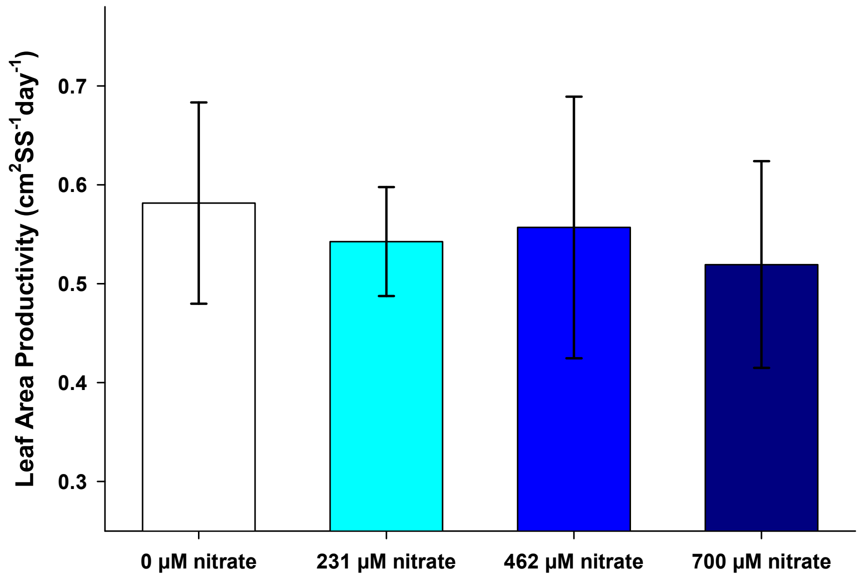

Figure 14 First Peristaltic Pump Experiment

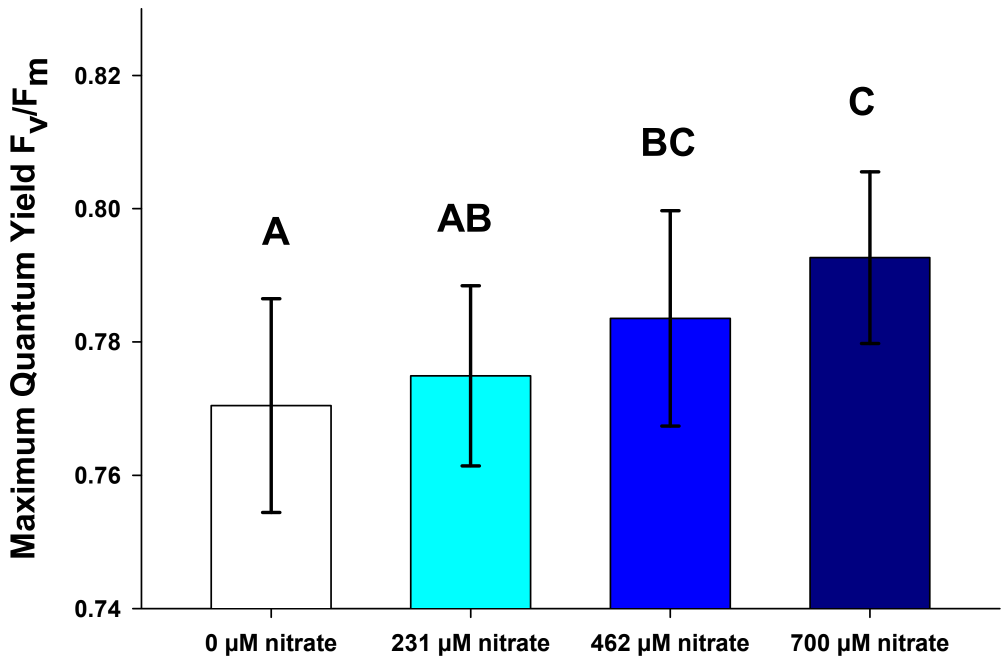

Figure 15 Second Peristaltic Pump Experiment

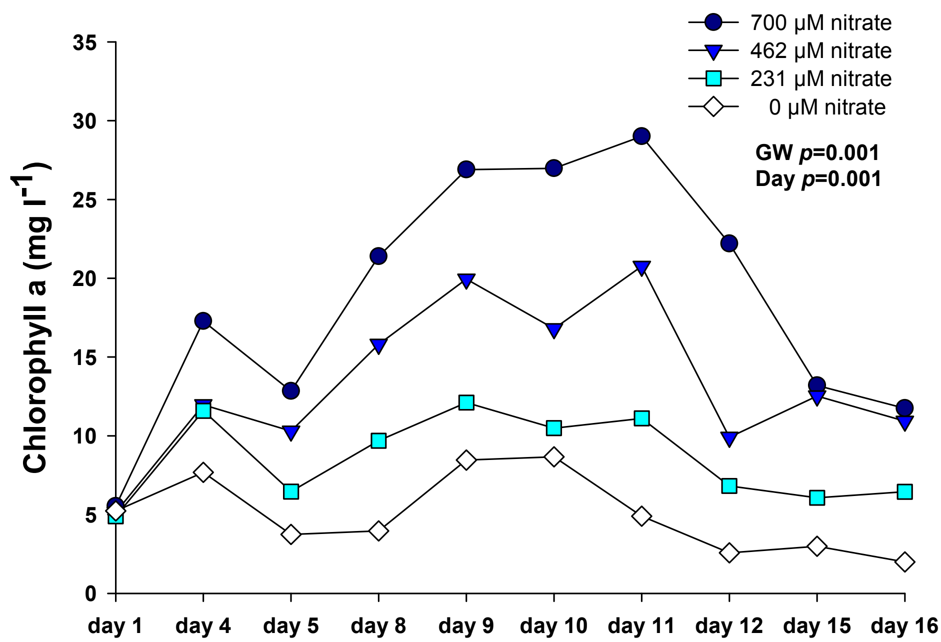

Figure 16 Second Peristaltic Pump Experiment

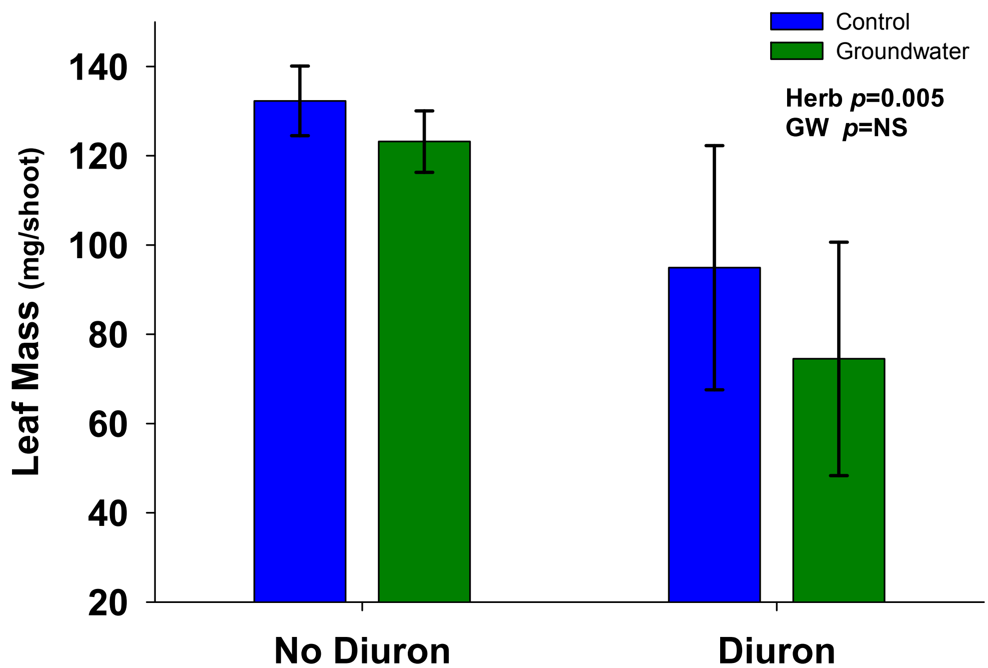

Figure 17 Second Peristaltic Pump Experiment

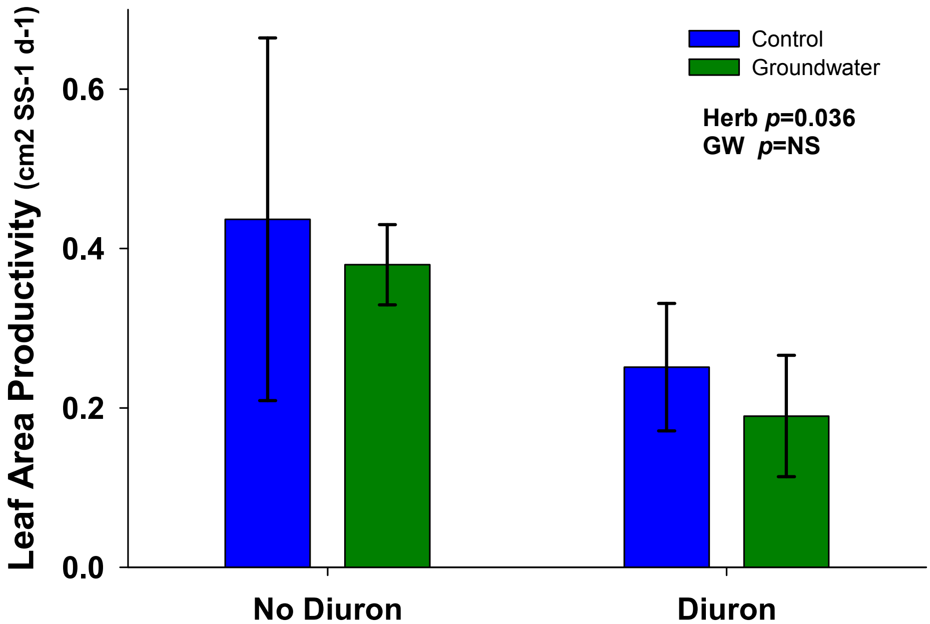

Figure 18 Second Peristaltic Pump Experiment

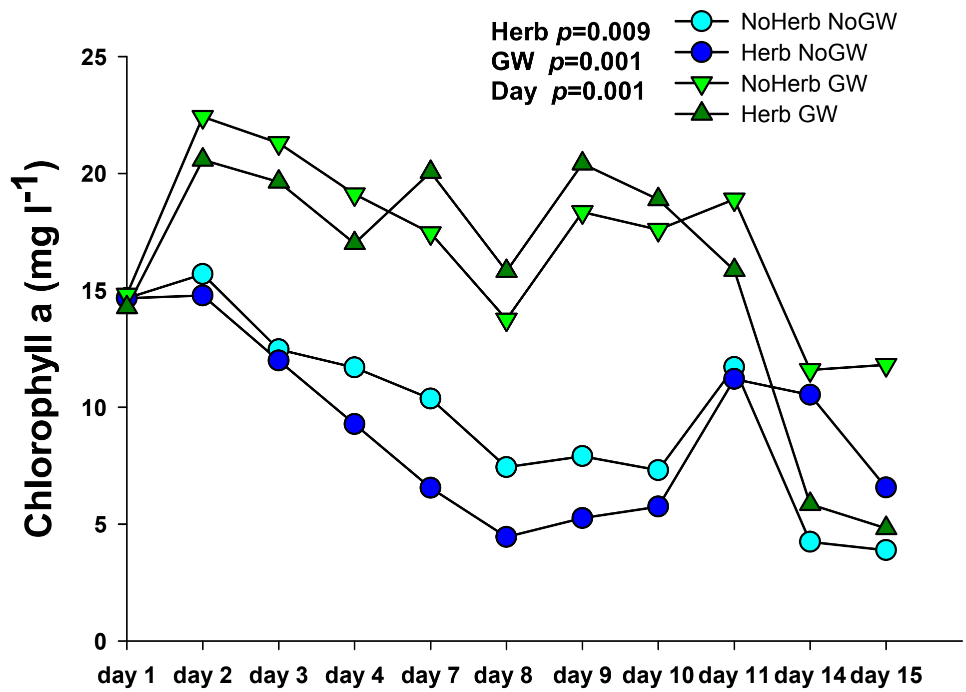

Figure 19 Second Peristaltic Pump Experiment

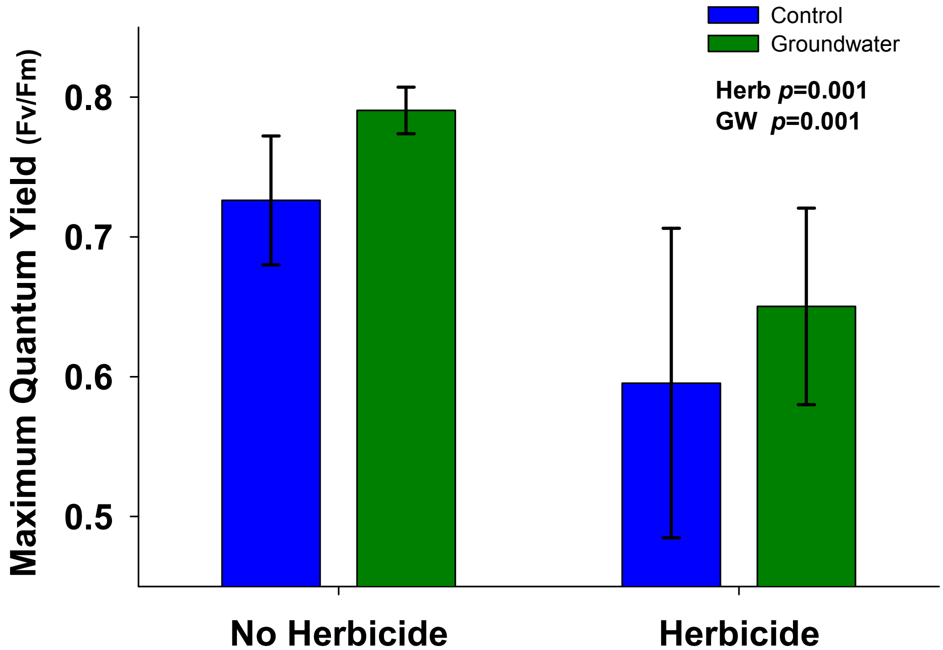

Figure 20 Second Peristaltic Pump Experiment