ABSTRACT

Title of Document: CREATING MARSHES WITH DREDGED MATERIAL ON A RESTORED ISLAND IN CHESAPEAKE BAY

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Directed By: Professor, Dr. J. Court Stevenson, Marine and Estuarine Environmental Science

Tidal marsh creation using dredged materials could compensate for losses due to a variety of anthropogenic activities, including higher rates of sea-level rise. However, initial seeding attempts failed in a newly created marsh in Poplar Island, MD. Hypotheses were that soils were too acid, too saline, too high in sulfides or seeds were not viable. In test plots containing mostly sand, amendments of dredged materials enhanced plant growth and survival. Furthermore pH was between 5.5 and 7, not low enough to inhibit growth of marsh grasses. Sulfides in pore water were very low (<20 μM). Soil moisture content limited production in plants growing under long photoperiods in summer conditions. Seed germination was zero in *Spartina patens* and decreased significantly in *Spartina alterniflora* at salinities greater than 10 and biomass was greatest in plants grown in low salinities (2.5 and 5).

CREATING MARSHES WITH DREDGED MATERIAL ON A RESTORED ISLAND IN CHESAPEAKE BAY

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2006

Advisory Committee:
Dr. J. Court Stevenson, Chair
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Dr. Evamaria W. Koch

Dedication

For Jeremy, your support, patience, love and belief in me made this possible. I am forever grateful.

Acknowledgements

I would like to thank my advisor Court Stevenson for all his guidance and generosity with his knowledge. Thank you also to my committee Jeff Cornwell and Evamaria Koch for their leadership over the course of my work. I am truly indebted to Lorie Staver for advice, support and assistance well above the call of duty. Thank you also to Erica Kiss, Sarah Rhoades, and Michael Owens for field and laboratory assistance, to Dave Miller for aerial photographs, and to Dave Kimmel for help with statistical interpretation. And I am grateful to my family for always believing in me. This work was supported by the U.S. Army Corps of Engineers, Maryland Environmental Service and Horn Point Laboratory.

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Introduction

Recent evidence from satellite imagery indicates over one half of the tidal marshes in Chesapeake Bay appear to be adversely affected by increasing rates of sea level rise (Kearney et al. 2002). This is unfortunate since coastal marshes are highly valued because they serve a variety of ecological functions, which include: improving water quality, controlling floods, diminishing droughts, stabilizing shorelines, and providing habitat for waterfowl and fur bearers; as well as a source of carbon for food chains in the bay (Kearney and Stevenson 1991; Marcus and Kearney 1991; Stevenson et al. 2002). Wetlands can also be highly productive ecosystems and because of low decomposition in anaerobic environments, marsh systems may be excellent "carbon sinks" taking CO_2 out of the atmosphere and storing it in living tissue. As a result marshes may serve an important function in addressing global climate change by removing greenhouse gasses from the atmosphere (Williams 1999). The economic and social benefits of intact wetlands have been estimated to exceed converted or drained areas by at least 60% (Costanza et al. 1997; Balmford et al. 2002). However sea level rise, coupled with land subsidence due to a variety of natural and anthropogenic factors has jeopardized the very health and survival of wetlands, particularly when sediment supplies are low and/or are declining (Stevenson et al. 1985; Delaney et al. 2000).

During periods of gradual sea level rise, mineral and organic matter was deposited along coastlines helping to build up present day coastal marshes (Redfield 1972). During the early part of the Holocene period (10,000 – 5,000 years ago) sea levels rose too rapidly for much marsh development. Marshes, however have flourished particularly after settlement along the Atlantic seaboard (Stevenson et al. 1988). Recent data show

that global warming is accelerating and higher rates of sea levels will exceed the accretionary potentials of marshes (Ward et al. 1998). Over the past 100 years global sea level has risen at a rate of 1-2 mm y^{-1} (Gornitz 1995; Douglass et al. 2001) and is projected to increase by as much as 88-100 cm by the year 2100 (IPCC 2001). If relative sea level rise reaches 1 cm per year, as has been suggested due to Greenland ice cap melting (Dowdeswell 2006), marshes would need 1.5-3 kg $m^{-2}yr^{-1}$ of organic deposition to keep abreast of sea level rise unless they are subsidized with inorganic sediment (Stevenson et al. 1985).

One dramatic example of erosion in Chesapeake Bay is Poplar Island located in Talbot County, south of Kent Island (Figure 1). Through the first half of the 17th century, the island consisted of 1,430 acres (Cronin 2005). During the Colonial period land use was largely agricultural with several woodlots and by the late 1800s other development included a post office, school and sawmill (Kearney and Stevenson 1991). By 1912 the island had been breached, producing an archipelago with three main islands, Poplar, Jefferson and Coaches, and was reduced to less than 500 acres (Tilghman 1915). Throughout the 19th and early 20th centuries Poplar Island was reduced to 134 acres, and by the 1960s only 80 acres remained. Poplar Island eroded to just over 4 acres by 1997 (Cronin 2005).

Island erosion leads to more sediment in the bay and its channels. Each year more than 2 million m^3 of uncontaminated coastal sediments are dredged from the Baltimore Harbor approach channels and either discharged into the water or deposited at land-based facilities (Maryland Environmental Service 1995). In the 1970s the first attempt was made to restore an island at the mouth of the Patapsco River. Expanding on the success

Location of Study Sites and Origin of Dredged Material

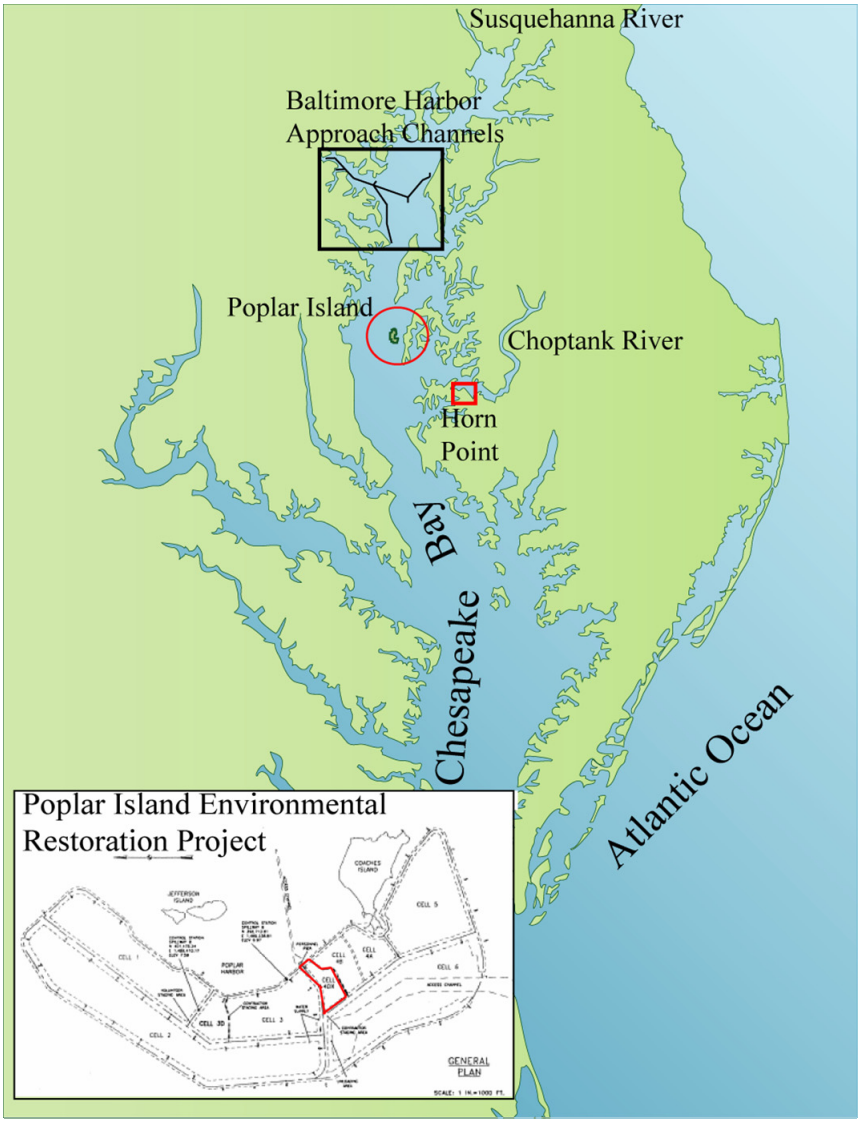

Figure 1: Study site map including the Susquehanna River, dredging channels that supply material to Poplar Island and the location of Poplar Island and Horn Point study sites. Inset is the footprint of the Poplar Island Environmental Restoration Project. Cell 4DX, containing experimental test plots 1-4 and the sand plot, is outlined in red.

of disposal of dredged material by this method, resource managers attempted to create new marsh habitat to replace that lost by erosion.

Uncontaminated dredged material had been used to create new wetlands or repair existing ones as early as 1969 in North Carolina (Seneca et al 1974; Woodhouse et al. 1974; Seneca et al 1985). By 1977, 24 dredged material sites along the east coast of the U.S. had been planted with *Spartina alterniflora* (Garbisch 1977). Despite these early projects, questions still persist over whether or not these marshes made from dredged material are truly successful in replacing all of the functions of lost natural marshes (Streever 2000).

Created marsh projects in North Carolina were studied over a number of years to determine the trajectory of establishment and assess overall success (Craft et al. 1999; Craft et al. 2002). Most studies were from high salinity *Spartina alterniflora* marshes and show a peak in aboveground biomass in dredged material within a few growing seasons. Webb and Newling (1985) had earlier found that the initial high above ground biomass decreased to that similar to a reference marsh. Broome et al. (1986) had earlier suggested that this surge is due to initial high levels of available nutrients (along with low levels of intraspecific competition). However, belowground biomass of *S. alterniflora* in dredged material wetlands is often observed to be less than half that of natural marshes even after the area has been established for 10 years or more (Craft et al. 2002) This could be explained by the young age of the sites in comparison to reference marshes. However Streever (2000) concluded that there is no strong evidence that dredged material marshes and natural marshes become more similar over time. This leads to an important question: should created marshes be expected to display all the characteristics of

reference marshes nearby (which may show numerous problems because of sea level rise)?

The success of initial establishment of vegetation on marshes created with dredged material does not guarantee that these wetlands are serving the same ecological function as nearby natural wetlands. This is a complex issue that requires long-term monitoring and comparison of vegetation, soil and water chemistry, benthic invertebrate and infauna communities, nekton communities, and bird communities. Based on their study of created marshes ranging in age from 3 to 19 years, Edwards and Proffitt (2003) agree that vegetation takes only a few years to resemble that of natural marshes provided that the marshes are set at the proper elevation. Streever's (2000) concern that belowground biomass of *S. alterniflora* in dredged material wetlands is often less than half that of natural marshes even after the area has been established for 10 years or more might be inconsequential. The lack of belowground biomass may be explained by physiological responses of plants to nutrients in the soil. High soil nutrients often produce low root:shoot ratios, since the plants do not need to scavenge nutrients (Valiela and Teal, 1974).

In 1996 a restoration project was funded to restore Poplar Island to its approximate size in 1847 (1,140 acres) using 30.6 million m^3 of uncontaminated material dredged during maintenance of the approach channels to Baltimore Harbor (Figure 1). The island was divided into cells designed to contain the dredged material. In order to compensate for the extensive losses of marshes in Chesapeake Bay, one half of the island was designated as intertidal marsh. The other half is designated as upland habitat. However initial seeding efforts in 2002 of over 20 halophytic and non-halophytic species

in one of the wetland cells (4DX) were completely unsuccessful. Subsequent transplantings of halophytic species (*Spartina alterniflora, Spartina patens*) and shrub species (*Iva frutescens,* and *Baccharis halimifolia*) in 2003 have been successful.

Although the reason for the failure of the initial seeding is still unclear, one possible explanation was low pH of the dredged material. When acid sulfate soils that contain pyrite (i.e. iron sulfides) are exposed to air and hydrated, sulfuric acid is produced (Saffigna and Dale 1999). Hydrogen sulfide, another potential end-product is especially toxic to seeds and young plants, especially when concentrations exceed 1-2 mM. The possibility of low pH in dredged materials at Poplar Island was a question that clearly needed to be addressed in order to successfully establish marsh vegetation.

The degree of saturation of marsh soils may also have a significant influence over how quickly and to what degree the dredged material comes to resemble wetland soil. Previous studies (Craft et al. 1999; Craft et al. 2002) have suggested that in frequently flooded or water saturated areas, decomposition is slowed due to anaerobic conditions. Not only does this allow organic matter to accumulate in the soils, but reduction of oxidized Fe and Mn occurs which consumes H^+ ions and decreases the acidity of the soils. We hypothesize that plant production will be increased in dredged material with a high moisture content.

Soil salinity is also a critical factor in establishing marshes in the mid-bay. Increases in local rates of sea level rise influence the type of vegetation in any given intertidal area. There is often a shift in species of oligohaline and fresh water marshes as salt water intrusions increase (Baldwin and Mendelssohn 1998). In coastal areas affected by disturbances such as fire, hurricanes and sediment deposition, regeneration of

vegetation is often highly dependant upon seedling recruitment (Baldwin and Mendelssohn 1998). Therefore, in order to understand and predict the fate of oligohaline and mesohaline marshes the effect that increased salinity has on seed germination and survival must be addressed.

Over the past decade the issue of salt water intrusion in oligohaline marshes has been the focus of several studies. In coastal Louisiana, Baldwin et al. (1996) found that while pulses of salt water intrusion due to storm events did not significantly affect seed viability in oligohaline marshes, long-term increases in salinity inhibit seedling emergence in several wetland species including *Eleocharis fallax, Phyla nodiflora* and *Saggitaria lancifolia.* In the salt marshes of the Iberian Peninsula, there was a reduction in germination of the halophytes *Sarcocornia perennis* and *Sarcocornia fruticosa* when exposed to increases in salinity. Decreased osmotic potential of the solution may have prevented seed hydration (Redondo et al. 2004) or there may have been direct toxic effects of sodium and chloride ions on the seeds (Soltani et al. 2004).

Short-term responses of *Spartina alterniflora* to root immersion in saline solutions were measured by Chambers et al. (1998) and uptake of nitrogen was reduced in salinities of 20 and 30. However *S. alterniflora* is rarely if ever found in salinities <2 (Anderson et al. 1968). The low survival of *S. alterniflora* in fresh water appears to be due to sulfate, an anion abundant in sea water (Stribling 1997). Although it has been shown that uptake of nitrogen by *S. alterniflora* is reduced at high salinities (>20) (Chambers et al. 1998) surprisingly little information has been found in the literature on the effects of increased salinity on *S. alterniflora* seed germination and it is not entirely clear at what soil salinity the nutrition of the plants actually suffers.

This study addresses the establishment of marsh vegetation on the newly built portion of Poplar Island by comparing how three plant species that are commonly planted on the east coast of the United States, *Spartina alterniflora, Spartina patens* and *Distichlis sipcata* grow in dredged material with and without the addition of sand. Different soil conditions were established to determine whether increasing the moisture content of this fine grained soil will maximize production in wetland plants and promote soil development. Pore water chemistry and sediment characteristics were evaluated and compared to an existing Chesapeake Bay marsh to determine whether dredged material incorporated into a sandy substrate provides a suitable habitat for marsh grasses. *Spartina alterniflora* plants were also grown in several salinities to investigate the effect that increases in salt concentrations have on these halophytes. The following experiments were designed to address four main questions. 1) Does the dredged material used on Poplar Island provide adequate nutrients to support growth of marsh plants compared to sandy substrates? 2) Is the pH of the dredged material low enough to inhibit seed germination and to be toxic to transplants? 3) Does water saturation of dredged material limit production in wetland plant species? 4) Does increased salinity affect the growth and nutrition of *Spartina alterniflora*?

Site Description and Methods

Site description

Sediment dredged from the channels approaching Baltimore Harbor (Figure 1) were applied over a sand base in a wetland cell (cell 4DX) at Poplar Island in 2002. Although some sediment may have originated from shore line erosion, the majority of

these materials entered the bay from the Susquehanna River (Figure 1). Sediment deposition in the shipping channels approaching Baltimore Harbor hinders navigation and makes maintenance dredging necessary (Figure 2). Sediment was slurried with bay water at Poplar Island, dried and applied to four experimental plots within cell 4DX (Figure 3). The sediment was incorporated into sandy substrates (which had been spread throughout the cell) in the four plots at the site (Figure 4). Plot 1 received 60 cm of dredged material (over sand) with no cultivation. Plot 2 contained 60 cm of dredged material (over sand) that was tilled prior to planting. In plot 3, 60 cm of dredged material (over sand) was covered with a layer of sand 15 cm deep and 60 cm of dredged material in plot 4 was covered with 30 cm of sand. A fifth site just to the east of the test plots that contained mostly sand was also sampled as a control (Figure 4). However it was later learned that there was some dredged material introduced in the high marsh of this control site. An unsuccessful seeding attempt in cell 4DX was made by contractors in the spring of 2002. Since none of the seeds germinated, live grass plugs were planted in 2003 (Environmental Concern, Inc. et al. 2001).

Horn Point (HP) marsh, located 4 km west of Cambridge, Maryland on the Choptank River (Figure 1) was chosen as an existing Chesapeake Bay marsh with which to compare marshes on Poplar Island. HP marsh has well developed vegetation zones characteristic to the mesohaline portion of Chesapeake Bay with *Spartina alterniflora* bordering the creek and a large zone of *Spartina patens* and *Distichlis spicata* in the higher elevations of the marsh. This portion of the Choptank has salinity and tidal ranges similar to Poplar Island (Figure 5).

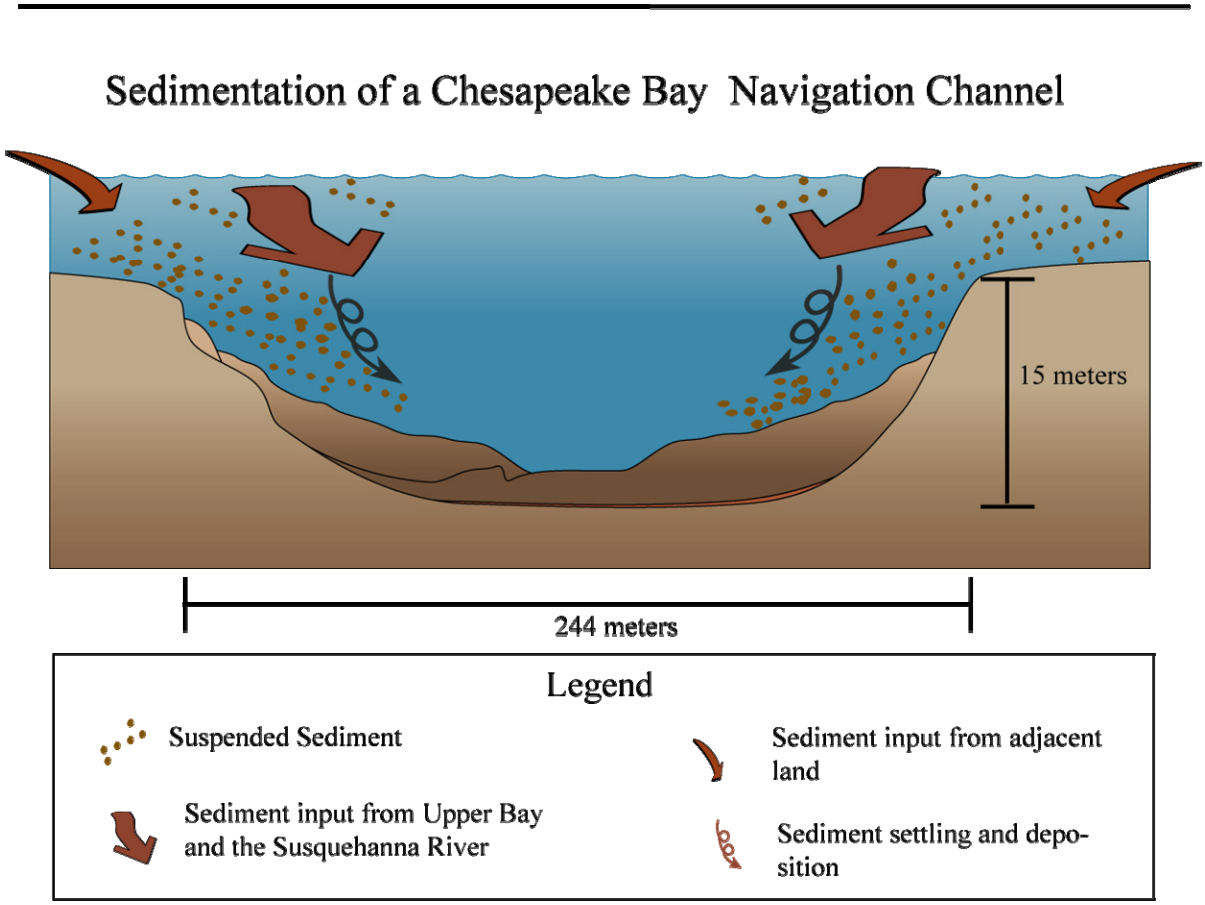

Figure 2: Conceptual model of sediment deposition in the Craighill Approach Channel to Fort McHenry, Maryland. As the bottom corners of the channels fill in maintenance dredging is a necessary means of keeping shipping routes open.

Managing Dredged Materials in Chesapeake Bay

Figure 3: Dredges removed deposited sediment from the Chesapeake Bay's shipping channels (top). The material was mixed with bay water to create a slurry which is pumped into various cells at the Poplar Island Environmental Restoration Project (bottom). Grading later occured to establish marsh hydrology within the cells. (Pictures courtesy of the U.S. Army Corps of Engineers, Baltimore District).

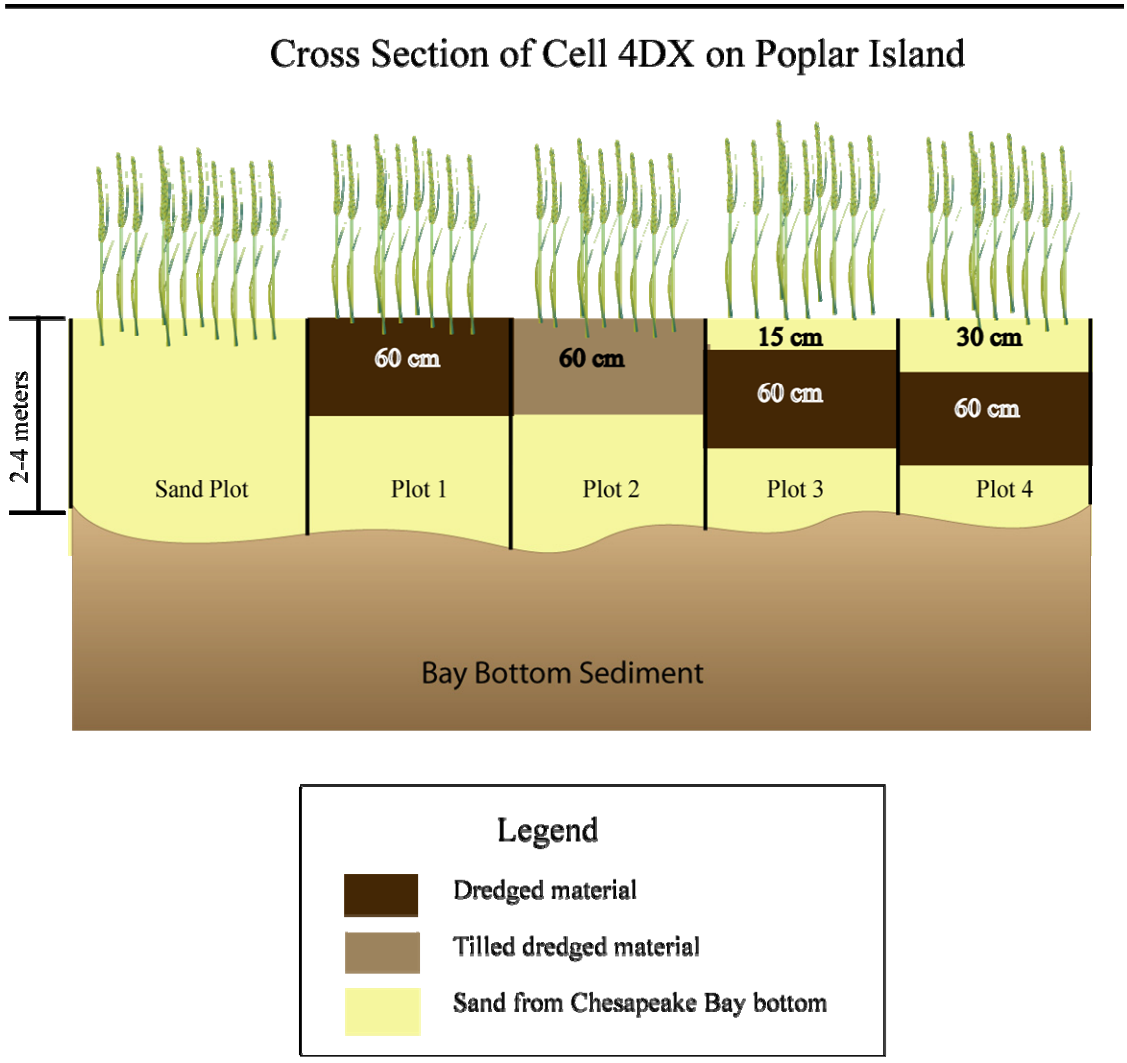

Figure 4: Cross sectional conceptual diagram of four experimental test plots constructed within cell 4DX on Poplar Island in which field measurements were taken. Included also, is an adjacent plot constructed only with sand.

Salinity Distribution in Chesapeake Bay

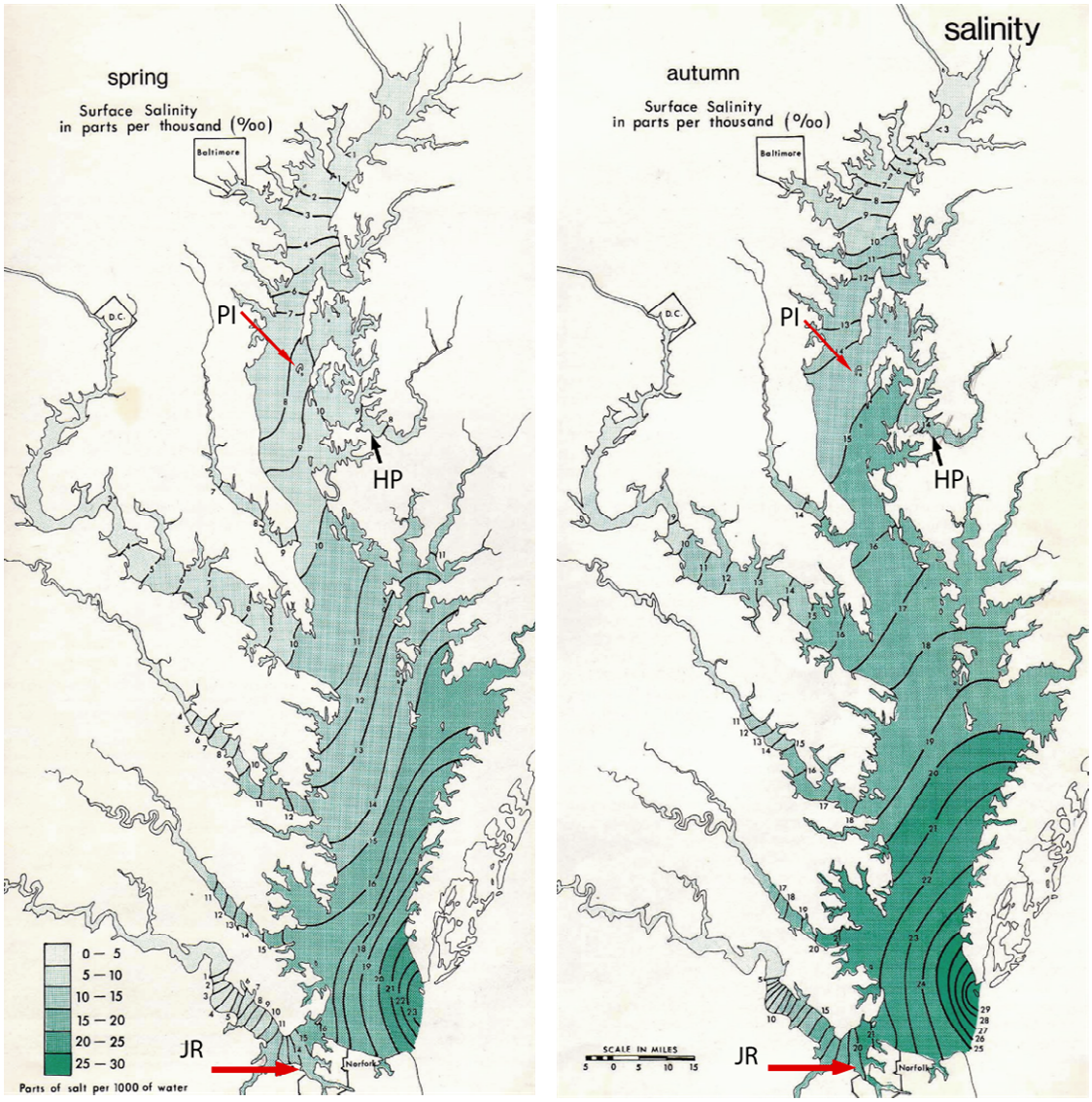

Figure 5: Horizontal salinity distribution for spring and autumn in Chesapeake Bay with each isohaline representing an increase of 1 in salinity. Study sites at Poplar Island (PI) and Horn Point (HP) and approximate collection site for James River (JR) seeds are indicated by arrows (Lippson 1973).

Sediment pore water field measurements

To determine the nutrient availability and pH of dredged material, sand and marsh sediments, pore water measurements were made in May, July and November 2004 in the four test plots using sediment pore water dialysis samplers (Figure 6) similar to Hesslein (1976). Two samplers were placed in each plot including the sand plot during each deployment, one in the low marsh receiving atmospheric exposure only during low tide and one in the high marsh being inundated only during the highest tides. Since the sediments were very compacted, a metal "pilot" was pounded into the substrate to make an opening for placement of the PVC pore water sampler (Figure 6). Samplers were allowed to equilibrate with the interstitial water (about 2 weeks) and processed in the field immediately following removal by drawing water from each well using a syringe and placing it in sample bottles. For analysis of dissolved iron, 100 µl 5N HCl was added to 0.5 ml of the sample to prevent any iron from precipitating. Samples were taken back to the laboratory where standard colorimetric analysis (Parsons et al. 1984) was used to determine the concentrations of ammonium (NH_4^+), soluble reactive phosphorus (SRP), and dissolved iron (Fe) (in J. C. Cornwell's Geochemistry Lab). Due to equipment difficulties, SRP was measured only during the May and November sampling periods. July 2003 measurements of SRP from prior dialysis sampling on Poplar Island are included in the reported data when available (Cornwell et al. 2005). Two additional dialysis samplers were deployed in Horn Point marsh (Figure 1) in the same manner and during the same periods as the Poplar Island deployments.

Figure 6: Sediment pore water dialysis samplers used to measure concentrations of ammonium, soluble reactive phosphorus, and iron in pore water on Poplar Island and at Horn Point Marsh and the metal "pilot" used to create an opening in the sediment. Peepers permitted measurements to be made up to 50 cm below the sediment surface (a). Holes were first made by pounding a metal "pilot" into harder sediments (b). After equilibration with interstitial water, syringes were used to withdraw water through a semi permeable membrane for analysis (c).

Vegetation field measurements

The above ground portions of *Spartina alterniflora* plants were collected from the low marsh zone of each experimental plot in cell 4DX on Poplar Island in May 2004 by clipping the culms at the sediment surface. Plant samples were also collected similarly from the Horn Point reference marsh. All plant samples were dried in a 60^{o}C oven for 72 hours. Whole plants were ground using a Wiley Mill and sent to A&L Eastern Agricultural Laboratories in Richmond, VA where macronutrient (nitrogen (N), potassium (K), sulfur (S), phosphorus (P), magnesium (Mg) and calcium (Ca)) and micronutrient (manganese (Mn), aluminum (Al), iron (Fe), zinc (Zn), boron (B) and copper (Cu) tissue concentrations were quantified (Mills and Jones 1996).

Seed germination and plant productivity in dredged material in environmental growth chamber experiments

In order to determine seed germination and plant growth in response to flooding of dredged material, a multi-factorial experiment was utilized. Newly placed dredged material was collected from Poplar Island and used to fill 72 columns made from 4" PVC pipe cut into 30 cm lengths. Seventy two additional columns were filled with sand. Six columns were placed in a tub which was subjected to one of three flooding regimes by filling each tub with 0, 2.5 or 15 cm of Choptank River water. Each tub received two *Spartina alterniflora* plants, two *Spartina patens* plants, and two *Distichlis spicata* plants, one of each species planted in dredged material and one in sand (Figure 7). All plants were obtained as 5 cm tall plugs from Environmental Concern in St. Michaels, MD and received an addition of 3 g of Osmocote 19-6-12 (N-P-K) slow-release fertilizer. Nine

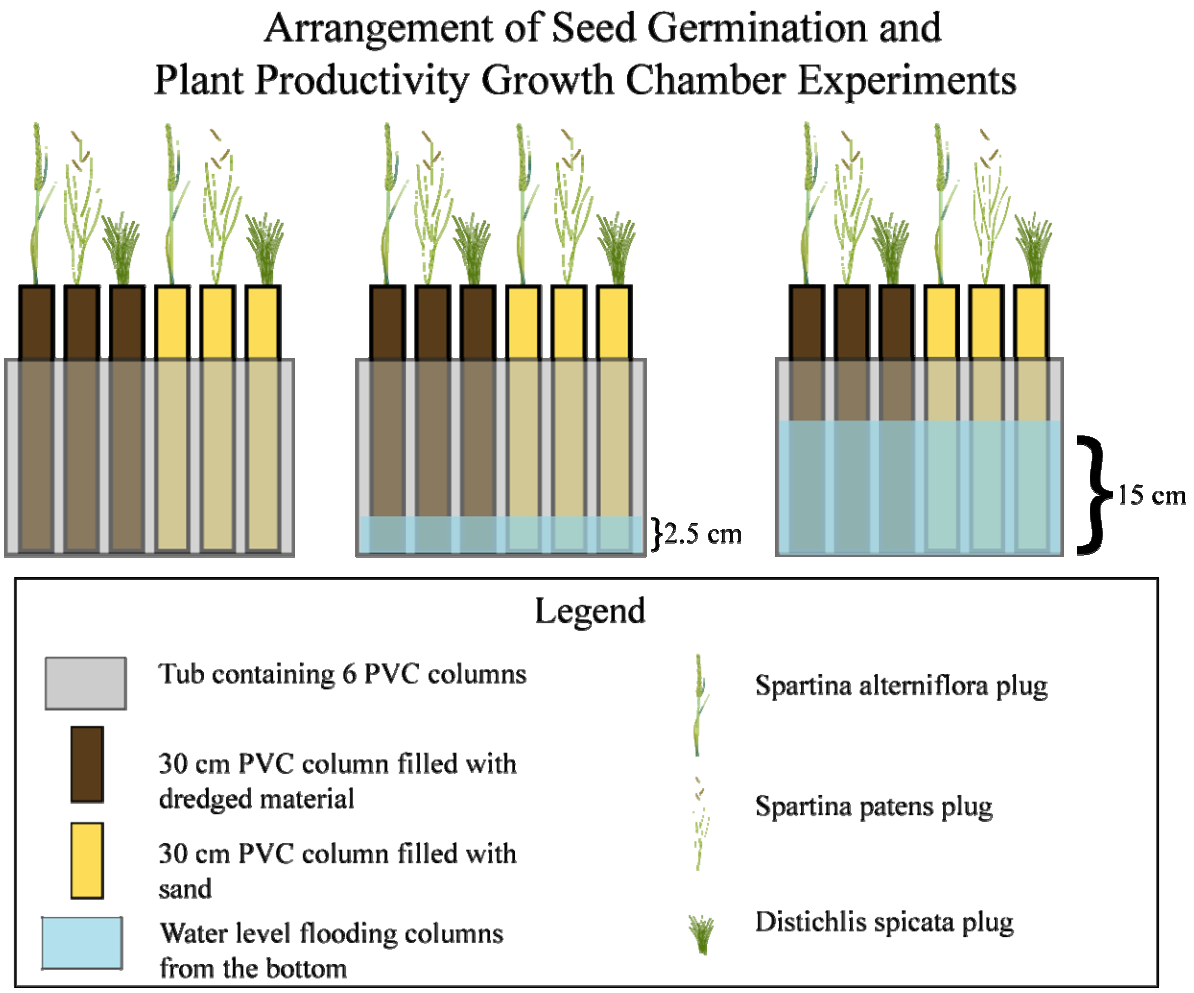

Figure 7: Diagram of the arrangement of plants growing in PVC columns filled with either sand or dredged material and subjected to one of three flooding regimes in a series of two environmental growth chamber (EGC) experiments. Three replicates of each tub were randomly placed in the EGC along with nine additional tubs prepared in the same fashion but planted with seeds of the same 3 species of marsh grasses.

additional tubs were planted in the same manner using seeds obtained from Pinelands Nursery in Toano, VA (Figure 8).

Before planting, *S. alterniflora* and *S. patens* seeds were sorted using a light table and those without an obvious embryo were not used. A sample set of 10 seeds of each species was placed on wet filter paper in Petri dishes and kept at 15^{o}C under full light intensity (1700 μE) for 12 hours per day for 2 weeks. Two replicates of each species were prepared in the same manner. Percent germination was noted to evaluate whether poor seed viability could be an explanation for low germination.

Tubs containing plants and seeds were randomly placed in a 3m x 2.5m x 2m walk-in M-96 style Environmental Growth Chamber (EGC) and were allowed to grow for a total of 10 weeks, being watered on a daily basis (Figure 8). Full light intensity (1700 μE) was simulated using 30 Osram Sylvania metal halide M400/U lamps and 30 Osram Sylvania LU400 high pressure sodium lamps for 12 hours of the day and total darkness for the remaining 12 hours. For the first three weeks the temperature was held at 15^{o}C during daylight and 10^{o}C at night. In order to simulate advancing spring conditions, during the fourth week, daytime temperatures were increased to 20^{o}C and nighttime temperatures to 15^{o}C. Day and night temperatures were then increased by 2^{o}C each week for the remainder of the experiment, up to 24^{o}C during the day and 19^{o}C at night.

At the end of the 10 week period, sediment and vegetation were removed from each column using a hydraulic extruder and each core was cut into three cross sectional 10 cm segments. Soil pH of each section was determined using a VWR SP20 pH meter which was placed in a slurry of sediment from the column and deionized water. A small

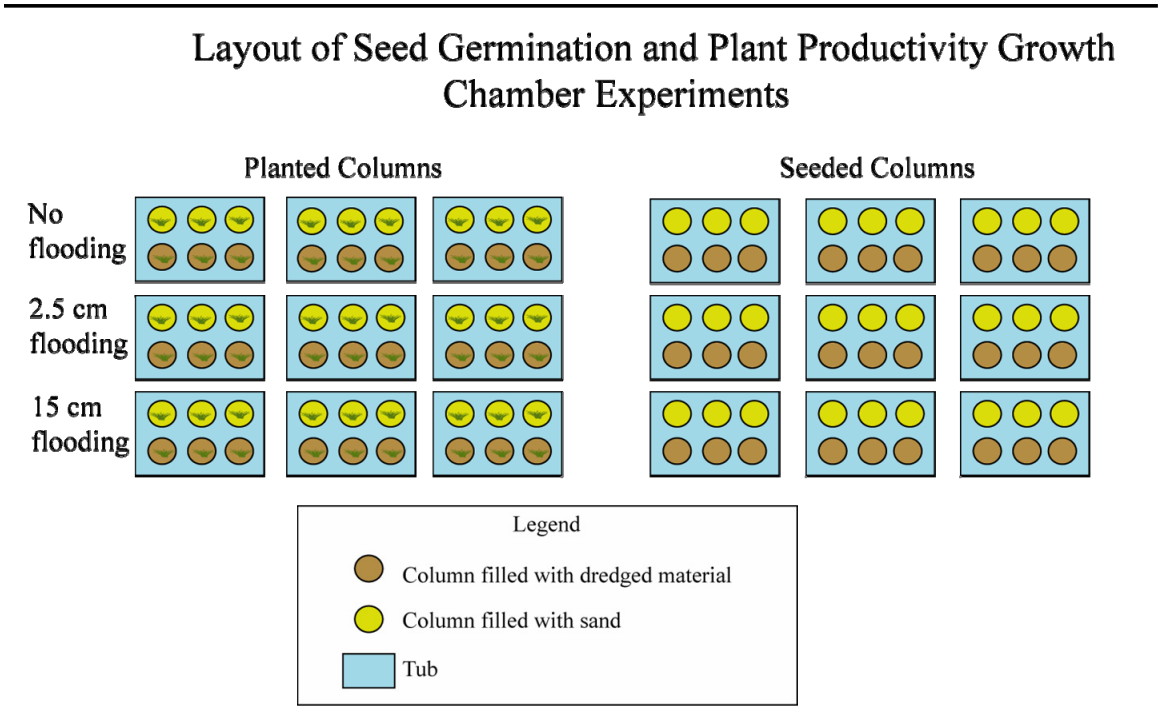

Figure 8: Diagram of the layout of environmental growth chamber (EGC) experiments. Tubs were randomly placed in the EGC.

amount of soil from each column was collected, weighed, dried at 60°C for 72 to 96 hours and reweighed to determine soil moisture content.

Aboveground stems were snipped off at the soil surface and root material was removed from the soil in each tube by rinsing the dredged material or sand through a 1 mm sieve. Both aboveground and belowground biomass was dried in a 60°C oven for a minimum of 72 h and weighed.

A second experiment was prepared in the same manner as described above to simulate more summer-like conditions. Day length for the second experiment was increased to 16 hours and the temperature was held at 23°C during the day and 18°C at night for the entire 10 weeks of the study. Aboveground and belowground biomass was determined for dried (60°C) plant material. Dry tissue of *Spartina alterniflora* plugs from the most flooded tubes (15 cm) in each of the two experiments was ground and analyzed to determine macronutrient (N, K, S, P, Mg and Ca) and micronutrient (Mn, Al, Fe, Zn, B and Cu) concentrations (Mills and Jones 1996) of the most productive plants.

Salinity and seed germination in environmental growth chamber experiments

In order to determine optimal salinity for germination, seven solutions of salinities 0-60 in increments of 10 were made by mixing Crystal Sea (Marine Enterprises International) with deionized water. *Spartina alterniflora* seeds were obtained from Pinelands Nursery in Toano, VA. These seeds were collected from a marsh at the mouth of the James River (Figure 5) where spring salinity is around 15 and fall salinity reaches 20. As with previous experiments, seeds were sorted on a light table and any without an obvious embryo were discarded. Fifty seeds were placed on paper filters that had been

watered with one of the seven salt concentrations. Each filter was sealed in a plastic bag to prevent water loss due to evaporation. This was replicated three times for each salt concentration. All the seeds were allowed to germinate in the same EGC used for the previous set of experiments. Lamps were set for 14 hours of daylight at 20^{o}C and 10 hours of dark at 15^{o}C for six days at which time the number of seeds that had germinated was counted and the three longest shoot heights in each sample was measured and recorded. The seeds were placed back in the chamber and germinated seeds were counted every three days. Final germination counts were made on day 12.

Salinity and seedling survival, growth and nutrition in environmental growth chamber experiments

To determine the effect of salinity on the growth and nutrition of *Spartina alterniflora* seedlings, three seedlings that had germinated in fresh water (0 salinity) were transferred to a two-quart container filled with sand. Six grams of Osmocote 19-6-12 (N-P-K) slow-release fertilizer was added to the sand to ensure availability of major nutrients to the seedlings. The pot was then watered with one of seven salt solutions (salinities of 0, 5, 10, 15, 20, 25, and 30). Three replicates were prepared for each solution. This procedure was then repeated using seedlings that had germinated in the solution with a salinity of 10. Constant salinity was maintained in each pot for 12 weeks by flushing each pot with its designated salt solution once every 2 days. At the end of the 12 weeks, plants were removed from the sand and whole plants were dried at 60^{o}C for 72 h and weighed to determine biomass in grams dry weight (gdw). The dried plants were then ground using a Wiley Mill and sent to A&L Eastern Agricultural Laboratories, Inc. in

Richmond, Va. for nutritional analysis of plant tissue macronutrients (N, K, S, P, Mg, and Ca) and micronutrients (Mn, Al, Fe, Zn, B and Cu) (Mills and Jones 1996). However there was not enough tissue available from the plants germinated in fresh water and grown in salinity 30 to analyze. In order to compare the James River seed with local seed, this experiment was then repeated using seeds that had been collected from Horn Point marsh in November 2004 and a treatment salinity of 2.5 was added. At the end of the second 12-week experiment there was not enough biomass available from any of the plants that grew in salinity 30 to perform a tissue analysis.

Statistical analysis

One way ANOVAs were performed on biomass data from the sediment moisture and salinity experiments on a Dell desktop computer with SAS 9.1 software (O'Rourke et al. 2005) using Levene's test for Homogeneity of Variances and pair wise comparisons were made using least squared means (LSM) (Ott and Longnecker 2001).

Results and Discussion

Sediment pore water field measurements

Pore water ammonium (NH_4^+), soluble reactive phosphorus (SRP), and dissolved iron (Fe) from the four experimental test plots on Poplar Island were compared to the sandy reference site on the island and the marsh at Horn Point. In the high marsh, NH_4^+ concentrations in the dredged material in plot 1 of Cell 4DX increased with depth with averages over the growing season (May to November) exceeding 1000 µM (up to 1520 µM) in the deepest sediments (Figure 9a). Because of high variability in the data, NH_4^+

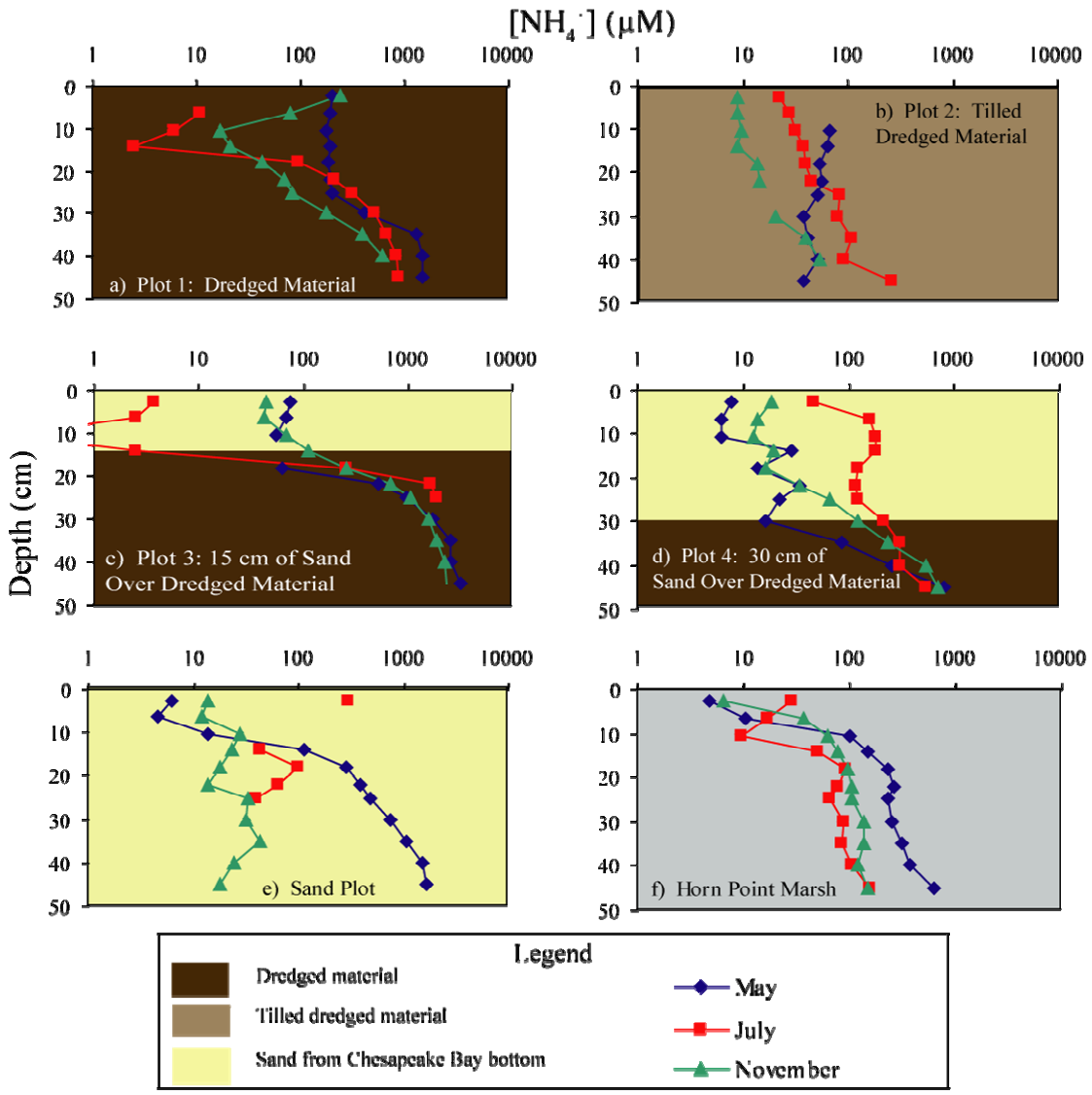

Figure 9a-f: Pore water profiles of ammonium (NH_4^+) concentrations measured in May, July and November 2004 in the high marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f).

concentrations are plotted on a log (base 10) scale. In plot 2, the tilled dredged material, NH_4^+ concentrations remained much lower in the high marsh (<265 μM) even in the deepest sediments (Figure 9b). Ammonium concentrations in the high marsh of plot 3, increased with depth and were dramatically higher in the deepest sediments, up to 3187 μM (Figure 9c). Like plots 1 and 3, NH_4^+ in plot 4's high marsh increased in the deeper sediments (Figure 9d). In the sand plot to the east of the test plots NH_4^+ in the high marsh was low to a depth of about 20 cm and increased gradually reaching 1628 μM, but only in May (Figure 9e). In November, NH_4^+ in the high marsh of the sand plot did not exceed 18 μM. No strong seasonal influence was detected in dredged material in the high marsh. Ammonium concentrations in the sediments of the high marsh at Horn Point were somewhat lower than in dredged material and did exceed 600 μM in the deep sediments (figure 9f).

Ammonium in the low marsh of plot 1 was similar to that of the high marsh, increasing with depth to over 1000 μM (Figure 10a). In the low marsh of plot 2 concentrations were higher in the deeper sediments, up to 933 μM (Figure 10b). Although enriched in NH_4^+, low marsh sediments in plot 3 and plot 4 did not exceed 1000 μM (Figure 10c, Figure 10d). In the low marsh of the sand plot, NH_4 concentrations increased greatly below 20 cm reaching a concentration of 2950 μM (Figure 10e). As in the high marsh this only occurred in May. By July, NH_4^+ was below 80 μM. In general NH_4^+ was most abundant in May in the low marsh of cell 4DX. The NH_4^+ at Horn Point was similar to most of the profiles at Poplar Island, with the exception of July where there was little depletion in the top 10 cm. This may reflect absence of plants.

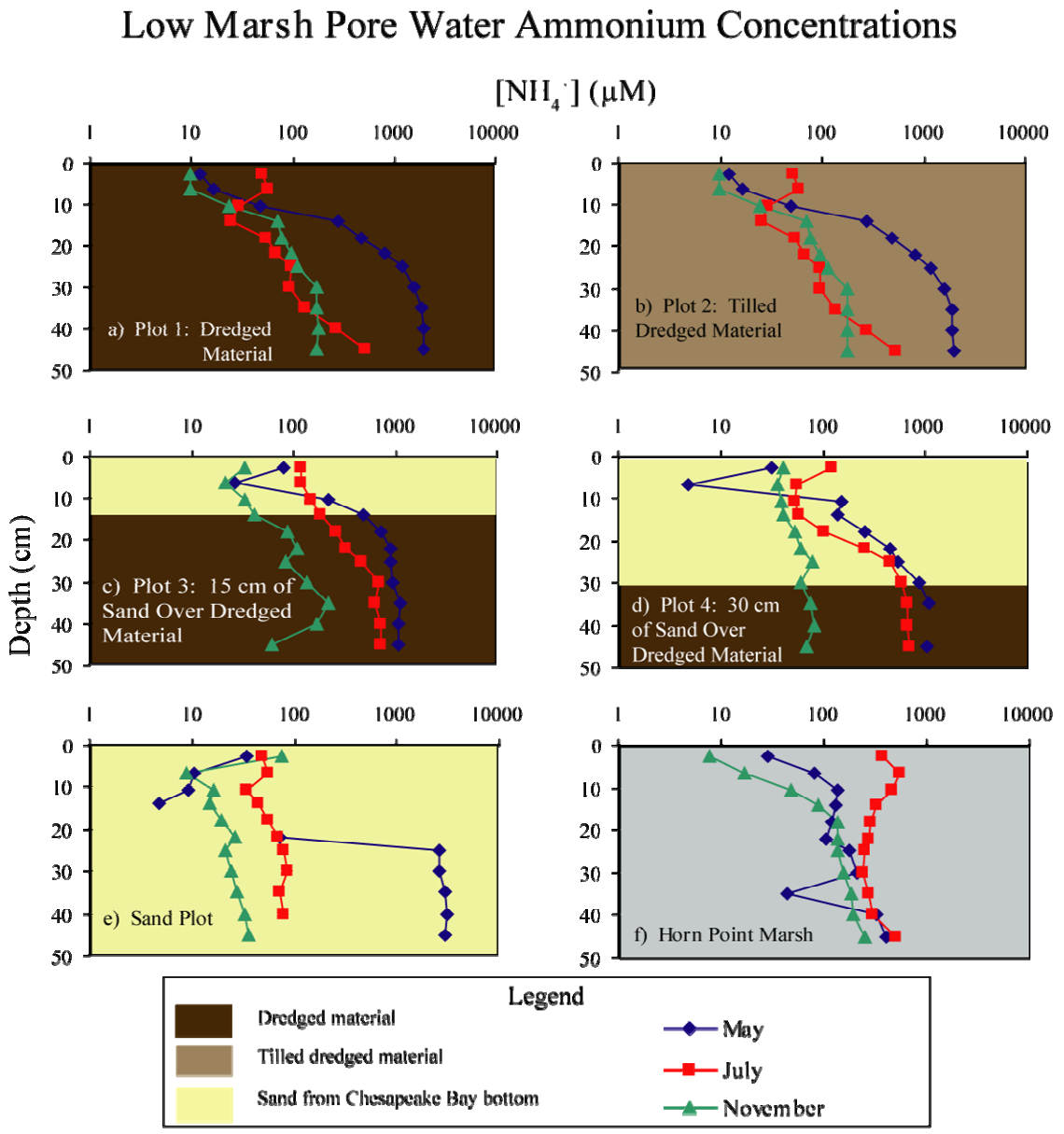

Figure 10a-f: Pore water profiles of ammonium (NH_4^+) concentrations measured in May, July and November 2004 in the low marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f).

In general, pore water ammonium concentrations in the experimental dredged material plots in cell 4DX were as high as or higher than those in the marsh at Horn Point in both the high marsh and the low marsh. Stribling and Cornwell (2001) measured monthly NH_4^+ concentrations in four marsh sites in Monie Bay, a brackish tidal system on the eastern shore of Chesapeake Bay. Maximum NH_4^+ concentrations were observed in the summer months and reached 147 μM within the first 23 cm of the sediment surface. With agriculture as the primary land use adjacent to Monie Bay, the area is considered to be enriched due to inputs from upland runoff. Since plants generally prefer NH_4^+ over nitrate (NO_3) as a nitrogen source particularly when soil redox is low (Marschner 1995), NH_4^+ concentrations exceeding 900 μM in all 4 of the dredged material sites in Cell 4DX suggest that nitrogen is available in abundance there for uptake by plants on Poplar Island. However there may be some N limitation in the sandy control area (which actually comprises most of the cell).

Soluble reactive phosphorus (SRP) was detected in the four dredged material plots in the high marsh zone but was lower than 10 μM in each (Figure 11 a-d). The SRP concentration increased below 10 cm in the high marsh of the sand plot in May and was almost negligible in November (Figure 11e). High marsh levels of SRP in Horn Point marsh were similar in May and November. Concentrations were very low at the surface and peaked at around 20 cm (Figure 11f) to 20 to 30 μM (just below the root zone).

Soluble reactive phosphorus concentrations in the low marsh of dredged material plots 1, 3 and 4 were similar to high marsh concentrations. In plot 2 however SRP

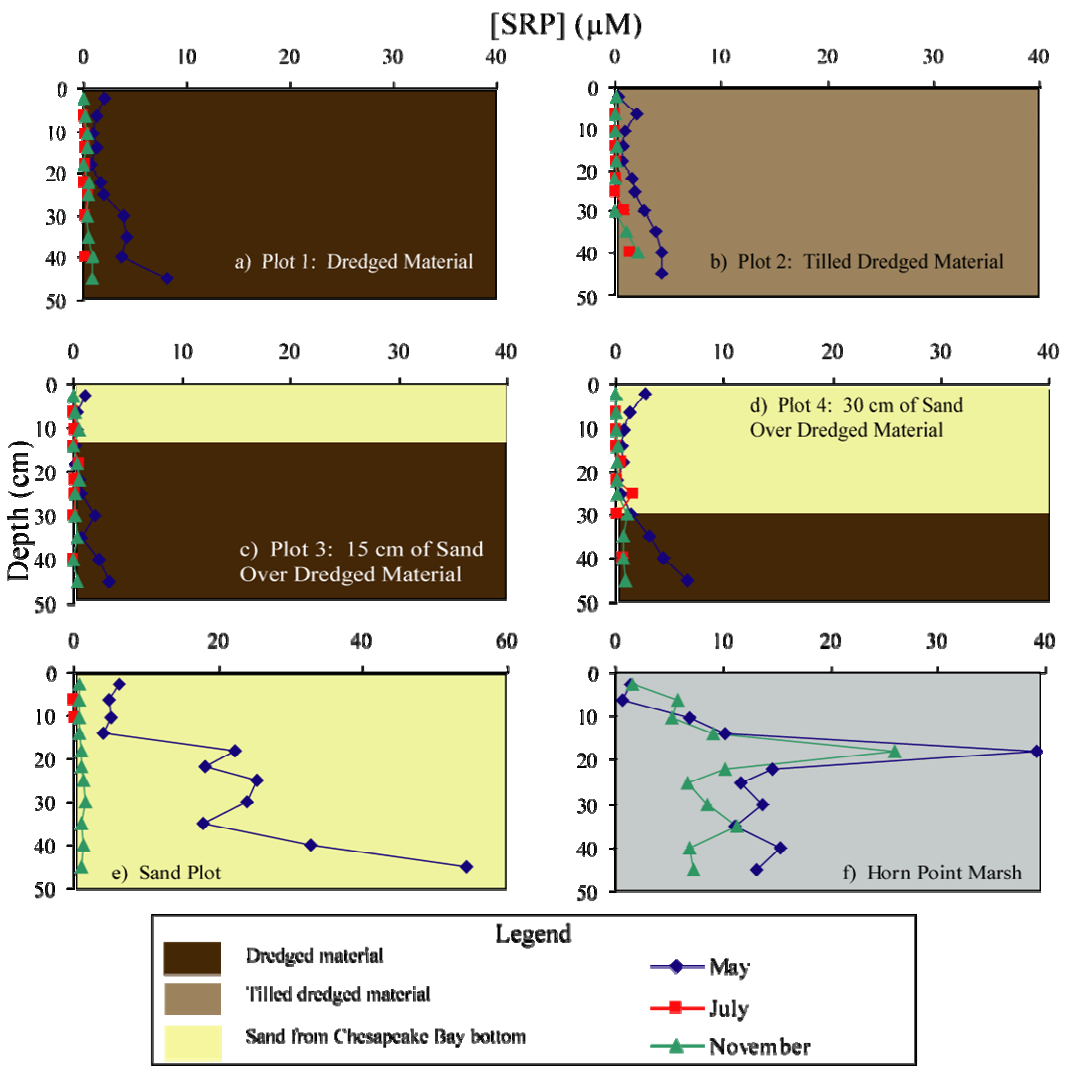

Figure 11a-f: Pore water profiles of soluble reactive phosphorus (SRP) concentrations measured in July 2003 and May and November 2004 in the high marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f). (Note: Scale for the sand plot differs from all others).

increased in the low marsh below 10 cm to nearly 100 μM in November (Figure 12 a-d). The amount of SRP measured in the low marsh in the sand plot adjacent to the test plots was very high (>50μM) below 20 cm in May and almost negligible in July and November (Figure 12e). Low marsh SRP concentrations were higher in the marsh at Horn Point in May than November reaching 40μM in the deepest sediments (Figure 12f).

The low concentrations of SRP in the high marsh zone of plots 1-4 is likely due to phosphate adsorption to iron minerals in the more oxidized sediments, as well as plant uptake. There is slightly more SRP in the low marsh zone of the dredged material plots. However, it is considerably lower than at Horn Point where all values were >15 μM at the beginning of the growing season (May) or that in Monie Bay (≥9 μM) (Stribling and Cornwell 2001). The abundance of SRP in both the high and low marsh of the sand plot in May followed by negligible amounts later in the year suggests that adsorption occurs very quickly in sandy soils. Although N limitation may not be of importance in launching marsh growth on dredged material, P fertilization may initially be necessary, at least until dredged material more closely resembles natural marsh soils.

In the high marsh in plots 1 and 2 on Poplar Island, dissolved Fe concentrations were highest in November reaching 40-60 ppm in the deeper sediments. In May and July however, significant quantities of Fe were only found in the deepest sediments and in much lower concentration (Figure 13a and b). In the high marsh of plot 3, extremely high Fe levels, (500 ppm at 30 cm) were evident compared to all other plots (Figure 13c). Plot 4 had the highest Fe in the pore water of its high marsh in November (Figure 13d) as did the sand plot (Figure 13e). By contrast, in the high marsh at Horn Point very little Fe was found (<10 ppm) (Figure 13f). Since all Fe was concentrated in the top 10 cm of

Low Marsh Pore Water Soluble Reactive Phosphorus Concentrations

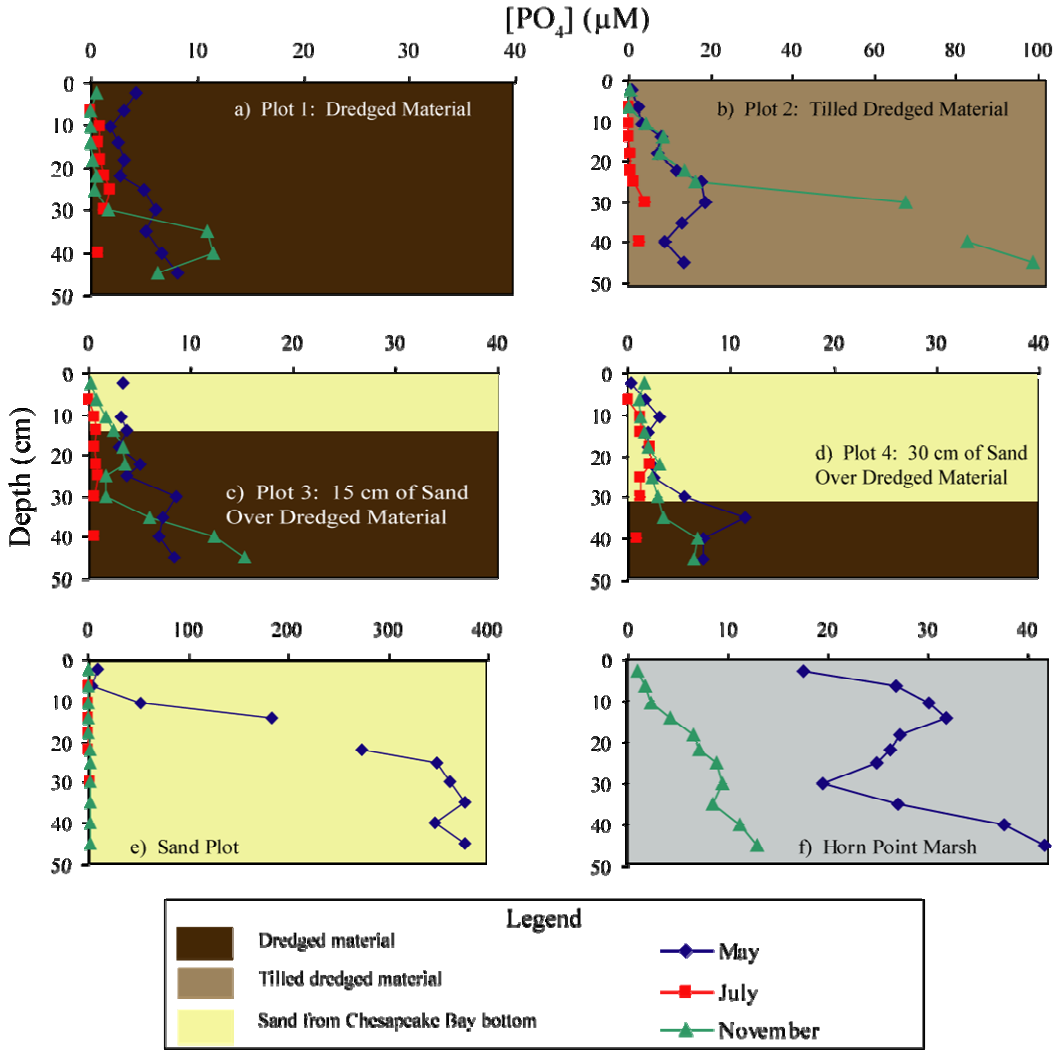

Figure 12a-f: Pore water profiles of soluble reactive phosphorus (SRP) concentrations measured in July 2003 and May and November 2004 in the low marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f). (Note: Scales for plot 2 and the sand plot differ from others).

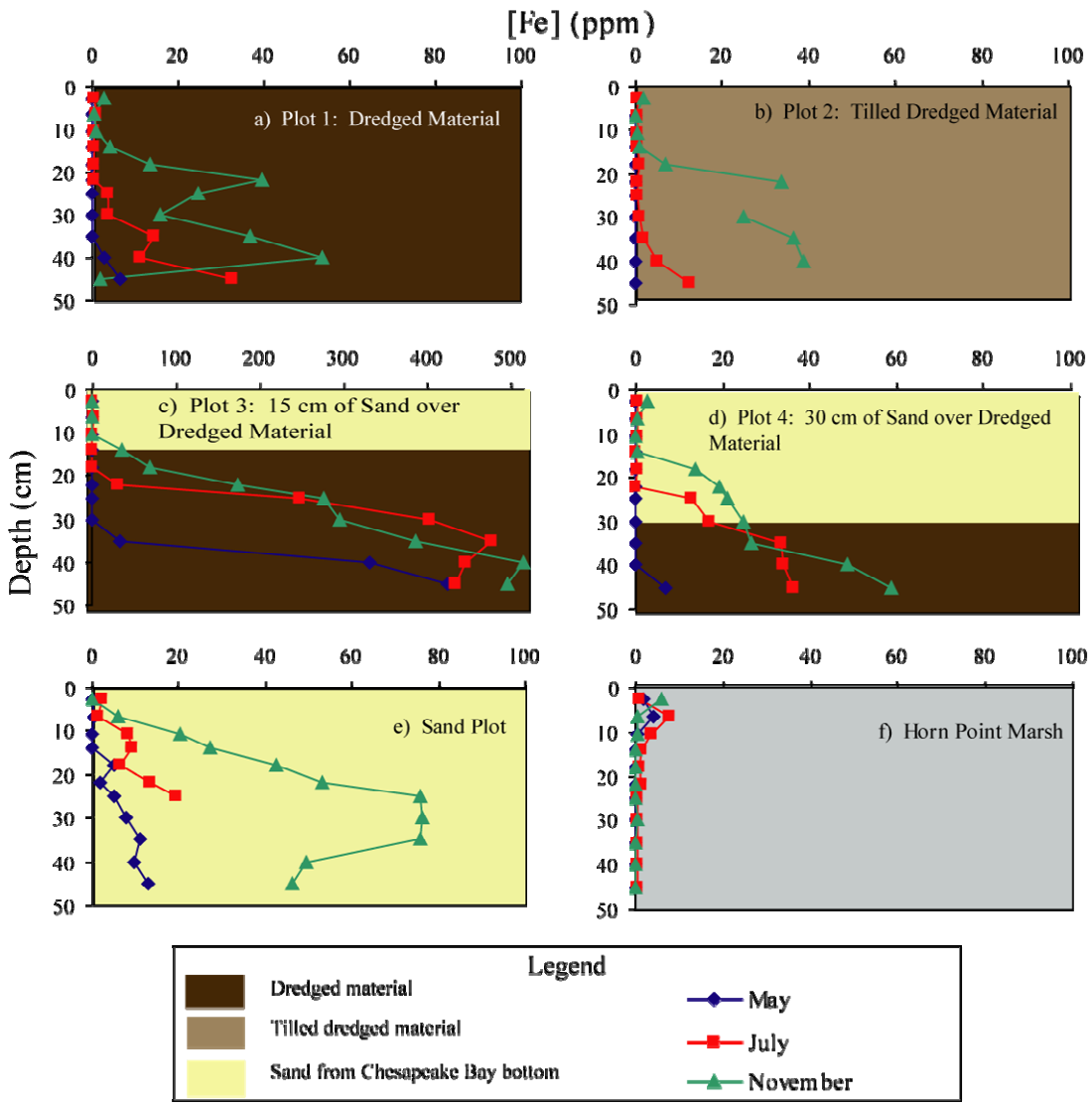

Figure 13a-f: Pore water profiles of dissolved iron (Fe) concentrations measured May, July and November 2004 in the high marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f). (Note: Scale for Plot 3 differs from all others).

sediment, it is likely associated with iron hydroxide plaques around the roots (Chambers et al. 1998).

Higher concentrations of Fe were found in the pore water of the low marsh in all four test plots. All four plots had concentrations exceeding 100 ppm in November (Figure 14a-d). Iron concentrations at the sand plot and Horn Point low marsh sites were much lower than the test plots. In the sand plot concentrations did not exceed 45 ppm (Figure 14e) and at Horn Point all measurements were less than 5 ppm (Figure 14f).

Iron, the fourth most abundant element in the continental crust, normally reaches estuarine sediments in its dissolved form via fluvial inputs (Haese 2000). Since most of the iron entering an estuary is trapped there, (only 10% reaches the open ocean), there is a potential for it to react with hydrogen sulfide, that is produced during sulfate reduction, to form iron sulfide compounds such as pyrite (FeS_2) (Kasten and Jorgensen 2000). When these compounds are exposed to air and rehydrated they may produce sulfuric acid which can drive soil pH to 3 or less (Saffigna and Dale 1999). This has been known to occur in sediments that are drained after being submerged under brackish or seawater for long periods of time (Dharmasri et al. 2004). During the submerged period, sulfides, generally in the form of iron sulfides, may build up to levels hundreds of times higher than what is found in upland soils. Upon draining, iron sulfides are quickly oxidized, forming sulfuric acid (Brady 1984). However in marsh soils at Poplar Island this has not extensively occurred and acidity does not appear to be a problem in Cell 4DX, where the amount of dredged material is small.

Low sediment pH can be detrimental to plants in several ways. At pH<4 soluble aluminum (Al) in soil exists as Al^{3+} which occupies the cation exchange sites of other

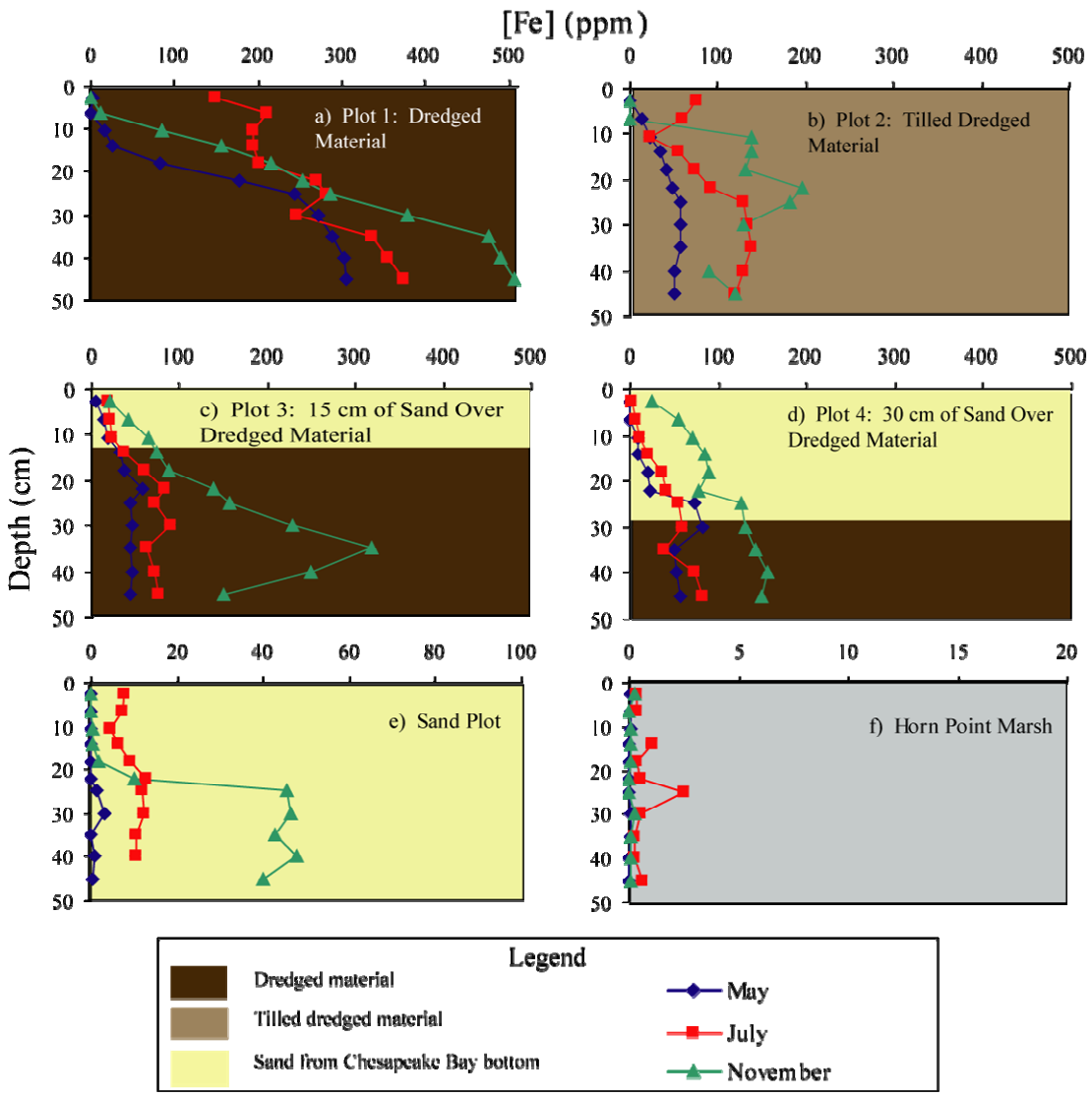

Figure 14a-f: Pore water profiles of dissolved iron (Fe) concentrations measured May, July and November 2004 in the low marsh of the four experimental test plots on Poplar Island (a-d), the reference sand plot to the east of the test plots on Poplar Island (e), and Horn Point Marsh (f). (Note: Scales for the sand plot and Horn Point Marsh differ from all others).

cations such as Ca^{2+} and Mg^{2+} and acts as an adsorber for phosphate (Marschner 1995), which limits the potential for PO_4 uptake by plants. In addition, enzyme activity in plant cells is optimal over a very narrow pH range, usually between pH 6-8 (Galston et al. 1980). Also, pH differences across chloroplast and mitochondrial membranes are necessary for generation of ATP. It was important to address the issue of acid sulfate soils in the experimental dredged material plots on Poplar Island to determine if wetland vegetation was able to be established in other cells on the island. If sulfide ions are present they would be buffered immediately as they react with iron (Brady 1984). Due to the abundance of dissolved iron in the pore water of the dredged material sites on Poplar Island compared to the marsh sediments at Horn Point, we can conclude that there is not an accumulation of sulfides in the dredged material used in cell 4DX (Haese 2000). Thus there is little risk of soils becoming too acid to support plants during dewatering. Since *Spartina alterniflora* has been shown to take up sulfide and oxidize it to sulfate (Carlson and Forrest 1982), it is unlikely that sulfide will become a problem to soils providing marsh vegetation remains healthy.

Vegetation field measurements

By the summer of 2004, when our measurements were taken, plant production in the low marsh (*Spartina alterniflora* zone) of the four experimental cells on Poplar Is. was visibly higher than the high marsh (*Spartina patens* zone) with *S. alterniflora* shoots over 1.5 m in height. Plants in the low marsh were also much greener in color than high marsh plants. In addition, plants growing in the dredged material plots were also taller and greener than those in the sandy reference plot to the east.

Plant tissue macronutrient concentrations suggest major differences between plants grown in dredged material, sand and Horn Point marsh sediment. Nitrogen (N) levels in plants from the four test plots on Poplar Island ranged from 1.51 to 1.82% and were somewhat higher than plants from both the sand plot (0.86%) and Horn Point marsh (1.15%) (Figure 15a). Although it is different for each plant species, the nitrogen content required for optimal growth is between 1.5 and 3% of the plant's dry weight (Marschner 1995; Epstein and Bloom 2005). Plants growing in the four experimental plots on Poplar Island all had a tissue N content near 1.5%. Although there is little actual information on a critical value for tissue N in *S. alterniflora* in particular, the plants in the Horn Point marsh have been stable for many years (but not very productive) (Cahoon 1975). It is our conclusion that the plants grown in cell 4DX are not N deficient in comparison to an existing Chesapeake Bay marsh.

The nutritional status for phosphorus (P) was poorer than for nitrogen in plants growing on Poplar Island, ranging from 0.12% to 0.22% in all 6 of the sampling sites with the highest concentration of P measured in plants from the tilled dredge site (plot 2) (Figure 15b). The critical tissue content range for optimal growth in higher plants is 0.3-0.5% (Marschner 1995). Plants collected from Poplar Island were somewhat lower than this range. However, there was no significant difference detected for plants growing in sand or in the marsh soil at Horn Point. Although our sediment pore water concentrations suggest that P limitation may be of some concern on Poplar Island, plants being grown in dredged material on the island did not appear to be more P deficient than those growing at Horn Point.

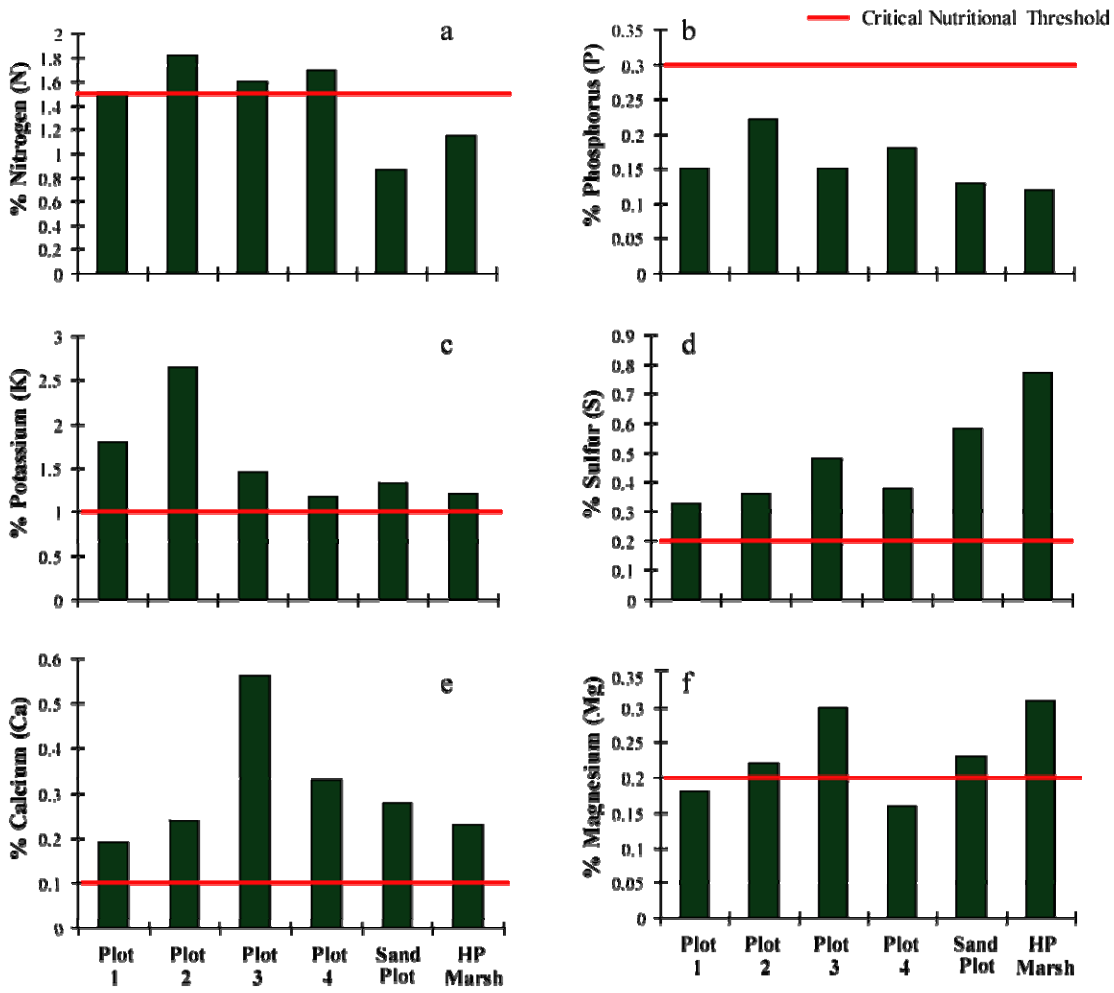

Figure 15a-f: Percentage of each macronutrient in tissue from *Spartina alterniflora* plants collected from the low marsh of each of the four experimental plots on Poplar Island, the sand plot on Poplar Island, and at Horn Point (HP) marsh in May, 2004. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

Since potassium (K) is abundant in seawater (380 mgl^{-1}) (Goldburg, 1966) it is usually not of concern to tidal salt marshes. In all of the areas we sampled, K levels in plants exceeded the 1% (Marschner 1995) threshold ranging from 1.18% to 2.64% in the four test plots with the highest levels in plants growing in dredged material (Figure 15c). Plants from the Horn Point marsh had 1.2% K, similar to the sandy sites on Poplar Island. However K tissue content was higher in the plants grown in plots 1 and 2 (without a sand layer on top of the dredged material) than all other sampling sites. Most likely the finely grained dredged material is able to retain more K from fertilizer additions than the sandy soils.

Levels of Sulfur (S) in plants from the four test plots ranged from 0.33% to 0.48% (Figure 15d). Sulfur was slightly elevated in the sand plot and at Horn Point (0.58% and 0.77%, respectively). Tissue levels of S also exceeded the 0.2% adequacy requirement for higher plants (Marschner 1995). It also corroborates our conclusion that sulfide is not abundant in dredged material being used on Poplar Island. *S. alterniflora* has been observed to have a higher S content than most plants (up to 1.2%) (Carlson and Forrest 1982; Stribling 1997) so the *S. alterniflora* at Poplar Island appears to be rather low in S, likely due to the fact that there is no free sulfide.

Magnesium (Mg) levels were slightly lower in plants from Poplar Island (0.16% to 0.23%) than those from Horn Point (0.31%), with the exception of plants from plot 3 (0.30%) (Figure 15e). Although Mg is very abundant in sea water uptake rate of Mg can be depressed by the presence of other cations (Heenan and Campbell 1981), there was concern of a Mg deficiency in plants growing in the created marsh cells. However since plants collected in the field had Mg tissue concentrations at or above the critical 0.2%

value (Epstein and Bloom 2005), it appears that uptake of Mg is adequate in dredged materials from the upper bay.

Another macronutrient critical for angiosperms is calcium. Tissue calcium levels were highest in plants from test plot 3 (0.56%) and ranged from 0.19% to 0.33% at all other sample sites (Figure 15f). The Ca content of plants varies greatly depending on the growing conditions and plant species but can range anywhere from 0.1 to 5.0% (Marschner 1995). Since the requirement for optimum growth is much lower for monocotyledons than dicotyledons (Marschner 1995), the calcium levels in plants growing in dredged material on Poplar Island indicate that they are receiving sufficient amounts of Ca. In some cases (plots 3 and 4) plants growing in dredged material had a higher tissue Ca content than Horn Point marsh's plants.

Micronutrient concentrations in plant tissue also varied across substrate types. Levels of zinc (Zn), manganese (Mn) and copper (Cu) (Figure 16b, c, and f) were much higher in the tissue of plants growing in dredged material than in those growing in the sandy plots on Poplar Island or at Horn Point marsh. In plots 1 and 2 Mn concentrations were 673 and 1415 ppm respectively while in all other treatments concentrations were 100 ppm and lower. The Mn toxicity threshold varies greatly depending on the species. For example, in maize levels of 200 ppm are toxic, whereas toxicity in sunflowers doesn't occur until tissue levels reach 5300 ppm (Marschner, 1995). Although there is little information available on the levels of Mn toxic to *S. alterniflora,* the levels in plants growing in dredge is higher than levels toxic to many other plants and may be of concern.

Zinc levels in plants from plot 1 and 2 were 58 and 56 ppm, more than twice levels in plants from any other site. Levels that are typically toxic to plants are between

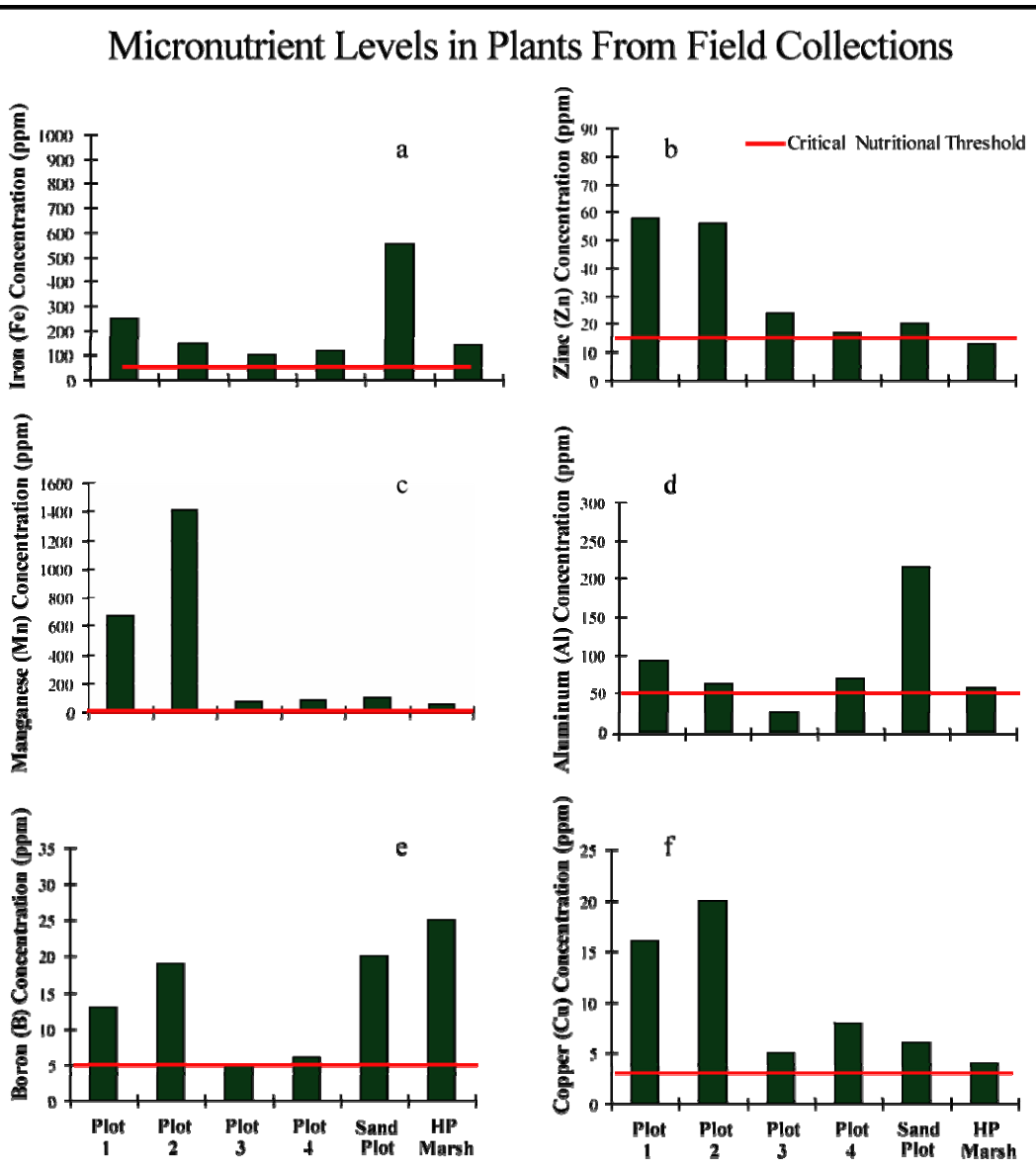

Figure 16a-f: Amount of each micronutrient in parts per million (ppm) in tissue from *Spartina alterniflora* plants collected in each of the four experimental plots on Poplar Island, the sand plot on Poplar Island, and at Horn Point (HP) marsh in May, 2004. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

100 and 300 ppm (Marschner, 1995), much higher than those found in our study sites. Copper was highest in plot 2 (20 ppm), followed by plot 1 (16 ppm). At all other sites plant tissue Cu levels were less than 10 ppm, but were more than the adequate level for plant nutrition (3 ppm) (Marschner 1995). Since Cu toxicity in most higher plants only occurs at levels higher than 20 (Marschner, 1995) it is unlikely that this should be a concern.

Iron (Fe) (Figure 16a) and aluminum (Al) (Figure 16d) were elevated in the sand plot to the east of the test plots on Poplar Island (553 ppm Fe and 215 ppm Al). Since the critical toxicity tissue concentration for iron is 500 ppm and above, this may become a problem to plants growing in sandy soils (Marschner, 1995). Iron levels in plants from the four test plots and Horn Point marsh were >250 ppm and Al levels were >95 ppm. While all plants contained adequate amounts of Fe, Al concentrations in plants from plot 3 were below the 50 ppm (Marschner 1995) threshold. Boron was lowest in plants collected from plots 3 and 4 (5 ppm and 6 ppm respectively) (Figure 15e) and was slightly higher in plants from the other four sites (13 to 25 ppm).

Tissue micronutrients in plants from Poplar Island suggest that adding a layer of sand lowers Mn, Zn, B and Cu compared to plants growing directly in dredged material. For plants in each of the sampling sites, levels of each of the micronutrients measured were similar to or higher than those in Horn Point marsh.

Seed germination and plant productivity in dredged material in environmental growth chamber experiments

Although Petri dish germination tests revealed 80 and 75% viability in *S. alterniflora* and *D. spicata* seeds respectively (*S. patens* failed to germinate), seed germination in dredged material was low. *Spartina alterniflora* seeds had highest germination under moderate (2.5 cm) flooding conditions while *D. spicata* seeds germinated only under the most flooded (15 cm) conditions in the first experiment (Figure 17). Seed germination in the second experiment under summer conditions was quite poor. No *S. patens* or *D. spicata* seeds germinated when planted in dredged material and a total of three *S. alterniflora* seeds germinated all of which were planted in dredge receiving 15 cm of flooding.

Seeds of most angiosperms are desiccation-tolerant and are able to withstand dry storage where their moisture content may fall below 4%. In some species however, seeds are unable to tolerate desiccation and are killed if their moisture content falls below 30%. (Berrie 1984). It may be that *Spartina patens* seed simply cannot tolerate desiccation. Since *Distichlis spicata* seeds that we used were stored dry and were highly viable, they are obviously tolerant of desiccation. *Spartina alterniflora* seeds were stored wet however, and dried out with the soil surface after planting. The failure of viable *S. alterniflora* and *D. spicata* seeds to germinate in dredged material suggests that the top layers of the soil, even when submersed in 15 cm of water and watered on a daily basis, became so dry that *S. alterniflora* seeds were killed by desiccation and *D. spicata* seeds had insufficient water available to imbibe and activate metabolic functions. Although seeding may be an effective way to establish plants in the low marsh where sediment

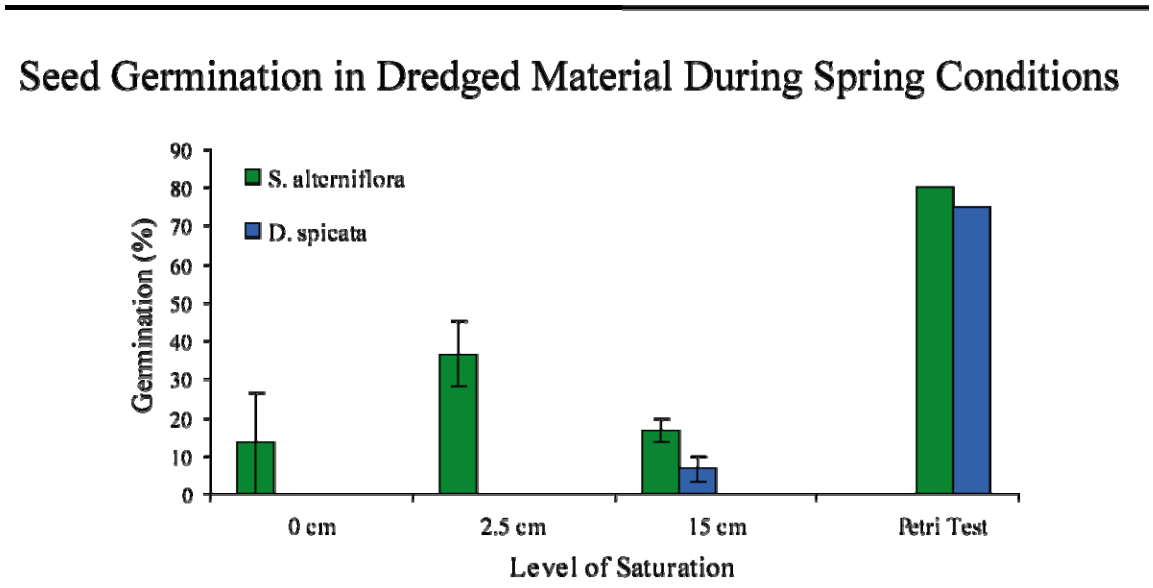

Figure 17: Percentage (± SE) of *Spartina alterniflora* and *Distichlis spicata* seeds that germinated in dredged material in the first of the two phytotron experiments (spring conditions) compared to the percentage of viable seeds as determined by a Petri dish test. *Spartina patens* seeds were not viable.

remains inundated, this dredged material dries out too quickly for seeds to be successful in the high marsh.

In the first of the two Experimental Growth Chamber (EGC) experiments (spring conditions), biomass of *Spartina patens* (Figure 18) was consistent across the three flooding levels in both sediments ranging in total dry weight between 21-23 g. No statistical differences were detected at $\alpha=0.05$. When day length was increased and plants were grown under summer conditions (second EGC experiment), biomass of *S. patens* was significantly higher in the most flooded dredged material than all other dredged material treatments at 56 gdw (total biomass) ($p<0.0001$), but biomass was also increased in all three of the sand conditions.

The biomass of *Spartina alterniflora* (Figure 19) was also fairly consistent across the levels of flooding and in both sediments in the spring experiment (experiment 1), ranging in total biomass from 12 to 21 gdw. None of these measurements differed significantly from one another ($\alpha=0.05$). The biomass of *S. alterniflora* was increased dramatically in the summer experiment (experiment 2) to 61 gdw (total biomass), but only in the most flooded dredged material. This measurement was significantly higher than all other treatments ($p<0.0001$).

In the first experiment (spring), *Distichlis spicata* biomass (Figure 20) was significantly greater in dredged material in 2.5 cm of flooding (41.5 gdw) than in non-flooded dredged material non-flooded sand or sand in 2.5 cm of flooding ($p\leq0.03$). However this biomass was not significantly different than biomass of *D. spicata* plants

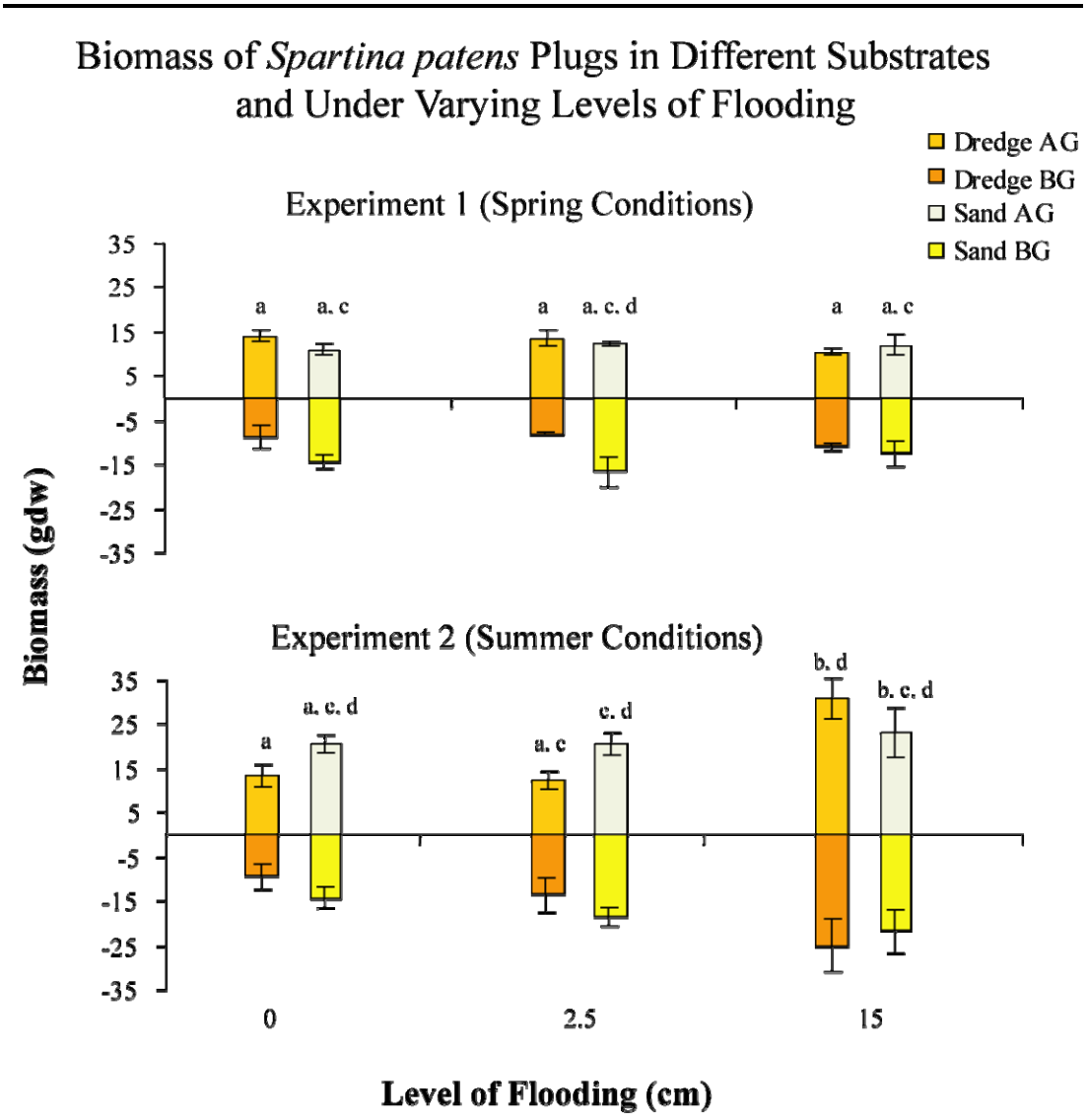

Figure 18: Biomass (± SE) of *Spartina patens* after 10 weeks of growth in one of three flooding levels in each of two environmental growth chamber (EGC) experiments. Above ground (AG) biomass of plants grown in dredged material and sand are presented as positive measurements while below ground (BG) biomass is presented as negative measurements. Bars with the same small letter above indicate no significant difference in total biomass between treatments at the α=0.05 level.

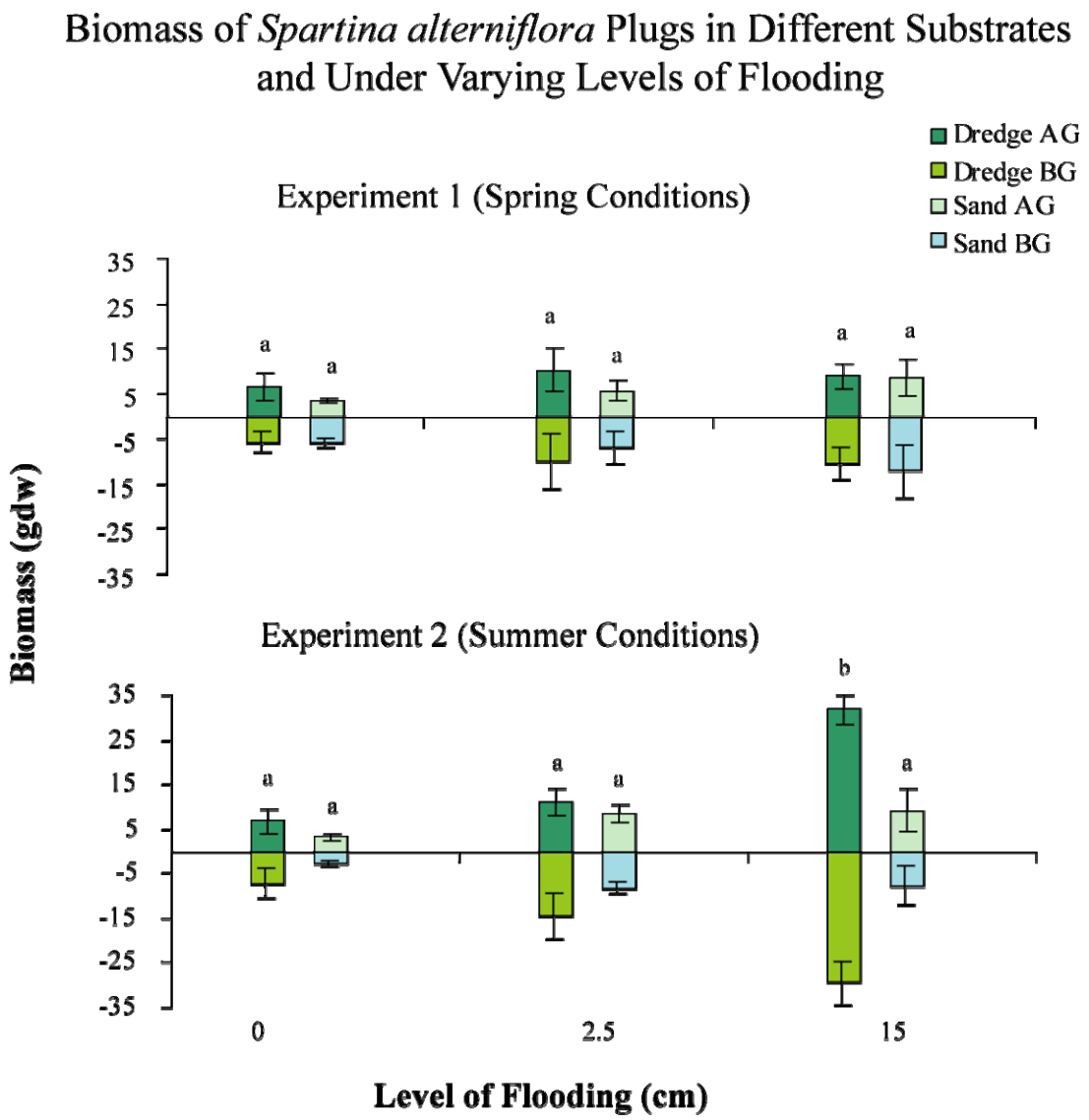

Figure 19: Biomass (± SE) of *Spartina alterniflora* after 10 weeks of growth in one of three flooding levels in each of two environmental growth chamber (EGC) experiments. Above ground (AG) biomass of plants grown in dredged material and sand are presented as positive measurements while below ground (BG) biomass is presented as negative measurements. Bars with the same small letter above indicate no significant difference in total biomass between treatments at the α=0.05 level.

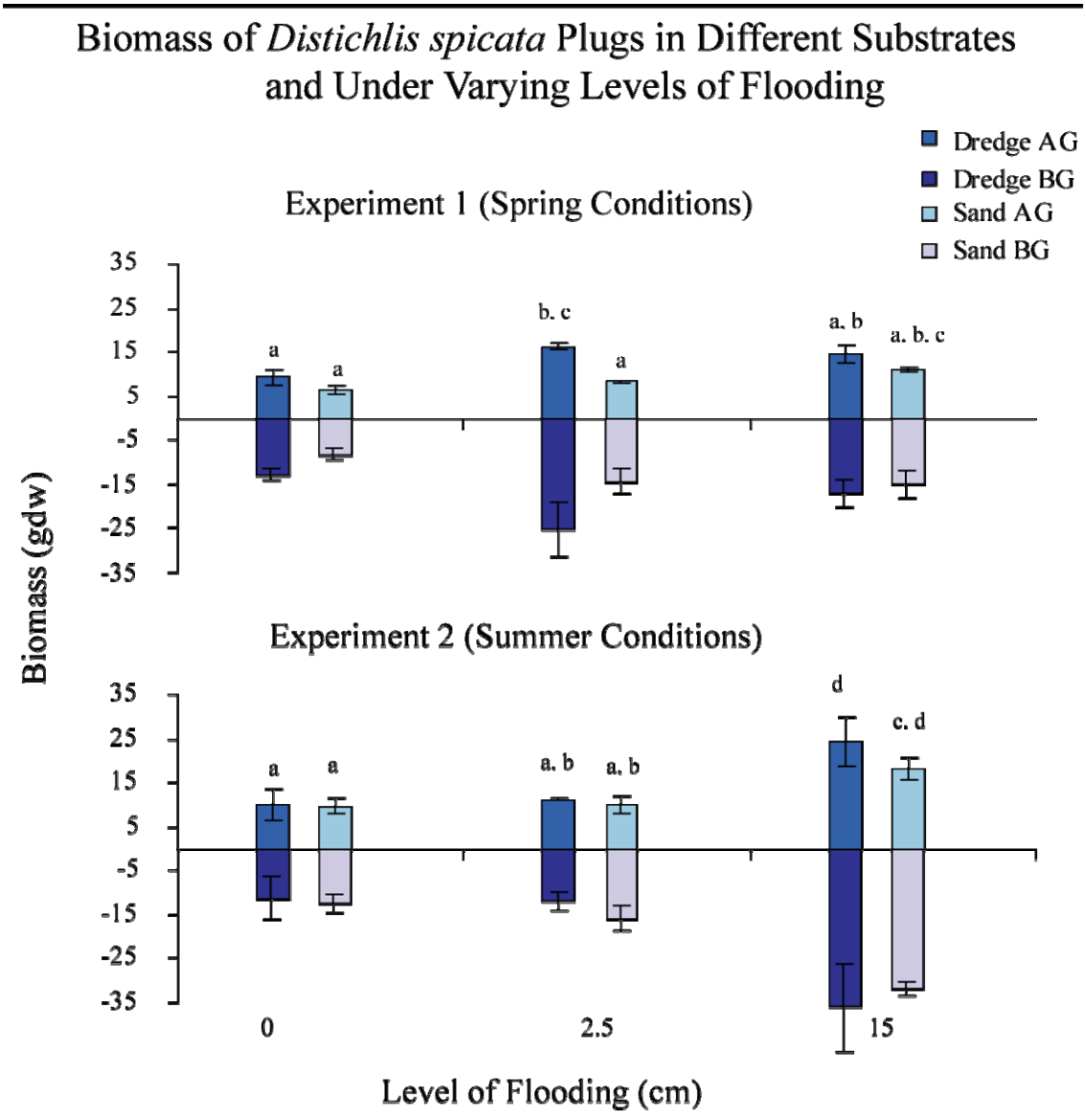

Figure 20: Biomass (± SE) of *Distichlis spicata* after 10 weeks of growth in one of three flooding levels in each of two environmental growth chamber (EGC) experiments. Above ground (AG) biomass of plants grown in dredged material and sand are presented as positive measurements while below ground (BG) biomass is presented as negative measurements. Bars with the same small letter above indicate no significant difference in total biomass between treatments at the α=0.05 level.

growing in either sediment under the most flooded conditions. When day length and temperature were increased in the second experiment, biomass of plants grown in 2.5 cm of flooding decreased and plants grown in both dredge and sand in the most flooded (15 cm) sediment was significantly increased.

The first environmental chamber experiment (spring conditions) failed to support our hypothesis that an adequate sediment moisture must be maintained in order to maximize production in marsh grass species growing in dredged material. However when plant production was maximized in all three species tested (*Spartina patens, S. alterniflora* and *Distichlis spicata*) by increasing temperature and light duration, low soil moisture did in fact limit production. The chamber experiments suggest that marsh establishment using transplants is most successful when planted in the summer. This is supported by controlled growth studies in which Seneca (1974) found that *S. alterniflora* seedlings that grew under short day conditions were shorter, contained less biomass, produced more culms and rhizomes, had less shoot moisture and contained higher chlorophyll concentrations than those grown under long day conditions. Seneca (1974) also found that the greatest rate of growth in the field in North Carolina, occurs in June under long day lengths.

Sediment pH of dredged material from the first experiment (spring conditions) ranged from 5.5 to 6.8 (Figure 21). The lowest pHs were found were in unvegetated dredged material with low moisture content. This supports the earlier observation that drying and hydration of the Poplar Island dredged material under the conditions in these experiments does not result in acid soils that will inhibit plant growth.

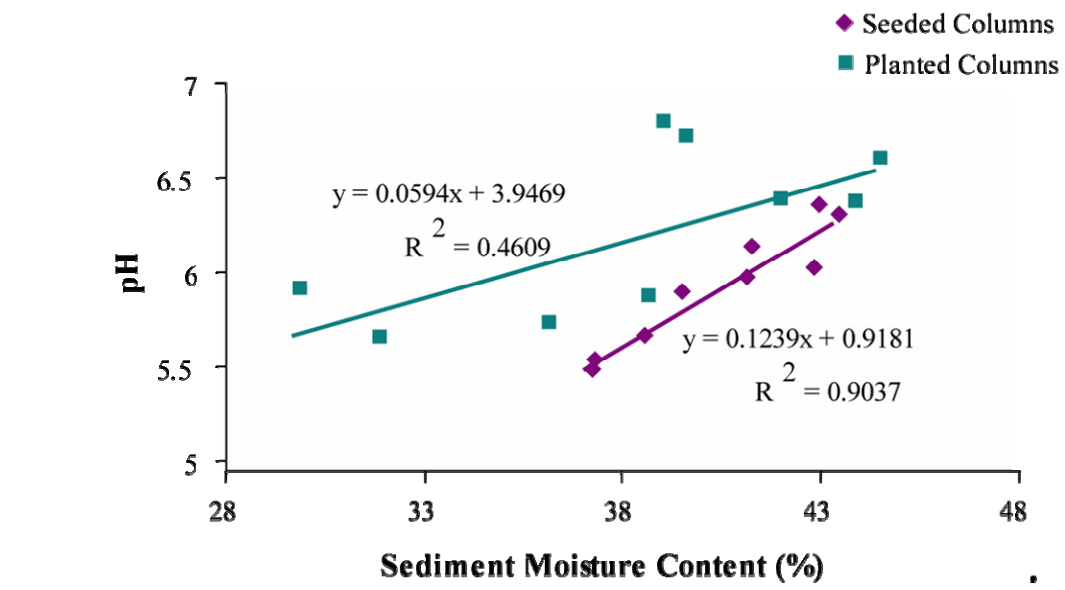

Figure 21: pH of dredged material of varying moisture contents with plants (planted columns) and without plants (seeded columns) during spring conditions.

Plant tissue macronutrient levels in plants grown in 15 cm of flooding in sand and dredged material in the two sediment moisture experiments varied somewhat for the two sediment types. Nitrogen (N) levels in plants from dredge in experiment 1 (spring conditions) and sand from both experiments were quite similar ranging from 1.19% to 1.30% (Figure 22a). Plants grown in dredged material in the second experiment (summer conditions) had much lower N levels (0.08%), clearly below the critical value of 1.5% (Marschner 1995). This is curious since no alterations were made in fertilizer additions and there was no N reduction in plants grown in sand.

Plant tissue phosphorus (P) was very similar (~0.14%) in all treatments except in the summer experiment the dredged material plant tissue P levels were significantly lower (0.09%) (Figure 22b). As in plants from field collections this is lower than the nutritional requirement of most higher plants (0.3%) (Marschner 1995) and appears to be another indication of P deficiency in these dredged sediments.

Plant tissue Potassium (K) levels in the four experimental treatments ranged from 0.90% to 1.41%, with the highest levels in plants grown in the dredged materials during the spring experiment, but did not vary significantly between the four treatments (Figure 22c). However, K levels in sand when grown under spring conditions were below the 1% critical threshold. Sulfur levels ranged from 0.28% to 0.36% in plants in dredged material and plants in sand from the summer experiment (Figure 22d). Sulfur was significantly lower (0.17%) in plants grown in sand during the spring experiment and did not reach the 0.2% critical value (Marschner 1995). Tissue calcium levels were similar across the four treatments (0.18% to 0.26%) (Figure 22e), and were more than adequate (>0.01%) (Marschner 1995).

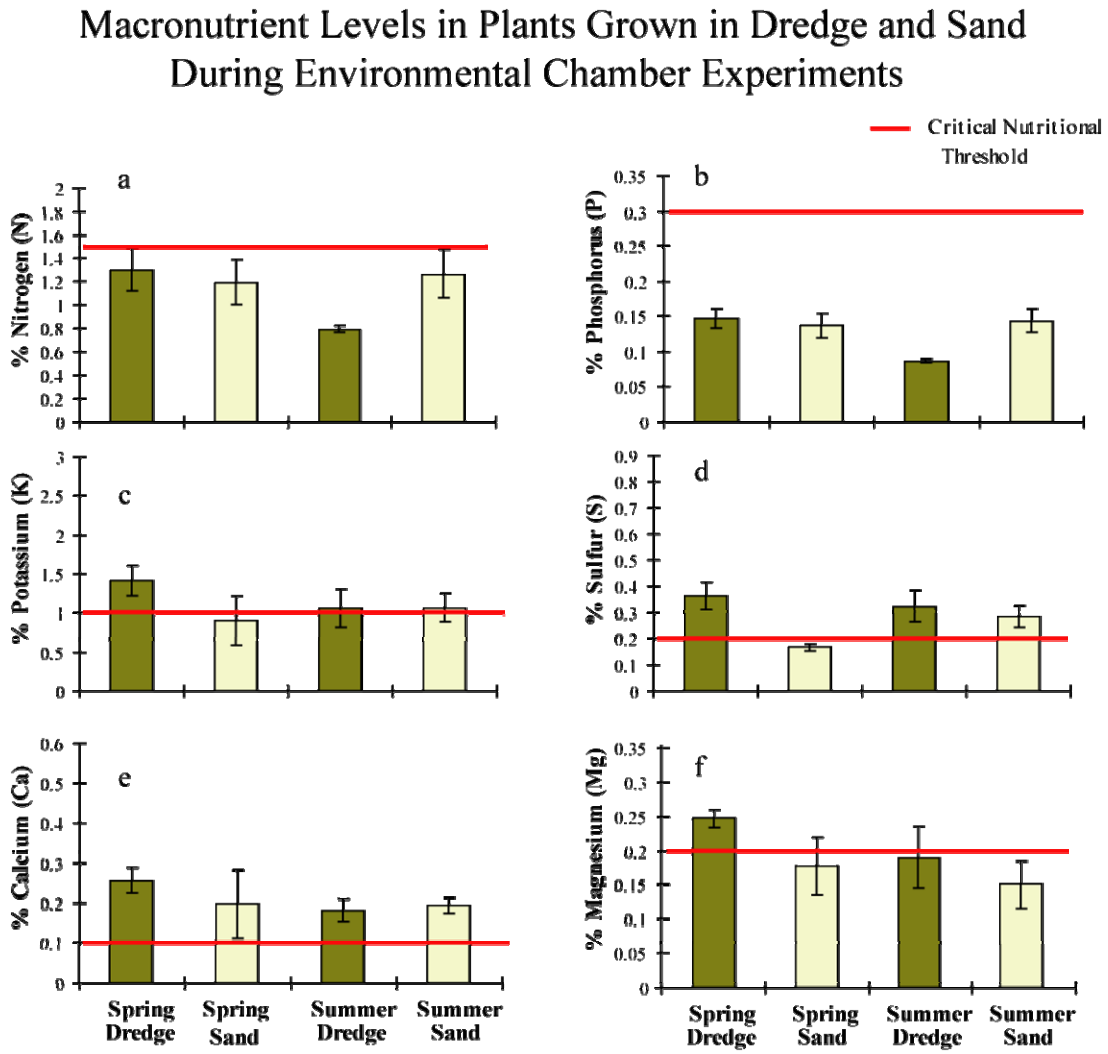

Figure 22a-f: Percentage (± SE) of each macronutrient in tissue from *Spartina alterniflora* plants grown during environmental growth chamber (EGC) experiment 1 (spring) and EGC experiment 2 (summer). Plants were 2 inch plugs when planted and grown for 10 weeks in either dredged material or sand that was subjected to 15 cm of flooding. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

Magnesium (Mg) levels were fairly consistent across the four treatments (0.15% to 0.25%) but were highest in the plants grown in dredged material during experiment 1 (spring) (Figure 22f). Magnesium levels of plants grown in sand in the EGC were below the critical nutritional threshold of 0.2% (Marschner 1995). Since the fertilizer formula used in these experiments did not contain any elements other than nitrogen, phosphorus and potassium, we can conclude that Mg is present in the dredged material and is not being provided to plants by sand.

Certain micronutrient concentrations in plant tissue varied more dramatically across substrate types in the two experiments (figure 23). Levels of iron (Fe), zinc (Zn) and manganese (Mn) (Figure 23a, b and c) were higher in the tissue of plants growing in dredged material than in those growing in sand. Aluminum (Al) (Figure 23d) was much higher in plants grown in dredged material than sand during the spring experiment. In fact the plants in sand were somewhat deficient (<50 ppm) (Marschner 1995). In the summer experiment, Al concentrations in plants growing in dredge were reduced and levels in plants grown in sand were increased. Aluminum becomes soluble in soils with pHs below 4 (Marschner 1995) and can be toxic to plants in high levels. The summer experiment yielding plants with lower accumulation of Al in their tissue, further supports our conclusion that these dredged sediments don't readily become acid, even when dried out quickly at high temperatures. Copper (Cu) (Figure 23f) plant tissue concentrations did not differ significantly across treatments and boron (B) (Figure 23e) levels were higher in plants growing in sand.

The growth chamber experiments further substantiated greater micronutrient availability in dredge as compared to sand, except in the case of boron which was below

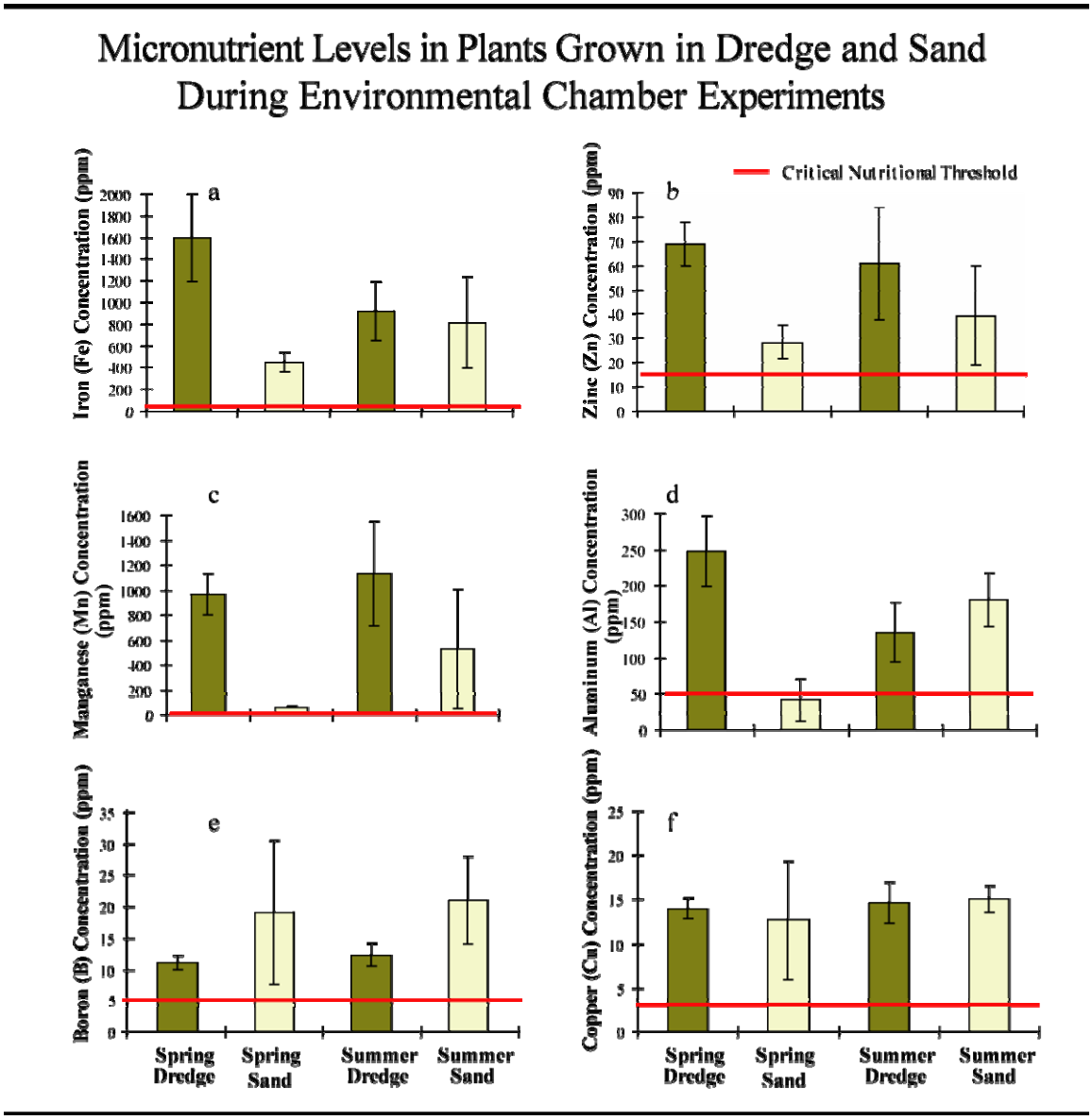

Figure 23a-f: Amount of each micronutrient in parts per million (ppm) in tissue from *Spartina alterniflora* plants grown during environmental growth chamber (EGC) experiment 1 (spring) and EGC experiment 2 (summer). Plants were 2 inch plugs when planted and grown for 10 weeks in either dredged material or sand that was subjected to 15 cm of flooding. (Error bars indicate standard error). The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

the adequacy threshold for most plants (20 ppm) (Epstein and Bloom 2005). Although the role of boron is the least understood of all of the plant mineral nutrients, it is postulated that it is important in many cellular functions including cell wall synthesis, carbohydrate metabolism, lignification, and respiration (Marschner 1995).

Salinity and seed germination in environmental growth chamber experiments

After 6 days, average shoot height of *S. alterniflora* seedlings in fresh water (salinity 0) was 3.2 cm (Figure 24). This was somewhat greater than seedlings in the lower salinity treatments (10-30) and significantly greater than those in the high salinity treatments (40-60). After 12 days roughly 80% of seeds in salinities of 0 and 10 had germinated (Figure 25). Percentage of seeds that germinated in salinities of 20 and 30 was significantly lower at around 60%, and seeds in salinities of 40 or greater had less than 20% germination.

Seed banks are important in contributing to the colonization of bare areas in marsh habitats. Although colonization can occur vegetatively, seeds can result in faster establishment of vegetation in large areas than rhizome growth (Wijte and Gallagher 1996a). *Spartina alterniflora* has been found growing in areas where salinity of interstitial water is often much higher than that of sea water (Wijte and Gallagher 1996a;

Wijte and Gallagher 1996b; Stribling 1997) as well as in marshes that are exposed to fresh water for part of the year, but is not found at salinities below 2 (Anderson et al. 1968). We found that *S. alterniflora* seeds germinated as well in fresh water as they did at a salinity of 10. This suggests that salt is not a requirement for germination. The slight reduction in germination at salinities of 20 and 30 and the significant drop thereafter is at

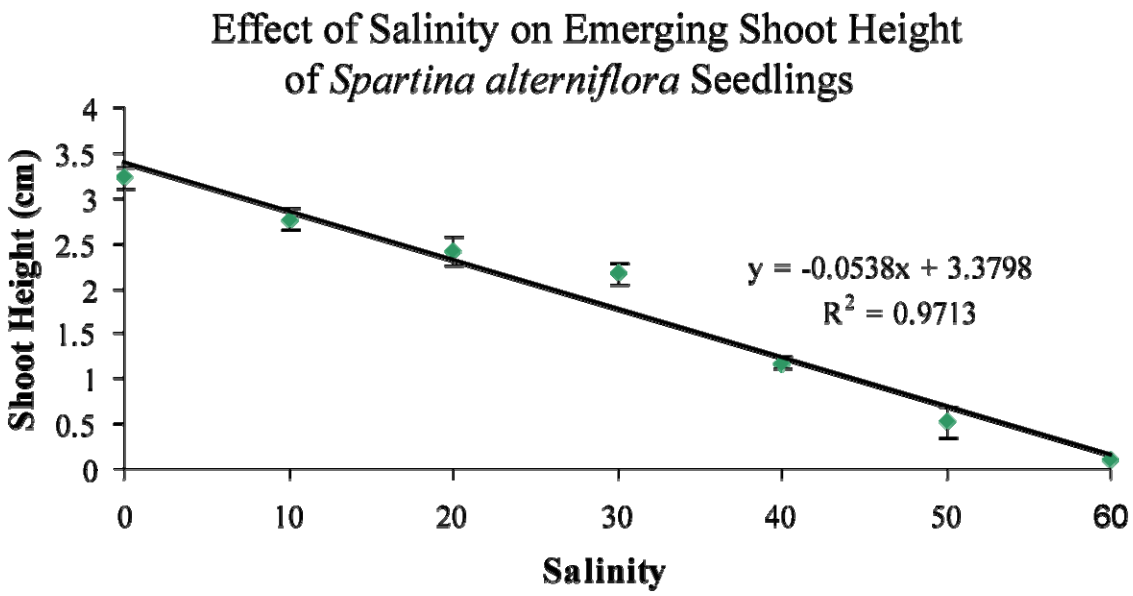

Figure 24: Average height (±SE) of the tallest shoots emerging from *Spartina alterniflora* seeds after 6 days in each salinity.

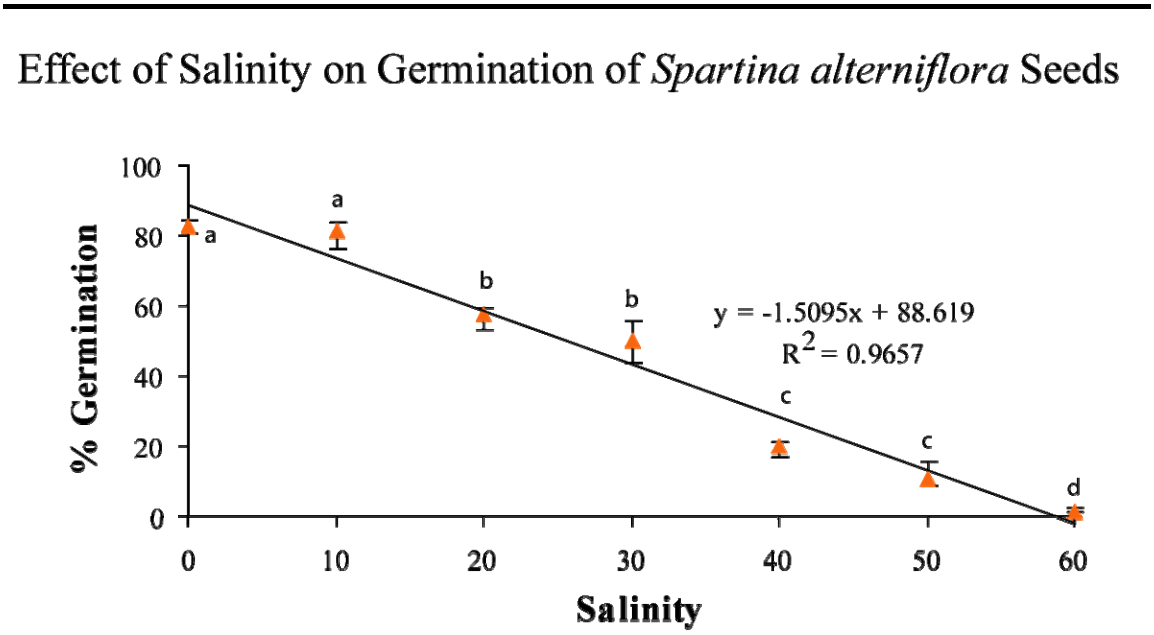

Figure 25: Percentage of *Spartina alterniflora* seeds (±SE) that germinated after 12 days in each salinity. Data points with the same small letter associated with it indicate no significant difference in germination between treatments at the α=0.05 level.

odds with Wijte and Gallagher's (1996a) findings that germination is not inhibited below salinities of 40. However they may have used seeds from higher salinity biotypes. Seed germination is stimulated by environmental factors such as water, oxygen and temperature. Once a seed imbibes water, the embryo mobilizes its nutrient reserves and gains osmotic pressure required to break through the seed coat (Epstein and Bloom 2005). Since temperature was held constant and seeds exposed to ambient oxygen levels during the 2 week germination experiment, we conclude that the water potential of seeds in salt water is higher than its surrounding substrate which decreases uptake of water by the seed and thus inhibits initiation of germination.

Salinity and seedling survival, growth and nutrition in environmental growth chamber experiments

The biomass of seedlings grown from the Lower James River seed was highest when grown in a salinity of 5 (Figure 26). Plants grown in fresh water did not differ significantly from plants grown in upper middle and high salinities ($\geq$15). All seedlings had a dry weight of 2 g or less after 12 weeks. There was a general trend that seeds germinated in fresh water and then transferred to a salt treatment were more productive than those that had germinated in salt water (except in the case of the fresh water treatment) (Figure 26). Seedlings germinated from James River seeds that had salt exposure during germination had slightly higher biomass when grown in fresh water than those which germinated with no contact with salt, although this difference is not significant ($p=0.74$).

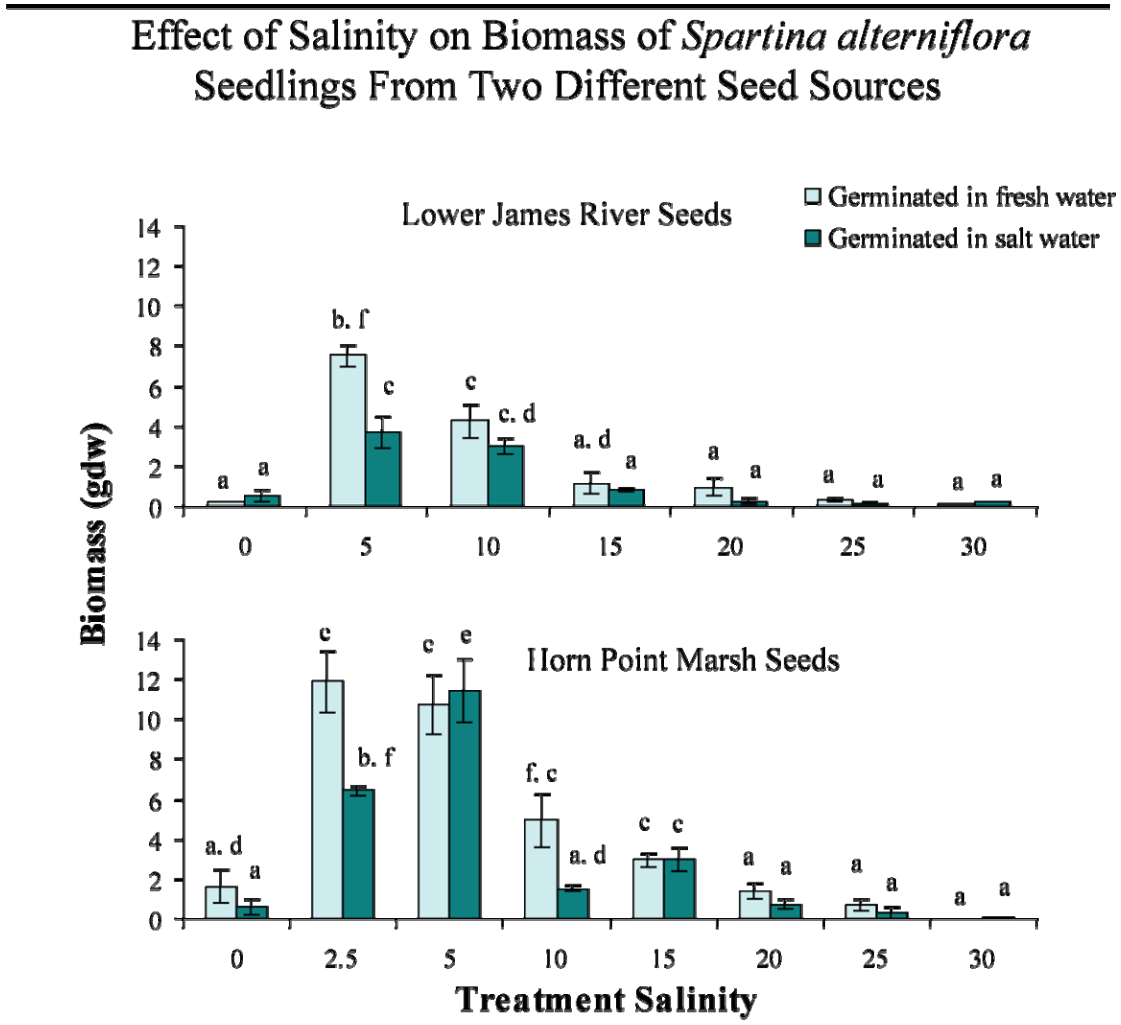

Figure 26: Biomass (± SE) of plants grown from 2 different seed sources (Lower James River and Horn Point marsh) for 12 weeks in an environmental growth chamber (EGC). Seeds germinated in either salt water (salinity of 10) or fresh water for 2 weeks before being grown for 12 additional weeks in one of eight treatment salinities. Bars with the same small letter above indicate no significant difference in total biomass between treatments at the α=0.05 level.

In general, seedlings that grew from seed collected from Horn Point marsh were more productive than James River seed but displayed a similar response to salinity (Figure 26). Plants grown in salinities of 2.5 and 5 were most productive with dry biomass between 6 and 12 g after 12 weeks. Horn Point seeds (like James River seeds) that germinated in fresh water had slightly higher biomass than seeds exposed to salt during germination except in the case of treatment salinities of 5 and 15 where there was no significant difference between the two germination techniques. Seedlings germinated in fresh water and grown in the same were slightly more productive than those which had salt exposure during germination and were transferred to the fresh water treatment. This is unlike what was observed in the seedlings grown from James River seed, but both were not statistically significant.

Nitrogen (N) levels in tissue of James River seedlings exceeded the minimum 1.5% requirement of most higher plants (Marschner 1986, Epstien and Bloom 2005) but was lowest in plants that had germinated in fresh water and grown in salinities of 5 and 10 (Figure 27a). In Horn Point seedlings, N levels in plants germinated in salt water and grown in fresh water were much higher (3.8%) than those of the James River seeds treated in the same manner (2.1%) (Figure 27a, Figure 28a). Nitrogen concentrations in the Horn Point marsh seeds grown in low salinities (2.5 and 5) were lower than for all other treatments (1.8-1.9%) (Figure 28a), although all plants were above the minimum requirement.

Phosphorus (P) concentrations were generally poorer in plants grown from James River seed (Figure 27b) than in plants grown from Horn Point marsh seed (Figure 28b), with the exception of seeds grown in salinity 5. The only James River seedlings that had

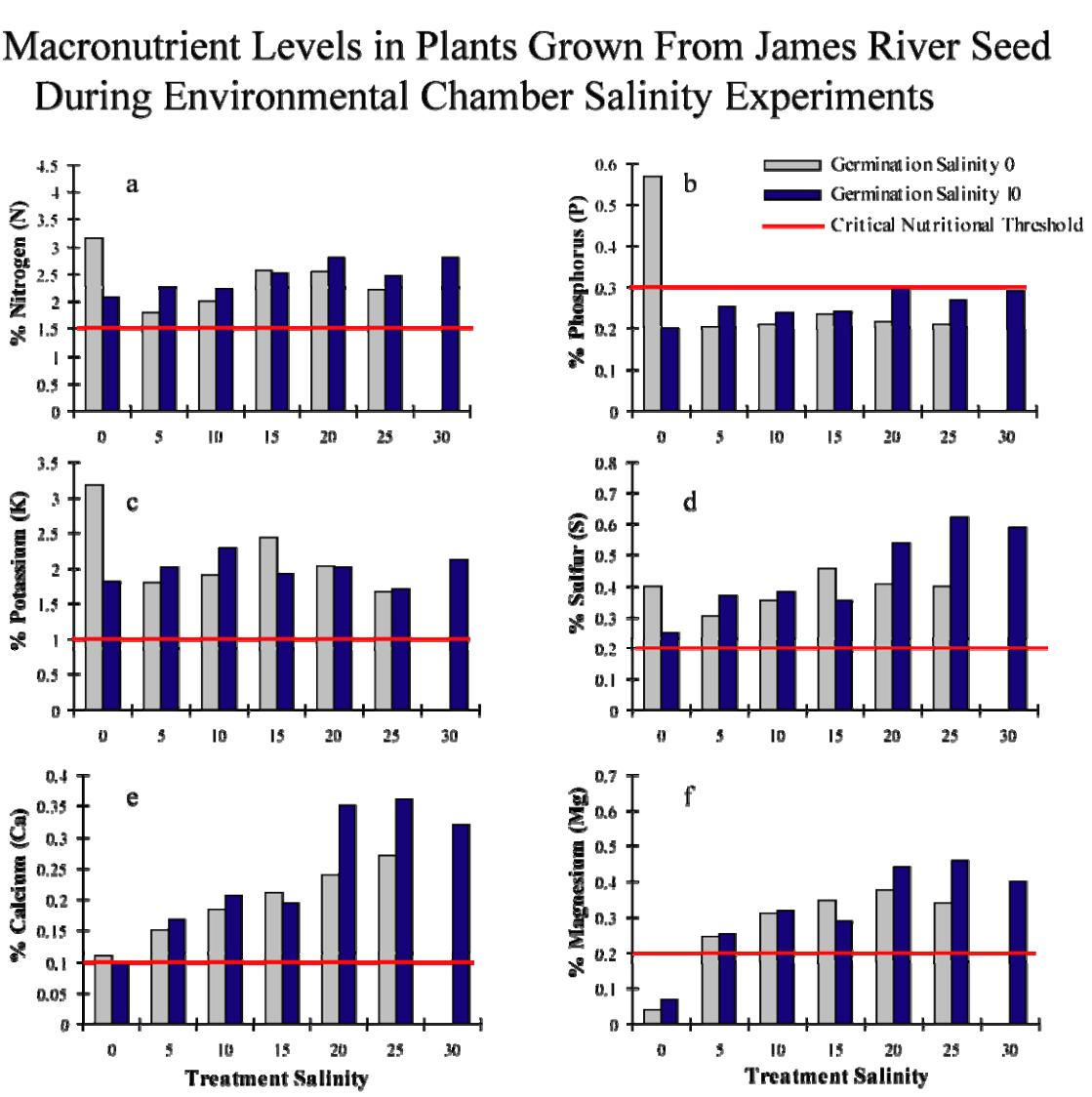

Figure 27a-f: Percentage of each macronutrient in tissue from *Spartina alterniflora* plants grown in one of 7 salinities for 12 weeks in an environmental chamber from seed collected from the Lower James River. Seeds germinated in either freshwater (salinity 0) or in saltwater (salinity 10) before being transferred to pots and receiving treatment. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

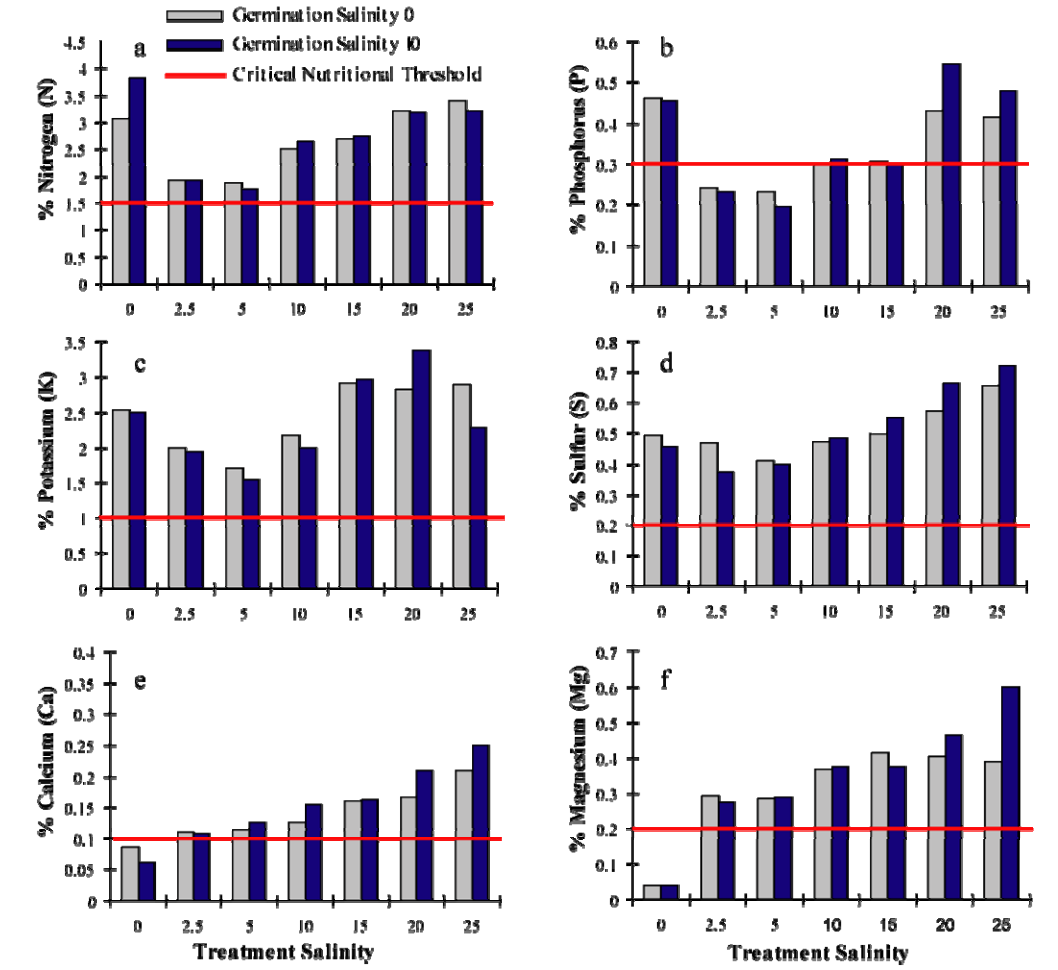

Figure 28a-f: Percentage of each macronutrient in tissue from *Spartina alterniflora* plants grown in one of 7 salinities for 12 weeks in an environmental chamber from seed collected from Horn Point Marsh. Seeds germinated in either freshwater (salinity 0) or in saltwater (salinity 10) before being transferred to pots and receiving treatment. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

P levels above the minimum requirement (0.3%) (Marschner 1995) were plants germinated and grown in fresh water with P levels of 0.58%. James River seedlings germinated in salt water and grown in salinities 20 and 30 had P levels just below the minimum requirement (0.29%) and all other treatments were quite P deficient (>0.27%). However the Horn Point seedlings, were P deficient in low salinity treatments (2.5 and 5) while the rest of the treatments were above the threshold (0.3%). Plants grown in fresh water (0) and high salinities (20 and 25) had the highest concentrations of phosphorus (0.46, 0.55 and 0.48% respectively).

Potassium (K) and Sulfur (S) levels were well above adequate for all plants grown from James River seed (Figure 27c and d) as well as those grown from Horn Point seed (Figure 28c and d). However at high salinities (15 to 25) K concentrations were higher in plants grown from Horn Point seeds. Calcium (Ca) levels were lowest in plants grown in fresh water for both sets of seeds (Figure 27e, Figure 28e) but were only deficient in plants grown in fresh water from Horn Point seed. Calcium concentrations in plant tissue increased with increasing treatment salinity and were highest in James River plants germinated in salt water and grown in salinities of 20 and higher. A similar trend was observed for plant tissue magnesium (Mg) levels (Figure 27f, Figure 28f). All plants grown in fresh water had Mg concentrations below the minimum requirement. All other treatments were similar for the two sets of seeds with the highest concentrations in plants grown from seeds germinated in salt water and grown in a treatment salinity of 25.

Concentrations of micronutrients in tissue of plants grown from James River seed and from Horn Point seed were adequate or nearly so for all measurements taken. Iron (Fe) levels did not vary across the treatment levels or between germination strategies for

James River seeds except in plants germinated in salinity 10 and grown in salinity 20 (Figure 29a) which had tissue Fe concentrations of 1085 ppm. Since there was not enough tissue available to perform replicate analyses, we cannot rule out that this may have been due to contamination in the sample. Iron levels in Horn Point seedlings were fairly consistent across the treatments ranging from 150 to 270 ppm.

Zinc (Zn) was most abundant in plants that were grown in fresh water for both batches of seeds (Figure 29b, Figure 30b) with the highest concentrations in plants whose seeds were germinated in fresh water (150 ppm for James River seedlings and 217 ppm for Horn Point seedlings). Zinc levels in plants from all other treatments were <100 but were all above the 15 ppm (Marschner 1995) threshold. Manganese in plants grown from James River seed (Figure 29c) was much higher than in those grown from Horn Point seed (Figure 30c) reaching 76 ppm when grown at salinity 20. All James River seedlings had Mn concentrations well above the 10 ppm (Marschner 1995) threshold while Horn Point seedlings that germinated in fresh water and grew in salinities of 5 and 10 just met this critical value. Aluminum (Al) concentrations were well above the nutritional threshold (50 ppm) (Marschner 1995) for higher plants in all treatments (Figure 29d, Figure 30d). However plants grown in the fresh and low salinity treatments had higher Al levels after having germinated in fresh water for both sets of seeds.

Boron (B) levels were lowest for all plants growing in fresh water (<7ppm) and increased with increasing treatment salinity, up to salinity 25 where B levels reached 38 ppm in James River seedlings and 36 ppm in Horn Point seedlings (Figure 29e, Figure 30e). With the exception of seedlings that grew in fresh water, all seedlings had B concentrations above the 5 ppm (Marschner 1995) threshold. Copper (Cu) concentrations

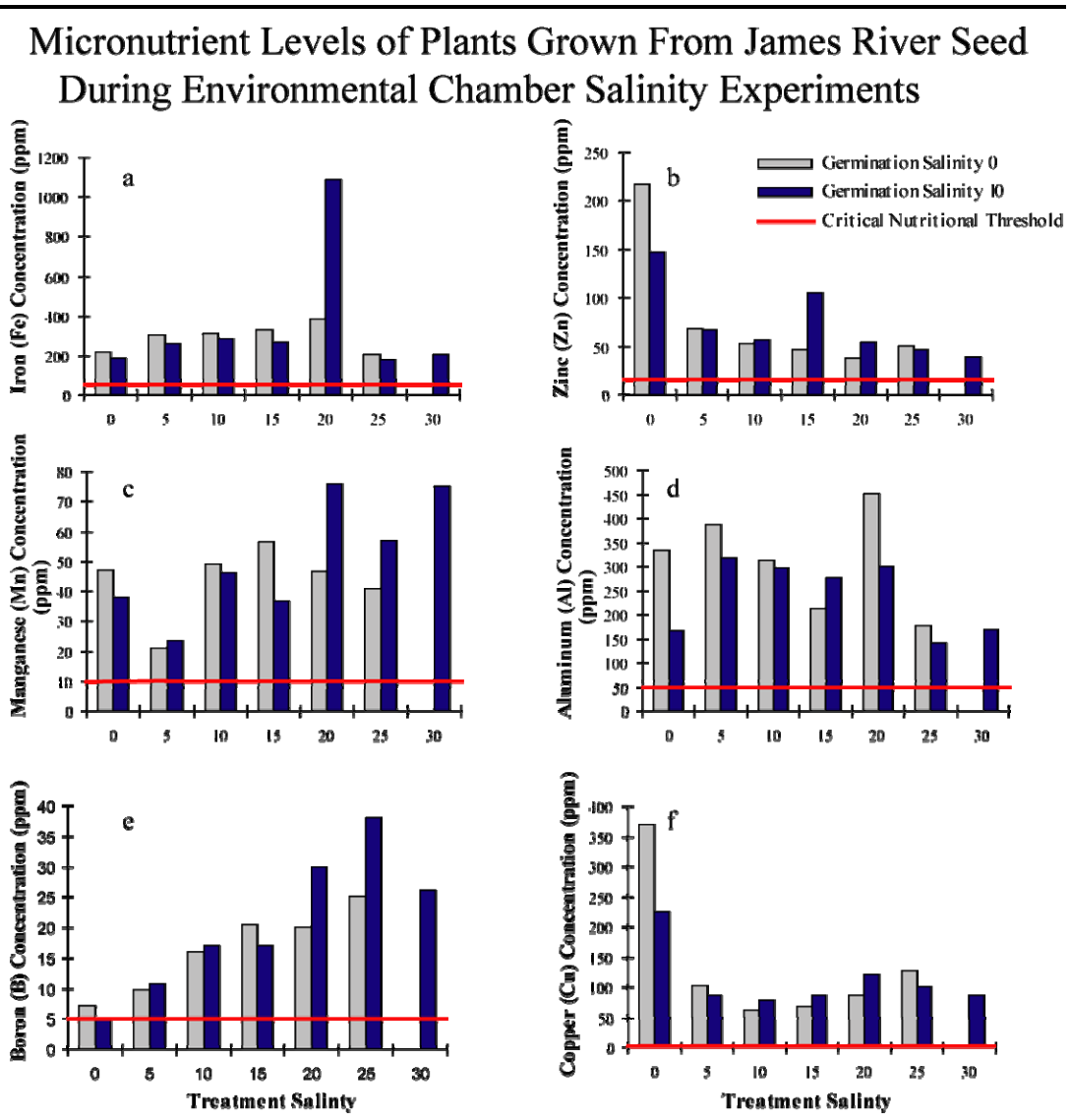

Figure 29a-f: Amount of micronutrients in parts per million (ppm) in tissue from *Spartina alterniflora* plants grown in one of 7 salinities for 12 weeks in an environmental chamber from seed collected from the Lower James River. Seeds germinated in either freshwater (salinity 0) or in saltwater (salinity 10) before being transferred to pots and receiving treatment. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

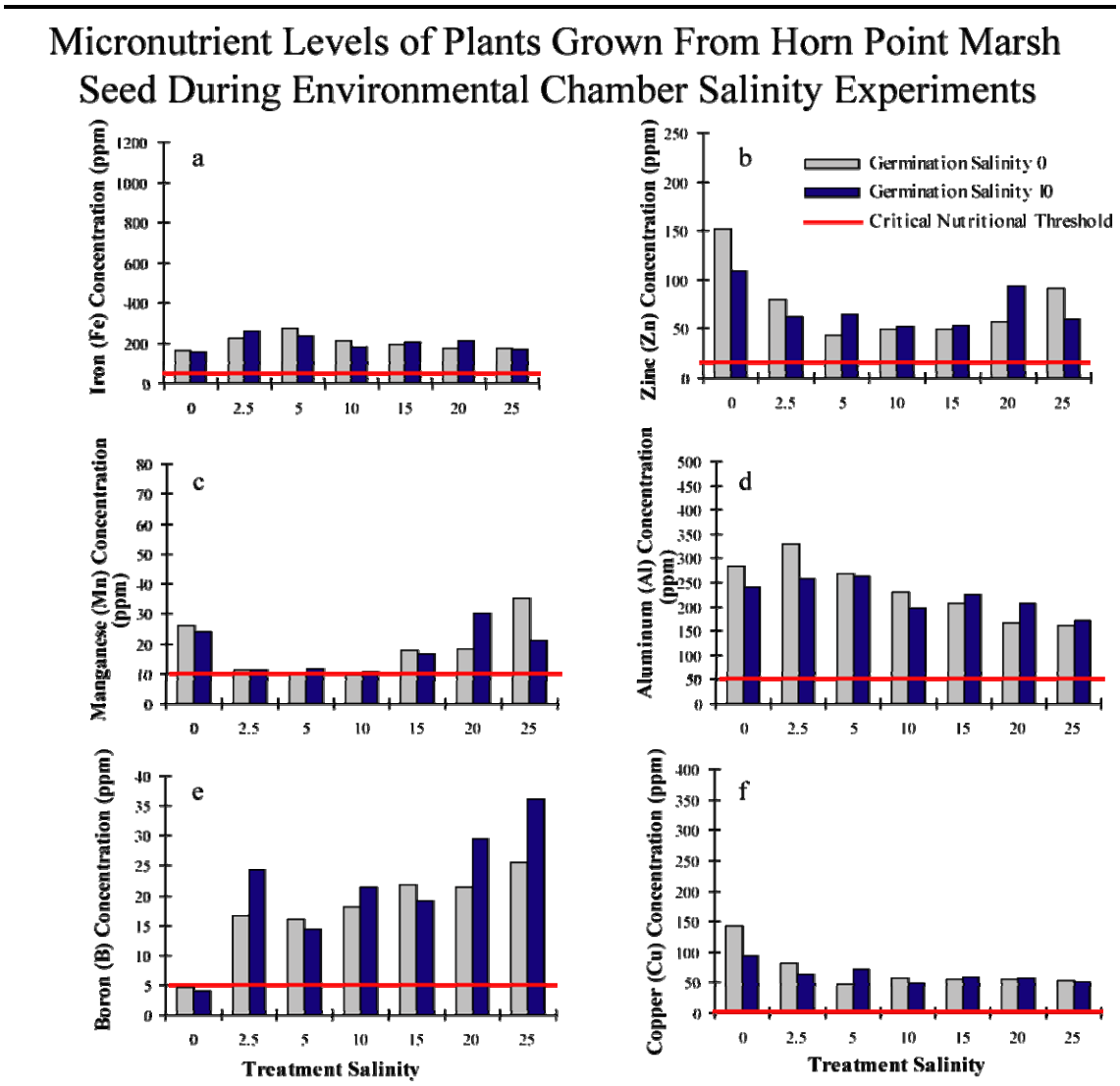

Figure 30a-f: Amount of micronutrient in parts per million (ppm) in tissue from *Spartina alterniflora* plants grown in one of 7 salinities for 12 weeks in an environmental chamber from seed collected from Horn Point Marsh. Seeds germinated in either freshwater (salinity 0) or in saltwater (salinity 10) before being transferred to pots and receiving treatment. The red line indicates the minimum amount of each nutrient required by most higher plants according to Marschner (1995) and Epstein and Bloom (2005).

were highest in plants that germinated and grew in fresh water for both sets of seeds (370 ppm for James River seedlings and 143 ppm for Horn Point seedlings) (Figure 29f, Figure 30f). Plants that were grown in salt solutions were somewhat lower but were similar to one another ranging from 60 to 120 in James River seedlings (Figure 29f) and 50 ppm to 80 ppm in Horn Point seedlings (Figure 30f). All seedlings contained well above the minimum amount of Cu (3 ppm) (Marschner 1995) required by most plants.

In *S. alterniflora* plants growing in different salinities, very little biomass production occurred in plants with no salt exposure. This is likely due to the fact that this species has a relatively high sulfur requirement that is satisfied by sulfate in sea salt. This was demonstrated by Stribling (1997) who measured increased shoot length and increased relative growth rates (RGR) in *S. alterniflora* plants exposed to increases in sulfate concentrations. In the growth chamber experiments the sulfur concentrations in plant tissue from plants growing in salinities of 20, 25 and 30 were higher than tissue concentrations of plants growing in lower salinities. Adams (1963) observed that *S. alterniflora* grown in fresh water became chlorotic and concluded the species a salt obligate that has the potential to invade upland areas if sea levels continue to rise. That may be so, but more interesting is the fact that it germinates best at the lowest salinities and may prosper after major freshets or some hurricanes that add substantial amounts of fresh water to marshes. However, since very little biomass accumulation occurred in plants grown in fresh water, we can concur that at least low levels of salt or sulfate are a requirement for *S. alterniflora.*

Although a difference in biomass production was higher in some of the seedlings that had germinated in freshwater than those that had germinated in salt water (James

River plants grown in salinities 5 and 10, and Horn Point plants grown in salinities 2.5 and 10), this was not consistent for all of the treatments. Biomass production was greatest at salinities of 5 for the James River seedlings and 2.5 and 5 for Horn Point seedlings and was decreased in all salinities above 5 for both sets of seeds. Since *S. alterniflora* is often found growing in areas where soil salinities can exceed that of sea water (>35) we had not anticipated biomass to be decreased at such low salt concentrations. Haines and Dunn (1976) found greater production in plants from a sandy beach at Sapelo Island, GA at salinities of 20 than salinities of either 5 or 40. Mooring et al. (1971) found optimum growth to occur near a salinity of 10 in plants from Ocracoke and Oak Islands, NC and Linthurst and Seneca (1981) determined that a salinity of 15 is optimum for biomass production. However Bradley and Morris (1992) measured greatest biomass in *S. alterniflora* plants from a salt marsh at North Inlet, SC that were grown in a salinity of 5 and a reduction in biomass as salinity increased when growing plants from rhizomes.

In halophytes, like glycophytes, cellular structures are sensitive to salt. Since accumulations of salt in the cytoplasm is toxic, high salt content of the cells is localized in the vacuoles requiring another osmotic component in the cytoplasm to lower the osmotic potential of the whole cell (Marschner 1995). Some species of plants such as tobacco and barley have been found to accumulate high amounts of the amino acid proline when exposed to water stress such as that caused by saline conditions. In *S. alterniflora* proline accumulation was initiated when substrate salinities neared sea water concentrations (Cavalieri and Huang 1979). Not only does this response to salinity increase the nitrogen requirement of the plants, but nitrogen being taken up may be going

towards production of nitrogen containing osmotic balancing compounds rather than new plant tissue (Bradley and Morris 1992). This may be an explanation of the lower biomass production in some experiments in plants growing in high salinities. However, analysis of plant tissue N concentrations did not confirm any significant increase in James River and Horn Point plants grown in high salinities; in fact they were lower.

In *Schoeneplectus triqueter* a European rush species that grows in marshes where average pore water salinities do not exceed 7, it was found that seedlings were more sensitive to the physiological stress of salinity than mature plants (Deegan et al. 2005). Likewise Haines and Dunn (1976) found that while maximum biomass accumulation in *Spartina alterniflora* seedlings occurred at salinities between 5 and 10, growth of well established *S. alterniflora* plants transplanted from a sandy beach at Sapelo Island, GA was unaffected at a salinity of 20. This may be an explanation of the low biomass production in seedlings growing in salinities of 10 and higher in our environmental chamber experiments. Perhaps the salt stress would not have been as great in more mature plants, where salt glands and other adaptive features have had more time to develop.

Another possible explanation of low biomass accumulation above salinities of 5 could be the seed source. While previous experiments (Haines and Dunn 1976; Mooring et al. 1971; Linthurst and Seneca 1981) were done with *Spartina alterniflora* plants obtained from coastal areas where salinities exceed 15, the seeds used for our experiment were obtained from marshes where salinities fall below 10. Possibly the plants used in our studies are an ecotype more adapted to lower salinity marshes. Gallgher et al. (1988) found that when tall and short forms of *S. alterniflora* were planted in a garden and left to

grow for several years, the differences in the two types were not as great as they were in the natural marsh. They suggest that morphological differences in growth forms of *S. alterniflora* in Delaware marshes could have several explanations. Either they are different genetic strains, not genetically different but influenced by the environment in which they are living, or they are fundamentally the same genetically but certain genetic characters are "turned on" at the seedling stage and persist even when exposed to another environment. Proffitt et al. (2003) suggest that the adaptive differences in growth rates, morphology and stress tolerance of different ecotypes of *S. alterniflora* may affect which ones maintain dominance under different environmental conditions. Using 5 genotypes of *S. alterniflora* from a Louisiana mudflat created with dredged material, they were unable to correlate morphological differences with salinity tolerance. However they did note a genotypic variance in the degree of leaf rolling in sea water strength salinity that suggested less tolerance of stressful salinities in some ecotypes.

Conclusions

Measurements of nutrients in the pore water of dredged material used in four experimental plots of Cell 4DX on Poplar Island show that nitrogen (as NH_4) is available in abundance for uptake by marsh plants. Tissue analysis of plants that were grown in this material show neither nitrogen deficiency nor any decreased nitrogen concentrations when compared to an existing Chesapeake Bay marsh. However N is deficient in sandy materials used throughout the cell and phosphorus was found to be in short supply in dredged material pore water as well as in plant tissue. To establish healthy marshes in dredged material on Poplar Island, the dredged material should be fertilized with

phosphorus. Plant tissue analyses also show that other macronutrients as well as essential micronutrients are made available to plants by dredged material. Although dredged material is high in iron, it is low in sulfides. As a result, acids are not likely to readily form in these soils as the material is oxidized and rehydrated. Although low pH is an issue to plant growth, the levels found in this study were not problematic. Saturation of the sediment however should be monitored carefully. Inadequate water supplies to surface sediments severely limits seed germination and seedling growth on dredged material and low sediment moisture limits growth in adult plants during times of maximum productivity. Salt water intrusions appear to limit the germination of *Spartina alterniflora* in mesohaline marshes and suggest that sexual reproduction may be limited to periods when salinities drop due to high precipitation and inflow. At Poplar Island the last major drop in salinity occurred during Hurricane Agnes in 1972. Therefore seeding of created marshes may not be feasible, except where it is possible to create brackish (salinities 2-10) conditions over a period of several weeks during spring and/or early summer.

Appendix

A&L Eastern Laboratories, Inc.

Standard Operating Procedures
For Plant Tissue Preparation and Analysis

Revised Date: April, 2003

INSTRUMENTS:

Wiley Mill Model 4 – Thomas Scientific Co., for large plant sample such as corn leaves
Cyclone Sample Mill – UDY Corporation, for small samples such as petioles
CEM Microwave Digestion System – Model: Mars 5.
Inductive Coupled Plasma Emission Spectrophotometer (ICP) – Fision, Model: Accuris.
LECO FP-42 Dry Combustion nitrogen analyzer

REAGENTS:

1. Nitric Acid – 70% ACS reagent grade.
2. Hydrochloric Acid – 37% ACS reagent grade.

QUALITY CONTROL REQUIREMENTS:

Analyze in house check sample each day samples are analyzed.
Analyze a blank sample each day samples are analyzed.
Monthly inter-laboratory check samples.
Outside performance samples are analyzed quarterly to evaluate accuracy.

PROCEDURE:

Sample Preparation:

Oven dry sample at 90-100^{o}C overnight, grind to pass 1mm sieve.

ICP (for elements other than N):

1. Weigh 0.2 g of sample into a 50 ml polypropylene centrifuge tube, add 2 ml of nitric acid and 0.5 ml of hydrochloric acid, tighten the screw type cap which has a small pre-drilled hole.
2. Microwave system is set at 5-minute ramp time to 103^{o}C and hold for 7 minutes. Power is set at 300W.
3. After microwave digestion is completed, let it stand for 5 minutes, add deionized water to 20 ml. The dilution factor is thus 100.
4. Analyze sample with ICP for S, P, K, Ca, Mg, Na, Zn, Mn, Fe, Cu, B, Al.

Nitrogen:

1. Run multiple blank and standard grade EDTA to set up LECO.
2. Weigh sample into a tin foil, wrap and drop in to the combustion tube, push start button to initiate the analytical process.

(Mills and Jones 1996)

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