2002

The combined effects of salinity and sulfide on the growth and physiology of the freshwater marsh plant Pancium hemitomon J.A. Schultes

James Wesley Pahl
Louisiana State University and Agricultural and Mechanical College

THE COMBINED EFFECTS OF SALINITY AND SULFIDE ON THE GROWTH AND PHYSIOLOGY OF THE FRESHWATER MARSH PLANT *PANICUM HEMITOMON* J.A. SCHULTES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

By
James Wesley Pahl
B.A., St. Mary's College of Maryland, 1993
December 2002

ACKNOWLEDGEMENTS

It might be typical and sappy, but first and foremost I have to acknowledge, and indeed I dedicate this dissertation to, my parents and my sister. They put a lot of effort into my formative years making sure I understood that, yes, it really was a good thing to develop and use my brain, though given my immense athletic and musical talents, I didn't really have much of a choice. Additionally, through it all (and they're probably blissfully ignorant of most of it) they never ceased to be anything but supportive of this endeavor.

Of course, it goes without saying that the opportunity for this endeavor would not have existed had it not been for the efforts of my advisor, Irv Mendelssohn. But it goes beyond simply providing an opportunity. In the past seven years I've developed into a scientist because of Irv's advisement and guidance. While I won't even begin to claim that my ideas about the way things should have been at times didn't ever conflict with his, at the end of it all I've come to appreciate the subtle nuances of his management style.

I would like to thank my committee, whom I regret to say I woefully underutilized during my tenure here: Drs. David Longstreth, Allan Biel, Ray Ferrell, Karen McKee, James Moroney and Charles Wilson.

I would especially like to thank the following research associates and graduate students, many of whom have now moved on to bigger and brighter degrees and careers of their own. Without the help of these people, much of this research would not have been possible: Chris Anastasiou (Southwest Florida Water Management District), Dr. Carlos Coronado Roman (South Florida Water Management District), Denise D'Abundo (LSU Department of Biological Sciences), Guerry Holm (LSU Coastal Ecology Institute), Nathan Kuhn (Texas Parks and Wildlife Department), Dr. Megan Lapeyre (US Geological Survey), Dr. Jeannine Lessmann (Eckerd College), Dr. Qianxin Lin (LSU Wetland Biogeochemistry Institute), Dr. Matt Poach (US Department of Agriculture), Gary Raulerson (Sarasota Bay National Estuary Program), Brenda Smith (University of Texas - Arlington) and Lee Stanton (LSU Department of Oceanography). In particular, thanks to Carlos, Denise and Lee for fighting a rather brutal heat during the middle of one of the worst droughts in south Louisiana's history to set up field sites, and to Chris for adroitly steering the boat through stormy seas that should've put the two of us on the bottom of Lake Salvador. Oh, and also to Megan, Gary and Brenda, for being players in a story about getting chased out of the water by an alligator that provided for an endearing bit of Irish wit (you'll have to ask me about it, since I probably can't write it in this document).

I'd also like to thank Kermit Coulon, Jr., and Jeff DeBlieux at Burlington Resources, Inc., and Clayton Hollier at Delta Farms for allowing the use of their respective properties on the shores of Bayou des Allemands and Lake Salvador for the field experimentation and for obtaining vegetation material for the growth chamber experimentation.

Never forget the bill payers. In addition to the numerous grants that were raided to pay for this research, I'd like to thank the Society of Wetland Scientists for providing a grant to help fund this work.

I'd like to finish by thanking the Academy …

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Vegetative response to saltwater intrusion into coastal freshwater wetlands is governed by the combined effects of increased salinity and porewater sulfide concentrations. I conducted a series of experiments to investigate the growth and physiological responses of the freshwater marsh plant *Panicum hemitomon* to increased salinity and sulfide, with the overall goal of understanding how these stresses operate in concert and individually. Specifically, the following questions were addressed:

1. What are the individual and combined effects of salinity and sulfide on the growth of *Panicum hemitomon*?
2. By what mechanisms are exposure to salinity and sulfide detrimental to the physiological processes affecting plant growth?
3. Are these stresses synergistic in their modification of such physiological processes, and which, if either, is dominant in its effect?

This research addressed the primary hypothesis that growth of *Panicum hemitomon* is adversely affected by an interaction between salinity and sulfide stresses associated with saltwater intrusion, and the mechanisms for decreased growth are alterations in the metabolic and morphological adaptations needed for a plant to survive in a flooded environment. Specific objectives were:

1. To determine the effect of salinity and sulfide stress on the growth of *Panicum hemitomon*, as defined by production of new biomass and culms.
2. To determine if the growth responses of *Panicum hemitomon* to salinity and sulfide are related to adaptations in plant morphology and fermentative and respiratory metabolism.
3. To determine if the results of controlled experiments on the effect of salinity and sulfide on *Panicum hemitomon* are accurate predictors of the growth and physiological response of plants under field conditions.

I exposed marsh sods to a factorial treatment arrangement of three salinities (0, 2, and 4 ppt) and three porewater sulfide concentrations (0, 0.5 and 1 mM) for 19 and 39 weeks. While salinity and sulfide both decreased relative growth rates in *P. hemitomon*, the salinity-induced growth inhibitions were more severe, particularly with regards to the belowground tissue. Additionally, there was a sulfide-induced stimulus in the production of adventitious tissue that was completely inhibited by elevated porewater salinities.

After 19 weeks, salinity at 4 ppt and elevated sulfide concentrations were deleterious to overall plant growth. A sulfide-induced growth stimulation in adventitious root production was inhibited at elevated salinities. After 39 weeks, elevated salinity at all concentrations was so stressful that the long-term effects of sulfide became inconsequential. Root cellular respiration under anaerobic conditions was higher under elevated sulfide, but this stimulation was also eliminated at higher salinity. A 12-week hydroponic exposure to elevated salinity and sulfide was subsequently initiated to further explore the response of physiological pathways associated with anaerobic fermentation capacity. Data from that experiment showed opposite effects of stressor treatment, with salinity stimulating and sulfide inhibiting root ethanol production.

A 3-month field experiment intended to validate the growth chamber experiments was complicated by drought-induced hypersaline conditions, but data did support the sensitivity of *P. hemitomon* belowground tissue to saltwater flooding, and reductions in the capacity to form aerenchymatous tissue for root tip aeration. These data led to the conclusion that the loss of *Panicum hemitomon* from the fresh marshes of coastal Louisiana is caused by both reduced growth and a reduced ability to adapt metabolically and morphologically to the highly-reduced edaphic conditions of a saltwater-flooded marsh.

INTRODUCTION

Sea-level rise (SLR), major storm events such as cold front and hurricane passages, and human-induced alterations to coastal hydrology can cause inland shifts in the relative position of the natural salinity gradient within the coastal zone. The resulting intrusion of saltwater threatens to alter the vegetative community structure of coastal ecosystems, where community structure is strongly influenced by this saltwater gradient (Warren and Niering, 1993; Latham et. al., 1994; Visser et. al. 1996). The vegetative character of specific marshes along this gradient is in large part governed by the ability of individual species to tolerate the stresses created by saltwater inundation. Natural events and human activities that result in the landward intrusion of saltwater thus threaten oligohaline and freshwater marsh plant communities that are not adapted to saline water exposure (Greenway and Munns, 1980).

Oligohaline marshes dominated by the grass *Panicum hemitomon* (maidencane) have been impacted by saltwater intrusion in the Northern Gulf of Mexico. Maidencane is a dominant species in many of the fresh marshes of the southeastern United States, and is normally considered salt-intolerant. It's replacement by more salt-tolerant species in Louisiana's coastal zone (Visser et. al., 1996) suggests that saltwater intrusion is the driving force behind these alterations.

Few field studies have attempted to separate the associated individual stressors from the overall intrusion phenomenon (Conner, 1994; Webb and Mendelssohn, 1996). While there has been an abundance of lab-based experimental research conducted on single component stressors associated with saltwater flooding, such as salinity or sulfide exposure (Bradley and Dunn, 1989; King et. al., 1992; Naidoo, 1994; Armstrong et. al., 1996a), studies that quantify both the individual and combined effects of component stressors associated with saltwater intrusion are comparatively rare (McKee and Mendelssohn, 1989; Naidoo and Mundree, 1993; Broome et. al., 1995). In particular, manipulative studies that have evaluated plant response to both elevated salinity and sulfide have been limited to brackish marsh species (Chambers et. al., 1998).

The oxygen content of flooded soils is quickly depleted by a combination of slower oxygen diffusion through water (Greenwood, 1961) and the continued biological oxygen demand of sediment microbes and plant root tissue (Turner and Patrick, 1968). When such conditions are associated with a steady source of organic carbon, anaerobic heterotrophic bacteria will use inorganic ions, such as SO_4^{2-}, as alternative terminal electron acceptors to O_2 during cellular respiration. Biochemical reduction of SO_4^{2-} produces H_2S, which can be toxic to plant growth at elevated concentrations (Havill et. al., 1985; Koch et. al., 1990; Armstrong et. al., 1996a). Freshwater plants are typically not exposed to concentrations of sulfide detrimental to plant growth (McKee and Mendelssohn, 1989; Pezeshki et. al. 1991) since sulfate levels in freshwater systems are commonly lower than 0.3 mM (Cole, 1988).

However, freshwater plants may be subjected to increased sulfide concentrations under saltwater intrusion events, where sulfate concentrations in saltwater may exceed 28 mM (Cole, 1988). Elevated concentrations of sulfide in the root zone can be detrimental to plant growth by scavenging interstitial oxygen and thereby forcing root tissue into anaerobic metabolism (Pearson and Havill, 1988), and through direct toxicity of the sulfide ion to plant cellular and biochemical processes (Ingold and Havill, 1985; Koch et. al., 1990). Saline water is also a direct stress on plant growth (Bradley and Morris, 1991; Drake and Gallagher, 1984; Feijtel et. al., 1989). Saline water has a lower water potential than both freshwater and many cellular tissues,

which can lead to initial problems with water uptake and loss in plants lacking the necessary metabolic and anatomical adaptations. Subsequent effects on vegetation result from direct exposure to seawater-borne ions such as sodium and chloride, which are directly toxic to plants and also interfere with nutrient uptake through competitive exclusion (Munns and Termaat, 1986).

Both salinity and sulfide are detrimental to plant growth (Bradley and Dunn, 1989; Feijtel et. al., 1989; Bradley and Morris, 1991; Rahman and Ungar, 1994; Chambers, 1997). Decreases in wetland plant growth can result from reductions in the metabolic and morphological adaptations needed to persist in a wetland environment. Enzymes associated with cellular aerobic respiration are susceptible to activity modification by environmental stressors such as salinity (Hernandez et. al., 1993) and sulfide (Allam and Hollis, 1972; Ingold and Havill, 1985; Beinert et. al., 1997). Anaerobic fermentation of fixed carbon often is an important adaptation in wetland plants where roots are subject to oxygen limitation during flooded conditions (Smith and ap Rees, 1979; Muench et. al., 1993; Bailey-Serres and Dawe, 1996). Primarily accomplished through the generation of ethanol via the enzyme alcohol dehydrogenase (ADH, EC 1.1.1.1), both the activity of ADH and the tissue generation of ethanol have been used as indicators of short-term stress response in flooded vegetation to root oxygen deficiency (Bertani et al., 1980; Mendelssohn et. al., 1981; Studer and Braendle, 1987; Chan and Burton, 1992).

Contradictory evidence of both stimulatory and inhibitory effects of both salinity (Naidoo et. al., 1992; Naidoo and Mundree, 1993; Misra and Dwivedi, 1995; Akhtar et. al., 1998) and sulfide on ADH activity illustrates the need for controlled experimentation to elucidate the exact response of an oligohaline species such as *P. hemitomon* to the physiological stresses associated with saltwater intrusion. Effects of sulfide on ADH activity may reflect the different modes by which sulfide may serve as an environmental stressor. Sulfide mimics or heightens waterlogging stress by scavenging oxygen from the rhizosphere, and should result in an increase in ADH activity as the plant compensates for root oxygen deficiency (Mendelssohn and McKee, 1987; Pearson and Havill, 1988). However, if sulfide-rich water infiltrates into root tissue (Carlson and Forrest, 1982; Koch and Mendelssohn, 1989; Raven and Scrimgeour, 1997), cellular components may be exposed to the direct toxicity of the sulfide ion (Dolferus et. al., 1997; Kleifeld et. al., 2000) and ADH activity may be inhibited (Koch et. al., 1990).

While alterations in metabolism may suffice in the short-term, longer-term changes in plant anatomy are necessary to allow wetland plants to tolerate the long-term soil anaerobiosis that can result from either frequent flooding or continuous waterlogging (Blom et. al., 1990; Jackson and Armstrong, 1999). Plants may respond to deleterious edaphic conditions by forming adventitious or water roots (Jackson, 1985) that emerge from stems above the soil surface to uptake oxygen and nutrients from a less inhospitable environment than the more highly-reduced and potentially phytotoxin-rich flooded soil. Adventitious root development is sensitive to salt stress (Huang et. al., 1995) in non-halophytic vegetation, though, and the possible loss of this adaptive ability may threaten the survival of salt-sensitive species and alter the community structure of coastal freshwater marshes that are subject to SLR.

Flooded plants may also develop aerenchymatous tissue in the roots, which allows for the supply of oxygen to root tips that would otherwise be oxygen-starved in the highly-reduced marsh soils. When the supply of oxygen to the roots is in excess exceeds f that needed by the root tissues, oxygen diffusion out into the surrounding rhizosphere may occur, resulting in an increase in E_h (Flessa and Fischer, 1992), the oxidation of reduced chemical species such as Fe^{2+}, Mn^{2+}, and S^{2-} in the soil or onto root plaques (Mendelssohn, 1993; Sunby et. al., 1998), thus

precipitating potential phytotoxins. The development of aerenchyma in flooded plants is sensitive to both elevated salinity (Naidoo and Mundree, 1993; Akhtar et. al., 1998) and sulfide (Armstrong et. al., 1996b).

While controlled laboratory or growth chamber investigations are necessary to quantify plant growth and physiological response to salinity and sulfide, results gained under such conditions may not accurately predict the state of field populations of freshwater marsh plants subjected to saltwater intrusion events. For example, while laboratory experiments have demonstrated both salinity and sulfide stress to the growth and physiology of common reed, *Phragmites australis* (Fürtig et. al., 1996; Adams and Bate, 1999; Armstrong and Armstrong, 2001), that species is persistent in the field under both high salinity and sulfide (Chambers et. al., 1998; Adams and Bate, 1999). Chambers et. al. (1998) suggested that the effects of sublethal stresses might leave *P. australis* susceptible to being out-competed in the field. This could be the case with *P. hemitomon*, which has demonstrated sublethal stress response to oligohaline conditions (McKee and Mendelssohn, 1989; Koch and Mendelssohn, 1989; Koch et al., 1990; Hester et. al., 1998). The deltaic plain of the Mississippi River, where relative SLR is an order of magnitude higher than the past century's eustatic mean, is an ideal laboratory to conduct field experimentation to determine if the results of our controlled experiments on the effect of salinity and sulfide on *Panicum hemitomon* are accurate predictors of plant performance in the natural environment.

OBJECTIVES AND EXPERIMENTATION

The goal of this dissertation was to determine the interactive effects of salinity and sulfide on the growth and physiology of *Panicum hemitomon*. The primary hypothesis of the research was that:

> The growth of *Panicum hemitomon* is adversely affected by an interaction between salinity and sulfide stresses associated with saltwater intrusion, and the mechanisms for decreased growth are alterations in the metabolic and morphological adaptations needed for a plant to survive in a flooded environment.

This dissertation contains several working objectives to address the primary hypothesis, organized into a series of controlled-environment growth chamber experiments and field manipulations. Those objectives were:

1. To determine the effect of salinity and sulfide stress on the growth of *Panicum hemitomon*, as defined by production of new biomass and culms.
2. To determine if the growth responses of *Panicum hemitomon* to salinity and sulfide are related to adaptations in plant morphology and fermentative and respiratory metabolism.
3. To determine if the results of controlled experiments on the effect of salinity and sulfide on *Panicum hemitomon* are accurate predictors of the growth and physiological response of plants under field conditions.

MATERIALS AND METHODS

EXPERIMENTS TESTING STRESSES ASSOCIATED WITH SALTWATER INTRUSION

Experiment One: Plant Growth Response in Intact Marsh Sods after 39 Weeks

Freshwater marsh sods were used as the growth substrate. PVC cylinders (15 x 60 cm) were used to collect *Panicum hemitomon* – dominated sods with intact vegetation from a tidal freshwater marsh on the western shore of Bayou des Allemands (29˚52'44"N, 90˚31'59"W) in the upper reaches of the Barataria Estuary in southeastern Louisiana on 18 December 1995. In the lab, all aboveground growth was removed, sods were cut to a depth of 35 cm from the peat surface, pea gravel was placed under the sod for drainage, and the cylinder units were capped and the bottom sealed. Three holes were drilled on each side of the PVC cylinders at 5, 15 and 25 cm below the surface of the sod, and rubber septa inserted to provide for injection of sulfide solutions into the sod. Interstitial water samplers (as per McKee et. al., 1988) were inserted to a depth of 20 cm below the sod surface to sample interstitial water from the center of the core. The experimental units were placed in a plant growth chamber (EGC Corp., Chagrin Falls, OH, USA) and *Panicum* culms were grown from the existing rootstock (Figure 1). Photoperiod inside the growth chamber was 14 hours at 500 µmol photons m^{-2} s^{-1}, with a 'daytime' temperature of 30˚C and a 'nighttime' temperature of 25˚C. Sods were held with 15 cm of overlying water to maintain an anaerobic soil environment.

Beginning 7 May 1996, *Panicum* sods were subjected to a 3x3 factorial treatment arrangement of salinity and sulfide for a period of 39 weeks to simulate a long-term saltwater intrusion event. Salinity levels of 0, 2 and 4 ppt, and sulfide concentrations of 0, 0.5, and 1 mM were established according to previous research identifying treatment levels sub-lethal to growth of *P. hemitomon* (McKee and Mendelssohn, 1989; Koch et. al., 1990). The salinity level of the overlying floodwater was adjusted to target concentrations in 2-ppt increments every three days. The saline floodwater used was a solution of an artificial sea salt lacking any sulfate salts that might increase sulfide concentrations in the anaerobic interstitial water environment (Table 1). Floodwater salinity was monitored throughout the duration of the experiment and adjusted as necessary through the addition of either distilled or saline water. Salinity was measured with a Model 2441 Salinity Refractometer (Atago Co., Ltd., Japan), zeroed with de-ionized H_2O.

Interstitial sulfide treatments were established by injecting an aqueous solution of 50-mM $Na_2S•9H_2O$ into the root zone. Sulfide concentrations were determined by mixing 5 ml of interstitial water, sampled from the center of the sod, with 5 ml of antioxidant buffer (0.8M salicylate, sodium salt, 1.1M NaOH, 0.2M ascorbate; Lazar Research Laboratories, Los Angeles, California, USA). Sulfide ion concentration was measured with a Lazar Model ISM-146 ion-specific microelectrode coupled to a Lazar Model DJM-146 reference electrode. A standard curve was prepared with $Na_2S•9H_2O$ and measured in the same manner as the samples. Sulfide concentrations were increased incrementally to $\pm$20% of the target concentrations and were maintained through subsequent injections of the same solution.

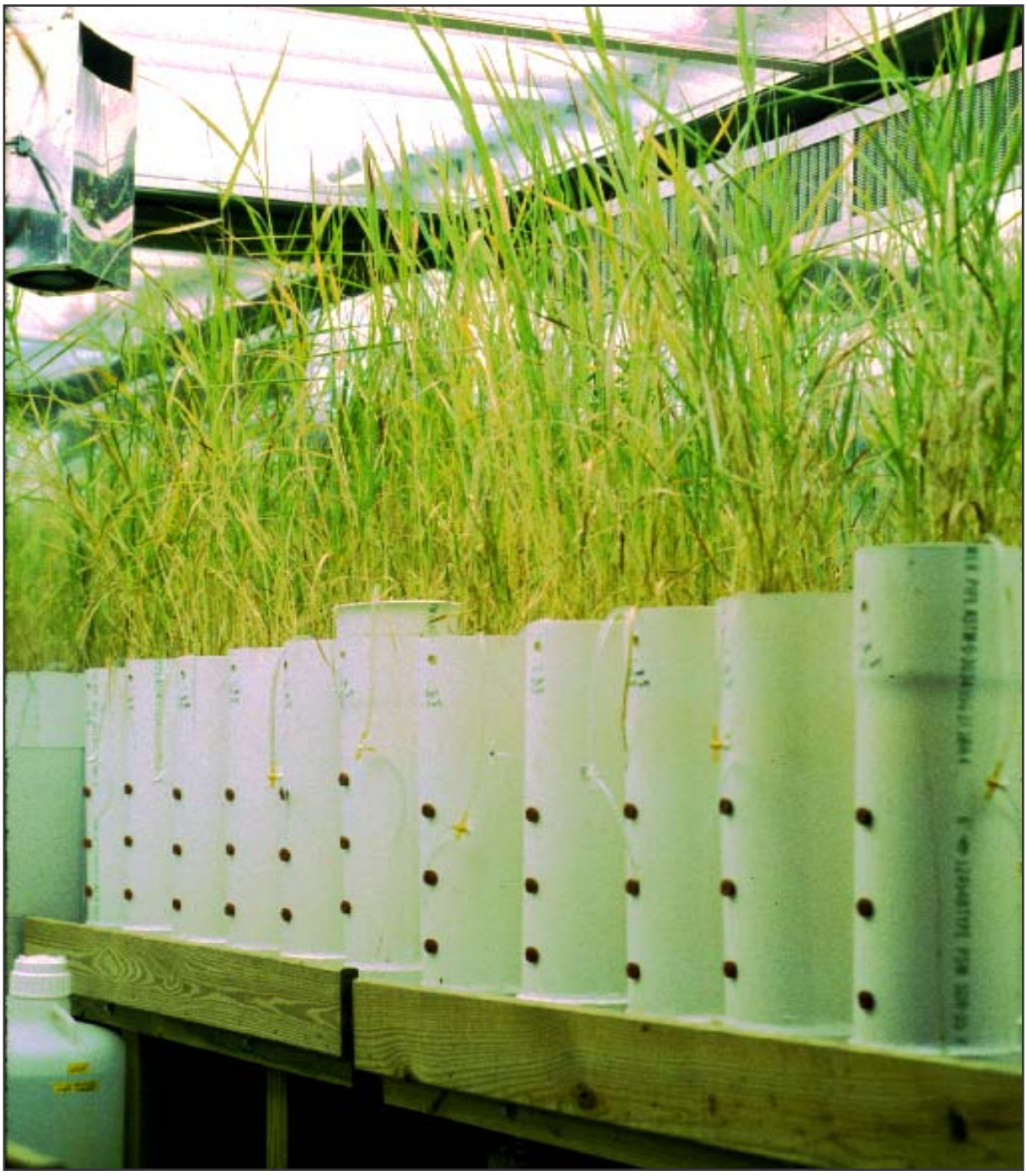

Figure 1. Photograph of EGC Corp. growth chamber during 39-week experiment, showing plants growing in experimental units.

Table 1. Modified version of Marine Biological Laboratory's trace solution formula for artificial seawater used to establish floodwater salinity concentrations in the 39-week experiment. MBL's trace solution formula according to Bidwell and Spotte (1985).

Salt Component	*Concentration*
NaCl	423 mM
$MgCl_2 \cdot 6H_2O$	22.9 mM
$Mg(NO_3)_2 \cdot 6H_2O$	12.8 mM
$MgHPO_4 \cdot 3H_3O$	12.8 mM
$CaCl_2 \cdot 2H_2O$	9.25 mM
KCl	8.32 mM
$NaHCO_3$	2.14 mM
KBr	0.748 mM
$SrCl_2 \cdot 6H_2O$	0.139 mM
H_3BO_3	0.388 mM
NaF	71.4 µM
EDTA-NaFe	0.179 µM

Soil oxidation-reduction potential (E_h) was measured on 17 August, 12 October and 18 November 1996 with bright platinum electrodes coupled to a calomel reference electrode (Dow Corning). Electrodes were checked prior to measurement with quinhydrone in a pH 4 buffer (218 mV at 25˚C). E_h measurements in the sods were calculated by adding 244 mV to account for the potential of the calomel electrode against a standard hydrogen electrode. Triplicate electrodes spaced randomly throughout the sod were read and values averaged within the experimental unit by depth.

Aboveground vegetation was clipped at the soil surface and segregated into live and dead stems. Adventitious roots, defined as those roots emerging from plant stems above the soil surface, were collected for separate biomass analysis. Dry mass was determined for all tissue types after drying to a constant mass in a 60˚C forced air oven. Growth rate of aboveground biomass and the percentage change in the total and live stem density between experiment initiation and termination were determined. Initial plant dry masses in the experimental units were predicted from a regression analysis of stem height vs. dry mass prior to initiation of the experiment. The mathematical relationship between *Panicum* culm height and aboveground dry mass was determined from field samples and the following polynomial regression was developed:

$$\text{Log}_{10}\,\text{Biomass} = -1.74 * (0.0038*SH) - (.00000205*SH^2) + (0.00000000038*SH^3)$$

where SH = stem height
$R^2 = 0.9263$, $P < 0.0001$
$n = 110$.

This regression was then used to predict the initial aboveground dry mass of the experimental plants based on the measurement of total stem height of those plants prior to imposition of the experimental treatments. The calculation of relative growth rate of aboveground biomass was:

$$RGR = \frac{(\ln(HarvestedAGDW)) - (\ln(InitialAGDW))}{time_{days}}$$

where AGDW is aboveground dry weight.

Percent changes in stem density were calculated by comparing the stem density at the time of harvest to that of the same plant measured prior to placement in the experimental unit. The biomass values used to calculate RGR are reported in the appendix.

Experiment Two: Plant Growth Response in Simulated Marsh Soil after 19 Weeks

An artificial soil mixture was used as the growing medium for the plants in this experiment, which was conducted for a shorter duration than Experiment One to limit the stress-induced mortality in the vegetation seen in some of the extreme salinity and sulfide treatments. Marsh peat from below the root zone was collected from a *Panicum hemitomon*-dominated fresh marsh on the western shore of Bayou des Allemands within the Barataria Estuary in Louisiana (29˚51'40"N, 90˚31'09"W) and mixed in a 2:1 ratio (v/v) with commercial potting soil (Southland Indoor/Outdoor Potting Soil, Southern Importers, Inc., Greensboro, NC, USA). This mixture was placed in 15-cm diameter PVC cylinders to a depth of 25 cm. *P. hemitomon* transplants were grown from rhizomes obtained from Horticultural Systems, Inc. (Parrish, FL, USA) in a soil medium that was a 2:1 mix (v/v) of Jiffy-Mix Plus (Jiffy Products of America, Inc., Batavia, IL, USA) to sand until an average of five culms reached a cumulative aboveground height of 500 cm. Plants were removed from the pots, washed of all soil, and placed in the soil within the PVC cylinders to establish artificial marsh sods. These cylinders were similar to those used in the 39-week experiment, except that only two septa were placed on each side of the cylinders at 5 and 15 cm below the soil surface for injection of the 50-mM $Na_2S \bullet 9H_2O$ solution. Interstitial water samplers were inserted to a depth of 15 cm as in the 39-week experiment. The experimental units were then placed in a growth chamber for the 19-week experiment. Growth chamber conditions were as described above.

The same 3x3 factorial treatment arrangement as described in the 39-week experiment (0, 2, and 4 ppt salinity, and 0, 0.5 and 1 mM sulfide) was initiated on 27 October 1998 with the exposure of the plants to saline floodwater, and the first injection of sulfide on 24 November 1998. The salinity level of the overlying floodwater was adjusted to target concentrations in 2-ppt increments every 3 days. The saline floodwater used was a solution of artificial sea salt (Instant Ocean, Aquarium Systems, Mentor, OH, USA). Both floodwater and interstitial water salinity were monitored throughout the experiment as described above and adjusted as necessary through the addition of either distilled or saline water to the overlying floodwater or injected into the root zone though septa on the side of the experimental unit. Interstitial sulfide concentrations were established and maintained as in the 39-week experiment. Oxidation-reduction potential (E_h) within the soil was measured on 30 December 1998, and 13 January, 21 March and 11 April 1999, at 20-cm soil depth. E_h measurements and samples for interstitial water chemistry were taken and analyzed as described above.

Vegetation within the cores was harvested either upon mortality of all aboveground material, or upon termination of the experiment on 12-14 April 1999. Aboveground material was cut at the soil surface and segregated into live and dead stems, and adventitious roots were sampled for separate biomass analysis. Belowground biomass was washed of all soil using tap water, and dry mass was determined for all tissue types after drying to a constant mass in a 60˚C oven.

Growth rates of aboveground, belowground, and total (aboveground + belowground) tissues between experiment initiation and termination were determined following the extrapolation of predicted initial dry weight of the plants prior to placement in the experimental units. Extra plants (n=22) were separated into aboveground and belowground tissues, and both fresh mass and dry mass were determined. The ratio of initial aboveground to belowground biomass was 1, and water content averaged 81.7% for the aboveground tissue and 84.3% for the belowground tissue. Based on these values, predicted initial aboveground and belowground dry masses were calculated. Relative growth rates and percentage change in stem density parameters were calculated as above. The biomass values used to calculate RGR are reported in the appendix.

At harvest, the terminal 10 cm of live belowground root tissue was sampled for respiratory analysis via carbon dioxide evolution. Approximately 1 g of live root material was placed in a 15-ml glass scintillation vial, which was incubated in a 30˚C water bath during the assay. Carbon dioxide exchange rate was determined using an ADC Corp. Model CLA2 Infrared Gas Analyzer, under both an atmosphere of ambient air for the measurement of aerobic respiration and of nitrogen for the measurement of anaerobic respiration.

Experiment Three: Plant Growth and Physiological Response in Hydroponics after 12 Days

This experiment utilized hydroponics as the growth media to allow for rapid sampling of belowground tissue. *Panicum hemitomon* rhizomes were obtained from a tidal fresh marsh on the western shore of Bayou des Allemands (29˚52'44"N, 90˚31'59"W) in the upper reaches of the Barataria Estuarine Basin in southeastern Louisiana on 16 February 2000. Rhizomes were washed, cut to a standard length of approximately 8 cm, and planted in a 2:1 mixture of Jiffy Mix (Jiffy Products of America, Inc., Batavia, IL, USA) and sand. New culms sprouted in a walk-in growth chamber (EGC Corp., Chagrin Falls, OH, USA) set to a 14-hour day length at 500 µmol photons m^{-2} s^{-1}, with a 'daytime' temperature of 30˚C and a 'nighttime' temperature of 25˚C.

After two months, when cumulative culm height above the rhizome reached approximately 800 mm, plants were washed of all soil material, all but one culm were clipped at the rhizome, and the plants were placed in groups of eight in 3-gallon hydroponic containers (Rubbermaid Roughtote, Rubbermaid Home Products, Wooster, OH, USA) to pre-condition the plants to aerobic, hydroponic conditions. The growth media was a modified ½-strength Hoagland's Solution (Table 2), constantly bubbled with air during the pre-conditioning phase and buffered with $CaCO_3$ to prevent acidification of the Hoagland's media resulting from root proton release due to the presence of ammonium salts.

Following two weeks of hydroponic pre-conditioning, plants were placed into the experimental units (Figure 2). Two 1000-ml Nalgene polypropylene specimen jars, stacked on

Table 2. Chemical constituents and their concentrations in the modified Hoagland's solution used in the 12-day experiment. The solution was buffered with 0.5 g $CaCO_3$ l^{-1} solution and pH was adjusted to 6.5.

Chemical Component	*Concentration*
NH_4Cl	0.4 mM
KH_2PO_4	0.5 mM
$MgSO_4 \cdot 7H_2O$	1.0 mM
$CaCl_2 \cdot 2H_2O$	2.5 mM
KCl	2.5 mM
H_3BO_3	22.5 µM
$MnCl_2$	4.5 µM
$ZnSO_4 \cdot 7H_2O$	0.5 µM
$CuSO_4 \cdot 5H_2O$	0.15 µM
MoO_3	0.07 µM

top of one another, were used for the experimental units. The exterior of the bottom chamber was painted silver to minimize the exposure of the hydroponic solution to light and prevent algal growth. Two 1.5-cm holes were drilled in the bottom chamber and rubber septa were inserted to provide for injection and sampling of sulfide solutions into the hydroponic solution. The upper chamber was formed by drilling out the bottom of the second 1000-ml sample jar, inverting it and sealing it to the upper rim of the lid of the lower chamber with silicone adhesive. A section of stiff airline connected the lower chamber with a scintillation vial taped to the apparatus exterior to serve as a gas trap. A hole drilled in the center of the lid of the bottom chamber allowed the plant to be positioned so that the roots and rhizomes would float midway in the bottom chamber, while the stem and leaves would emerge into the upper chamber. The plant was fixed into position using a non-toxic silicone caulk (GE Translucent RTV 128 Silicone Rubber Adhesive Sealant, GE Silicones, Waterford, New York, USA) to seal the hole in the lid of the lower chamber.

The experiment utilized a factorial arrangement of three salinity (0, 2 and 4 ppt) and sulfide (0, 0.3 and 0.6 mM) treatments. The lower chamber of the experimental unit was meant to replicate the anaerobic conditions of a flooded soil. Unbuffered 1/2 strength Hoagland's Solution growth media (as above) was bubbled with nitrogen gas prior to plant introduction into the lower chamber, which was quickly sealed following placement of the plant in the lid. Salinity of the growth media was increased in 2-ppt increments every two days until the final target salinity was reached using additions of NaCl. Sulfide concentrations in the bottom chamber were raised in 0.3-mM increments every two days until final target concentration was achieved. Interstitial sulfide concentrations were established by injecting an aqueous solution of 50-mM $Na_2S \cdot 9H_2O$ into the root zone through the septa on the side of the lower chamber. Sulfide concentrations were increased incrementally until within ±0.06 mM in the 0.3-mM

Figure 2. Photograph showing hydroponic setup used in 12-day experiment. Description for apparatus is in Materials and Methods.

treatments and ±0.12 mM in the 0.6-mM treatments, and were then maintained within that range through subsequent injections of the same $Na_2S•9H_2O$ solution. Sample aliquots of the lower chamber hydroponic solution were obtained through the septum using a syringe, and sulfide concentrations were determined as described in Experiment One. Once the salinity and sulfide treatments were set in the lower chamber, the upper chamber was flooded in 5-cm increments per day with a NaCl solution matching the salinity in the lower chamber. Treatment was initiated once plants were flooded to a depth of ten centimeters. Plants were exposed to the salinity*sulfide treatment combination for twelve days.

Plant gas exchange parameters were measured prior to harvest. Net photosynthesis, transpiration rate and stomatal conductance were measured using a CID Inc. Model CI-301 CO_2 Portable Photosynthesis System (CID Inc., Camas, WA, USA). At the end of the experiment, plants were harvested for physiological and growth parameters. Aboveground vegetation was clipped where the culm emerged from the rhizome and segregated into live and dead tissue. Belowground tissue was identified as the remaining roots and rhizome. The terminal five centimeters of live roots were taken from the bottom chamber under a flow of nitrogen gas, and either immediately frozen in liquid nitrogen for assaying alcohol dehydrogenase activity or placed on moistened filter paper for immediate analysis of ethanol production (both as per Chabbi et. al., 2000). Percent recovery of internal standard for the assay was 116±8% (n=4). The remaining plant material was separated into aboveground (stem and leaf) and belowground (root and rhizome) compartments, dried at 65˚C and dry mass taken for biomass production analysis. The biomass values used to calculate RGR are reported in the appendix.

Field Experiment

Panicum hemitomon rhizomes were collected from a tidal fresh marsh on the western shore of Bayou des Allemands in the middle reaches of the Barataria Estuary on 16 February 2000 (29˚52'44"N, 90˚31'59"W; shown as "des Allemands" in Figure 3). Rhizomes were washed, cut to a standard length of approximately eight centimeters, planted in a 2:1 mixture of Jiffy-Mix (Jiffy Products of America, Batavia, IL, USA) and sand, and subsequently allowed to sprout new culms in a walk-in growth chamber (EGC Corp., Chagrin Falls, OH, USA). Photoperiod inside the growth chamber was set at 14 hours at 500 µmol photons m^{-2} s^{-1}, with a 'daytime' temperature of 30˚C and a 'nighttime' temperature of 25˚C. Plants were then removed to a greenhouse and allowed to grow in 8-liter plastic pots in the same soil mix until used in the field manipulation. On the sides of the pots were drilled twelve 2.5-cm diameter holes so that ambient groundwater could circulate through the plant's root zone once placed at the field sites. Interstitial water samplers (McKee et. al., 1988) were inserted to a depth of 15 cm below the soil surface for sampling of interstitial water from the center of the pot.

Field manipulations involving the potted plants were performed in the summer and fall of 2001 within the Barataria Estuarine Basin in coastal Louisiana. I intended to reproduce the factorial design of salinity and sulfide treatments used in the growth chamber experiments described above by transplanting pots into different salinity marshes, and by manipulating transplant elevation within those marshes. On 20 July 2001, the "des Allemands" site was established in a *P. hemitomon*-dominated tidal freshwater marsh in the upper freshwater reaches of the basin, on the western shore of Bayou des Allemands. This was the same *P. hemtimon*-dominated marsh where the source rhizomes were obtained in February. One 22 July 2001, the "Salvador" site was established in an oligohaline marsh dominated by *Spartina patens* and

Sagittaria lancifolia, along the southeastern shore of Lake Salvador (29˚39'44"N, 90˚16'07"W; Figure 3).

At each marsh, a weedeater was used to remove aboveground vegetation from a 20-meter x 5-meter area, which was then organized into five 2-meter x 5-meter blocks, separated by 2-meter buffers. Within each marsh, replicate pots were transplanted into the marsh peat so that the soil surface of the pot was either equal to that of the ambient marsh or 15 cm below the marsh surface. Webb and Mendelssohn (1996) demonstrated that decreasing the elevation of a *S. lancifolia*-dominated marsh sod by 15 cm resulted in a decrease in redox potential and an increase in interstitial sulfide concentrations. Eight plants (four replicates of each elevation) were planted in each block, for a total of forty plants per site.

Plants were harvested at the des Allemands marsh on 29 August, 23 September and 9 November, representing 34, 65 and 112 days after site establishment, respectively. Plants were harvested at the Salvador marsh on 31 August, 24 September and 10 November, representing 34, 64 and 111 days after site establishment, respectively. Soil oxidation-reduction potential (E_h) was measured at the time of harvest as described above at both 2-cm and 20-cm soil depths. Interstitial water samples were taken from the center of the pot at harvest for nutrient and elemental analysis using the interstitial water samplers.

Aboveground plant material was clipped and sorted by live and dead upon return to the lab, and dry weight determined following drying in a 65˚C oven to a constant mass. Belowground material was washed on site in either ambient bayou or lake water for the des Allemands or Salvador marsh site, respectively. Root material 5-10 cm distal from the root tip were clipped and placed on wetted filter paper in petri dishes for porosity analysis upon return to the lab. *Panicum hemitomon* roots do not develop maximum aerenchyma until 5 cm distal from the root tip (Pahl, unpublished data). Root porosity was determined as per Burdick (1989). Remaining belowground material was washed in tap water, and dry weight determined following drying in a 65˚ C oven to a constant mass.

Relative growth rates of aboveground, belowground, and total (aboveground + belowground) tissues between experiment initiation and termination were determined following the extrapolation of predicted initial dry weight of the plants prior to placement in the experimental units. Initial plant dry masses in the experimental units were predicted from a regression analysis of stem height vs. dry mass prior to initiation of the experiment. The biomass values used to calculate RGR are reported in the appendix. The mathematical relationship between *P. hemitomon* culm height and aboveground dry mass was determined from a combination of field samples and extra potted plants and the following polynomial regressions developed:

$$Aboveground\ Biomass = 0.02876 + 0.00046*SH$$

where SH = stem height
Adjusted $R^2 = 0.4800$, $P < 0.0001$
$n = 60$,

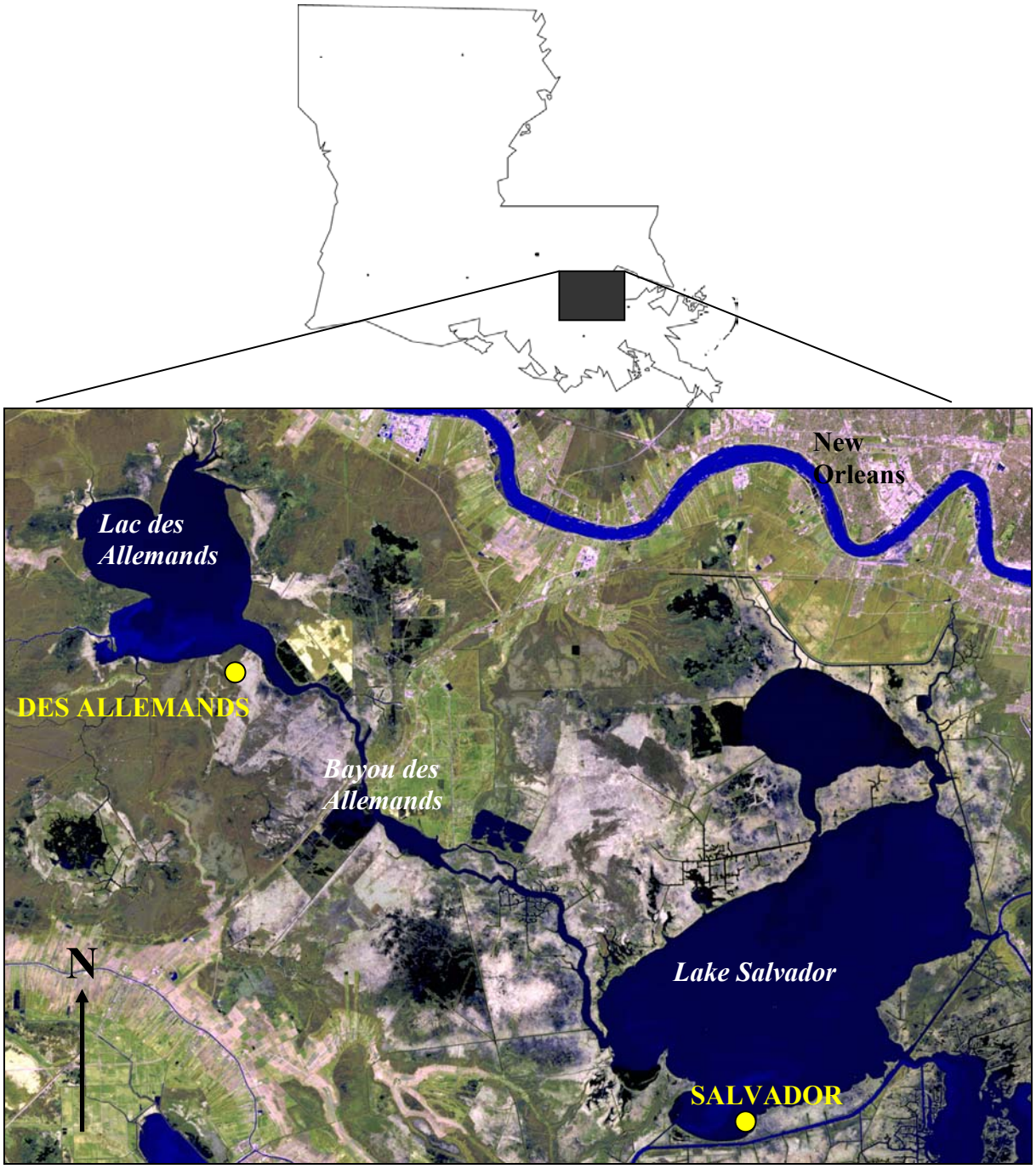

Figure 3. Map of upper Barataria Basin in coastal Louisiana, showing the location of the tidal freshwater (des Allemands) and oligohaline (Salvador) marshes used in the field experiment. Note that the des Allemands marsh was also the source of vegetative material for Experiments One and Three, as well as the field experiment. Image courtesy of the Louisiana Oil Spill Coordinator's Office.

*Belowground Biomass = 0.30092 + 0.00057*SH*

where SH = stem height
Adjusted R^2 = 0.4216, P < 0.0001
n = 60.

These regressions were then used to predict the initial aboveground dry mass of the experimental plants based on the measurement of total stem height of those plants prior to imposition of the experimental treatments. The calculation of relative growth rate of plant biomass was:

$$RGR = \frac{(\ln(HarvestedDW)) - (\ln(InitialDW))}{time_{days}}.$$

STATISTICAL ANALYSIS

Statistical analyses were performed using the General Linear Models procedure of the SAS v.6.12 statistical package for the Macintosh (SAS Institute, Cary, North Carolina, 1996). For Experiments One through Three, growth and physiological response data, with the exception of E_h, were analyzed using a completely randomized design with a factorial treatment arrangement, with salinity and sulfide as the main effects. For Experiment One, response variables were segregated into four subsets. Soil oxidation-reduction potential (E_h) data were analyzed through a repeated-measures model using the factorial arrangement as the main plot and time as the subplot. Interstitial water elemental chemistry data and the biological response variables, organized into biomass-related and stem density – related subsets, were analyzed using multivariate analyses of variance (MANOVA). Data were transformed when necessary to satisfy the assumptions of normality and homogeneity of variance.

For Experiment Two, interstitial chemistry and growth response variables were analyzed as above. Physiological response variables were aggregated into a data set consisting of aerobic and anaerobic respiration, change in respiration, and alcohol dehydrogenase activity. The data set for Experiment Three consisted of alcohol dehydrogenase activity, root ethanol production, and three gas exchange variables; net photosynthesis, transpiration rate and stomatal conductance. Additionally, a data set of growth response variables from Experiment Three was established. Data sets were analyzed using multivariate analyses of variance (MANOVA). Data were transformed when necessary to satisfy the assumptions of normality and homogeneity of variance.

E_h data for Experimients One and Two were analyzed using a repeated measures analyzsis. For the 39-week experiment, a repeated measures analysis was conducted on the redox potential data, utilizing data from all nine treatment combinations measured on17 August and 12 October, with the analysis run on 41 sods for which data was available. On both 30 December 1998 and 13 January 1999, 44 of 45 experimental units (EUs) contained live vegetation and were suitable for E_h measurements during Experiment Two. On 21 March, 42 EUs were still alive, while on 11 April there were 40 live EUs available for analysis. In all cases, these represented replicates of the nine combinations of salinity and sulfide; the repeated-measures analysis included the 40 EUs in which observations were available for all four dates.

Statistical analyses were performed using the General Linear Models procedure of the

SAS v.6.12 statistical package for the Macintosh (SAS Institute, Cary, NC, USA, 1996). Response variables were segregated into two subsets; interstitial chemistry and plant growth response. Each subset was analyzed using multivariate analyses of variance (MANOVA). Data were analyzed using a randomized-block design with the following treatment arrangement:

Model = Site
Block(site)
Elevation
Site*Elevation
Time
Site*Time
Elevation*Time
Site*Elevation*Time.

Data were transformed when necessary to satisfy the assumptions of normal and homogeneous distribution of residuals of the ANOVA.

Treatment effects indicated as significant by the four MANOVA Test Criteria statistics (Wilk's Lambda, Lillai's Trace, Hotelling-Lawley Trace, and Roy's Greatest Root) were used in *a-posteriori* univariate analyses of variance (ANOVAs) to determine statistical differences between means within response variables. Statistical differences between the main effect treatment levels were analyzed using a post-hoc Tukey-Kramer Honestly Significant Difference (HSD) test. Statistical differences between treatment combinations were analyzed using post-hoc LSMeans comparisons, employing a Tukey-Kramer adjustment of the overall error rate. Significant differences were reported at $\alpha = 0.05$, unless otherwise noted. Means and standard errors presented here are for the raw data, while statistical differences between the means are based on the transformed data, when transformations were utilized. Summary ANOVA tables for all response variables used in the MANOVAs are listed in the appendix.

RESULTS AND DISCUSSION

RESPONSE OF *PANICUM HEMITOMON* TO SALINITY AND SULFIDE STRESS IN GROWTH CHAMBER EXPERIMENTS

Treatment Conditions in the Experimental Units

Averaged over the duration of the 39-week experiment, salinities were 0.12±0.02 ppt, 2.68±0.09 ppt, and 5.16±0.21 ppt, for the 0-ppt, 2-ppt and 4-ppt treatment levels, respectively. Salinities were slightly higher than intended for the elevated salinity treatments due to the repeated injections of $Na_2S•9H_2O$ for sulfide concentration maintenance. Based on results from Koch and Mendelssohn (1989), it was expected that after the intended sulfide treatment levels were reached, minimum inputs of $Na_2S•9H_2O$ would be necessary to maintain treatment concentrations. However, sulfide levels continued to decline in the sods throughout the experiment, requiring frequent injections of $Na_2S•9H_2O$ solution to hold sulfide concentrations within the targeted 20% range around the desired sulfide concentration. Interstitial sulfide concentrations, averaged over the duration of the experiment, were 0.06±0.03 mM, 0.42±0.03 mM, and 0.68±0.07 mM for the 0-mM, 0.5-mM and 1-mM treatment levels, respectively. Elevated sulfide treatment significantly reduced E_h at both 1-cm and 25-cm soil depths compared to the 0-mM sulfide treatment (Table 3). At neither depth were there significant differences in E_h between the two elevated sulfide treatments. Neither salinity nor time had a significant influence on E_h at either 1 cm or 25 cm below the soil surface.

Table 3. Interstitial oxidation-reduction potential (E_h) at both 1-cm and 25-cm soil depth during the 39-week experiment. Values are means ± 1 SE, and are an aggregate of data collected on 17 August and 12 October 1996. Note that letters denote significant differences between means only within a particular soil depth.

	Oxidation-Reduction Potential (mV)	
Sulfide Treatment	*1 cm Soil Depth*	*25 cm Soil Depth*
0 mM	33.4±14.7 a	84.5±10.2 a
0.5 mM	-103.4±10.3 b	-45.4±13.1 b
1 mM	-126.6±10.8 b	-74.1±15.5 b

During the 19-week experiment, interstitial water salinities, when averaged across sulfide treatments, were 2.2 ± 0.2, 3.1 ± 0.1 and 4.0 ± 0.1 ppt in the 0-ppt, 2-ppt and 4-ppt salinity treatments, respectively. Sulfide treatment also had a significant effect on interstitial water salinity (P = 0.0001), with higher salinities in the two elevated sulfide treatments (3.2±0.2 ppt and 3.3±0.2 ppt for 0.3-mM and 0.6 mM sulfide treatments, respectively) as compared to that at 0-mM sulfide (2.6±0.2 ppt). As in the 39-week experiment, this was due to repetitive injections of $Na_2S•9H_2O$ solution to hold sulfide concentrations within a 20% range around the desired

target sulfide concentration. Interstitial sulfide concentrations, averaged over the duration of the experiment, were 0.02 ± 0.01, 0.31 ± 0.04, and 0.64 ± 0.08 mM for the 0-mM, 0.5-mM and 1-mM treatment levels, respectively. E_h at 20-cm soil depth was significantly reduced at elevated sulfide compared to the sulfide-free treatments. Mean E_h at 0-mM sulfide was 47±41 mV, significantly higher than means of –80±69 mV or –99±62 mV at 0.5 mM or 1 mM sulfide, respectively. Although the date of measurement was also an overall significant influence on E_h, there were no significant differences between mean values of E_h by date.

Growth Parameter Response to Multivariate Analysis

Multivariate analyses of variances (MANOVAs) run on biomass response variable subsets suggest an important temporal component to the sensitivity of *Panicum hemitomon* to the stresses associated with saltwater intrusion. When analyzed as a group in the MANOVA, the biomass response parameters were significantly influenced by salinity and sulfide both as main factors and in combination after 39 weeks. The *a-posteriori* ANOVAs run on this data were then structured to include both the main treatment effects as well as combined salinity*sulfide. In comparison, MANOVA showed that only salinity and sulfide as main treatment effects significantly influenced the biomass response parameters after 19 weeks, with no significant interaction between the two. Therefore, only the main treatment effects were used in the *a-posteriori* ANOVAs. MANOVA performed on growth response variables did not indicate any significant effect of either salinity or sulfide as main treatment effects or an interaction between salinity and sulfide after twelve days. MANOVA performed on stem density response variables showed an opposite pattern than that of biomass variables after the 39- and 19-week experiments. After 19 weeks, both salinity and sulfide, and combinations of the individual stressors, were overall significant influences on stem density; therefore, the full model was used in the *a-posteriori* ANOVAs. After the 39-week experiment only salinity was significant on stem density response, and thus only salinity was used as a treatment effect in the *a-posteriori* ANOVAs on that data. Growth response parameters were not significantly effected by treatment conditions after the 12-day hydroponics exeperiment.

Aboveground Growth Response

Sulfide was more deleterious than salinity to aboveground tissue development after 19 weeks within the confines of the treatment levels used in this experiment. While elevated sulfide did not have a significant effect on the relative growth rate of aboveground tissue (RGR_{AG}), there were reductions in percent live biomass to well below 50% in both the 0.5-mM (26.8±5.8%) and 1-mM treatments (33.7±5.2%) from that at 0-mM (69.9±5.4%), and a significant decrease in the generation of new live culms at 0.5-mM sulfide. In the elevated sulfide treatments, the majority of stems were dead, regardless of salinity, and elevated salinity did not significantly reduce the percentage of live stems in the sulfidic treatments (Figure 4C). Sulfide inhibited aboveground production in *Panicum* (Feijtel et. al., 1989; Koch and Mendelssohn, 1989), oligohaline marsh species such as *Sagittaria lancifolia* (Webb and Mendelssohn, 1996) and *Phragmites australis* (Armstrong et. al., 1996; Chambers, 1997) and halophytic plants such as *Atriplex patula*, *Festuca rubra* and *Puccineliia maritima* (Havill et. al., 1985). RGR_{AG} was significantly lower at 4-ppt salinity (0.011±0.001 g g^{-1} day^{-1}) compared to 0-ppt and 2-ppt salinity (0.014±0.001 g g^{-1} day^{-1}

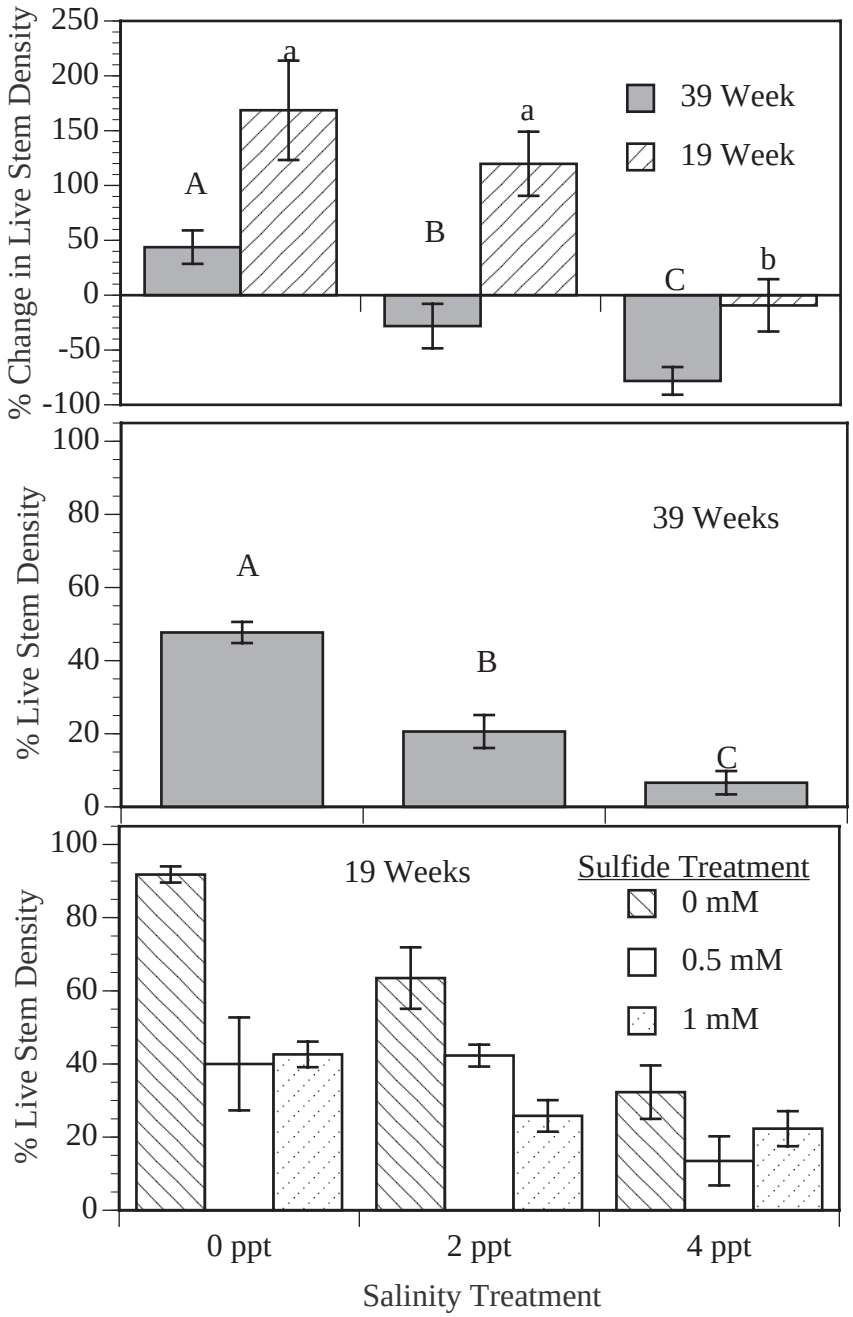

Figure 4. Response of stem density parameters to exposure to elevated salinity treatment in 19- and 39-week experiments. (Upper pane) Percent change in live stem density between experiment initiation and experiment termination. (Middle pane) Live stem density as a percentage of total stem density in response to salinity treatment after 39-week exposure. (Lower pane) Live stem density as a percentage of total stem density decreased in response to combined salinity and sulfide treatments after 19-week exposure. Capital letters indicate significant differences in percent change in total stem density between treatments. Lower-case letters indicate significant differences in percent change in live stem density between treatments.

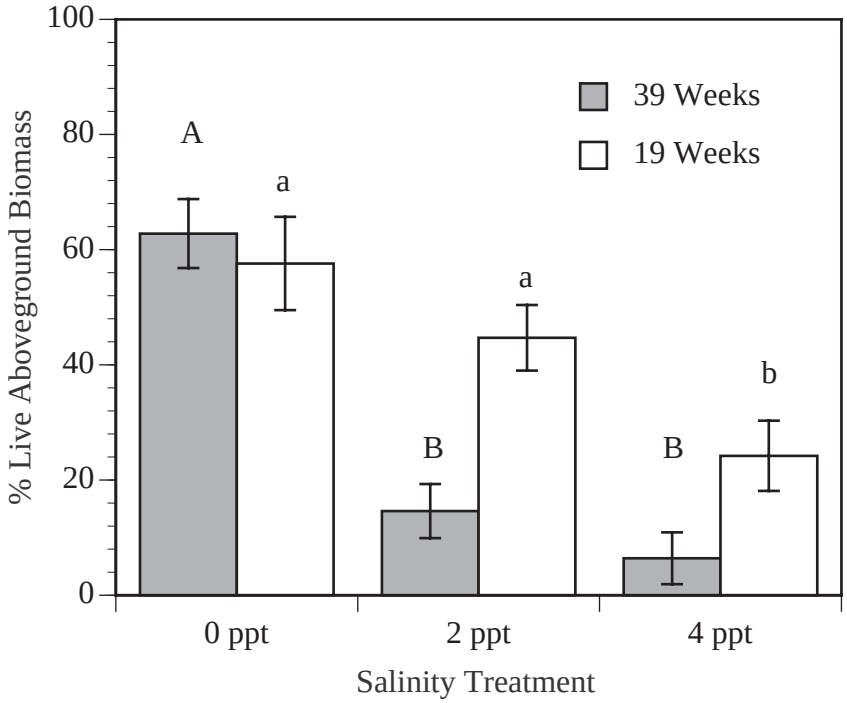

Figure 5. Decrease in the percent contribution of live aboveground biomass to total aboveground (live + dead) biomass with increasing salinity treatment after both 39- and 19-weeks. Letters indicate significant differences between treatments.

for both) after 19 weeks. Elevated salinity also lead to reductions in percent live biomass of aboveground tissue (Figure 5), a net loss of live culms after 19 weeks (Figure 4A), and a trend towards a reduction in the percentage of live stems (Figure 4C).

However, elevated salinity at both 2 ppt and 4 ppt was so stressful after 39 weeks that the long-term effects of sulfide on aboveground tissue development became largely inconsequential. After 39 weeks most of the aboveground biomass was dead at elevated salinity (Figure 5), while the majority was live in the 0-ppt treatments. Correspondingly, there were net losses of live stems at both 2-ppt and 4-ppt salinity (Figure 4A), and the frequency of live stems was significantly lower at elevated salinity than at 0 ppt (Figure 4B). Sulfide-induced effects on percent live aboveground biomass (15.2±5.7% and 21.4±8.3% at 0.5-mM and 1-mM sulfide, respectively, vs. 40.3±9.3% at 0-mM sulfide) were still significant after 39 weeks, but the magnitude of growth inhibition due to elevated sulfide was less than that by elevated salinity and there were no longer-term effects of sulfide on stem density response. The sensitivity of aboveground tissue to saltwater exposure has also been seen in crops such as *Curcurbita pepo* (Huang et. al., 1995) and *Triticum aestivum* (Gorham et. al., 1985; Salama et. al., 1994), as well as non-halophytic wetland plants such as *Echinochloa crus-galli* (Rahman and Ungar, 1994), *Sagittaria lancifolia* (Howard and Mendelssohn, 1999a), and *Eleocharis palustris* (Howard and Mendelssohn, 2000).

Additionally, these results bolster much of the literature on *P. hemitomon's* sensitivity to both these stresses (Pezeshki et. al., 1987a; Koch and Mendelssohn, 1989; Flynn et. al., 1994; Howard and Mendelssohn, 1999b). The sensitivity of aboveground growth may involve which

adaptive mechanisms *Panicum* and other glycophytic grasses use to physiologically adapt to saltwater exposure. Many glycophytic plants, incapable of preventing the uptake of ions associated with saltwater during intrusion events, will sequester those ions in older leaves that are then discarded. In glycophytes that are particularly sensitive to salt, there may be an inhibition of leaf growth that leads to a decrease in the volume of new tissue into which these ions can be accumulated (Neumann, 1997), leading to an earlier buildup in salt concentrations and an earlier exposure of critical tissues and the cellular biochemical machinery to the direct ion toxicity then the physiological tolerance of the plant cellular machinery becomes critical.

Belowground Growth Response after 19 Weeks

Belowground tissue in *Panicum hemitomon* appears to be particularly sensitive to saltwater flooding stress. Both salinity and sulfide significantly reduced the relative growth rate of belowground tissue (RGR_{BG}) after 19 weeks (Figures 6 and 7, respectively). Koch and Mendelssohn (1989) found a significant decrease in both belowground root and rhizome biomass in intact *P. hemitomon* sods exposed to a mean concentration of 0.63- mM sulfide for four months, to the extent that only a few shallow roots remained viable in their plants. In this research, however, while belowground growth in the two elevated sulfide treatments was almost half of that which occurred in the absence of sulfide stress at the end of the 19-week experiment (Figure 7), salinity was the dominant stress on belowground tissue production. Belowground tissue growth rate at 2 ppt was 63% that in the absence of salinity, and was reduced by 76% under 4-ppt salinity (Figure 6). Root biomass development was sensitive to salt exposure in glycophytic crops such as squash (*Curcurbita pepo*) (Huang et. al., 1995) and wheat (*Triticum aestivum*) (Gorham et. al., 1985; Salama et. al., 1994) as well as the halophytic wetland species *Triglochin striata* (Naidoo 1994).

Total Biomass Growth Response and Biomass Partitioning

Salinity was the dominant stressor on total plant production as compared to sulfide in the 19-week experiment. Total relative growth rate decreased 33% with an increase in interstitial water salinity from 0 ppt to 4 ppt (Figure 6A), and decreased 20% with an increase in interstitial water sulfide from 0 mM to 0.5 mM (Figure 7A). Both stressors reduced the root-to-shoot biomass ratio to similar values between 0.2 and 0.3 (Figures 6B and 7B, respectively), reaffirming that both salinity and sulfide were more stressful to belowground tissue development than aboveground in *Panicum hemitomon*. This agrees with Linthurst's (1979) observation of decreased root:shoot ratio in *Spartina alterniflora* in response to elevated sulfide, but is in contrast to the greater reduction in wheat aboveground biomass due to salinity observed by Gorham et. al. (1985), and the hypothesis that aboveground tissues are more sensitive to salt stress than belowground tissues (Munns and Teraat (1986).

The lack of any significant growth differences between treatments in the hydroponic experiment suggests that 12 days was too short a time period for the expression of any growth alterations. Interestingly, McKee and Mendelssohn (1989) suggested that *P. hemitomon* was more salt-tolerant than *Sagittaria lancifolia*, in comparison to Visser et. al.'s (1996) conclusion that *S. lancifolia* is more tolerant of oligohaline salinities on the basis of ecological distributions in a coastal Louisiana estuary. However, Howard and Mendelssohn (1999) suggested that the delayed expression of visual stress indicators in *P. hemitomon* as compared to *S. lancifolia.*

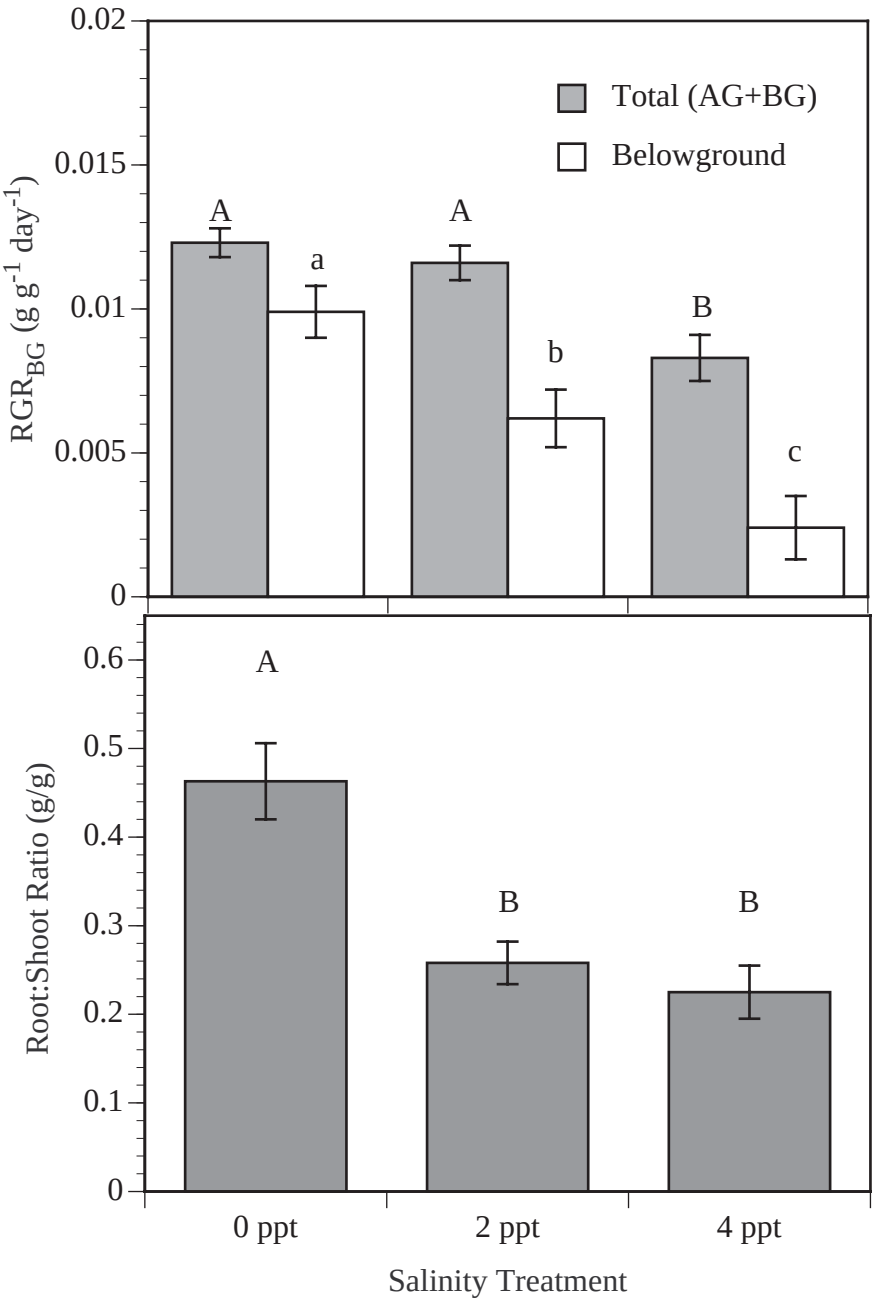

Figure 6. Belowground tissue and root:shoot ratio responses after 19-week exposure to elevated salinity treatment. Values are averaged across sulfide treatments (Top panel) Relative growth rate of total and belowground biomass. (Bottom pane) Ratio of root biomass vs. shoot biomass. Different letters in each panel indicate significant differences between treatment means for a particular response factor.

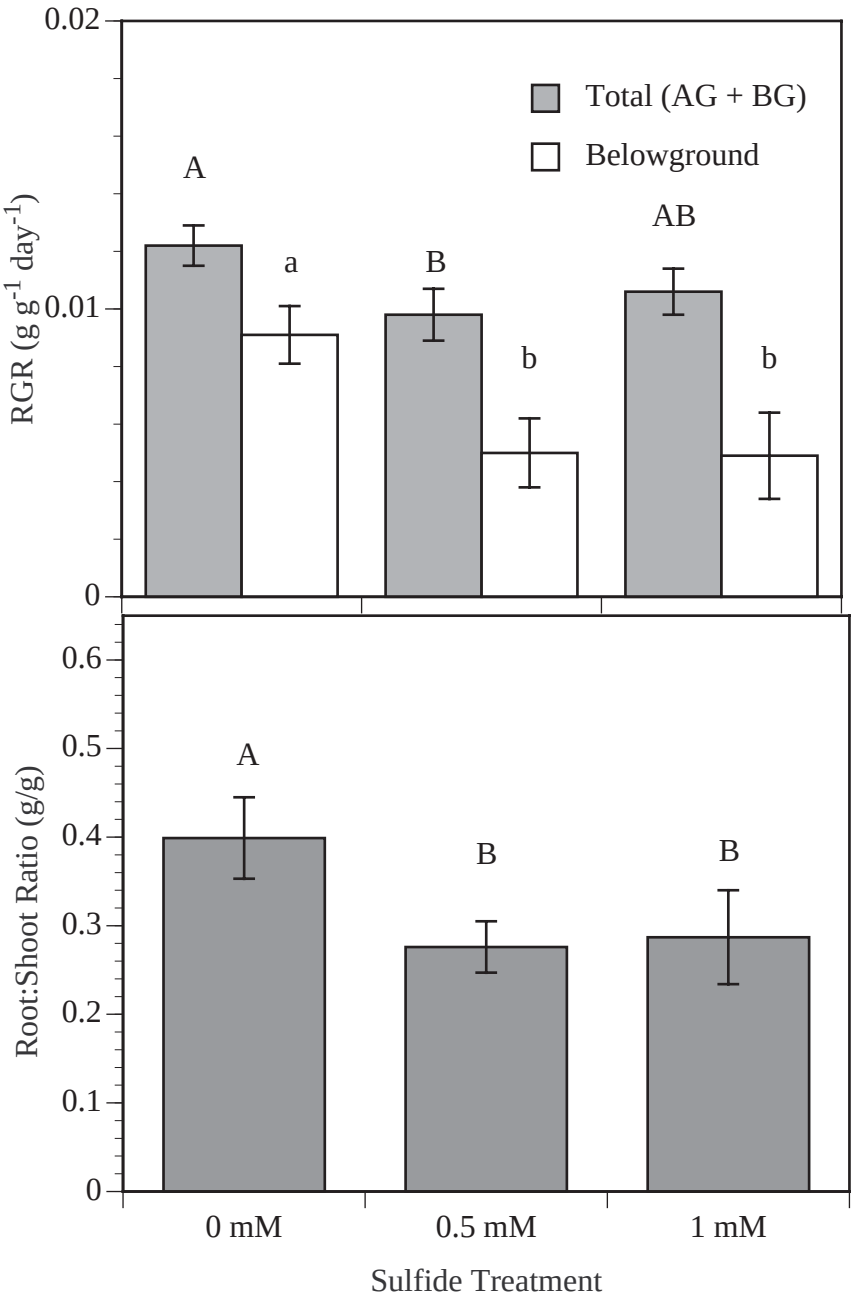

Figure 7. Belowground tissue and root:shoot ratio responses after 19-week exposure to elevated sulfide treatment. Values are averaged across salinity treatments. (Top panel) Relative growth rate of total and belowground biomass. (Bottom pane) Ratio of root biomass vs. shoot biomass. Different letters in each panel indicate significant differences between treatment means for a particular response factor.

Morphological Adaptations Underlying Plant Growth Response - Adventitious Root Tissue Production

Plants may respond to waterlogged or reduced soil conditions by forming adventitious or water roots (Jackson, 1985; Blom et. al., 1990), which emerge from the stems just above the soil surface to uptake oxygen and nutrients from a less inhospitable environment than themore highly-reduced, phytotoxin-rich soil interstitial zone and to maintain an anchor into the soil. Elevated sulfide stimulated adventitious tissue development after both the 19- and 39-week experiments, while salinity proved inhibitory. When averaged across salinity treatments, after 19 weeks percent adventitious root biomass was significantly higher at 1-mM sulfide (3.5$\pm$0.5%) than at 0-mM sulfide (2.0$\pm$0.3%). Percent total adventitious biomass at 0.5-mM sulfide (3.4$\pm$0.5%) was not statistically different from the other sulfide treatments. However, elevated salinity proved lethal to these sulfide-induced tissues. After 19 weeks percent live adventitious tissue at 4 ppt (0.6$\pm$0.1%) was less than half that at either 0 ppt or 2 ppt (1.6$\pm$0.3% in both).

This trend was further developed after 39 weeks, where at 4 ppt there was a complete inhibition of the sulfide-induced stimulation in total adventitious tissue production that occurred at 0 ppt (Figure 8). Within the 1-mM sulfide treatment, specifically, there was an 83% decrease in total adventitious tissue from 0 ppt to 4 ppt, where there were no significant differences between sulfide treatments in percentage contribution of adventitious roots to total biomass. Huang et. al. (1995) also found that 100 mol m^{-3} NaCl inhibited adventitious root production in waterlogged *Curcurbita pepo*. In contrast, the halophyte *Sporobolus virginicus* (Naidoo and Mundree, 1993) and three halophytic species of *Trifolium* (Rogers and West, 1993) developed new adventitious root tissue under saline conditions. It may be that an important adaptation in halophytes is a reduced sensitivity of adventitious bud primordia to salinity stress.

Indicators Of Short-Term Metabolic Responses To Different Roles Of Sulfide As An Environmental Stressor – Root Tissue Respiration after 19 Weeks and Root Anaerobic Metabolism (ADH/EtOH) after 12 Days

Physiological responses to elevated interstitial sulfide were more variable than those due to elevated salinity *per se*, which may have been due to sulfide's two possible modes of stressor action; either an oxygen scavenger or a direct phytotoxin. The primary effect of sulfide in the 19-week experiment was apparently as an oxygen scavenger that exacerbated flooding-induced soil anoxia. After 19 weeks there was an increase in root respiratory rates in plants subject to elevated sulfide concentrations in the absence of salinity (Figure 9). This is consistent with Cizkova and Bauer's (1998) conclusion that increased rhizome respiration in *Phragmites australis* resulted from a greater oxygen deficiency from either lower oxidation-reduction potential (E_h) or higher BOD of a flooded organic soil. E_h was significantly lower in sulfide-treated sods in this experiment. Houle et. al. (2001) suggested that higher respiration rates that would have consumed the available fixed carbon and prevented its incorporation into structural tissue, thereby explaining observations of reduced growth in *Aster laurentianus*.

While excessive variation masks any significant differences between means, the overall significance of salinity treatment on respiration suggests a trend towards the inhibition of the sulfide-induced stimulation (Figure 9). The combined effects of salinity and sulfide on CO_2 evolution from root tissue were similar under both aerobic and anaerobic atmospheres. There is contradictory evidence in the literature for both reduced (Hamada, 1996; Epron et. al., 1999) and increased respiration in salt-exposed plants (Reuveni et. al., 1997; Bouraoui et. al., 1998). The

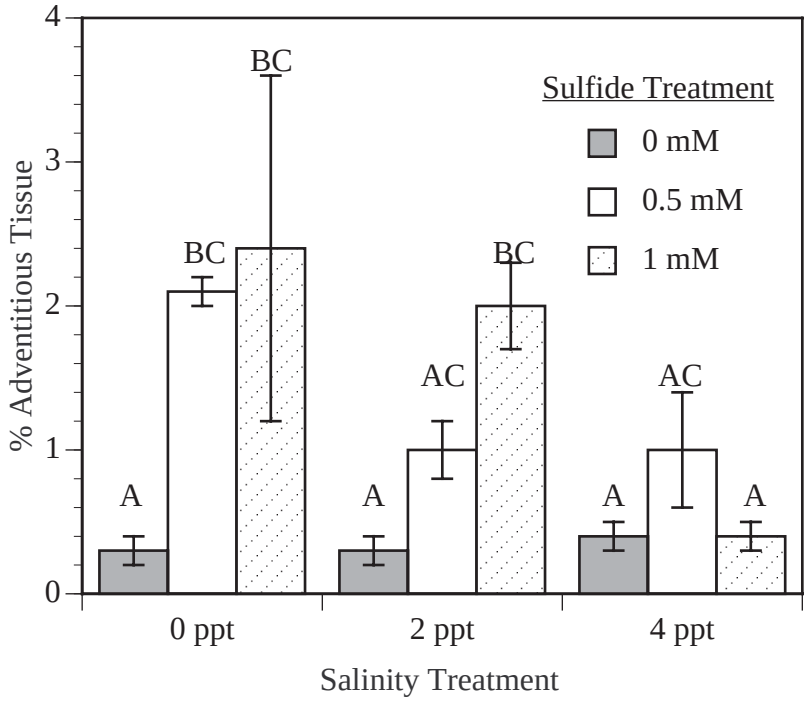

Figure 8. Inhibition by elevated salinity of the sulfide-induced stimulus on production of adventitious root tissue after 39 weeks. Different letters indicate significant differences between treatments.

activity of the respiratory enzyme cytochrome oxidase has similarly demonstrated both negative (Akhtar et. al., 1998) and positive responses to salt exposure (Hernandez et. al., 1993). Admittedly, gas exchange analysis is a measure of overall CO_2 evolution and cannot differentiate between aerobic respiration and anaerobic fermentation (Lehninger et. al., 1993).

Interstitial sulfide penetration into root tissue following inhibition of root oxygen transport may allow for the direct metabolic phytotoxicity of sulfide (Carlson and Forrest, 1982; Koch et. al., 1990). Root fermentation response in the 12-day hydroponic experiment is suggestive of this mode of action. Alcohol dehydrogenase activity response to combined salinity and sulfide was highly variable but overall significant, and the data suggest that elevated salinity may stimulate ADH activity (Figure 10). This would be consistent with salinity-induced stimulations in ADH activity in wheat (Akhtar et. al., 1998) and the marsh plant *Sporobolus virginicus* (Naidoo and Mundree, 1993), and is supported by the stimulation in root ethanol production after 12 days of elevated salinity treatment. Salinity and sulfide had opposing effects on root ethanol generation, with salinity stimulating ethanol production at 4 ppt (Figure 11) while both elevated sulfide concentrations resulted in significantly lower ethanol production (Figure 12). There were no significant effects of combined salinity and sulfide on root ethanol production. The sulfide-induced inhibition of ethanol production is consistent with the sensitivity of metallo-enzymes such as ADH to sulfide (Cossins et. al., 1968; Allam and Hollis, 1972; Ingold and Havill, 1985), seen previously in *Panicum hemitomon* by Koch et. al. (1990) and in *Phragmites australis* by Fürtig et. al. (1996).

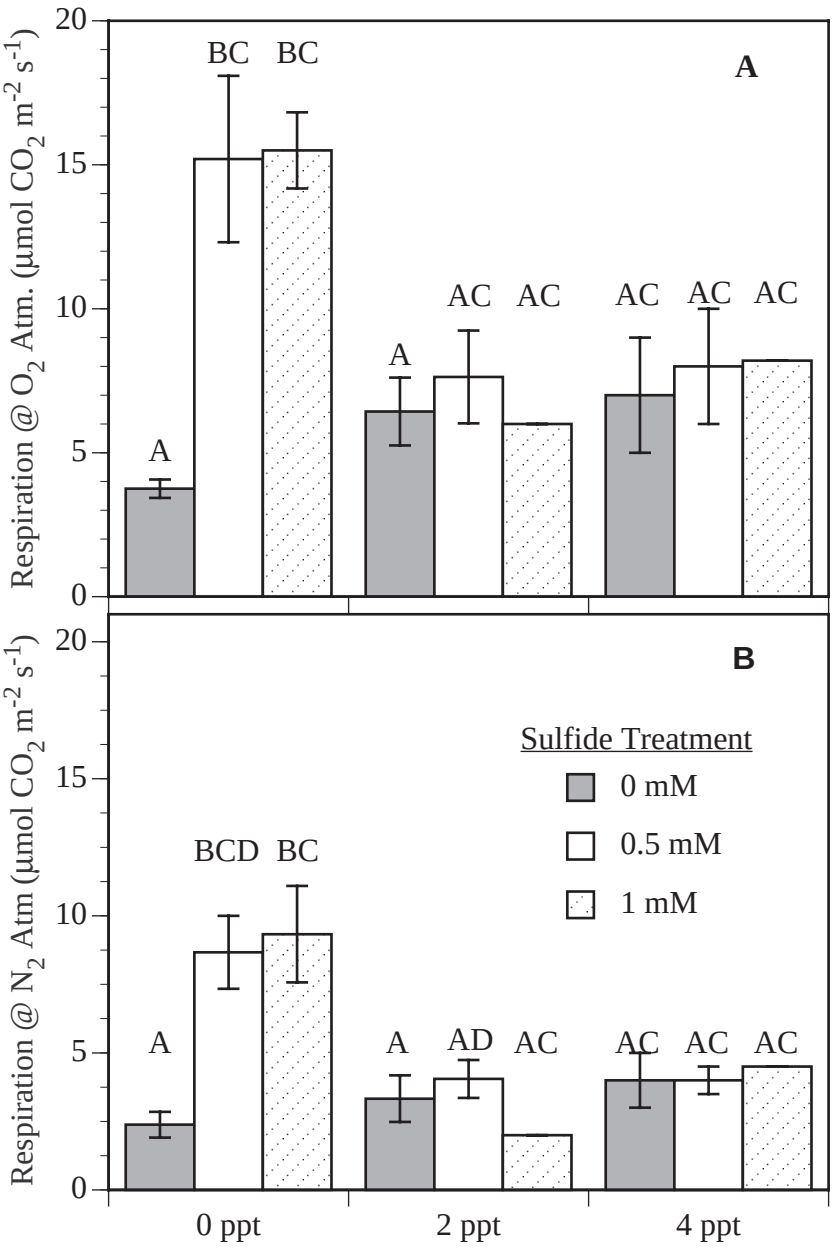

Figure 9. Root respiration response under ambient (A) and nitrogen atmospheres (B) following 19-week exposure to elevated salinity and sulfide treatment. Values are mean±1 SE. Different letters within each graph indicate significant differences between treatment combination means.

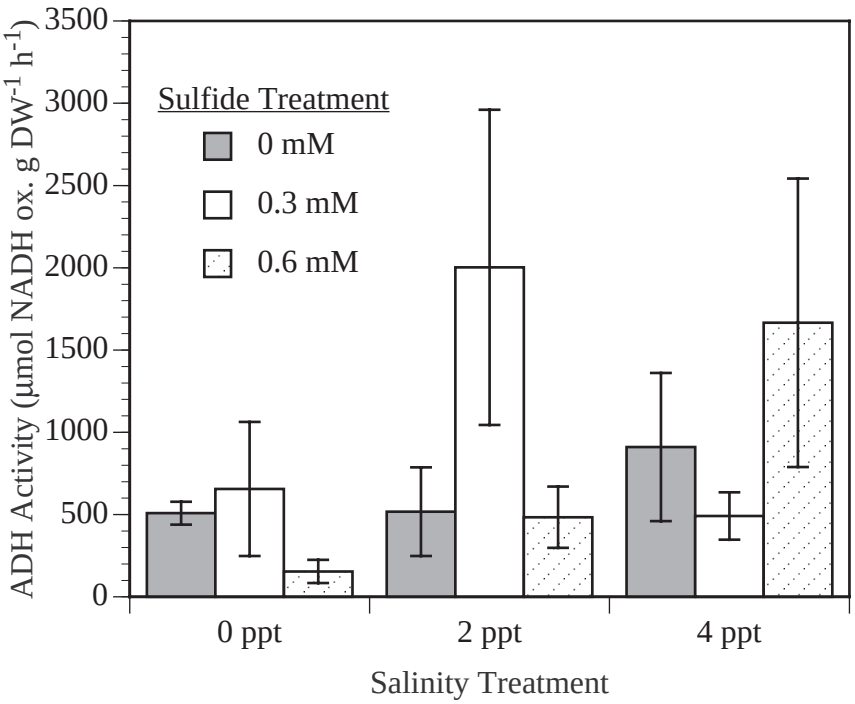

Figure 10. Root alcohol dehydrogenase activity in response to combined salinity and sulfide treatments after 12 days. Values shown are mean±1 SE.

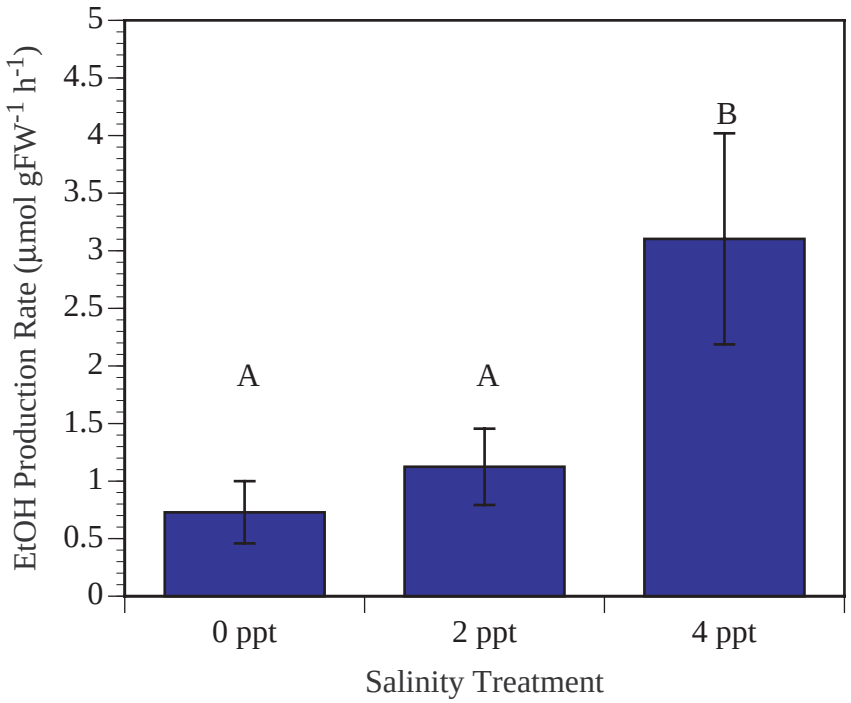

Figure 11. Response of root ethanol production assay to elevated salinity treatment after 12 days. Shown are means±1 SE. Letters indicate significant differences between mean values.

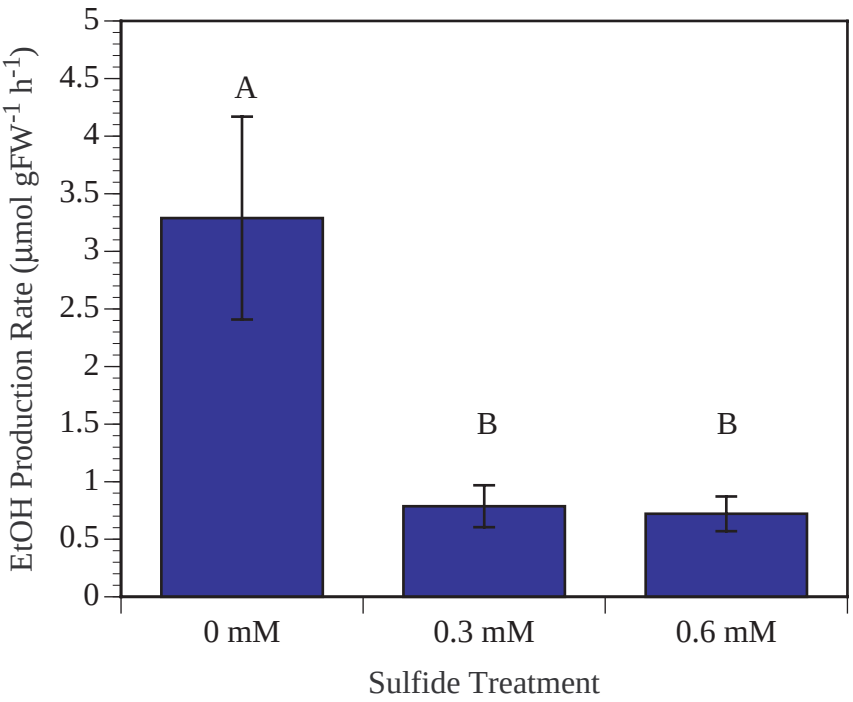

Figure 12. Response of root ethanol production assay to elevated sulfide treatment after 12 days. Shown are means±1 SE. Letters indicate significant differences between mean values.

The assay of ADH activity is a measure of potential pathway utilization, while the assay of tissue ethanol release is a measure of realized activity. There was no significant correlation between root ethanol production and alcohol dehydrogenase activity. This suggests that there was no *de novo* production of ADH enzyme in response to salinity, and instead that there may have been an activation of pre-existing protein. Alcohol dehydrogenase activity was highly correlated to the percentage of live aboveground biomass ($r = 0.4946$), and ethanol production was significantly correlated with photosynthesis (data not shown; $r = 0.4519$). This latter correlation suggests that realized fermentation may depend on photosynthetic substrate availability. Alterations to photosynthetic ability have been noted to result from both salinity and sulfide ((Kuramoto and Brest, 1979; Pan and Pezeshki, 1991; Allen et. al., 1997; Youssef and Saenger, 1998).

Physiological results suggest that the exact effect of combined salinity and sulfide may be dependent on the individual mechanism of sulfide stress. When sulfide acts as an oxygen scavenger, as it seemed to have in the 19-week experiment, then salinity inhibition of respiration may underlie observations of reduced growth that will be described next. If instead sulfide acts as a direct phytotoxin, as appears to have occurred in the 12-day hydroponic experiment, then the observed short-term antagonism of sulfide with salinity-induced stimulation of fermentation, as suggested by the correlation of root ethanol production with net photosynthesis (data not shown), may lead to substrate limitation of fermentation and prevent maintenance of existing tissue. Further experimentation is required to confirm this hypothesis.

GROWTH AND PHYSIOLOGICAL RESPONSE OF *PANICUM HEMITOMON* TO SIMULATED SALTWATER INTRUSION IN THE FIELD

Environmental Conditions During the Study

The initiation of this experiment coincided with the final summer of a severe three-year drought in southern Louisiana driven by the 1997-2000 El Niño / La Niña cycle. Low water levels both at the experiment initiation and the 34-day harvest (Table 4) make it reasonable to assume that early in the study growth response was primarily driven by porewater conditions. As the study progressed, and water levels rose to flood conditions, growth response of the plants became increasingly influenced by the more saline floodwater from the adjacent water bodies and the concomitant changes in porewater chemistry resulting from that flooding. While the process of slowing metabolism overlaid these responses and growth as the season progressed and the plants initiated annual senescence patterns, a pattern of sensitivity in both the aboveground and belowground tissues to flooding emerged, particularly at the oligohaline (Salvador) marsh.

Table 4. Floodwater depths at the des Allemands and Salvador treatment marshes during the three sampling dates of the field experiment. Values are mean ± 1 SE, n = 3. BS indicates that water level was below the marsh surface.

Days from Initiation	*Water Depth (mm)*			
	des Allemands		*Salvador*	
	Ambient	*-15 cm*	*Ambient*	*-15 cm*
34 Days	BS	BS	BS	BS
64/65 Days	122±3	234±12	109±3	195±7
111/112 Days	218±4	327±5	173±7	287±10

Multivariate analysis of variance (MANOVA) indicated that only marsh site (Site) and harvest period (Time) were significant main treatment effects on interstitial chemistry, and the only significant interaction was Site*Time. Neither Block(Site) nor elevation treatment (Elevation) were significant as main treatment effects, nor were any interactions associated with Elevation. Accordingly, *a-posteriori* ANOVAs performed on the individual response variables used the following restricted design:

Site
Time
Site*Time.

Marsh site was not a significant influence on redox potential, though time of harvest and the combination of site and time were significant. Although water levels increased to flooding conditions by the end of the study, E_h both at the surface and at 20-cm depth remained above –50 mV at both sites regardless of sampling date (Table 5), and above 0 mV during all dates at the

Table 5. Interstitial chemistry of experimental units at time of harvest for the field experiment as a function of combined site and elevation treatment. Values are means ± 1 SE, in interstitial water. Letters indicate significant differences between treatment means within a particular response variable.

Treatment					
Marsh Site	*Days from Initiation*	*E_h (mV)* *1 cm*	*20 cm*	*NH_4^+ (μM)*	*S (ppm)*
des Allemands	34 days	25.9±13.3a	47.6±8.4a	53.24±18.59ab	1.88±0.86ab
	65 days	20.3±24.4a	15.1±18.8ac	20.93±6.35b	0.14±0.03a
	112 days	6.6±13.2a	34.3±15.2a	76.26±10.95ab	0.79±0.22ab
Lake Salvador	34 days	21.4±12.8a	43.5±8.5a	33.13±11.88b	0.42±0.29a
	64 days	159.7±27.8b	145.1±18.0b	63.78±18.88ab	0.18±0.04a
	111 days	-43.1±18.5a	-28.7±18.9c	100.21±7.55a	1.66±0.44b

des Allemands site. After 111 days, mean E_h at the Salvador marsh decreased to below 0 mV both at the surface and at 20-cm depth following heavy rains two days prior to site visit to values significantly lower than during the first harvest.

Reduced freshwater inputs to the upper reaches of the Barataria Estuary associated with the drought allowed for the upstream movement of estuarine water so that salinities, when averaged across harvest date, exceeded 4 ppt at both sites (Table 6). Salinity also increased over time when averaged between sites (Table 7). Sulfide concentrations remained low in both marshes regardless of time (Table 6). While both salinity and sulfide differed significantly by site, the ecological relevance of the small differences is questionable.

Table 6. Interstitial chemistry of field experimental units as a function of marsh site, with data averaged across date of harvest. Values are ± 1 SE, in interstitial water. Letters indicate significant differences between treatment means within a particular response variable.

Chemical Species	*Site*	
	des Allemands	*Lake Salvador*
Salinity (ppt)	4.0±0.2a	4.5±0.2b
Sulfide (mM)	0.05±0.01a	0.07±0.01b
Ca (mM)	4.50±0.22a	5.63±0.24b
K (mM)	2.93±0.10a	3.37±0.10b
Mg (mM)	6.38±0.29a	8.51±0.33b
Mn (μM)	31.49±1.82a	40.23±2.18b
Na (mM)	44.00±1.95a	56.90±2.53b

Table 7. Interstitial chemistry of field experimental units at the time of harvest as a function of marsh site. Values are ± 1 SE, in interstitial water. Letters indicate significant differences between treatment means within a particular response variable.

Chemical Species	*Days from Initiation*		
	34 days	*64/65 days*	*111/112 days*
Salinity (ppt)	3.7±0.3a	4.4±0.2ab	4.7±0.2b
Fe (mM)	0.10±0.01ab	0.19±0.06a	0.10±0.01b
Na (mM)	44.09±2.59a	54.11±3.21b	54.15±3.18b

The principle cations associated with salinity stress (Na, K, Ca, Mg) were all significantly higher at the Salvador marsh as compared to the des Allemands marsh (Table 5), when averaged across , but were influenced by, harvest date. Porewater Mn responded similarly to the salinity-associated cations (Table 6), while interstitial concentrations of Fe were not influenced by site, but did vary by time, with elevated concentrations at the 64/65-day harvests (Table 7). Among the cations, only Na varied significantly over time, and was lower during the 34-day harvest than during either the 64/65- or 111/112-day harvests (Table 7). Both interstitial NH_4^+ and sulfur increased during the study period at the Salvador marsh, but did not vary significantly at the des Allemands site (Table 5). Interstitial P did not vary significantly across either site or time during this study.

Growth Parameter Response to Multivariate Analysis

MANOVA indicated that only marsh site and harvest period were significant main treatment effects on plant growth response, and that all interactions involving time of harvest (Site*Time, Elevation*Time, and Site*Elevation*Time) were significant. Neither Block(Site) nor elevation treatment were significant as main treatment effects, nor was the Site*Elevation interaction. Accordingly, *a-posteriori* ANOVAs performed on the individual response variables used the following design:

Site
Time
Site*Time
Elevation*Time
Site*Elevation*Time.

Aboveground Growth Response

Relative growth rate of aboveground tissue (RGR_{AG}) was slightly but significantly higher at the Salvador site as compared to that at the des Allemands marsh (0.0297±0.0027 and 0.0252±0.0024 g g^{-1} day^{-1}, respectively). RGR_{AG} decreased over time, and though there was an effect of combined elevation and time of harvest, the pattern of declining RGR_{AG} during the course of the study did not vary significantly between elevation treatments (Figure 13). The

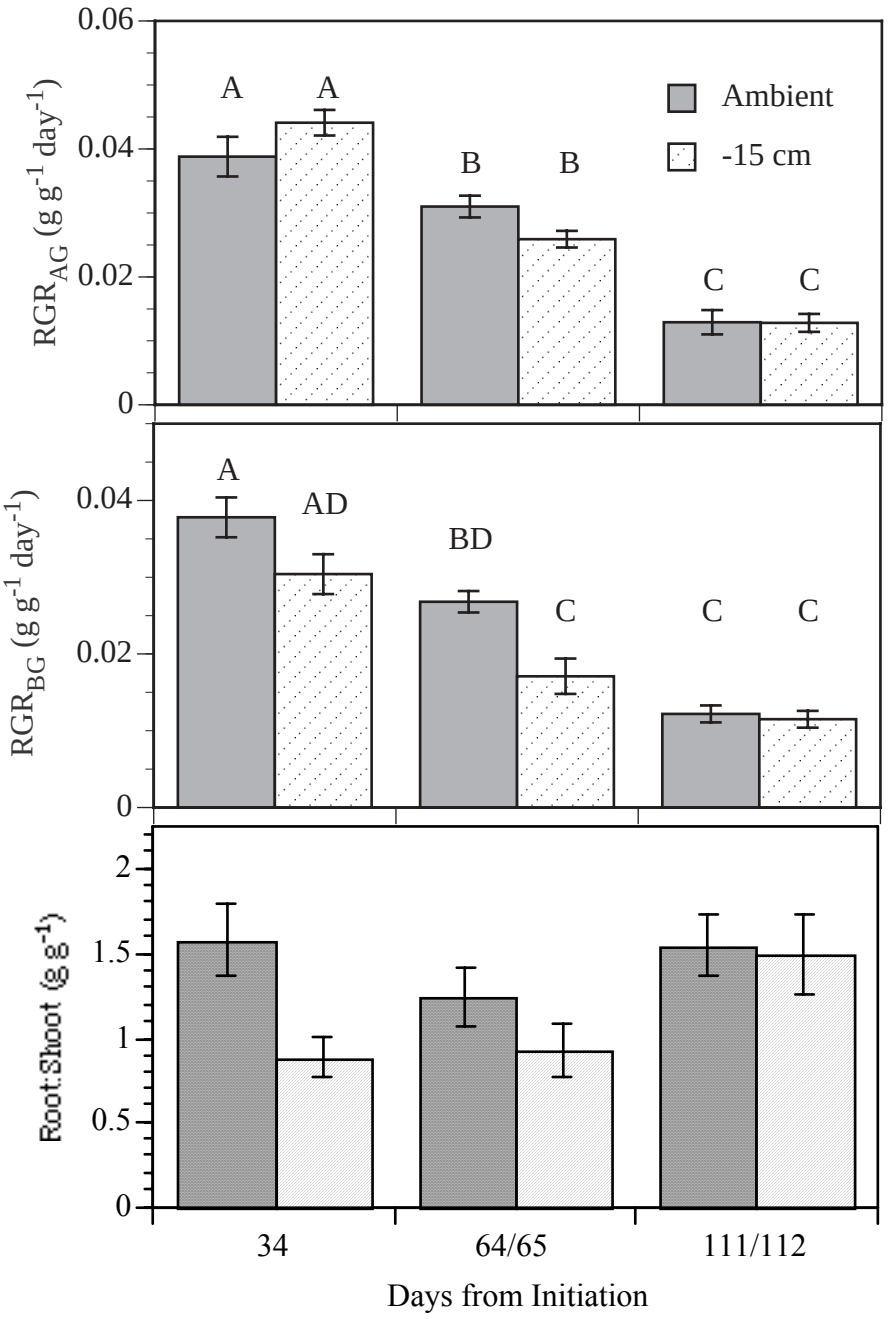

Figure 13. Growth response of *Panicum hemitomon* by elevation treatment throughout the duration of the field experiment. Shown are values ± 1 SE. (top pane) Relative growth rate of aboveground biomass declined significantly over time but did not vary by elevation treatment. (middle pane) Relative growth rate of belowground tissue declined significantly over time, but was significantly lower at 65 days in the ambient-15 cm treatment as compared to the ambient treatment. (bottom pane) Root:shoot biomass was not significantly different between treatments or dates at $P = 0.05$, but there was a significantly lower root:shoot ratio at 34 days between elevation treatments at $P = 0.0648$.

decline in RGR_{AG} of *Panicum hemitomon* over time was consistent with the senescence of aboveground vegetation as the study progressed through the early fall (Sasser and Gosselink, 1984). The pattern of RGR_{AG} for aboveground tissue was significantly correlated with E_h, both at the surface and at 20-cm depth, and negatively correlated with interstitial salinity, NH_4^+ and sulfur (Table 8). Analysis of covariance (ANCOVA) found that the negative linear relationship between RGR_{AG} and interstitial NH_4^+ was significant at ambient elevation, but not at the –15 cm elevation (Table 9).

Table 8. Correlations between plant growth responses and soil chemical variables determined at the time of harvest in the field experiment. * indicates significance at $P = 0.05$, and ** indicates significance at $P = 0.01$. Significance of correlations from Steele and Torrie (1980). ^{T} indicates transformed data used in correlation analysis. See Appendix for variable transformation information.

Soil Chemical Variable	*Growth Response Variable*				
	RGR		*Root:Shoot*^{T}	*Live DW as % Total DW*^{T}	*Root Porosity*^{T}
	Aboveground	*Belowground*			
E_h (1 cm)	0.309*	0.255	-0.154	0.257	0.317*
E_h (20 cm)	0.391**	0.259	-0.234	0.440**	0.430**
pH	-0.043	0.051	0.032	-0.351**	-0.086
Salinity	-0.306	-0.153	0.214	-0.461**	-0.215
Sulfide^T	0.110	-0.030	-0.209	0.160	0.029
NH_4^+	-0.505**	-0.616**	0.068	-0.596**	-0.152
Ca	0.201	0.276*	0.115	-0.064	0.112
Fe^T	0.038	-0.105	-0.194	0.037	0.145
K	0.036	0.172	0.250	-0.170	0.059
Mg	0.080	0.115	0.089	-0.168	0.132
Mn^T	0.108	0.004	-0.114	-0.049	0.301*
Na^T	-0.224	-0.092	0.200	-0.265	-0.091
P	0.010	-0.084	-0.137	-0.218	-0.044
S^T	-0.410**	-0.273*	0.410**	-0.381**	-0.145

The fraction of aboveground biomass that was live tissue (%LiveAG) responded to a 3-way interaction between site, elevation and time (Figure 14). Values declined significantly from the 64/65-day harvests to the 111/112-day harvests consistent with fall senescence of aboveground tissue. However, there was a significant decrease in % LiveAG after 64 days in the lower elevation treatment at the Salvador marsh that did not occur either in the ambient treatment at that site or at the des Allemands marsh regardless of elevation. Plants retained live, turgid roots during this time and while mortality cannot be ignored as a source of the reduction in %LiveAG, these results may also suggest an acceleration of aboveground tissue senescence, such as that seen in water stressed plants (Sharma and Singh, 1989; Lin and Kao, 1998).

Table 9. Linear relationships between abiotic and growth response variables in the field experiment at the time of harvest as a function of elevation treatment, determined by analysis of covariance.

Biotic Response	*Interstitial NH_4^+*
RGR_{AG}	
Ambient	$= 0.04016-(0.00027*NH_4^+)$ $P < 0.0001$
-15 cm	$= 0.03168-(0.00006*NH_4^+)$ $P = 0.3142$
RGR_{BG}	
Ambient	$= 0.03619-(0.00022*NH_4^+)$ $P < 0.0001$
-15 cm	$= 0.02613-(0.0001*NH_4^+)$ $P = 0.0313$

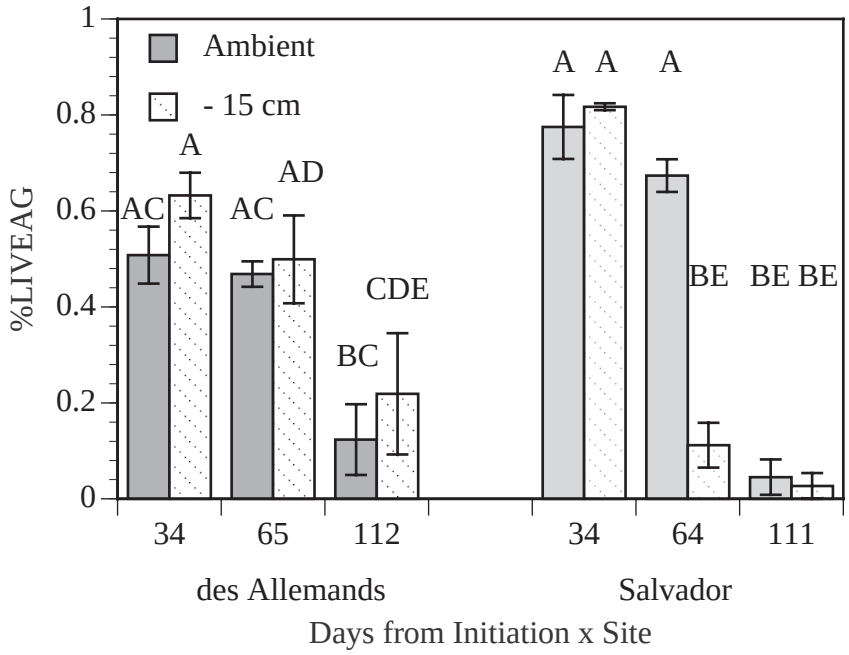

Figure 14. Live aboveground biomass as a fraction of final aboveground biomass in the field experiment responded to a three-way interaction between site, elevation and time of harvest.

%LiveAG was significantly correlated with E_h at 20-cm depth, and negatively correlated with pH, salinity and interstitial NH_4^+ , Na and S (Table 8). ANCOVAs run on %LiveAG vs. interstitial NH_4^+ and sulfur showed highly significant negative relationships at the Salvador marsh that were not seen at the des Allemands marsh (Table 10). Interstitial NH_4^+ and %LiveAG at the Salvador marsh were more negatively correlated in the –15 cm treatments than in the ambient treatments (Table 10). This could indicate uptake by the vegetation, and would explain why NH_4^+ was not significantly correlated to salinity. The latter relationship would have been expected if marsh flooding by saline water stimulated the mineralization of decomposing organic nitrogen to NH_4^+ in the absence of denitrification. Interstitial NH_4^+ concentrations were not sufficient to suggest toxicity as seen in some systems (Magalhaes et. al., 1995; Barker, 1999; Britto et. al., 2001). The negative correlation between %LiveAG and salinity is consistent with the sensitivity of *P. hemitomon* to salinity shown in the growth chamber experiments reported in this dissertation, as well as that demonstrated by other authors (Pezeshki et. al., 1987a; McKee and Mendelssohn, 1989; Howard and Mendelssohn, 1999).

Table 10. Linear relationships between abiotic and growth response variables in the field experiment at the time of harvest as a function of marsh site, determined by analysis of covariance.

Biotic Response	*Interstitial Chemical*	
	NH_4^+	*S*
% Live AG BM (for $\log_{10}$(%LiveAG)		
des Allemands	$= 0.77101-(0.00272*NH_4^+)$ $P = 0.0740$	$= 0.76103-(0.11118* \log_{10}S)$ $P = 0.2912$
Salvador	$= 1.09747-(0.00753*NH_4^+)$ $P < 0.0001$	$= 1.11368-(0.46602*\log_{10}S)$ $P = 0.0094$

Similar to Neumann's (1997) assertion of leaf ion accumulations influencing aboveground tissue growth, Kuramoto and Brest (1979) speculated that grasses may be more susceptible to salinity stress than more succulent species that rely on vacuolar storage of ions. They stated that if with the adoption of exudation mechanisms grasses probably have a higher exposure of chloroplasts to salt. While leaf adaptation seems to be more developed in halophytic grasses (Longstreth and Strain, 1977), within the freshwater-to-oligohaline environment this could underlie the competitive edge that *Sagittaria* seems to demonstrate over *Panicum* under saltwater intrusion events (Lapeyre et. al., 2001).

Belowground Growth Response

Relative growth rate of belowground tissue (RGR_{BG}) decreased significantly overtime, but more rapidly at the lower elevation as compared to at ambient elevation (Figure 7). At the

time of the 64/65-day harvest, RGR_{BG} at the –15 cm elevation was significantly lower than at the ambient elevation, and was not significantly different from RGR_{BG} at 111 days, which corresponded to senescent vegetation. These results suggest an increased sensitivity of belowground tissue, particularly at lower soil depths, to the stresses of saltwater flooding, consistent with similar observations from the growth chamber experiments reported above. This may also represent an acceleration of senescence in the belowground tissue at the lower elevation as seen by Snapp and Shennan (1994) in tomato. RGR_{BG} was negatively correlated with interstitial NH_4^+ and S (Table 8). ANCOVA showed a more negative linear relationship between RGR_{BG} vs. interstitial NH_4^+ at ambient elevation than at the –15 cm elevation (Table 9). This also likely reflects reduced growth, and concomitant reduced nutrient uptake, allowing for the accumulation of interstitial NH_4^+. RGR_{BG} was not correlated to salinity *per se*, but it is important to remember that salinity was elevated in both treatment marshes. Interestingly, RGR_{BG} was positively correlated to interstitial calcium. Supplements of calcium were shown to mollify salinity stress in *Arabidopsis* (Epstein, 1998; Liu and Zhu, 1998), wheat (Kinraide, 1998) and tomato (Navarro et. al., 2000), and the significant correlation between calcium and RGR_{BG} in *P. hemitomon* (Table 8) is consistent with this beneficial effect.

Biomass Partitioning and Total Biomass Growth Response

Root:shoot biomass ratio was significantly higher at the des Allemands marsh (1.48±0.13) as compared to at the Salvador marsh (1.13±1.0). There was also a significant effect of combined elevation and time of harvest on root:shoot ratio. Although there were no significant differences between treatment means, there was a trend of towards increasing root:shoot ratio in the lower elevation treatments between the first and last harvests (Figure 13). Root:shoot ratio was significantly correlated to interstitial S (Table 8).

Morphological Adaptations Underlying Plant Growth Response – Aerenchymatous Tissue Development

While there were both site and time-of-harvest effects on root porosity, significant effects of combined site and harvest period indicated differential responses between sites to the temporal declines in root porosity. Specifically, root porosity decreased significantly after 65 days at the des Allemands site, as compared to after 111 days at the Salvador site (Figure 15). Root porosity was positively correlated with E_h at both the surface and at 20-cm depth, and also with interstitial Mn (Table 8).

Higher root porosities early in the field study was unexpected. Prior to initiation of the experiment, the plants were raised in drained conditions. In the absence of an environmental factor such as root hypoxia that would serve as a stimulus for the development of aerenchymatous tissue, root porosity should have been low. Root aerenchyma development between experiment initiation and the 34-day harvest may have been stimulated by the low interstitial NH_4^+ concentrations (Drew, 1989) that were associated with low water levels. However, the lack of correlation between NH_4^+ and root porosity would argue against the increase in interstitial NH_4^+ driving the decrease in root porosity seen in later harvests.

Instead, consider that root porosity was performed only on live root tissue. Rapid death of the non-porous old roots due to flooding-induced anaerobiosis, and a correspondingly rapid production of highly porous new tissue (Vartapetian and Jackson, 1997) would explain the high

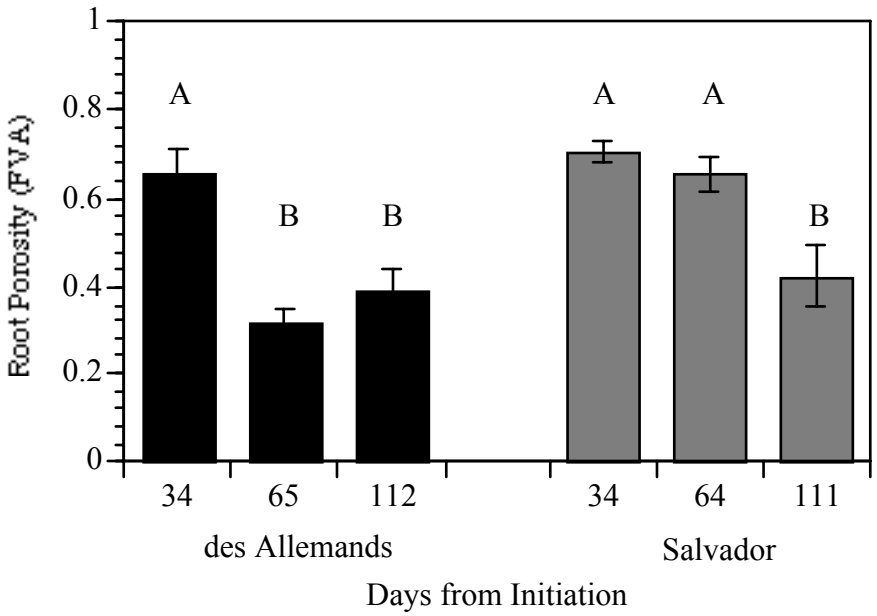

Figure 15. Root aerenchymatous tissue development decreased less quickly over time for plants at the Salvador site as compared to those at the des Allemands site in the field experiment. Shown are values for root fraction of aerenchyma, means ± 1 SE. Different capital letters indicate significant differences between treatment means.

porosities seen during the 34-day harvests. However, if production of aerenchyma in new roots was inhibited by either elevated salinity or waterlogging, then porosity would be expected to decline over time if the initial roots died. The positive correlation between root porosity and E_h, which is contrary to the idea that root aerenchyma develops in response to root zone hypoxia, may instead be suggestive of impacts on root tissue resulting from changes in the dominant redox couples , and the generation of phytotoxic reduced chemical species. The decrease in root porosity over time at both marshes would then reflect chronic effects of elevated salinity and waterlogging on the ability of the plants to adapt to a waterlogged environment.

SUMMARY OF *PANICUM HEMITOMON* RESPONSE TO COMBINED SALINITY AND SULFIDE

These results provide evidence that a mechanism for the loss of *Panicum hemitomon* from the fresh marshes of coastal Louisiana in response to saltwater intrusion is both reduced growth and a reduced ability to adapt metabolically and morphologically to the highly-reduced edaphic conditions of a saltwater-flooded marsh. Aerobic respiration in roots was sensitive to salinity, contributing to reductions in the growth rate of aboveground and more importantly belowground tissues. As roots suffered reduced growth and tissue necrosis, short-term metabolic and long-term morphological adaptations became critical for maintaining adequate nutrient and water uptake as well as the generation of metabolic energy. However, elevated salinity depressed the generation of adventitious root tissue and may have had a role in reduced aerenchymatous tissue generation in the roots. The failure of these morphological adaptations

forced the plants to rely on anaerobic fermentation to provide the biochemical energy needed for tissue maintenance and growth. Realized root fermentation, vis-à-vis tissue ethanol accumulation, was reduced under sulfidic conditions, though, thus preventing even short-term tolerance of saltwater stress. The depression of both short-term metabolic and long-term anatomical adaptive mechanisms, when combined with reduced aboveground and belowground growth, would lead to reduced vigor and threaten competitive ability under oligohaline salinity and sulfide levels and direct mortality under more extreme conditions.

APPLICATION OF RESULTS TO THE ECOLOGY OF *PANICUM HEMITOMON* IN THE SOUTHEASTERN UNITED STATES

While ranging throughout the southeastern United States (Tiner, 1993), the most detailed descriptions of *Panicum hemitomon* distribution are those in the Mississippi River deltaic plain of coastal Louisiana. The tidal freshwater reaches of the estuarine basins in this system are typified by extensive floating marshes, in which *P. hemitomon* is often dominant (Evers et. al., 1996). These mats are formed by the more resilient fractions of belowground and adventitious tissue produced by component vegetation, and the thickest freshwater mats are associated with *P. hemitomon* – dominated communities (Sasser et. al., 1996). Sasser et. al. (1995) found that *P. hemitomon* on average accounts for 76% of the biomass of marshes that it dominates. According to an estimate by O'Neil (1949), *P. hemitomon* – dominated floating marshes once covered 100,000 hectares (ha) of the Louisiana coastal zone. By 1990, 75,600 hectares identified as floating freshwater marsh within the Barataria and Terrebonne Estuarine Basins were still either dominated or co-dominated by *P. hemitomon* (Evers et. al., 1996), a decline of approximately 24,000 ha in *P. hemitomon* habitat from historical values. From 1968 to 1990, the percent of fresh-to-oligohaline marsh area within Louisiana's Barataria Estuary Complex occupied by *P. hemitomon* - dominated communities decreased from 44% to 18% (Visser et. al., 1996), while within the Terrebonne Estuary Complex, *Panicum*-dominated communities decreased from 67% to 19% during the same time period (Visser et. al., 1999).

Concurrent increases in communities dominated by *Eleocharis spp*. and *Sagittaria lancifolia*, the latter of which is characterized by a higher mean salinity of 1.5 ± 1.0 ppt (Visser et. al., 1996) suggest that the loss of *P. hemitomon* from these systems is due to saltwater intrusion into the upper reaches of the estuaries accompanying high rates of relative sea level rise (Penland and Ramsey., 1990). Much of the structural integrity of the floating marsh mats is owed to the belowground and adventitious tissue production of *P. hemitomon* and the persistence of that material in the peat. This dissertation demonstrated reduced production of the very tissues needed for continued stability of the floating marsh mat, and thus the mechanism by which the loss of this species from the mat and subsequent replacement by more oligohaline species such as *Eleocharis* spp. and *S. lancifolia* can lead to structurally weaker floating marsh (Sasser et. al., 1996). Mat thinning or decay due to stressor-induced decreases in root production and/or species replacement and the corresponding declines in belowground production jeopardize the persistence of the floating marsh. When graminoid dominants such as *P. hemitomon* are lost from a freshwater marsh, the highly organic peat becomes more susceptible to loss by tidal flushing. The elevation of the marsh may decrease by 10 to 20 cm before more salt tolerant species can become established, exacerbating the problem of saltwater intrusion (Chabreck, 1982) and threatening to accelerate conversion of vegetated wetland to open water.

Sasser et. al. (1995) discussed that the temporal stability of a floating marsh may owe to the constant presence of plant roots in a saturated soil environment, thus insulating the ecosystem against changes resulting from drought and flood conditions. However, in the event of either acute or chronic saltwater intrusion, this advantage becomes problematic by not allowing the roots to escape the saline rooting environment that results. Additionally, as discussed by both Gagliano and Wicker (1989) and Sasser et. al. (1995), there may be a risk of increased subsurface erosion of the floating marsh system with higher water levels. The risk of erosion is all the more possible when this water is saline and/or sulfidic, thus both inhibiting new belowground production and phytotoxic to existing belowground biomass.

The near-monoculture stands of *P. hemitomon* that characterize the tidal freshwater marshes of coastal wetland complexes give way to mixed *P. hemitomon/Sagittaria lancifolia* and then *S. lancifolia*-dominated communities as one travels seaward. *Panicum hemitomon* was unable to maintain growth at elevated salinity when also exposed to competition with *S. lancifolia* and *Spartina patens* (Lapeyre et. al., 2001), and it may be that competition with a more salt-tolerant species under saltwater intrusion conditions aggravates stress-induced growth reductions and removes the species from the community mosaic at salinities as low as 1.5 ppt, as observed by Visser et. al. (1996).

The two marshes used in the field study exhibited semi-buoyant tendencies and were subject to variable water levels on the marsh. Within Louisiana's Chenier Plain, on the southwestern coast, *P. hemitomon*-dominated marshes are commonly attached to the underlying substrate (Visser et. al., 2000). The fresh marshes of southwestern Louisiana typically have a higher soil mineral content (52% as opposed to 14% as in the deltaic plain), owing to the influence of discharge from the Atchafalaya River (Brupbacher et. al., 1973). Holm et. al. (2000) found that oligohaline marshes closer to sediment sources, and having a greater mineral content, were less buoyant than more organic inland marshes.

Panicum hemitomon has an extensive range throughout the southeastern United States, and in most of that range it is also not floating. In the Florida Everglades, *P. hemitomon* is common in the wet prairies in the northern and central Everglades between the *Cladium jamaicense*-dominated marshes and the sloughs, and in the sloughs themselves. Inundation of the vegetation can be significant in both these environments, approaching one meter in the sloughs during the rainy season, with hydroperiods commonly exceeding 11 months (Lodge, 1994). Far from being stressed, *P. hemitomon* appears to thrive under such conditions, and maximum productivity has been shown to occur with a frequency of inundation between 85 and 90% (Lowe, 1986). However, those are freshwater conditions. The results of this dissertation suggest that *P. hemitomon* in semi-buoyant floating marshes and fixed marshes with variable flooding depths may be highly susceptible to the stresses associated with saltwater intrusion events. Specifically, the sensitivity of adventitious and belowground tissues to salinity and sulfide may allow for a deleterious positive feedback loop similar to that described by Nyman et. al. (1993) for *Spartina alterniflora*. Marshes along the northern Gulf Coast depend on organic production to ensure adequate vertical accretion of the marsh surface to counter a rise in sea-level (Nyman et. al., 1993; Warren and Niering, 1993). Reductions in either belowground and adventitious root production may depress accretion rates, thereby exacerbating the flooding stress to which the plants are subjected.

MANAGEMENT IMPLICATIONS

Wetland loss in coastal Louisiana is extensive, with almost 1085 square kilometers of coastal wetlands having been lost during the 12 year period between 1978 and 1990 (Barras et. al., 1994). Recent GIS analysis of land loss from 1930 to 1990 indicates that the majority of land loss has occurred in interior marshes, and that the process responsible for the most land loss has been submergence (Penland et. al., 1998). The causes as outlined in the Introduction, include both natural factors related to the deltaic environment as well as anthropogenic hydrologic alterations to the coastal zone. In addition to wetland loss, I have already described data that suggest that a shift in the community composition in much of the remaining fresh marsh has also occurred (Visser et. al., 1996) that is being driven by saltwater intrusion into the oligohaline and freshwater marshes.

The need to ameliorate coastal land loss has been the impetus behind both functioning and planned large-scale river diversions, which are intended to nourish the marshes behind the Mississippi River levee with fresh water and sediments and push back saltwater intrusion. The most recent of these projects is the Davis Pond Freshwater Diversion (Figures 15), a $120 million structure intended to directly preserve or indirectly benefit approximately 810,000 acres of marshes and bays (USGS, 2002). When operational, Davis Pond is expected to increase water levels slightly in the northern reaches of the Barataria Estuary and establish enough of a hydraulic head to push the salinity isopleths several kilometers seaward.

The results of this dissertation and the literature discussed in it would predict that *Panicum hemitomon* should benefit from the project and would be expected to expand its range within the estuary. With seasonal pulses of freshwater and sediments, the marshes around Lake Catahouatche and northern Lake Salvador would be expected to perhaps more closely resemble the more mineral fresh marshes of the Terrebonne Estuarine Basin as opposed to the highly organic marshes that presently characterize the upper reaches of the present Barataria Estuary. While the depth of flooding may increase slightly, the increase in freshwater and oligohaline salinities in the marshes affected by the project should easily prove more of a subsidy than a stress on the vegetation. Sulfide may potentially still be problematic if the river water used to flood the estuary contains elevated sulfate concentrations from agricultural runoff or municipal sources. In such situations sulfide generation in the soils may occur independent of a saltwater source (Feng and Hsieh, 1998; Lamers et. al., 1998). Given the deleterious growth and physiological responses to flooding with saline water seen in *P. hemitomon* in this dissertation and in studies by others (McKee and Mendelssohn, 1989; Flynn et. al., 1994; Howard and Mendelssohn, 1999b), the alleviation of salt stress would be expected to benefit this species both in terms of physiology and growth as well as potential competitive ability with other prevalent oligohaline species such as *Eleocharis* spp., *Sagittaria lancifolia* and *Spartina patens*.

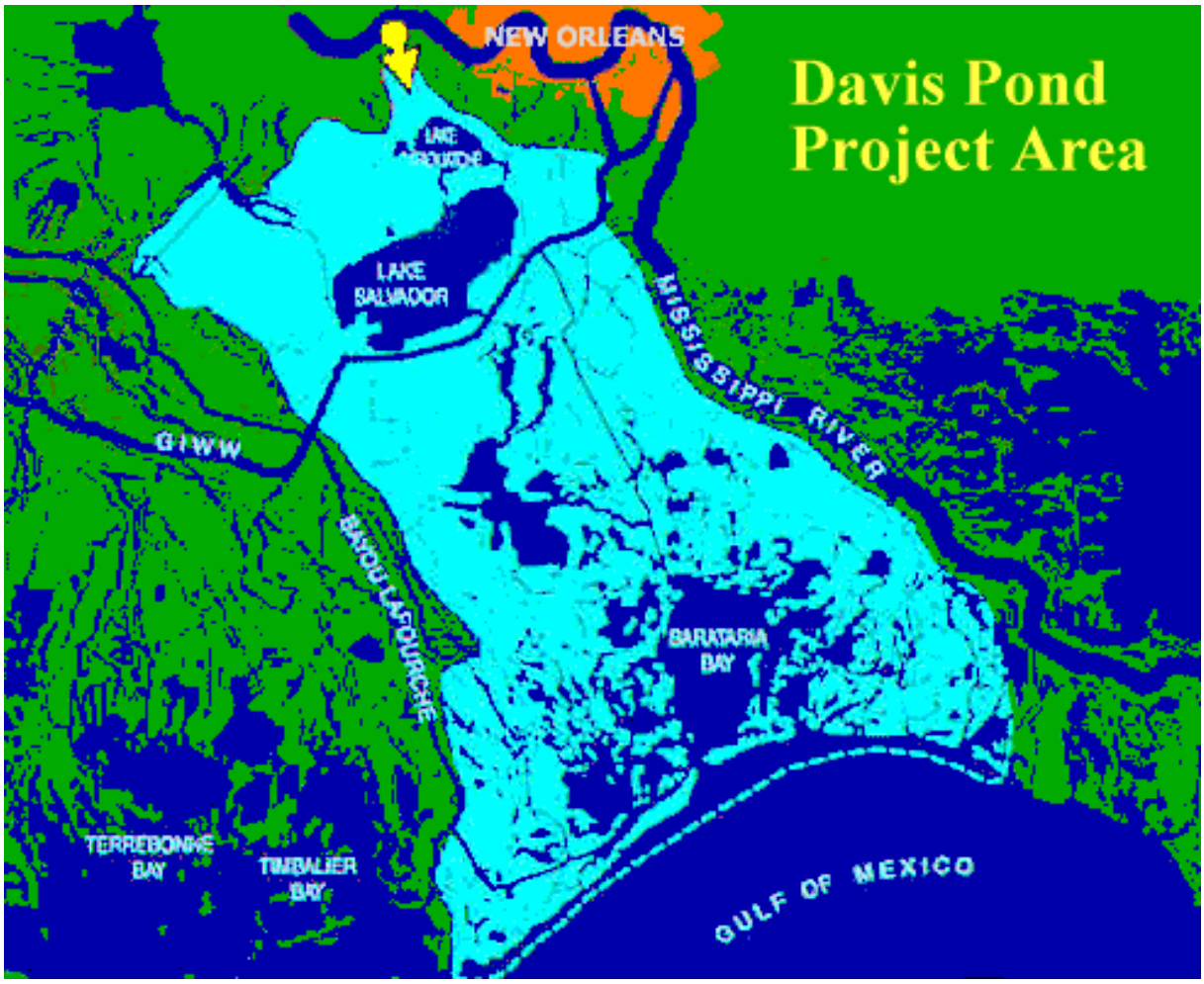

Figure 16. Map of the area of the Barataria Estuary predicted to benefit either directly or indirectly from operations of the Davis Pond Freshwater Diversion in southeastern Louisiana. Image courtesy of the U.S. Army Corps of Engineers (http://www.mvn.usace.army.mil/pao/dpond/davispond.htm).

REFERENCES

Adams, J.B., and G.C. Bate. 1999. Growth and photosynthetic performance of *Phragmites australis* in estuarine waters: a field and experimental evaluation. Aquatic Botany **64**: 359-367.

Akhtar, J., J. Gorham, R.Q. Qureshi and M. Aslam. 1998. Does tolerance to wheat to salinity and hypoxia correlated with root dehydrogenase activities or aerenchyma formation? Plant and Soil **201**: 275-284.

Allam, A.I., and J.P. Hollis. 1972. Sulfide inhibition of oxidases in rice roots. Phytopathology **62**: 634-639.

Allen, J.A., J.L. Chambers and S.R. Pezeshki. 1997. Effects of salinity on baldcypress seedlings: physiological responses and their relation to salinity tolerance. Wetlands **17**: 310-320.

Armstrong, J., and W. Armstrong. 2001. Rice and *Phragmites*: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. American Journal of Botany **88**: 1359-1370.

Armstrong, W., J. Armstrong, and P.M. Beckett. 1996a. Pressurised aeration in wetland macrophytes: some theoretical aspects of humidity-induced convection and thermal transpiration. Folia Geobotanica et Phytotaxonomica **31**: 25-36.

Armstrong, J., W. Armstrong, J. Wu and F. Afreen-Zobayed. 1996b. A role for phytotoxins in the *Phragmites* die-back syndrome? Folia Geobotanica et Phytotaxonomica **31**: 127-142.

Bailey-Serres, B., and R.K. Dawe. 1996. Both 5' and 3' sequences of maize adh1 mRNA are required for enhanced translation under low-oxygen conditions. Plant Physiology **112**: 685-695.

Barker, A.V. 1999. Foliar ammonium as an index of stress in plants. Commun soil sci plant anal **30**: 167-174.

Barras, J.A., P.E. Bourgeois, and L.R. Handley. 1994. Land Loss in Coastal Louisiana 1956-90. National Biological Survey, National Wetlands Research Center Open File Report 94-01. 4 pp. 10 color plates.

Beinert, H., R.H. Holm and E. Münck. 1997. Iron-sulfur clusters: nature's modular, multipurpose structures. Science **277**: 652-659.

Bertani, A., I. Brambilla and F. Menegus. 1980. Effect of anaerobiosis on rice seedlings: growth, metabolic rate, and fate of fermentation products. Journal of Experimental Botany **31**: 325-331.

Bidwell, J.P., and S. Spotte. 1985. Artificial Seawaters: Formulas and Methods. Jones and Bartlett Publishers, Inc., Boston. 329 pp.

Blom, C.W.P.M., G.M. Bögemann, P. Laan, A.J.M. van der Sman, H.M. van de Steeg and L.A.C.J. Voesenek. 1990. Adaptations to flooding in plants from river areas. Aquatic Botany **38**: 29-47.

Bouraoui, N., C. Grignon and E. Zid. 1998. Effect of NaCl on root growth and root respiration in triticale (X-triticosecale Wittmack). Cahiers Agricultures **7**: 372-376.

Bradley, P.M., and E.L. Dunn. 1989. Effects of sulfide on the growth of three salt marsh halophytes of the southeastern United States. American Journal of Botany **76**: 1707-1713.

Bradley, P.M., and J.T. Morris. 1991. Relative importance of ion exclusion, secretion and accumulation in *Spartina alterniflora* Loisel. Journal of Experimental Botany **42**: 1525-1532.

Britto, D.T., M.Y. Siddiqi, A.D.M. Glass and H.J. Kronzucker. 2001. Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proceedings of the National Academies of Sciences, USA **98**: 4255-4258.

Broome, S.W., I.A. Mendelssohn and K.L. McKee. 1995. Relative growth of *Spartina patens* (Ait.) Muhl. And *Scirpus olneyi* Gray occurring in a mixed stand as affected by salinity and flooding depth. Wetlands **15**: 20-30.

Brupbacher, R.H., J.E. Sedberry and W.H. Willis. 1973. The coastal marshlands of Louisiana : chemical properties of the soil materials. Bulletin - Louisiana Agricultural Experiment station, No. 672. 34 p.

Burdick, D.M. 1989. Root aerenchyma development in *Spartina patens* in response to flooding. American Journal of Botany **76**: 777-780.

Carlson, P.R., Jr., and J. Forrest. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: evidence from natural sulfur isotope abundance ratios. Science **216**: 633-635.

Chabbi, A., K.L. McKee and I.A. Mendelssohn. 2000. Fate of oxygen losses from *Typha domingensis* (Typhaceae) and *Cladium jamaicense* (Cyperaceae) and consequences for root metabolism. American Journal of Botany **87**: 1081-1090.

Chambers, R.M. 1997. Porewater chemistry associated with *Phragmites* and *Spartina* in a Connecticut tidal marsh. Wetlands **17**: 360-367.

Chambers, R.M., T.J. Mozdzer, J.C. Ambrose. 1998. Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. Aquatic Botany **62**: 161-169.

Chan, J.W.Y., and R.S. Burton. 1992. Variation in alcohol dehydrogenase activity and flood tolerance in white clover, *Trifolium repens*. Evolution **46**: 721-734.

Cízková, H., and V. Bauer. 1998. Rhizome respiration of Phragmites australis: effect of rhizome age, temperature, and nutrient status of the habitat. Aquatic Botany **61**: 239-253.

Cole, G.A. 1988. Textbook of Limnology: Third Edition. Waveland Press, Prospect Heights, 401 pp.

Conner, W. H. 1994. The effect of salinity and waterlogging on growth and survival of baldcypress and Chinese tallow seedlings. Journal of Coastal Research **10**: 1045-1049.

Cossins, E.A., L.C. Kopala, B. Blawacky and A.M. Spronk. 1968. Some properties of a higher plant alcohol dehydrogenase. Phytochemistry **7**: 1125-1134.

Dolferus, R., M. Ellis, G. De Bruxelles, B. Trevaskis, F. Hoeren, E.S. Dennis and W.J. Peacock. 1997. Strategies of gene action in *Arabidopsis* during hypoxia. Annals of Botany **79 (Suppl. A)**: 21-31.

Drake, B.G., and J.L. Gallagher. 1984. Osmotic potential and turgor maintenance in *Spartina alterniflora* Loisel. Oecologia **62**: 368-375.

Drew, M.C. 1989. Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphate-starvation in adventitious roots of *Zea mays* L. Plant Physiology **91**: 266-271.

Epron, D., M.L. Toussaint and P.M. Badot. 1999. Effects of sodium chloride salinity on root growth and respiration in oak seedlings. Annals of Forest Science **56**: 41-47.

Epstein, E. 1998. How calcium enhances plant salt tolerance. Science **280**: 1906-1907.

Evers, D.E., G.O. Holm, and C.E. Sasser. 1996. Digitization of the Floating Marsh maps of the Barataria and Terrebonne Basins, Louisiana. Barataria-Terrebonne National Estuary Program, BTNEP Publication No. 28, Thibodaux, Louisiana.

Feijtel, T.C., J.P.A. Moore, K.L. McKee and I.A. Mendelssohn. 1989. Salinity and flooding level as determinants of soil solution composition and nutrient content in *Panicum hemitomon*. Plant and Soil **114**: 197-204.

Feng, J., and Y.P. Hsieh. 1998. Sulfate reduction in freshwater wetland soils and the effects of sulfate and substrate loading. Journal of Environmental Quality **27**: 968-972.

Flessa, H., and W.R. Fischer. 1992. Plant-induced changes in the redox potentials of rice rhizospheres. Plant and Soil **143**: 55-60.

Flynn, K.M., K.L. McKee nd I.A. Mendelssohn. 1994. Recovery of freshwater marsh vegetation after a saltwater intrusion event. Oecologia **103**: 63-72.

Fürtig, K., A. Rüegsegger, C. Brunold and R. Brändle. 1996. Sulfide utilization and injuries in hypoxic roots and rhizomes of common reed (*Phragmites australis*). Folia Geobotanica et Phytotaxinomica **31**: 143-151.

Gagliano, S.M. 1973. Canals, dredging and land reclamation in the Louisiana coastal zone. Hydrological and Geological studies of Coastal Louisiana. Report No. 14, Coastal Resources Unit, Center for Wetland Resources, LSU, Baton Rouge, LA.

Gorham, J., R.G. Wyn Jones and E. McDonnell. 1985. Some mechanisms of salt tolerance in crop plants. Plant and Soil **89**: 15-40.

Greenway, H., and R. Munns. 1980. Mechanisms of Salt Tolerance in Nonhalophytes. Annual Review of Plant Physiology **31**: 149-190.

Greenwood, D.J. 1961. The effect of oxygen concentration on the decomposition of organic materials in soil. Plant and Soil **14**: 360-376.

Hamada, A.M. 1996. Effect of NaCl, water stress or both on gas exchange and growth of wheat. Biologia Plantarum **38**: 405-412.

Havill, D.C., A. Ingold and J. Pearson. 1985. Sulfide tolerance in coastal halophytes. Vegetatio **62**: 279-285.

Hernadez, J.A., M.S. Almansa, L. del Rio and F. Sevilla. 1993. Effect of salinity on metalloenzymes of oxygen metabolism in two leguminous plants. Journal of Plant Nutrition **16**: 2539-2554.

Hester, M.W., I.A. Mendelssohn, and K.L. McKee. 1998. Intraspecific variation in salt tolerance and morphology in *Panicum hemitomon* and *Spartina alterniflora* (Poaceae). International Journal of Plant Science **159**: 127-138.

Holm, G.O., Jr., C.E. Sasser, G.W. Peterson, and E.M Swenson. 2000. Vertical movement and substrate characteristics of oligohaline marshes near a high-sediment, riverine system. Journal of Coastal Research **16**: 164-171.

Houle, G., L. Morel, C.E. Reynolds and J. Siégel. 2001. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). American Journal of Botany **88**: 62-67.

Howard, R.J., and I.A. Mendelssohn. 1999a. Salinity as a constraint on growth of oligohaline marsh macrophytes. I. Species variation in stress tolerance. American Journal of Botany **86**: 785-794.

Howard, R.J., and I.A. Mendelssohn. 1999b. Salinity as a constraint on growth of oligohaline marsh macrophytes. II. Salt pulses and recovery potential. American Journal of Botany **86**: 795-806.

Howard, R.J., and I.A. Mendelssohn. 2000. Structure and composition of oligohaline marsh plant communities exposed to salinity pulses. Aquatic Botany **68**: 143-164.

Huang, B., D.S. NeSmith, D.C. Bridges, and J.W. Johnson. 1995. Reponses of squash to salinity, waterlogging, and subsequent drainage: II. Root and shoot growth. Journal of Plant Nutrition **18**: 141-152.

Ingold, A., and D.C. Havill. 1985. Some effects of sulfide in vitro on selected enzymes, oxygen uptake and ion uptake in four salt marsh plant species. Comparative Physiology and Ecology **10**: 1-6.

Jackson, M.B., and W. Armstrong. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biology **1**: 274-287.

King, G.M., M.J. Klug, R.G. Wiergert, and A.G. Chalmers. 1982. Relation of soil water movement and sulfide concentration to *Spartina alterniflora* production in a Georgia salt marsh. Science **218**: 61-63.

Kinraide, T.B. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. Plant Physiology **118**: 513-520.

Kleifeld, O., A. Frenkel, O. Bogin, M. Eisenstein, V. Brumfeld, V. Burnstein and I. Sagi. 2000. Spectroscopic studies of inhibited alcohol dehydrogenase from *Thermoanaerobacter brockii*: proposed structure for the catalytic intermediate state. Biochemistry **39**: 7702-7711.

Koch, M.S., and I.A. Mendelssohn. 1989. Sulfide as a soil phytotoxin: Differential responses in two marsh species. Journal of Ecology **77**: 565-578.

Koch, M.S., I.A. Mendelssohn, and K.L. McKee. 1990. Mechanism for the hydrogen sulfide - induced growth limitation in wetland macrophytes. Limnology and Oceanography **35**: 399-408.

Kuramoto, R.T., and D.E. Brest. 1979. Physiological response to salinity by four salt marsh plants. Botanical Gazette **140**: 295-298.

Lamers, L.P.M., H.B.M. Tomassen and J.G.M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science and Technology **32**: 199-205.

Lapeyre, M.K., J.B. Grace, E. Hahn, and I.A. Mendelssohn. 2001. The importance of competition in regulating plant species abundance along a salinity gradient. Ecology **82**: 62-69.

Latham, P. J., L. G. Pearlstine, W.M. Kitchens. 1994. Species association changes across a gradient of freshwater, oligohaline, and mesohaline tidal marshes along the lower Savannah River. Wetlands **14**: 174-183.

Lehninger, A.L., D.L. Nelson, M.M. Cox. 1993. Principles of Biochemistry. Worth Publ., New York, 1013 pp.

Lin, J.N., and C.H. Kao. 1998. Water stress, ammonium, and leaf senescence in detached rice leaves. Plant Growth Regulation **25**: 165-169.

Liu, J., and J.-K. Zhu. 1998. A calcium sensor homolog required for plant salt tolerance. Science **280**: 1943-1945.

Linthurst, R.A. 1979. The effect of aeration on the growth of *Spartina alterniflora* Loisel. American Journal of Botany **66**: 685-691.

Lodge, T.E., and M.S. Douglas. 1996. The Everglades Handbook: Understanding the Ecosystem. St. Lucie Press, 248 pp.

Longstreth, D.J., and B.R. Strain. 1977. Effects of salinity and illumination on photosynthesis and water balance in *Spartina alterniflora*. Oecologia **31**: 191-200.

Lowe, E.F. 1986. The relationship between hydrology and vegetational pattern within the floodplain marsh of a subtropical, Florida lake. Florida Scientist **49**: 213-233.

Magalhaes, J.R., A.T. Machado and D.M. Huber. 1995. Similarities in response of maize genotypes to waterlogging and ammonium toxicity. Journal of Plant Nutrition **18**: 2339-2346.

McKee, K.L., and I.A. Mendelssohn. 1989. Response of a freshwater marsh plant community to increased salinity and increased water level. Aquatic Botany **34**: 301-316.

McKee, K.L., I.A. Mendelssohn, and M.W. Hester. 1988. Reexamination of pore water sulfide concentrations and redox potentials near the aerial roots of *Rhizophora mangle* and *Avicennia germinans*. American Journal of Botany **75**: 1352-1359.

Mendelssohn, I.A. 1993. Factors Controlling the Formation of Oxidized Root Channels: A Review and Annotated Bibliography. Wetlands Research Program Technical Report WRP-DE-5. 36 pp.

Mendelssohn, I.A., and K.L. McKee. 1987. Root metabolic response of *Spartina alterniflora* to hypoxia. Pages 239-253 in R.M.M. Crawford (ed.). Plant Life in Aquatic and Amphibious Habitats. Blackwell Scientific Publishers, Oxford.

Mendelssohn, I.A., K.L. McKee, and W.H. Patrick, Jr. 1981. Oxygen deficiency in *Spartina alterniflora* roots: metabolic adaptation to anoxia. Science **214**: 439-441.

Misra, N., and U.N. Swivedi. 1995. Carbohydrate metabolism during seed germination and seedling growth in green gram under saline stress. Plant Physiology and Biochemistry **33**: 33-38.

Muench, D.G., O.W. Archibold and A.G. Good. 1993. Hypoxic metabolism in wild rice (*Zizania palustris*): enzyme induction and metabolite production. Physiologia Plantarum **89**: 165-171.

Munns, R., and A. Termaat. 1986. Whole-plant responses to salinity. Australian Journal of Plant Physiology **13**: 143-160.

Naidoo, G. 1994. Growth, water and ion relationships in the coastal halophytes *Triglochin bulbosa* and *T. striata*. Environmental and Experimental Botany **34**: 419-426.

Naidoo, G., and S.G. Mundree. 1993. Relationship between morphological and physiological responses to waterlogging and salinity in *Sporobolus virginicus* (L.) Kunth. Oecologia **93**: 360-366.

Navarro, J.M., V. Martínez and M. Carvajal. 2000. Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions. Plant Science **157**: 89-96.

Neumann, P. 1997. Salinity resistance and plant growth revisited. Plant, Cell and Environment **20**: 1193-1198.

Nyman, J.A., J.C. Callaway, and R.D. DeLaune. 1993. Case study of a rapidly submerging coastal environment: Relationships among vertical accretion, carbon cycling and marsh loss in Terrebone Basin, Louisiana. Pgs. 452-457 In Proceedings of the Hilton Head Island, South Carolina, U.S.A. International Coastal Symposium, Vol. 2.

O'Neil, T. 1949. The muskrat in the Louisiana coastal marshes. Louisiana Department of Wildlife and Fisheries, New Orleans. 152 pp.

Pan, S., and S.R. Pezeshki. 1991. Effect of sulfide on photosynthesis of *Oryza sativa.* Plant Physiology Communications **27**: 356-358.

Pearson, J., and D.C. Havill. 1988. The effect of hypoxia and sulfide on culture-grown wetland and non-wetland plants. II. Metabolic and physiological changes. Journal of Experimental Botany **39**: 431-439.

Penland, S., and K.E. Ramsey. 1990. Relative sea-level rise in Louisiana and the Gulf of Mexico: 1908-1988. Journal of Coastal Research **6**: 323-342.

Penland, S., L. Wayne and L.D. Britsch. 1998. GIS analysis of natural and human causes of coastal land loss in the Mississippi River delta plain. in Recent Research in Coastal Louisiana: Natural System Function and Response to Human Influences. 3-5 February 1998 (Abstract).

Pezeshki, S.R., R.D. DeLaune, and S.Z. Pan. 1991. Relationship of soil hydrogen sulfide level to net carbon assimilation of *Panicum hemitomon* and *Spartina patens.* Vegetatio **95**: 159-166.

Pezeshki, S. R., R. D. DeLaune, and W.J. Patrick, Jr. 1987. Response of the freshwater marsh species, *Panicum hemitomon* Schult., to increased salinity. Freshwater Biology **17**: 195-200.

Rahman, M. and I.A. Ungar. 1994. The effect of competition and salinity on shoot growth and reproductive biomass of *Echinochloa crus-galli*. Aquatic Botany **48**: 343-353.

Reuveni, J., J. Gale and M. Zeroni. 1997. Differentiating day from night effects of high ambient CO_2 on the gas exchange and growth of *Xanthium strumarium* L. exposed to salinity stress. Annals of Botany **79**: 191-196.

Rogers, M.E., and D.W. West. 1993. The effects of rootzone salinity and hypoxia on shoot and root growth in *Trifolium* species. Annals of Botany **72**: 503-509.

Salama, S., S. Trivedi, M. Busheva, A.A. Arafa, G. Garab, and L. Erdei. 1994. Effects of NaCl salinity on growth, cation accumulation, chloroplast structure and function in wheat cultivars differing in salt tolerance. Journal of Plant Physiology **144**: 241-247.

Sasser, C.E., and J.G. Gosselink. 1984. Vegetation and primary production in a floating freshwater marsh in Louisiana. Aquatic Botany **20**: 245-255.

Sasser, C. E., J. G. Gosselink, E.M. Swenson, C.M. Swarzenski, and N.C. Leibotwitz. 1996. Vegetation, substrate and hydrology in floating marshes in the Mississippi River delta plain wetlands, USA. Vegetatio **122**: 129-142.

Sasser, C.E., J.M. Visser, D.E. Evers and J.G. Gosselink. 1995. The role of environmental variables on interannual variation in species composition and biomass in a subtropical minerotrophic floating marsh. Canadian Journal of Botany **73**: 413-424.

Sharma, S.N., and G. Singh. 1989. Limitations of gas exchanges in intact leaves during ontogeny of field-grown chickpeas. Journal of Experimental Botany **40**: 1399-1406.

Smith, A.M. and T. ap Rees. 1979. Pathways of carbohydrate fermentation in the roots of marsh plants. Planta **146**: 327-334.

Snapp, S.S., and C. Shennan. 1994. Salinity effects on root growth and senescence in tomato and the consequences for severity of phytophthora root rot infection. American Society of Horticultural Sciences, Journal **119**: 458-463.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach, Second Edition. McGraw-Hill Book Co., New York.

Studer, C., and R. Braendle. 1987. Ethanol, acetaldehyde, ethylene release and ACC concentration of rhizomes from marsh plants under normoxia, hypoxia and anoxia. Pages 293-301 *in* R.M.M. Crawford (ed.). Plant Life in Aquatic and Amphibious Habitats, Special Publication Number 5 of the British Ecological Society. Blackwell Scientific Publishers, Oxford.

Sunby, B., C. Vale, I. Caçador, F. Catarino, M.-J. Madureira and M. Caetano. 1998. Metal-rich concretions on the roots of salt marsh plants: mechanism and rate of formation. Limnology and Oceanography **43**: 245-252.

Turner, F.T., and W.H. Patrick, Jr. 1968. Chemical changes in waterlogged soils as a result of oxygen depletion. Transactions of the 9th International Congress of Soil Sciences **4**: 53-56.

USGS. 2002. Davis Pond Freshwater Diversion Project. http://www.lacoast.gov/programs/DavisPond/index.htm

Vartapetian, B.B., and M.B. Jackson. 1997. Plant adaptations to anaerobic stress. Annals of Botany **79** (Suppl. A): 3-20.

Visser, J.M., C.E. Sasser, R.H. Chabreck and R.G. Linscombe. 1996. Marsh vegetation types of the Mississippi River deltaic plain. Estuaries **21**: 818-828.

Visser, J.M., C.E. Sasser, R.H. Chabreck and R.G. Linscombe. 1999. Long-term vegetation change in Louisiana tidal marshes, 1968-1992. Wetlands **19**: 168-175.

Visser, J.M., C.E. Sasser, R.H. Chabreck, and R.G. Linscombe. 2000. Marsh vegetation types of the Chenier Plain, Louisiana, USA. Estuaries **23**: 318-327.

Warren, R.S., and W.A. Niering. 1993. Vegetation change on a northeast tidal marsh: Interaction of sea-level rise and marsh accretion. Ecology **74**: 96-103.

Webb, E.C., and I. A. Mendelssohn. 1996. Factors affecting vegetation dieback of an oligohaline marsh in coastal Louisiana: field manipulation of salinity and submergence. American Journal of Botany **83**: 1429-1434.

Youssef, T., and P. Saenger. 1998. Photosynthetic gas exchange and accumulation of phytotoxins in mangrove seedlings in response to soil physio-chemical characteristics. Tree Physiology **18**: 317-324.

APPENDIX – ANOVA TABLES

The following pages are the ANOVA tables that were run a posteriori of the MANOVA for each set of response variables analyzed for each experiment. The ANOVA tables shown are for the factors for which statistical relationships were reported in the dissertation, therefore they may represent either untransformed or transformed data, depending on if it was necessary to satisfy the assumptions of the MANOVA. The following superscripts are used to indicate the state of data transformation:

^{U} signifies untransformed data
^{L} signifies $\log_{10}$-transformed data
^{S} signifies square-root transformed data
^{A} signifies arcsine (square-root) transformed data

Appendix Table 1. 39-Week Experiment – Abiotic Factors, pg. 1

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
E_h (1 cm)^U	Salinity	2	1733.78	0.32	0.7294
	Sulfide	2	202314.31	37.19	0.0001
	Salinity*Sulfide	4	7005.84	1.29	0.2955
	Error	32	5440.66		
	Time	1	2.41	0.00	0.9807
	Time*Salinity	2	1769.50	0.44	0.6510
	Time*Sulfide	2	323.47	0.08	0.9237
	Time*Salinity*Sulfide	4	2716.51	0.67	0.6189
	Error (Time)	32	4066.57		
E_h (25 cm)^U	Salinity	2	5880.06	1.31	0.2844
	Sulfide	2	202606.62	45.08	0.0001
	Salinity*Sulfide	4	4615.94	1.03	0.4084
	Error	32	4494.57		
	Time	1	2062.28	0.40	0.5298
	Time*Salinity	2	13401.40	2.62	0.0882
	Time*Sulfide	2	3181.21	0.62	0.5429
	Time*Salinity*Sulfide	4	1876.59	0.37	0.8301
	Error (Time)	32	5109.82		
Ca	Model	8	0.92	60.17	0.0001
	Salinity	2	3.03	197.86	0.0001
	Sulfide	2	0.35	22.74	0.0001
	Salinity x Sulfide	4	0.10	6.40	0.0009
	Error	28	0.02		
Fe	Model	8	4.26	47.31	0.0001
	Salinity	2	0.17	1.87	0.1733
	Sulfide	2	12.19	135.42	0.0001
	Salinity x Sulfide	4	1.75	19.49	0.0001
	Error	28	0.09		
K	Model	8	3.81	98.24	0.0001
	Salinity	2	10.14	261.70	0.0001
	Sulfide	2	1.56	40.18	0.0001
	Salinity x Sulfide	4	0.59	15.22	0.0001
	Error	28	0.04		

Appendix Table 1. 39-Week Experiment – Abiotic Factors, pg. 2

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Mg	Model	8	1.99	97.30	0.0001
	Salinity	2	7.39	362.00	0.0001
	Sulfide	2	0.31	15.03	0.0001
	Salinity x Sulfide	4	0.08	3.81	0.0136
	Error	28	0.02		
Mn	Model	8	0.77	15.37	0.0001
	Salinity	2	1.52	30.48	0.0001
	Sulfide	2	1.06	21.29	0.0001
	Salinity x Sulfide	4	0.16	3.27	0.0256
	Error	28	0.01		
Na	Model	8	1.88	161.15	0.0001
	Salinity	2	4.41	377.37	0.0001
	Sulfide	2	0.82	70.37	0.0001
	Salinity x Sulfide	4	0.52	44.59	0.0001
	Error	28	0.01		
NH_4^+	Model	8	421.95	20.24	0.0001
	Salinity	2	348.96	16.74	0.0001
	Sulfide	2	1155.94	55.44	0.0001
	Salinity x Sulfide	4	14.64	0.70	0.5970
	Error	28	20.85		
P	Model	8	1.27	14.85	0.0001
	Salinity	2	1.89	22.14	0.0001
	Sulfide	2	2.58	30.20	0.0001
	Salinity x Sulfide	4	0.06	0.75	0.5649
	Error	28	0.09		
S	Model	8	1.87	11.44	0.0001
	Salinity	2	0.03	0.21	0.8153
	Sulfide	2	6.98	42.65	0.0001
	Salinity x Sulfide	4	0.17	1.06	0.3956
	Error	28	0.16		

Appendix Table 2. 39-Week Experiment – Biotic Factors

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Relative Growth Rate (Aboveground)[U]	Model	8	0.0000064	1.38	0.2466
	Salinity	2	0.0000067	1.43	0.2557
	Sulfide	2	0.0000053	1.13	0.3388
	Salinity x Sulfide	4	0.0000069	1.47	0.2379
	Error	28	0.0000047		
Live as % Total BM[A]	Model	8	0.57	10.67	0.0001
	Salinity	2	1.69	31.60	0.0001
	Sulfide	2	0.18	3.30	0.0516
	Salinity x Sulfide	4	0.07	1.25	0.3113
	Error	28	0.05		
Adv. As % Final BM[A]	Model	8	0.006	6.09	0.0001
	Salinity	2	0.005	5.11	0.0128
	Sulfide	2	0.013	13.98	0.0001
	Salinity x Sulfide	4	0.003	3.40	0.0219
	Error	28	0.001		
Note that for stem density responses, only salinity was indicated as a significant factor by the MANOVA					
%Δ Total SD[A]	Model	2	0.11	5.52	0.0084
	Salinity	2	0.11	5.52	0.0084
	Error	34	0.02		
%Δ Live SD[A]	Model	2	0.25	13.74	0.0001
	Salinity	2	0.25	13.74	0.0001
	Error	34	0.02		
Live as % Total SD[A]	Model	2	1.02	20.78	0.0001
	Salinity	2	1.02	20.78	0.0001
	Error	34	0.05		

Appendix Table 3. 39-Week Experiment – Raw Productivity Values #1: Predicted Initial Live Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	9.1±1.1	12.4±0.4	6.5±1.6
2 ppt	8.5±1.0	10.0±1.7	6.4±1.3
4 ppt	10.2±2.0	10.8±1.4	11.9±2.0

Appendix Table 4. 39-Week Experiment – Raw Productivity Values #2: Final Aboveground Live Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	23.5±4.0	21.9±1.6	24.4±3.7
2 ppt	14.0±4.0	2.3±0.9	1.7±0.7
4 ppt	2.0±2.0	0.9±0.8	5.7±5.7

Appendix Table 5. 39-Week Experiment – Raw Productivity Values #3: Final Aboveground Total Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	30.6±3.9	47.0±4.0	46.6±7.3
2 ppt	44.6±5.2	34.4±2.6	32.1±3.5
4 ppt	26.0±9.0	35.8±2.0	29.0±5.7

Appendix Table 6. 39-Week Experiment – Raw Productivity Values #4: Final Adventitious Root Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	0.1±0.0	1.0±0.3	1.2±0.7
2 ppt	0.1±0.0	0.4±0.1	0.6±0.1
4 ppt	0.1±0.1	0.4±0.2	0.1±0.0

Appendix Table 7. 39-Week Experiment – Raw Productivity Values #5: Stem Density Variables

Response Variable	*Salinity Treatment*		
	0 ppt	*2 ppt*	*4 ppt*
Initial SD	16±1	17±1	18±1
Final Live SD	23±3	11±3	3±2
Final Total SD	47±5	36±2	29±2

Appendix Table 8. 19-Week Experiment – Abiotic Factors, pg. 1

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
E_h^U	Salinity	2	5593	0.69	0.5106
	Sulfide	2	314805	38.66	0.0001
	Salinity*Sulfide	4	1539	0.19	0.9424
	Error	31	8143		
	Time	3	11249	5.43	0.0017
	Time*Salinity	6	1307	0.63	0.7050
	Time*Sulfide	6	3097	1.49	0.2868
	Time*Salinity*Sulfide	12	2510	1.21	0.2868
	Error (Time)	93	2071		
pH^L	Model	8	0.0004	8.06	0.0001
	Salinity	2	0.0020	4.36	0.0233
	Sulfide	2	0.0121	26.38	0.0001
	Salinity x Sulfide	4	0.0004	0.94	0.4578
	Error	26	0.0005		
Ca^U	Model	8	5616.70	65.91	0.0001
	Salinity	2	5409.45	63.48	0.0001
	Sulfide	2	16686.95	195.82	0.0001
	Salinity x Sulfide	4	956.34	11.22	0.0001
	Error	26	85.22		
Fe^L	Model	8	2.54	62.97	0.0001
	Salinity	2	0.01	0.32	0.7255
	Sulfide	2	9.85	243.86	0.0001
	Salinity x Sulfide	4	0.16	3.96	0.0122
	Error	26	0.04		
K^U	Model	8	251.14	26.74	0.0001
	Salinity	2	687.85	73.25	0.0001
	Sulfide	2	307.63	32.76	0.0001
	Salinity x Sulfide	4	32.31	3.44	0.0220
	Error	26	9.39		

Appendix Table 8. 19-Week Experiment – Abiotic Factors, pg. 2

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Mg^{U}	Model	8	1698.03	45.19	0.0001
	Salinity	2	2239.90	59.61	0.0001
	Sulfide	2	4578.18	121.83	0.0001
	Salinity x Sulfide	4	234.15	6.23	0.0012
	Error	26	37.58		
Mn^{L}	Model	8	0.43	51.62	0.0001
	Salinity	2	0.10	12.05	0.0002
	Sulfide	2	1.58	191.68	0.0001
	Salinity x Sulfide	4	0.03	3.33	0.0250
	Error	26	0.01		
Na^{U}	Model	8	314727	73.69	0.0001
	Salinity	2	509541	119.31	0.0001
	Sulfide	2	635345	148.77	0.0001
	Salinity x Sulfide	4	22050	5.16	0.0034
	Error	26	4271		
$NH_4^{+(L)}$	Model	8	3.22	116.43	0.0001
	Salinity	2	0.07	2.39	0.1112
	Sulfide	2	12.23	441.91	0.0001
	Salinity x Sulfide	4	0.21	7.66	0.0003
	Error	26	0.03		
P^{L}	Model	8	0.49	42.63	0.0001
	Salinity	2	0.01	0.58	0.5682
	Sulfide	2	1.82	158.69	0.0001
	Salinity x Sulfide	4	0.05	4.45	0.0072
	Error	26	0.09		
S^{L}	Model	8	1.08	3.73	0.0050
	Salinity	2	0.59	2.03	0.1522
	Sulfide	2	3.12	10.75	0.0004
	Salinity x Sulfide	4	0.24	0.82	0.5248
	Error	26	0.29		

Appendix Table 9. 19-Week Experiment – Biomass Response Variables

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Note that for biomass responses, only salinity and sulfide as main treatment effects were indicated as significant factors by the MANOVA					
Live as % Tot AG BM[A]	Model	4	0.67	20.75	0.0001
	Salinity	2	0.47	14.55	0.0001
	Sulfide	2	0.80	24.75	0.0001
	Error	30	0.03		
Adv. As % Final BM[A]	Model	4	0.007	3.97	0.0106
	Salinity	2	0.011	6.17	0.0057
	Sulfide	2	0.003	1.52	0.2342
	Error	30	0.002		
Adv. As % Tot AG BM[A]	Model	4	0.005	2.10	0.1055
	Salinity	2	0.001	0.54	0.5882
	Sulfide	2	0.008	3.75	0.0352
	Error	30	0.002		
Root vs. Shoot Ratio[U]	Model	4	0.009	9.74	0.0001
	Salinity	2	0.014	14.75	0.0001
	Sulfide	2	0.004	4.12	0.0262
	Error	30	0.001		
RGR: Aboveground[U]	Model	4	0.000023	4.42	0.0063
	Salinity	2	0.000036	6.86	0.0035
	Sulfide	2	0.000008	1.63	0.2129
	Error	30	0.000005		
RGR: Belowground[U]	Model	4	0.000108	10.99	0.0001
	Salinity	2	0.000151	15.41	0.0001
	Sulfide	2	0.000055	5.55	0.0089
	Error	30	0.000010		
RGR: Total (AG + BG)[U]	Model	4	0.000033	7.16	0.0004
	Salinity	2	0.000050	10.65	0.0003
	Sulfide	2	0.000014	3.00	0.0650
	Error	30	0.000005		

Appendix Table 10. 19-Week Experiment – Raw Productivity Values #1: Predicted Initial Aboveground Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	1.0±0.2	1.3±0.4	1.6±0.1
2 ppt	1.1±0.2	1.4±0.3	1.2±0.3
4 ppt	1.7±0.4	1.4±0.2	1.4±0.4

Appendix Table 11. 19-Week Experiment – Raw Productivity Values #2: Predicted Initial Belowground Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	0.9±0.2	1.1±0.3	1.4±0.1
2 ppt	0.9±0.1	1.2±0.3	1.0±0.2
4 ppt	1.5±0.3	1.2±0.1	1.2±0.3

Appendix Table 12. 19-Week Experiment – Raw Productivity Values #3: Predicted Initial Total Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	1.9±0.4	2.4±0.7	3.0±0.3
2 ppt	2.1±0.3	2.6±0.6	2.2±0.5
4 ppt	3.2±0.7	2.5±0.3	2.6±0.7

Appendix Table 13. 19-Week Experiment – Raw Productivity Values #4: Final Aboveground Live Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	0.30±0.07	0.19±0.07	0.18±0.05
2 ppt	0.21±0.10	0.15±0.05	0.21±0.03
4 ppt	0.10±0.04	0.03±0.02	0.05±0.01

Appendix Table 14. 19-Week Experiment – Raw Productivity Values #5: Final Aboveground Total Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	0.31±0.07	0.44±0.14	0.46±0.12
2 ppt	0.25±0.11	0.48±0.10	0.48±0.09
4 ppt	0.21±0.01	0.25±0.09	0.25±0.04

Appendix Table 15. 19-Week Experiment – Raw Productivity Values #6: Final Live Aboveground Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	11.5±1.01	4.6±1.4	7.5±0.9
2 ppt	9.4±1.3	4.6±1.2	3.5±0.7
4 ppt	6.5±0.8	0.9±0.7	1.8±0.8

Appendix Table 16. 19-Week Experiment – Raw Productivity Values #7: Final Total Aboveground Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	13.2±1.6	12.8±1.0	16.4±1.2
2 ppt	14.3±1.0	13.5±1.8	10.0±1.3
4 ppt	12.8±2.0	7.0±1.1	9.1±1.3

Appendix Table 17. 19-Week Experiment – Raw Productivity Values #8: Final Total Belowground Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	7.1±0.6	4.0±0.6	8.1±0.6
2 ppt	4.7±0.4	3.2±0.5	2.0±0.4
4 ppt	3.4±0.2	1.7±0.5	1.5±0.5

Appendix Table 18. 19-Week Experiment – Raw Productivity Values #9: Final Total (Aboveground+Belowground) Biomass (g)

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	20.3±1.9	16.9±1.0	24.6±1.3
2 ppt	19.0±1.3	16.7±2.2	12.0±1.3
4 ppt	16.2±2.0	8.8±1.3	10.6±1.7

Appendix Table 19. 19-Week Experiment - Stem Density Response Variables

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
%Δ Total SD[A]	Model	8	0.004	0.96	0.4880
	Salinity	2	0.004	0.98	0.3998
	Sulfide	2	0.007	1.85	0.1774
	Salinity x Sulfide	4	0.001	0.28	0.8863
	Error	26	0.004		
%Δ Live SD[A]	Model	8	0.09	4.25	0.0023
	Salinity	2	0.17	8.25	0.0017
	Sulfide	2	0.11	5.41	0.0109
	Salinity x Sulfide	4	0.02	0.77	0.5563
	Error	26	0.02		
Live as % Total SD[A]	Model	8	0.32	13.93	0.0001
	Salinity	2	0.51	22.17	0.0001
	Sulfide	2	0.50	21.93	0.0001
	Salinity x Sulfide	4	0.08	3.46	0.0214
	Error	26	0.02		

Appendix Table 20. 19-Week Experiment – Raw Productivity Values #10: Initial Stem Density

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	6±1	5±1	8±1
2 ppt	6±0	8±1	6±1
4 ppt	9±1	6±0	8±1

Appendix Table 21. 19-Week Experiment – Raw Productivity Values #11: Final Live Stem Density

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	20±2	10±4	14±2
2 ppt	17±2	16±5	10±3
4 ppt	12±1	2±1	7±3

Appendix Table 22. 19-Week Experiment – Raw Productivity Values #12: Final Total Stem Density

Salinity Treatment	*Sulfide Treatment*		
	0 mM	*0.5 mM*	*1 mM*
0 ppt	21±2	24±8	35±9
2 ppt	29±7	39±8	38±6
4 ppt	42±8	17±2	29±5

Appendix Table 23. 19-Week Experiment - Physiological Response Variables

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Aerobic Respiration	Model	8	50.38	6.01	0.0015
	Salinity	2	49.42	5.89	0.0129
	Sulfide	2	50.97	6.08	0.0117
	Salinity x Sulfide	4	39.85	4.75	0.0112
	Error	15	8.39		
Anaerobic Respiration	Model	8	18.93	5.81	0.0017
	Salinity	2	27.70	8.50	0.0034
	Sulfide	2	13.16	4.04	0.0396
	Salinity x Sulfide	4	15.02	4.61	0.0126
	Error	15	3.26		
Change in Respiration Between Aerobic and Anaerobic	Model	8	8.32	2.29	0.0791
	Salinity	2	3.38	0.93	0.4159
	Sulfide	2	12.28	3.38	0.0612
	Salinity x Sulfide	4	6.57	1.81	0.1793
	Error	15	3.63		

Appendix Table 24. 12-Day Experiment – Physiological Response Variables

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Note that for growth response variables, neither salinity nor sulfide as main treatment effects nor the interaction between salinity and sulfide were indicated as significant by the MANOVA.					
Net photosynthesis[U]	Model	8	0.6791	2.37	0.0427
	Salinity	2	0.1162	0.40	0.6707
	Sulfide	2	0.8360	2.91	0.0703
	Salinity x Sulfide	4	0.8734	3.04	0.0328
	Error	29	0.2870		
Transpiration[U]	Model	8	0.1099	21.76	0.0001
	Salinity	2	0.2846	56.36	0.0001
	Sulfide	2	0.1049	20.76	0.0001
	Salinity x Sulfide	4	0.0095	1.88	0.1400
	Error	29	0.0051		
Stomatal conductance[U]	Model	8	193.95	13.10	0.0001
	Salinity	2	521.58	35.24	0.0001
	Sulfide	2	181.44	12.26	0.0001
	Salinity x Sulfide	4	17.43	1.18	0.3412
	Error	29	14.80		
Root ethanol production[L]	Model	8	0.8307	4.27	0.0013
	Salinity	2	1.9212	9.87	0.0004
	Sulfide	2	1.2309	6.32	0.0046
	Salinity x Sulfide	4	0.0466	0.24	0.9142
	Error	34	0.1947		
Root ADH activity[L]	Model	8	0.4601	2.56	0.0323
	Salinity	2	0.3461	1.92	0.1656
	Sulfide	2	0.2080	1.16	0.3298
	Salinity x Sulfide	4	0.5386	2.99	0.0363
	Error	27	0.1799		

Appendix Table 25. Field Experiment – Abiotic Response Variables, pg. 1

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Note that for abiotic responses, only Site and Time as main treatment effects and the interaction of Site x Time were indicated as significant factors by the MANOVA.					
E_h (1 cm)^U	Model	5	45705	13.20	0.0001
	Site	1	11628	3.36	0.0725
	Time	2	55651	16.08	0.0001
	Site x Time	2	45229	13.07	0.0001
	Error	52	3461		
E_h (20 cm)^U	Model	5	32561	14.73	0.0001
	Site	1	6325	2.86	0.0967
	Time	2	28393	12.84	0.0001
	Site x Time	2	45476	20.57	0.0001
	Error	52	2211		
pH^U	Model	5	0.5013	1.52	0.2002
	Site	1	0.0013	0.00	0.9511
	Time	2	0.0078	0.02	0.9768
	Site x Time	2	1.2461	3.77	0.0295
	Error	52	0.3302		
Salinity^U	Model	5	3.3291	2.89	0.0225
	Site	1	3.9690	3.44	0.0693
	Time	2	4.7288	4.10	0.0222
	Site x Time	2	1.5212	1.32	0.2762
	Error	52	1.1534		
Sulfide^L	Model	5	0.2345	2.90	0.0222
	Site	1	0.6932	8.56	0.0051
	Time	2	0.1355	1.67	0.1977
	Site x Time	2	0.0792	0.98	0.3828
	Error	52	0.0810		
$NH_4^{+\,(U)}$	Model	5	7762	4.29	0.0024
	Site	1	3489	1.93	0.1708
	Time	2	13505	7.47	0.0014
	Site x Time	2	5005	2.77	0.0721
	Error	52	1809		

Appendix Table 25. Field Experiment – Abiotic Response Variables, pg. 2

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Ca^{U}	Model	5	7726	2.80	0.0258
	Site	1	29636	11.49	0.0013
	Time	2	2537	0.98	0.3808
	Site x Time	2	586	0.23	0.7976
	Error	52	2579		
Fe^{L}	Model	5	0.1318	2.10	0.0801
	Site	1	0.0487	0.78	0.3823
	Time	2	0.2165	3.45	0.0392
	Site x Time	2	0.0666	1.00	0.3533
	Error	52	0.0627		
K^{U}	Model	5	1022.0	2.00	0.0942
	Site	1	4144.1	8.11	0.0063
	Time	2	291.8	0.57	0.5686
	Site x Time	2	104.7	0.20	0.8155
	Error	52	511.3		
Mg^{U}	Model	5	8314	4.73	0.0012
	Site	1	38452	21.88	0.0001
	Time	2	240	0.14	0.8726
	Site x Time	2	1187	0.68	0.5134
	Error	52	1757		
Mn^{L}	Model	5	0.0515	2.72	0.0295
	Site	1	0.1690	8.93	0.0043
	Time	2	0.0142	0.75	0.4784
	Site x Time	2	0.0324	1.71	0.1906
	Error	52	0.0189		
Na^{L}	Model	5	0.0613	5.77	0.0003
	Site	1	0.1808	17.03	0.0001
	Time	2	0.0556	5.23	0.0085
	Site x Time	2	0.0024	0.22	0.7999
	Error	52	0.0106		

Appendix Table 25. Field Experiment – Abiotic Response Variables, pg. 3

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
P^U	Model	5	5.5271	1.60	0.1776
	Site	1	9.7773	2.82	0.0989
	Time	2	5.8828	1.70	0.1929
	Site x Time	2	2.2721	0.66	0.5230
	Error	52	3.4624		
S^L	Model	5	1.2687	5.09	0.0007
	Site	1	0.0019	0.01	0.9307
	Time	2	2.2871	9.18	0.0004
	Site x Time	2	0.9078	3.65	0.0330
	Error	52	0.2490		

Appendix Table 26. Field Experiment – Biotic Response Variables, pg. 1

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Note that for growth response variables, only Site and Time as main treatment effects, and all interactions involving Time as a component effect, were indicated as significant by the MANOVA.					
Relative Growth Rate: Aboveground Tissue[U]	Model	11	0.00078	26.08	0.0001
	Site	1	0.00027	8.93	0.0046
	Time	2	0.00383	128.06	0.0001
	Time x Site	2	0.00006	1.99	0.1488
	Time x Elevation	3	0.00008	2.63	0.0619
	Time x Site x Elevation	3	0.00006	1.89	0.1456
	Error	44	0.00003		
Relative Growth Rate: Belowground Tissue[U]	Model	11	0.00050	12.80	0.0001
	Site	1	0.00001	0.34	0.5618
	Time	2	0.00232	59.12	0.0001
	Time x Site	2	0.00002	0.53	0.5928
	Time x Elevation	3	0.00023	5.95	0.0017
	Time x Site x Elevation	3	0.00001	0.29	0.8341
	Error	44	0.00004		
Relative Growth Rate: Total (AG + BG)[U]	Model	11	0.00061	29.21	0.0001
	Site	1	0.00008	4.03	0.0511
	Time	2	0.00306	147.60	0.0001
	Time x Site	2	0.00002	0.90	0.4138
	Time x Elevation	3	0.00008	3.88	0.0153
	Time x Site x Elevation	3	0.00002	0.92	0.4402
	Error	44	0.00002		
LIVE AS % TOTAL Aboveground DW[A]	Model	11	0.6979	18.70	0.0001
	Site	1	0.0156	0.42	0.5220
	Time	2	2.8865	77.32	0.0001
	Time x Site	2	0.2904	7.78	0.0013
	Time x Elevation	3	0.1519	4.07	0.0125
	Time x Site x Elevation	3	0.1800	4.82	0.0056
	Error	44	0.0373		

Appendix Table 26. Field Experiment – Biotic Response Variables, pg. 2

Factor	*Source*	*df*	*Mean Square*	*F Value*	*P*
Note that for growth response variables, only Site and Time as main treatment effects, and all interactions involving Time as a component effect, were indicated as significant by the MANOVA.					
Root:Shoot[A]	Model	11	0.0162	2.67	0.0105
	Site	1	0.0240	3.95	0.0532
	Time	2	0.0218	3.60	0.0360
	Time x Site	2	0.0118	1.94	0.1562
	Time x Elevation	3	0.0212	3.50	0.0234
	Time x Site x Elevation	3	0.0063	1.03	0.3878
	Error	44	0.0061		
Root Porosity[U]	Model	11	0.1299	6.15	0.0001
	Site	1	0.2217	10.50	0.0025
	Time	2	0.3641	17.25	0.0001
	Time x Site	2	0.1043	4.94	0.0125
	Time x Elevation	3	0.0176	0.84	0.4829
	Time x Site x Elevation	3	0.0256	1.21	0.3188
	Error	44	0.0211		

VITA

"Jim Pahl grew up the son of a poor sharecropper (dueling banjos …)" No, Jim was born in Washington, D.C., and grew up in central Maryland. After high school, Jim's life o'academia began at St. Mary's College of Maryland, where he earned a Bachelor of Arts in Biology in 1993. Jim worked like a real person for a year afterwards in environmental restoration and consulting, but a particularly foul winter spent in Philadelphia in 1994 convinced him that the offer of a doctoral program in Baton Rouge (they said it didn't often snow there) was not worth passing up. He's been there ever since. He leaves south Louisiana with a knowledge for the important things in life; namely, recipes for gumbo, jambalaya, red beans, boiled crawfish and shrimp, and deep-fried turkey. Oh, he also learned a thing or two about wetland plant ecology and physiology and coastal ecosystem management. For these and other sterling accomplishments, Louisiana State University has decided to award Jim the Doctor of Philosophy degree. How about that.