# DEVELOPING TOOLS TO IDENTIFY FACTORS THAT LIMIT PRODUCTION IN COASTAL MARSHES

A Dissertation

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**TABLE OF CONTENTS**

**LIST OF TABLES**

**LIST OF FIGURES**

**ABSTRACT**

Marsh loss is a problem in many areas around the world. In Louisiana's coastal marshes, where *Spartina patens* is the most common plant, restoration and management seek to slow wetland loss rates that average approximately 77.4 $km^2$/year. To combat the problem, scientists and managers require tools to determine local causes and evaluate the effectiveness of management techniques. Current methods for identifying factors that limit productivity in marshes are too time-consuming or expensive for wide-spread, regular use. Critical values of elemental concentrations in plant tissue are widely used to diagnose mineral deficiencies and toxicities in agricultural crops, however. I used the chemical composition of leaf tissue from *S. patens* grown under controlled conditions to develop critical values of C:N ratio and concentrations of Na, Mn, and Ca to identify N limitation, salinity stress, and flooding stress, respectively. I tested these critical values and identified seasonal changes in leaf tissue chemistry in a field experiment where all three limiting factors fluctuated naturally. I also compared the leaf tissue chemistry of *S. patens* and *Spartina alterniflora*, the second most common plant in Louisiana's coastal marshes, to facilitate comparisons between the species. Finally, I investigated the effects of flooding stress on above- and belowground biomass of *S. patens*. Aboveground biomass was reduced by low N-uptake, high salinity, and high flooding. Belowground biomass was reduced by increased flooding. In leaf tissue, C:N decreased with increasing N-availability and [Na] increased with increasing salinity. [Mn] and [Ca] in leaf tissue decreased with increased flooding. In the field study, C:N increased seasonally. Biomass was most highly correlated with [Na] in spring and with both [Na] and C:N in summer. In fall, leaf tissue composition appeared unrelated to biomass. Managers should take seasonal differences in leaf tissue composition and nutritional requirements into account when diagnosing the causes of limited production and when creating management plans. Leaf tissue should be collected in summer to diagnose limiting factors. To have the most impact, flooding should be used in late spring or early summer to reduce salinity and increase N-availability.

# CHAPTER 1.
# GENERAL INTRODUCTION

## Background

Land loss, particularly loss of coastal marshes, is a serious problem in coastal Louisiana and estimates of land loss rates range from approximately 66 $km^2$ to 90 $km^2$ per year (Gagliano et al. 1981, Britsch and Dunbar 1993, Barras et al. 2003). Many factors cause loss of coastal wetlands, including subsidence and sea level rise, which reduce the elevation of marshes relative to sea level. Hydrologic alterations to marshes resulting from sea level rise and anthropogenic projects such as construction of protection levees, digging navigation canals, and draining land for agriculture reduce the resiliency of marshes by intensifying stress factors such as high salinity, low nutrient-availability, and flooding. Increased stress reduces plant productivity, and because vegetative growth of marsh plants controls rates of vertical accretion in Louisiana's coastal marshes (Nyman et al. 2006), increased stress also reduces the ability of marshes to keep up with relative sea level rise. Managers require methods for selecting management strategies that combat the causes of limited productivity and for evaluating the effectiveness of strategies they choose to implement.

An ideal bioindicator would rapidly identify different factors that induce stress in marsh plants (Ewing et al. 1997) and would be simple and inexpensive enough to use regularly and across a large area, ideally an entire coastline. Several methods for estimating productivity exist. For example, managers can use changes in above-ground biomass to identify sites that differ in productivity (e.g. Burdick et al. 1989, Ewing et al. 1997). Plant biomass is a practical indicator because it integrates many biogeochemical processes and physiological responses (Ewing et al. 1995). However, this method of estimating productivity requires intense sampling over a short period of time; thus it is too costly to be used regularly or over a large area. Shoot elongation varies with plant growth (Ewing et al. 1997) but this technique requires repeated visits to sites and locating previously tagged stems. Also, while these techniques may identify areas where productivity is limited, they cannot identify the factors that limit

production. Identifying the causes of limited production can improve management plans by suggesting possible remedies. For example, in a marsh where low N availability limits production, treatment plans designed to lower salinity will not increase production unless they also increase N-availability. Methods such as leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration can be used to identify limiting factors because they vary with salinity stress or nutrient starvation (Ewing et al. 1995, 1997). Although these methods can be used to directly identify limiting factors, they are too costly for use on large geographic or temporal scales.

Elemental concentrations in plant tissue have been used as indicators of growing conditions and nutrient limitation for both agricultural crops (e.g., Fageria et al. 2008, McKee and McKelvin 1993) and wetland plants (e.g., Gusewell 2002 and 2004, Koerselman and Meuleman 1996, Patrick and DeLaune 1976). Leaf tissue testing as a means of identifying concentrations of elements in plant tissue is the most widely-used method to diagnose mineral deficiencies in agricultural crop plants (Epstein and Bloom 2005). By comparing concentrations of elements in the leaf tissue of crops to critical values and sufficiency ranges that are developed in greenhouse studies, farmers can determine which elements limit production and alter fertilizer applications to improve crop yields. For example, N, K, and Ca are important indicators of limitation in agricultural crops and have been included in diagnosis and recommendation integrated systems (e.g., Walworth and Sumner 1987). In wetland ecology, tools are just beginning to be developed that will allow management and restoration professionals to diagnose the causes of limited production in marsh ecosystems. For example, several studies have investigated the use of N:P ratios to identify nutrient limitation by N or P (Koerselman and Meuleman 1996, Stribling and Cornwell 2001, Guesewell and Koerselman 2002). Also, C:N ratios may be used to indicate N-limitation. Where salinity is low, increasing N availability increases productivity and decreases C:N ratios of *Spartina patens* leaf tissue (Foret 2001, Crain 2007). Concentrations of Na in leaf tissue may indicate limitation by salinity stress because in some species, [Na] in leaf tissue increases with increasing salinity

(McKee and Mendelssohn 1989; Bradley and Morris 1991). However, some nutrient concentrations in plant tissue and other indicators of limiting factors change during the growing season as a result of changing requirements for growth (Ewing et al. 1997). Thus, the seasonal timing of comparisons may change how elemental composition should be interpreted.

In this dissertation, I focus mainly on *Spartina patens* and include comparisons with *Spartina alterniflora* because they make up 25% and 13% of the vegetation in coastal Louisiana, respectively (Chabreck 1970). These species also occur throughout the Gulf of Mexico and Atlantic coasts of the United States so they are commonly used in restoration and management plans for coastal marshes. Understanding how the mineral requirements of *S. patens* and how they differ from the mineral requirements of *S. alterniflora* could improve the effectiveness of such plans. This information could also help formulate plans that would facilitate removal of *S. patens* or *S. alterniflora* in places where they have become invasive, such as the San Francisco Bay area in California.

## Research Objectives

The overall objectives of this dissertation are to (1) describe changes in biomass production of *S. patens* to common stress factors, (2) develop indicators in leaf tissue of *S. patens* that can be used to diagnose the causes of limited production, and (3) validate those indicators in a field setting. As part of the validation process, I also examined seasonal changes in leaf tissue composition to determine if certain times of year were more appropriate than others for diagnosing limiting factors. Also, I compared the leaf tissue of *S. patens* to *S. alterniflora* because critical values are species specific and these species are commonly compared in scientific literature.

## Study Area

For field-based portions of this study, I collected samples at eight sites in saline and intermediate marshes along the coast of Louisiana. I selected fresher and more saline sites on the Chenier plain at Cameron Prairie National Wildlife Refuge and Rockefeller Refuge and on the Mississippi

Delta near the mouth of the Atchafalaya River at Marsh Island Wildlife Refuge and marsh adjacent to Fourleague Bay. Flooding studies were carried out at the Rockefeller Refuge and Marsh Island sites. Following Penfound and Hathaway's (1938) classification system for coastal marshes, fresher sites were chosen to include species that indicated intermediate marsh such as *Sagittaria lancifolia* and *Scirpus olneyi*. More saline sites were chosen to include species that indicate saline marsh such as *Spartina alterniflora*. The purpose of this method of site selection was to sample marshes over a broad range of salinity conditions and riverine influence under which *S. patens* grows. Maps of field sites and details about site selection can be found in the following chapters.

**Synopsis of Chapters**

In the first two research chapters, I develop critical values that can be used to diagnose limitation by N starvation, salinity stress, and flooding stress by growing *S. patens* under conditions where the factors of interest were controlled. Chapter 2 used a greenhouse experiment to examine the interacting effects of N availability and salinity stress on production and leaf tissue chemistry. Chapter 3 used a field experiment where *S. patens* was grown at varying levels above and below local marsh elevation, but where other factors were allowed to vary naturally, to develop indicators of flooding stress. Chapters 4 and 5 describe the leaf tissue chemistry of plants collected from field sites. In Chapter 4, I compare the leaf tissue chemistry of *S. patens* and *S. alterniflora* growing in similar conditions. The purpose of this comparison was to determine how these species, which are often compared in literature, differ so that more accurate comparisons can be made in the future. Critical values developed under controlled conditions may not be useful in field conditions because of differences in ranges of nutrient availability, salinity, and/or flooding. In Chapter 5, I validate the tools I developed in Chapters 2 and 3 by analyzing *S. patens* leaf tissue collected at sites across Louisiana's coast. In this chapter I also examine seasonal changes in leaf tissue. Management decisions based on aboveground biomass will also affect belowground biomass. It is important for managers to anticipate

how their management decisions will alter belowground biomass. In Chapter 6, I show the effect of flooding stress on belowground biomass of *S. patens* and determine the relationship between indicators of flooding stress in leaf tissue and limitation of belowground biomass by flooding stress.

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## CHAPTER 2.
## IMPROVING MARSH RESTORATION: LEAF TISSUE CHEMISTRY IDENTIFIES FACTORS LIMITING PRODUCTION IN *SPARTINA PATENS*

### Introduction

Marsh loss is a problem in many areas of the world. In coastal Louisiana, 77.4 $km^2$/year of marsh converted to open water between 1978 and 2000 (Barras et al. 2003). Marshes convert to open water because of many factors, including sea-level rise, sediment starvation, and changes in hydrology and soil chemistry. Fresh water and sediment input are critical factors in combating coastal marsh loss (Day 2000). Mineral sediments help maintain marsh elevation by increasing soil elevation, plant production through nutrient delivery, and organic matter accumulation (DeLaune et al. 1979). Increased soil organic matter accumulation alone has also been associated with increasing marsh elevation (Nyman 2006, Craft 2007). Increasing marsh elevation is essential for countering global sea-level rise and local subsidence. Determining potential causes of marsh loss is difficult because although reducing salinity and increasing nutrients can increase biomass production in *Spartina patens* (Ait.) Muhl (marsh hay, cordgrass), a perennial wetland grass (DeLaune et al. 2005), current techniques to determine which factor limits growth are both time-consuming and expensive.

Many management techniques have been developed to combat marsh loss; however managers often lack tools (1) to make informed decisions about which restoration technique to use or (2) to evaluate results of a technique that has been implemented. Several methods for estimating productivity currently exist; however, none is feasible for regular, wide-spread use for various reasons. For example, managers can use changes in above-ground biomass to identify sites that differ in productivity (e.g. Burdick et al. 1989, Ewing et al. 1997). This method of estimating productivity requires intense sampling over a short period of time; thus it is too costly to be used regularly. Shoot elongation varies with plant growth (Ewing et al. 1997) identifying limitation in this manner requires repeated visits to sites and locating previously tagged stems. Also, while these techniques may identify areas where

production is limited, they cannot identify the factors that limit production. Methods such as leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration vary with salinity stress or nutrient starvation (Ewing et al. 1995, 1997). Although these attributes can be used to directly identify limiting factors, they are too costly for widespread annual use. By developing a simple, inexpensive tool to determine which factors limit plant growth across large, heterogeneous areas, I can improve the evaluation of freshwater introductions and other marsh restoration techniques. Although the tool that I describe here is specific for *S. patens* in coastal Louisisana, our methods could be applied to other species and in other systems.

Nutrient ratios in plant tissue may provide a way to predict limitation of production due to high salinity and/or low nutrient availability. The Redfield Ratio (C:N:P of live algae cells = 106:16:1; Redfield et al. 1963) is used worldwide to determine which nutrient limits algae production (Day et al. 1989, p. 169). While the Redfield Ratio itself only applies to algae, the concept can be used to identify limiting factors in vascular plants and forest productivity as well. Nutrient ratios in plant tissue are crucial in the management of numerous agricultural crops (Campbell 2000) but have yet to used as a diagnostic tool to pinpoint nutrient deficiencies or stress in wetland plants. Increasing nutrient availability increases production and decreases C:N ratios of *S. patens* leaf tissue where salinity is low (Foret 2001, Crain 2007). Nutrient ratios is less expensive and more widely accessible technique for to managers to identify limitation because it requires only a single visit to a site where investigators collect a few grams of live plant tissue. The plant tissue must be rinsed, oven dried, ground, and analyzed with standard chemical analyses that are available commercially.

The objectives of this study were to determine the feasibility of identifying the factors that limit plant productivity in coastal marshes with leaf chemical characteristics and to provide a basis for interpreting nutrient ratios of samples taken in the field. In this paper I show how the leaf chemistry of *S. patens* responds to changes in salinity stress and nutrient availability under controlled nutrient and

salinity conditions in a greenhouse. I use these data to determine chemical signatures in *S. patens* leaf tissue that may be used as references to indicate factors that limit productivity in coastal marshes. I focus on *S. patens* because it is the most common plant species in coastal Louisiana (Chabreck 1970).

## Methods

I grew *S. patens* plants in a greenhouse under varying levels of salinity and nutrients in a balanced four by four factorial design with four replications (128 experimental units). I obtained two populations of *S. patens* that differed in salinity tolerance from Dr. Mark Hester (currently Associate Professor at University of Louisiana, Lafayette). The lethal salinity levels (50% death of above-ground tissue) for these two populations were 66 ppt for population "k" and 81 ppt for population "i" (Hester et al. 1996). I used plants from two populations with documented phenotypic differences to represent random variation rather than to investigate the effects of population on leaf chemistry. I initially grew the plants clonally in separate bedding trays containing sand, water, and commercial fertilizer (Peters 20-20-20 N-P-K; elemental N-P-K = 20-8.72-16.6).

I made experimental soils from a homogeneous mixture of 90% commercial play sand and 10% potter's clay to which I added one of four combinations of 19-5-8 (elemental N-P-K = 19-2.18-6.64) and 35-0-0 encapsulated (slow-release, non-water soluble) fertilizer. Specific nutrient treatments were chosen to approximate 25%, 75%, 125%, and 200% of the N (0.49, 1.46, 2.43, and 3.89 gN/L soil respectively) and phosphorus levels (0.024, 0.073, 0.12, and 0.19 gP/L soil respectively) of unmanaged, *S. patens*-dominated marshes at Rockefeller Wildlife Refuge (approximately 29° 37' N, 92° 36' W; Foret 2001). The average nutrient levels of these marshes at Rockefeller Wildlife Refuge were approximately 1.96 gN/L soil and 0.096 gP/L (Foret 2001). The actual levels of N achieved in the experimental soils were 6.6%, 19.8%, 32.9%, and 52.6% and the actual levels of phosphorus achieved were 2.9%, 8.8%, 14.5%, and 22.9% of nutrient levels at Rockefeller Refuge. I planted two stems of the same population ("i" or "k") in each one-gallon pot. I placed two pots, one containing each population, in 64 14-gallon

randomly arranged tubs and flooded the tubs with well water to the soil surface inside the pots. Plants were allowed to grow for twenty-six days before I raised the salinity level of the water in the tubs.

I raised the salinity in the tubs with Forty Fathoms marine mix (bioassay grade) in five installments over a 10-day period until the water in the tubs reached the target salinity. Target salinities were 2, 6, 18, and 36 ppt. Mean actual salinites achieved were 2, 5, 17, and 38 ppt. I replaced water lost to evapotranspiration twice weekly to keep the pots flooded to the soil surface. To reduce build up of salt in the soil I poured water from the tubs over the soil surface. I collected pore water samples from a randomly-selected sub-sample of 16 pots every three to four weeks and measured conductivity and salinity in the pore water and tub water. The experiment lasted 144 days from the time I began the nutrient treatments. Merino et al. (2010) tested the hypothesis that the response of growth to nutrient availability did not vary with salinity. They found that growth varied most in response to nutrient availability at low salinity, but did not vary at all at high salinity (Figure 1).

At the conclusion of the experiment, I harvested above- and below-ground tissue over a three-day period. I washed the below-ground tissue and dried both above- and below-ground tissue at 60° to a constant weight and weighed it to determine biomass. Because above- and below-ground biomass were linearly correlated ($R^2 = 0.981649$, $p = 0.0001$), I added them together to estimate total biomass (Merino et al. 2010). Using the average biomass of pots grown under specific nutrient and salinity conditions, I classified treatment combinations in terms of factors that limit productivity.

I classified pots into four groups by limiting factor: nitrogen, salinity, both, or neither (Table 1). Pots with N treatments > 30% N and salinities < 10 ppt were classified as neither-limited because the high biomass of plants in these treatments (Figure 1) suggested that a factor other than salinity or N limited growth. Pots that had an average porewater salinity of less than 10 ppt and N treatment of 30%

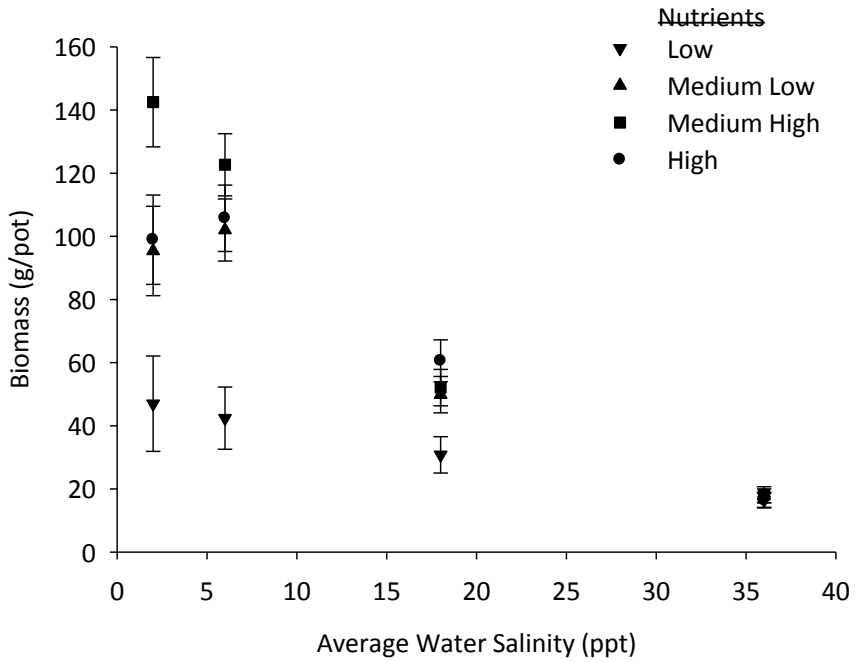


Figure 1: Mean biomass (±1 SD) of *Spartina patens* leaf tissue from plants grown under various nutrient and four salinity treatments. Nutrient levels (g/L soil) were low (0.129 g/L N, 0.003 g/L P), medium low (0.384 g/L N, 0.008 g/L P), medium high (0.639 g/L N, 0.014 g/L P), and high (1.024 g/L N, 0.022 g/L P). Nutrient treatments were created by mixing 19-5-8 and 35-0-0 slow-release fertilizer (Osmocote) into soils consisting of 90% sand and 10% clay. Salinity treatments were created by adding Forty Fathoms Marine mix into water in the tubs. Adapted from Merino et al. (2010).

N (Figure 1) were classified as N-limited because of their low biomass combined with low N availability. I reasoned that salinity was not limiting growth in these pots because the same salinity treatments did not limit growth in the neither-limited pots. Although biomass was too similar in plants grown at higher salinities to use it to identify limiting factors, I applied the same logic I used for the lower salinity pots. Pots with average salinities higher than 10 ppt and N treatments > 30% were classified as salinity-limited. The remaining pots (i.e. those with salinity > 10 ppt and N treatment of 30% N) were classified as both-limited (Figure 1). This classification resulted in an unequal number of pots in each limiting factor group.

Above-ground tissue samples from each pot were ground with a Wiley mill to produce a homogeneous tissue sample for chemical analysis. C concentration was determined with a CHN analyzer in the lab at University of Louisiana, Lafayette. I sent ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Lab (STPAL, LSU, Baton Rouge, LA) to determine N, phosphorus, and Na concentrations in leaf tissue. The STPAL used dry combustion by Leco N analyzer to determine N content. They used ICP analysis to determine concentrations of Na and phosphorus.

Table 1: Combinations of nutrient and salinity treatments included in each limiting factor group. Nutrient treatments were created by mixing 19-5-8 and 35-0-0 slow-release fertilizer (Osmocote) into soils consisting of 90% sand and 10% clay. Salinity treatments were created by adding Forty Fathoms Marine mix into water in the tubs.

| Nutrients | | | | Salinity | |
|---|---|---|---|---|---|
| Intended (g N/L soil) | Intended (g P/L soil) | Actual (g N/L soil) | Actual (g P/L soil) | Intended (ppt) | Mean Actual (ppt) |
| Neither-Limited | | | | | |
| 1.46 | 0.073 | 0.384 | 0.008 | 2 | 2 |
| 1.46 | 0.073 | 0.384 | 0.008 | 6 | 5 |
| 2.43 | 0.12 | 0.639 | 0.014 | 2 | 2 |
| 2.43 | 0.12 | 0.639 | 0.014 | 6 | 5 |
| 3.89 | 0.19 | 1.024 | 0.022 | 2 | 2 |
| 3.89 | 0.19 | 1.024 | 0.022 | 6 | 5 |
| Nutrient-Limited | | | | | |
| 0.49 | 0.024 | 0.129 | 0.003 | 2 | 2 |
| 0.49 | 0.024 | 0.129 | 0.003 | 6 | 5 |
| Salinity-Limited | | | | | |
| 1.46 | 0.073 | 0.384 | 0.008 | 18 | 17 |
| 1.46 | 0.073 | 0.384 | 0.008 | 36 | 38 |
| 2.43 | 0.12 | 0.639 | 0.014 | 18 | 17 |
| 2.43 | 0.12 | 0.639 | 0.014 | 36 | 38 |
| 3.89 | 0.19 | 1.024 | 0.022 | 18 | 17 |
| 3.89 | 0.19 | 1.024 | 0.022 | 36 | 38 |
| Both-Limited | | | | | |
| 0.49 | 0.024 | 0.129 | 0.003 | 18 | 17 |
| 0.49 | 0.024 | 0.129 | 0.003 | 36 | 38 |

Data were analyzed as a one-way ANOVA with four groups (neither-, N-, salinity-, and both-limited) in PROC MIXED in SAS. PROC MIXED has the capability to handle unbalanced sample sizes within groups, as in our analysis. I used contrasts within the ANOVAs to compare N:P ratios, C:N ratios, and Na concentrations of plants grown at high salinity with those of plants grown at low salinity. I used LSMeans to obtain a mean for each of the groups. To determine boundaries for the tool to evaluate limiting factors, I averaged the means of the high and low salinity groups. I used the same procedures to make comparisons between plants grown at high and low N levels. Pearson's correlation coefficients were used to determine correlations. I determined significance for all tests using an alpha level of 0.05.

**Results**

There was a significant difference in N:P ratios among the four limiting factors ($F_{3,103} = 22.53$, $p < 0.0001$). Plants that were not N-limited had lower N:P ratios (mean = 40.77, SE = 2.10) than plants that were N-limited (mean = 53.62, SE = 3.62; $F_{1,103} = 14.05$, $p = 0.0003$; Figure 2a). Plants that were salinity-limited had lower N:P ratios (mean = 32.74, SE = 2.29) than plants were not salinity-limited (mean = 54.28, SE = 2.18; $F_{1,103} = 45.90$, $p < 0.0001$; Figure 2a). Also, plants with higher [Na] in leaf tissue had lower N:P ratios (Figure 2b).

There was a significant difference in C:N ratios among limiting factors ($F_{3,104} = 12.38$, $p < 0.0001$). Plants that were not N-limited had lower C:N ratios than plants that were N-limited ($F_{1,104} = 36.69$, $p < 0.0001$; Figure 3). The mean C:N ratio for non-N-limited plants was 42.07 ± 2.27 whereas the mean C:N ratio for N-limited plants was 69.94 ± 3.94. The average of the mean C:N ratio overall was 56. C:N ratios of plants that were salinity-limited were not significantly different from C:N ratios of plants that were not salinity-limited (mean = 49.04, SE = 2.28; $F_{1,104} = 0.12$, $p = 0.7285$).

There was a significant difference in Na concentration among limiting factors ($F_{3,103} = 22.53$, $p < 0.0001$). Plants that were not N-limited had higher Na concentrations (mean = 1.13, SE = 0.04) than

plants that were N-limited (mean = 0.93, SE = 0.07; $F_{1,122} = 14.13$, $p = 0.0003$, Figure 4). Na concentrations were higher in plants that were salinity-limited (mean = 1.38, SE = 0.03) than plants that were not salinity-limited (mean = 0.79, SE = 0.03; $F_{1,122} = 131.75$, $p < 0.0001$). The mean Na concentration for salinity-limited plants was 1.4%. The mean Na concentration for non-salinity-limited plants was 0.8%. The average of the mean Na concentration overall was 1.1%. Na concentrations in plants was correlated with water salinity ($r = 0.811$, $p < 0.0001$).

## Discussion

Biomass measurements alone could not be used to determine the cause of the limitation of production because intermediate levels of biomass developed where growth was salinity limited, N limited, and co-limited by high salinity and low N availability (Figure 1). The large difference in biomass between plants grown in limited and unlimited conditions highlights the importance of determining

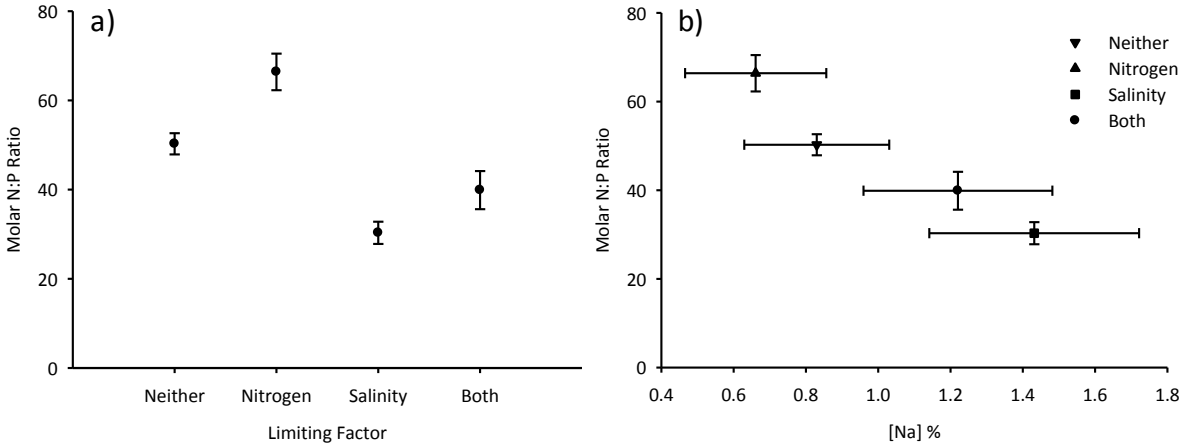


Figure 2: Mean molar N:P ratios (±1 SE) of *Spartina patens* leaf tissue (a) grown under various limiting conditions and (b) relative to mean (±1 SE) Na concentrations in leaf tissue. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Nutrient treatments were created by mixing 19-5-8 and 35-0-0 slow-release fertilizer (Osmocote) into soils consisting of 90% sand and 10% clay. Salinity treatments were created by adding Forty Fathoms Marine mix into water in the tubs.

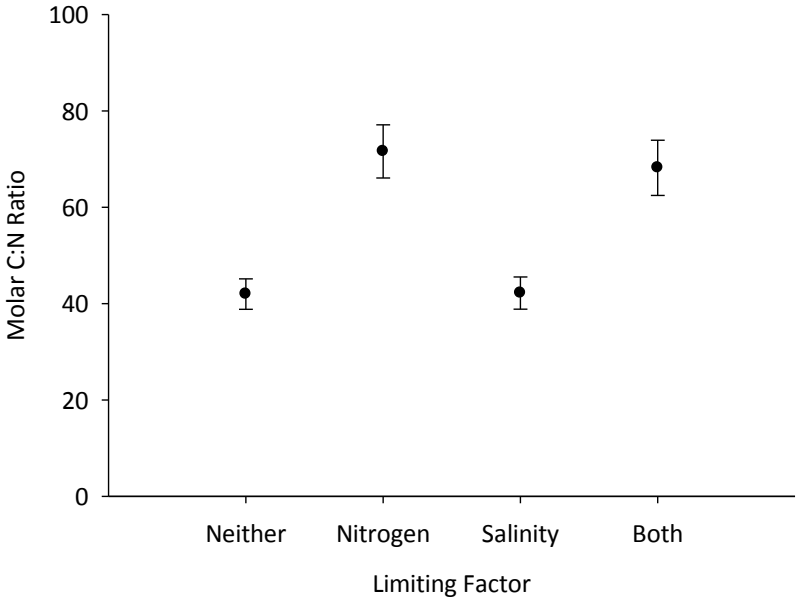


Figure 3: Mean molar C:N ratios (±1 SE) of *Spartina patens* leaf tissue grown under various nutrient and salinity conditions. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Neither- limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.

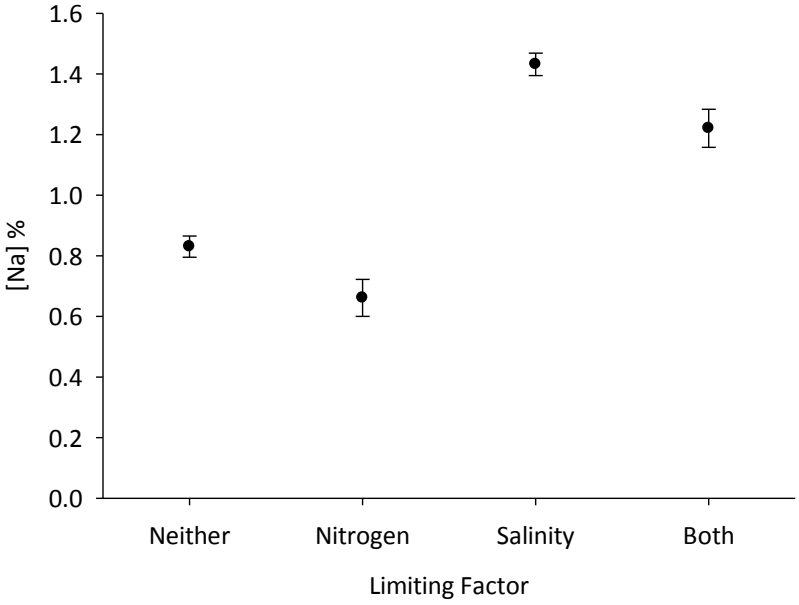


Figure 4: Mean Na concentrations (±1 SE) of *Spartina patens* leaf tissue grown under various nutrient and salinity conditions. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Neither-limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.

limiting factors for improving the health of degrading marshes. Merino et al. (2010) found that maximum biomass for *S. patens* occurred when plants grew in water low in salinity and soil high in nutrients.Al

though previous studies appear to disagree on the growth response of *Spartina spp.* to changes in salinity, the results of our study show that the range of salinities under which tests were conducted could have influenced the results of these studies. For instance, DeLaune et al. (2005) showed that for *S. alterniflora* grown where salinity was less than 8 ppt, adding nutrients had a bigger effect on growth than decreasing salinity. Our results suggest that these lower salinities likely do not produce conditions that limit production in *Spartina spp*. A study (Foret 2001) found that *S. patens* had large differences in growth responses to salinity where salinity differed from 15 ppt to near 0 ppt. The change in growth in this study was likely due to reducing salinity stress on the plants.

N:P ratios in leaf tissue could not be used to identify N or salinity limitation because N:P ratios were affected by both changes in N and salinity levels. Phosphorus content in leaves did not vary much and was generally high relative to N. N:P ratios (range: 20.57 to 104.85, mean: 44.01) were somewhat higher than the ranges reported for *Spartina spp*. in previous studies. Foret (2001) found N:P ratios between 18 and 32 for *S. patens*. Stribling and Cornwell (1992) found N:P ratios between 7.4 and 25 (converted to molar ratios from the reported mass ratios) for *S. alterniflora*. The highest N:P ratios in this study occurred at the lowest salinity treatments and in plants with the lowest leaf tissue [Na] (Figure 2). This could be because soils have a higher phosphate sorption capacity in freshwater than in saline conditions (Sundareshwar and Morris 1999). Based on the standard of N:P ratios > 35 to indicate P-limitation in multiple species (molar ratio converted from mass ratio; Koerselman and Mueleman 1996), average N:P ratios of limiting factor groups suggest that all groups were P-limited except the salinity-limited group. It appears that this N:P ratio may be somewhat too high to be a useful indicator of P-limitation in *S. patens*, however. If P strongly limited production in nearly all plants as this ratio

suggests, there would not have been evidence of limitation by high salinity. There are too few reports of N:P ratios from the field to determine if the high N:P ratios that observed at low salinities in this study are common. Further study of the effects of P availability and salinity on the uptake of P by *S. patens* are necessary to adjust the ratio of N:P as an indicator of P-limitation for this species.

C:N ratios were useful in identifying N limitation because C:N ratios varied predictably with N levels. Higher C:N ratios indicated limitation of productivity by N starvation. Our C:N ratios (range: 19.84 to 138.88, mean: 49.04) were within the ranges reported for *Spartina spp*. in previous studies. Foret (2001) reported C:N ratios between 40 and 120 for *S. patens*. Bradley and Morris (1992) reported C:N ratios between 30 at high salinity and 90 at low salinity for above-ground tissue of *S. alterinflora*. Our findings also agree with previous studies reporting that enhanced N decreased the C:N ratio of *Spartina spp.* leaf tissue (Foret 2001, Bradley and Morris 1992). In contrast to Foret's findings that increased nutrient availability reduced C:N ratios only where salinity was low, in our study, C:N ratios also decreased with higher N availability where salinity was high. Our findings agree with Bradley and Morris's (1992) finding that the internal N supply needed to maintain growth in *Spartina alterniflora* increased with increasing salinity.

Na concentration in leaf tissue was a useful tool for identifying salinity stress. While changes in both salinity and N levels affected Na concentration, the effect of salinity on Na concentration was much greater than the effect of N variations on Na concentration. Plants that grew in water with higher salinity had higher Na concentrations in their leaf tissue. Na concentrations in leaf tissue of other marsh species have also been shown to increase with increases in water salinity level (McKee and Mendelssohn 1989, Bradley and Morris 1991). The high correlation between leaf tissue Na and water salinity suggests that a single measurement of leaf tissue salinity is a better indicator of salinity exposure than a single measurement of water salinity because of the dynamic nature of water salinity in coastal marshes.

Our findings confirm that the chemical composition of the leaf tissue of *S. patens* can be used to determine if low N availability or high salinity limit productivity. A combination of the response of C:N ratios and Na concentration in plant tissue to variations in the conditions under which the plants were grown can be used to distinguish plants grown under different limiting conditions (Figure 5). This tool (Figure 6) could eliminate much speculation about methods for improving production in degrading coastal marshes by allowing managers to more easily test their assumptions about which factors limit production. Analyzing small samples of leaf tissue to determine leaf chemistry also has the potential to be more cost-effective than current methods for identifying limiting factors via measuring biomass because it is less time-consuming. The type of elemental analysis I used for this study is relatively inexpensive and available through agriculture and extension offices throughout the United States. Studies are needed to confirm that this tool can identify limiting factors under field conditions for *S. patens* and other species.

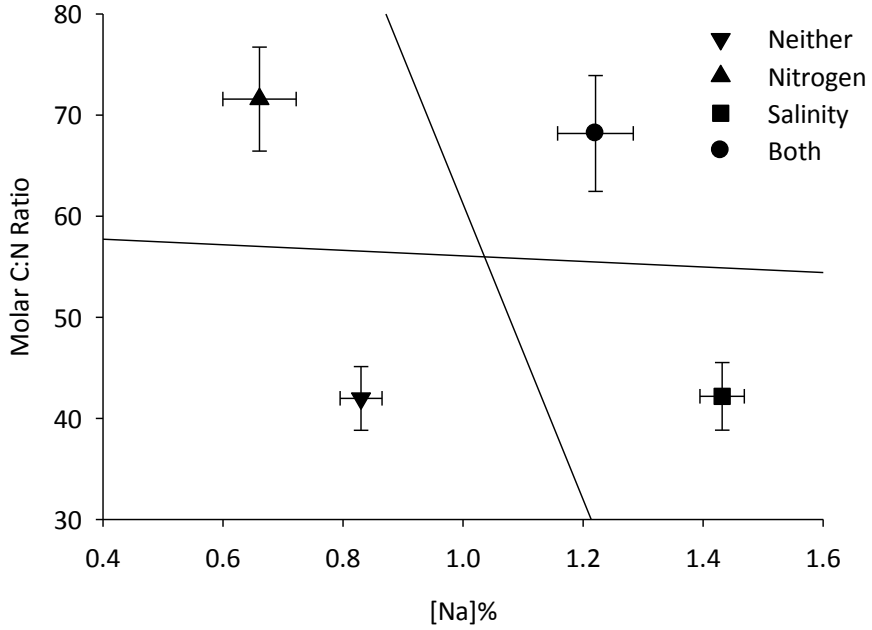


Figure 5: Mean molar C:N ratio and Na concentrations (±1 SE) in *S. patens* leaf tissue. Nutrient-limited indicates low nutrients limited productivity. Salinity-limited indicates high salinity limited productivity. Neither-limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.

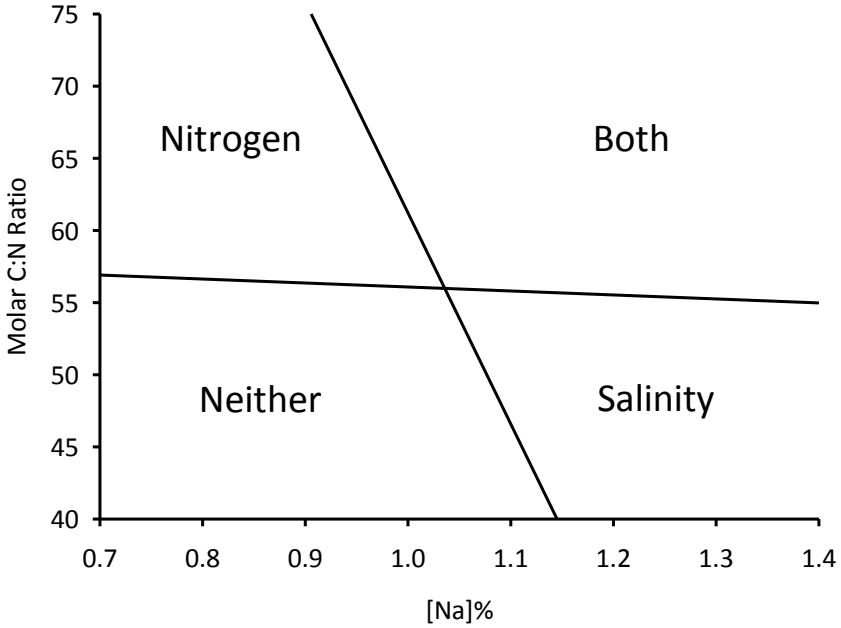


Figure 6: Na concentrations and C:N ratios in *Spartina patens* leaf tissue used as a signature to identify conditions limiting biomass production. This tool shows that C:N ratios in *S. patens* greater than 56 indicate limitation by low N availability and Na concentrations greater than 1.1% indicate limitation by high salinity.

A potential issue with applying results of this study directly to management of marshes is that salinity tolerance in *S. patens* varies throughout the Gulf of Mexico coast. It is not reasonable to assume that plants growing in higher salinity environments have higher salinity tolerances than plants growing in lower salinity environments (Hester et al. 1996). The lethal salinity for population "i" was similar to the average lethal salinity of 80-83 ppt across the Gulf Coast and the lethal salinity for population "k" was somewhat lower (Hester et al. 1996). Differences in salinity tolerances of *S. patens* growing in field conditions is an important reason to test relationships observed in this greenhouse experiment in a field setting.

One limitation of this study is that these N and Na signatures do not reflect changes in C:N ratios and Na concentrations that may result from variations in flooding stress. Future experiments will identify both the chemical signatures that can be used to identify marshes that are stressed by flooding

and the effects flooding may have on the signatures I have already identified. A second limitation of this study is that vegetation responses to stress under constant, controlled conditions may not accurately reflect responses to natural variations in marshes. Future efforts will focus on field experiments to test whether the relationships I observed in this greenhouse experiment apply to plants growing in the field.

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## CHAPTER 3.
## DEVELOPING CRITICAL VALUES TO IMPROVE DIAGNOSIS AND MANAGEMENT OF FLOOD STRESS IN *SPARTINA PATENS* MARSHES

### Introduction

Identifing concentrations of elements in plant tissue via leaf tissue testing is the most widely-used method to diagnose mineral deficiencies in agricultural crop plants (Epstein and Bloom 2005). Critical values and sufficiency ranges that are developed in greenhouse studies can be directly applied by farmers to improve crop yields. For example, concentrations of N < 2.8-3.6% in the leaf tissue of rice indicate N-limitation, depending on the cultivar and the growth stage of the plant (Brandon and Wells 1986). N concentrations in plant tissue below these levels indicate that fertilizing plants would improve productivity. This information is commonly provided to farmers by agencies such as a state's department of agriculture and university agricultural extension offices (e.g. Bell and Kovar 2000).

In wetland ecology, tools are just beginning to be developed that will allow management and restoration professionals to diagnose the causes of limited production in marsh ecosystems. Several studies have investigated the use of N:P ratios to identify nutrient limitation by N or P (Koerselman and Meuleman 1996, Stribling and Cornwell 2001, Guesewell and Koerselman 2002). I previously developed chemical signatures that indicate salinity stress and N-limitation in *Spartina patens* Ait. Mulh (Tobias et al. 2010).

Although recent research to diagnose limited production in wetland species has focused on nutrient limitation or salinity stress, flooding stress is at least as important for controlling production of *S. patens* as either of these factors. *S. patens* is generally more productive at higher elevation and lower salinities in Louisiana marshes (Broome et al. 1995). In a greenhouse experiment, *S. patens* was most productive when drained slightly (water depth = -10 cm) and least productive when constantly flooded (water depth = +20 cm; Spalding and Hester 2007). In some locations, soil hypoxia may be the primary factor controlling nutrient uptake and growth of *S. patens* (Bandyopadhyay et al. 1993). For example,

dieback in Louisiana's coastal marshes that are dominated by *S. patens* and *S. alterniflora* was found to be caused by submergence rather than high salinity, and aboveground biomass of *S. patens* was higher when it was grown with less flooding (Webb et al. 1995).

Here, I develop critical values that can be used to identify limitation of production by flooding stress in *S. patens.* I focus on *S. patens* because it is the most common plant in coastal Louisiana marshes (Chabreck 1970) and because it also occurs throughout the Gulf of Mexico and Atlantic coastal marshes. Previous studies have observed that [Mn], [Fe], [Ca] , or [Mg] in the tissue of wetland plants vary with changes in flooding, but none have developed guidelines for interpreting concentrations in leaf tissue as a means of improving restoration and management practices. Also, critical values for flooding stress in agricultural crops do not appear to have been developed at this time. Mn and Fe become mobile, and thus more available to plants, in acidic soils and under anoxic conditions. The Mn and Fe content of leaf tissue increases with increased flooding in *Leersia oryzoides* (Pierce et al. 2009). Leaf tissue [Mn] of *Spartina alterniflora* grown in anoxic soils was more elevated than in plants grown in aerated soil (Bandyopadhyay et al. 1993). Rice (*Oryza sativa*) may develop Fe toxicity as a result of low Eh and/or acidic soil conditions (Fageria et al. 2008). The Ca content of plant tissue also increases in plants grown under drained conditions (Lissner et al. 2003). Flooding stress can reduce leaf Mg content (McKee and Mendelssohn 1989). Mn and Mg content of *S. alterniflora* tissue has been reported to correlate with plant productivity across a gradient from well drained marsh to poorly drained marsh, while [Ca] was not correlated to productivity (DeLaune and Pezeshki 1988). Also, more productive stands of S. alterniflora contained higher [Mn] (DeLaune et al. 1981).

The purpose of this experiment was to determine which elemental concentrations or ratios could be used as an indicator of flooding stress in *Spartina patens* growing in a range of flooding levels. Here, I evaluate the utility of several elements as indicators of flooding stress. I also propose guidelines for these elements to be used to diagnose limitation of production due to flooding stress.

Concentrations of each of these elements were determined via ICP analysis, which is inexpensive, commercially available through university agricultural extension offices, and is commonly used to detect mineral deficiencies or toxicities in agricultural crops.

## Methods

Manipulating flooding stress traditionally has utilized greenhouse studies (e.g., Howard and Mendelssohn 1999) or three levels of flooding in the field (e.g., Webb et al. 1995), but I used a recently developed field-based technique that creates six levels of flooding stress (Morris 2007). These installations are termed "marsh organs" because they resemble the pipes on a pipe organ. Marsh organs were constructed from thirty-six 15.2-cm-diameter PVC pipes, which were bolted together for stability. Each marsh organ consisted of six rows of six pipes in each row (Figure 7). The pipes were cut to lengths of 122, 107, 91, 76, 61, and 46 cm. For the purposes of this paper, rows are defined as the set of six pipes of equal elevation within a marsh organ. I identified rows by numbers such that "row one" was the tallest (least flooded) and "row six" was the shortest (most flooded). Columns are defined as a set of contiguous pipes consisting of one pipe of each elevation within a marsh organ. I identified columns using letters such that column A is to the west and column F is to the east.

I installed a total of four marsh organs in coastal marshes in the summer of 2007. Sites were selected to represent a range of conditions experienced by *S. patens* in Louisiana's coastal marshes. Marshes at Marsh Island Wildlife Refuge (29°34'47" N, 92°00'40" W and 29°34'42" N, 91°49'29" W) receive fresh water and sediment from the Atchafalaya River. Soils at Rockefeller Refuge sites (29°37'54" N, 92°38'18" W and 29°37'12" N, 92°34'11" W) developed without direct riverine influences. Following Penfound and Hathaway's (1938) classification system for coastal marshes, I installed one marsh organ in a saline area where the surrounding marsh was dominated by *Spartina alterniflora* and one marsh organ in an intermediate marsh where the surrounding marsh was dominated by *S. patens* and

contained some *Sagittaria lancifolia* and/or *Typha domingensis* at each refuge. *S. patens* in adjacent marshes at all sites ranged from rare to dominant.

Marsh organs were installed in shallow ponds or lakes within marsh sites. I oriented the organs so that the tallest pipes were to the north to maximize sun exposure for all pipes. Organs were dug into the soil to a level such that the fourth row from the top of the organ was even with the level of the local marsh. This resulted in row 1 being approximately 46 cm above local marsh elevation and row 6 being approximately 30 cm below local marsh elevation. I adjusted each marsh organ to ensure that the rows were level following installation.

I filled the pipes with a mixture of local pond sediment and marsh soil to the top of each pipe. I planted each pipe with approximately ten stems of *Spartina patens* collected from the adjacent marsh. In spring of 2008 most of the pipes had lost some soil elevation (min = -5, max = 40, avg = 11 cm for all four organs). To re-establish soil elevation to the intended levels, I lifted the plants out of the pipes, refilled the pipes with pond sediment, and replaced the plants. Care was taken to avoid breaking stems

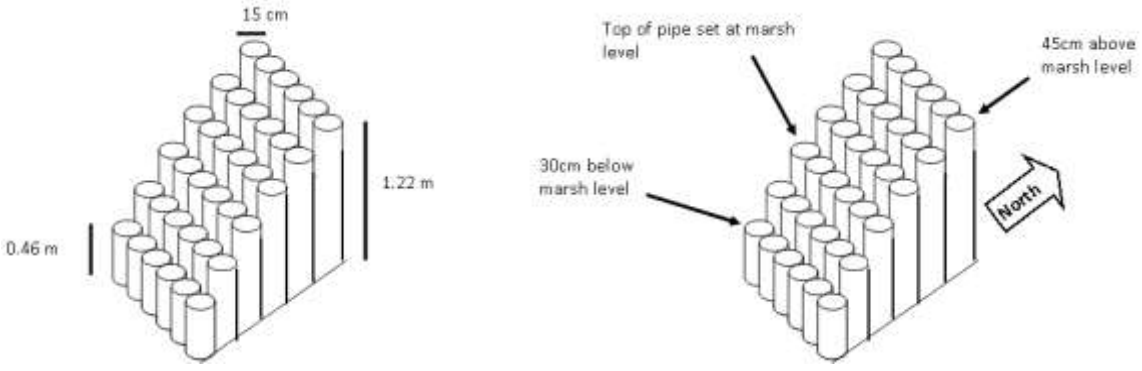


Figure 7: Shape, size, and orientation of marsh organs. Organs consist of six rows and six columns of 15 cm diameter PVC pipe. Heights of rows are 123, 107, 91, 76, 61, and 46 cm from the bottom of the pipes. Marsh organs are set into the pond sediment such that the top of the fourth row is at local marsh level. Note: Diagrams are not to scale.

or damaging roots. At the time of refilling, I also replaced any plants that were completely missing or showed no signs of live tissue with 20 new stems collected from the adjacent marsh. I replaced all plants in rows 5 and 6, except for three plants that were able to survive in these rows in the saline marsh organ at Rockefeller Refuge. I also replaced seven plants in rows 1-4. I replaced plants with 20 stems rather than 10 stems, as in the original planting, because I wanted the size of the replacement plants to be of a size similar to the plants that had been growing in the pipes rather than the original size of the plants. The increase in size of replacement plants over the original plantings was intended to reduce potential effects of the reduced time between planting and harvest that replacement plants experienced relative to original plantings.

I harvested half of the pipes from each marsh organ in summer 2008 (columns B, D, and F) and half in fall 2008 (columns A, C, and E). Although care was taken to select only *S. patens* for planting, a few pipes included other species at the time of harvest. In the lab, I sorted stems according to species and whether they were alive or dead. Live *S. patens* tissue was rinsed to remove any soil or salt on the leaf surface. All tissue was oven-dried at 60° to a constant weight and the dry weight was recorded as above-ground biomass. After weighing, samples of leaf tissue were taken from *S. patens* plants. Leaf tissue was selected from the top 15 cm of the plant only and no stems were included in tissue samples. Leaf tissue was ground in a coffee grinder (Black and Decker Smartgrind) and submitted to the LSU AgCenter's Soil Testing and Plant Analysis Lab (STPAL; Baton Rouge, LA, USA) to determine N, P, K, Na, Mn, Fe, Mg, and Ca concentrations in leaf tissue. The STPAL used dry combustion by Leco N analyzer to determine N and C content. They used ICP analysis to determine concentrations of all other elements.

I attempted to collect porewater from within the pipes. This was not possible, however, because despite lengthy attempts to extract porewater at 10, 15, and 20 cm below the soil surface, there simply was not enough porewater in the pipes to conduct any tests. Instead, I collected porewater from the nearby marsh and made the assumption that porewater conditions in the nearby

marsh were similar to those in the pipes. With a syringe, I collected porewater at 10 cm below the soil surface. I measured salinity, conductivity, and pH with a hand-held salinity meter (YSI model 63). I also collected porewater samples for nutrient analysis. These samples were filtered through 0.45 um nylon filters (Watman) to remove particles. I stored porewater samples on ice until they could be analyzed. I determined the concentrations of ammonia-N using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et al. 1998).

I measured soil redox potential (Eh) at 10 cm below the soil surface with Pt electrodes, a calomel reference electrode (accumet), and a pH/mV/temperature meter ("Oyster 10" by Extech Instruments). Prior to use, Pt electrodes were cleaned with souring powder and a brush. Pt electrodes were also tested by measuring Eh of a solution of quinhydrone in standard pH 4 and pH 7 solutions. Taking multiple Eh measurements for each pipe would have been ideal, but because of limited space inside the pipes, only one electrode of each type could be inserted into the soil. This resulted in a single measurement of Eh for each pipe at the time plants were harvested.

I measured soil elevation loss inside the pipes and the depth of pond water relative to the top of each pipe at the time of each harvest. Hourly water level data were obtained from water level loggers at Coastwide Reference Monitoring System (CRMS) sites near marsh organs (stations 0523, 0530, 0608, and 0610; LDNR 2008). Distances between CRMS stations and marsh organs ranged from 0.2-6.9 km. Hourly CRMS water level data and water level and soil elevation measurements taken immediately prior to harvesting plants were used to calculate the mean depth of water relative to the soil for each pipe for two weeks prior to harvest. In summer 2008, the pond surrounding the fresh marsh organ at Rockefeller Refuge had completely dried. Because of this, I could not accurately calculate average flooding depths for that organ. The data from the fresh marsh organ at Rockefeller Refuge was therefore excluded from any statistical analyses relating to water level for the summer harvest. The pond contained water in fall 2008 and I was able to calculate water depths for the fall sampling.

Although all of the marsh organs were completely submerged by storm surge from Hurricane Ike on September 13, 2008, minimal damage to the installations was observed following the hurricane. One exception was that the saline marsh organ at Rockefeller Refuge had tilted slightly and visual inspection indicated that relatively large amounts of soil elevation had been lost via undercutting in some of the pipes in this organ. The resulting soil elevations that have been measured may therefore have been unrepresentative of the growing conditions during fall.

I preformed all statistical analyses in SAS (SAS Institutes, Inc., Cary, N.C.). I tested for associations among elemental concentrations, above-ground biomass, and depth of flooding using Pearson correlation coefficients, estimated with PROC CORR. I used PROC REG to quantify the relationships between selected variables that were highly correlated. I identified the y-intercept of the linear regression of average water depth and [Mn] as a critical value. This critical value is the value of [Mn] that the linear model predicts a plant would have if it grew in marsh soils that were flooded to the soil surface (i.e. average water level = 0). I did the same analyses for [Ca]. I used these two models to classify plants as having grown in soil that was flooded above or below the surface based on the concentrations of Mn or Ca in their leaf tissue.

**Results**

Salinity levels rose substantially in the vicinity of the marsh organs at fresher sites following Hurricane Ike (Table 2). Salinity was already high at the saline sites and it did not change much following the hurricane. Orthophosphate and ammonium-N concentrations followed a similar pattern to that of salinity. Porewater was generally neutral to slightly acidic in the adjacent marsh for all locations and all sampling periods (Table 2). As expected, there was a trend toward decreasing Eh with increasing flooding (Figure 8). I suspect, however, that the magnitude of Eh reported by our equipment was biased for summer samples. Although Eh appears to suggest that soils were far too oxidized to contain reduced Mn during summer sampling, visual inspection of soils showed clearly that iron was reduced at 10 cm

below the soil surface in most pipes in rows 4 through 6. Roots in these reduced soil zones appeared to have accumulated plaques of oxidized iron on their surfaces. Eh values in fall appeared reasonable, given the visual evidence of reducing conditions. I chose not to use Eh in my analysis, however, because I suspected that Eh measurements were biased in summer.

Pipes that received the least flooding lost the most soil elevation by the summer harvest (Figure 9a). Some pipes that were consistently flooded accumulated small amounts of sediment on top of the soil surface. In the fall, soil elevation loss followed a pattern similar to summer elevation loss in three out of four marsh organs (Figure 9b). The substantial elevation loss at the Rockefeller Saline site apparently resulted from undermining of the pipes by storm surge from Hurricane Ike. Neither the above- or below-ground portions of the plants appeared to have been damaged by the hurricane, but the structure of the marsh organ itself appeared to have been undermined and the back side was warped slightly downward. No other marsh organ appears to have sustained such damage, but it was impossible to separate the effects of biogeochemical processes from possible effects of erosion for fall measurements.

Table 2: Chemistry of porewater extracted from 10 cm below the marsh surface adjacent to marsh organ installations. No means or standard errors are included because porewater chemistry presented here represents single measurements taken in each adjacent marsh.

| | Summer | | | | Fall | | | |
|---|---|---|---|---|---|---|---|---|
| | Rockefeller | | Marsh Island | | Rockefeller | | Marsh Island | |
| | Fresher | Saline | Fresher | Saline | Fresher | Saline | Fresher | Saline |
| Temperature (°C) | 32.10 | 28.60 | 31.00 | 33.70 | 13.20 | 16.80 | 23.10 | 20.80 |
| Conductivity (mS) | 16.70 | 27.05 | 3.77 | 13.49 | 21.16 | 26.29 | 18.77 | 12.12 |
| Salinity (ppt) | 9.70 | 16.50 | 1.80 | 7.60 | 12.70 | 16.10 | 11.20 | 6.90 |
| pH | 6.65 | 6.89 | 6.41 | 5.69 | 6.93 | 7.26 | 6.47 | 6.49 |
| Orthophosphate (mg/L) | 1.44 | 8.40 | 1.86 | 5.34 | 3.48 | 7.62 | 4.80 | 2.52 |
| Ammonium-N (mg/L) | 0.78 | 27.52 | 0.84 | 2.16 | 4.62 | 24.75 | 0.78 | 0.90 |

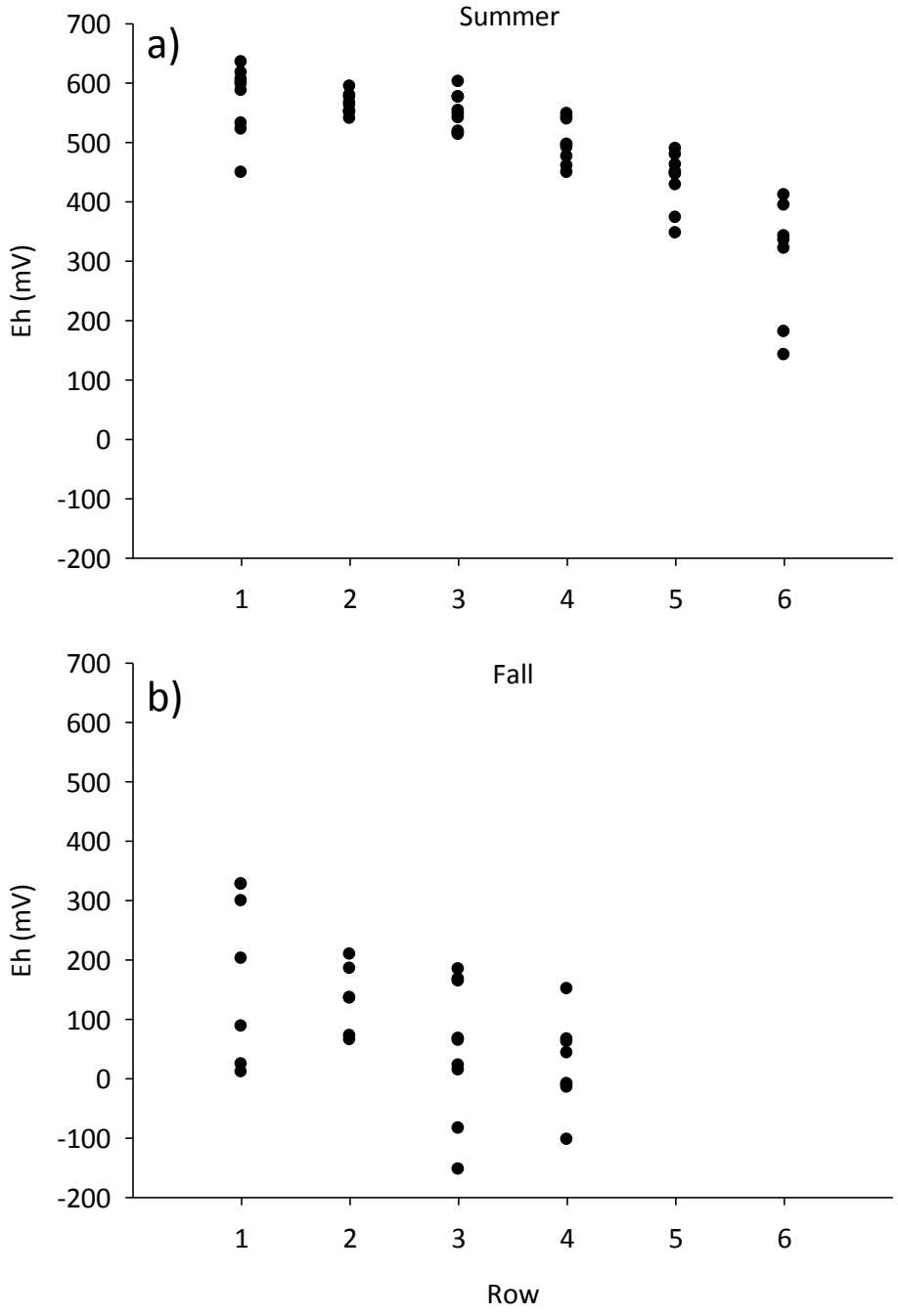


Figure 8: Soil Eh measured at 10 cm below the surface of soils within pipes at the time plants were harvested in (a) summer and (b) fall. Row 1 was the most drained (approx. 45 cm above the local marsh surface) and row 6 was the most flooded (approximately 30 cm below the local marsh surface).

There was a significant linear relationship between total live above-ground biomass and the two-week average depth of flooding in summer but not in fall (Table 2). Biomass was higher in pipes that received less flooding (Figure 10). Samples taken from the most flooded pipes generally showed signs of decay and few plants from these rows showed signs of growth. In some cases no plants could be found in these pipes. Plants grown at elevations close to marsh level or at higher elevations generally appeared healthy. Plants in row 1, at 46 cm above marsh level, clearly were not stressed by flooding. Pipes in this row almost always contained the highest biomass.

In the leaf tissue harvested during the summer, the only elements that correlated highly ($|r| > 0.50$) with both total live biomass and average flooding depth were Mn and Ca (Table 4). In the fall, no elements correlated highly with total live biomass and average flooding depth. The [Mn] in leaf tissue was lower in plants harvested during the summer that received more flooding. The linear model of the relationship between [Mn] and the two-week average depth of flooding for plants harvested in the summer indicates that the relationship is strong (Table 3) and predicts that leaf tissue will contain 223 ppm Mn when the average water level is at the soil surface (i.e. average water depth = 0 cm; Figure 11). This concentration of Mn in leaf tissue represents a reasonable separation point between plants that are flood-stressed and those that are not. Where [Mn] < 223 ppm live biomass was consistently low (Figure 12a). Where [Mn] > 223 ppm, however, the range of live biomass was approximately 1.6 times the range of biomass for plants with [Mn] < 223 ppm. When applied to the plants used in the linear model, [Mn] correctly identified the flooding condition under which the plants grew for 45/52 pipes (87%).

The response of [Ca] in leaf tissue was similar to that of [Mn] for summer and fall samples respectively (Figure 13). [Ca] in leaf tissue decreased with increasing flooding during the summer and the linear relationship was stronger than the relationship of [Mn] to flooding depth (Table 3). Where the average water level is at the soil surface (i.e. water depth = 0 cm), the linear model predicts that [Ca] = 0.26%. There was slightly more variation in live biomass for plants with [Ca] > 0.26% (Figure 14a) but it

was not nearly as pronounced as the increase in variation in biomass in response to [Mn] > 223 during the summer. When applied to the plants used in the linear model, [Ca] correctly identified the flooding conditions under which the plants grew for 49/52 pipes (94%).

None of the patterns I observed in the summer data were observed in the fall data. [Mn] in leaf tissue varied over three times more than it did during the summer (Figure 12b) and there was little relationship, if any, between water depth and above-ground biomass (Figure10b). In the fall, the relationship between [Ca] and water depth was weak, as was the relationship between [Ca] and live biomass (Table 3).

**Discussion**

Although I was unable to test porewater chemistry from the soil within the pipes, porewater from the adjacent marsh verified that the plants in this study were grown in a broad range of salinity and nutrient conditions. I intentionally designed this experiment to control only the height of plantings relative to the marsh surface while allowing salinity, nutrients, and water levels to fluctuate as they would in a natural marsh. The salinity levels I measured in the adjacent marsh are similar to those reported by other studies of *Spartina* dominated marshes (e.g., Nyman et al. 2009), as are the porewater nutrient levels (e.g., Mendelssohn 1979).

Loss of soil elevation may have been caused by a combination of factors. The soils I used were highly organic so I would expect them to oxidize upon draining. Also, as soils dried they may have compacted. It is beyond the scope of this paper to determine which might be responsible here, however. Regardless of the cause, however, loss of elevation following drainage of wetland soils is an important consideration for management and restoration plans.

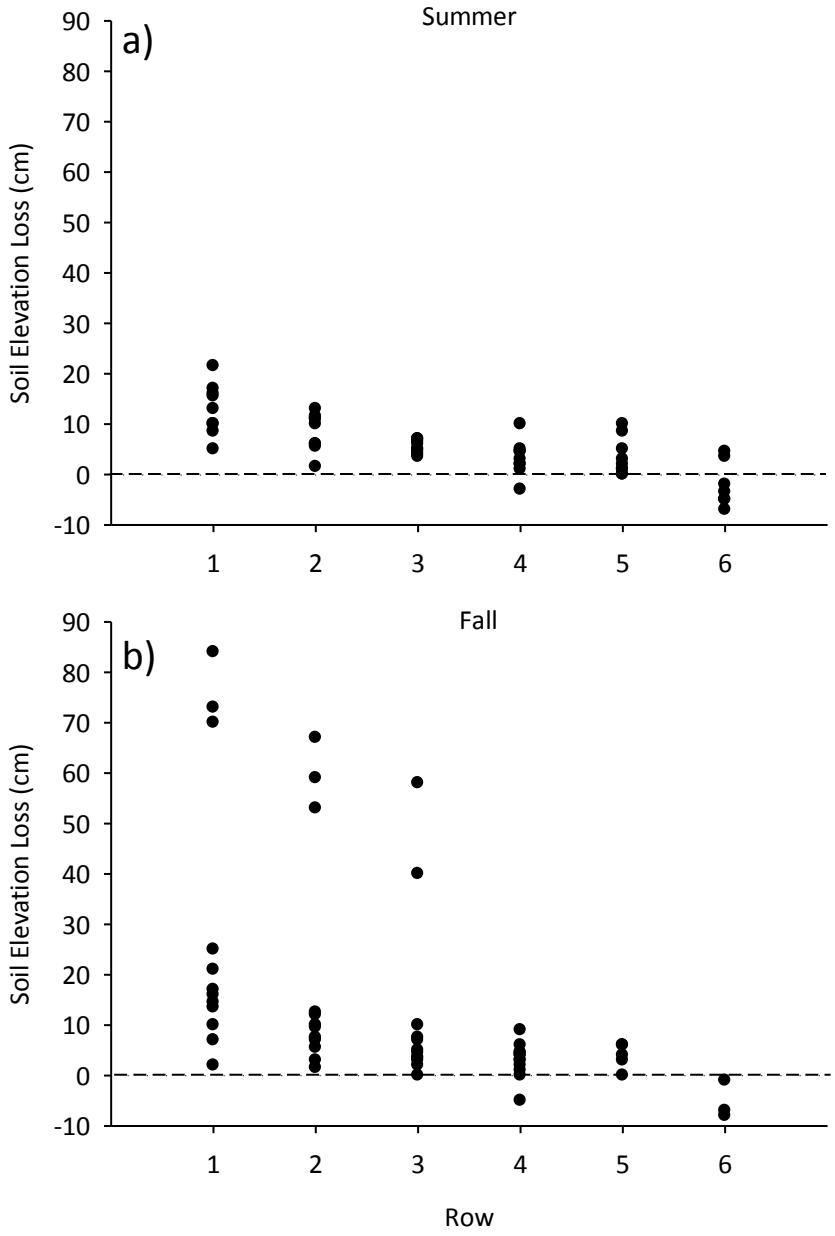


Figure 9: Elevation loss (cm) for soils within pipes in (a) summer and (b) fall. Positive loss values indicate that soil levels were below the top of the pipe at the time of harvest. Negative loss indicates accumulation of sediment above the top of the pipe at the time of harvest. Row 1 was the most drained (approx. 45 cm above the local marsh surface) and row 6 was the most flooded (approximately 30 cm below the local marsh surface).

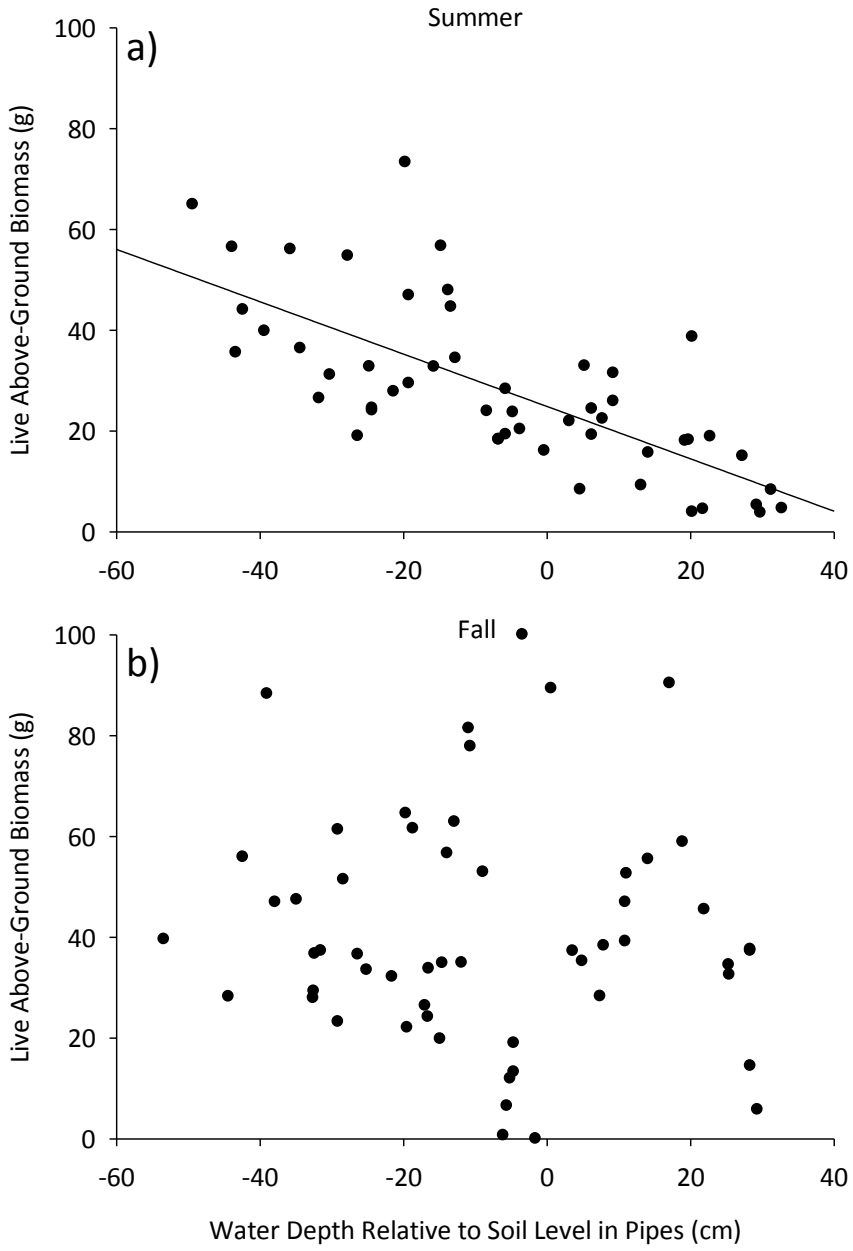


Figure 10: Live above-ground biomass (g) of all species of plants grown with varying level of flooding harvested in (a) summer and (b) fall. Water depth is an average depth of flooding above the soil surface, calculated for each pipe with data from hourly data from water level recorders at the nearest CRMS station.

Table 3: Linear regressions between average depth of water relative to the soil surface in each pipe (cm) and total live biomass, leaf tissue [Mn], and leaf tissue [Ca] of *Spartina patens*. Linear regressions were estimated using PROC REG (SAS).

| | Summer | | | | | | | Fall | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | Variable | DF | Mean | SE | t | p | $R^2$ | Variable | DF | Mean | SE | t | p |
| Total Live Biomass (g) | 0.5148 | Intercept | 1 | 24.87 | 1.65 | 15.05 | <.0001 | 0.0044 | Intercept | 1 | 40.38 | 3.33 | 12.14 | <.0001 |
| | | Slope | 1 | -0.52 | 0.07 | -7.28 | <.0001 | | Slope | 1 | -0.07 | 0.14 | -0.49 | 0.6283 |
| [Mn] (ppm) | 0.5586 | Intercept | 1 | 222.93 | 17.64 | 12.64 | <.0001 | 0.2034 | Intercept | 1 | 648.31 | 64.36 | 10.07 | <.0001 |
| | | Slope | 1 | -6.05 | 0.76 | -7.95 | <.0001 | | Slope | 1 | 9.88 | 2.66 | 3.71 | 0.0005 |
| [Ca] (%) | 0.6656 | Intercept | 1 | 0.27 | 0.01 | 26.82 | <.0001 | 0.0169 | Intercept | 1 | 0.32 | 0.01 | 24.06 | <.0001 |
| | | Slope | 1 | 0.00 | 0.00 | -9.98 | <.0001 | | Slope | 1 | 0.00 | 0.00 | 0.96 | 0.3393 |

Table 4: Pearson correlation coefficients for live biomass, average water depth, and leaf tissue concentrations of various elements in leaf tissue of *Spartina patens* grown with varying levels of flooding. Correlation coefficients were estimated with PROC CORR (SAS). Italics indicate $\alpha<0.05$ and bold indicates $\alpha<0.01$.

| | Summer | | | Fall | | |
|---|---|---|---|---|---|---|
| | Live Biomass | | Avg. Water | Live Biomass | | Avg. Water |
| | *S. patens* | All Species | Depth | *S. patens* | All Species | Depth |
| S. patens | - | 0.97 | -0.75 | - | ***0.98*** | -0.09 |
| All Species | 0.97 | - | -0.72 | ***0.98*** | - | -0.07 |
| Avg. Water Depth | -0.75 | -0.72 | - | -0.09 | -0.07 | - |
| Al | -0.19 | -0.20 | 0.32 | -0.21 | -0.24 | ***0.37*** |
| As | ***-0.38*** | ***-0.39*** | ***0.44*** | -0.10 | -0.11 | *0.30* |
| B | ***0.41*** | ***0.39*** | *-0.33* | -0.17 | -0.16 | ***0.75*** |
| Cd | - | - | - | - | - | - |
| Ca | ***0.67*** | ***0.64*** | ***-0.82*** | 0.05 | 0.07 | 0.13 |
| C | *-0.29* | -0.25 | ***0.44*** | ***0.39*** | ***0.41*** | -0.06 |
| Cu | 0.02 | 0.01 | 0.01 | ***-0.37*** | ***-0.41*** | ***0.37*** |
| Fe | -0.09 | -0.10 | 0.21 | -0.19 | -0.21 | *0.53* |
| Pb | -0.23 | -0.24 | *0.29* | ***-0.34*** | ***-0.38*** | *0.30* |
| Mg | 0.14 | 0.10 | -0.06 | ***-0.36*** | ***-0.36*** | ***0.44*** |
| Mn | ***0.57*** | ***0.54*** | ***-0.75*** | -0.24 | -0.19 | ***0.45*** |
| Mo | ***0.50*** | ***0.44*** | ***-0.73*** | *-0.32* | *-0.34* | -0.06 |
| Ni | -0.16 | -0.17 | 0.27 | *-0.33* | ***-0.36*** | ***0.41*** |
| Ni | -0.20 | -0.24 | 0.14 | ***0.39*** | ***0.41*** | -0.06 |
| P | 0.05 | 0.06 | 0.01 | 0.26 | *0.27* | 0.05 |
| K | 0.33 | 0.32 | -0.49 | 0.09 | 0.08 | ***-0.70*** |
| Se | *-0.33* | *-0.33* | ***0.36*** | - | - | - |
| Na | *0.03* | *0.02* | ***-0.13*** | -0.23 | -0.24 | ***0.53*** |
| S | 0.13 | 0.10 | -0.24 | *-0.32* | *-0.33* | ***0.39*** |
| Zn | -0.07 | -0.09 | 0.11 | -0.12 | -0.15 | *0.31* |
| C:N | 0.11 | 0.14 | -0.05 | 0.04 | 0.06 | -0.07 |

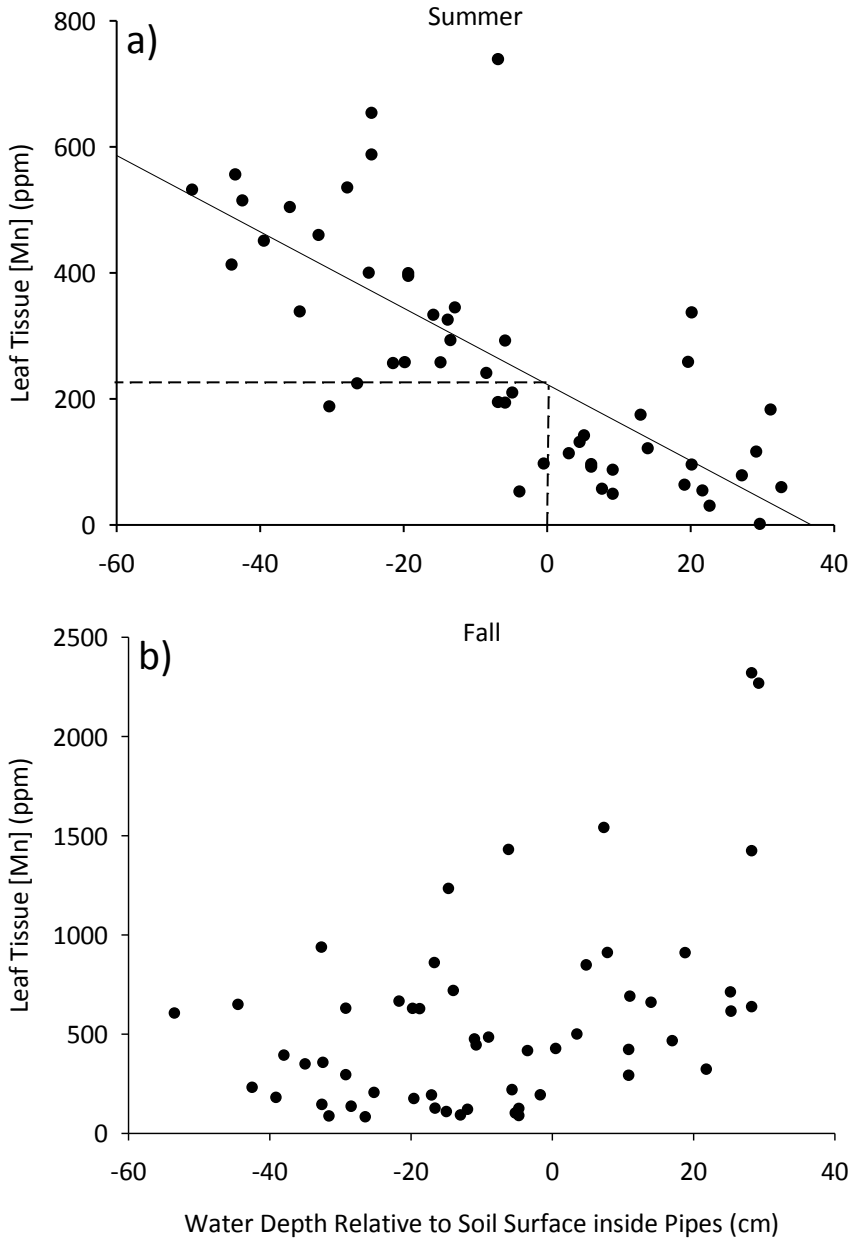


Figure 11: Mn concentrations in leaf tissue of *Spartina patens* plants grown with varying levels of flooding in the field and harvested in (a) summer and (b) fall. Water depth is an average depth of flooding above the soil surface, calculated for each pipe with data from water level recorders at the nearest CRMS station. The dashed line in (a) indicates that a linear regression predicts that the leaf tissue of *S. patens* growing in soil that is flooded to the soil surface will have 223 ppm Mn.

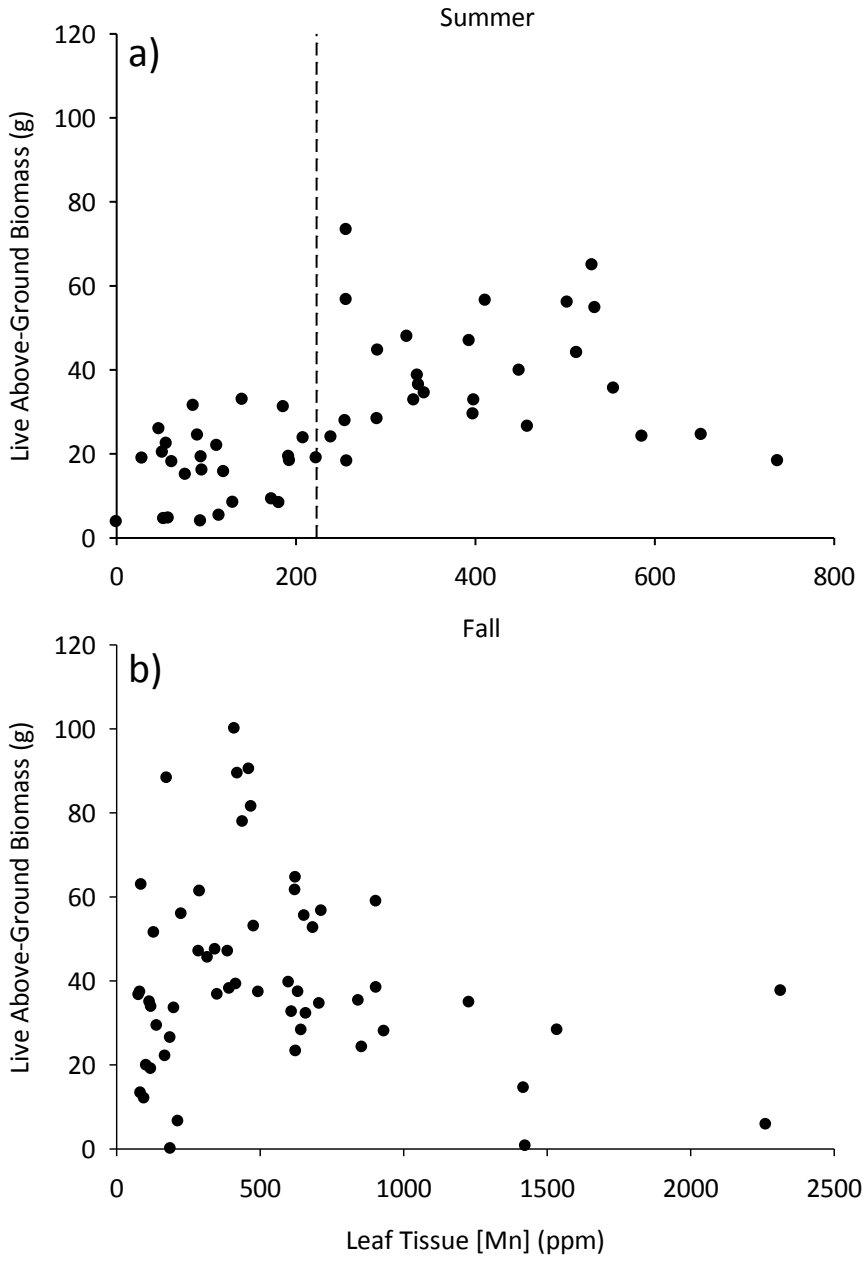


Figure 12: Live biomass (g) for *Spartina patens* plants grown at varying levels of flooding stress in the field, relative to Mn concentrations (ppm) in *S. patens* leaf tissue harvested in (a) summer and (b) fall. The dashed line in (a) indicates the critical value predicted by the linear model shown in figure 12(a).

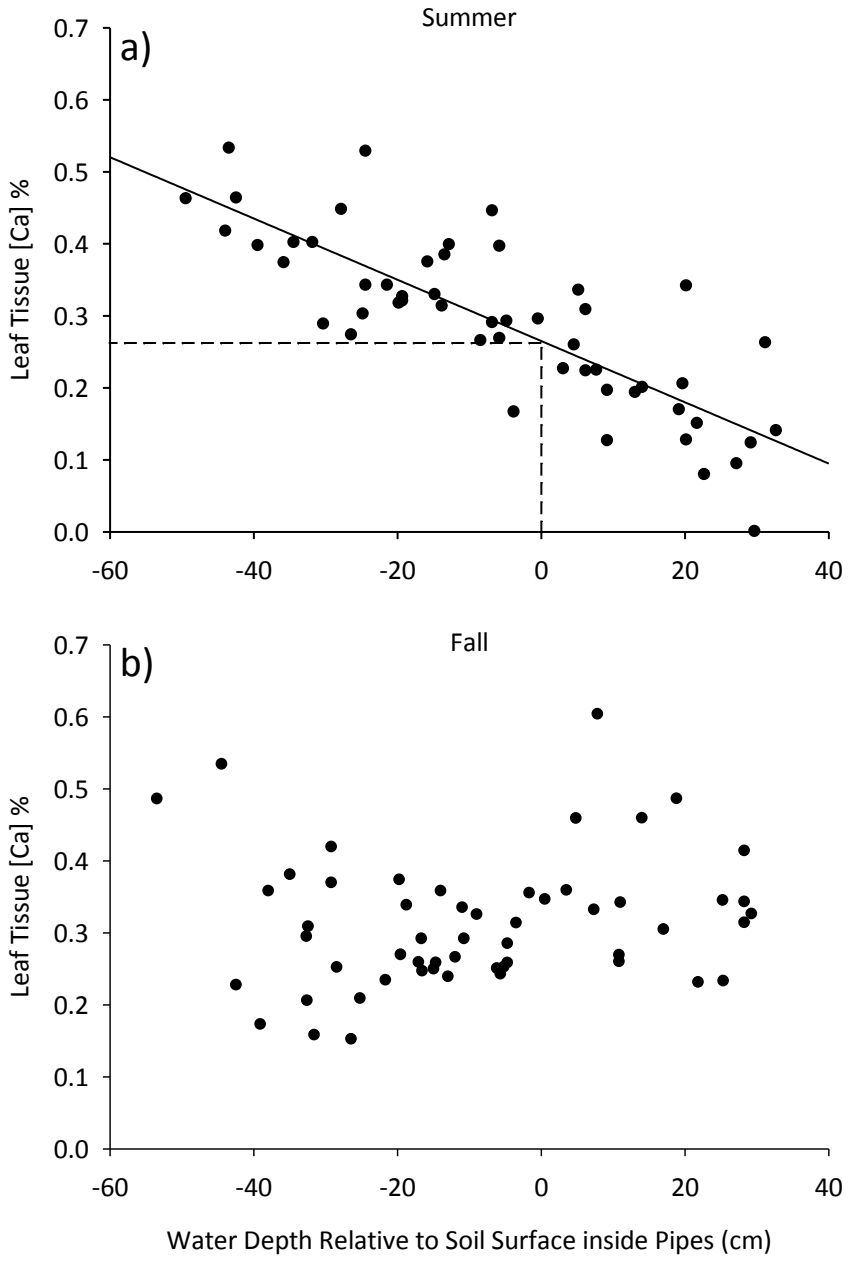


Figure 13: Ca concentrations in leaf tissue of *Spartina patens* plants grown with varying levels of flooding in the field and harvested in (a) summer and (b) fall. Water depth is an average depth of flooding above the soil surface, calculated for each pipe with data from water level recorders at the nearest CRMS station. The dashed line in (a) indicates that a linear regression predicts that the leaf tissue of *S. patens* growing in soil that is flooded to the soil surface will have 0.26 % Ca.

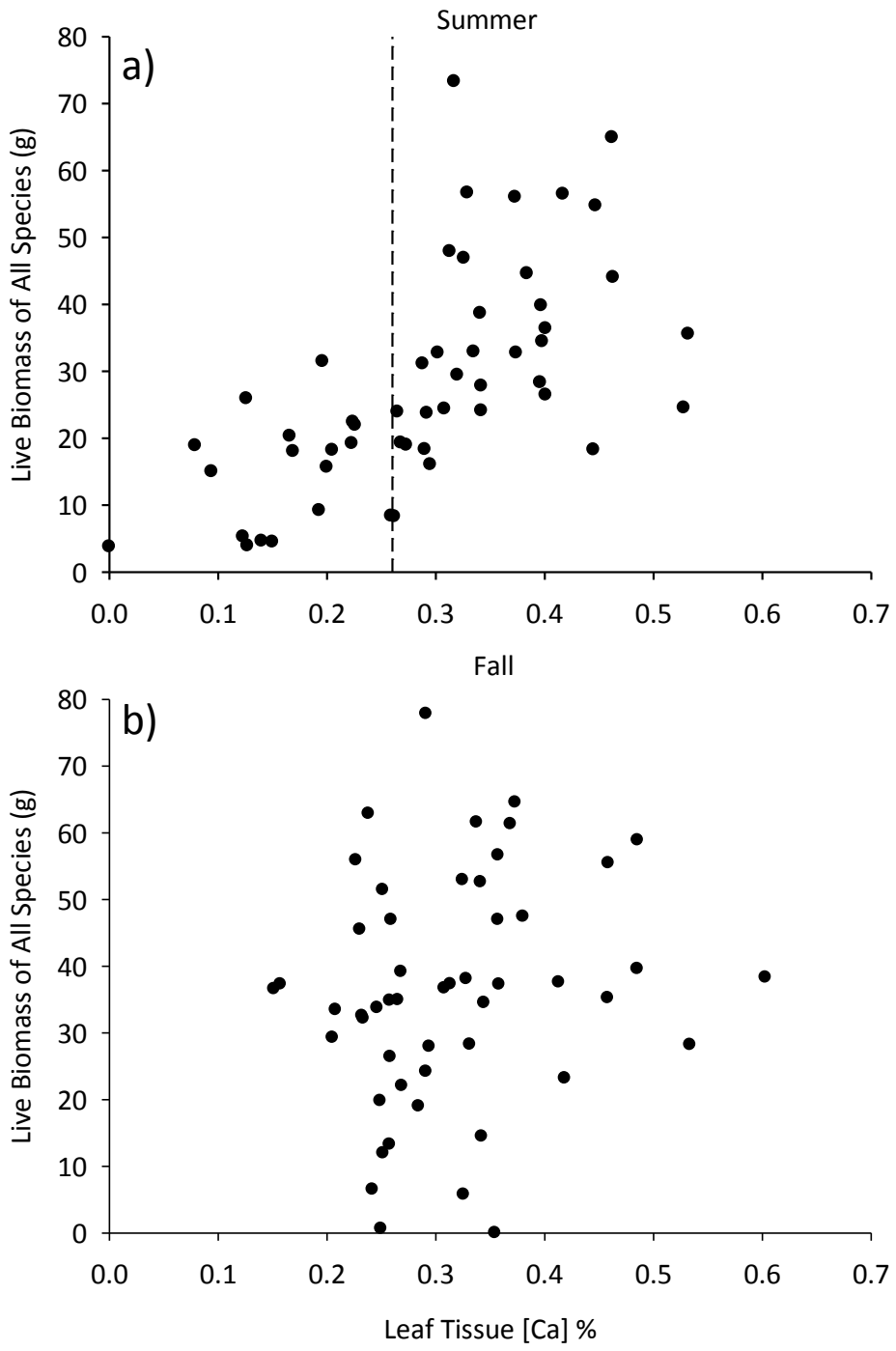


Figure 14: Live biomass (g) for plants grown at varying levels of flooding stress in the field, relative to Ca concentrations (%) in *Spartina patens* leaf tissue, harvested in (a) summer and (b) fall. The dashed line in (a) indicates the critical value predicted by the linear model shown in figure 14(a).

In this study, *S. patens* was more productive when soil elevation was higher, resulting in less flooding stress. These results contrast with a previous study, which showed that in a South Carolina marsh, increased flooding stimulated production of *S. alterniflora* to an optimum point (Morris et al. 2002). I observed no evidence of an optimal elevation for *S. patens* productivity in my study. Differences between my study and that conducted on the Atlantic coast may result from differences in tidal amplitude, marsh elevation relative to mean high tide, and the development of hypersaline conditions in South Carolina marshes. Tidal amplitudes on the coast of South Carolina are approximately 4-5 times those in Louisiana. The elevation of marshes in Louisiana is at mean high tide (Nyman et al. 2009), whereas the marsh platform in Atlantic coast marshes is below daily mean high tide and is superoptimal for marsh production (i.e. increased flooding stimulates production of *S. alterniflora*; Morris et al. 2002). Another possible reason for contrasts between these two studies is that elevation of the marsh platform in these regions appears to be controlled by different mechanisms in these locations. Elevation on the Atlantic coast is determined by mineral sedimentation (Morris et al. 2002). Elevation in Louisiana's coastal marshes is controlled by vegetative growth (Nyman et al. 2006).

There are several reasons why biomass was expected to be similar to original plants with similar flooding levels. Original plants were installed after the seasonal peak in biomass production, which for *S. patens* is in July (Ewing et al. 1997), so the majority of time they spent in pipes prior to the installation of replacement plants was during the dormant season. Because of this, original plants grew slowly and accumulated little biomass between the time of planting and following spring when plants that had died were replaced. Plant death in less flooded rows was more likely to have been caused by transplant shock than effects of the water levels they experienced. If plant death was caused by flooding stress, I reasoned that replacement plants would also likely die prior to harvest because they would be unable to sustain the rapid growth that occurs in spring. As expected, replacement plants in row 6, which experienced the highest water levels relative to soil surfaces, generally died prior to summer harvest.

Most pots in this row were empty or contained standing dead plant material at the time of harvest. For these reasons, I decided it was more appropriate to include measurements of biomass for replacement plants in analyses than to include measurements on original plants (i.e. zeros). Biomass of replacement plants was similar to original plants that received similar amounts of flooding; thus replacing plants in early spring had little, if any, effect on results. If replacement plants did have lower biomass at the time of harvest, this would only make differences in biomass among water levels more difficult to detect. As a strong relationship between water level and biomass was detected, the apparent effect of replacing relatively few plants was small.

Relatively large elevation losses measured at the fall harvest only at the saline marsh organ at Rockefeller Refuge were probably the result of erosion from Hurricane Ike's storm surge. Such losses were likely an artifact of the marsh organ structure because I did not observe similar erosion in the adjacent marsh.

For *S. patens*, tissue analysis conducted in summer may better reflect deficiencies or toxicities that limit growth than tissue analysis conducted at the end of the growing season. Indicators of plant stress may be seasonal in their ability to identify plants with limited productivity (Ewing et al. 1997). In *S. patens*, most growth occurs by July with less production occurring afterwards (Ewing et al. 1997). Our fall sampling period may have been after growth had begun to slow; thus factors limiting growth then may have little influence on biomass production. In fall, flooding may have affected biomass production less than early season flooding conditions when the plants were actively growing. However, plants apparently had not completely senesced when our fall samples were taken, as it appears that leaf tissue chemistry was influenced by saline storm surge from Hurricane Ike. The higher correlation of Na in the leaf tissue with flooding level in the fall supports this interpretation.

Seasonal patterns in water level may have contributed to differences in Mn availability and uptake by *S. patens*. Mn concentrations in porewater rise quickly following flooding of soils and peak at approximately 25 days of flooding (McKee and McKelvin 1993). Higher water levels in fall than in summer may have increased Mn availability and thus uptake of Mn. This could account for the higher and more variable [Mn] in leaf tissue for fall samples.

[Mn] and [Ca] in the leaf tissue of *S. patens* harvested during the summer can be used to differentiate plants that were grown with average water levels above the soil surface from plants that were grown with average water levels below the soil surface. However, the relationship between [Mn] and live *S. patens* biomass was more indicative of a limiting factor relationship than the relationship between [Ca] and biomass (Figures 6 and 8). In a typical plot of growth responses to changes in nutrients, growth would be expected to increase up to a point when the concentration of the nutrient reached an adequate level. Beyond that point, growth would not longer increase and would remain stable unless toxicity developed (Epstein and Bloom 2005). These curves are typically developed in controlled greenhouse situations, however. In our experiment salinity and nutrients were allowed to vary with environmental conditions; thus when flooding was no longer limiting, biomass production was controlled by these other factors. Where productivity was not limited by flooding, variation in growth would be expected because salinity and nutrient availability vary among our sites. Production in plants with leaf tissue [Mn] > 223 ppm is limited by something other than flooding, such as high salinity or low N availability, as indicated by the wider variation in productivity for these plants.

Several studies have shown that in greenhouse conditions, wetland plants exhibit increased [Mn] in above-ground tissue when stressed by flooding (e.g., Pierce et al. 2009, Bandyopadhyay 1993). A few studies have shown the opposite, however, for plants grown in flooded soils that are subsequently drained. For example, the Mn content of common carpet grass (*Axonopus affinis*) and centipedegrass (*Ermochloa ophuiroides*) was also higher in plants that were drained following flooded

conditions than in plants that were flooded but not drained (Bush et al. 1999). Another study (Lissner et al. 2003) found that *Cladium jamaicense* had higher [Mn] at Eh = +600 mV than at either +150 or -150 mV, where P was not limiting. The authors suggest that the $Mn^{2+}$ with which they amended their experimental soils was not oxidized following draining of the soil.

In this experiment it is more likely, however, that $Mn^{2+}$ availability was primarily controlled by pH or organic matter content of the soil rather than redox potential. Because of this, redox potential may be somewhat irrelevant to the availability of Mn in drained wetland soils (Gotoh and Patrick 1972). At low pH, as often occurs in drained wetland soils, such as the the local soil I used, most of the Mn in soils is expected to be soluble $Mn^{2+}$, which is more easily taken up by plants, rather than insoluble $Mn^{4+}$. Such conditions would not be likely to develop in commonly used experimental soils that are a mix of clay and sand. Craft et al. (1991) showed that [Mn] in porewater of created marshes was approximately 26 times that of porewater of natural marshes in North Carolina. This supports the suggestion that Mn was more available in the most drained pipes of the marsh organs than in the most flooded pipes because created marshes in this study were made from previously flooded materials. Created marshes in the study by Craft et al. also had significantly lower porewater pH than natural marshes. It is possible that $Mn^{2+}$ was available to plants in all of our flooding treatments as $Mn^{2+}$ can be available throughout a wide range of Eh because it can make complexes with organic matter (Reddy and DeLaune 2008, p.425).

Without direct measurements of soil pH, it is not possible to verify that changes in pH rather than redox potential were responsible for the pattern of Mn uptake by *S. patens* observed in this study. Future studies should employ pH probes directly on the soil where limited porewater is available, rather than attempting to measure porewater pH. In future studies, soil analyses should be conducted to characterize the soil material used to fill the pipes. Measuring pH of the soil in each pipe would verify chemical changes that are suspected to have occurred in soils and conducting soil analyses could account for any differences in uptake among plants in differed marsh organs.

A second reason that further studies should conduct soil analyses is that they would have been useful in accounting for differences in [Mn] of leaf tissue among the marsh organs. Although [Mn] in leaf tissue was similar among plants grown in different marsh organs, it is possible that Mn content of the soil used to fill the pipes differed among the marsh organs. Regional differences in Mn content of soil have not been reported for Louisiana's marshes. One study of the effects of inundation by Mississippi River water showed that marshes that receive freshwater and sediment from the Mississippi River had significantly higher Mn in sediments than those that were away from direct river influence (DeLaune et al. 2003). This may suggest that the soils used to fill marsh organs at Marsh Island may have had higher Mn content than the soils used at Rockefeller Refuge.

[Mn] in leaf tissue may have been influenced by poor root growth in the most flooded pipes. Wetland plants are adapted to flooded conditions, but extended flooding can disrupt physiological functioning and nutrition (DeLaune et al. 1998). Lack of oxygen in the soil may reduce root growth and the ability of the roots to take up nutrients. The most flooded pipes contained entirely anoxic soil and less flooded pipes contained at least some anoxic soil, as evidenced by dark colored soil and iron plaques on roots. Although *S. patens* roots are able to tolerate low Eh conditions once established, they are less able to grow into reduced soils than oxidized ones (Pezeshki et al. 1991). Even established roots that have developed extensive arenchyma tissue cannot survive in anoxic soils indefinitely (Pezeshki 2001). Soon after soils become hypoxic, metabolism in plant roots switches to fermentation (Drew 1997). Even if $Mn^{2+}$ was more available in the most flooded pipes, the roots would be unable to take it up because anaerobic respiration produces less energy than aerobic respiration so plants lose the ability to absorb nutrients or translocate them to stems or leaves (Epstein and Bloom 2005). Plants in the tallest pipes likely developed larger root systems because the taller pipes contained a greater volume of oxidized soil and thus a greater volume of suitable rooting conditions than in shorter, more flooded

pipes. This also could have contributed to the increased [Mn] in plants growing at higher elevations. Studies of Mn uptake are needed to understand the mechanisms responsible for these observations.

Like [Mn], [Ca] in the leaf tissue of *Cladium jamaicense* has also been shown to increase with increasing Eh (Lissner et al. 2003). Although [Ca] was better able to predict flooding levels than [Mn], it may less useful as an indicator of flooding stress because the variation in the relationship between [Ca] and total live biomass was relatively constant across the range of [Ca] I observed. If [Ca] were used as an indicator of flooding stress, the constant variation in the relationship between [Ca] and biomass would suggest that production in all plants was limited by flooding stress. This was not the case in our study, as the variation in the relationship between average flooding depth and biomass becomes more variable when plants experience less flooding.

A second reason [Ca] may not be an ideal indicator of flooding stress is that it may be influenced by other factors that are not directly related to flooding. [Ca] in leaf tissue may also be controlled by N-availability (Jones 1998) and/or salinity (Epstein and Bloom 2005). Because [Ca] may be influenced by N-availability, salinity, and flooding level, [Ca] may be a better indicator of overall production than of flooding stress alone. More productive plants with higher rates of transpiration also have higher [Ca] in their leaf tissue (Jones 1998). This also suggests that low [Ca] could indicate general limitation of production. [Ca] has been used to indicate the overall degree of limitation by environmental factors in diagnosis and recommendation integrated systems (DRIS; Bailey et al. 1997). More research may be necessary to identify interactions between these factors and flooding.

I found that [Ca] < 0.26% and [Mn] < 223 ppm in leaf tissue were useful for identifying limitation in general and limitation by flooding stress respectively in *S. patens* for plant tissue harvested during the summer, but could not be used to identify flooding stress in plants harvested during the fall. The seasonal nature of these results suggests that making comparisons among studies relating to the tissue

of plants harvested at different times during the growing season may not be possible. These cutoff values correctly identified the growing conditions of 94 and 87% of plants sampled, respectively. I therefore recommend [Mn] or [Mn] in combination with [Ca] rather than [Ca] alone to identify flooding stress in *S. patens*. Ideally, these values will be verified with data that were not included in the regression models. I plan to verify these values by analyzing tissue samples from an ongoing field experiment. It is uncertain whether the results of this study could be applied to marshes outside the Gulf of Mexico. Differences between this study and previous research conducted in Atlantic Coast marshes suggest that the results of this study may only be applicable to marshes with small tidal amplitude and low mineral soil. Further studies must be done to determine whether productivity in other systems is controlled by flooding stress as it is in Louisiana's coastal marshes.

Results of this study may be more applicable to newly created marshes than mature marshes. Relatively small plugs of plants, which were similar to plugs used in restoration projects, were used rather than intact marsh sods so root disturbance may have affected plant growth. Also, soil and porewater chemistry are likely to be more similar to newly created marshes because marsh organ pipes were filled with local soil that subsequently drained.

If results of this study are applied to mature marshes, the large elevation losses in response to drainage that I observed suggest that managers and restoration professionals should limit the use of drainage to improve marsh productivity. Draining marsh soils increased productivity of *S. patens* in our study but it also caused increased loss of soil elevation. In extremely drained marsh soils *S. patens* was the most productive, but the plants in the most drained soils were unable to keep up with the rates of soil elevation loss. I hypothesize that short, shallow drawdowns early it the growing season may have a positive effect on production without causing the major losses of soil elevation that occurred in our most drained pipes. Further research is necessary to test this hypothesis.

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## CHAPTER 4.
## A COMPARISON OF THE ELEMENTAL COMPOSITION OF LEAF TISSUE OF *SPARTINA PATENS* AND *SPARTINA ALTERNIFLORA* IN LOUISIANA'S COASTAL MARSHES

### Introduction

Elemental concentrations in plant tissue have been used to diagnose limiting conditions in agricultural crops. For example, concentrations of N in the leaf tissue of rice below 2.8-3.6% indicate that fertilization may improve production (Brandon and Wells 1986) and [Mn] > 4000 ppm is toxic to rice (Adriano 1986). Information such as this on the chemical composition of wetland plant tissue could be used to improve or evaluate restoration and management plans for coastal marshes, as it is used to improve management of agricultural crops to increase plant productivity. I focus on *Spartina alterniflora* Loisel. and *Spartina patens* (Ait.) Muhl because they make up 13% and 25% of the vegetation in coastal Louisiana, respectively (Chabreck 1970). These species also occur throughout the Gulf of Mexico and Atlantic coasts of the United States so they are commonly used in restoration and management plans for coastal marshes. Understanding how the mineral requirements of these two common plants differ could improve how they are used in such plans. This information could also help formulate plans that would facilitate their removal in places where they have become invasive, such as the San Francisco Bay area in California.

Some guidelines for diagnosing nutrient limitation and salinity stress in wetland plants have been developed. Mass N:P ratios > 16 in plant tissue have been used to diagnose P-limitation (e.g., Koerselman and Meuleman 1996). C:N and [Na] have been used to diagnose N-limitation and salinity stress, respectively, in *Spartina patens* (Tobias et al. 2010). These guidelines may not apply to all wetland species, however, because even when different species experience the same nutrient availability, their tissue chemistry can vary widely (McJannet et al. 1995). For example, in several *Carex spp.* ranges of [N] and [P] in leaf tissue did not even overlap (Güsewell and Koerselmann 2002). If *Spartina* species have differing elemental concentrations in their leaf tissue under similar growing

conditions, basing management decisions on interspecific comparisons would be inaccurate, particularly if their nutrient uptake mechanisms differ in their susceptibility to salinity, anoxia, or sulfides. *S. alterniflora* and *S. patens* may have different nutrient requirements, and thus different concentrations of some elements in their leaf tissue, because they are adapted to different environments. *S. alterniflora* is found in more flooded (Bertness 1991) and more saline marshes than *S. patens* (Visser et al. 1998, 2000) so it is reasonable to suspect that these species might have different mechanisms for tolerating stressful conditions and therefore may have different nutrient requirements. In fact, *S. alterniflora* is more salinity tolerant and shows higher ion selectivity than *S. patens* (Hester et al. 2001).

Several potential indicators have proven useful for identifying limited production. Changes in above-ground biomass can be used to identify sites that differ in productivity (e.g., Burdick et al. 1989, Ewing et al. 1997), as can shoot elongation (Ewing et al. 1997). Salinity stress and nutrient starvation can be identified with measurements of leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration (Ewing et al. 1995, 1997). Many of these require specialized equipment, are time-consuming, or are expensive, which makes them difficult to use over large spatial or temporal scales. Tests to determine elemental composition of plant tissue and soil, however, are inexpensive and commercially available and collecting samples for such tests requires little time.

Elemental concentrations in plant tissue may be a more effective means of identifying factors that limit plant production than elements available in the soil because elemental concentrations in plant tissue reflect how plants react to all environmental factors simultaneously. Some conditions, such as flooding or high salinity levels, can reduce the ability of plants to take up nutrients. When this happens, although the necessary elements may be available in the soil, nutrient uptake and plant productivity are still limited. For example, sulfide toxicity resulting from soil anoxia limits the ability of plants to take up ammonium from soil porewater; thus productivity in plants that are stressed by flooding may be N-limited although there are sufficient levels of ammonium in soil (Mendelssohn and Morris 2000). Even

when sulfide concentrations are low soil testing may not accurately reflect the amount of nutrients available to flood-stressed plants because fine roots often die as a result of oxygen deficiency (Larcher 2003).

The purpose of this paper is to quantify differences in leaf tissue chemistry between *S. alterniflora* and *S. patens* by addressing the following questions: (1) Does the leaf tissue chemistry differ between these species, and if it does, which elements are different? (2) How does porewater chemistry affect the leaf tissue chemistry of each species? (3) Are there seasonal patterns leaf tissue chemistry? I focus on C:N, [Na], [Mn], and [Ca] because these elements have been previously used to identify causes of limited production in *S. patens* (Tobias et al. 2009, Tobias et al. in review). I also include [K] and Na:K because maintaining a high K:Na is an essential factor for salinity tolerance in halophytes (Maathuis and Amtmann 1999). N, K, and Ca have also been identified as being important indicators of limitation in agricultural crops and have been included in diagnosis and recommendation integrated systems (e.g., Walworth and Sumner 1987). I report concentrations of other elements as well, however, because they may be of interest for purposes other than mine. I examine seasonal patterns because some nutrient concentrations in plant tissue and other indicators of limiting factors change during the growing season as a result of changing requirements for growth (Ewing et al. 1997). Thus, the seasonal timing of comparisons may change how elemental composition should be interpreted.

## Methods

I collected leaf tissue samples from *S. alterniflora* and *S. patens* growing together in intermediate to saline marshes across the coast of Louisiana. Samples were taken at Cameron Prairie National Wildlife Refuge, Rockefeller Wildlife Refuge, Marsh Island Wildlife Refuge, and Fourleague Bay. At each location, I sampled at two sites: one fresher and one more saline. Following Penfound and Hathaway's (1938) classification system for coastal marshes, fresher sites were chosen to include species that indicated intermediate marsh such as *Sagittaria lancifolia* and *Scirpus olneyi* and more

saline sites were chosen to include species that indicate brackish marsh such as *Spartina alterniflora*. Samples were taken seasonally during the growing season (spring, summer, and fall) from May 2007 to November 2008.

I collected porewater samples at 10 cm below the marsh surface at each point with a syringe connected to a piece of rigid tubing. The tubing was sealed at the end and holes were drilled along the sides to approximately 2 cm from the end. I pre-filtered porewater with a piece of nylon stocking fitted over the end of the tubing to exclude large soil particles. I measured salinity, conductivity, and pH of porewater with a handheld meter (YSI Model 63). For nutrient analysis, I filtered water samples with 0.45 µm nylon syringe filters (Whatman) to remove sediment. These water samples were transported to the lab on ice and kept cold until nutrient analyses could be performed. I determined the concentrations of ammonium-N using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et a. 1998).

Leaf tissue was collected from the top 10-15 cm of plants growing away from the edge of a bayou or lake. I defined an edge as being the area adjacent to a water body where vegetation was visibly different from the adjacent marsh (usually 3-5 meters). Care was taken to harvest tissue samples from plants growing in similar soil conditions at each site. I placed tissue samples in zip-top bags and stored them on ice until I returned to the lab. Samples were rinsed to remove soil and salt that may have been present on leaf surfaces before drying them at 60°C to a constant weight. Tissue samples were then ground with a coffee grinder (Black and Decker Smartgrind) or Wiley Mill. The grinders were cleaned between samples with compressed air to remove particles. I submitted dried and ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL; Baton Rouge, LA)

to determine their elemental composition. C and N content was determined by dry combustion by CHN Analyzer. Concentrations of all other elements were determined by ICP analysis.

I tested for differences in the overall elemental composition of the leaf tissue of the two species collected on the same day, at the same site with a multivariate paired t-test. For this test, I only used data from sites where I collected both *S. alterniflora* and *S. patens*, resulting in 54 pairs of tissue samples. To perform this test in SAS, I used the MANOVA option in PROC GLM to test for differences between species, while treating each pair of samples as a block. I further explored differences between individual elemental concentrations using the ANOVA tests that are also produced by the code for the multivariate t-test. I used Pearson correlation coefficients (PROC CORR) to explore relationships between porewater chemistry and elemental concentrations in leaf tissue. I used a p-value of 0.05 as the cut-off for significance in all statistical tests.

Seasonal comparisons of the concentrations of Na, Mn, and K as well as ratios of C:N and Na:K were made *a priori*, and were thus made independently of the results of statistical tests. I considered the effects of each discrete sampling period separately, rather than pooling data by season over both years because weather patterns were extremely different between the two years of this study. Spring flooding on the Mississippi River was extremely high in spring 2008 and in fall 2008, storm surge from Hurricane Ike inundated all of our study sites with saline water to a depth of approximately 2.5 m.

**Results**

Overall, the leaf tissue chemistry of *S. alterniflora* and *S. patens* collected on the same day, at the same site was different ($F_{15,39} = 46.58$, $p < 0.0001$). Concentrations of Ca, C, Mg, Mn, N, P, K, and Zn differed by species (Table 5). Molar C:N ratio was also different between species. Ca, Mg, Mn, N, P, K, Zn were significantly higher in *S. alterniflora* than in *S. patens* (Table 6). [C] and C:N ratio were significantly higher in *S. patens* than *S. alterniflora*. Concentrations of Al, B, Cu, Fe, Na, and S did not differ between species. Molar N:P and Na:K also did not vary by species.

Higher concentrations of ammonium-N and higher salinity in porewater were associated with lower C:N in both species (Tables 7 and 8). For *S. patens*, but not *S. alterniflora*, higher salinity was associated with higher [Na] and [Mn] was negatively associated with porewater pH. Porewater salinity was negatively correlated with [Ca] and C:N in *S. patens* (Table 8). The pH of porewater was negatively correlated with [Mn] in *S. patens*. Porewater ammonium-N was negatively correlated with C:N in both species and was positively correlated with [Na] in *S. patens*. Orthophosphate was positively correlated with C:N ratio in *S. alterniflora* and negatively correlated with [Ca] in *S. patens* (Tables 7 and 8). Ammonium-N in porewater was weakly associated with porewater salinity and orthophosphate in porewater was weakly associated with pH (Table 9). Porewater salinity was lowest during summer sampling periods and higher during spring and fall sampling (Table 10). On average, pH was generally neutral to slightly acidic. Ammonium-N was substantially higher in summer and fall 2008 than in previous sampling periods. Average porewater ammonium-N for spring 2007 through spring 2008 ranged from approximately 0.8 - 2.7 mg/L. There were no apparent seasonal patterns in porewater concentrations of orthophosphate and concentrations of orthophosphate remained > 1 mg/L throughout the study.

Average C:N ratios in *S. patens* were greater than C:N ratios in *S. alterniflora* for every sampling period (Figure 15). C:N ratios were lower in both species in spring and fall 2008 than spring and fall 2007, respectively. [Na] in *S. alterniflora* was similar to [Na] in *S. patens* in 2007 but not in 2008 (Figure 16). [Na] in both species generally followed the same seasonal pattern as porewater salinity and was substantially higher in fall 2008 than in other seasons. In most seasons, average Na:K was higher in *S. patens* than in *S. alterniflora* (Figure 17). Ratios of Na:K increased in *S. alterniflora* and decreased in *S. patens* throughout both growing seasons, causing their Na:K ratios to converge in the fall. Ratios of Na:K were most similar in fall 2008 (Figure 17). Patterns in [K] mirrored patterns in Na:K ratios. The ratio of Na:K was higher in *S. alterniflora* and generally decreased in *S. alterniflora* throughout the growing season, except in fall 2008. The ratio of Na:K increased in *S. patens* throughout both growing

seasons and the Na:K ratios of the two species converged in both fall sampling periods (Figure 18). [Na] was correlated with [K] in *S. patens* ($r = 0.552$, $p < 0.0001$) but not in *S. alterniflora* ($r = 0.086$, $p = 0.5384$). [Na] and [K] appear to be most related in *S. patens* where [Na] in leaf tissue was high (Figure 19). There appears to be a weak seasonal effect on [Mn] in *S. alterniflora*, but not on *S. patens* (Figure 20). [Mn] was always higher in *S. alterniflora* than in *S. patens*. [Mn] in *S. patens* was consistently below 140 ppm, while [Mn] in *S. alterniflora* was rarely that low. [Ca] was consistently higher in *S. alterniflora* than in *S. patens* (Figure 21). In both years, the difference in [Ca] between the two species was smaller the spring and became larger throughout the growing season. While [Ca] consistently increased in *S. alterniflora* throughout the growing season, [Ca] decreased substantially in the fall of 2007 and in the summer of 2008, relative to their respective previous seasons.

**Discussion**

[Na] was similar among paired samples of *S. patens* and *S. alterniflora*. This observation suggests that Na uptake in these species respond similarly to changes in porewater salinity at the range of salinity I observed (0.5-19.2 ppt). [Na] in leaf tissue of *S. patens* increases with increasing salinity, and an average [Na] of 1.1% suggests that *S. patens* was limited by salinity stress (Tobias et al. 2010). For plants that are salinity stressed, indicators of salinity stress such as [Na] and [K] should correlate with porewater salinity. In contrast, although increased salinity reduces *S. alterniflora* productivity, [Na] in leaf tissue peaks for plants grown at 15-20 ppt salinity and at flooding levels similar to those experienced by plants at our sampling sites (Brown et al. 2006). Another study showed that mean [Na] in leaf tissue of *S. alterniflora* for plants growing in salinities of 10, 20, and 30 ppt was not different and [Na] was only slightly higher for plants growing in a salinity of 40 ppt (Bradley and Morris 1991). These studies support the interpretation that *S. alterniflora* was not likely to be stressed by high salinity at my study sites.

Table 5: Results of ANOVAs indicating differences in individual elemental concentrations in Spartina *alterniflora* and *Spartina patens* leaf tissue. ANOVAs were performed using PROC GLM (SAS). "Species" effects indicate differences between *S. alterniflora* and *S. patens*. "Block" effects indicate differences among pairs of samples. All ratios are molar; units for elemental concentrations are given.

| | Model | | Species | | Block | |
|---|---|---|---|---|---|---|
| Element | $F_{54,53}$ | p | $F_{1,53}$ | p | $F_{53,53}$ | p |
| Al (ppm) | 1.36 | 0.1349 | . | . | . | . |
| B (ppm) | 1.08 | 0.3912 | . | . | . | . |
| Ca (%) | 1.93 | 0.0091 | 54.27 | <0.0001 | 0.94 | 0.5923 |
| C (%) | 1.70 | 0.027 | 17.45 | 0.0001 | 1.41 | 0.1084 |
| Cu (ppm) | 2.91 | <0.0001 | 0.09 | 0.7693 | 2.96 | <0.0001 |
| Fe (ppm) | 1.62 | 0.0407 | 0.22 | 0.6421 | 1.65 | 0.0364 |
| Mg (%) | 2.66 | 0.0002 | 61.86 | <0.0001 | 1.55 | 0.0579 |
| Mn (ppm) | 2.24 | 0.0019 | 35.67 | <0.0001 | 1.61 | 0.0427 |
| N (%) | 7.24 | <0.0001 | 60.83 | <0.0001 | 6.23 | <0.0001 |
| P (%) | 3.33 | <0.0001 | 55.01 | <0.0001 | 2.36 | 0.0011 |
| K (%) | 2.13 | 0.0032 | 7.06 | 0.0104 | 2.04 | 0.0052 |
| Na (ppm) | 3.13 | <0.0001 | 3.98 | 0.0511 | 3.30 | <0.0001 |
| S (%) | 2.65 | 0.0003 | 2.76 | 0.1028 | 2.65 | 0.0003 |
| Zn (ppm) | 4.50 | <0.0001 | 111.12 | <0.0001 | 2.49 | 0.0006 |
| C:N | 4.89 | <0.0001 | 88.68 | <0.0001 | 3.31 | <0.0001 |
| N:P | 2.31 | 0.0013 | 0.02 | 0.8910 | 2.35 | 0.0011 |
| Na:K | 1.37 | 0.1288 | . | . | . | . |

Table 6: Least squares means of elemental concentrations in the leaf tissue of *Spartina alterniflora* and *Spartina patens* calculated with PROC GLM (SAS). All ratios are molar; units for elemental concentrations are given.

| | *Spartina alterniflora* | | *Spartina patens* | |
|---|---|---|---|---|
| | Mean | Std. Error | Mean | Std. Error |
| Al (ppm) | 67.13 | 12.75 | 113.33 | 12.75 |
| B (ppm) | 5.20 | 0.61 | 6.71 | 0.61 |
| Ca (%) | 0.42 | 0.02 | 0.22 | 0.02 |
| C (%) | 44.33 | 0.18 | 45.39 | 0.18 |
| Cu (ppm) | 1.62 | 0.19 | 1.55 | 0.19 |
| Fe (ppm) | 118.87 | 10.80 | 111.74 | 10.80 |
| Mg (%) | 0.31 | 0.01 | 0.19 | 0.01 |
| Mn (ppm) | 172.35 | 9.89 | 88.85 | 9.89 |
| N (%) | 1.42 | 0.03 | 1.07 | 0.03 |
| P (%) | 0.13 | 0.00 | 0.09 | 0.00 |
| K (%) | 0.80 | 0.03 | 0.67 | 0.03 |
| Na (%) | 1.05 | 0.04 | 1.15 | 0.04 |
| S (%) | 0.48 | 0.05 | 0.59 | 0.05 |
| Zn (ppm) | 9.21 | 0.31 | 4.64 | 0.31 |
| C:N | 38.72 | 1.41 | 57.46 | 1.41 |
| N:P | 25.71 | 0.70 | 25.58 | 0.70 |
| Na:K | 2.48 | 0.16 | 3.22 | 0.16 |

Table 7: Pearson correlation coefficients (r) and p-values (p), describing the relationships between porewater chemistry and concentrations of various elements in leaf tissue of *Spartina alterniflora*. Porewater was collected at 10 cm below the marsh surface. Leaf tissue was collected from leaves originating in the top 15 cm of the plant's stem. Correlations were calculated with PROC CORR (SAS).

| | | Al | B | Ca | C | Cu | Fe | Mg | Mn | Mo |
|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (ppt) | r | -0.15 | 0.12 | 0.05 | 0.09 | 0.24 | -0.40 | 0.16 | -0.37 | 0.11 |
| | p | 0.3181 | 0.4137 | 0.7511 | 0.5586 | 0.2418 | 0.0053 | 0.2739 | 0.011 | 0.5918 |
| pH | r | -0.16 | 0.00 | 0.27 | -0.18 | -0.31 | -0.29 | 0.36 | -0.14 | -0.29 |
| | p | 0.3089 | 0.9847 | 0.0793 | 0.2501 | 0.1274 | 0.0536 | 0.0175 | 0.3502 | 0.1552 |
| Orthophosphate (ppm) | r | -0.15 | -0.10 | 0.23 | 0.17 | -0.45 | -0.21 | 0.22 | -0.06 | -0.40 |
| | p | 0.3232 | 0.4904 | 0.1128 | 0.2512 | 0.0232 | 0.1591 | 0.1384 | 0.6933 | 0.045 |
| Ammonium-N (ppm) | r | -0.16 | 0.06 | 0.26 | 0.19 | -0.28 | -0.27 | 0.07 | -0.12 | -0.15 |
| | p | 0.2873 | 0.6852 | 0.0711 | 0.1979 | 0.1711 | 0.0686 | 0.6388 | 0.4003 | 0.4859 |

| | | Ni | N | P | K | Na | S | Zn | C:N | N:P | Na:K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (ppt) | r | 0.04 | 0.27 | -0.16 | 0.02 | 0.16 | 0.32 | 0.04 | -0.13 | 0.46 | 0.12 |
| | p | 0.8583 | 0.0713 | 0.2807 | 0.9153 | 0.2778 | 0.0268 | 0.8127 | 0.3995 | 0.0013 | 0.4096 |
| pH | r | 0.10 | -0.29 | -0.48 | -0.29 | 0.13 | 0.00 | -0.40 | 0.42 | 0.10 | 0.56 |
| | p | 0.6506 | 0.0581 | 0.0011 | 0.0534 | 0.3971 | 0.9924 | 0.0071 | 0.0049 | 0.5105 | <0.0001 |
| Orthophosphate (ppm) | r | 0.27 | -0.26 | -0.24 | -0.29 | -0.15 | 0.09 | -0.37 | 0.38 | -0.06 | 0.08 |
| | p | 0.1974 | 0.0706 | 0.0947 | 0.0477 | 0.3192 | 0.5415 | 0.0107 | 0.0082 | 0.6798 | 0.5843 |
| Ammonium-N (ppm) | r | 0.03 | 0.39 | 0.02 | 0.23 | 0.31 | 0.64 | -0.09 | -0.34 | 0.42 | -0.03 |
| | p | 0.8907 | 0.0064 | 0.9013 | 0.1118 | 0.0311 | <.0001 | 0.5646 | 0.0168 | 0.0029 | 0.5843 |

Table 8: Pearson correlation coefficients (r) and p-values (p), describing the relationships between porewater chemistry and concentrations of various elements in leaf tissue of *Spartina patens*. Porewater was collected at 10 cm below the marsh surface. Leaf tissue was collected from leaves originating in the top 15 cm of the plant's stem. Correlations were calculated with PROC CORR (SAS).

| | | Al | B | Ca | C | Cu | Fe | Mg | Mn | Mo |
|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (ppt) | r | -0.07 | -0.12 | -0.54 | -0.34 | 0.19 | -0.07 | 0.24 | -0.13 | -0.09 |
| | p | 0.6194 | 0.4289 | <.0001 | 0.0211 | 0.2017 | 0.6568 | 0.1051 | 0.4008 | 0.5297 |
| pH | r | -0.27 | -0.29 | -0.27 | 0.08 | -0.01 | -0.25 | 0.16 | -0.34 | 0.12 |
| | p | 0.0811 | 0.0528 | 0.0804 | 0.5903 | 0.9701 | 0.1003 | 0.3058 | 0.0224 | 0.4199 |
| Orthophosphate (ppm) | r | 0.17 | -0.15 | -0.31 | 0.13 | -0.46 | 0.13 | 0.13 | -0.11 | -0.27 |
| | p | 0.24 | 0.3027 | 0.0315 | 0.3606 | 0.001 | 0.3963 | 0.3686 | 0.4607 | 0.0686 |
| Ammonium-N (ppm) | r | -0.12 | -0.03 | -0.14 | -0.55 | 0.02 | -0.12 | 0.41 | -0.03 | -0.63 |
| | p | 0.4114 | 0.8552 | 0.3273 | <.0001 | 0.8796 | 0.3988 | 0.0036 | 0.8582 | <.0001 |

| | | Ni | N | P | K | Na | S | Zn | C:N | N:P | Na:K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (ppt) | r | -0.13 | 0.39 | 0.13 | 0.50 | 0.41 | 0.48 | 0.32 | -0.33 | 0.53 | -0.23 |
| | p | 0.3868 | 0.0061 | 0.3713 | 0.0003 | 0.0046 | 0.0006 | 0.0264 | 0.0232 | 0.0001 | 0.1166 |
| pH | r | 0.07 | -0.11 | -0.20 | 0.03 | 0.12 | 0.12 | -0.25 | 0.17 | 0.04 | 0.04 |
| | p | 0.6308 | 0.4592 | 0.1903 | 0.8652 | 0.4449 | 0.4433 | 0.0994 | 0.2713 | 0.8041 | 0.7840 |
| Orthophosphate (ppm) | r | -0.25 | -0.05 | -0.02 | -0.03 | -0.03 | 0.11 | -0.15 | 0.14 | -0.04 | 0.00 |
| | p | 0.0921 | 0.7341 | 0.887 | 0.828 | 0.8363 | 0.4453 | 0.3031 | 0.3262 | 0.7707 | 0.9799 |
| Ammonium-N (ppm) | r | -0.75 | 0.61 | 0.36 | 0.74 | 0.65 | 0.77 | 0.46 | -0.50 | 0.53 | -0.15 |
| | p | <.0001 | <.0001 | 0.0112 | <.0001 | <.0001 | <.0001 | 0.001 | 0.0003 | 0.0001 | 0.2879 |

Table 9: Pearson correlation coefficients (r) and p-vaules (p) describing the relationships among porewater chemical values. Porewater was collected at 10 cm below the marsh surface. Correlation coefficients and p-values were estimated with PROC CORR (SAS).

| | | Salinity (ppt) | pH | Orthophosphate (ppm) |
|---|---|---|---|---|
| pH | r | 0.251 | | |
| | p | 0.100 | | |
| Orthophosphate (ppm) | r | 0.242 | 0.309 | |
| | p | 0.101 | 0.041 | |
| Ammonium-N (ppm) | r | 0.399 | 0.023 | 0.153 |
| | p | 0.006 | 0.881 | 0.299 |

Table 10: Summary of porewater chemistry by season. Porewater was collected at 10 cm below the marsh surface. Means and standard errors were calculated using PROC MEAS (SAS). N represents the number of samples taken.

| | | | Salinity (ppt) | | Ammonium-N (ppm) | | Orthophosphate (ppm) | | pH | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Season | N | Mean | Std. Error | Mean | Std. Error | Mean | Std. Error | Mean | Std. Error |
| 2007 | Spring | 5 | 13.88 | 2.82 | 0.77 | 0.15 | 5.48 | 2.19 | 6.37 | 0.27 |
| | Summer | 7 | 7.73 | 1.37 | 1.06 | 0.37 | 2.63 | 0.99 | 7.12 | 0.21 |
| | Fall | 11 | 15.13 | 1.49 | 2.66 | 0.62 | 5.41 | 1.15 | 8.89 | 1.07 |
| 2008 | Spring | 10 | 12.65 | 1.22 | 1.80 | 0.68 | 2.32 | 0.65 | 6.52 | 0.13 |
| | Summer | 12 | 11.89 | 1.54 | 7.32 | 2.40 | 6.55 | 1.48 | 6.74 | 0.07 |
| | Fall | 9 | 15.44 | 1.02 | 12.42 | 3.28 | 5.54 | 0.93 | 6.91 | 0.10 |

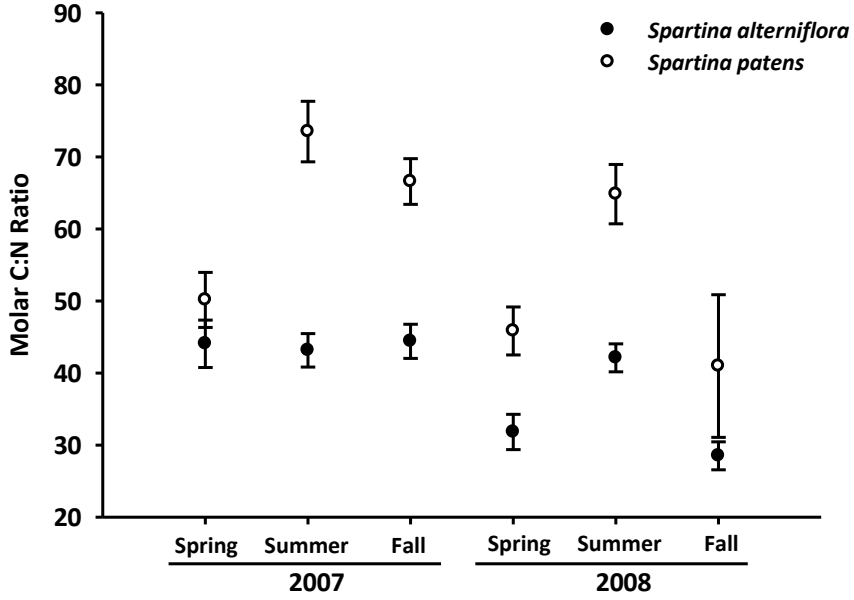


Figure 15: Molar C:N ratios (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

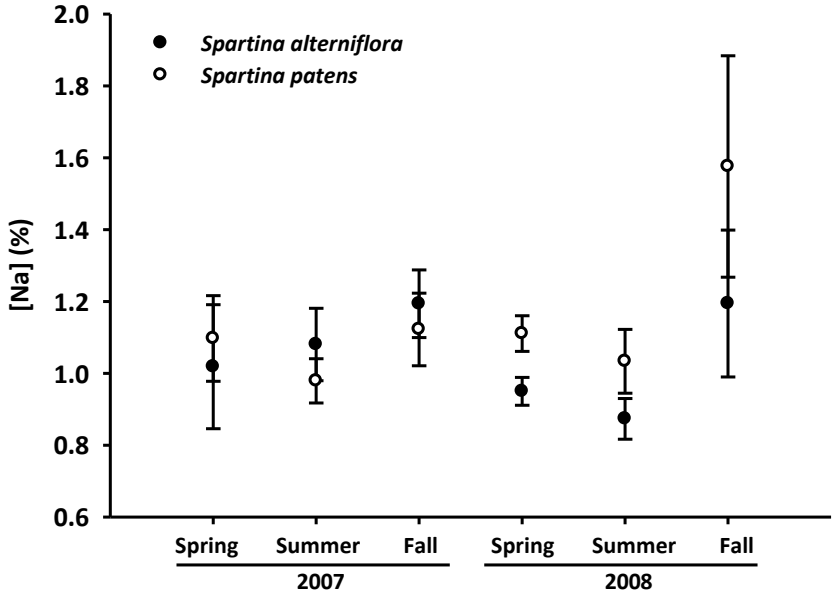


Figure 16: Na concentrations (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

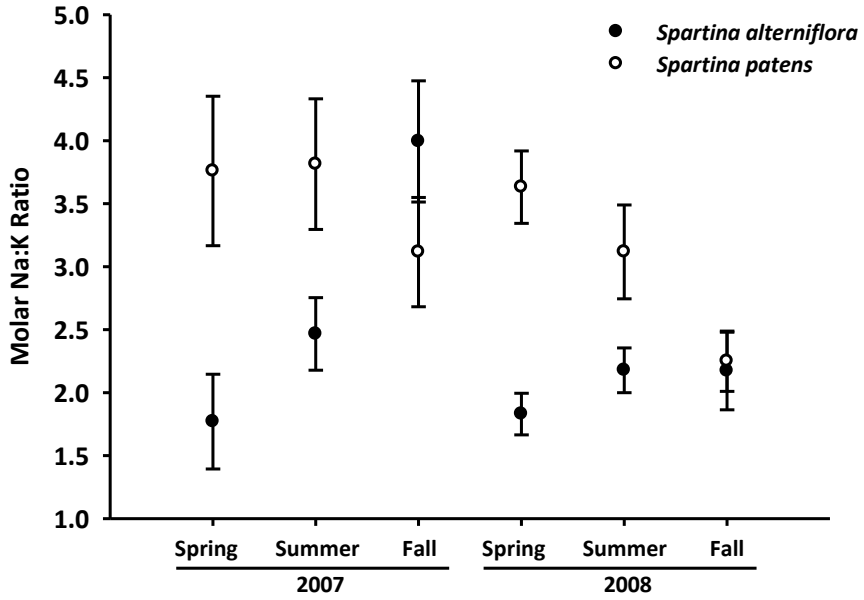


Figure 17: Molar Na:K ratios (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

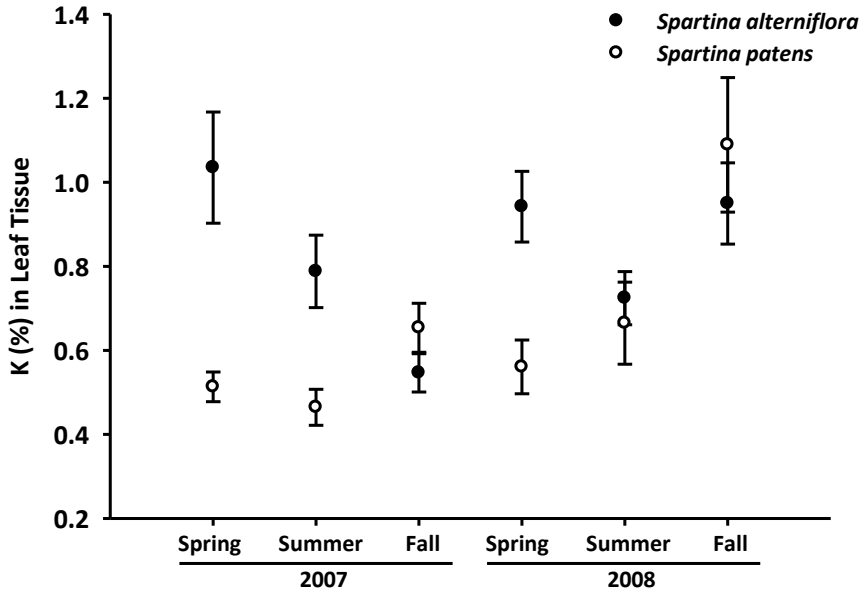


Figure 18: Concentrations of K (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

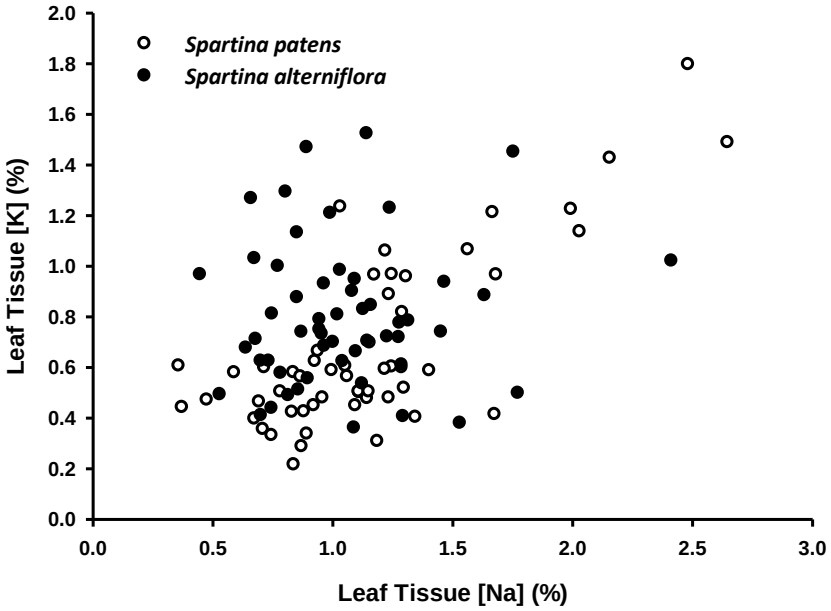


Figure 19: Leaf tissue [Na] and [K] of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

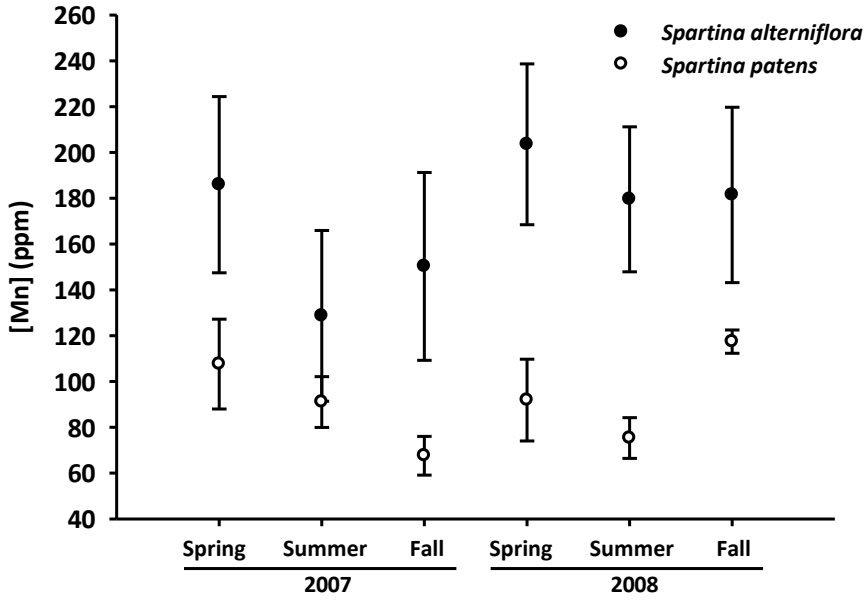


Figure 20: Mn concentrations (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

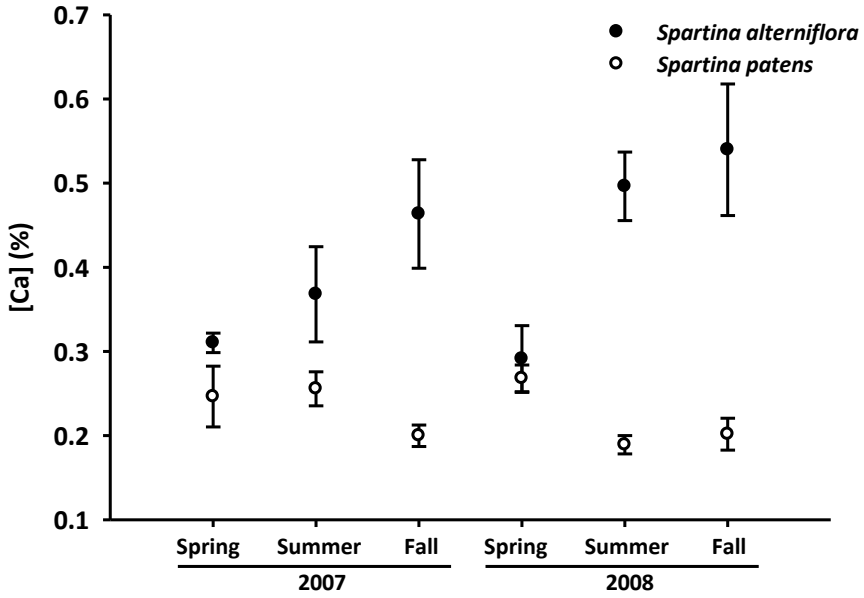


Figure 21: Ca concentrations (±1 SE) in leaf tissue of *Spartina alterniflora* and *Spartina patens* growing together in saline and intermediate marshes in Louisiana over two growing seasons (2007-2008).

*S. alterniflora* and *S. patens* have similar [Na] but are known to have different salinity tolerances. Higher [K] in *S. alterniflora* than in *S. patens* in paired samples may account for the similarity in [Na]. This suggests that *S. alterniflora* exhibits more ion selectivity than *S. patens* (Hester et al. 2001) because *S. alterniflora* is able to take up more K than *S. patens* when they grow under the same conditions. When salinity is low, halophytes can accumulate high [K] in their tissue (Flowers and Colmer 2008). When salinity is high, high $K^+$ availability may block the influx of $Na^+$ into roots (Zhang et al. 2010). The correlation of porewater salinity with [Na] and [K] in the leaf tissue of *S. patens* but not in the leaf tissue of *S. alterniflora* also suggests that while *S. patens* is salinity stressed, *S. alterniflora* growing in the same location is not. The correlation of [K] and [Na] in *S. patens* ($r = 0.552$, $p < 0.0001$) but not in *S. alterniflora* ($r = 0.086$, $p = 0.5384$) suggests that *S. patens* is unable to take up K without also taking up Na in high salinity environments, while *S. alterniflora* is able to exclude Na. More research is needed to describe how salinity affects Na and K uptake dynamics in these species and to identify elemental concentrations or ratios that may identify salinity limitation in *S. alterniflora*.

Although both species increase their uptake of N to improve exclusion of Na in their roots, *S. alterniflora* may be more efficient at the process than *S. patens*. When growing under the same porewater conditions, *S. patens* incorporates less N on average into its leaf tissue than *S. alterniflora*. On average, productivity in the *S. patens* sampled in our study was limited by both low N and high salinity (mean C:N = 57.46, mean [Na] = 1.07) based on a tool to diagnose limitation of production by N-limitation and salinity stress (Tobias et al. 2010). On average, *S. alterniflora* that I collected was not N-limited, however, because the average [N] in our *S. alterniflora* (mean [N] = 1.42%, mean salinity = 10.5 ppt) was higher than the critical N concentrations reported in two separate studies. In *S. alterniflora* growing in mesocosms with 15 ppt salinity, the critical [N] was 7.3 ±0.7 gN/kg (0.73%; Smart and Barko 1980). Similarly, another study also found that that at 20 ppt salinity critical N concentration was around 8.2 gN/kg (0.82%; Bradley and Morris 1992).

C:N in both species was correlated with porewater ammonium-N and salinity. High C:N in *S. patens* was more strongly associated with low N availability than it was for *S. alterniflora* (Tables 7 and 8). This supports the conclusion that on average *S. patens* is N-limited but *S. alterniflora* is not, or that N-uptake is more susceptible to salinity stress in *S. patens* than in *S. alterniflora*. Lower C:N ratios in the fall of 2008 illustrates how the leaf tissue chemistry of these plants reacts to flooding with high salinity water. Plants may have taken up N in response to Na from saline storm surge water. *S. alterniflora* requires more N in its tissue when grown in more saline conditions (Bradley and Morris 1992). If plants were severely flood stressed they might be unable to take up N (Mendelssohn and Morris 2000). Soil hypoxia may influence nutrient uptake more than salinity level for *S. patens* (Bandyopadhyay et al. 1993). The latter study was conducted with salinity levels on the low end of the range observed in the current study, however. Plants in our study were either not stressed by the relatively short duration of flooding by storm surge or they recovered quickly and were able to take up N that built up in the porewater during the flooding.

[Mn] in *S. alterniflora* was higher on average than in *S. patens* for each sampling period. This supports the conclusion that *S. alterniflora* is more flood tolerant than *S. patens*. [Mn] in *S. alterniflora* leaf tissue increased when marsh elevation was raised by adding sediment (DeLaune et al. 1990). Similarly, [Mn] in *S. patens* leaf tissue decreases with increased flooding in organic marsh soils, and [Mn] < 223 ppm indicates that *S. patens* grew in soils that are flooded above the soil surface (Tobias et al., in review). This [Mn] may not indicate flooding stress in *S. alterniflora* as is does in *S. patens*, however, because production in *S. alterniflora* is stimulated by moderate flooding (Morris 2002) and may have different mechanisms for Mn uptake. Biomass measurements would be necessary to determine what [Mn] indicate flooding stress in *S. alterniflora*. On average, *S. patens* in this study was moderately flood stressed because [Mn] was always below 223 ppm and increased flooding from Hurricane Ike did not decrease [Mn] in the leaf tissue. The observation that plants were still able to take up N from porewater suggests that the plants were not severely stressed by flooding, however. Potential seasonal patterns in the [Mn] in *S. alterniflora* should be investigated further. For *S. patens*, although [Mn] in summer leaf tissue reflects recent flooding conditions, fall leaf tissue may not (Tobias et al. in review). I assumed that paired tissue samples came from plants that were experiencing similar redox conditions because flooding levels appeared similar. However, soil Eh tends to have high spatial variability and I did not measure soil Eh near where each tissue sample was collected.

Early in the growing season, comparisons between *S. patens* and *S. alterniflora* may be more accurate than comparisons made toward the end of the growing season because [Ca] diverge starting in the summer. Ca is not translocated from older plant tissue into new plant tissue as the plant grows (Jones 1998), therefore [Ca] in new leaves, such as those collected for this study, reflect conditions that the plant experienced recently. [Ca] may be influenced somewhat by conditions earlier in the growing season, however, because plants with greater root biomass are more able to take up Ca. [Ca] in *S. patens* leaf tissue is unaffected by changes in Eh (Bandyopadhyay et al. 1993) and [Ca] in leaf tissue of

both species is unaffected by changes in salinity (Bradley and Morris 1991, Bandyopadhyay et al. 1993). Thus, I attribute changes in [Ca] to seasonal changes in plant production rather than to plant reactions to changes in porewater conditions caused by storm surge. Sharp decreases in [Ca] in *S. patens* in fall 2007 and summer 2008 may indicate that production had ceased between these and the previous sampling periods. [Ca] in *S. alterniflora* continued to increase throughout both growing seasons. This may indicate that *S. alterniflora* has a longer growing season than *S. patens* growing under the same conditions. Previous studies have indicated that while *S. alterniflora* biomass increases from March to September (Darby and Turner 2008), *S. patens* productivity, as measured by leaf elongation, declines after June (Ewing et al. 1997). Differences in the length and timing of the growing season for these species suggest that although the best time to take samples for tissue analysis to diagnose limitation in *S. patens* is summer (Tobias et al. unpublished data), fall may be the best time to diagnose the causes of limited production in *S. alterniflora*.

Making comparisons between the leaf tissue chemistry of *S. alterniflora* and *S. patens* should be undertaken with caution because concentrations of certain elements differ significantly between these two species. When growing under the same porewater conditions, *S. patens* incorporates less N, Mn, and Ca on average into its leaf tissue than *S. alterniflora*. The time of the year in which samples were taken should be taken into account because C:N ratios, [Ca], and [Mn] exhibit seasonal patterns that may be related to seasonal changes in plant production and/or climate patterns. Large weather events such as storms, spring floods, and possibly droughts should also be taken into account because *S. patens* and *S. alterniflora* react differently to environmental conditions. The use of pulsed flooding as a management tool to reduce salinity and increase N may be beneficial to both species. Flooding events of short durations deliver N subsidies to marshes without negatively affecting N uptake in either species or increasing [Mn] in leaf tissue.

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# CHAPTER 5.
# VALIDATING AND APPLYING TOOLS FOR IMPROVING COASTAL RESTORATION AND MANAGEMENT

## Introduction

Land loss, particularly loss of coastal marshes, is a serious problem in coastal Louisiana. From the 1930s through 1990s Louisiana lost an average of approximately 66 $km^2$ of coastal marsh per year (Britsch and Dunbar 1993) land loss rates in the 1980s were estimated to be as high as approximately 90 $km^2$ per year (Gagliano et al. 1981). Many factors cause loss of coastal wetlands, including subsidence and sea level rise, which reduce the elevation of marshes relative to sea levels. Sea level rise increases flooding stress and salinity stress on marshes. Hydrologic alterations resulting from anthropogenic projects such as construction of protection levees, digging navigation canals, and draining land for agriculture also reduce the resiliency of marshes to relative sea level rise by intensifying stress factors such as high salinity, low nutrient-availability, and flooding.

Increased stress reduces plant productivity, and because vegetative growth of marsh plants controls rates of vertical accretion in Louisiana's coastal marshes (Nyman et al. 2006), increased stress also reduces the ability of marshes to keep up with relative sea level rise. To deal with the problem of coastal wetland loss, managers must implement plans that increase plant productivity, and thus the ability of marshes to vertically accrete and keep up with sea level rise. For example, freshwater diversions deliver fresh water and sediment that are important factors in reducing marsh loss (Day et al. 2000). However, managers require methods for selecting management strategies that address the causes of limited productivity and for evaluating the effectiveness of strategies they choose to implement.

Several methods for estimating productivity currently exist. For example, managers can use changes in above-ground biomass to identify sites that differ in productivity (e.g. Burdick et al. 1989, Ewing et al. 1997). Plant biomass is a practical indicator because it integrates many biogeochemical

processes and physiological responses (Ewing et al. 1995). However, this method of estimating productivity requires intense sampling over a short period of time, thus it is too costly to be used regularly or over a large area. Shoot elongation varies with plant growth (Ewing et al. 1997) but this technique requires repeated visits to sites and locating previously tagged stems. Also, while these techniques may identify areas where productivity is limited, they cannot identify the factors that limit production.

Identifying the causes of limited production can improve management plans by suggesting possible remedies. For example, in a marsh where low N availability limits production, management plans designed to lower salinity will not increase production unless they also increase N-availability. Methods such as leaf spectral reflectance, carbon dioxide uptake, leaf expansion, and leaf proline concentration can be used to identify limiting factors because they vary with salinity stress or nutrient starvation (Ewing et al. 1995, 1997). Although these methods can be used to directly identify limiting factors, they are too costly for use on large geographic or temporal scales. Elemental concentrations in plant tissue have been used as indicators of growing conditions and nutrient limitation for both wetland plants (e.g., Gusewell 2002 and 2004, Koerselman and Meuleman 1996, Patrick and DeLaune 1976) and agricultural crops (e.g., Fageria et al. 2008, McKee and McKelvin 1993). For example, where salinity is low, increasing N availability increases productivity and decreases C:N ratios of *S. patens* leaf tissue (Foret 2001, Crain 2007). In other marsh species, [Na] in leaf tissue increases with increasing salinity (McKee and Mendelssohn 1989; Bradley and Morris 1991).

An ideal bioindicator would rapidly identify different factors that limit growth (Ewing et al. 1997), and would be simple and inexpensive enough to use regularly and across a large area, ideally an entire coastline. To improve the ability to identify limitation of productivity in *S. patens* by salinity stress, N-limitation, and flooding stress I previously developed tools that use analytical methods based on the chemical content of leaf tissue and that are commercially available and commonly used in

agriculture. These tools were developed in two experiments: one in a controlled greenhouse setting with constant levels of N availability and salinity stress (Tobias et al. 2010), and a second in a field setting in which *S. patens* was grown at varying heights above and below local marsh level where water levels, salinity, and N-availability were allowed to fluctuate naturally (Tobias et al. in review). In the greenhouse experiment (Tobias et al. 2010), I identified ranges of C:N and [Na] in leaf tissue that identify limitation by salinity stress, N-limitation, and a combination of both these factors (Figure 22). In the field study, I determined that [Mn] < 223 ppm and/or [Ca] < 0.26 % may be used to identify plants growing in soil that was flooded above the soil surface (Tobias et al. in review).

Because of the constant conditions in the greenhouse experiment and the wide range of flooding levels in the field experiment it was necessary to evaluate whether these tools can be applied

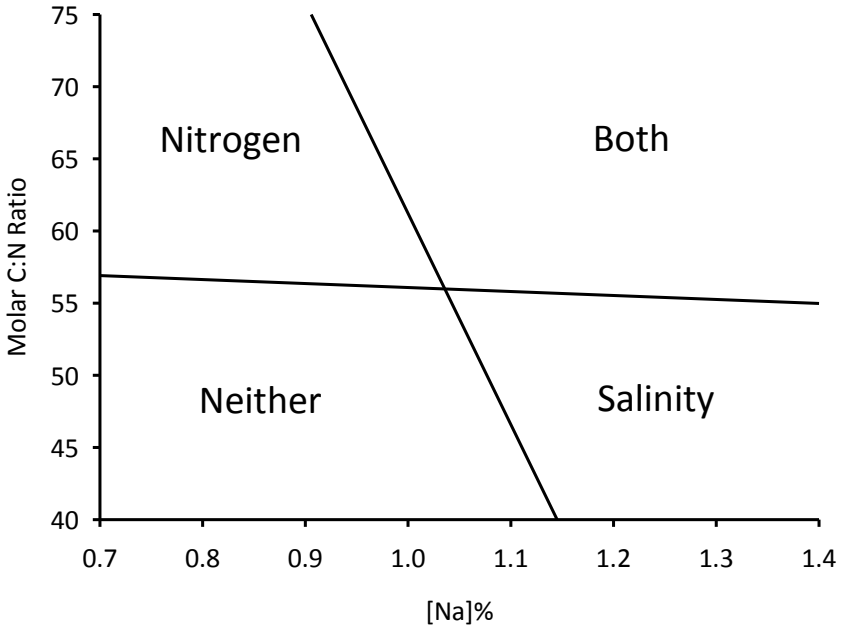


Figure 22: Na concentrations and C:N ratios in *Spartina patens* leaf tissue used as a signature to identify conditions limiting biomass production. This tool shows that C:N ratios in *S. patens* greater than 56 indicate limitation by low N availability and Na concentrations greater than 1.1% indicate limitation by high salinity. (Adapted from Tobias et al. 2010.)

where natural variations in N availability, salinity, and flooding exist. To do this, I collected tissue samples from a large, heterogeneous area of marsh across the coast of Louisiana. I also looked for seasonal patterns in these indicators to determine if certain times of year were more or less appropriate to apply the tools. Although the tools I describe here are specific for *S. patens* in coastal Louisiana, these methods could be applied to other species and other systems.

**Methods**

I collected data at four locations, each with saline and intermediate marshes, along the coast of Louisiana. I selected a fresher and a more saline site at each location. Locations on the Chenier Plain were Cameron Prairie National Wildlife Refuge (29°50'51" N, 93°14'24" W and 29°51'15" N, 93°07'58" W) and Rockefeller Refuge (29°37'47" N, 92°38'12" W and 29°37'13" N, 92°32'19" W), and locations on the Mississippi Delta, near the mouth of the Atchafalaya River, were Marsh Island Wildlife Refuge (29°34'46" N, 92°00'51" W and 29°34'44" N, 91°49'31" W) and marsh adjacent to Fourleague Bay (29°21'59" N, 91°10'26" W and 29°17'04" N, 91°07'35" W). Following Penfound and Hathaway's (1938) classification system for coastal marshes, fresher sites were chosen to include species that indicated intermediate marsh such as *Sagittaria lancifolia* and *Scirpus olneyi*. More saline sites were chosen to include species that indicate saline marsh such as *Spartina alterniflora*. All sites contained *S. patens*. The purpose of this method of site selection was to sample marshes over a broad range of salinity conditions and riverine influence under which *S. patens* grows. I classified these eight sites into four groups based on marsh type and geomorphic region: Intermediate Atchafalaya (IA), Intermediate Chenier (IC), Saline Atchafalaya (SA), and Saline Chenier (SC).

At each of the eight sites, I took samples at three plots approximately 100 meters apart. I visited the same general area on each trip but plot locations were selected haphazardly. I collected samples in spring, summer, and fall of 2007 and 2008. I only visited four sites in Spring 2007 because of permitting and time constraints. In Fall 2008, weather prevented sampling at Fourleague Bay.

I collected vegetation from 0.25 $m^2$ clip plots at each plot to estimate biomass and compare productivity among sampling sites. I cut vegetation at the marsh surface and transported the aboveground portion to the lab. In the lab I sorted stems by species and whether they were alive or dead. I defined live stems as those that had any visibly green tissue on the stem or leaves. Stems that appeared dead were broken and the inside of the stem was examined as well. Biomass samples were then dried to a constant weight in a 60°C oven before weighing. I collected porewater samples at 10 cm below the marsh surface at each plot with a syringe connected to a piece of rigid tubing. The tubing was sealed at the end and holes were drilled along the sides to approximately 2 cm from the end. I pre-filtered porewater with a piece of nylon stocking fitted over the end of the tubing to exclude large soil particles. I measured salinity, conductivity, and pH with a handheld meter (YSI Model 63). For nutrient analysis, I filtered water samples with 0.45 µm nylon syringe filters (Whatman) to remove sediment. These water samples were transported to the lab on ice and kept cold until nutrient analyses were performed. I determined the concentrations of ammonium-N using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et al. 1998).

Researchers have suggested the collection of many different parts of a plant for analysis; however, I collected leaf tissue because it is easy to collect and leaf tissue may be more indicative of limitation of the entire plant than stem tissue because is more sensitive to nutritional deficiencies than other plant parts (Smith 1962). I collected leaf tissue from the top 10-15 cm of *S. patens* stems and no stems were included in tissue samples. I stored tissue samples on ice until they could be processed in the lab. I rinsed tissue samples with deionoized water to remove salt and/or sediment from leaf surfaces before drying in a 60°C oven to a constant weight. Once dried, I ground tissue in a coffee grinder (Black and Decker Smartgrind). The grinder was cleaned with compressed air to remove debris between samples. I submitted dried and ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL) to determine their elemental composition. C and N content were

determined by dry combustion by Leco CN Analyzer. Concentrations of all other elements were determined by ICP analysis. To identify limitation of production by N starvation and/or salinity stress at each site, I used a tool developed by Tobias et al. (2010). This tool uses molar C:N ratios and Na concentrations in *S. patens* tissue to diagnose limitation of production as being caused by N starvation, salinity stress, a combination of both of these factors, or neither of these factors. I also identified flooding stress with [Mn] and [Ca] described by Tobias et al. (in review). Molar ratios of Na:K were used to indicate salinity tolerance because halophytes take up excess K from soil to block the uptake of Na (Maathuis and Amtmann 1999). I used hourly water level data from Coastwide Reference Monitoring System (CRMS) stations near our sites to calculate an estimate of the average depth of flooding at each site for two weeks prior to sampling.

I performed regression analyses with PROC REG in SAS. I used a principal components analysis with an orthogonal rotation (SAS PROC FACTOR, method=prin rotate=varimax) to identify which indicators of limited production were most highly associated with plant biomass. I retained only principal components with eigenscores > 1 for interpretation.

**Results**

Concentrations of ammonium-N in porewater averaged 3.84 mg/L and ranged from undetectable levels to over 26 mg/L through the course of this study. Orthophosphate in porewater averaged 4.46 mg/L and varied from undetectable levels to nearly 16 mg/L. Porewater salinity averaged 10.25 ppt over all sites and varied from 0.5 to over 22.1 ppt (Table 11).

Average surface water pH was generally neutral to slightly acidic and average surface water salinity ranged from 1.18 – 15.69 ppt (Table 12). Ammonium-N was higher at intermediate sites than saline sites in the spring but was much higher at saline sites in summer and fall. At saline sites, orthophosphate increased in summer and remained high through fall. Salinity generally was lower at

intermediate sites than saline sites. Within marsh types, average salinity was lower at Atchafalaya sites than Chenier sites, however average ammonium-N was not different (Table 11). Species richness was generally higher at fresh sites than their more saline counterparts (Table 13). Intermediate sites were dominated by *Spartina patens* or co-dominated by *S. patens* and *Scirpus olneyi* or *Sporobolus virginicus* (Table 14). Saline sites were usually co-dominated by *S. patens* and *S. alterniflora*, although some saline sites were also co-dominated by *Juncus roemerianus* (Table 14). Saline sites were generally more salinity-limited than intermediate sites, particularly in spring (Figure 23). In summer, intermediate sites became more salinity-limited than they were in spring. There was no evidence of P-limitation throughout this study (Figure 23).

Higher C:N ratios and [Na] and lower [Mn] were associated with low biomass (i.e. limited productivity). At lower C:N ratios and [Na] and higher [Mn] biomass was more variable. I interpreted this relationship to mean that at higher C:N ratios and [Na] and lower [Mn], production was limited by the stressor indicated. At lower C:N ratios and [Na] and higher [Mn], the wide range of biomass indicated that some other factor or factors controlled production. The relationship between total live biomass and Na:K followed a limiting factor pattern in spring and fall but not summer (Figure 27). Na:K was lower at intermediate sites than at saline sites in spring, but did not follow the same pattern in summer or fall (Figure 27).

In this study porewater ammonium-N was not a good predictor of molar C:N ratio in *S. patens* leaf tissue under field conditions. Although there was a significant linear relationship between porewater ammonium-N and leaf tissue C:N ratio, little of the variation in C:N was explained (PROC REG; $R^2 = 0.09$ $F_{1,106} = 10.57$, $p = 0.0015$). Leaf tissue C:N ratios did appear related to porewater ammonium-N where salinity was low, however (Figure 28). There was also a significant linear relationship between porewater salinity and [Na] in leaf tissue, but porewater salinity explained little of the variation in leaf tissue [Na] (PROC REG; $R^2 = 0.19$ $F_{1,106} = 25.02$, $p < 0.0001$). Relationships of total live biomass with C:N

ratio, [Na], and [Mn] in *S. patens* leaf tissue exhibited non-linear patterns that I expect to see in indicators of limited production (Figures 24, 25, 26).

Almost all sites in all seasons were classified as flooded based on [Mn] < 223 ppm. Although [Mn] appeared not to be related to average depth of flooding (Figure 29), further investigation indicated that water levels recorded by water level recorders did not coincide with actual water levels on the marsh surface. For example, at the site with average [Mn] of approximately 543 ppm surface, water was not 12 cm deep when I sampled it, as indicated by water level recorder data. In fact, soil at this site was

Table 11: Mean and standard error of salinity, pH, ammonium-N, and orthophosphate of porewater at 10 cm below the marsh surface at sampling sites, over two growing seasons (May-December, 2007-2008). Sampling sites were classified as intermediate Atchafalaya (IA), intermediate Chenier (IC), saline Atchafalaya (SA), or saline Chenier (SC). The number of samples is represented by "n." Means and standard errors were calculated with PROC MEANS (SAS).

| | | | Salinity (ppt) | | pH | | Ammonium-N (mg/L) | | Orthophosphate (mg/L) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Season | Group | n | Mean | Standard Error | Mean | Standard Error | Mean | Standard Error | Mean | Standard Error |
| Spring | IA | 7 | 3.63 | 0.54 | 6.47 | 0.18 | 3.85 | 1.25 | 2.49 | 0.66 |
| | IC | 9 | 5.90 | 0.51 | 6.04 | 0.17 | 1.04 | 0.37 | 1.65 | 0.73 |
| | SA | 7 | 9.36 | 0.55 | 6.42 | 0.13 | 1.15 | 0.23 | 3.79 | 0.94 |
| | SC | 9 | 16.17 | 0.77 | 6.54 | 0.16 | 2.17 | 0.90 | 4.18 | 1.67 |
| Summer | IA | 12 | 3.20 | 0.34 | 6.76 | 0.18 | 1.48 | 0.62 | 0.93 | 0.31 |
| | IC | 9 | 9.96 | 1.18 | 6.83 | 0.12 | 1.65 | 0.30 | 2.95 | 1.02 |
| | SA | 12 | 8.82 | 0.55 | 6.67 | 0.12 | 2.64 | 0.96 | 7.36 | 1.30 |
| | SC | 11 | 16.15 | 0.42 | 6.97 | 0.07 | 8.80 | 2.86 | 7.09 | 1.59 |
| Fall | IA | 9 | 5.77 | 0.81 | 6.59 | 0.09 | 2.03 | 0.83 | 4.03 | 1.48 |
| | IC | 12 | 11.13 | 1.33 | 6.76 | 0.10 | 4.95 | 1.00 | 4.82 | 0.95 |
| | SA | 9 | 11.50 | 1.88 | 6.65 | 0.07 | 1.31 | 0.34 | 5.33 | 1.14 |
| | SC | 12 | 16.94 | 0.51 | 7.09 | 0.07 | 11.28 | 2.44 | 6.14 | 1.27 |

Table 12: Mean and standard error of pH and salinity of surface water at sampling sites, over two growing seasons (May-December, 2007-2008). Sampling sites were classified as intermediate Atchafalaya (IA), intermediate Chenier (IC), saline Atchafalaya (SA), or saline Chenier (SC). The number of samples is represented by "n." Means and standard errors were calculated with PROC MEANS (SAS).

| | | | pH | | Salinity (ppt) | |
|---|---|---|---|---|---|---|
| Season | Group | n | Mean | Standard Error | Mean | Standard Error |
| Spring | IA | 7 | 6.52 | 0.36 | 3.75 | 3.19 |
| | IC | 9 | 6.86 | 0.40 | 5.97 | 1.38 |
| | SA | 7 | 4.64 | 1.73 | 6.70 | 2.69 |
| | SC | 9 | 6.82 | 0.22 | 14.10 | 0.77 |
| Summer | IA | 12 | 7.47 | 0.23 | 1.18 | 0.43 |
| | IC | 8 | 7.68 | 0.35 | 5.84 | 2.01 |
| | SA | 12 | 7.08 | 0.19 | 3.43 | 0.80 |
| | SC | 11 | 7.09 | 0.13 | 11.50 | 2.00 |
| Fall | IA | 8 | 7.27 | 0.36 | 5.30 | 1.10 |
| | IC | 12 | 7.53 | 0.06 | 10.61 | 2.44 |
| | SA | 10 | 7.24 | 0.20 | 15.20 | 0.00 |
| | SC | 12 | 7.16 | 0.29 | 15.69 | 1.87 |

Table 13: Mean and standard error of biomass of *Spartina patens*, total biomass, and species richness (per 0.25 $m^2$ clip plot). Samples were taken over two growing seasons (May-December, 2007-2008). Sampling sites were classified as intermediate Atchafalaya (IA), intermediate Chenier (IC), saline Atchafalaya (SA), or saline Chenier (SC). The number of samples is represented by "n." Means and standard errors were calculated with PROC MEANS (SAS).

| | | | Live *S. patens* Biomass (g/plot) | | Total *S. patens* Biomass (g/plot) | | Live Biomass of All Species (g/plot) | | Total Biomass of All Species (g/plot) | | Species Richness (spp/plot) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Season | Group | n | Mean | Standard Error | Mean | Standard Error | Mean | Standard Error | Mean | Standard Error | Mean | Standard Error |
| Spring | IA | 7 | 197.79 | 94.05 | 289.82 | 144.22 | 369.18 | 54.31 | 604.59 | 131.70 | 3.56 | 0.44 |
| | IC | 9 | 322.40 | 65.46 | 464.81 | 93.16 | 403.51 | 37.04 | 631.07 | 59.36 | 1.83 | 0.27 |
| | SA | 7 | 89.89 | 29.93 | 132.49 | 47.05 | 263.27 | 30.23 | 420.58 | 48.48 | 3.00 | 0.33 |
| | SC | 9 | 195.30 | 46.35 | 275.36 | 56.81 | 249.28 | 38.13 | 394.07 | 47.44 | 1.58 | 0.15 |
| Summer | IA | 12 | 174.80 | 50.18 | 237.99 | 70.76 | 330.99 | 41.70 | 501.26 | 60.03 | 3.67 | 0.24 |
| | IC | 8 | 344.72 | 95.73 | 364.14 | 98.61 | 486.24 | 67.90 | 383.10 | 206.56 | 2.22 | 0.46 |
| | SA | 12 | 50.53 | 18.99 | 64.18 | 24.12 | 199.64 | 19.88 | 354.93 | 40.78 | 3.00 | 0.17 |
| | SC | 11 | 299.06 | 48.62 | 423.09 | 56.32 | 330.72 | 38.66 | 470.77 | 45.86 | 1.89 | 0.26 |
| Fall | IA | 9 | 93.81 | 31.29 | 169.86 | 64.89 | 161.62 | 32.13 | 375.43 | 33.67 | 4.00 | 0.37 |
| | IC | 12 | 389.86 | 86.39 | 349.93 | 116.47 | 536.41 | 48.37 | 829.14 | 90.76 | 2.00 | 0.41 |
| | SA | 9 | 39.01 | 28.10 | 100.21 | 43.89 | 159.46 | 23.10 | 294.50 | 39.28 | 2.83 | 0.37 |
| | SC | 12 | 174.95 | 40.13 | 365.10 | 79.03 | 229.45 | 36.19 | 384.27 | 43.67 | 1.36 | 0.20 |

Table 14: Species composition of saline and intermediate marshes, based on average biomass of each species over two growing seasons. Samples were collected from 0.25 $m^2$ clip plots over two growing seasons (May-December, 2007-2008). Sampling sites were classified as intermediate Atchafalaya (IA), intermediate Chenier (IC), saline Atchafalaya (SA), or saline Chenier (SC).

| Percent of Total Biomass | IA | | IC | | SA | | SC | |
|---|---|---|---|---|---|---|---|---|
| | **West** | **East** | **West** | **East** | **West** | **East** | **West** | **East** |
| >50 | | *Spartina patens* | *Sporobolus virginicus* | *Spartina patens* | | *Spartina alterniflora* | *Spartina patens* | *Spartina Patens* |
| 20-50 | *Spartina patens*<br>*Scirpus olneyi* | | *Spartina patens* | | *Spartina patens*<br>*Juncus roemerianus*<br>*Spartina alterniflora* | *Spartina patens* | *Spartina alterniflora* | |
| 10-20 | *Distichlis spicata* | *Sporobolus virginicus* | | | *Distichlis spicata* | *Distichlis spicata* | | |
| 5-10 | | *Spartina alterniflora*<br>*Scirpus olneyi* | *Paspalum vaginatum* | | | | | *Distichlis spicata* |
| Present | *Aster tenuifolius*<br>*Eleocharis sp.*<br>*Juncus roemerianus*<br>*Lythrum lineare*<br>*Paspalum vaginatum*<br>*Sagittaria lancifolia*<br>*Scirpus robustus*<br>*Sporobolus virginicus* | *Distichlis spicata*<br>*Eleocharis sp.*<br>*Juncus roemerianus*<br>*Paspalum vaginatum*<br>*Scirpus robustus* | *Amaranthus sp.*<br>*Distichlis spicata*<br>*Pluchea foetida*<br>*Scirpus robustus*<br>*Vigna luteola* | *Distichlis spicata*<br>*Scirpus robustus* | *Aster tenuifolius*<br>*Spartina cynosuroides*<br>*Scirpus robustus* | *Aster tenuifolius*<br>*Scirpus olneyi*<br>*Scirpus robustus* | *Scirpus robustus*<br>*Sporobolus virginicus* | *Spartina alterniflora* |

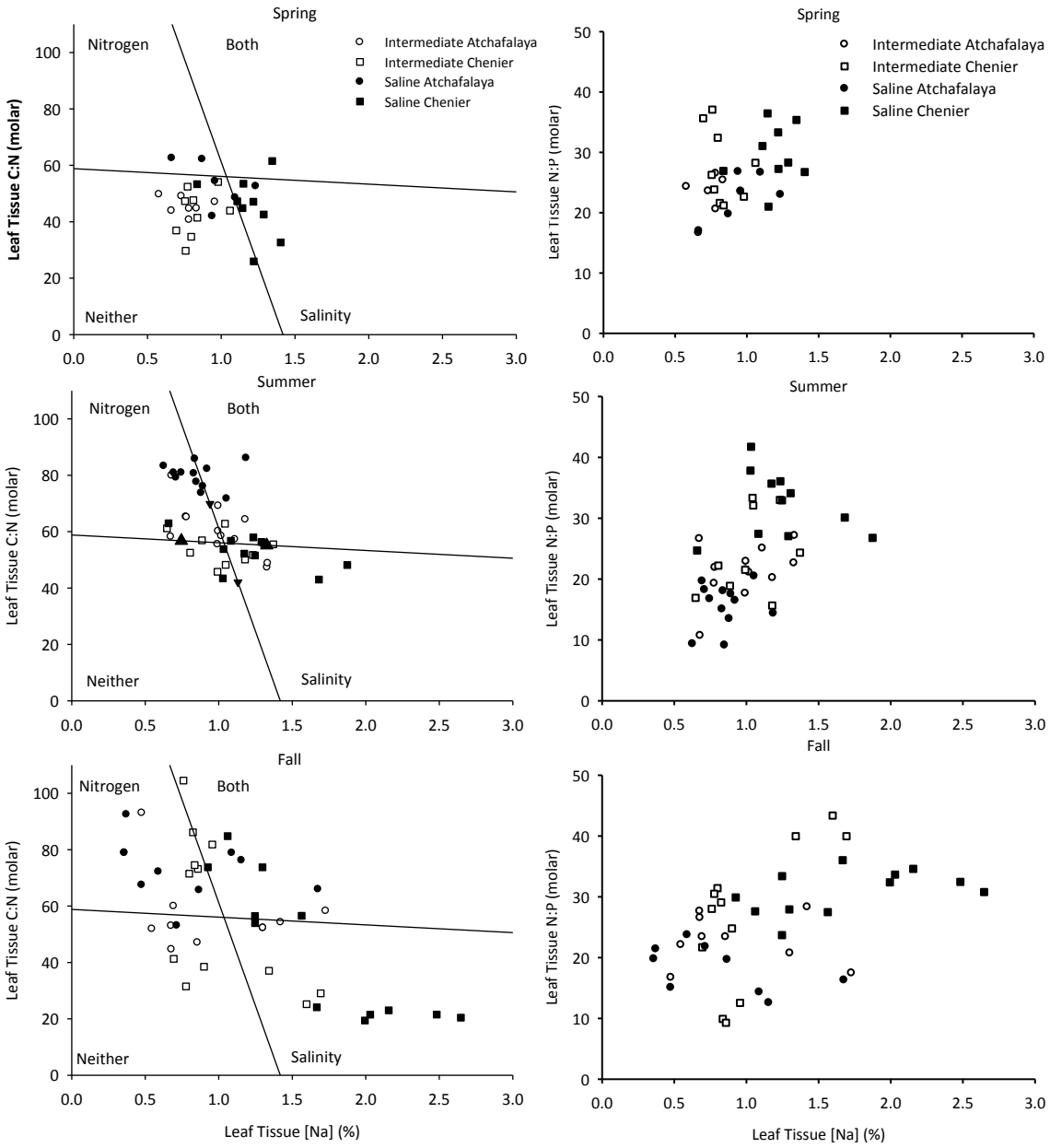


Figure 23: Seasonal patterns in molar C:N ratios (left), N:P ratios (right), and [Na] (%) in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem. Limiting factor labels and the lines dividing their respective regions were adapted from Tobias et al. (2010). Nitrogen-limited indicates low nitrogen availability limited productivity. Salinity-limited indicates high salinity limited productivity. Neither-limited indicates plants received high nutrients and low salinity. Both-limited indicates plants received low nutrients and high salinity.

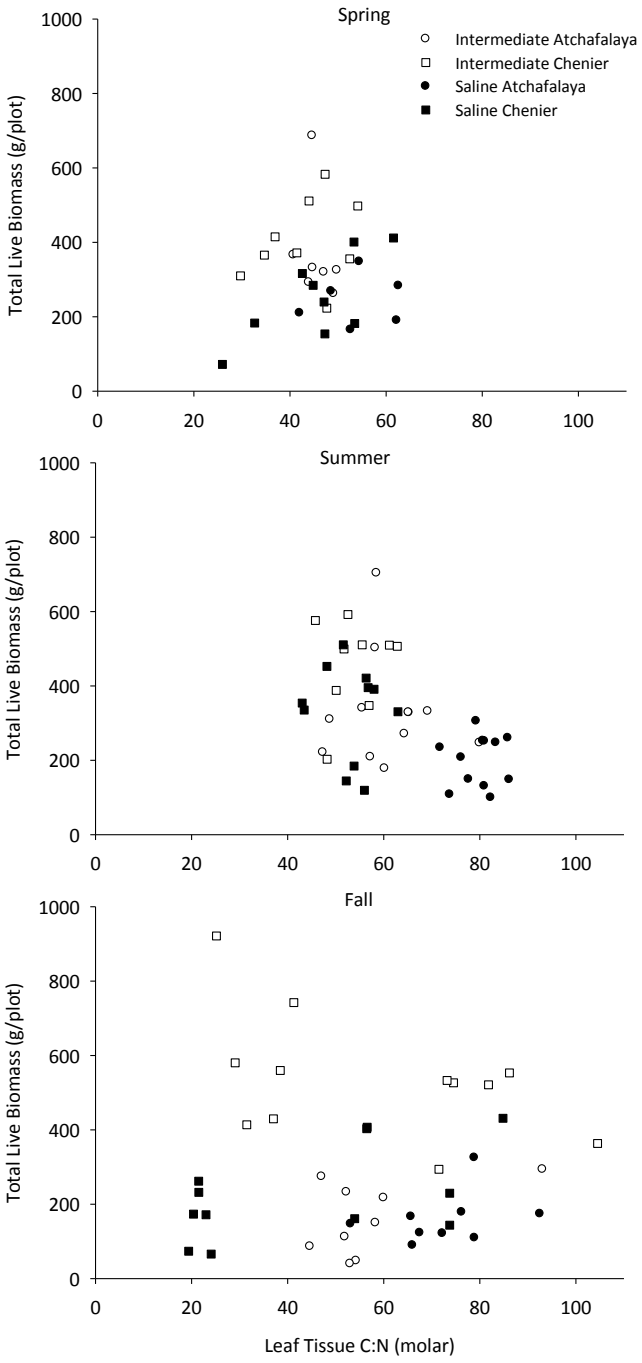


Figure 24: Relationship between total biomass of all species collected in a plot and the molar C:N ratio in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem. Low variation in biomass within the high range of C:N ratios indicates that N-uptake controlled productivity within that range.

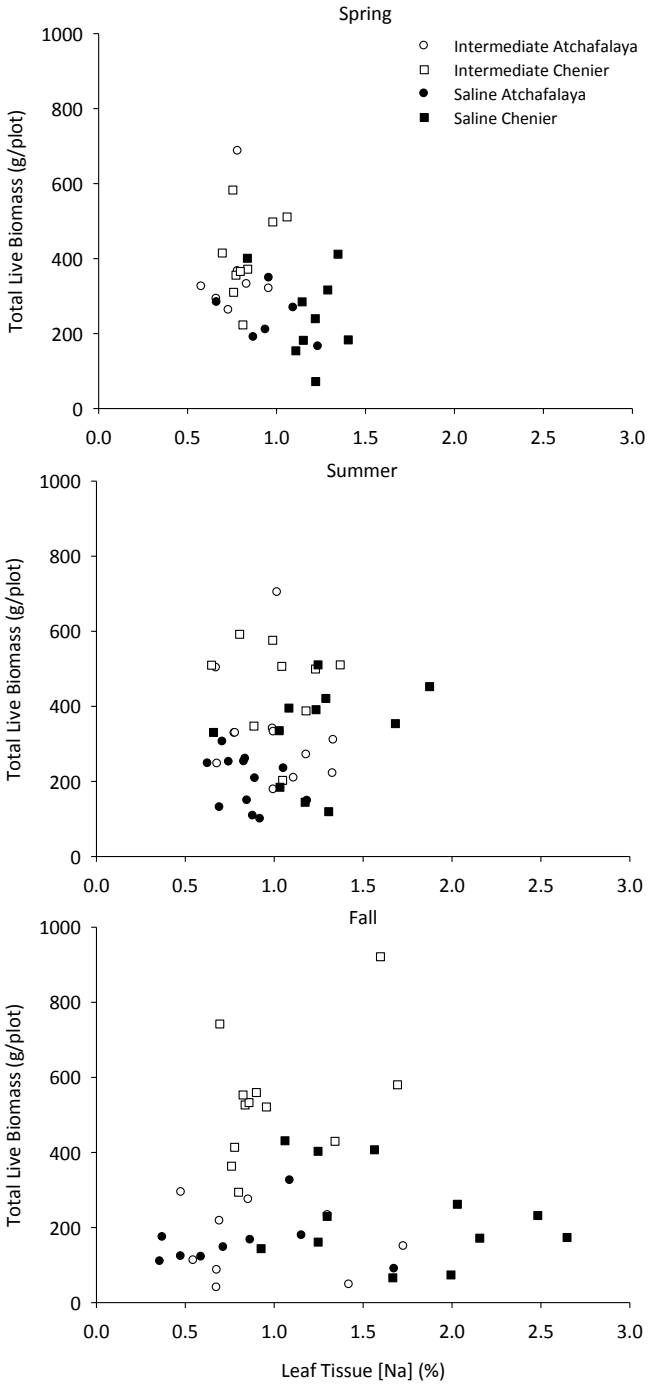


Figure 25: Relationship between total biomass of all species collected in a plot and the Na concentration in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem. Low variation in biomass within the high range of [Na] indicates that salinity controlled productivity within that range.

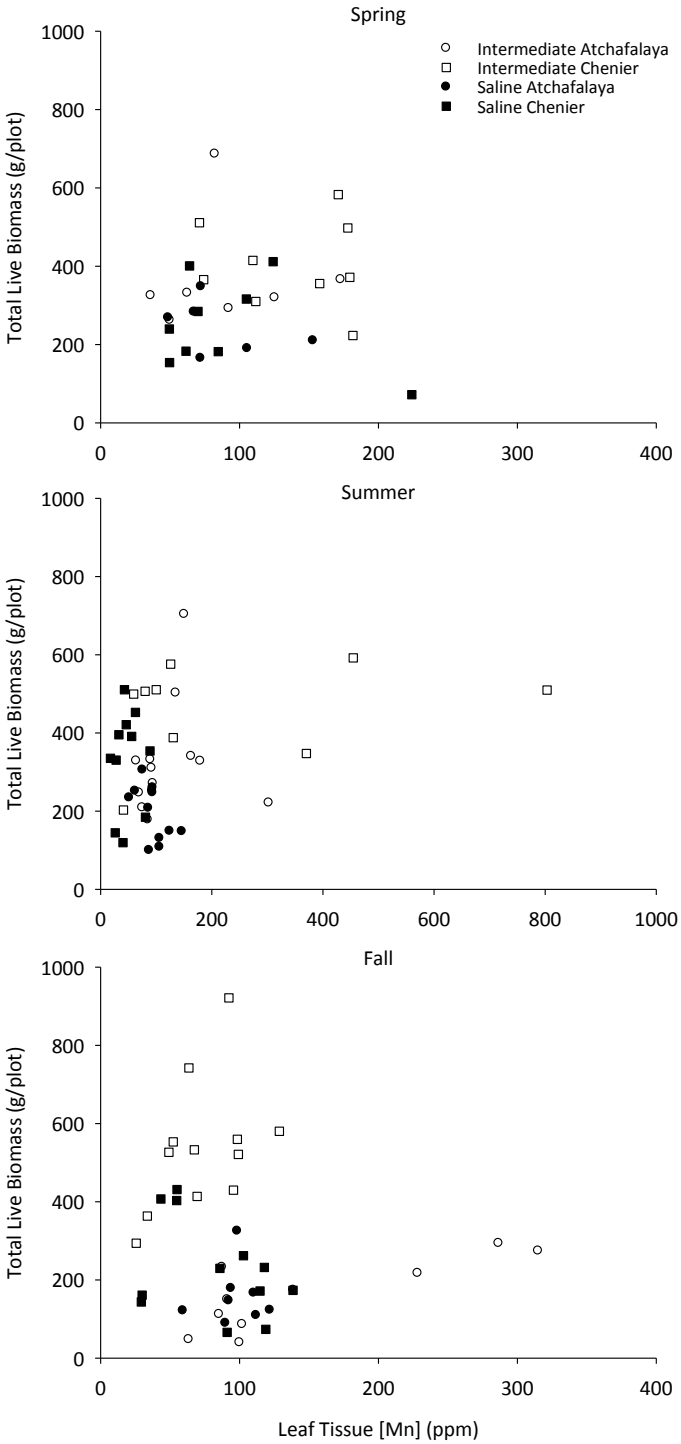


Figure 26: Relationship between total biomass of all species collected in a plot and the Mn concentration in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem. Low variation in biomass within the low range of [Mn] indicates that flooding controlled productivity within that range.

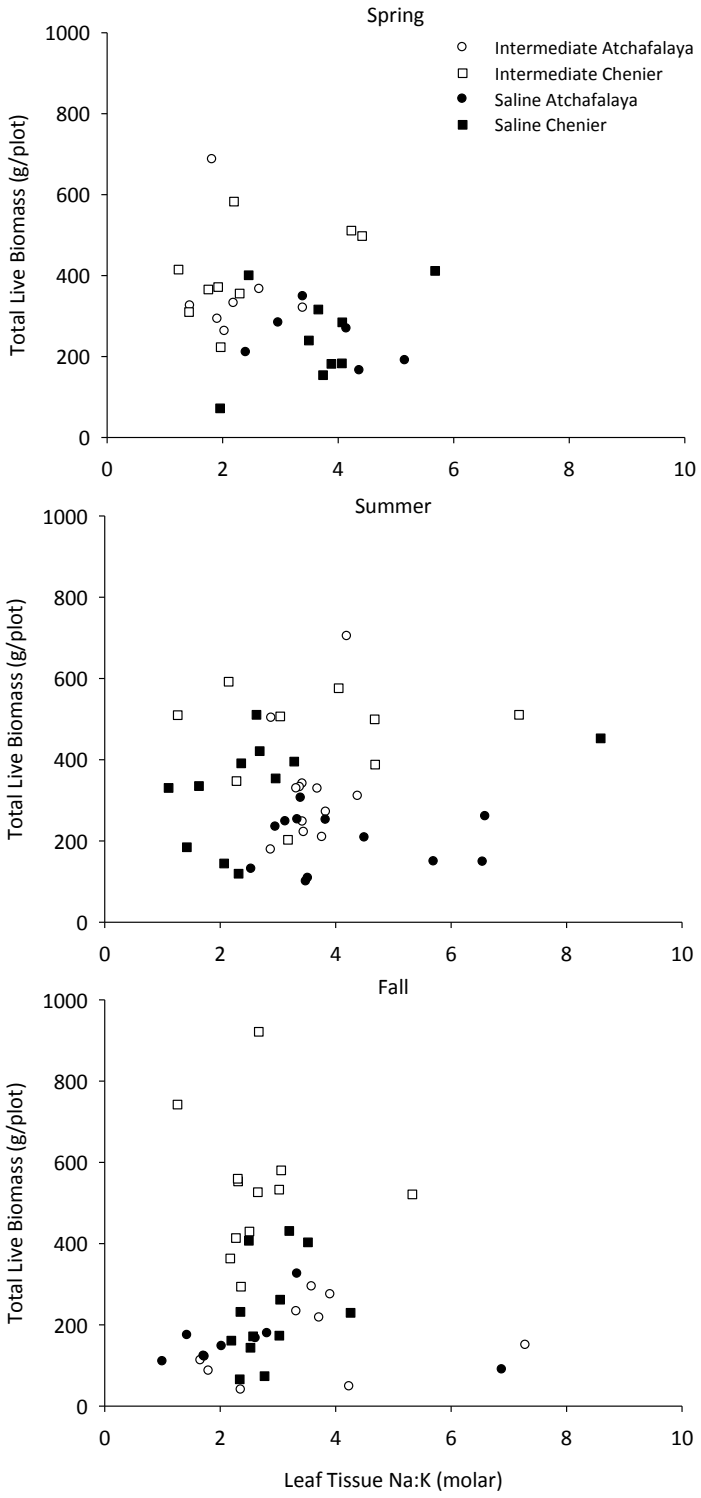


Figure 27: Relationship between total biomass of all species collected in a plot and the molar ratio of Na to K in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem.

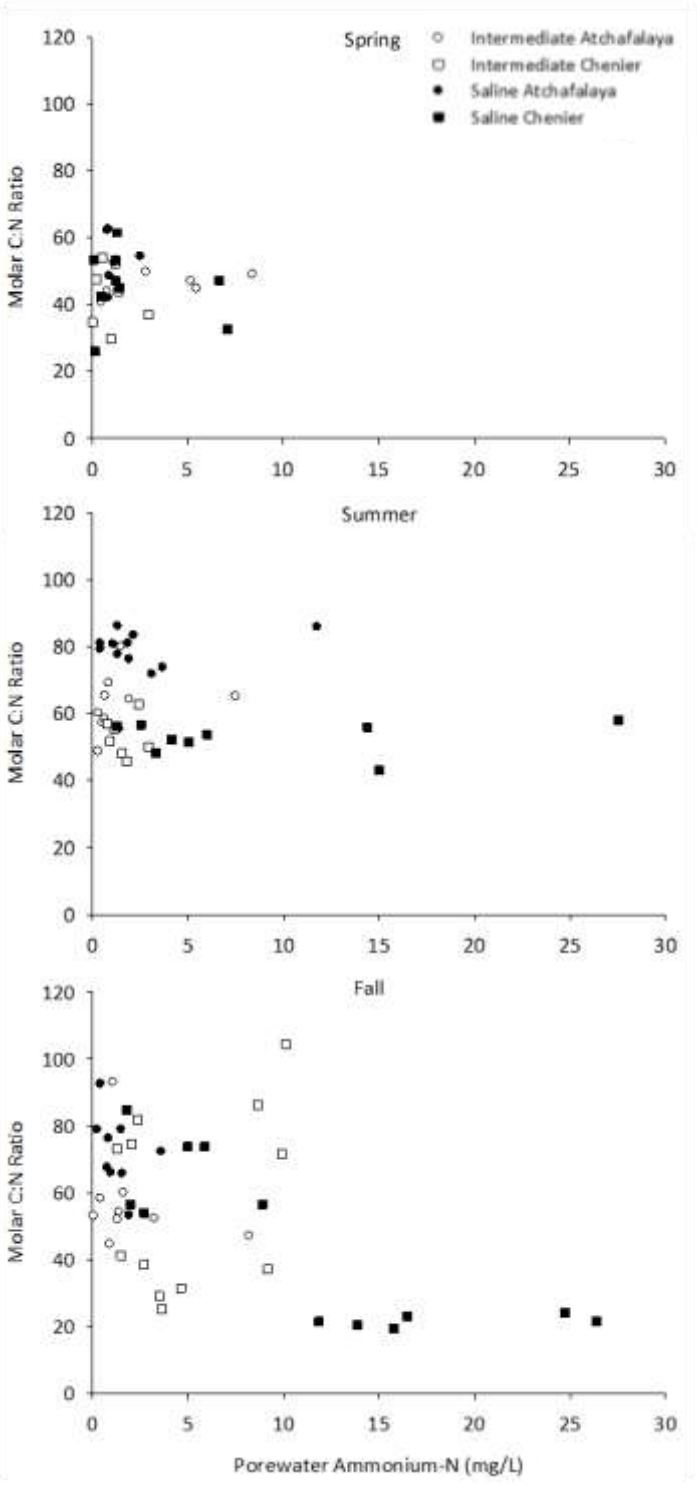


Figure 28: Seasonal relationships between ammonium-N in porewater and molar C:N ratio in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem. Porewater was extracted from soil at 10 cm below the marsh surface.

so dry that I was unable to extract porewater at that time. Furthermore, in many cases where water level recorders indicated that average water depth was below the marsh surface, I measured several cm of water ponded at our sampling plotss. However, at most sites where water levels were > 0 cm, [Mn] < 223 ppm and at sites that were known to be dry at the time of sampling [Mn] was much higher. More sites were correctly identified as having flooding levels below the soil surface at the time of harvest when [Ca] < 0.26% was used as an indicator of flooding stress. Plants showed the greatest range of [Ca] during the summer and it appears that saline sites were more likely to be classified as flood stressed than intermediate sites (Figure 30).

Average ammonium-N concentrations for each season were similar (Table 11). Average orthophosphate was generally lowest in spring and remained low in intermediate sites during summer (Table 11). Porewater salinity was variable at each site, but increased substantially at most sites in the fall of 2008 following Hurricane Ike. There was a significant interaction between season and group on

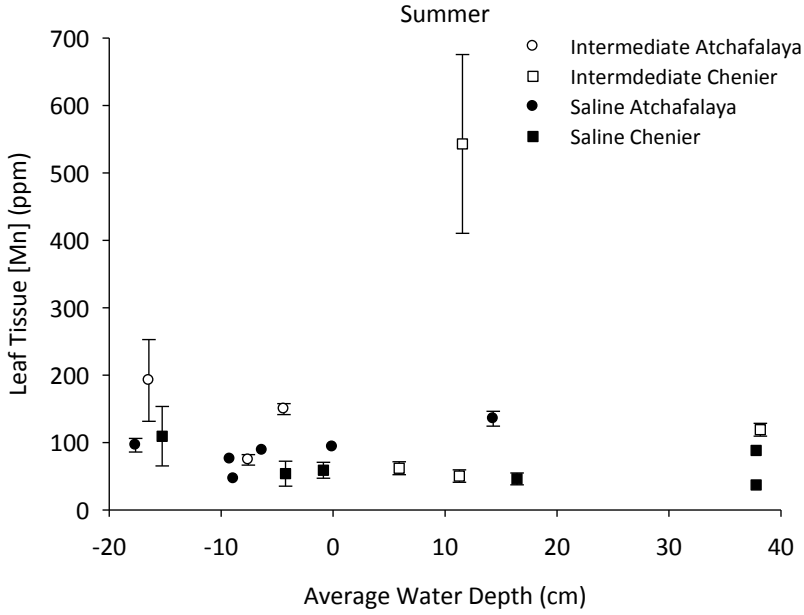


Figure 29: Leaf tissue [Mn] (ppm) of *Spartina patens* relative to the two-week average water depth at the nearest CRMS station. Samples were collected over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem.

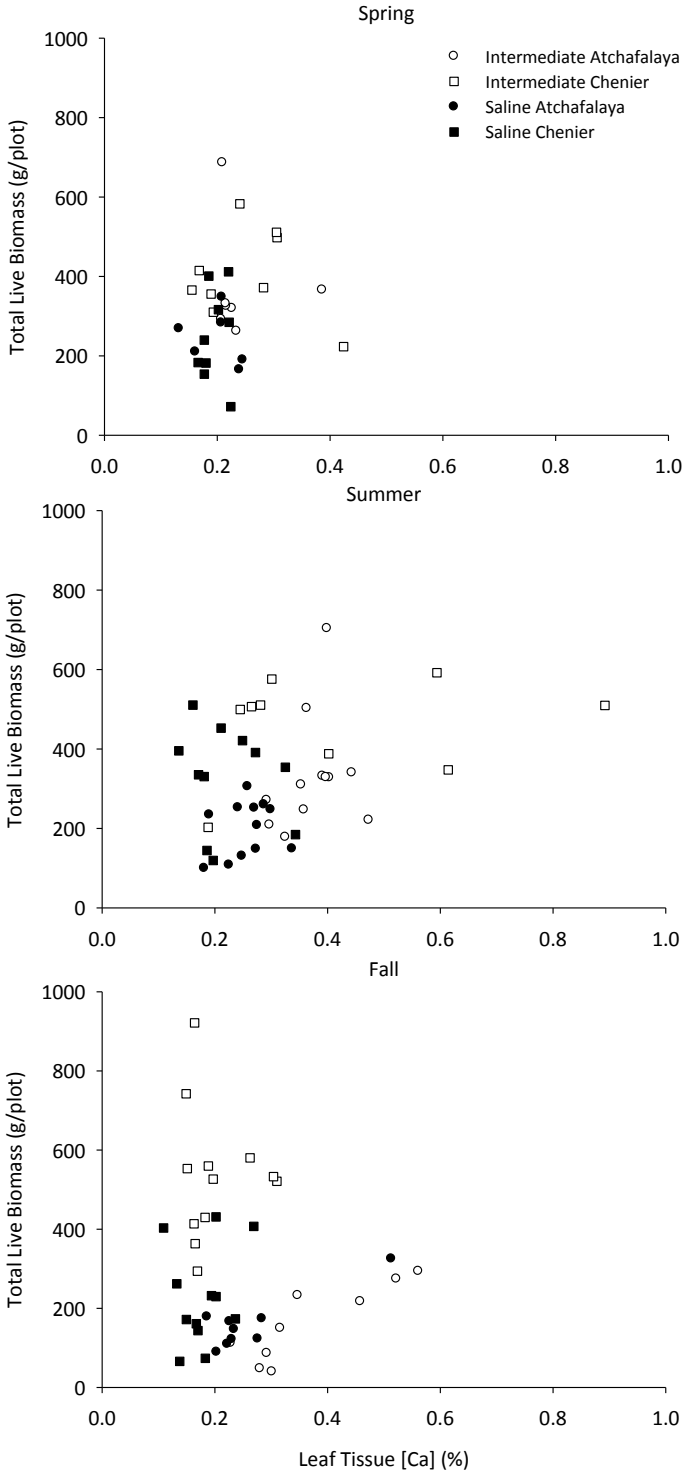


Figure 30: Relationship between total biomass of all species collected in a plot and the Ca concentration in *Spartina patens* leaf tissue at each plot over two growing seasons (2007-2008). Leaf tissue samples were taken from leaves originating within the top 15 cm of a plant's stem.

total live biomass ($F_{6,106} = 4.06$, $p = 0.0011$). Biomass increased from spring to summer but, with the exception of intermediate Chenier sites, total live biomass in the fall was similar to summer biomass or declined (Table 13).

In a principal components analysis (PCA) of biomass and elemental data, the first three principal components (PCs) had eigenvalues > 1 in spring and summer, but in fall, only the first two PCs had eigenvalues >1. These PCs were retained for interpretation and they explained 80% of the variation in samples in spring, 86% of variation in summer, and 61% of the variation in fall (Table 15). In spring, total live biomass loaded highly on the first PC, as did [Na] and Na:K. C:N and Na:K loaded highly on PC2, and [Mn] and [Ca] loaded highly on PC3. In summer, [Mn] and [Ca] loaded highly on PC1; total live biomass , C:N, and [Na] loaded highly on PC2; and Na:K loaded highly on PC3. In fall, total live biomass did not load highly on either PC. [Mn] and [Ca] loaded highly on PC1 and [Na] loaded highly on PC2, however.

Plants had higher C:N ratios (i.e. they became more N-limited) as the growing season progressed (Figure 23). In spring, fresh sites were limited by neither low N-availability nor high salinity, whereas saline sites were usually salinity-limited. Regardless of differences in initial limiting conditions, all sites became more N-limited in the summer than they were in the preceding spring. Fresh sites continued to become more N-limited into the fall of 2007, as did saline sites on the Chenier plain. Saline Atchafalaya sites became less N limited in the fall of 2007 than in summer 2007. In fall of 2008, C:N in Chenier sites was lower than in samples from the same sites in 2007.

## Discussion

Porewater ammonium-N in this study was similar to average ammonium-N for *Spartina*-dominated marshes in other studies (e.g., Mendelssohn 1979, Craft et al. 1991). The range of porewater salinities observed in this field experiment (0.05-22.1 ppt) was about half of the range in a similar greenhouse experiment (Tobias et al. 2010, range=0.05-45ppt) but the range of Na concentrations was

Table 15: Eigenvalues, proportion of variance explained, and variable loadings for the first three principal components of a six variable principal components analysis of total live biomass and selected elemental components of leaf tissue of *Spartina patens* collected over spring, summer, and fall over two growing seasons (May – December, 2007 – 2008). Leaf tissue was collected from leaves originating in the top 15 cm of stems. Variable loadings are multiplied by 100 and high loadings are bolded to ease interpretation. Ratios are molar; units for elemental concentrations are given.

| SPRING | | | |
|---|---|---|---|
| Principal Component | Eigenvalue | Proportion of Variance | Cumulative Proportion of Variance |
| 1 | 2.08 | 0.35 | 0.35 |
| 2 | 1.42 | 0.24 | 0.58 |
| 3 | 1.32 | 0.22 | 0.80 |
| | | | |
| Variable | PC1 | PC2 | PC3 |
| Total Live Biomass (g/plot) | -70 | 23 | 13 |
| C:N | -15 | 91 | -7 |
| [Na] (%) | 91 | 19 | -2 |
| [Mn] (ppm) | 2 | -27 | 86 |
| [Ca] (pph) | -17 | 18 | 86 |
| Na:K | 62 | 75 | 0 |

| SUMMER | | | |
|---|---|---|---|
| Principal Component | Eigenvalue | Proportion of Variance | Cumulative Proportion of Variance |
| 1 | 2.26 | 0.38 | 0.38 |
| 2 | 1.84 | 0.31 | 0.68 |
| 3 | 1.04 | 0.17 | 0.86 |
| | | | |
| Variable | PC1 | PC2 | PC3 |
| Total Live Biomass (g/plot) | 47 | -60 | 9 |
| C:N | -4 | 94 | 21 |
| [Na] (%) | -32 | -74 | 42 |
| [Mn] (ppm) | 94 | 1 | -10 |
| [Ca] (pph) | 95 | -2 | -9 |
| Na:K | -8 | 0 | 99 |

| FALL | | | |
|---|---|---|---|
| Principal Component | Eigenvalue | Proportion of Variance | Cumulative Proportion of Variance |
| 1 | 2.01 | 0.33 | 0.33 |
| 2 | 1.68 | 0.28 | 0.61 |
| | | | |
| Variable | PC1 | PC2 | |
| Total Live Biomass (g/plot) | -30 | -3 | |
| C:N | 4 | -84 | |
| [Na] (%) | 6 | 94 | |
| [Mn] (ppm) | 85 | -4 | |
| [Ca] (pph) | 86 | -36 | |
| Na:K | 55 | 29 | |

much larger (Tobias et al. 2010, range=0.7-1.4). The highest [Na] observed occurred (> 2.0 %) in samples taken after Hurricane Ike. I suspect that high [Na] in these samples may result from flooding with saline storm surge.There were no large differences in porewater ammonium-N between sites on the Chenier Plain and those that receive water from the Atchafalaya River, probably because all sites had highly organic soils. Seasonal averages of ammonium-N for each group were generally similar to previously reported levels for similar marsh types in Louisiana (2.33 mg/L, Burdick et al. 1989; 1-1.5 mg/L, DeLaune et al. 1980). At some sites, ammonium-N levels were much higher than previous reports, however. The highest average ammonium-N levels coincided with the highest average salinity levels. This suggests that plants may have been stressed by high salinity, thus they were unable to take up high levels of N that were available in porewater. Salinity tended to be lower and plants were less likely to be salinity-limited at Atchafalaya sites than Chenier sites. Mean species richness at my study sites was somewhat lower than those reported by Visser et al. (1998 and 2000). Results of this study also show that despite their higher biomass, Chenier marshes had somewhat lower species richness than marshes that receive water from the Atchafalaya River with similar dominant species.

Porewater orthophosphate concentrations measured in this study may be underestimates of actual orthophosphate present in porewater because samples were not acidified upon collection. It is possible that some orthophosphate could have adhered to the collection vial or precipitated with Fe in the water samples before they were analyzed. Even though lack of acidification may have caused me to underestimate orthophosphate availability, the range of porewater phosphorus availability measured in this study is similar to phosphorus availability measured in the porewater of marsh sods taken from a Louisiana marsh (0.02 mmol P/L, Broome et al. 1995; vs. range = 0.01 – 0.08 mmol P/L in this study). Marshes in this study were limited by N rather than P, however.

Storm surge from Hurricane Ike may have increased nutrient concentrations as well as salinity in porewater during fall because seawater is higher in $Na^+$, N, and P than river water (Day et al. 1989).

Sediments deposited on marshes during hurricanes may also provide a nutrient subsidy to marshes. In addition to direct subsidies of nutrients in storm surge water and sediment, plants that are stressed by flooding may be unable to take up nutrients (Mendelssohn and Morris 2000). Thus, porewater nutrient concentrations would not be depleted by plant uptake following a hurricane. Also, plant tissue that died as a result of high salinity or flooding stress provides releases nutrients to marsh soils as organic nutrients in plant tissues are mineralized during the decomposition process.

Marsh productivity was consistently low at sites where any single stress indicator was high and productivity was most variable where any single stress factor was low. The pattern of lower and less variable biomass in plants with higher levels of stress indicators (Figures 24-28) supports the conclusion that these indicators can be used to diagnose the causes of limited production in marshes. For example, there was less variation in biomass when C:N ratios were higher than there was when C:N ratios were lower in summer (Figure 24); the same was true for [Na] during fall (Figure 28) and [Mn] during summer and fall (Figure 26). Under controlled conditions where only the limiting factor of interest varies, a plot of how growth responds to changes in nutrient availability shows that growth increases until the concentration of the nutrient reaches an adequate level; beyond that point, growth would remain stable unless toxicity developed (Epstein and Bloom 2005). In our study salinity, nutrients, and flooding were allowed to vary with environmental conditions; thus when one factor was no longer limiting, biomass production was controlled by other factors. Where productivity was not limited by a particular factor, variation in growth would be expected because of variability in the levels of stress induced by other factors. For example, Merino et al. (2010) showed that when salinity was high *S. patens* biomass was consistently low, but when salinity was low biomass varied with nutrient availability.

Although a previous study (Tobias et al. 2010) showed that C:N ratio in *S. patens* was related to N-availability in porewater in a controlled setting, in this study porewater ammonia was not a good predictor of molar C:N ratio. Ammonium-N may appear to be unrelated to C:N because C:N in plant

tissue likely reflects long-term average N availability at a site, while the porewater N analysis provides only a snapshot of conditions at the time the tissue samples were collected. Also, high salinity or flooding stress may reduce the ability of plants to use all the available N in porewater (Mendelssohn and Morris 2000), so C:N may be elevated in plants growing in marshes with abundant N. Thus, C:N ratio in leaf tissue would only be expected to relate to porewater ammonium-N when salinity is low.

Summer is the most appropriate time to use C:N ratio and [Na] to diagnose N- and salinity-limitation because temporal patterns that I observed and statistical analysis suggest that N starvation begins to limit production in summer and because the effect of the interaction of salinity and N-availability on production is most evident in summer. Based on seasonal patterns in the results of this field experiment, it is likely that guidelines for interpreting C:N will be less informative for samples taken early in the growing season, as all sites will appear to have adequate N. Furthermore, *S. patens* productivity, as measured by leaf elongation, declines after June (Ewing et al. 1997). In this study, the lack of increase in *S. patens* biomass between summer and fall samples agrees with Ewing et al. (1997). Because plants grow slowly in fall, elemental concentrations in fall tissue samples may not accurately reflect the causes of limited production in *S. patens* throughout the bulk of biomass accumulation. Our data show that as plants senesce in the fall, C:N ratios increase; thus collecting tissue samples in fall could result in inaccurate indications that N starvation limits production for samples taken too late in the growing season.

Further investigation of potential indicators of flooding stress is needed. The use of [Mn] or [Ca] as an indicator of flooding stress could not be tested rigorously in this study because water level recorder data apparently did not accurately reflect conditions at our sampling sites. Future studies should sample nearer to water level recorders to reduce the effects of topography that likely caused the inaccuracies I observed. The indication by PCA that [Mn] was more variable in summer supports the hypothesis that [Mn] is most useful as an indicator in the summer. Our site specific observations also

lend some support to the use of [Mn] as an indicator of flooding stress. Most sites where average water level was > 0 cm had [Mn] < 223 ppm and sites that were known to be dry had high [Mn]. The fact that most of the sites I sampled had [Mn] < 223 ppm suggests that flooding stress is the main factor limiting productivity throughout this landscape. Principal components analysis suggested that [Na] and C:N ratios accounted for more of the variation in biomass than [Mn]. This likewise supports the conclusion that moderate flood stress was common at all sites. In microtidal systems, such as these marshes, the elevation of the marsh platform varies little and is located within a centimeter of average daily high water (Nyman et al. 2009). This also supports the finding that marshes generally all experienced similar, but moderate levels of flooding stress. It is unlikely that all sites were severely flood-stressed because if they were, plants would unable to take up nutrients because extended flooding can disrupt nutrition (DeLaune et al. 1998, Mendelssohn and Morris 2000).

In the results of the PCA, [Ca] always loaded highly with [Mn] and never loaded highly with total live biomass. This suggests that [Ca] is more related to flooding levels than overall production, as previously hypothesized (Tobias et al. in review). In summer at least, [Ca] < or > 0.26% was better able to discriminate between sites that were known to be flooded and those that were known to be relatively dry, respectively. Also, because Ca is not translocated in plant tissue (Jones 1998), it may more accurately reflect more recent flooding conditions than Mn, which can be translocated from older leaves to new growth. One potential problem with [Ca] as an indicator of flooding stress is that its uptake may be influenced by several factors other than flooding such as N-availability (Jones 1998) or salinity (Epstein and Bloom 2005). As with [Mn], however, more research is needed and more appropriate water level data would be necessary to make firm conclusions about the usefulness of [Ca] as an indicator of flooding stress.

Where N starvation or salinity stress is the primary cause of limited productivity, increasing production can be seen as an issue of extending the growing season or increasing growth rates.

Reducing salinity early in the growing season can increase growth rates and increasing N-availability later in the growing season can increase overall biomass. The results of PCA on indicators of limiting factors and biomass suggest that some indicators are more strongly related to productivity in some seasons than in others. This is consistent with other studies that also found that indicators of the causes of limited production may change throughout the growing season as the nutrient requirements of plants change (Ewing et al. 1995). For example, one study found that indicators of salinity limitation were effective only in spring and summer while indicators of nutrient-limitation were effective in fall (Ewing et al. 1997). In this study, high [Na] is the most important controller of biomass production in spring. N availability is secondarily important, but flooding only accounted for a small proportion of the variability. This suggests that during spring, reducing salinity should be a primary management objective. Although the availability of N ultimately determines biomass accrual in *S. alterniflora* and *Distichlis spicata*, elevated sediment salinity reduces growth rates (Smart and Barko 1980). This supports our finding that salinity levels are more important for controlling biomass production in *S. patens* marshes in the spring. In spring, flooding stress from short flooding durations should have little impact on biomass production. Because I was unable to determine accurate amounts of flooding for each site and because prolonged flooding may reduce root biomass, and thus the ability of plants to take up N, I recommend pulsed flooding events rather than prolonged flooding as a tool to lower salinity.

During summer, N starvation becomes an important factor in reduced biomass production where salinity stress is also low. This is not surprising because Valiela et al (1976) found that growth rate was highest in summer, as was the depletion of porewater-N. Rapid growth would deplete N, making it a limiting factor. The effect of the interaction of salinity and N availability on productivity may be stronger in summer than in spring such that N demand increases with salinity because the internal N supply required in support of growth for *S. alterniflora* increases with salinity (Bradley and Morris 1992). In summer, although indicators of flooding stress accounted for most of the variation in our leaf tissue

chemistry, biomass was most highly associated with variation of C:N and [Na] in leaf tissue. At high salinity levels *S. patens* biomass was low regardless of N-availability, a finding that is consistent with the findings of Merino et al. (2008).

Seasonal patterns in C:N ratios suggest that the timing of freshwater introductions is critical. To have maximum impact on production, freshwater introductions should be timed for late spring and early summer such that C:N ratios of *S. patens* are beginning to increase. Adding N too early in the growing season, when N is still available in excess, may have little to no effect on production. The seasonal shift in C:N ratios from low to high during the growing season probably results from a combination of physiological factors and seasonal changes in N availability. Early in the growing season, plants are small and apparently find as much N as they need from mineralization of soil organic matter. Spring floods also deliver N-rich water to marshes that are hydrologically connected to rivers. This combination of factors leads to low C:N ratios in *S. patens* in the spring. Later in the growing season, plants demand more N to supply their larger biomass and to produce osmotica to block $Na^+$ uptake (Bradley and Morris 1992). This combination of factors makes N starvation more likely toward the end of the growing season.

Na:K in leaf tissue was most closely related to productivity during spring. If Na:K is to be interpreted as an indicator of salinity tolerance (Maathuis and Amtmann 1999), this supports the hypothesis that controlling salinity levels is most important early in the growing season. It would be important for plants to increase their [K] in spring to reduce [Na] uptake and keep growth rates as high as possible. Intermediate sites likely have lower Na:K than saline sites because plants exhibit luxury uptake of K when salinity levels are low and at high salinity [Na] may enter roots through an alternate pathway that is not blocked by high [K] (Flowers and Colmer 2008).

Identifying limiting factors can be useful for managers whose goal is to increase biomass production in coastal marshes and for those who use stress to achieve other goals such as replacing invasive species with natives. Seasonal patterns in biomass production and elemental concentrations in leaf tissue suggest that summer is the most appropriate time to collect tissue samples of *S. patens* to diagnose limiting factors in coastal Louisiana. The combination of C:N and [Na] can be used to diagnose the interacting effects of N-limitation and salinity stress. Seasonal patterns also suggest that decreasing salinity in spring and increasing N-availability in summer would be an effective strategy for increasing production. [Mn] and [Ca] appear to indicate flooding stress, but further study is needed to refine guidelines for their use. The guidelines presented in this paper have only been tested for *S. patens* in Louisiana's coastal marshes. While a similar approach would likely be an effective way to diagnose the causes of limited production in other species or *S. patens* in other locations, further study is necessary before these guidelines are used to inform management decisions for these situations. In particular, the most appropriate time to take tissue samples may be earlier in the year for *S. patens* marshes at higher latitudes because they have shorter growing seasons and peak production is likely to be earlier. Also, different species have different nutrient requirements and stress tolerances so separate guidelines should be developed for different species.

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# CHAPTER 6.
# ABOVEGROUND INDICATORS OF FLOODING STRESS IN BELOWGROUND BIOMASS OF *SPARTINA PATENS*

## Introduction

Root productivity in marshes in coastal Louisiana is essential for maintaining stability against factors that contribute to marsh loss. Vegetative growth of roots controls vertical accretion in these marshes, making productivity an important factor in the ability of marshes to keep up with sea level rise (Nyman et al. 2006). Live root material contributes to the strength of marsh soils (McGinnis 1997), thus increasing their resistance to erosion (Nyman et al. 1995). In this paper I investigate factors that affect root productivity of *Spartina patens* because it is the most common plant species in coastal Louisiana (Chabreck 1970).

Numerous studies have examined the effects of flooding on aboveground biomass of *Spartina spp*. (e.g., Broome et al. 1995, Bandyopadhyay et al. 2003, Visser 2006, Morris 2007). Flooding has been shown to reduce above-ground production in *S. patens* (Webb et al. 1995, Tobias et al. in review). Relatively few studies have attempted to quantify belowground productivity in *Spartina spp.* and most of these have focused on the effects of nutrient additions on root productivity. These studies have generally concluded that while N-fertilization may increase aboveground biomass, it either decreases (Swarzenski et al. 2008, Valiela et al. 1976) or it has no effect on belowground production (Wigand et al. 2004, Darby and Turner 2008b). Even fewer studies have been conducted on the effects of flooding on the belowground biomass of *Spartina spp*. and these have generally found that flooding inhibits root productivity (Valiela et al. 1976, Howes et al. 1981, Nyman et al. 1995). None of these studies have specifically manipulated flooding levels, but have made observations of relative flooding levels in a field setting.

The primary purposes of this paper are to examine the effects of flooding on (1) below-ground biomass of *S. patens* and (2) biomass partitioning between above- and belowground portions of the

plant. I also consider relationships of indicators of N-limitation and salinity stress in aboveground biomass (C:N and [Na] in leaf tissue, respectively; Tobias et al. 2010) with belowground biomass. I expected that belowground biomass would decrease as average depth of flooding increases because flooding above the soil surface reduces aboveground biomass in *S. patens* in summer (Tobias et al. in review). If flooding stress limits belowground biomass, I also expect that belowground biomass will be negatively associated with [Mn] and [Ca] in leaf tissue, as is the case with aboveground biomass (Tobias et al. in review). I examine the ratio of live belowground biomass to live aboveground biomass (root:shoot ratio) because it has been shown to increase with increased flooding levels (Knox et al. 1986). I hypothesize that plants that are N-limited will have higher root biomass than plants that are not N-limited because increased N-availability has been shown to reduce overall belowground biomass (Swarzenski et al. 2008). I expected that root:shoot ratio would increase with increasing N-limitation because aboveground biomass is limited by N, while belowground biomass does not increase with N-fertlization (Darby and Turner 2008). I expected that increased salinity stress would reduce belowground biomass and made no predictions for how salinity stress would affect root:shoot ratio.

I examined relationships between belowground biomass and leaf tissue chemistry, rather than root tissue chemistry because such comparisons would allow managers to use leaf tissue chemistry to infer which factor or factors limit belowground production. My previous studies haved suggested the use of leaf tissue chemistry as a tool for indentifying factors limiting aboveground production and it is important to understand how management decisions based on these tools will affect belowground production as well.

**Methods**

Manipulating flooding stress traditionally has utilized greenhouse studies (e.g., Howard and Mendelssohn 1999) or three levels of flooding in the field (e.g., Webb et al. 1995), but I used a recently developed field-based technique that creates six levels of flooding stress (Morris 2007). These

installations are termed “marsh organs” because they resemble the pipes on a pipe organ. Marsh organs were constructed from 36 15.2-cm-diameter PVC pipes that were bolted together for stability. Each marsh organ consisted of six rows of six pipes in each row (Figure 31). The pipes were cut to lengths of 122, 107, 91, 76, 61, and 46 cm. For the purposes of this paper, rows are defined as the set of six pipes of equal elevation within a marsh organ. I identified rows by numbers such that “row one” was the tallest (least flooded) and “row six” was the shortest (most flooded). Columns are defined as a set of contiguous pipes consisting of one pipe of each elevation within a marsh organ. I identified columns using letters such that column A is to the west and column F is to the east.

I installed marsh organs at four locations in coastal Louisiana in the summer of 2007. Locations were selected to represent a range of conditions experienced by *S. patens* in Louisiana’s coastal marshes. *S. patens* in adjacent marshes at all sites ranged from rare to dominant. Marshes at Marsh Island Wildlife Refuge (29°34’47” N, 92°00’40” W and 29°34’42” N, 91°49’29” W) receive fresh water and sediment from the Atchafalaya River. Soils at Rockefeller Refuge sites (29°37’54” N, 92°38’18” W and 29°37’12” N, 92°34’11” W) developed without direct riverine influences. Following Penfound and Hathaway’s (1938) classification system for coastal marshes, I installed one marsh organ in a saline area where the surrounding marsh was dominated by *Spartina alterniflora* and one marsh organ in an intermediate marsh where the surrounding marsh was dominated by *S. patens* and contained some *Sagittaria lancifolia* and/or *Typha domingensis* at each refuge.

Marsh organs were installed in shallow ponds or lakes within marshes. I oriented the organs so that the tallest pipes were to the north to allow maximum sun exposure for all pipes. Organs were dug into the soil to a level such that the fourth row from the top of the organ was even with the surface of the adjacent marsh. This resulted in row 1 being approximately 46 cm above local marsh elevation and row 6 being approximately 30 cm below local marsh elevation. I adjusted each marsh organ to ensure that the rows were level following installation.

I filled the pipes with a mixture of local pond sediment and marsh soil to the top of each pipe. I planted each pipe with approximately ten stems of S. patens collected from the adjacent marsh in 2007. Although care was taken to select only S. patens for planting, a few pipes included other species when harvested in 2008. In spring of 2008 most of the pipes had lost some soil elevation (min = -5, max = 40, avg = 11 cm for all four organs). To re-establish soil elevation to the intended levels, I lifted the plants out of the pipes, refilled the pipes with pond sediment, and replaced the plants. Care was taken to avoid breaking stems or damaging roots. At the time of refilling, I also replaced any plants that were completely missing or showed no signs of live tissue with 20 new stems collected from the adjacent marsh. I replaced all plants in rows 5 and 6, except for three plants that were able to survive in these rows in the saline marsh organ at Rockefeller Refuge. I also replaced seven plants in rows 1-4. I replaced plants with 20 stems rather than 10 stems, as in the original planting, because I wanted the size of the replacement plants to be of a size similar to the plants that had been growing in the pipes rather than the original size of the plants.

I harvested half of the pipes from each marsh organ in summer 2008 (columns B, D, and F) and half in fall 2008 (columns A, C, and E). Above- and below-ground biomass were separated at the soil

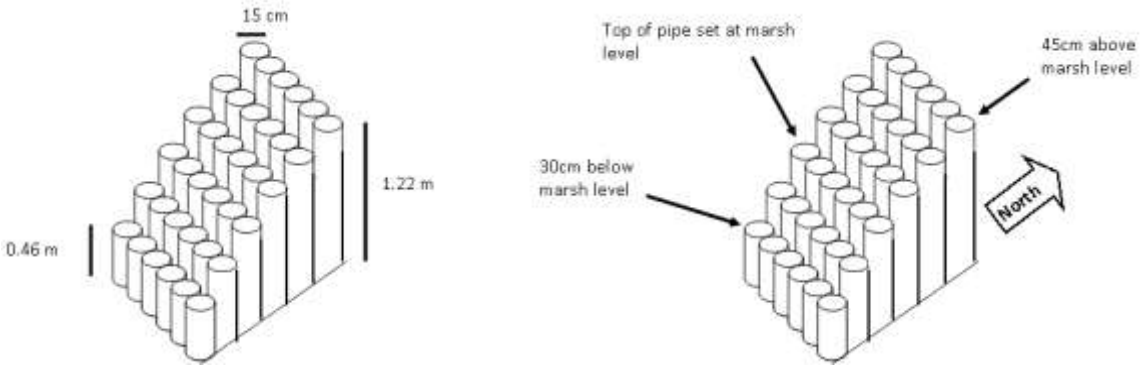


Figure 31: Shape, size, and orientation of marsh organs. Organs consist of six rows and six columns of 15 cm diameter PVC pipe. Heights of rows are 123, 107, 91, 76, 61, and 46 cm from the bottom of the pipes. Marsh organs are set into the pond sediment such that the top of the fourth row is at local marsh level. Note: Diagrams are not to scale.

surface with a sharp serrated bread knife and above- and below-ground portions of plants were transported to the lab in separate bags.

I attempted to collect porewater from within the pipes prior to harvest. This was not possible, however, because despite lengthy attempts to extract porewater at 10, 15, and 20 cm below the soil surface, there simply was not enough porewater in the pipes to conduct any tests. Instead, I collected porewater from the nearby marsh. I collected porewater at 10 cm below the soil surface with a syringe. I measured salinity, conductivity, and pH with a hand-held salinity meter (YSI model 63). I also collected porewater samples for nutrient analysis. These samples were filtered with 0.45 µm nylon filters (Watman) to remove particles. I stored porewater samples on ice until they could be analyzed. I determined the concentrations of ammonia-N using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et al. 1998).

I measured soil redox potential (Eh) at 10 cm below the soil surface with Pt electrodes, a calomel reference electrode (accumet), and a pH/mV/temperature meter ("Oyster 10" by Extech Instruments). Prior to use, Pt electrodes were cleaned with souring powder and a brush. Pt electrodes were also tested by measuring Eh of a solution of quinhydrone in standard pH 4 and pH 7 solutions. Taking multiple Eh measurements for each pipe would have been ideal, but because of limited space inside the pipes, only one electrode of each type could be inserted into the soil. This resulted in a single measurement of Eh for each pipe at the time plants were harvested.

I measured soil elevation loss inside the pipes and the depth of pond water relative to the top of each pipe at the time of each harvest. Hourly water level data were obtained from water level loggers at Coastwide Reference Monitoring System (CRMS) sites near marsh organs (stations 0523, 0530, 0608, and 0610; LDNR 2008). Distances between CRMS stations and marsh organs ranged from 0.2-6.9 km. Hourly CRMS water level data and water level and soil elevation measurements taken immediately prior

to harvesting plants were used to calculate the mean depth of water relative to the soil for each pipe for two weeks prior to harvest.

Although all of the marsh organs were completely submerged by storm surge from Hurricane Ike, which made landfall on September 13, 2008, minimal damage to the installations was observed following the hurricane. One exception was that the saline marsh organ at Rockefeller Refuge had tilted slightly and visual inspection indicated that relatively large amounts of soil elevation had been lost via undercutting in some of the pipes in this organ. The resulting soil elevations that have been measured may therefore have been unrepresentative of the growing conditions during fall.

In the lab, I washed all sediment from roots with tap water. Root masses were broken apart to remove sediment that was trapped between roots. Roots were considered "clean" when water passing through them remained clear. All material (root and peat) that was retained by a 2 mm standard test sieve (Fisher Scientific no. 10) was then sorted. I placed washed roots in a tub filled with 3-4 cm of water. With tweezers and a magnifying glass, I separated live roots from dead roots and peat material. I assumed that roots that were turgid, had root hairs attached, and were light orange to white in color were alive. Roots that met this description will henceforth be referred to as "live roots." Roots that were gray in color, squishy, and/or retained no root hairs were assumed to be dead, as was the partially decomposed material that comprised the peat. Roots that met this description will henceforth be referred to as "dead roots." I also considered any live rhizomes and stem material that was below the soil surface when the plants were harvested to be live belowground biomass. Live biomass was dried to a constant weight at 60°C and weighed.

I developed a subsampling method to reduce sorting time for large root samples. First, I removed all large pieces of live material from the sample. These were dried and weighed as previously stated. When subsequent sorting yielded a minimal amount of live root material (< approximately 0.5 g

wet live root material in one hour), the remaining roots were mixed in the tub to produce a uniform mix of live and dead material. I separated this mix into 16 pieces of uniform area. I randomly selected two of these pieces to be completely sorted. For these two "subsamples" I dried and weighed both the live and dead portions of the subsample. The remaining 7/8 of the mixed live and dead material was dried and weighed together. To calculate the amount of live root biomass of the unsorted portion of a sample, I multiplied the average proportion of live:dead material in the two subsamples by the dry weight of the unsorted mixed portion. To calculate the total live below-ground biomass of the pot, I added the calculated live biomass of the unsorted portion, the live biomass of the two subsamples, and the biomass of the large pieces of live material that were originally sorted out of the sample.

I calculated the root:shoot ratio for each pipe by dividing the total live root and rhizome biomass for each pipe by the live aboveground biomass of all species. I used all species rather than only *S. patens* because I was unable to identify roots to species. Also, pipes with species other than *S. patens* were extremely rare, and in most cases only a few stems were present.

Although I installed four marsh organs, data presented in this paper are from only three. The pond in which I installed the marsh organ in the intermediate marsh at Rockefeller Refuge was completely drained at the time of harvest in summer. I was unable to calculate average depths of flooding for this marsh organ because I was unable to measure water levels at the time of harvest. Examination of data from water level recorders suggests that the pond had been drained for at least two weeks. The draining of the pond indicated that there was little to no gradient in flooding stress imposed on plants in this marsh organ.

I used multisource regression (SAS PROC MIXED) to identify factors that affected the relationships of flooding level with live root biomass and the ratio of live root biomass to live aboveground biomass (root:shoot ratio). A second regression (SAS PROC REG) or ANOVA (SAS PROC

GLM) was run as indicated by the results of the multisource regression, with only the variables that were significant, to determine regression coefficients or least squares means of groups. I used the type 3 (partial) sums of squares reported by PROC GLM to estimate the percent of the variability in root:shoot ratio explained by significant terms in the model. I used Pearson correlations (SAS PROC CORR) to look for associations between elemental components of leaf tissue and below-ground biomass and root:shoot ratio. Correlations were estimated separately for each season because previous analysis showed that the elemental composition of leaf tissue differed seasonally (Tobias et al. in review). Root:shoot ratio was log transformed to improve normality.

**Results**

In summer, porewater salinity was higher at saline sites than intermediate sites, as were N and phosphorus availability (Table 16). In fall, after Hurricane Ike, salinity was similar at all sites and nutrient availability was higher in intermediate sites than in summer.

In a multisource regression analysis, the relationship between average flooding level and live root biomass was not significantly different among locations or between seasons (Table 17). Live root biomass decreased with increased flooding (Figure 32). There was no significant effect of flooding level on the log transformed root:shoot ratio (Table 18). There was, however, a significant effect of the interaction of season and location on the log transformed root:shoot ratio ($F_{2,57} = 3.73$, $p = 0.0301$). This interaction only explained approximately 9.7% of the variation in the log transformed root:shoot ratio, however. Root:shoot ratio was higher in fall than summer for all three locations (Figure 33).

In summer, increased live root biomass was significantly associated with higher concentrations of Mn and Ca in leaf tissue (Table 19). Log transformed root:shoot ratio was significantly associated with [Ca] and [Mn] in leaf tissue in summer (Table 20). I report correlations during fall to facilitate comparisons with other studies that have reported end of season tissue concentrations. I do not

interpret these associations, however, because I found in previous studies that the elemental composition of leaf tissue in the fall is not a good indicator of growing conditions for *S. patens* (Tobias et al. in review, Tobias et al unpublished data). Root:shoot ratio was not associated with total biomass (above- + below-ground biomass) in summer ($r = 0.16700$, $p = 0.3376$) or fall ($r = -0.29351$, $p = 0.0974$).

**Discussion**

In summer, sites with higher porewater salinity also had higher nutrient availability. Higher salinity in fall than in spring at intermediate sites was caused by inundation of our sites with saline storm surge from Hurricane Ike. The increase in nutrient availability at intermediate sites in fall is also likely related to the effects of storm surge. Nutrient availability increases when plants are stressed by other factors such as high salinity or flooding because stressed plants grow more slowly and are unable to take up nutrients that are available. Conversely, in places where plants are not stressed by other factors, they deplete nutrients in porewater and this becomes the factor that limits plant production.

Salinity, water level, or latitudinal differences may be more important than nutrient availability for determining belowground production of *Spartina alterniflora* Darby and Turner (2008c). Our results suggest that water level is the most important factor controlling production of belowground biomass of *S. patens*, although I did not test the effects of latitudinal climate differences. Increasing water levels significantly reduced root biomass, regardless of location or season. Elemental analysis of leaf tissue showed that [Mn] and [Ca] in leaf tissue, which vary with flooding stress (Tobias et al. in review), also vary with below-ground biomass in this study. Plants whose belowground biomass is limited by flooding stress take up less Ca because rates of Ca absorption are governed by the size of the root system (Loneragan and Snowball 1968). Increasing water levels did not affect root:shoot ratio, but root:shoot ratio varied by season and location. This suggests that flooding affects above- and belowground

Table 16: Chemistry of porewater extracted from 10 cm below the marsh surface adjacent to marsh organ installations. No means or standard errors are included because porewater chemistry presented here represents single measurements taken in each adjacent marsh.

| | Summer | | | | Fall | | | |
|---|---|---|---|---|---|---|---|---|
| | Intermediate | | Saline | | Intermediate | | Saline | |
| | Rockefeller Refuge | Marsh Island | Rockefeller Refuge | Marsh Island | Rockefeller Refuge | Marsh Island | Rockefeller Refuge | Marsh Island |
| Conductivity (mS) | 16.7 | 3.77 | 27.05 | 13.49 | 21.16 | 18.77 | 26.29 | 12.12 |
| Salinity (ppt) | 9.7 | 1.8 | 16.5 | 7.6 | 12.7 | 11.2 | 16.1 | 6.9 |
| pH | 6.65 | 6.41 | 6.89 | 5.69 | 6.93 | 6.47 | 7.26 | 6.49 |
| Orthophosphate (mg/L) | 1.44 | 1.86 | 8.40 | 5.34 | 3.48 | 4.80 | 7.62 | 2.52 |
| Ammonium-N (mg/L) | 0.78 | 0.84 | 27.52 | 2.16 | 4.62 | 0.78 | 24.75 | 0.90 |

Table 17: Results of a multisource regression analysis of the effects of water level, location, and season of harvest on live root biomass of *Spartina patens*. Water level is the average water level (cm) for two weeks prior to harvest, which was calculated from hourly water level measurements at nearby Coastwide Reference Monitoring System (CRMS) stations. Site indicates one of three marsh organs (Rockefeller saline, Marsh Island saline, and Marsh Island intermediate). Season indicates the time of harvest (summer or fall).

| Effect | Num DF | Den DF | F | p |
|---|---|---|---|---|
| Water Level | 1 | 59 | 29.68 | <0.0001 |
| Site | 2 | 59 | 1.44 | 0.2454 |
| Season | 1 | 59 | 0.00 | 0.9676 |
| Water Level X Site | 2 | 59 | 0.66 | 0.5201 |
| Water Level X Season | 1 | 59 | 0.09 | 0.7688 |
| Season X Site | 2 | 59 | 0.1 | 0.9013 |
| Water Level X Season X Site | 2 | 59 | 2.82 | 0.0676 |

Table 18: Multisource regression analysis of the effects of water level, location, and season of harvest on the ratio of live root biomass:live shoot biomass of *Spartina patens*. Water level is the average water level (cm) for two weeks prior to harvest, which was calculated from hourly water level measurements at nearby Coastwide Reference Monitoring System (CRMS) stations. Site indicates one of three marsh organs (Rockefeller saline, Marsh Island saline, and Marsh Island intermediate). Season indicates the time of harvest (summer or fall).

| Effect | Num DF | Den DF | F | p |
|---|---|---|---|---|
| Water Level | 1 | 51 | 0.3 | 0.5891 |
| Location | 2 | 51 | 3.58 | 0.0352 |
| Season | 1 | 51 | 1.91 | 0.1726 |
| Water Level X Location | 2 | 51 | 0.05 | 0.9559 |
| Water Level X Season | 1 | 51 | 0.21 | 0.647 |
| Season X Location | 2 | 51 | 4.9 | 0.0113 |
| Water Level X Season X Location | 2 | 51 | 1.86 | 0.1666 |

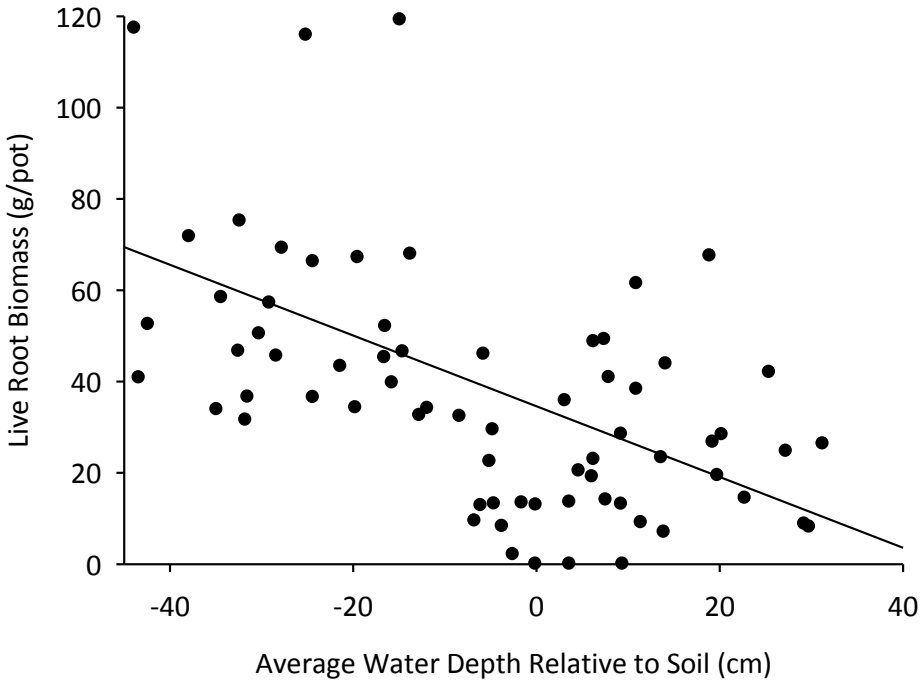


Figure 32: Relationship between the average depth of flooding relative to soil within each pot for two weeks prior to plant harvest and live root biomass of *Spartina patens* grown at varying levels above and below local marsh elevation. Flooding depth was calculated with hourly water level data from the nearest Coastwide Reference Monitoring System (CRMS) station. Regression equation: slope = -0.67351 ($p < 0.0001$), intercept = 33.00305 ($p < 0.0001$), $R^2 = 0.3004$ (PROC MIXED; SAS)

Table 19: Correlations of leaf tissue chemistry with belowground biomass of *Spartina patens* grown under varying levels of flooding. Leaf tissue was collected from leaves originating from the top 15 cm of a plant's stem. Correlation coefficients (r) and p-values (p) were estimated with PROC CORR (SAS). The number of samples included in the analysis is represented by "n."

| | Summer | | | Fall | | |
|---|---|---|---|---|---|---|
| | r | p | n | r | p | n |
| C:N | 0.03573 | 0.8410 | 34 | 0.25332 | 0.2323 | 24 |
| N:P | -0.22570 | 0.2066 | 33 | 0.49491 | 0.0102 | 26 |
| Na:K | -0.07071 | 0.6958 | 33 | -0.37194 | 0.0613 | 26 |
| [Ca] | 0.59987 | 0.0002 | 34 | -0.07154 | 0.7284 | 26 |
| [Mn] | 0.57493 | 0.0004 | 34 | -0.16199 | 0.4292 | 26 |
| [N] | -0.11758 | 0.5078 | 34 | 0.26267 | 0.1948 | 26 |
| [P] | 0.12482 | 0.4818 | 34 | -0.18847 | 0.3565 | 26 |
| [Na] | 0.20534 | 0.2440 | 34 | -0.3831 | 0.0534 | 26 |

Table 20: Correlations of leaf tissue chemistry with log transformed root:shoot ratio of *Spartina patens* grown under varying levels of flooding. Leaf tissue was collected from leaves originating from the top 15 cm of a plant's stem. Correlation coefficients (r) and p-values (p) were estimated with PROC CORR (SAS). The number of samples included in the analysis is represented by "n."

| | Summer | | | Fall | | |
|---|---|---|---|---|---|---|
| | r | p | n | r | p | n |
| C:N | 0.07467 | 0.6699 | 35 | 0.09246 | 0.6674 | 24 |
| N:P | -0.05131 | 0.7732 | 34 | -0.41470 | 0.0393 | 25 |
| Na:K | 0.24214 | 0.1677 | 34 | 0.13735 | 0.5127 | 25 |
| [Ca] | 0.38409 | 0.0227 | 35 | -0.23109 | 0.2664 | 25 |
| [Mn] | 0.37827 | 0.0251 | 35 | 0.40739 | 0.0432 | 25 |
| [N] | -0.06607 | 0.7061 | 35 | -0.49101 | 0.0127 | 25 |
| [P] | 0.01095 | 0.9502 | 35 | -0.12178 | 0.5620 | 25 |
| [Na] | 0.22596 | 0.1918 | 35 | -0.04772 | 0.8208 | 25 |

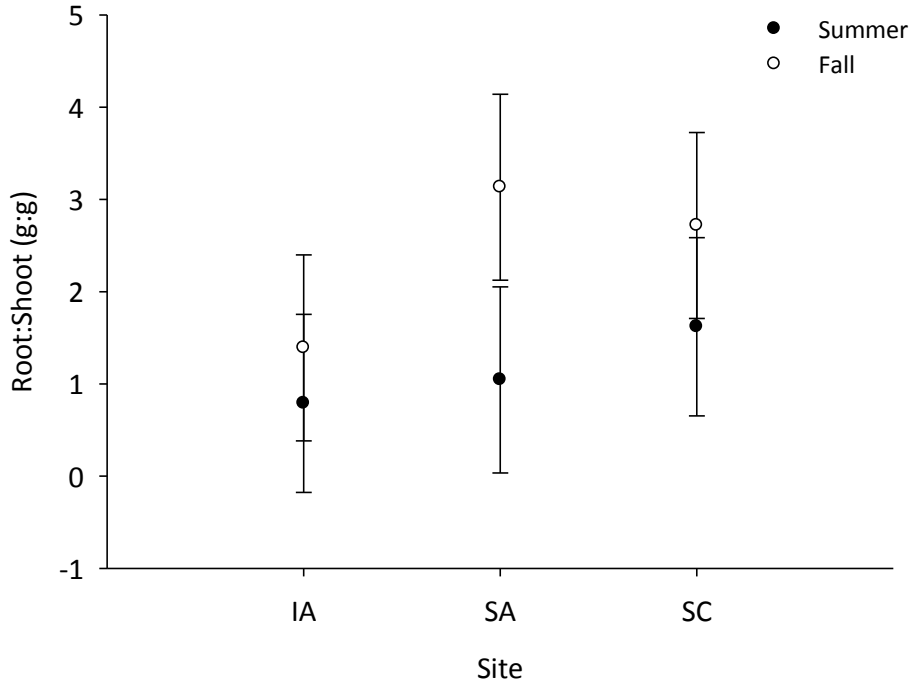


Figure 33: Differences in root:shoot ratio of *Spartina patens* grown at varying heights above and below local marsh elevation by season and sample site. Sample sites were located in intermediate Atchafalaya (IA), saline Atchafalaya (SC), and saline Chenier Plain (SC) marshes.

biomass in a similar manner, but that changes in nutrient availability, salinity, and/or other, local conditions that were not measured in this study may alter the allocation of biomass between roots and shoots. However, the interacting effects of season and location explained a very small proportion of the variation in root:shoot ratio. In contrast to our results, high root:shoot ratios have been observed in *S. alterniflora* growing in unfavorable soil conditions (Knox 1986) and high root:shoot ratios have been interpreted as evidence of flooding stress (Nyman et al. 1995).

Nutrient uptake, as measured by C:N, N:P, [N] and [P] in leaf tissue, had little, if any, relationship with belowground biomass or root:shoot ratio in this study. Numerous studies have shown that increased N-availability increases aboveground biomass of *S. patens* (e.g., Foret 2001, DeLaune et al. 2003, Merino et al. 2010). Previous research has also shown that while N-fertilization stimulates *S. patens* to increase above-ground production, it does not necessarily increase root and rhizome biomass (Wigand et al. 2004, Darby and Turner 2008b). Our finding that N:P ratio and [P] in leaf tissue were not correlated with belowground biomass or root:shoot ratio during summer contrasts with previous studies that found that P-fertilization reduced belowground biomass in *S. alterniflora* (Darby and Turner 2008 a and b). Plants in our study did not exhibit these patterns. I did not fertilize plants, however, so the difference in results between these studies could be related to the difference in ranges in nutrient availability. Decomposition of root material may also be stimulated by increased P-availability that stimulates microbial activity in salt marsh soils (Sundareshwar et al. 2003).

Although I did find associations of N:P with belowground biomass and root:shoot ratio in fall, leaf tissue concentrations of nutrients in *S. patens* do not accurately reflect factors that limit production in the fall (Tobias et al. unpublished data). If these associations were to be interpreted, however, they would suggest that N-uptake rather than P-uptake controls root:shoot ratio, and that it does so by disproportionately increasing aboveground biomass relative to belowground biomass rather than by reducing belowground biomass because increased N:P ratios in leaf tissue are associated with increased

belowground biomass and decreased root:shoot ratio. Also, in a study of the aboveground portions of the same plants used in this study, increased N in leaf tissue was associated with increased shoot biomass in fall (Tobias et al. unpublished data). Our interpretation does not exclude the possibility that increased P-uptake relative to N-uptake reduces root:shoot ratio in fall. However, I suggest that this scenario is irrelevant to overall biomass production because does not hold during periods of active plant growth and that it is unlikely given that there is evidence to suggest that during fall increased N-uptake increases aboveground biomass relative to belowground biomass.

The differences in results between our study and previous studies could also result from differences in nutrient requirements among species. *S. patens* incorporates less N and P into leaf tissue than *S. alterniflora* growing under the same conditions and differences in leaf tissue N are greater in the fall than in spring or summer (Tobias et al. unpublished data). *S. alterniflora* translocates resources from roots and rhizomes to above-ground tissue in spring and fall to support rapid spring growth and fall inflorescence production that results in a decrease in below-ground biomass during these times (Darby and Turner 2008). Increased root:shoot ratio in fall relative to summer in *S. patens* could suggest that plants reallocate biomass to roots during fall as a result of senescence. This interpretation of our results should be undertaken with caution, however, because the change in root:shoot ratio in fall samples could also result from shoot death in response to high salinity following Hurricane Ike, rather than from normal seasonal changes in biomass allocation.

The lack of correlation between belowground biomass and C:N ratio and [Na] in aboveground tissue may result from interacting effects of N availability and high salinity. Because increased nutrient availability and high salinity were confounded in this study, as they are in many coastal wetlands, high salinity and, thus high [Na] in leaf tissue, may have dampened the effects of increased nutrient availability on root biomass. Increased nutrient availability does not increase aboveground biomass of *S. patens* when salinity is high in a controlled setting (Merino et al. 2010) and it seems likely that the

interaction of salinity and nutrient availability would have similar effects on belowground biomass. Also, I would expect plants with healthier root systems (i.e. those that were not stressed by flooding in this study) to be able to regulate [Na] uptake, and thus [Na] in leaf tissue more effectively, but there was no evidence that [Na] or Na:K ratio in leaf tissue was related to flooding levels or belowground biomass.

The inability to obtain porewater samples from each pipe in the organ prevented me from evaluating possible interactive effects of nutrient availability, salinity stress, and flooding stress on biomass production. The variation in the relationship between average water depth and live belowground biomass suggests that factors other than depth of flooding contribute to belowground biomass, even when plants experience high levels of flooding. This contrasts with previous research that showed that at high levels of flooding, aboveground biomass increases linearly with decreasing flooding when plants are flooded above the soil surface, but that the relationship is much more variable when water levels are lower (Tobias et al. unpublished data). Also, average water depth explained less of the variation in live belowground biomass (approximately 30%) than in aboveground biomass (approximately 51%; Tobias et al. unpublished data). The contrast in effects of flooding on above- and belowground biomass suggests that increased availability of nutrients and/or low salinity may provide some protection from flooding stress for belowground biomass that it does not provide for aboveground biomass. Studies conducted with controlled levels of these three factors would be necessary to evaluate such interactions, however.

Although many studies show the effects of increased nutrient availability (e.g., Valiela et al. 1976, Wigand et al. 2004, Darby and Turner 2008 a, b, and c), studies are necessary to determine the response of root growth to reduced nutrient availability and the effects of interacting nutrient availability and flooding stress on root biomass. It could be that plants growing in oxidized soil are able to rapidly increase root production to forage for N or P as necessary, but that plants growing in anoxic soils cannot. This seems possible because root biomass is more sensitive to nutrient availability than

rhizome biomass and because peak root biomass occurs when porewater N is lowest (Valiela et al. 1976). This is likely not possible in highly reduced soils, however, so there is probably some benefit to plants having increased aboveground biomass when nutrient levels are high. Although N-fertilization does not increase root biomass, increased above-ground production in *S. alterniflora* resulting from N-fertilization has been shown to increase soil redox potential (Howes et al. 1981). This could result in an increase in decomposition of the peat surrounding the plants, but could also increase the ability of roots to forage for N if necessary because it is easier for roots to grow into oxidized soil than reduced soil.

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## CHAPTER 7.
## SUMMARY AND CONCLUSION

In this dissertation, I examined the response of the leaf tissue chemistry of *Spartina patens* to salinity stress, N starvation, and flooding stress. With these responses, I developed and tested guidelines for interpreting leaf tissue chemistry to identify causes of limited production in Louisiana's coastal marshes. Although tools for similar purposes have previously been developed, none were specifically for *S. patens* and none were inexpensive and convenient enough for use over large spatial scales and for repeated sampling over long time periods.

First I developed chemical signatures, or critical values, in relatively controlled settings. I examined the interacting effects of salinity and N availability on growth and the concentration of Na and ratio of C:N in *S. patens* leaf tissue in a greenhouse experiment. Plants grown under more saline conditions had higher concentrations of Na in their leaf tissue. Plants that were grown where N was limiting had higher ratios of C:N in their leaf tissue. On average, plants that were N limited had C:N ratios > 56 and plants that were salinity limited had Na concentrations > 1.1 %. I also determined the effects of flooding on *S. patens* growth and leaf tissue chemistry in a field experiment where plants were grown under various levels of flooding. Plants that experienced more flooding had less Mn and Ca in their leaf tissue than plants that experienced less flooding. Plants with average flooding levels above the soil surface had Mn concentrations < 223 ppm and Ca concentrations < 0.26 %.

Second, I compared the leaf tissue chemistry of *S. patens* and *S. alterniflora* growing under the same conditions in the field. The purpose of this experiment was to facilitate accurate comparisons of the leaf tissue chemistry of these species. Because data are not always available for both species, comparisons of leaf tissue chemistry are often made between these species to validate research results. Results of this study show that overall the leaf tissue chemistry of these species is different. Some elemental concentrations are similar between the species, however, and some elemental

concentrations vary seasonally. These direct comparisons provide a point of reference for determining which elements are comparable and in what seasons comparisons can and cannot be made.

Third, I conducted a field experiment to determine whether the chemical signatures I developed under controlled settings can be used in the field where water levels, salinity, and N availability fluctuate naturally. A second goal of this study was to determine whether there were seasonal changes in chemical signatures that should be taken into account when diagnosing the causes of limited production in *S. patens*. The relationship between live aboveground biomass and leaf tissue concentrations of Na and Mn and the ratio of C:N followed patterns expected in a limiting factor situation. I was unable to rigorously test the use of Mn and Ca concentrations to identify flooding stress, but I found no indications that they should not be used. Seasonal changes in leaf tissue chemistry indicate that factors that limit productivity change throughout the growing season. Salinity stress, as indicated by Na concentrations in leaf tissue, is the most important control on biomass production in spring. Both salinity stress and low N availability, as indicated by high C:N ratios in leaf tissue, are important in summer. Overall, summer is the most appropriate time of the year to use leaf tissue chemistry to diagnose limiting factors for *S. patens* in Louisiana. Based on seasonal patterns in productivity and leaf tissue composition, an effective management strategy for increasing aboveground biomass in *S. patens* marshes would be to reduce salinity in spring and increase N availability in summer.

Finally, I investigated the effects of flooding stress on belowground biomass of *S. patens* and relationships between belowground biomass and concentrations of elements in leaf tissue. The purpose of correlating aboveground chemical signatures and belowground biomass was to develop chemical signatures in leaf tissue that could diagnose limited root growth. Also, understanding how leaf tissue chemistry relates to root growth would help managers predict how management decisions based on leaf tissue would affect the growth of roots in *S. patens* marshes. Increased flooding reduced belowground biomass regardless of season or location. Concentrations of Mn and Ca in leaf tissue can be used to

identify reduced belowground biomass resulting from flooding. There was no relationship between belowground biomass and N or P in leaf tissue. These results suggest that leaf tissue concentrations of Mn and Ca, which can be used to identify limitation of growth in aboveground biomass, can also be used to identify limitation of growth in belowground biomass. This is important because it gives managers a simple way to test whether management plans involving flooding adversely affect belowground biomass.

When considered together, the results of these studies show that elemental concentrations in leaf tissue can be used to identify factors that limit productivity in above and belowground biomass of *S. patens*. In addition to being able to identify limiting factors in controlled and field settings, these tools are relatively inexpensive and efficient to implement because they rely on commercially available testing procedures. Although these tools cannot be applied directly to *S. alterniflora*, differences among the leaf tissue chemistry of these species appears predictable, and thus comparisons between the species can be made for some elements, if the time of year in which samples are taken is considered.

These studies also show that any of the three factors studied in this dissertation can limit biomass when the stress it induces is sufficiently high. The overall increase in biomass at lower levels of a stress factor depends on the intensity of other stressors. Managers need to keep these interactions in mind when developing plans to increase productivity by managing stressors on marshes. Also, other factors may be present that limit productivity, such as the availability of other nutrients, shading, competition with other plant species, and/or herbivory. Managers should also consider that seasonal changes in plant physiology and chemistry will impact the success of monitoring and management plans. Leaf tissue testing to determine limiting factors in *S. patens* biomass should be undertaken in summer because plants begin to show signs of N limitation during that time and because indicators of flooding stress can only be used at this time. Additionally, seasonal testing indicates that late spring and early

summer is the most effective time of year to introduce flooding to reduce salinity stress and increase N uptake.

These studies suggest several paths for future research. Although I developed tools to identify N limitation and salinity limitation that take into account the interacting effects of N availability and salinity, this dissertation does not quantify the effects of interactions between flooding stress and N availability and/or salinity. Additional research will be necessary to identify possible interactions among all three potential stressors. Another possible path for future research would be to use the tools I have developed to study spatial patterns in limiting factors. These spatial data could be used to improve models that predict the response of marshes to events such as sea level rise or tropical storm surge. They could also be used to identify places where specific restoration practices would be most effective and to monitor the response of marshes to restoration or management plans.

## APPENDIX A:
## MONTHLY WEATHER DATA

Table A1: Average monthly air temperature and precipitation for January 2007-December 2008 at Lake Charles, LA. These data were downloaded from the Louisiana Office of State Climatology (http://www.losc.lsu.edu/cgi-bin/newsmonthly.py). Data were downloaded for weather station "Lake Charles LCH."

| | Air Temperature | | | | | | | | | Precipitation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Averages | | | | | Extremes | | | | 1day | | 2day | |
| Month | Max | Min | Ave | Nrm | DFN | Max | Min | Total | DFN | Max | Dt | Max | Dt |
| Jan-07 | 58 | 44 | 51 | 51 | 0 | 76 | 29 | 8.79 | 3.27 | 2.77 | 4 | 2.77 | 4 |
| Feb-07 | 62 | 42 | 52 | 54 | -2 | 77 | 27 | 0.67 | -2.61 | 0.35 | 24 | 0.35 | 24 |
| Mar-07 | 73 | 53 | 63 | 61 | 2 | 81 | 30 | 4.39 | 0.85 | 1.53 | 14 | 1.53 | 14 |
| Apr-07 | 74 | 54 | 64 | 67 | -3 | 84 | 37 | 2.96 | -0.68 | 1.64 | 10 | 1.64 | 10 |
| May-07 | 84 | 67 | 76 | 75 | 1 | 93 | 54 | 11.33 | 5.27 | 2.86 | 3 | 2.86 | 3 |
| Jun-07 | 89 | 73 | 81 | 81 | 0 | 93 | 66 | 5.31 | -0.76 | 1.56 | 16 | 1.56 | 16 |
| Jul-07 | 89 | 75 | 82 | 83 | -1 | 93 | 69 | 11.43 | 6.3 | 3.36 | 6 | 4.46 | 5 |
| Aug-07 | 93 | 76 | 85 | 82 | 3 | 102 | 72 | 2.81 | -2.04 | 1.71 | 17 | 1.71 | 17 |
| Sep-07 | 89 | 71 | 80 | 78 | 2 | 93 | 63 | 5.71 | -0.24 | 1.97 | 13 | 3.08 | 12 |
| Oct-07 | 83 | 61 | 72 | 70 | 2 | 91 | 44 | 3.45 | -0.49 | 2.24 | 17 | 2.26 | 17 |
| Nov-07 | 72 | 53 | 62 | 60 | 2 | 84 | 38 | 6.18 | 1.57 | 1.7 | 21 | 2.62 | 24 |
| Dec-07 | 69 | 48 | 58 | 53 | 5 | 82 | 31 | 3.14 | -1.46 | 1.27 | 26 | 1.27 | 26 |
| Jan-08 | 60 | 42 | 51 | 51 | 0 | 77 | 24 | 5.1 | -0.42 | 1.26 | 16 | 1.5 | 15 |
| Feb-08 | 70 | 47 | 58 | 55 | 3 | 82 | 35 | 2.27 | -1.12 | 1.2 | 12 | 1.2 | 12 |
| Mar-08 | 72 | 49 | 61 | 61 | 0 | 90 | 34 | 3.05 | -0.49 | 2.09 | 10 | 2.09 | 10 |
| Apr-08 | 79 | 58 | 69 | 67 | 2 | 86 | 40 | 3.62 | -0.02 | 1.98 | 4 | 1.98 | 4 |
| May-08 | 85 | 68 | 76 | 75 | 1 | 92 | 54 | 3.12 | -2.94 | 2.43 | 15 | 2.44 | 14 |
| Jun-08 | 91 | 74 | 83 | 81 | 2 | 96 | 69 | 2.76 | -3.31 | 0.86 | 16 | 0.9 | 16 |
| Jul-08 | 92 | 74 | 83 | 83 | 0 | 96 | 68 | 5.36 | 0.23 | 4.44 | 15 | 4.44 | 15 |
| Aug-08 | 90 | 74 | 82 | 82 | 0 | 96 | 71 | 5.44 | 0.59 | 1.87 | 5 | 2.07 | 20 |
| Sep-08 | 86 | 68 | 77 | 78 | -1 | 92 | 57 | 5.35 | -0.6 | 1.39 | 1 | 1.83 | 1 |
| Oct-08 | 80 | 56 | 68 | 70 | -2 | 89 | 36 | 1.45 | -2.49 | 0.6 | 15 | 0.81 | 15 |
| Nov-08 | 72 | 47 | 60 | 60 | 0 | 84 | 33 | 3.41 | -1.2 | 1.65 | 12 | 1.65 | 12 |
| Dec-08 | 66 | 45 | 56 | 53 | 3 | 78 | 30 | 2.99 | -1.61 | 0.91 | 28 | 1.31 | 27 |

Table A2: Average monthly air temperature and precipitation for January 2007-December 2008 at Morgan City, LA. These data were downloaded from the Louisiana Office of State Climatology (http://www.losc.lsu.edu/cgi-bin/newsmonthly.py). Data were downloaded for weather station "Morgan City."

| | Air Temperature | | | | | | | | | Precipitation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Averages | | | | | Extremes | | | | 1day | | 2day | |
| Month | Max | Min | Ave | Nrm | DFN | Max | Min | Total | DFN | Max | Dt | Max | Dt |
| Jan-07 | 61 | 47 | 54 | 52 | 2 | 77 | 34 | 6.17 | 0.36 | 2.01 | 28 | 2.02 | 27 |
| Feb-07 | 63 | 44 | 53 | 55 | -2 | 80 | 31 | 0.9 | -3.49 | 0.56 | 21 | 0.56 | 21 |
| Mar-07 | 74 | 54 | 64 | 61 | 3 | 84 | 37 | 2.62 | -2.08 | 2.29 | 15 | 2.32 | 15 |
| Apr-07 | 77 | 56 | 67 | 67 | 0 | 87 | 37 | 2.41 | -1.81 | 0.83 | 11 | 1.08 | 10 |
| May-07 | 84 | 66 | 75 | 75 | 0 | 90 | 59 | 3.41 | -1.97 | 1.46 | 13 | 1.46 | 13 |
| Jun-07 | 89 | 72 | 80 | 80 | 0 | 94 | 65 | 6.31 | 0.5 | 1.38 | 10 | 1.74 | 19 |
| Jul-07 | 89 | 75 | 82 | 82 | 0 | 95 | 71 | 5.48 | -2.12 | 0.93 | 28 | 1.21 | 17 |
| Aug-07 | 92 | 76 | 84 | 81 | 3 | 101 | 73 | 8.09 | 0.69 | 1.48 | 16 | 1.83 | 16 |
| Sep-07 | 87 | 73 | 80 | 78 | 2 | 91 | 66 | 3.51 | -2.98 | 1.12 | 14 | 1.27 | 13 |
| Oct-07 | 81 | 64 | 72 | 70 | 2 | 89 | 47 | 5.95 | 2.29 | 2.77 | 23 | 2.77 | 23 |
| Nov-07 | 72 | 53 | 62 | 61 | 1 | 81 | 39 | 0.6 | -4.47 | 0.36 | 26 | 0.39 | 25 |
| Dec-07 | 68 | 50 | 59 | 55 | 4 | 79 | 34 | 5.95 | 1 | 1.71 | 21 | 1.71 | 21 |
| Jan-08 | 61 | 42 | 52 | 52 | 0 | 76 | 26 | 6.99 | 1.18 | 1.74 | 16 | 3.2 | 16 |
| Feb-08 | 70 | 47 | 58 | 55 | 3 | 80 | 34 | 1.09 | -3.45 | 0.65 | 1 | 0.65 | 1 |
| Mar-08 | 72 | 50 | 61 | 61 | 0 | 83 | 34 | 1.7 | -3 | 0.57 | 4 | 0.57 | 4 |
| Apr-08 | 78 | 60 | 69 | 67 | 2 | 85 | 45 | 2.94 | -1.28 | 2.37 | 27 | 2.37 | 27 |
| May-08 | 83 | 68 | 75 | 75 | 0 | 89 | 56 | 3.28 | -2.1 | 1.31 | 4 | 1.44 | 3 |
| Jun-08 | 87 | 73 | 80 | 80 | 0 | 91 | 66 | 3.77 | -2.04 | 0.93 | 16 | 1.33 | 26 |
| Jul-08 | 90 | 74 | 82 | 82 | 0 | 94 | 68 | 2.44 | -5.16 | 0.76 | 25 | 0.99 | 24 |
| Aug-08 | 87 | 73 | 80 | 81 | -1 | 95 | 69 | 10.8 | 3.4 | 2.28 | 9 | 2.32 | 13 |
| Sep-08 | 85 | 69 | 77 | 78 | -1 | 92 | 63 | 3.02 | -3.47 | 1.3 | 13 | 1.3 | 13 |
| Oct-08 | 80 | 60 | 70 | 70 | 0 | 89 | 40 | 0.29 | -3.37 | 0.16 | 23 | 0.16 | 23 |
| Nov-08 | 70 | 48 | 59 | 61 | -2 | 81 | 38 | 2.53 | -2.54 | 1.32 | 30 | 1.35 | 29 |
| Dec-08 | 67 | 46 | 57 | 55 | 2 | 80 | 31 | 1.98 | -2.97 | 0.49 | 29 | 0.8 | 28 |

**APPENDIX B:**
**MAPS OF SAMPLING LOCATIONS**

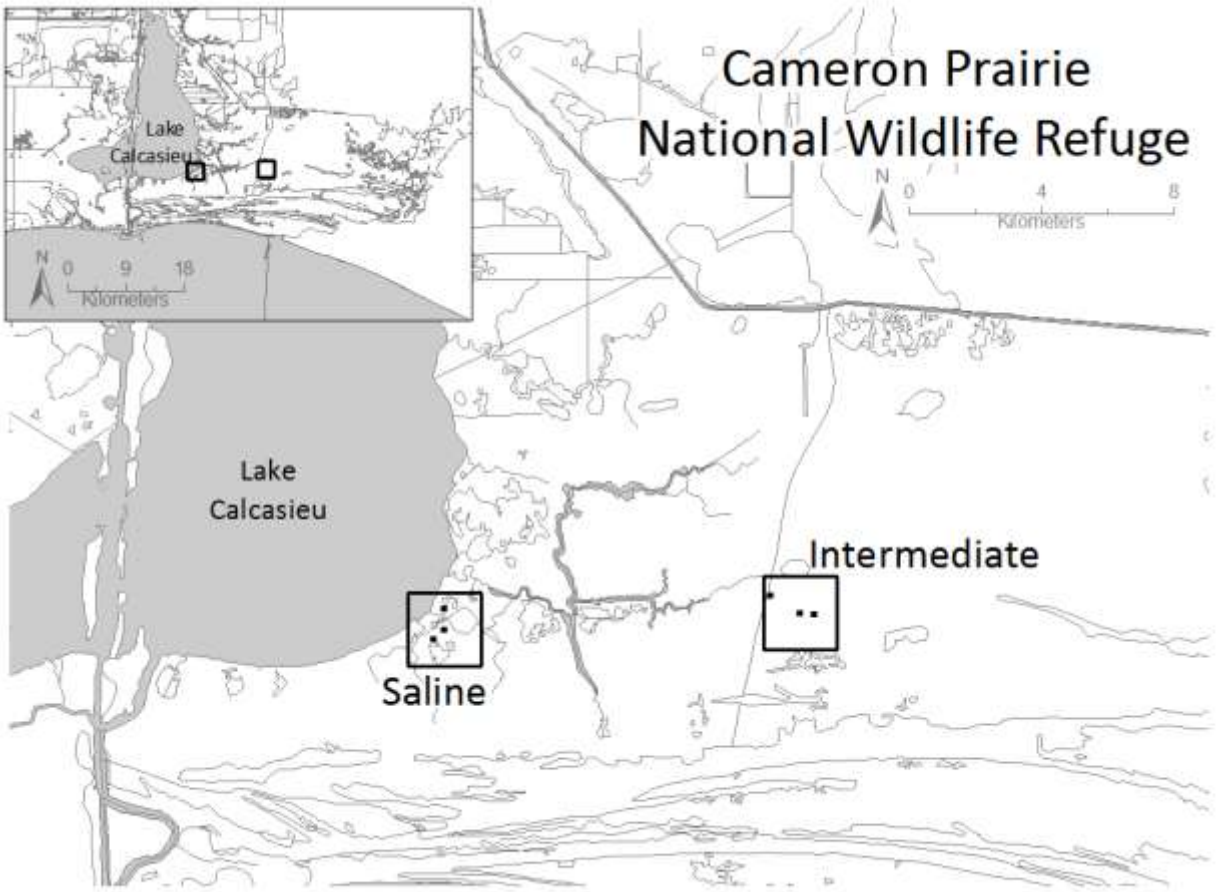


Figure B1: Map of sampling sites (hollow boxes) and plots (small black boxes) in saline and intermediate marshes in the vicinity of Cameron Prairie National Wildlife Refuge. Hydrographic data represented in this map were downloaded from the United States Geological Survey's National Hydrography Dataset website (http://nhd.usgs.gov/data.html).

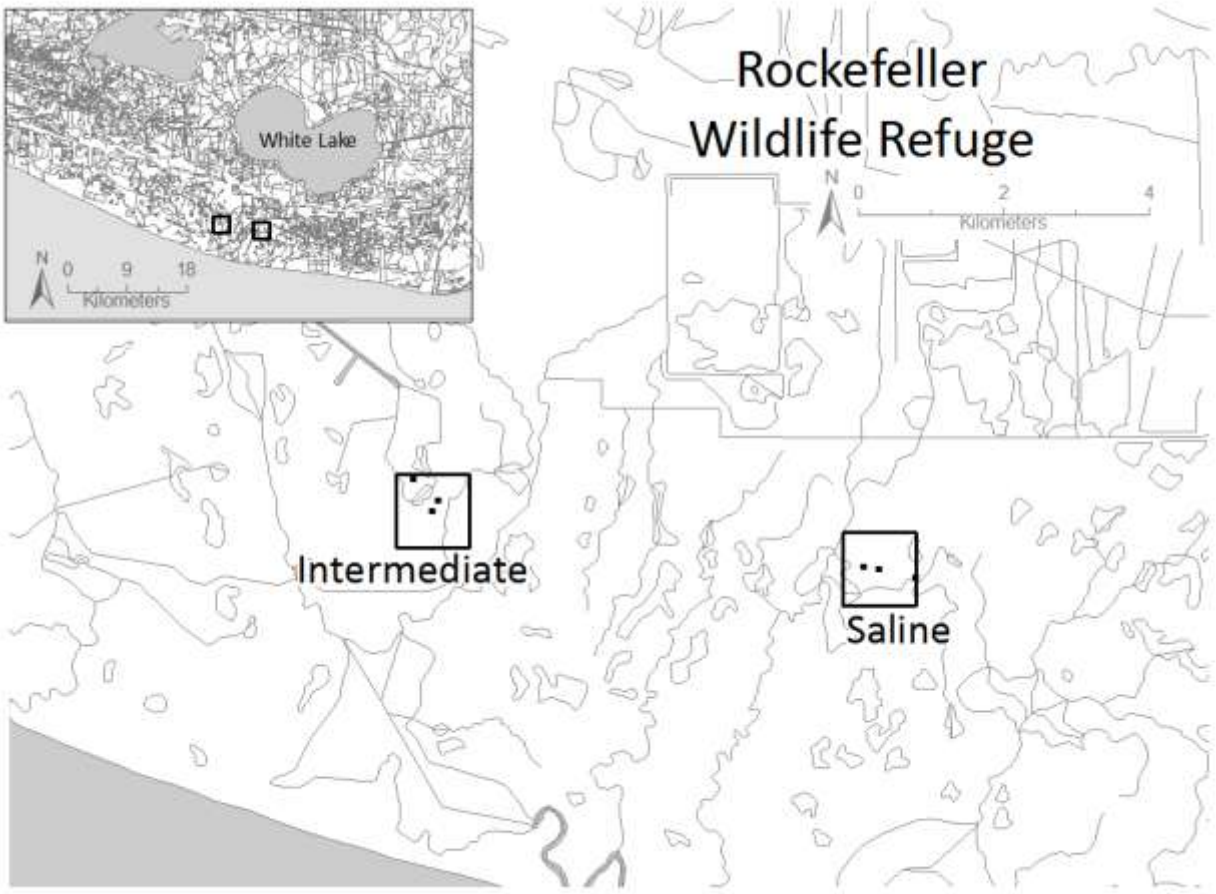


Figure B2: Map of sampling sites (hollow boxes) and plots (small black boxes) in saline and intermediate marshes at Rockefeller Wildlife Refuge. Hydrographic data represented in this map were downloaded from the United States Geological Survey's National Hydrography Dataset website (http://nhd.usgs.gov/data.html).

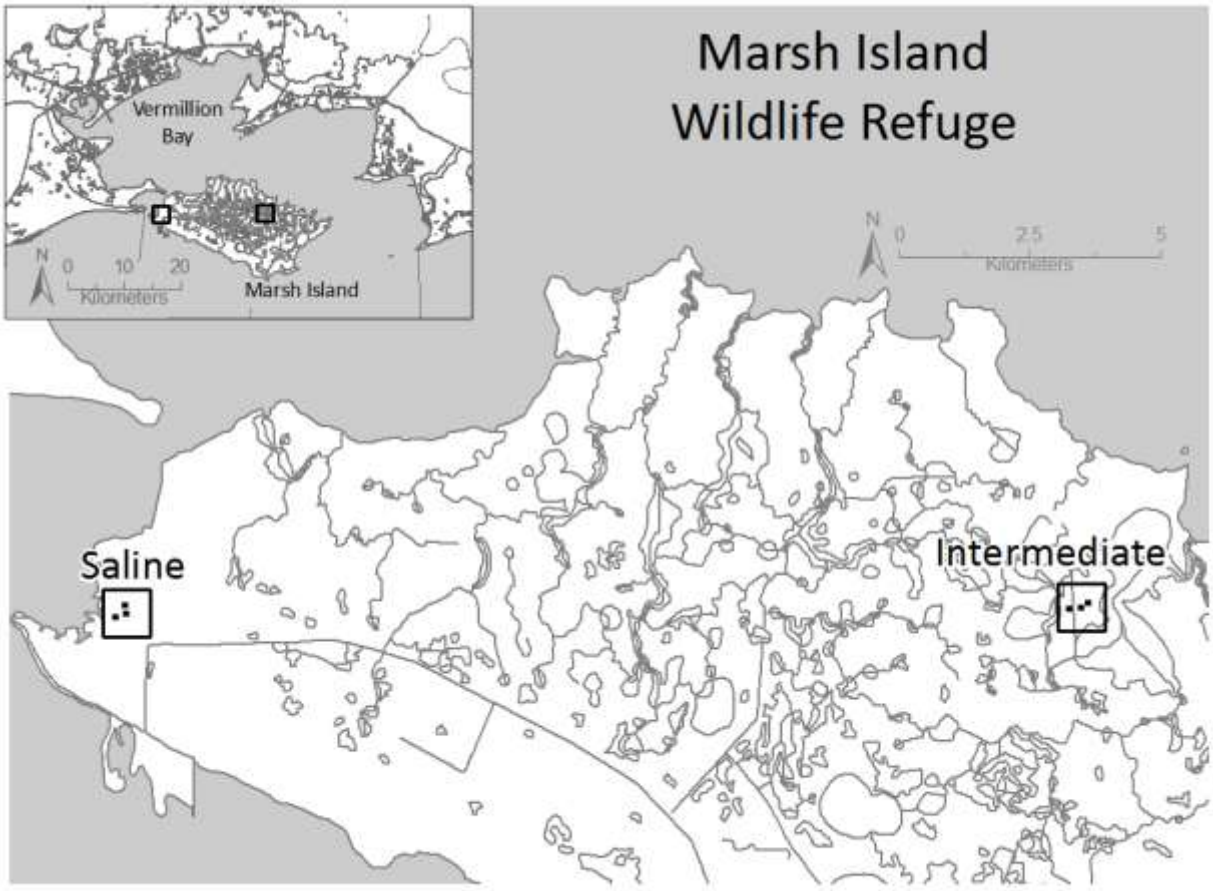


Figure B3: Map of sampling sites (hollow boxes) and plots (small black boxes) in saline and intermediate marshes at Marsh Island Wildlife Refuge. Hydrographic data represented in this map were downloaded from the United States Geological Survey's National Hydrography Dataset website (http://nhd.usgs.gov/data.html).

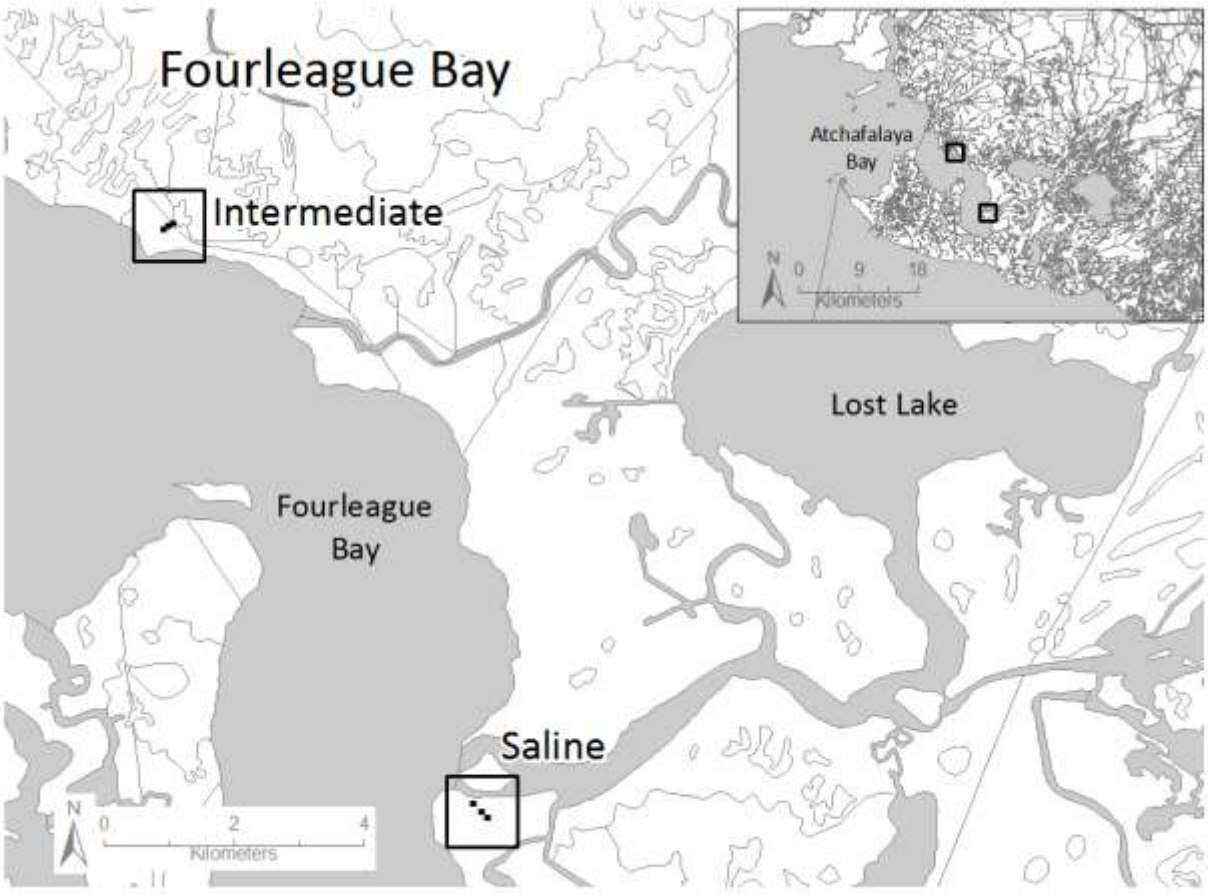


Figure B4: Map of sampling sites (hollow boxes) and plots (small black boxes) in saline and intermediate marshes near Fourleague Bay. Hydrographic data represented in this map were downloaded from the United States Geological Survey's National Hydrography Dataset website (http://nhd.usgs.gov/data.html).

**APPENDIX C:**
**ADDITIONAL FIGURE FROM GREENHOUSE EXPERIMENT**

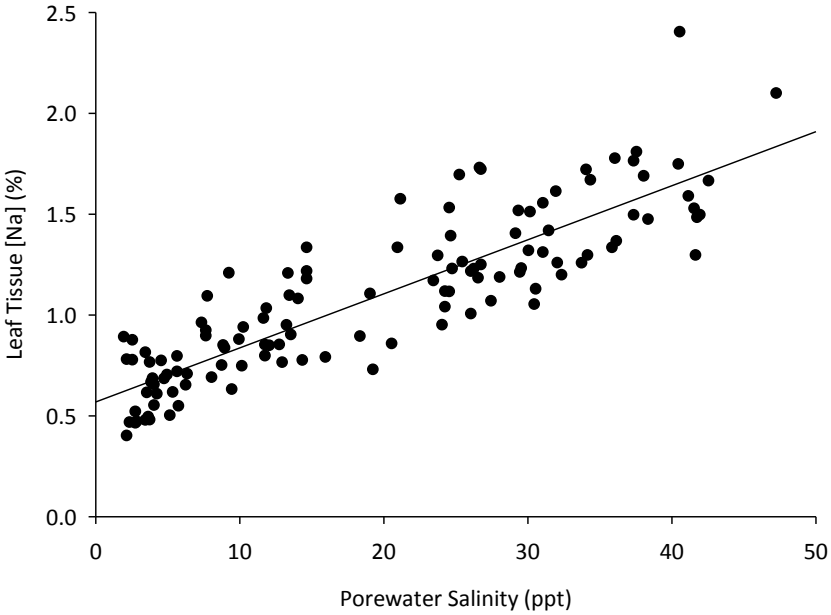


Figure C1: Relationship between porewater salinity and sodium concentration in leaf tissue of *Spartina patens* grown under controlled conditions in a greenhouse experiment. The equation of the regression line is $y = 0.57 + 0.03x$ ($R^2 = 0.75$, $p < 0.001$; SAS PROC REG).

## VITA

Vanessa Danielle Tobias was born in 1981 in Camarillo, California. She is the daughter of Christine and Robert Tobias and has a twin sister, Michele. She graduated from Adolfo Camarillo High School in 1999. Vanessa attended the University of California, Los Angeles, where she received a Bachelor of Arts degree with majors in biogeography and German language and literature in 2003. After graduating from UCLA, Vanessa spent a year working as a naturalist with the Resource Conservation District of the Santa Monica Mountains and volunteering as a docent at Point Mugu State Park in Malibu, California.

Vanessa received a Master of Science degree from the University of Michigan's School of Natural Resources and Environment in 2006. Her master's thesis related the distribution of steelhead trout in Topanga Creek in Topanga, California, to groundwater resources in the creek. Vanessa is currently a doctoral candidate in the School of Renewable Natural Resources at Louisiana State University.