

Developing a tool to map coastal wetlands
affected and unaffected by freshwater introductions,
LUMON Project No. 674139,
Interagency Agreement No. CREST07-10

Final Report

from

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June, 2009

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EXECUTIVE SUMMARY

Coastal wetland restoration and management often entail introducing freshwater into estuaries to increase emergent plant production by increasing nutrient availability, reducing salinity stress, and/or reducing flooding stress. Examples in Louisiana occur in the Mississippi River Deltaic Plain (e.g., Myrtle Grove Siphon) as well as the Louisiana Chenier Plain (e.g., Freshwater Introduction South of Highway 82). Assessing and managing freshwater impacts requires accurate identification of affected and unaffected areas. An ideal tool would be inexpensive and require only a single site-visit. The most direct way to identify the affected areas is to map variability in the response of plant production to changes in nutrient availability and water salinity. However, estimating plant production is too expensive for annual use at many sites. Likewise, leaf spectral reflectance, CO₂ uptake, leaf expansion, leaf proline concentration, soil nutrient ratios, and soil elemental concentrations are not suitable for routine use by wetland restoration planners and managers.

The goal of this project (LUMCON Project No. 674139, Interagency Agreement No. CRSET07-10) was to evaluate the feasibility of modifying the Redfield Ratio concept to spatially delineate the effects of freshwater introductions on emergent plant growth. The Redfield Ratio is the ratio of the atomic weights of the elements C:N:Si:P (106:16:15:1). It has been used worldwide to determine if N, Si, or P limit growth of oceanic and estuarine algae. We hypothesized that assaying for a similar group of elements in emergent plants would provide an index of nutrient, salinity, and flooding stress that can be measured over large areas to map areas where freshwater introductions increase plant growth. We focused on marshes dominated by *Spartina patens* because it is the most common emergent plant in coastal Louisiana, and because most wetland restoration projects that use freshwater

introductions target marshes dominated by it. Previous research by a variety of scientists for a variety of purposes suggests that the most promising elements to include in a modified Redfield Ratio are C, N, P, Na, Mg, K, and Mn. Carbon, and N can be determined with a CHN analyzer; all other elements can be determined via ICAP. Those assays are relatively inexpensive. If such data were collected however, the data could not yet be used for restoration or management because the concentrations and ratios of these elements from living plants grown under known conditions are lacking. We therefore (1) analyzed plant tissue from a previously conducted greenhouse experiment to develop a modified Redfield Ratio for *S. patens*, and (2) verified the modified ratio with a new field experiment. The field experiment also was needed to determine the optimum period during the growing season for collection of emergent plant tissue.

We used the chemical composition of leaf tissue from *S. patens* grown under controlled conditions to create a simple and inexpensive tool to identify salinity stress and nutrient limitation. Biomass measurements alone could not be used to determine the cause of the limitation of production because low levels of biomass developed where growth was salinity limited, nutrient limited, and co-limited by high salinity low nutrient availability We found that C:N ratios and Na concentrations of *S. patens* leaf tissue can be used to classify factors that limit production in *S. patens* (Figure 1).

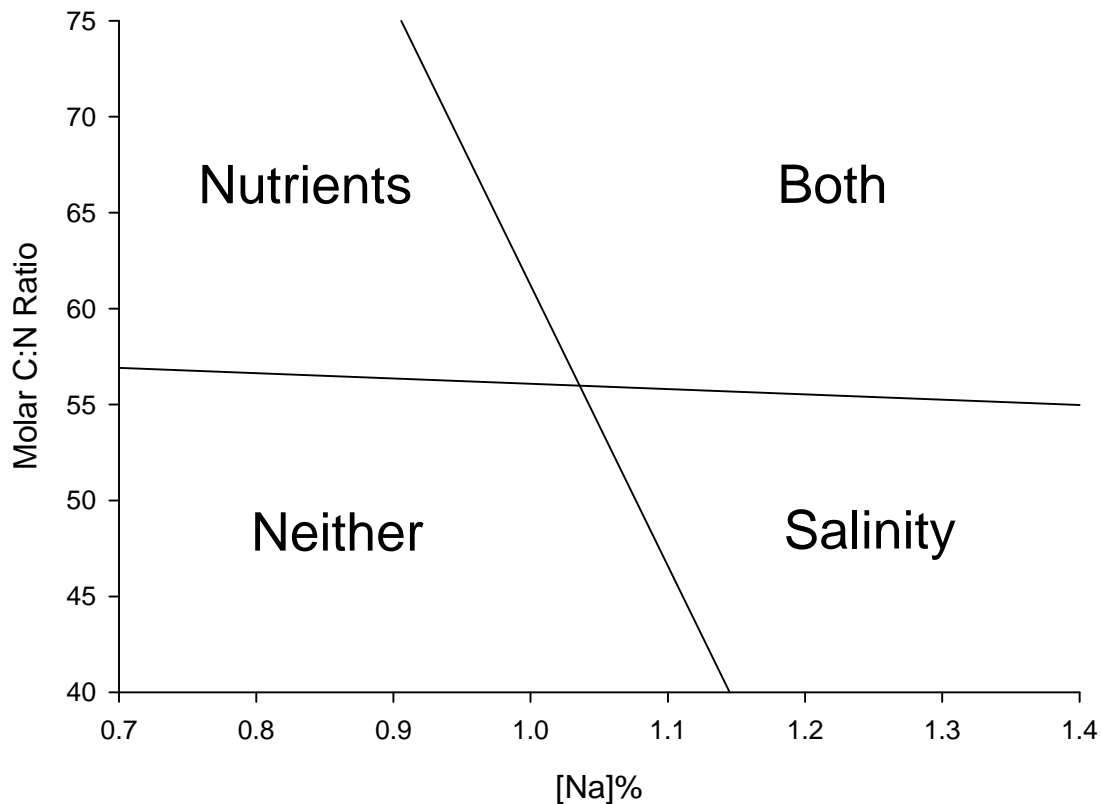


Figure 1: Sodium concentrations and C:N ratios in *Spartina patens* leaf tissue used as a signature to identify conditions limiting biomass production. Using this tool, C:N ratios in *S. patens* greater than 56 generally indicate limitation by low nutrient availability and sodium concentrations greater than 1.1% generally indicate limitation by high salinity.

In the field study, we confirmed that these signatures are valid but only if the leaf tissue is collected in the middle of the growing season (July, early August). This tool could be valuable to coastal wetland restoration planners involved in planning and evaluating freshwater introduction if applied regularly to large outfall areas and used to delineate areas where plant growth is limited by salinity stress, low nitrogen availability, a combination of both those factors, or by neither of those factors.

**Chapter 1: Improving marsh restoration: leaf tissue chemistry identifies factors
limiting greenhouse production in *Spartina patens***

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Abstract

Marsh loss is a problem in many areas around the world. To combat the problem, scientists and managers need tools to determine its cause and evaluate the effectiveness of management techniques. Current methods for estimating productivity and identifying factors that limit productivity are too time-consuming or expensive for wide-spread, regular use, however. In coastal Louisiana, where *Spartina patens* (Ait.) Muhl is the most common plant, restoration seeks to slow wetland loss rates that averaged approximately 77.4 km²/year between 1978 and 2000. We used the chemical composition of leaf tissue from *S. patens* grown under controlled conditions to create a simple and inexpensive tool to identify salinity stress and nutrient limitation. By growing *S. patens* at varying nutrient availability and salinity levels, we found that C:N ratios and Na concentrations can be used to classify factors that limit production in *S. patens*.

Keywords: marsh, restoration, wetlands, *Spartina patens*, chemistry, nitrogen, salinity, sodium, phosphorus

Introduction

Marsh loss is a problem in many areas of the world. In coastal Louisiana, 77.4 km²/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 to (1) make informed decisions about which restoration technique to use or (2) 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) but using this method requires repeated visits to sites and locating previously tagged stems. Also, while these methods 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 methods 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 we can improve the evaluation of freshwater introductions and other marsh restoration techniques. Although the tool that we describe here is specific for *S. patens* in coastal Louisiana, 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. Increasing nutrient availability increases production and decreases C:N ratios of *S. patens* leaf tissue where salinity is low (Foret 2001, Crain 2007). This method is less expensive and more widely accessible to managers because it requires only a single visit to a site where investigators collect a few grams of live plant material. The plant material must be rinsed, oven dried, ground, and analyzed with standard chemical analyses that are available commercially.

Our objectives were to determine the feasibility of using leaf chemical characteristics to identify the factors that limit plant productivity in coastal marshes and provide a basis for interpreting nutrient ratios of samples taken in the field. In this paper we 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. We use this data to propose chemical signatures in *S. patens* leaf tissue to indicate factors that limit productivity in coastal marshes. We focus on *S. patens* because it is the most common plant species in coastal Louisiana (Chabreck 1970).

Methods

We 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). We obtained two populations of *S. patens* that differed in salinity tolerance from Mark Hester (University of New Orleans, New Orleans, LA). 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). We used plants from two populations with documented phenotypic differences to represent random variation rather than to investigate the effects of population on leaf chemistry. We initially grew the plants clonally in separate bedding trays containing sand, water, and commercial fertilizer (Peters 20-20-20 N-P-K).

We made experimental soils from a homogenous mixture of 90% commercial play sand and 10% potter’s clay to which we added one of four combinations of 19-5-8 and 35-0-0 encapsulated (slow-release, non-water soluble) fertilizer. We chose specific nutrient treatments to approximate 25%, 75%, 125%, and 200% of the nitrogen (4.90×10^{-4} , 1.46×10^{-3} , 2.43×10^{-3} , and 3.89×10^{-3} gN/cm³ respectively) and phosphorus levels (2.4×10^{-5} , 7.3×10^{-5} , 1.2×10^{-4} , and 1.9×10^{-4} gP/cm³ 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 1.96×10^{-3} gN/cm³ and 9.6×10^{-5} gP/cm³ (Foret 2001). The actual levels of nitrogen achieved in the experimental soils were 30%, 95%, 130%, and 205% and the actual levels of phosphorus achieved were 125%, 375%, 620%, and 980% of nutrient levels at Rockefeller Refuge. We planted two stems of the same population (“i”

or “k”) in each one-gallon pot. We 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 we raised the salinity level of the water in the tubs.

We raised the salinity in the tubs using 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 salinities achieved were 2, 5, 17, and 38 ppt. We 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 we poured water from the tubs over the soil surface. We 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 we began the nutrient treatments. Merino et al. (in revision) 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, we harvested above- and below-ground tissue over a three-day period. We washed the below-ground tissue and dried both above- and below-ground tissue at 60° and weighed it to determine biomass. Because above- and below-ground biomass were linearly correlated ($R^2=0.981649$, $p=0.0001$), we added them together to estimate total biomass (Merino in revision). Using the average biomass of pots grown under specific nutrient and salinity conditions, we classified treatment combinations in terms of factors that limit productivity.

We classified pots into four groups by limiting factor: nutrient, salinity, both, or neither (Table 1). Pots with nutrient 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 nutrients limited growth. Pots that had an average porewater salinity of less than 10 ppt and nutrient treatment of 30% N (Figure 1) were classified as nutrient-limited because of their low biomass combined with low nutrient availability. We 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, we applied the same logic we used for the lower salinity pots. Pots with average salinities higher than 10 ppt and nutrient treatments $> 30\%$ were classified as salinity-limited. The remaining pots (i.e. those with salinity < 10 ppt and nutrient treatment of 30% N) were classified as both-limited (Figure 1).

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

Data were analyzed using PROC MIXED in SAS as a one-way ANOVA with four groups (neither-, nutrient-, salinity-, and both-limited). We used contrasts within the

ANOVAs to compare N:P ratios, C:N ratios, and Na concentrations of plants grown at high salinity to those of plants grown at low salinity. We used LSMeans to obtain a mean for each of the groups. To determine boundaries for the tool to evaluate limiting factors, we averaged the means of the high and low salinity groups. We used the same procedures to make comparisons between plants grown at high and low nutrients. Correlations were performed using Pearson's correlation coefficient. We 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 nutrient-limited had lower N:P ratios than plants that were nutrient-limited ($F_{1,103} = 14.05$, $p = 0.0003$; Figure 2). Plants that were salinity-limited had higher N:P ratios than plants were not salinity-limited ($F_{1,103} = 45.90$, $p < 0.0001$; Figure 2).

There was a significant difference in C:N ratios among limiting factors ($F_{3,104} = 12.38$, $p < 0.0001$). Plants that were not nutrient-limited had lower C:N ratios than plants that were nutrient-limited ($F_{1,104} = 36.69$, $p < 0.0001$; Figure 3). The mean C:N ratio for non-nutrient-limited plants was 42.07 whereas the mean C:N ratio for nutrient-limited plants was 69.94. 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 ($F_{1,104}=0.12$, $p=0.7285$).

There was a significant difference in Na concentration among limiting factors ($F_{3,103} = 22.53$, $p < 0.0001$). Plants that were not nutrient-limited had higher Na concentrations than plants that were nutrient-limited ($F_{1,122} = 14.13$, $p = 0.0003$, Figure 4). Sodium concentrations were higher in plants that were salinity-limited than plants that were not salinity-limited ($F_{1,122} = 131.75$, $p < 0.0001$). 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%.

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, nutrient limited, and co-limited by high salinity low nutrient availability (Figure 1). The large difference in biomass between plants grown in limited versus unlimited conditions highlights the importance of determining limiting factors for improving the health of degrading marshes. Merino et al. (in review) found that maximum biomass for *S. patens* occurred when plants grew in water low in salinity and soil high in nutrients.

Although 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.* Another 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.

No clear effect of either nutrients or salinity could be shown among the four limiting factor groups using N:P ratios in plant tissue as an indicator. Phosphorus content in leaves did not vary much and was generally high. Although plants were subjected to relatively high levels of phosphorus in all treatment soils, this is unlikely to have

impaired productivity because unlike nitrogen, excess phosphorus has not been shown to damage plants. Our 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*. Our highest N:P ratios occurred at our lowest salinities (Figure 2), which could be because soils have a higher phosphate sorption capacity in freshwater than in saline conditions (Sundareshwar and Morris 1999). There are too few reports of N:P ratios from the field to determine if the high N:P ratios that we observed at low salinities are common. It is likely that such high N:P ratios would rarely develop in the field where soil P is high because in unlike in our experimental soils, dissolved organic matter would compete with P for clay binding sites in addition to Cl⁻.

C:N ratios were useful in identifying nutrient limitation because C:N ratios varied predictably with nutrient levels. Higher C:N ratios indicated limitation of productivity by nitrogen 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. alterniflora*. Our findings also agree with previous studies reporting that enhanced nutrients 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 nutrient availability where salinity was high. Our findings agree with Bradley and

Morris's (1992) finding that the internal nitrogen supply needed to maintain growth in *Spartina alterniflora* increased with increasing salinity.

Sodium concentration in leaf tissue was a useful tool for identifying salinity stress. While changes in both salinity and nutrient levels affected sodium concentration, the effect of salinity on Na concentration was much greater than the effect of nutrient variations on Na concentration. Plants that grew in water with higher salinity had higher sodium concentrations in their leaf tissue. Sodium 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).

Our findings confirm that the chemical composition of the leaf tissue of *S. patens* can be used to determine if low nutrient availability or high salinity limit productivity. Using a combination of the response of C:N ratios and sodium concentration in plant tissue to variations in the conditions under which the plants were grown, it is possible 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. Using small samples of leaf tissue to determine leaf chemistry also has the potential to be more cost-effective than current methods for estimating production via measuring biomass because it is less time-consuming. The type of elemental analysis we 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.

One limitation of this study is that these nutrient and sodium signatures do not reflect changes in C:N ratios and sodium 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 we 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 we observed in this greenhouse experiment apply to plants growing in the field.

Acknowledgements

Funding for this study was provided by and by the Coastal Restoration and Enhancement Through Science and Technology Program (contract no. CREST 07-10). We thank D. Price for providing tissue samples used for tissue analysis and portions of the data used in this study. We also thank J. Merino for providing data.

Literature Cited

- Barras, J., S. Beville, D. Britsch, S. Hartley, S. Hawes, J. Johnston, Q. Kinler, A. Martucci, J. Porthouse, D. Reed, K. Roy, S. Sapkota, and J. Suhayda. 2003. Historical and Projected Coastal Louisiana Land Changes: 1978-2000. USGS Open File Report 03-334, 39 p. (Revised January 2004.)
- Bradley, P.M. and J.T. Morris. 1991. Relative importance of ion exclusion, secretion, and accumulation in *Spartina alterniflora* Loisel. *Journal of Experimental Botany* 42: 1525-1532.
- Bradley, P.M. and J.T. Morris. 1992. Effect of salinity on the critical nitrogen concentration of *Spartina alterniflora* Loisel. *Aquatic Botany* 43: 149-161.
- Burdick, D.M., I.A. Mendelssohn, and K.A. McKee. 1989. Live standing crop and metabolism of the marsh grass *Spartina patens* as related to edaphic factors in a brackish, mixed marsh community in Louisiana. *Estuaries* 12(3): 195-204.
- Chabreck, R.H. 1970. Marsh zones and vegetative types of the Louisiana coastal marshes. Ph.D. Dissertation. Louisiana State University, Baton Rouge, Louisiana.
- Craft, C. 2007. Freshwater input structures soil properties, vertical accretion, and nutrient accumulation of Georgia and U.S. tidal marshes. *Limnology and Oceanography* 52(3):1220-1230.
- Crain, C.M. 2007. Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries and Coasts* 30(1):26-34.

- Day, J.W., Jr., C.A.S. Hall, W.M. Kemp, A. Yáñez-Arancibia. 1989. *Estuarine Ecology*. John Wiley and Sons, New York, NY, USA.
- Day, J.W. et al. 2000. Pattern and process of land loss in the Mississippi Delta: a spatial and temporal analysis of wetland habitat change. *Estuaries* 23(4): 425-438.
- DeLaune, R.D., R.J. Buresh, and W.H. Patrick, Jr. 1979. Relationship of soil properties to standing crop biomass of *Spartina alterniflora* in a Louisiana marsh. *Estuarine and Coastal Marine Science* 8:477-487.
- DeLaune, R.D., R. Pezeshki, and A. Jugsujinda. 2005. Impact of Mississippi River freshwater introduction on *Spartina patens* marshes: responses to nutrient input and lowering of salinity. *Wetlands* 25(1): 155-161.
- Ewing, E., K.L. McKee, I.A. Mendelssohn, and M.W. Hester. 1995. A comparison of indicators of sub-lethal nutrient stress in the salt marsh grass *Spartina patens*. *Environmental and Experimental Botany* 35(3):331-343.
- Ewing, K. and K.L. McKee. 1997. A field comparison of indicators of sublethal stress in the salt-marsh grass *Spartina patens*. *Estuaries* 20(1):48-65.
- Foret, J.D. 2001. Nutrient limitation of tidal marshes of the Chenier Plain, Louisiana. Ph.D. Dissertation, University of Louisiana at Lafayette, Lafayette, Louisiana.
- Hester, M.W., I.A. Mendelssohn, and K.L. McKee. 1996. Intraspecific variation in salt tolerance and morphology in the coastal grass *Spartina patens* (Poaceae). *American Journal of Botany* 83: 1521-1527.
- McKee, K.L. and I.A. Mendelssohn. 1989. Response of a freshwater marsh plant community to increased salinity and increased water level. *Aquatic Botany* 34: 301-316.

- Merino, J., D. Huval, and J. Nyman. Unpublished. An application of the concept of limiting factors to environmental restoration: nutrients, salinity, and emergent vegetation in coastal marshes.
- Nyman, J.A. et al. 2006. Marsh vertical accretion via vegetative growth. *Estuarine, Coastal, and Shelf Science* 69: 370-380.
- Redfield, A.C., B.H. Ketchum, and F.A. Richards. 1963. The influence of organisms on the composition of sea-water. p.26-77. *In* N.M. Hill(ed.). *The Sea*. Wiley-Interscience, New York, NY, USA.
- Stribling, J.M. and J.C. Cornwell. 2001. Nitrogen, phosphorus, and sulfur dynamics in a low salinity marsh system dominated by *Spartina alterniflora*. *Wetlands* 21(4): 629-638.
- Sundareshwar, P.V. and J.T. Morris. 1999. Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient. *Limnology and Oceanography*. 44(7) 1693-1701.

Table

Table 1: Combinations of treatments included in each limiting factor group.

Nutrients		Salinity	
Intended (%)	Mean Actual (%)	Intended (ppt)	Mean Actual (ppt)
Neither-Limited			
75	95	2	2
75	95	6	5
125	130	2	2
125	130	6	5
200	205	2	2
200	205	6	5
Nutrient-Limited			
25	30	2	2
25	30	6	5
Salinity-Limited			
75	95	18	17
75	95	36	38
125	130	18	17
125	130	36	38
200	205	18	17
200	205	36	38
Both-Limited			
25	30	18	17
25	30	36	38

Figures

Figure 1: Mean biomass (± 1 SD) of *Spartina patens* leaf tissue from plants grown under various nutrient and four salinity treatments. Adapted from Merino et al. (in review).

Figure 2: Mean molar N:P ratios (± 1 SD) 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 3: Mean molar C:N ratios (± 1 SD) 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 molar sodium concentrations (± 1 SD) 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 5: Mean molar C:N ratio and Na concentrations (± 1 SD) 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: Sodium concentrations and C:N ratios in *Spartina patens* leaf tissue used as a signature to identify conditions limiting biomass production. Using this tool, C:N ratios in *S. patens* greater than 56 indicate limitation by low nutrient availability and sodium concentrations greater than 1.1% indicate limitation by high salinity.

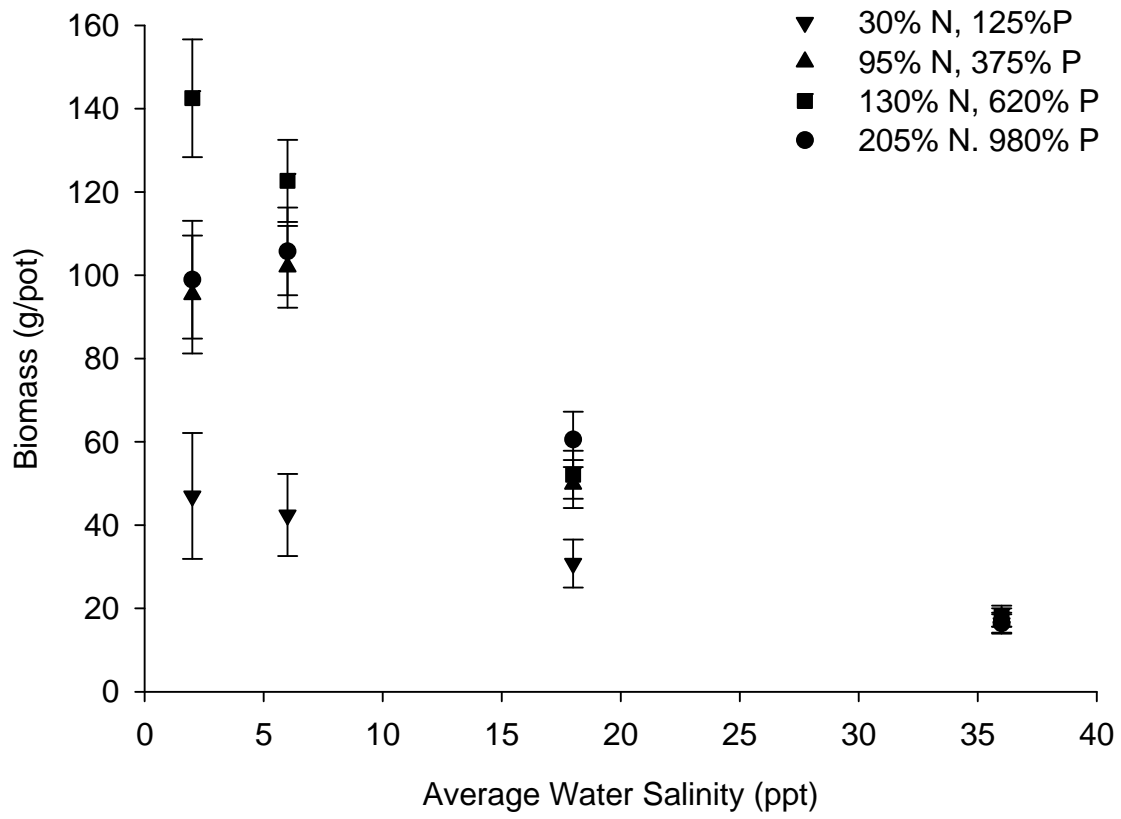


Figure 1

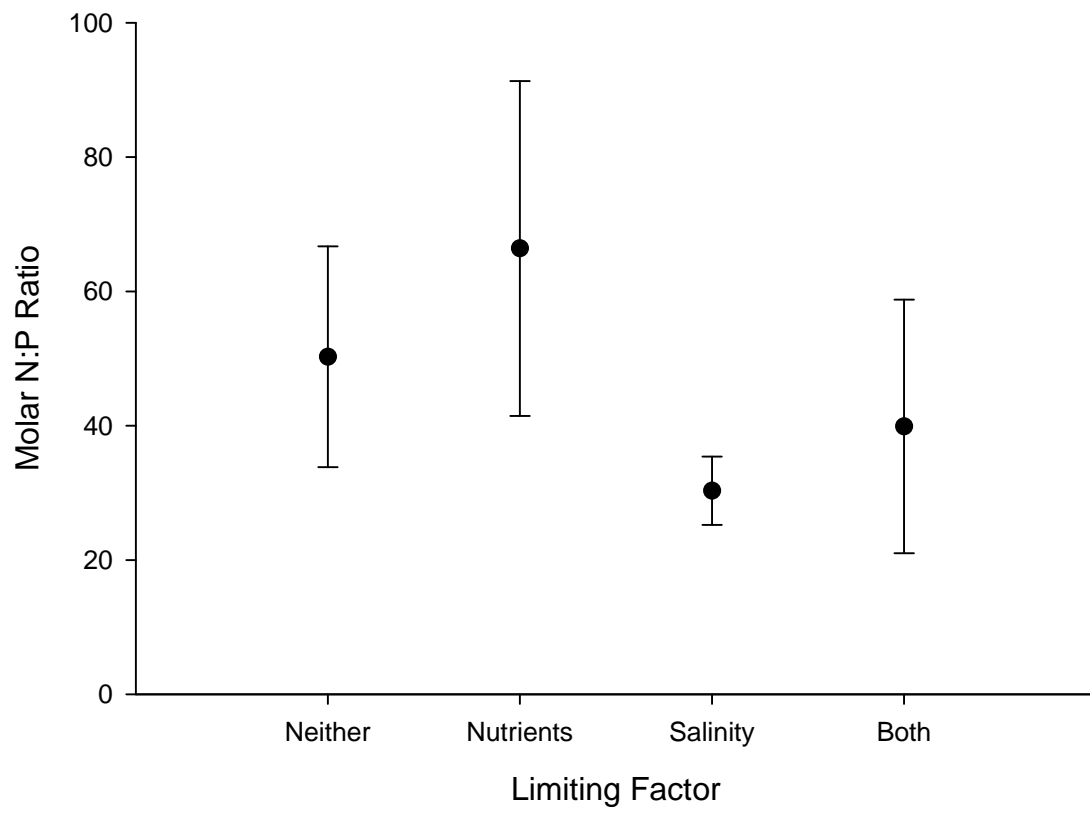


Figure 2

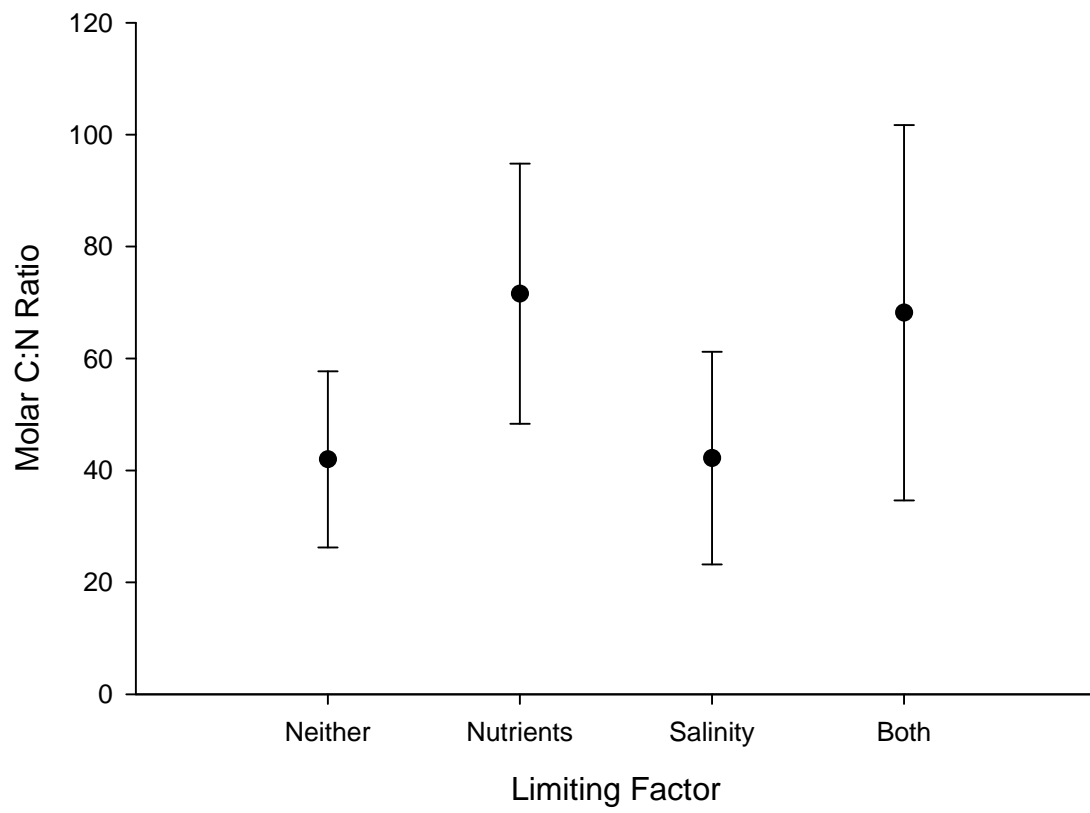


Figure 3

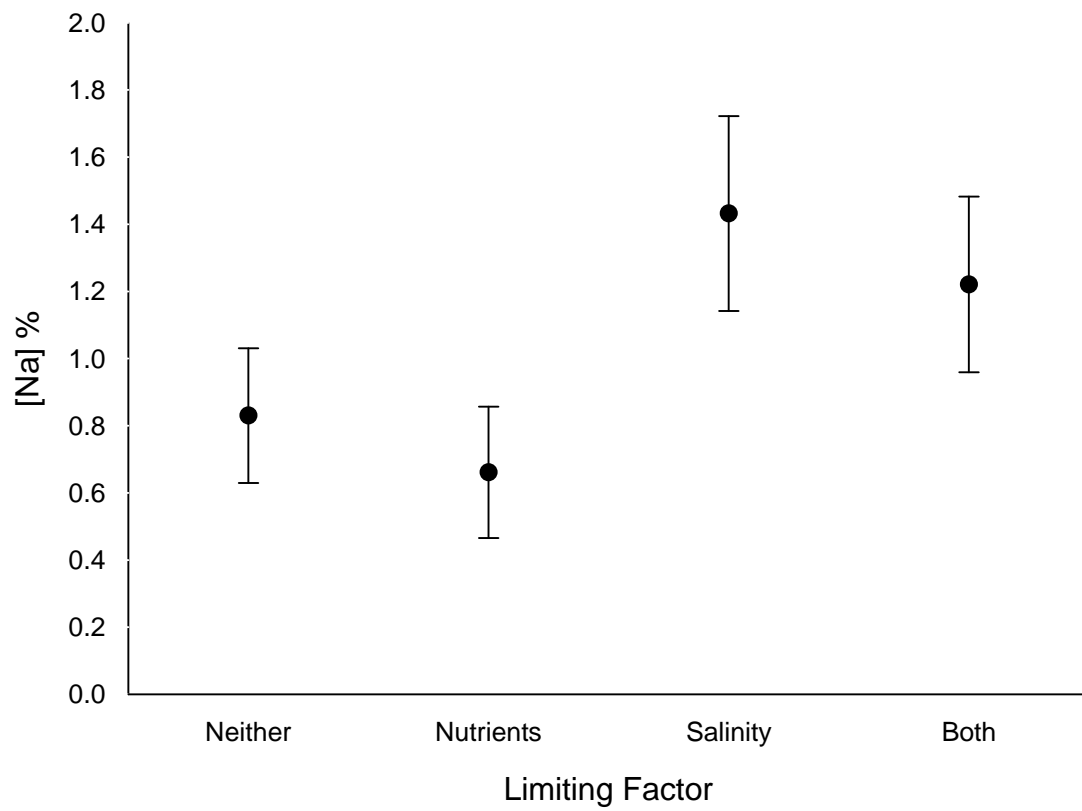


Figure 4

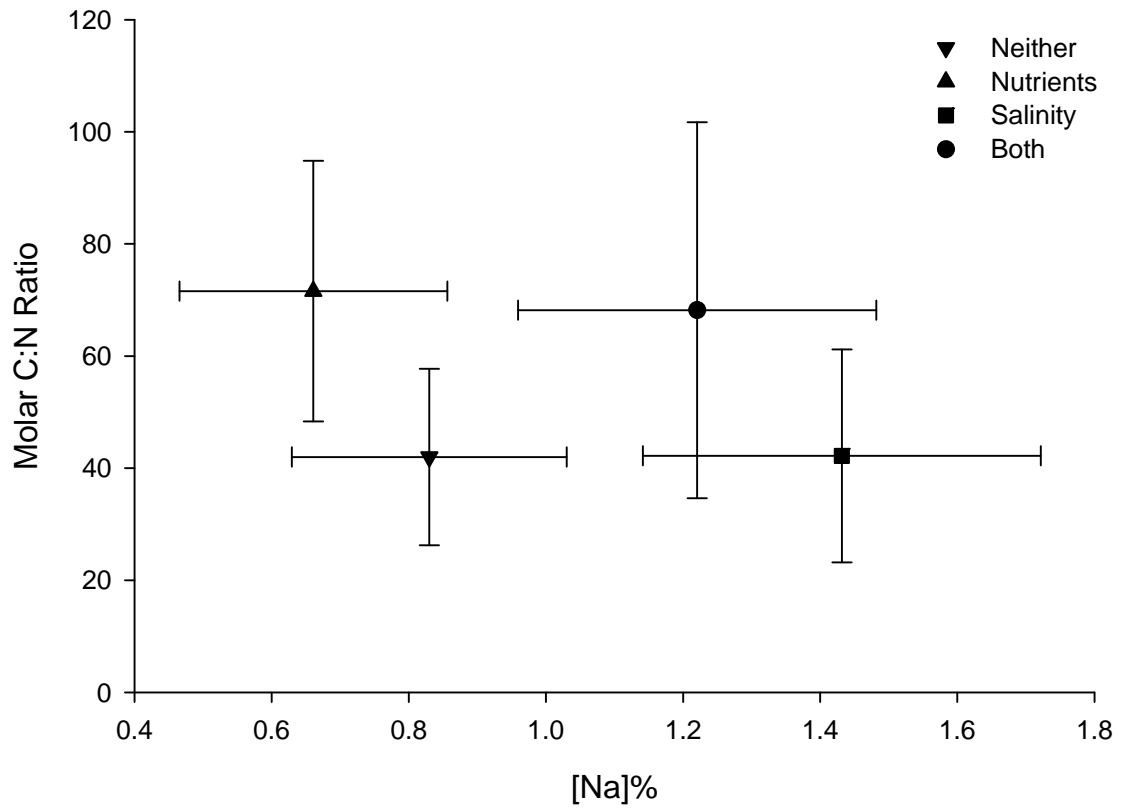


Figure 5

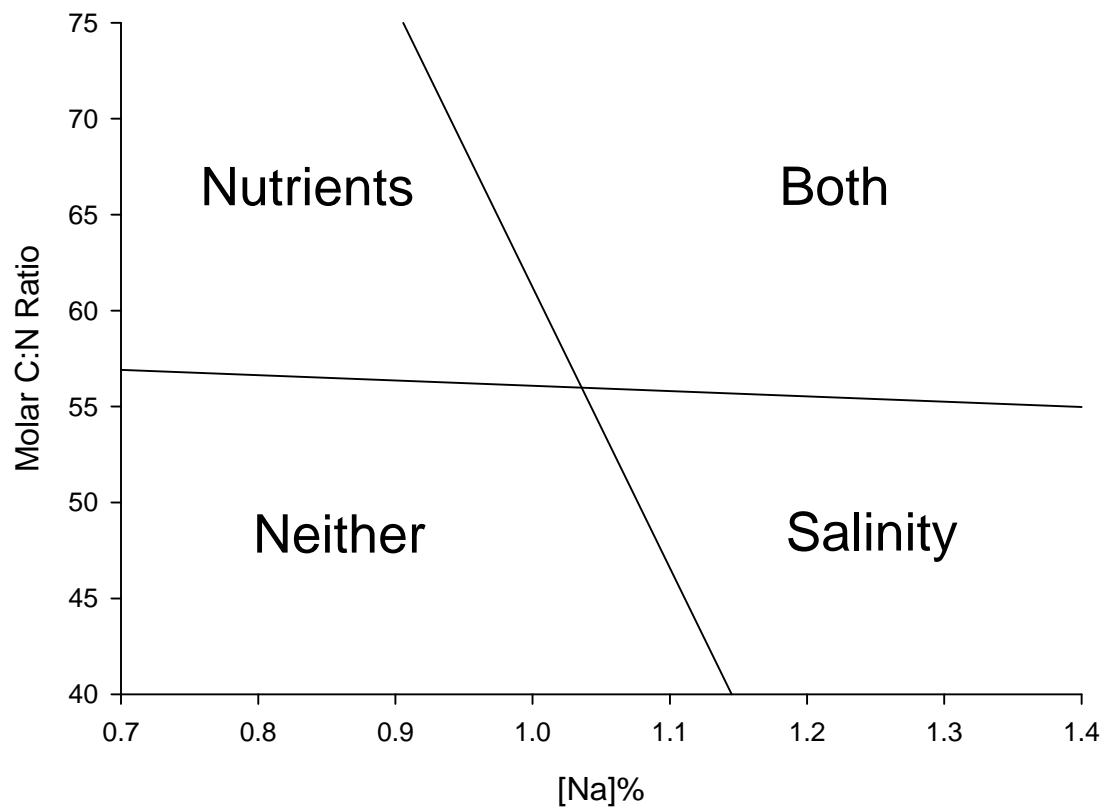


Figure 6

Chapter 2: Field studies to validate chemical signatures in *Spartina pates* leaf tissue of nutrient limitation and salinity stress.

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Introduction

Wetland management plans can be implemented to achieve a variety of goals. In Louisiana, maintaining or increasing productivity of marsh vegetation is often a management goal. In some cases maintaining productive marshes is specifically done to reduce marsh loss, combat sea-level rise, reduce coastal erosion, or prevent the invasion by non-native species. For example, freshwater diversions deliver fresh water and sediment that are important factors in controlling marsh loss (Day et al. 2000). In other cases managers control these factors to manage habitats for wildlife or fish. In all cases, managers require methods for selecting management strategies that address the cause 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). 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) but using this method requires repeated visits to sites and locating previously tagged stems. While these methods may identify areas where limited production may cause problems, they cannot identify the factors that limit production.

Identifying the causes of limited production can improve management plans by suggesting possible remedies. For example, a marsh with production limited by low nitrogen availability will not be improved by treatment plans designed to lower salinity. 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 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 & 2003, Koerselman & Meuleman 1996, Patrick & DeLaune 1976) and agricultural crops (e.g. Campbell 2000). Where salinity is low, increasing nitrogen availability increases productivity and decreases C:N ratios of *S. patens* leaf tissue (Foret 2001, Crain 2007). In other marsh species, Na concentration has been shown to increase with increasing salinity (McKee and Mendelssohn 1989; Bradley and Morris 1991).

To improve the ability to identify limitation of productivity in *S. patens* by salinity stress and/or nitrogen-limitation we developed a tool that uses analytical methods that are commercially available and commonly used in agriculture. This tool was developed in a controlled greenhouse setting with constant levels of nitrogen availability and salinity stress and using tissue samples taken from plants at the end of the growing season. Because of the constant conditions in the greenhouse study, one of our objectives was to evaluate whether it is feasible to use this tool where natural variations in nitrogen availability and salinity levels exist by applying it to large, heterogeneous areas of the Louisiana coast. We also looked for seasonal patterns in C:N ratio and/or [Na]% to determine if some times of year were more or less appropriate to apply the tool. Although the tool that we describe here is specific for *S. patens* in coastal Louisiana, our methods could be applied to other species and in other systems.

Methods

We collected samples at eight sites in brackish and intermediate marshes along the coast of Louisiana. We selected paired fresher and more saline sites at Cameron Prairie National Wildlife Refuge, Rockefeller Refuge, Marsh Island Wildlife Refuge, and marsh adjacent to Four League Bay (Figure I). 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 brackish marsh such as *Spartina alterniflora*. The purpose of this method of site selection was to sample marshes over a broad range of salinity conditions under which *S. patens* grows. Sites near Fourleague Bay were chosen to be accessible by outboard motor boat (17 foot Boston Whaler). Sites at all other locations were accessible by airboat.

We collected samples in Spring, Summer, and Fall of 2007 and 2008. We only visited four sites in Spring 2007 because of permitting and time constraints. In Fall 2008 weather-related issues prevented sampling at Four League Bay.

At each location, we took samples at three points, approximately 100 meters apart. We visited the same general area on each trip but the sampling location was selected randomly. We collected porewater samples at 10 cm below the marsh surface at each point using 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. We pre-filtered porewater using a piece of nylon stocking fitted over the end of the tubing to exclude large soil particles. We measured salinity, conductivity, and pH using a handheld meter (YSI Model 63). For nutrient analysis, we filtered water samples using 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. We determined the concentrations of ammonia-nitrogen using the Nessler method and reactive phosphorus (orthophosphate) using the ascorbic acid method (Clesceri et al. 1998).

We collected vegetation from 0.25 m² clip plots at each point to estimate biomass and compare productivity among sampling sites. We cut vegetation at the marsh surface and

transported it to the lab. In the lab we sorted stems by species and whether they were alive or dead. We 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.

To determine limitation of production by nitrogen starvation and/or salinity stress at each site we used a tool developed by Tobias et al. (year). This tool uses molar C:N ratios and Na concentrations in *S. patens* tissue to diagnose limitation of production as being caused by nitrogen starvation, salinity stress, a combination of both of these factors, or neither of these factors. We collected leaf tissue from the top 10-15 cm of *S. patens* stems and no stems were included in tissue samples. We stored tissue samples on ice until they could be processed in the lab. We rinsed tissue samples with deionized water to remove salt and/or sediment from leaf surfaces before drying in a 60°C oven for several days. Once dried, we ground tissue in a coffee grinder (Black and Decker Smartgrind). The grinder was cleaned using compressed air to remove particles between samples. We submitted dried and ground tissue samples to the LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL) to determine their elemental composition. Carbon and nitrogen content was determined using dry combustion by Leco CN Analyzer. Concentrations of all other elements were determined using ICP analysis. We performed regression analyses using PROC REG in SAS.

Results

Species richness was generally higher at fresh sites than their more saline counterparts (Table 1). Fresh sites were dominated by *Spartina patens*. Saline sites were usually co-dominated by *S. patens* and *S. alterniflora*, although saline sites at Marsh Island that were dominated by *S. patens* in 2007 became dominated by *Juncus roemerianus* in 2008.

Total biomass for all species in a 0.25 m² clip plot was highest at sites with lower salinity but was also more variable at these sites than those with higher salinity, where biomass was generally low (Figure D). Samples with the lowest salinity (<5 ppt) were from near the Atchafalaya river during the spring and summer. Sites with the highest biomass and highest porewater salinity were identified as measurements from fresher sites in the fall of 2008 that had been impacted by storm surge from Hurricanes Gustav and Ike. The effect of elevated salinity appears to have been more prolonged in sites on the Chenier Plain because we did not observe extremely elevated salinity at the fresher sites we visited that receive water from the Atchafalaya River.

Similar relationships exist between total biomass of all species in a plot and molar C:N ratio, sodium concentration, and manganese concentration (Figures E,F,G). Higher values of these indicators were associated with low biomass (i.e. limited productivity). At lower values of these indicators, we observed varied biomass values. We interpreted this relationship to mean that at high values of the indicators, production was limited by the stressor indicated. At lower values of the indicator, the wide range of biomass indicated that some other factor or factors controlled production.

Concentrations of ammonia in porewater ranged from undetectable levels to over 26 mg/L over the course of this study. Ammonia concentrations were lowest in spring samples and most variable in fall (Figure A). Orthophosphate varied from undetectable levels to nearly 16

mg/L. Orthophosphate was lowest in porewater in spring 2008 and remained low in fresher sites during summer 2008 (Figure B). Porewater salinity varied from 0.5 to over 22.1 ppt. Porewater salinity was somewhat variable at each site, but the only consistent pattern was an increase in porewater salinity in the fall of 2008 (Figure C). Paired sites generally showed similar patterns in changes in porewater salinity levels.

There were seasonal variations in C:N ratios. Plants had higher C:N ratios (i.e. they became more nitrogen-limited) as the growing season progressed (Figure H). All fresh sites started out nitrogen-limited in both springs. Salty sites were salinity-limited in both springs except in spring 2008 MISW was nitrogen-limited and BHB was neither-limited. Despite the differences in initial limiting conditions, all sites became more nitrogen-limited in the summer than they were in the preceding spring. Fresh sites continued to become more nitrogen-limited into the fall of 2007, as did salty sites on the Chenier plain. Riverine salty sites became less nitrogen limited in the fall of 2007. Although a previous study (Tobias et al. year) showed that C:N ratio in *S. patens* was related to nitrogen availability in a controlled setting, in this study porewater ammonia was not a good predictor of molar C:N ratio in *S. patens* leaf tissue under field conditions (PROC REG $F_{1,87} = 0.17$, $p = 0.6851$). Sodium concentrations did not show a seasonal pattern.

In samples taken following the hurricanes in September 2008, C:N ratios decreased at all sites, indicating that production was less limited by nitrogen availability. Sodium concentrations in samples from the Chenier Plain were higher than in summer 2008, while sites at Marsh Island had lower sodium concentrations than in the summer. We were unable to sample at Four League Bay in the fall of 2008 because of poor weather conditions.

Discussion

We found the highest productivity at sites with lowest stress; however productivity was also most variable where stress factor was low. The relationships between total biomass in clip plots and porewater salinity, sodium concentration, and C:N ratio each followed a similar pattern to that identified by Merino et al. (in press) in a greenhouse experiment, with the exception of sites with elevated porewater salinity following the hurricanes of September 2008. The pattern of lower and less variable biomass at higher salinities supports the hypothesis that salinity controls biomass where salinity is high but at low salinity, nitrogen availability controls biomass.

Porewater salinity increased at all sites in fall of 2008, but only half of our sites were salinity stressed based on the sodium concentrations in their leaf tissue. In areas with low porewater salinity, variations in the amount of spring flood water, and thus the amount of nitrogen delivered to plants, controls early season biomass. 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 (Chapter 1, range=0.05-45ppt) but the range of sodium concentrations was much larger (Chapter 1, range=0.7-1.4).

Samples with both high biomass and high porewater salinity were determined to be fresher sites that were impacted by storm surge from Hurricanes Gustav and Ike. These apparent anomalies in the relationship between porewater salinity and biomass point to the care that must be taken in using porewater chemistry to diagnose the causes of limitation of production in coastal marshes. Lower porewater salinity in spring and summer samples suggest that the plants had likely accumulated biomass earlier in the season and were done growing by the time storm surge elevated porewater salinity. Thus elevated salinity did not impact production. This also suggests that production early in the growing season is important for marshes to withstand the effects of hurricanes. We suspect that salinity effects of the hurricanes were mitigated at Marsh

Island because of their connectivity to the Atchafalaya River. Sites on the Chenier Plain have no direct connection to riverine flooding.

We used porewater ammonia concentrations as an indicator of nitrogen availability for this study. Ammonia may appear to be unrelated to C:N ratio for several reasons. First, although ammonia is a preferred form of nitrogen for plants, other forms of nitrogen such as nitrate are also available for uptake. We used ammonia because we assumed reducing conditions at 10 cm below the marsh surface would dictate that no nitrate would be available. We did not measure redox potential, however, so this assumption cannot be tested. Second, C:N ratios in plant tissue likely reflect long-term average nitrogen availability at a site. The porewater ammonia analysis we performed only provides a snapshot of conditions at the time the tissue samples were collected. Third, *S. patens* may not be able to effectively take up ammonia under reduced conditions (Mendelssohn and Morris 2000) or high salinity stress. The highest ammonium availability was at the sites with the highest salinity stress. This suggests that plants that are highly salinity stressed are unable to take up nitrogen, a finding supported by the review of Mendelssohn and Morris (2000).

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 nitrogen availability. Early in the growing season, plants are small and can find as much nitrogen as they need to grow. Spring floods also deliver nitrogen-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 nitrogen to supply their larger biomass but less nitrogen is available (Bradley and Morris 1992). This leads to nitrogen starvation towards the end of the growing season.

Increasing production can be seen as an issue of extending the growing season where nitrogen starvation is the problem. By increasing n-availability at appropriate times, managers may be able to improve productivity. C:N ratios and sodium concentrations in *S. patens* can be used to diagnose limitation of production in Louisiana's coastal marshes. However, using C:N ratios to diagnose limitation of production due to nitrogen-starvation for management purposes should be undertaken with caution. Because there is a seasonal pattern, managers must use C:N ratio data in conjunction with information on when samples were taken. Summer is probably the most appropriate time to use this tool because our results suggest that nitrogen starvation begins to limit production at this time. The boundaries in Figure H were developed using tissue samples taken from *S. patens* grown under controlled conditions at the end of a growing season. Based on the results of this field experiment, it is likely that these guidelines will not be informative for samples taken early in the growing season. Also, as plants senesce in the fall, C:N ratios increase. Thus, samples taken too late in the growing season may inaccurately indicate that nitrogen starvation limits production.

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 such that C:N ratios of *S. patens* are beginning to increase because adding nitrogen too early in the growing season, when nitrogen is still available in excess, may have little to no effect on production.

C:N ratios and sodium concentrations can also be used to identify the effects of large meteorological events such as storm surge from tropical storms, as shown by elevated leaf tissue Na after the 2008 hurricanes, spring floods, as shown by fewer sites being nitrogen limited in 2008 than in 2007 probably because of the larger spring flood of 2008. In fall 2008, following

storm surge from Hurricanes Gustav and Ike, the sites most impacted by the storms were extremely salinity-limited and showed no signs of nitrogen-limitation.

Literature Cited

Bradley, P. M. and J. T. Morris. 1991. Relative importance of ion exclusion, secretion, and accumulation in *Spartina alterniflora* Loisel. *Journal of Experimental Botany* 42(245): 1525-1532.

Bradley, P. M. and J. T. Morris. 1992. Effect of salinity on the critical nitrogen concentration of *Spartina alterniflora* Loisel. *Aquatic Botany* 43: 149-161.

Burdick, D.M., I.A. Mendelsohn, and K.A. McKee. 1989. Live standing crop and metabolism of the marsh grass *Spartina patens* as related to edaphic factors in a brackish, mixed marsh community in Louisiana. *Estuaries* 12(3): 195-204.

Campbell, C.R. 2000. Reference sufficiency ranges for plant analyses in the southern region of the United States. *Southern Cooperative Series Bulletin #394*.
<http://www.ncagr.gov/agronomi/saaesd/scsb394.htm>

Day, J.W. et al. 2000. Pattern and process of land loss in the Mississippi Delta: a spatial and temporal analysis of wetland habitat change. *Estuaries* 23(4): 425-438.

Clesceri, L. S., A. E. Greenberg, A. D. Eaton, and M. H. Franson, eds. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20 ed. Washington, D.C.: American Public Health Association, American Water Works Association, and Water Environment Federation.

Day, J. W., G. P. Shaffer, L. D. Britsch, J. D. Reed, S. R. Hawes, and D. Cahoon. 2000. Pattern and process of land loss in the Mississippi delta: a spatial and temporal analysis of wetland habitat change. *Estuaries* 23(4): 425-438.

- Ewing, E., K.L. McKee, I.A. Mendelssohn, and M.W. Hester. 1995. A comparison of indicators of sub-lethal nutrient stress in the salt marsh grass *Spartina patens*. *Environmental and Experimental Botany* 35(3):331-343.
- Ewing, K. and K.L. McKee. 1997. A field comparison of indicators of sublethal stress in the salt-marsh grass *Spartina patens*. *Estuaries* 20(1):48-65.
- Foret, J.D. 2001. Nutrient limitation of tidal marshes of the Chenier Plain, Louisiana. Ph.D. Dissertation, University of Louisiana at Lafayette, Lafayette, Louisiana.
- Gusewell S., and M. Koerselman. 2002. Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology Evolution and systematic.* 5:37-61.
- Gusewell S., W. Koerselman, and J.T.A. Verhoeven. 2003. Biomass N : P ratios as indicators of nutrient limitation for plant populations in wetlands. *Ecological Applications* 13:372-384.
- Koerselman W., and A.F.M. Meuleman. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33:1441-1450.
- McKee, K. L. and I. A. Mendelssohn. 1989. Response of a freshwater marsh plant community to increased salinity and increased water level. *Aquatic Botany* 37: 301-316.
- Mendelssohn, I. A. and J. T. Morris. 2000. Eco-physiological controls on the productivity of *Spartina alterniflora* Loisel. In *Concepts and Controversies in Tidal Marsh Ecology*, edited by M. P. Weinstein and D. A. Kreeger. Dordrecht, Netherlands: Kluwer Academic Publishers.
- Patrick, Jr., W.H., and R.D. DeLaune. 1976. Nitrogen and Phosphorus utilization by *Spartina alterniflora* in a salt-marsh in Barataria Bay, Louisiana. *Estuarine and Coastal Marine Science* 1976:59-64.

Penfound, W. T., and E. S. Hathaway. 1938. Plant communities in the marshlands of southeastern Louisiana. *Ecological Monographs* 8:1-56.

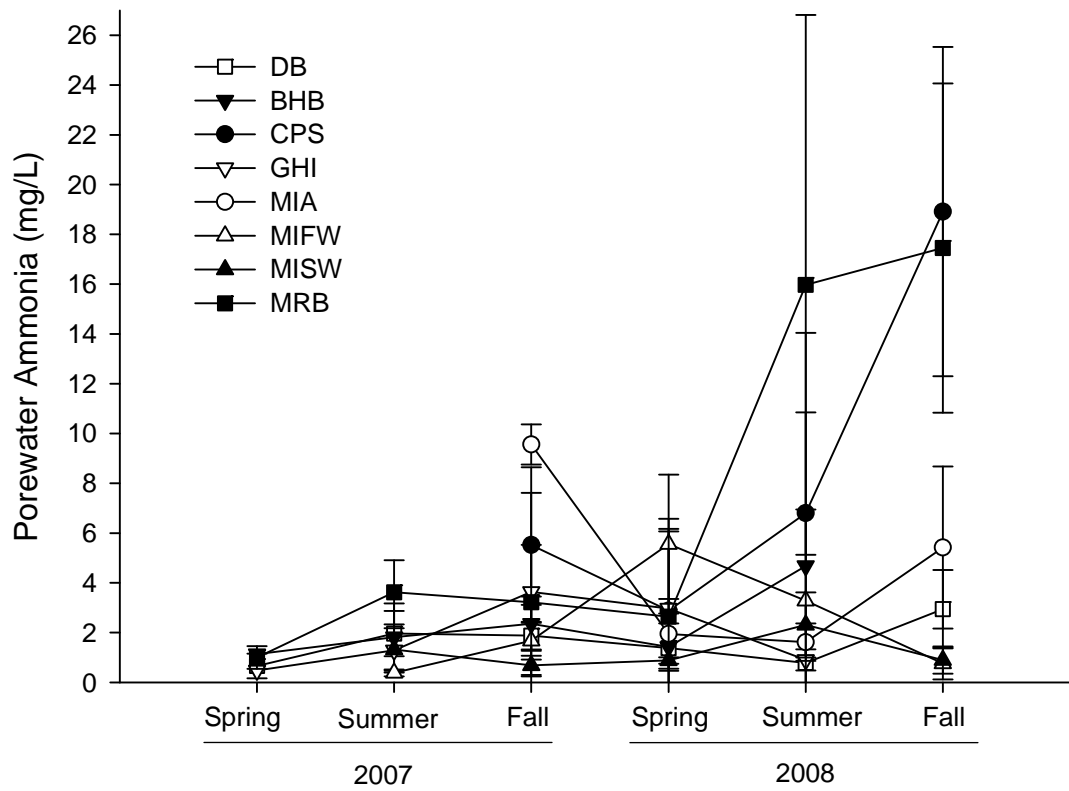


Figure A: Ammonia concentrations (mg/L) in porewater taken at 10 cm below marsh surface.

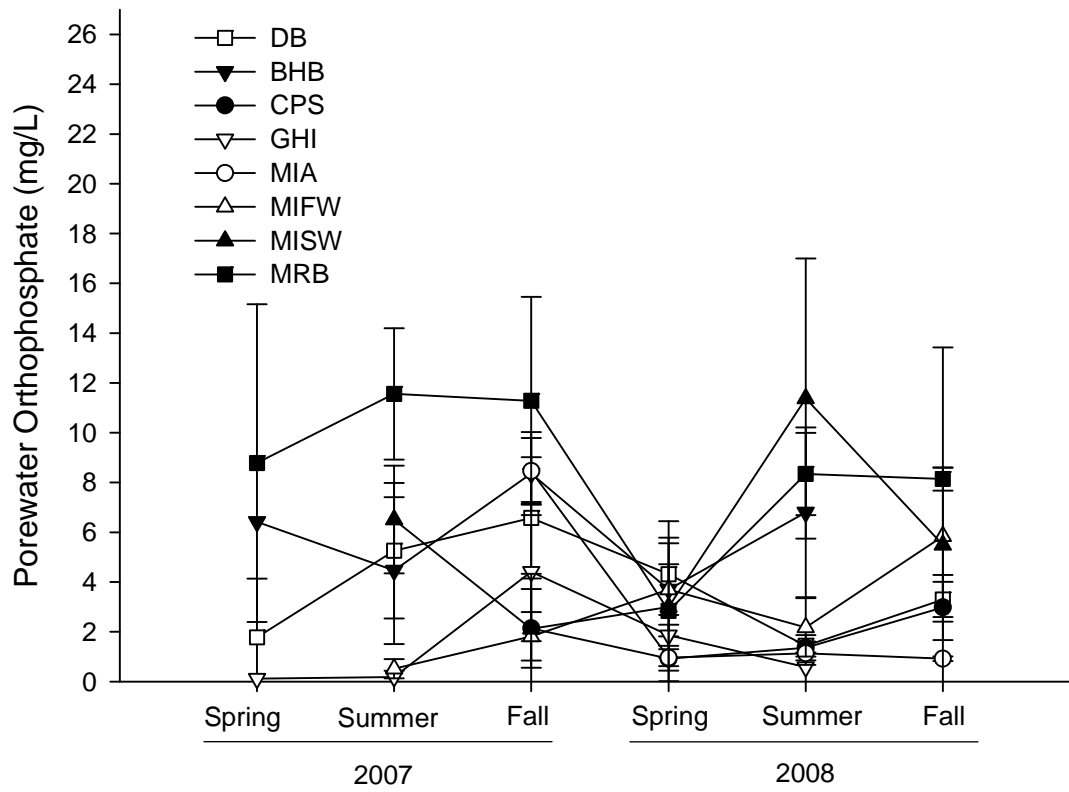


Figure B: Orthophosphate concentrations (mg/L) in porewater taken at 10 cm below marsh surface.

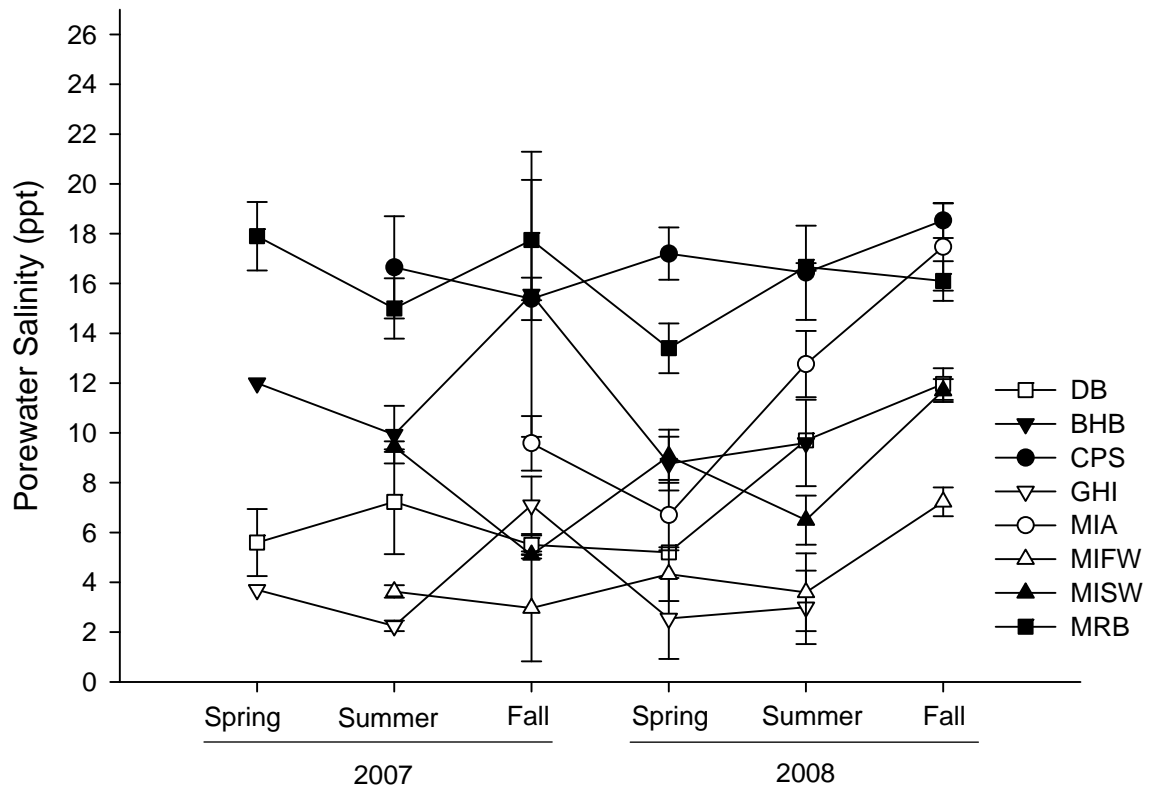


Figure C: Salinity levels (ppt) in porewater taken at 10 cm below marsh surface.

Spring 2007- Fall 2008

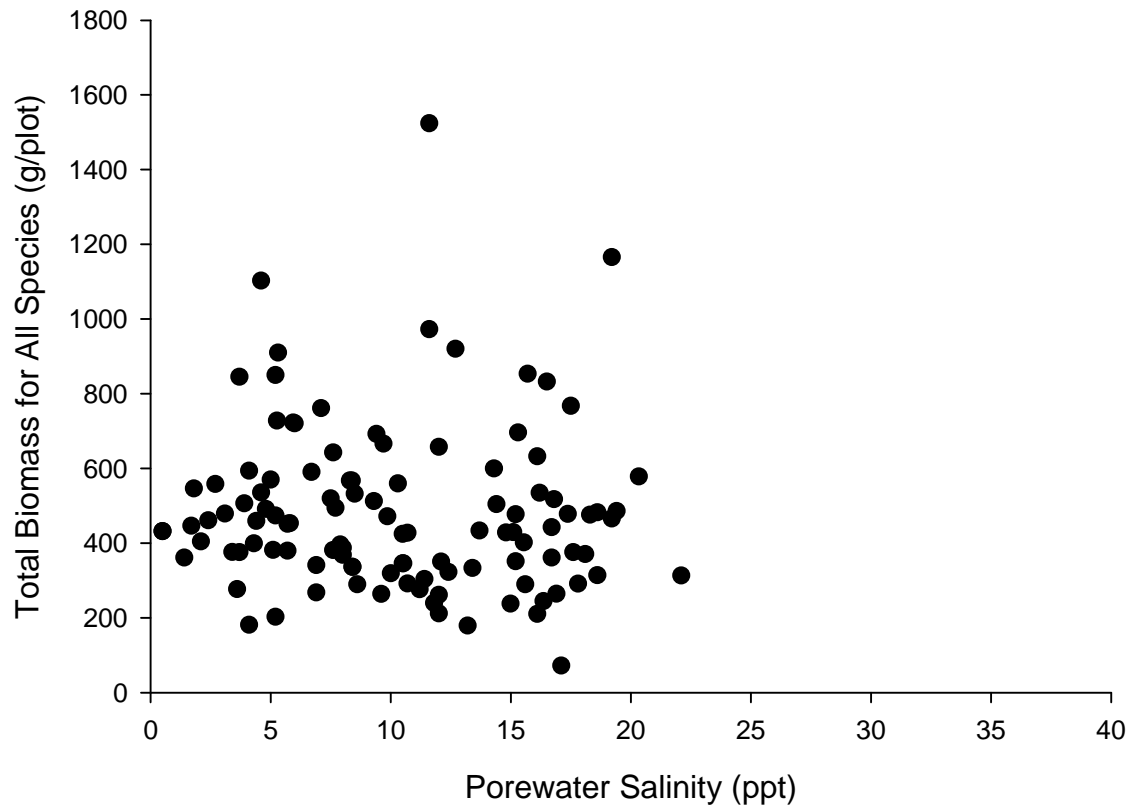


Figure D: Relationship between total biomass (g) for all species in a 0.25 m² clip plot and porewater salinity (ppt) taken at 10 cm below marsh surface at the time clip plots were harvested.

- Redo this graph w/ different symbols for F & S sites?
- Make different symbols for sites with elevated salinity from the hurricanes?

Spring 2007-Fall2008

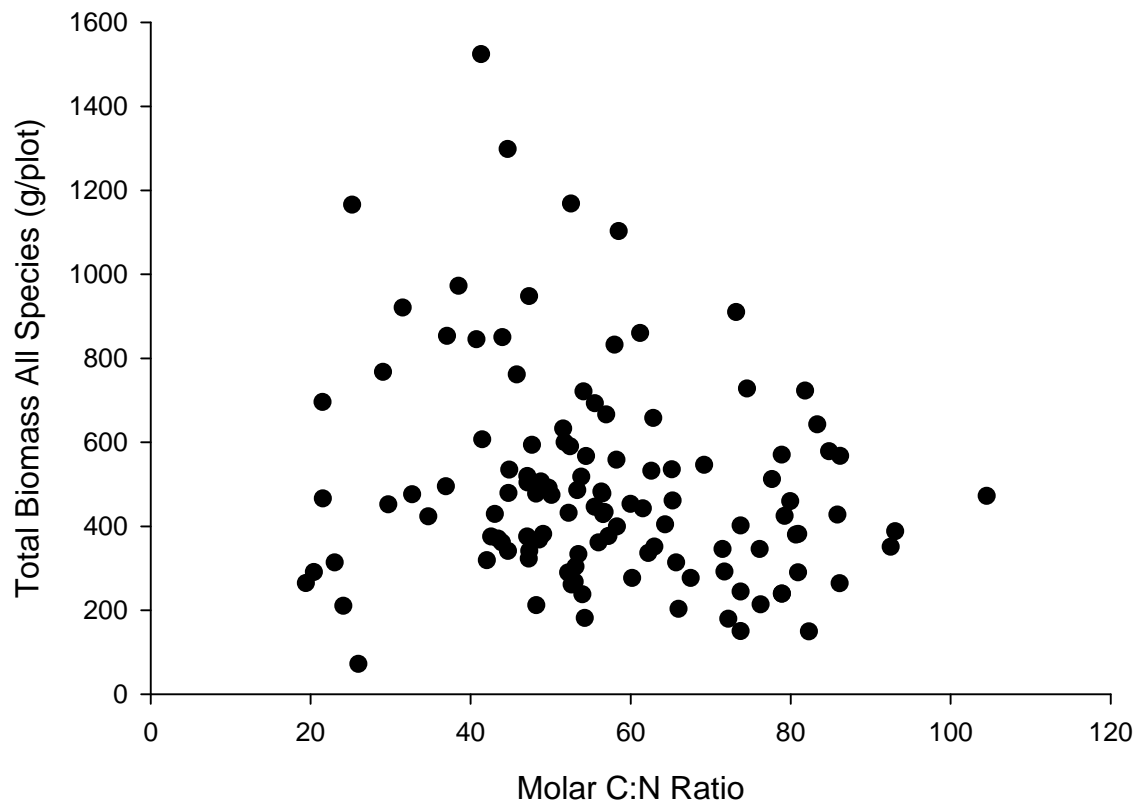


Figure E: Relationship between total biomass of all species collected in a plot and the molar C:N ratio of *Spartina patens* leaf tissue at each plot.

Spring 2007-Fall 2008

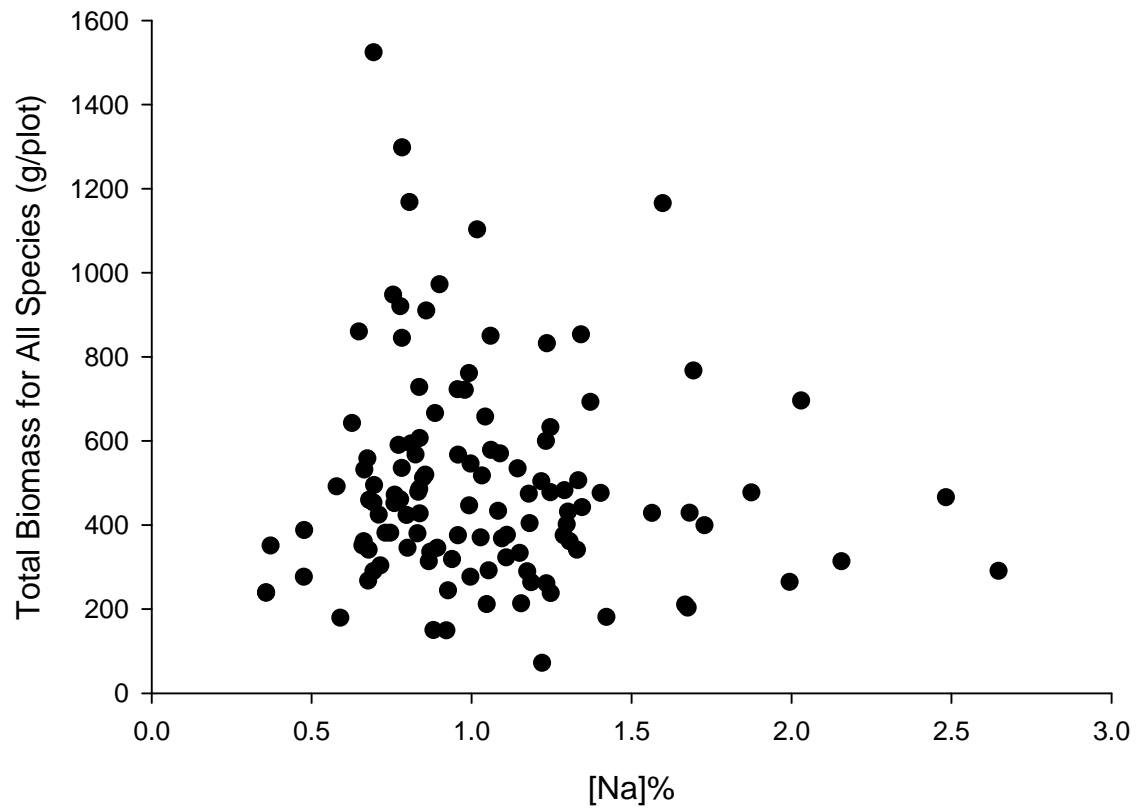
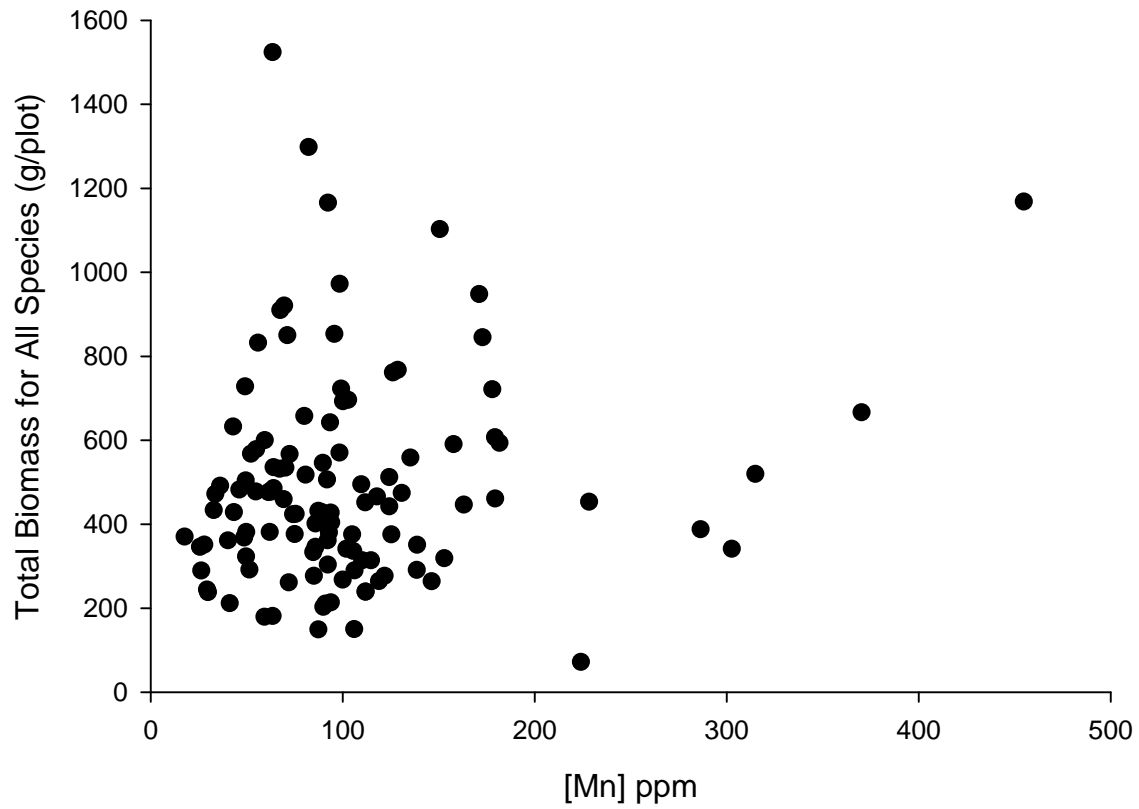


Figure F: Relationship between total biomass of all species collected in a plot and the sodium concentration of *Spartina patens* leaf tissue at each plot.

Spring 2007- Fall 2008



+ one outlier at approx. (800, 900)

Figure G: Relationship between total biomass of all species collected in a plot and the manganese concentration of *Spartina patens* leaf tissue at each plot.

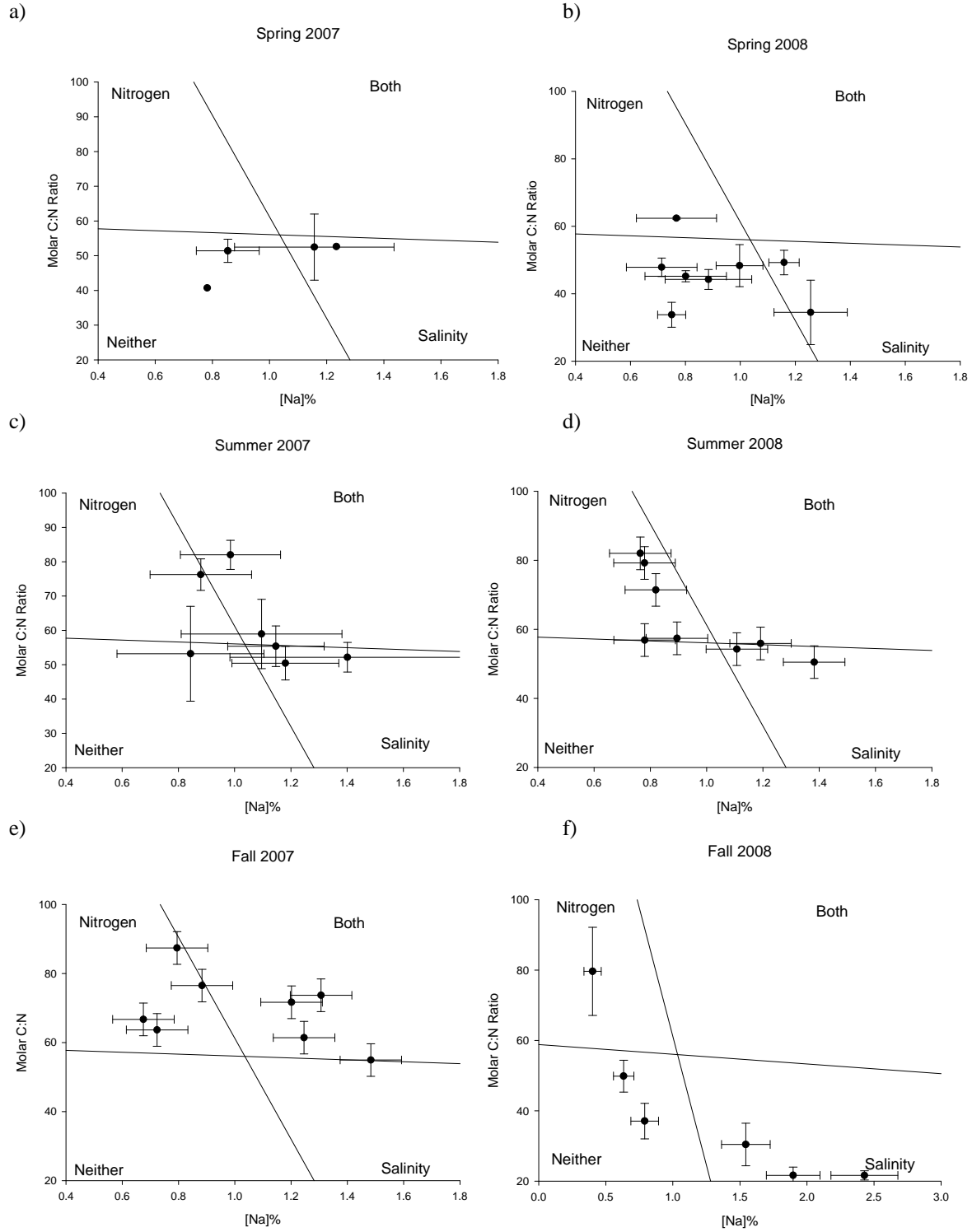


Figure H: Molar C:N ratios and [Na]% in *S. patens* leaf tissue collected over a two-year sampling period.

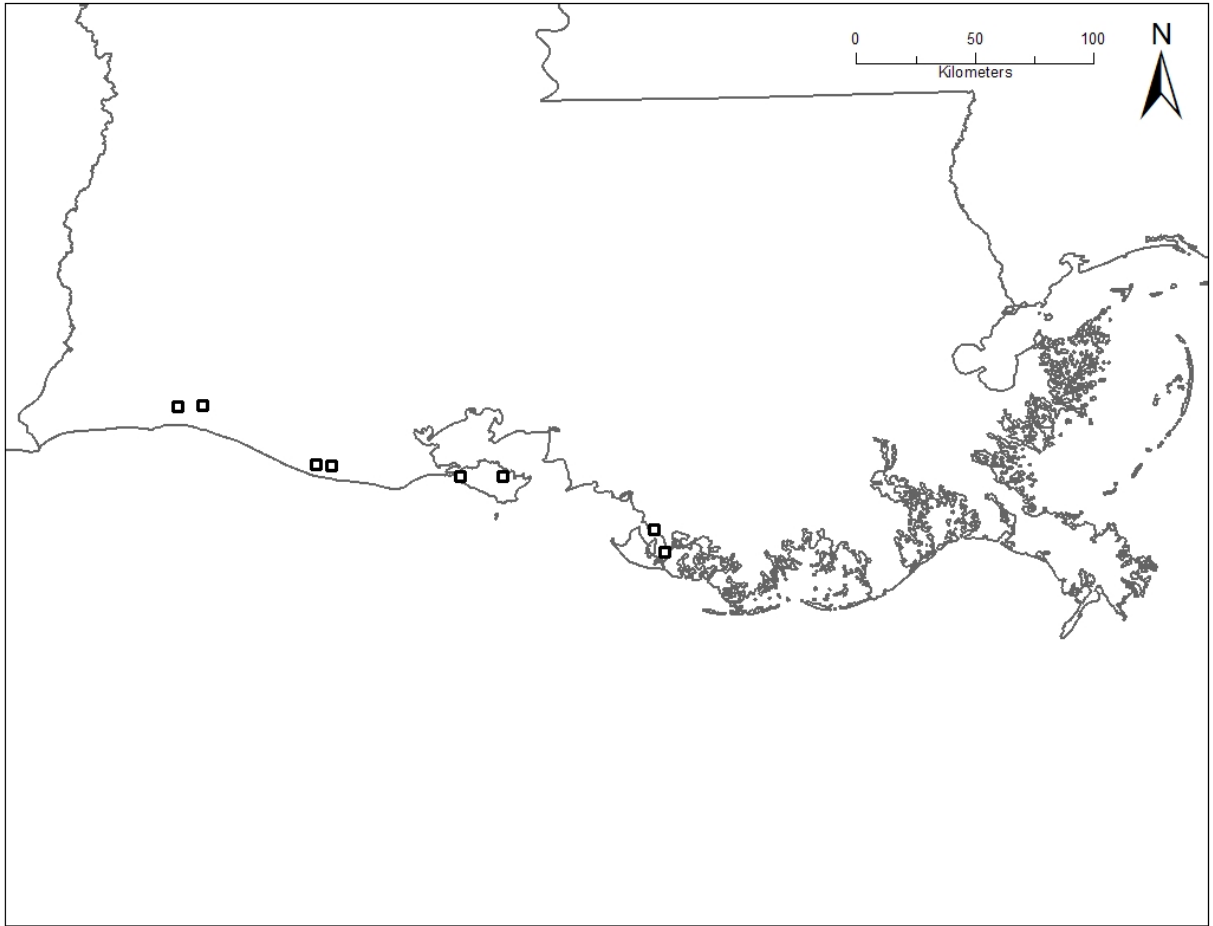


Figure I: Location of sampling sites along the coast of Louisiana.

	Spring2007								Summer2007								BHB	CPS	DB	G		
	BHB	CPS	DB	GHI	MIA	MIFW	MISW	MRB	BHB	CPS	DB	GHI	MIA	MIFW	MISW	MRB						
algae spp.	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Spartina alterniflora	171.4	.	0	0	.	.	.	4	174.8	146.9	0	0	.	0	63.7	0	154.8	27.6	0	0	0	0
Amaranthus sp.	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Aster tenuifolius	0	.	0	0	.	.	.	0	0	0	0	0	.	24.9	0	0	0	0	0	0	0	0
Spartina cynosuroides	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Distichlis spicata	18.8	.	0	0	.	.	.	31.3	35.6	0	0	6	.	38.7	17.7	0	20.8	0	0	0	0	2
Eleocharis spp.	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Juncus roemerianus	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Lythrum lineare	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Paspalum sp.	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Spartina patens	69.9	.	477.4	506.9	.	.	.	466.5	44.3	148.6	655.0	225.8	.	198.9	89.5	603.7	17.8	284.4	696.2	17.8	284.4	696.2
Pluchea foetida	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Sagittaria lancifolia	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Scirpus olneyi	0	.	0	9	.	.	.	0	0	0	0	61.9	.	29.0	0	0	0	0	0	0	0	0
Scirpus robustus	0	.	1	0	.	.	.	0	0	0	0	0	.	0	6	0	0	4	5	0	0	0
Solidago sp.	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Sporobolus virginicus	0	.	0	0	.	.	.	0	0	0	0	70.6	.	18.3	0	0	0	0	0	0	0	12
Unidentified species	0	.	0	20.0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	1	0	0	0
Vigna luteola	0	.	0	0	.	.	.	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0
Total Live Biomass	260.1	.	478.3	536.0	.	.	.	501.7	254.7	295.8	655.0	364.7	.	309.9	176.4	603.7	193.4	316.2	702.3	193.4	316.2	702.3
Total Dead Biomass	482.3	.	1105.2	1730.1	.	.	.	233.4	472.6	75.4	605.4	515.0	.	616.2	704.7	247.0	482.1	266.1	1040.9	482.1	266.1	1040.9
Species Richness	3	.	2	3	.	.	.	3	3	3	1	5	.	6	4	1	4	3	3	4	3	3

	Spring2008								Summer2008								BHB	CPS	DB	G		
	BHB	CPS	DB	GHI	MIA	MIFW	MISW	MRB	BHB	CPS	DB	GHI	MIA	MIFW	MISW	MRB						
algae spp.	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spartina alterniflora	74.4	156.0	0	90.7	0	0	28.0	0	263.8	6.5	0	126.8	0	0	90.4	0	0	65.8	0	0	0	0
Amaranthus sp.	0	0	0	0	0	0	0	0	0	0	0	0	1.4	0	0	0	0	0	0	0	0	0
Aster tenuifolius	0	0	0	0	0	0	0	0	1.1	0	0	0	0	7.2	0.2	0	0	0	0	0	0	0
Spartina cynosuroides	0	0	0	0	0	0	7.9	0	0	0	0	0	0	0	3.4	0	0	0	0	0	0	0
Distichlis spicata	92.1	0	0	2.0	0	32.2	42.5	24.4	24.0	0	0	22.0	0	42.5	51.2	1.2	0	0	1.2	0	0	0
Eleocharis spp.	0	0	0	3	0	1.9	0	0	0	0	0	0	0	2.3	0	0	0	0	0	0	0	0
Juncus roemerianus	0	0	0	0	0	0.0	200.4	0	0	0	0	14.1	0	0	36.2	0	0	0	0	0	0	0
Lythrum lineare	0	0	0	0	0	0	0	0	0	0	0	0	0	7.6	0	0	0	0	0	0	0	0
Paspalum sp.	0	0	0	0	119.6	0	0	0	0	0	0	48.4	0	0	0	0	0	0	0	0	0	0
Spartina patens	201.0	83.5	646.0	293.6	166.2	152.8	92.1	231.3	49.1	402.2	612.0	352.0	81.1	155.6	86.5	307.6	0	190.6	761.1	0	0	0
Pluchea foetida	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0
Sagittaria lancifolia	0	0	0	0	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scirpus olneyi	0	0	0	88.6	0	220.1	0	0	0	0	0	80.6	0	188.1	0	0	0	0	0	0	0	0
Scirpus robustus	0	0	5.3	18.3	0	1.2	7.1	0	5.2	0	31.9	34.0	8.5	0	2.4	0	0	0	0	0	0	0
Solidago sp.	0	0	0	0	10.5	0	0	0	0	0	0	0	28.5	0	0	0	0	0	0	0	0	0
Sporobolus virginicus	0	0.4	0	81.1	182.0	0	0	0	0	0	0	9.4	417.1	0	0	0	0	0	0	0	0	0
Unidentified species	0	0	0	0	5.4	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	0
Vigna luteola	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Live Biomass	367.5	239.8	651.3	577.3	484.5	408.6	378.1	255.7	363.2	408.6	643.9	687.2	537.2	403.5	270.4	308.8	0	256.4	762.4	0	0	0
Total Dead Biomass	570.4	723.8	1252.3	981.2	373.3	577.3	769.6	780.3	555.0	375.8	1661.2	748.3	347.6	844.8	752.4	1355.8	0	658.9	2269.3	0	0	0
Species Richness	3	3	2	7	7	6	2	2	5	2	2	8	6	7	7	2	2	2	2	2	2	2

Table 1: Biomass extrapolated to 1 m² plots and species richness for each site. Biomass numbers presented here were calculated by averaging the biomass of each species from all subsites and multiplying by four.

[This table needs major surgery. Probably needs to be retyped because these numbers disappeared from excel.]

Reformat numbers

Fix Species Richness numbers!