1998

Intraspecific Variation in Three Marsh Grasses in Response to Increased Flooding and Salinity.

Jeannine Marie Lessmann
Louisiana State University and Agricultural & Mechanical College

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INTRASPECIFIC VARIATION IN THREE MARSH GRASSES IN RESPONSE TO INCREASED FLOODING AND SALINITY

A Dissertation

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

in

The Department of Oceanography and Coastal Sciences

by
Jeannine Marie Lessmann
B.S., University of Maryland, Baltimore County, 1991
May 1998

Acknowledgments

I would like to first thank Dr. Irving Mendelssohn. His advice and guidance were invaluable to the success of my doctoral program. His commitment to excellence in research and teaching and his professionalism ensured quality in my development and inspired me in my pursuit of an academic career. Additionally, I would like to thank the members of my committee: Drs. Karen McKee, David Longstreth, Denise Reed, Mary Musgrave, and Robert Gambrell. I have been fortunate to have each member directly involved with my progress throughout this program and to have benefited from their individual strengths and support.

Many other people contributed to the success of this dissertation. Completing five greenhouse experiments and a field study required the help of many fellow graduate students, whose hard work and shared enthusiasm were greatly encouraging. Their varied backgrounds contributed significantly to broadening my perspectives both in research and in teaching. I also want to thank those who shared my sense of adventure in experiencing life during this unique time and in this unique culture. I have gained so much more through their excitement and insights.

Individually, I would like to thank Cheryl Hagn and Bill Keogh for their love, laughs, and well timed diversions. Jason and Vanessa Lessmann were each witness and friend, and I am pleased to always have them to look back with and share the memories. Richard Lessmann, though at a great distance, always gave me his love and support. I have never forgotten. Finally, my deepest love and gratitude go to my mother and best friend, Marie Lessmann. She has always been my greatest inspiration and teacher.

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Major: Oceanography and Coastal Sciences
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Dissertation directed by Professor Irving A. Mendelssohn
Pages in dissertation, 98. Words in abstract, 148.

ABSTRACT

Intraspecific variation in response to moderate levels of flooding and salinity stress was identified in *Spartina patens*, *S. alterniflora*, and *Panicum hemitomon* . This analysis enabled genotypes of each species to be selected for desired traits to enhance their function in restored systems, such as increased belowground biomass to stabilize soil substrates or increased aboveground biomass to enhance sedimentation. It was also determined that increased flood tolerance conferred a greater advantage for plant growth at moderate stress levels than did increased salt tolerance. However, this growth advantage was overridden by high salinity stress, and was greatly reduced by excessive flooding stress. Populations identified as more flood-tolerant were characterized as having greater total biomass, better ability to maintain relatively constant concentrations of nutrients with changing stress levels, and higher nutrient use efficiencies for nitrogen and phosphorous. Therefore, the use of the more flood-tolerant populations in deteriorating marshes would potentially restore high productivity and self-sustainability, both functions which are critical to the long-term success of restoration projects.

Chapter 1

Introduction

In the last few decades the depth and frequency of flooding has increased in many coastal areas around the world, with global sea level rise estimated at 0.3-3 mm/yr (Jelgersma *et al.* 1993; Gornitz 1995). In coastal Louisiana relative sea level rise is estimated to be 1.04-1.19 cm/yr, a rate dramatically increased by rapid subsidence of the Mississippi deltaic plain (Penland and Ramsey 1990). Accretion of the marsh surface through accumulation of inorganic sediment and organic matter at a rate sufficient to effectively keep pace with the relative rise of sea level is critical for wetland survival. Wetland vegetation facilitates vertical marsh accretion by trapping sediment and producing organic material. The construction of artificial levees, the closure of some distributaries, and dredging of canals have interrupted the natural supply of sediments (Turner *et al.* 1984; Cahoon and Reed 1995). Therefore, the role of vegetation in building the marsh surface is all the more important. However, vegetation alone is usually insufficient to maintain the necessary accretion rates, and excessive inundation of the marsh surface often results.

Artificial levees, closed distributaries, and dredged canals have also disrupted hydrology critical to wetland function and structure by restricting freshwater flow from the Mississippi River. This has lead to intrusion of saltwater into previously freshwater and brackish areas (Salinas *et al.* 1986; Nyman *et al.* 1993). Plants in these areas fail to adjust to excessive waterlogging and increased salinity, leading to plant death and marsh deterioration. Furthermore, subsequent recolonization is prevented by the inundated, reduced, and more saline soil conditions (Mendelssohn and McKee 1992; Boesch *et al.* 1994; Flynn *et al.* 1995; Baldwin *et al.* 1996). In Louisiana, these factors have resulted in the highest wetland land loss rates in the United States (Boesch *et al.* 1994).

Recovery and restoration of these deteriorated areas can often be initiated or accelerated by replanting healthy vegetation. Epstein *et al.* (1980) proposed that an

"engineering approach" to environmental problems was no longer adequate alone and that it should be combined with a "genetic approach." The possibility that the genetic approach might be feasible is supported by the identification of genetic differentiation among populations within wetland plant species, with certain populations being better adapted than others to specific environmental stressors (Keeley 1979; Silander and Antonovics 1979; Davies and Singh 1983; Eleuterius 1989; Jefferies and Rudmik 1991; Hester 1995; Krauss 1997). By utilizing this genetic variation, plant stocks can be selected that are more flood and salt tolerant than existing vegetation, thereby improving the quality of transplant material and enhancing stress response by vegetation to overcome the barrier to recolonization. More tolerant populations would benefit deteriorating areas through increasing productivity and stem density, enhancing sedimentation and peat formation, further facilitating vertical marsh accretion. As a result, stress-tolerant genotypes may enhance the rate by which created and restored marshes reach the functional equivalency of healthy, natural systems and accelerate recovery that is persistent and self-sustaining. Furthermore, planting of improved stocks while local vegetation still remains, prior to areas deteriorating fully to open water, may facilitate establishment of more tolerant transplants and maintain integrity of the soil substrate. Such early intervention, only made possible by new plant stock availabilities, could reduce the need for large scale restoration operations and minimize land losses in certain areas.

This research has examined the potential for population differentiation in *Panicum hemitomon* Schultes, *Spartina patens* (Aiton) Mulh., and *Spartina alterniflora* Loisel., dominant perennial species in Louisiana's freshwater, brackish, and salt marshes, respectively (Chabreck and Linscombe 1982). *Panicum hemitomon* ranges in the U.S. on the Atlantic coast from New Jersey to Florida and westward along the northern Gulf of Mexico to Texas, and in southern Africa. *Spartina patens* is found along the Atlantic coast from Quebec to south Florida and westward to Texas, salt marshes of the Great

Lakes, West Indies, southern Europe, and northern Africa. *Spartina alterniflora* extends along the Atlantic coast from New Foundland to south Florida and westward to Texas (Godfrey and Wooten 1979). *Spartina* species have been used extensively around the world in coastal restoration programs, including the U.S. Pacific Coast (Mumford *et al.* 1990; Callaway and Josselyn 1992; Daehler and Strong 1996), China (Chung 1993), and New Zealand (Bascand 1970).

Although these three species exhibit flood tolerance, and *S. alterniflora* and *S. patens* exhibit salt tolerance, elevated salinities and prolonged flooding have been shown to adversely affect their growth and survival (Gleason and Zieman 1981; Morris 1984; Mendelssohn and McKee 1988; Feijtel *et al.* 1989; McKee and Mendelssohn 1989). Under reduced conditions, plant roots must respire anaerobically, at a considerable loss of energy yield to the plant (Drew 1983; Crawford 1992). This energy loss can result in the disruption of metabolic and transport processes by affecting shoot-water relations and transport of nutrients and important regulatory phytohormones to aboveground tissues (Drew 1983; Koch *et al.* 1990; Armstrong *et al.* 1994). Phytotoxins, such as sulfides, may accumulate in waterlogged soil, damaging belowground tissue and resulting in decreased nutrient uptake from the soil by the roots (Allam and Hollis 1972; Pearson and Havill 1988; Koch *et al.* 1990).

In an effort to avoid hypoxia/anoxia belowground and buffer soil phytotoxins, many species develop air spaces in their tissues called aerenchyma. This response has been clearly demonstrated to be an important long term adaptation in maintaining flood tolerance and competitive ability in waterlogged sediments (Gleason and Zieman 1981; Schat 1984; Burdick and Mendelssohn 1987; Laan *et al.* 1989a). Aerenchyma is interconnecting gas-filled spaces in the cortex produced by either the breakdown of existing cells (lysigenous) or cell separation (schizogenous). Aerenchyma reduces both the oxygen demand of respiring tissue and physical resistance to diffusion along the diffusion pathway (Armstrong *et al.* 1994). Greater aerenchyma formation allows for

greater root length (Justin and Armstrong 1987), higher apical oxygen concentrations (Armstrong *et al.* 1994), and the development of an oxidized rhizosphere (Coutts and Armstrong 1976; Armstrong *et al.* 1994). Under flooded conditions these anatomical changes in the root can be effected within several weeks or months (Das and Jat 1977). Aerenchyma development in *S. alterniflora* was found already maximized independently of soil reducing levels (Arenovski and Howes 1992), in contrast to a development period of 25 days in *S. patens* (Burdick 1989). Smirnoff and Crawford (1983) distinguished flood tolerant from intolerant species at a root porosity of 10%. Root porosities as great as 50% of total root volume were determined in *S. patens* under increased flooding (Burdick and Mendelssohn 1987; Naidoo *et al.* 1992).

Three main stresses associated with salinity affect plant growth and metabolism, (Briens and Larher 1982; Lance and Rustin 1984; Marler and Zozor 1996). Osmotic stress results from a lower osmotic potential in the rooting medium than in plant tissue. This inhibits water uptake into the plant producing a "physiological drought," which can cause stomatal closure, reduced photosynthesis, and reduced cell elongation (Jefferies 1981; Rozema *et al.* 1985a). Toxic ion stress results from the uptake and accumulation of Na^+ and Cl^- from seawater to levels toxic to plant functioning (Flowers *et al.* 1977; Gorham *et al.* 1985). In addition, salt stress inhibits the uptake of critical nutrient ions due to competitive inhibition by Na^+ and Cl^- ions (Linthurst and Blum 1981; Huang and Redmann 1995) creating a nutrient deficiency. In response to these stresses, halophytes such as *Spartina*, exclude ions at the root-saltwater interface, accumulate ions in their tissues through compartmentalization of salts in their vacuoles, or secrete salts from their leaf tissue (Flowers 1985; Bradley and Morris 1991). These mechanisms allow plants to effectively control tissue concentrations of total ions and maintain needed levels of nutrients. Therefore, ions such as Na^+ are expected to be lower in plants better able to regulate uptake or exclusion of ions from the soil under salinity stress or higher when compartmentalization is the strategy for osmotic balance (Bradley and Morris 1991; Wang

et al. 1992; Reimann and Breckle 1995). Translocation and compartmentalization of ions into vacuoles (Flowers 1985; Naidoo 1994) prevents the buildup of salts to toxic levels in the cell cytoplasm (Flowers *et al.* 1977; Cavalieri and Huang 1979; van Diggelen *et al.* 1986). To prevent osmotic imbalance between the cytoplasm and the vacuole, compatible osmotic solutes are produced in the cytoplasm. In *Spartina*, the compatible osmoticum proline as well as other organic solutes accumulate. However, proline accumulation has been demonstrated to have a salinity threshold level ranging from 15 to 30 ppt (Cavalieri 1983; Ewing *et al.* 1997).

As the first part of my dissertation research, comparative greenhouse studies were conducted to differentiate flood-tolerant populations of *P. hemitomon*, *S. patens* and *S. alterniflora* collected along the Texas and Louisiana coast. These populations were first propagated in a greenhouse under drained conditions for four or more vegetative generations to remove environmental influences of the collection site. All three species were then flooded for approximately two months, and intraspecific variation in leaf elongation and biomass partitioning were measured and used as an indicator of relative flood tolerance. This chapter resulted in the selection of populations for each species ranging from greater flood tolerance to lesser flood tolerance, of which the *S. patens* populations were investigated further. This chapter has been published in the journal Ecological Engineering.

The differentially flood-tolerant *S. patens* populations, which were also evaluated for differential salt tolerance in a separate study (Hester 1995), were used in field and greenhouse studies examining multiple stressor effects of increased flooding and salinity under both natural and controlled conditions. Little research has focused on genotypic response to the interaction of increased flooding and salinity or tested differentially flood- and salt-tolerant populations under deteriorating field conditions. Chapter 3 describes the field study and focuses on growth and ion relations in determining genotypic differences to flooding and salinity. This research provided additional insight into the relative

significance of improved flood tolerance versus improved salt tolerance in conferring greater success of different genotypes in more stressed areas. Further, the results pointed to a realized potential for use of these more stress-tolerant populations in the restoration of different types of deteriorating marshes.

Chapter 4 was designed to test similar hypotheses regarding the differentially stress-tolerant populations under controlled conditions and to identify specific response mechanisms underlying the differential population tolerances. By comparing such responses in plants that are closely related and similar in ecological distribution, the results can contribute to a greater understanding of the relative roles of each mechanism in stress-tolerance. This understanding would also aid in better matching greater stress-tolerant populations and site conditions, as well as in delineating the role of intraspecific variation in stress tolerance in predicting community responses to natural or anthropogenic changes in the environment. Chapter 5 summarizes the conclusions drawn from this research and discusses possible future research directions. The research chapters are to stand alone for publication. As a result, there is some redundancy in chapter contents.

Chapter 2

Population Variation in Growth Response to Flooding of Three Marsh Grasses[1]

INTRODUCTION

Coastal wetlands in Louisiana are suffering severe land loss rates due to excessive inundation of the marsh surface in a rapidly subsiding deltaic plain as well as from human impacts (Turner *et al.* 1984; Penland and Ramsey 1990; Reed 1991). Failure of plants to adjust to waterlogging leads to plant death and marsh deterioration with subsequent recolonization impeded or prevented by the inundated soil surface and reduced soil conditions (Mendelssohn and McKee 1989). Therefore, the need exists for wetland plants that display superior flood tolerance for use in restoring deteriorating marshes. Additionally, as sea level rise accelerates due to global warming and the submergence tolerance of wetland plant species is surpassed, the identification of more flood tolerant stocks will attain world-wide importance.

Genetic differentiation among populations within a plant species is well documented, with certain populations being better adapted than others to specific environmental stressors (Keeley 1979; Silander and Antonovics 1979; Davies and Singh 1983; Schat 1984; Chung 1989). This natural genetic variation provides the potential for selection of plant populations exhibiting improved stress tolerance. The development of superior stocks of wetland species could yield improved productivity in high stress areas and potentially facilitate vertical accretion of the marsh surface through increased mineral accretion and peat formation. As a result, these plants may: 1) enhance the rate by which created and restored marshes reach the functional equivalency of healthy, natural systems, 2) reverse the trend of increasing inundation, and 3) accelerate recovery that is persistent and self-sustaining. To my knowledge, no attempts

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have been made to select populations of coastal vegetation for greater flood tolerance and, as a result, superior genotypes are not currently available for wetland restoration.

Comparative greenhouse studies were conducted to evaluate intraspecific variation in flood tolerance for selected populations of *Panicum hemitomon* Schultes, *Spartina patens* (Aiton) Mulh., and *Spartina alterniflora* Loisel., which are dominant species in Louisiana's freshwater, brackish, and salt marshes, respectively. Although these species exhibit flood tolerance, excessive flooding has been shown to adversely affect their growth and survival (Mendelssohn and Seneca 1980; Morris 1984; Mendelssohn and McKee 1988; McKee and Mendelssohn 1989). The goal of this research was to develop methodology to identify and select genotypes of these species that exhibit superior flood tolerance. My results show for the first time that these important wetland species do exhibit population-specific differences in flood tolerance and that the potential exists for the selection of greater flood-tolerant genotypes for use in coastal restoration. Thus, the ability to identify superior flood-tolerant populations of these species could have important implications for the future success of wetland creation and restoration projects.

MATERIALS AND METHODS

Plant material and experimental design

Eighteen populations of *P. hemitomon*, 19 populations of *S. patens*, and 25 populations of *S. alterniflora* were collected along the Louisiana and Texas coasts and propagated in a greenhouse under drained conditions for 4-6 vegetative generations to remove environmental influences of the collection site. Separate experiments for the three species were conducted. For each species, two or more stems of uniform height from each population were selected and planted into 215 cm^3 pots containing a 50:50 mixture of Jiffy Mix, which had been waterlogged for approximately 90 days to create reduced soil conditions, and Jiffy Mix Plus (4.2 kg/m^3 of 7-18-5-12, N-P-K-Mg; commercial potting mediums, Jiffy Mix Products, West Chicago, Illinois). A layer of small (3-5 mm) gravel approximately 0.5 cm in depth was placed over the soil surface to reduce the buoyancy of

the pots while submerged. Five replicates of the populations were placed into 50 x 50 x 100 cm Nalgene tanks in a randomized block design (for a total of 90 pots of *P. hemitomon*, 95 pots of *S. patens*, and 125 pots of *S. alterniflora*).

Populations for each species were first established under drained, but moist soil conditions (26, 59, and 24 days for *P. hemitomon*, *S. patens*, and *S. alterniflora*, respectively). To avoid initially shocking the plants, flooding levels in the tanks were raised gradually from the soil surface to the top of the tank (flooding levels did not serve as treatments). Populations of *P. hemitomon* were flooded, at weekly intervals, to the soil surface, 13 cm above the soil surface, and 26 cm above the soil surface, then for six weeks at 39 cm above the soil surface (tank top), for a total of 58 days. Populations of *S. patens* were flooded, at four-week intervals, to the soil surface and 39 cm above the soil surface, for a total of 57 days. Populations of *S. alterniflora* were flooded, at two-week intervals, to the soil surface, 13 cm above the soil surface, and 26 cm cm above the soil surface, then for three weeks to 39 cm above the soil surface, for a total of 67 days.

Sampling was conducted at either weekly (*P. hemitomon*) or biweekly (*S. alterniflora* and *S. patens*) intervals. For each sampling period, two stems from each pot were selected for young, elongating leaves. Elongation rates (cm/day) were measured by placing a ruler behind the youngest leaf and measuring the distance from ligule to leaf tip. Also, redox potentials at 1-cm and 7-cm depths were measured in each pot with bright platinum electrodes. The potential of a standard calomel reference electrode (+244 mV) was added to the millivolt reading to obtain Eh. Mean Eh during each sampling period and for each depth was calculated for each population and over all populations for each species.

At the end of the experiment and final flooding level, plant material of each population was harvested at the water level (above-water biomass), at the soil surface (below-water biomass) and below the soil surface (belowground biomass). Biomass was further partitioned into stems, leaves, roots, and rhizomes. Dead aboveground biomass was also

determined. Dead belowground biomass was minimal and not separated from live belowground biomass for *P. hemitomon* and *S. patens*. For some populations of *S. alterniflora* however, the initial root system as well as some stems that were alive at the time of planting died upon flooding. For these populations, dead belowground biomass was separated from live belowground biomass. Dry weights of the harvested plant material was recorded after drying at 80°C for 3 days.

Statistical Analyses

Analyses of covariance were performed for leaf elongation and final biomass data for each species, with initial cumulative height and initial plant weight, respectively, as covariates (SAS Institute, Inc. 1985). The covariates were not significant and were not used in the final analysis. Assumptions of normality and homogeneity of variance were tested (Shapiro-Wilk test) and found to be valid. Average leaf elongation rates for each population were analyzed with repeated measures analysis of variance (ANOVA) in a split plot design. Significant population effects for soil redox potential were also tested with an ANOVA. When significant population effects were found ($p \leq 0.05$), multiple comparisons of the means were conducted with Tukey's Honestly Significant Difference (HSD).

The original 15 to 21 biomass variables were analyzed initially with a MANOVA ($p=0.0001$), followed by univariate analysis of each variable (PROC GLM). ANOVA analysis however, failed to yield consistent results in the population rankings for the biomass variables, thereby precluding population selection. Correlation analysis (PROC CORR) was also performed, but all variables were highly correlated and therefore, a rotated principal components analysis (PCA) was used to reduce the number of variables for final analysis (PROC FACTOR) (Manly 1986; Johnson and Wichern 1988). PCA was used to generate uncorrelated, linear combinations of the original biomass variables. Rotation of the principal axes resulted in each variable having a high loading on one principal component and medium to low loadings on the remaining principal components

(Johnson and Wichern 1988). The principal components describing the greatest proportion of variation for each species were retained and an ANOVA performed on the population principal component scores to determine significant population effects. Where significant population differences within a species were detected ($p \leq 0.05$), population scores were ranked and multiple comparisons of population scores were conducted with Tukey's HSD.

RESULTS

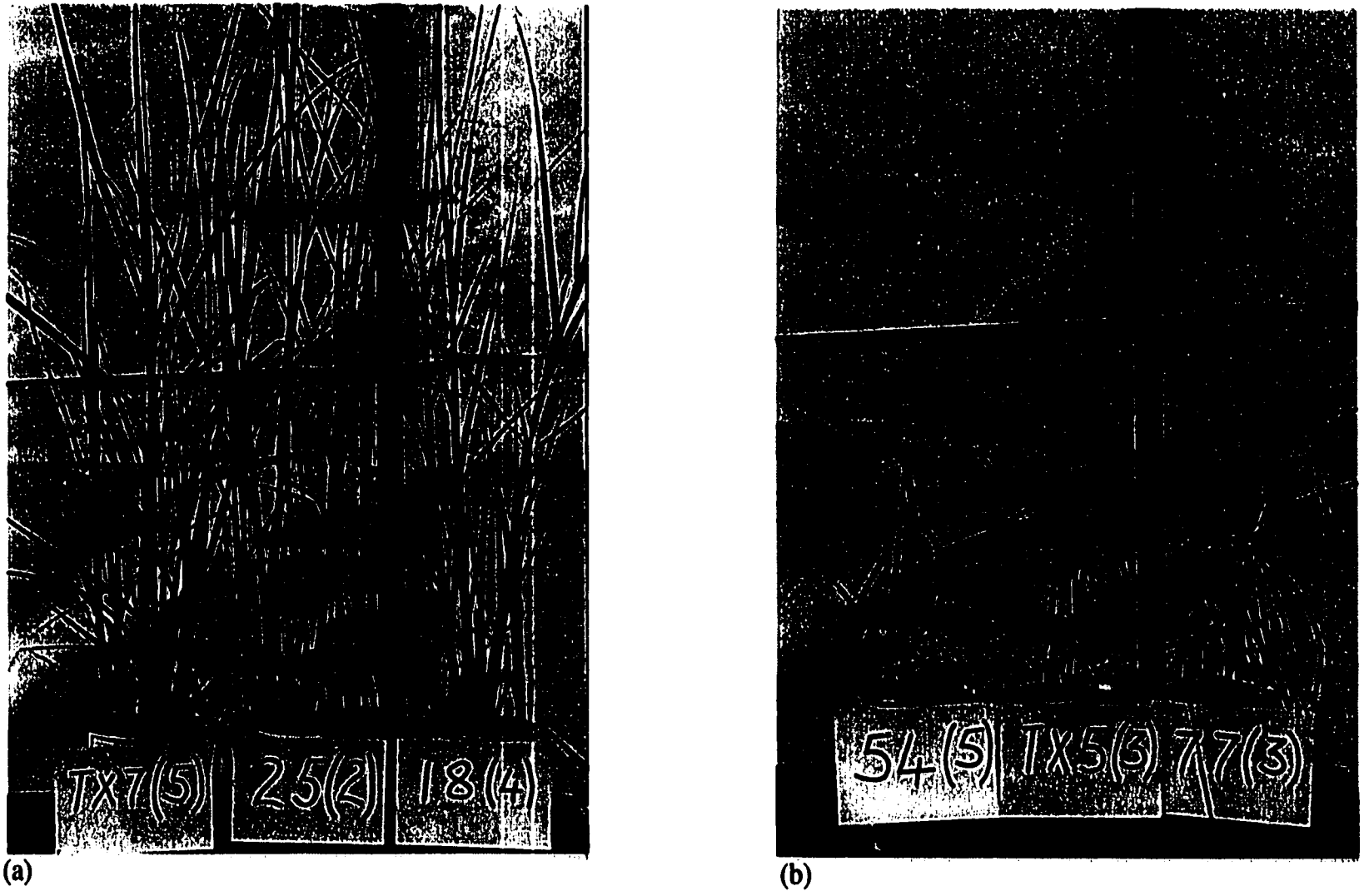
Redox potentials decreased over the flooding periods and ultimately reached levels that ranged from -37 to -138 mV (*S. alterniflora.*), +219 to +28 mV (*S. patens*), and +29 to -46 mV (*P. hemitomon*) (Table 2.1). Thus, soil conditions were reducing and did not differ significantly among populations within a species. Growth and biomass production among populations in response to the flooding treatment did differ significantly and these differences were visually evident for each species (Figure 2.1)

Population selections were made according to PCA score rankings, with the highest scores identifying the most flood-tolerant populations. For all three species, the first principal component accounted for the majority of the sample variance (44.3%-46.5%) and therefore, was weighted more heavily in the identification procedure. For *S. alterniflora*, the first three principal components retained from PCA analysis accounted for 73.2% of the variation among the populations (Table 2.2). Principal component 1 (PC1), which explained 46.2% of the variation, included a high loading for total biomass, and population effects were found to be significant ($p \leq 0.0001$). Principal component 2 (PC2) had high loadings for dead belowground biomass variables, and principal component 3 (PC3) had high loadings for the root to shoot ratio variables. PC2 and PC3, which accounted for 27.1% of the variation, also had significant population effects ($p \leq 0.001$ and $p \leq 0.05$, respectively); however, Tukey's comparisons revealed too few population differences for PC2 and PC3 to be useful and they were not used in further analysis. The top three scoring populations for PC1 were 7, 49, and 89, and were identified as the

Table 2.1 Redox potential during each sampling period. Measurements were taken at a depth of 1 cm and 7 cm for each population in each species (mean±S.E.).

Spartina alterniflora			*Spartina patens*			*Panicum hemitomon*		
Days Flooded	Eh (mV) 1 cm depth	Eh (mV) 7 cm depth	Days Flooded	Eh (mV) 1 cm depth	Eh (mV) 7 cm depth	Days Flooded	Eh (mV) 1 cm depth	Eh (mV) 7 cm depth
14	-38±12	-37±13	0	219±10	134±13	3	25±12	-40±10
30	-131±11	-138±11	21	137±11	43±10	9	ND	ND
46	-99±12	-79±13	39	123±12	28±11	15	-46±15	-37±13
67	-119±9	-138±9	57	104±11	61±11	58	29±10	-36±9

ND=not determined



(a) (b)

Figure 2.1 Differential growth response of *S. alterniflora* populations under flooding stress; populations pictured in A exhibited greater growth response and populations pictured in B exhibited poorer growth.

Table 2.2 Rotated principal component patterns for each species. Variables which loaded highly on a principal component are indicated in bold.

Biomass	*Spartina alterniflora*			*Spartina patens*			*Panicum hemitomon*		
variables[a]	PC[b] 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
AGBIO	**0.9693**	-0.0578	-0.1738	**0.7395**	0.6411	-0.1494	**0.7483**	0.6402	0.0855
BGBIO	**0.9370**	-0.0667	0.1869	**0.9468**	-0.0735	0.2419	**0.8299**	0.0969	0.4859
TOTBIO	**0.9892**	-0.0627	-0.0569	**0.8754**	0.4779	-0.0408	**0.8283**	0.5126	0.2197
AWST	**0.7062**	-0.2046	-0.2460	0.2168	**0.9055**	-0.0993	0.1601	**0.8243**	0.0696
AWLF	**0.7111**	-0.1872	-0.2786	0.3549	**0.8026**	-0.0993	0.3701	**0.7767**	-0.0003
BWST	**0.9575**	-0.0665	-0.1749	**0.8893**	0.3422	0.0937	**0.8711**	0.2376	0.0828
BWLF	**0.8359**	0.0693	-0.0460	0.2684	-0.5901	0.2373	0.2994	-0.1904	-0.3330
ROOT	**0.9036**	-0.1280	0.1252	**0.9057**	-0.1138	0.2536	**0.8828**	-0.0976	0.1395
RHZM	0.6622	0.1796	0.3839	0.0386	0.0786	**0.9349**	0.2426	0.2262	**0.9196**
BGST	**0.8231**	-0.2104	-0.0184	**0.9046**	-0.0842	-0.2284	**0.7379**	0.0020	-0.2350
AWBIO	**0.7267**	-0.1970	-0.2754	0.2932	**0.9319**	-0.1071	0.2469	**0.8947**	0.0536
BWBIO	**0.9537**	-0.0135	-0.1300	**0.9236**	0.1940	-0.1438	**0.8723**	0.2363	0.0807
RTSHT2	-0.1086	-0.0392	**0.9544**	0.0579	-0.4700	**0.8687**	0.1486	0.3603	**0.9024**
RTSHTA	0.0174	-0.1823	**0.7767**	0.2497	-0.7387	0.4496	0.2614	-0.8009	0.0547
RTSHTB	-0.1593	0.0517	**0.8617**	-0.1447	-0.0454	**0.9510**	0.0197	0.0447	**0.9691**
DRT	-0.0706	**0.7638**	-0.0803						
DRH	-0.0246	0.5560	0.0224						
DBGS	-0.2364	0.5612	0.0820						
AGDEAD	0.6694	0.2275	0.2147						
BGDEAD	-0.1674	**0.9378**	-0.0727						
TOTDEAD	0.4814	**0.7354**	0.1436						
% Variance	46.2%	13.8%	13.3%	46.5%	26.4%	15.6%	44.3%	20.3%	15.9%

[a]AGBIO=aboveground biomass, BGBIO=belowground biomass, AWBIO=above-water biomass, BWBIO=below-water biomass, TOTBIO=total biomass, RHZM=rhizome, BGST=belowground stem, LF=leaf, ST=stem, RTSHT2=(root+rhizome):shoot, RTSHTA=root:shoot, RTSHTB=rhizome:shoot, DRT=dead root, DBGS=dead belowground stems. [b]PC=principal component.

most flood tolerant populations, whereas the lowest scoring populations, 35, 54, and 3 were identified as the least flood tolerant populations (Figure 2.2).

For *S. patens*, the first three principal components retained from the PCA analysis accounted for 88.4% of the sample variance (Table 2.2). PC1 included a high loading in total biomass, as well as in the below-water and belowground variables, PC2 had high loadings in above-water variables, and PC3 had high loadings for the rhizome variables. PC1, PC2, and PC3 all had highly significant population effects ($p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.01$, respectively). Tukey's comparisons, however, revealed too few population differences for PC3 to be useful and it was not used further for selection. Populations 26, 38, and 14 exhibited high PC1 and PC2 scores and, consequently, were identified as the most flood tolerant populations for *S. patens*. Populations 69, 4, and 1 scored low for PC1 and PC2 and were identified as the least flood tolerant populations. Population 2 was not chosen as least flood tolerant because it had the highest PC2 score (Figure 2.3).

For *P. hemitomon*, the first three principal components retained from the PCA analysis accounted for 80.6% of the sample variance (Table 2.2) and loadings were similar to those in the *S. patens* analysis. PC1 had a high loading for total biomass as well as in the below-water and belowground variables. PC2 had high loadings for above-water variables, whereas the rhizome variables were most closely associated with PC3. A highly significant population effect was found for PC1 and PC2 ($p \leq 0.0001$); the population effect for PC3 was not significant. Populations 97, 89, and 86 had high ranking scores for PC1 and PC2 and were identified as the most flood tolerant populations (Figure 2.4). Population 88 was not included in this group because it scored high only for PC1, the PC2 score was low. Populations 78, 81, and 94 had the lowest scores for PC1 and PC2 and were identified as the least flood tolerant populations. Population 96 was not included because it had the second highest PC2 score. In general, population differentiation based on PCA analysis was in agreement with the original biomass response variables (Tables 2.3- 2.5).

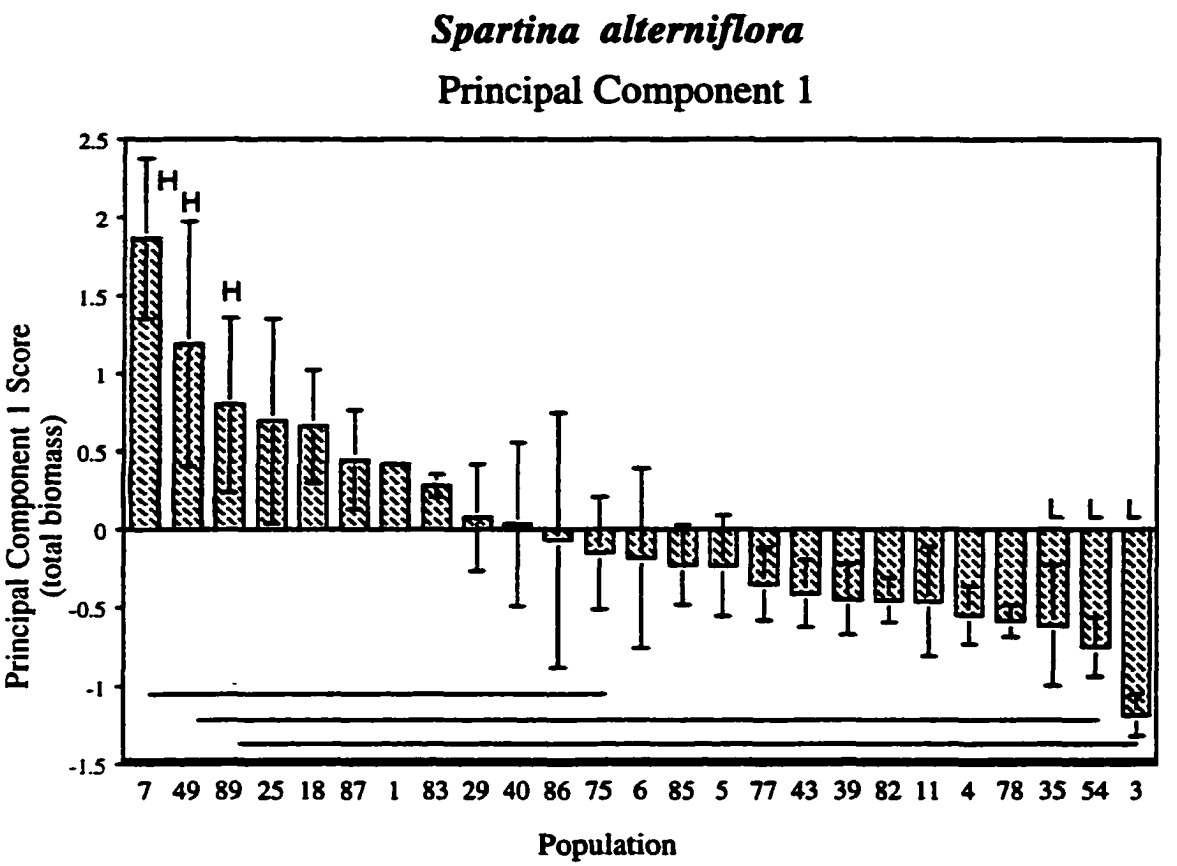


Figure 2.2 *Spartina alterniflora* population scores ranked for principal component 1. The results of Tukey's Honestly Significant Difference comparison are indicated by the horizontal lines. (H) indicates populations identified as more flood tolerant; (L) indicates populations identified as less flood tolerant. Absence of bar indicates SE smaller than symbol.

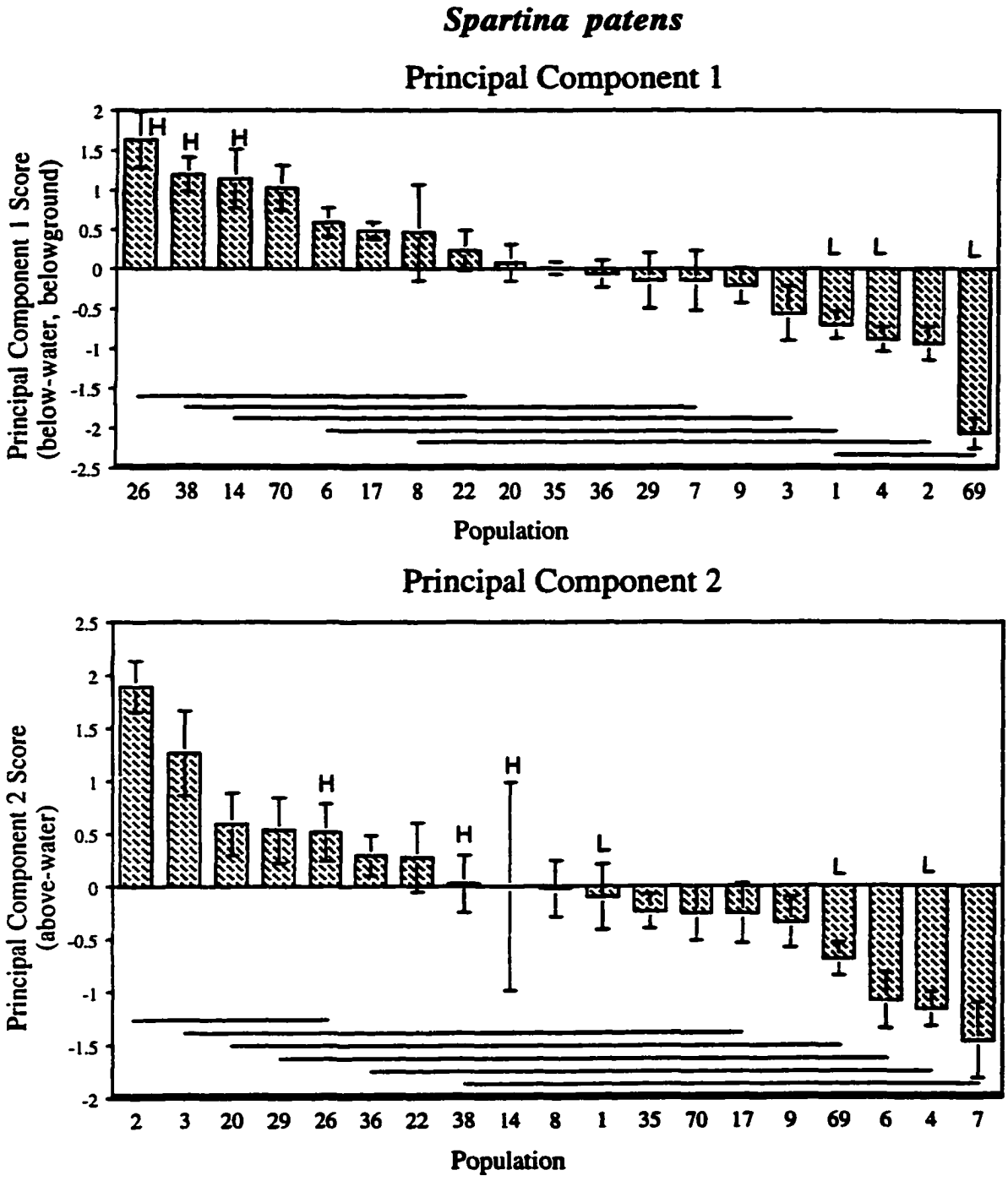


Figure 2.3 *Spartina patens* population scores ranked for principal component 1 and principal component 2. The results of Tukey's Honestly Significant Difference comparison are indicated by the horizontal lines. (H) indicates populations identified as more flood tolerant; (L) indicates populations identified as less flood tolerant.

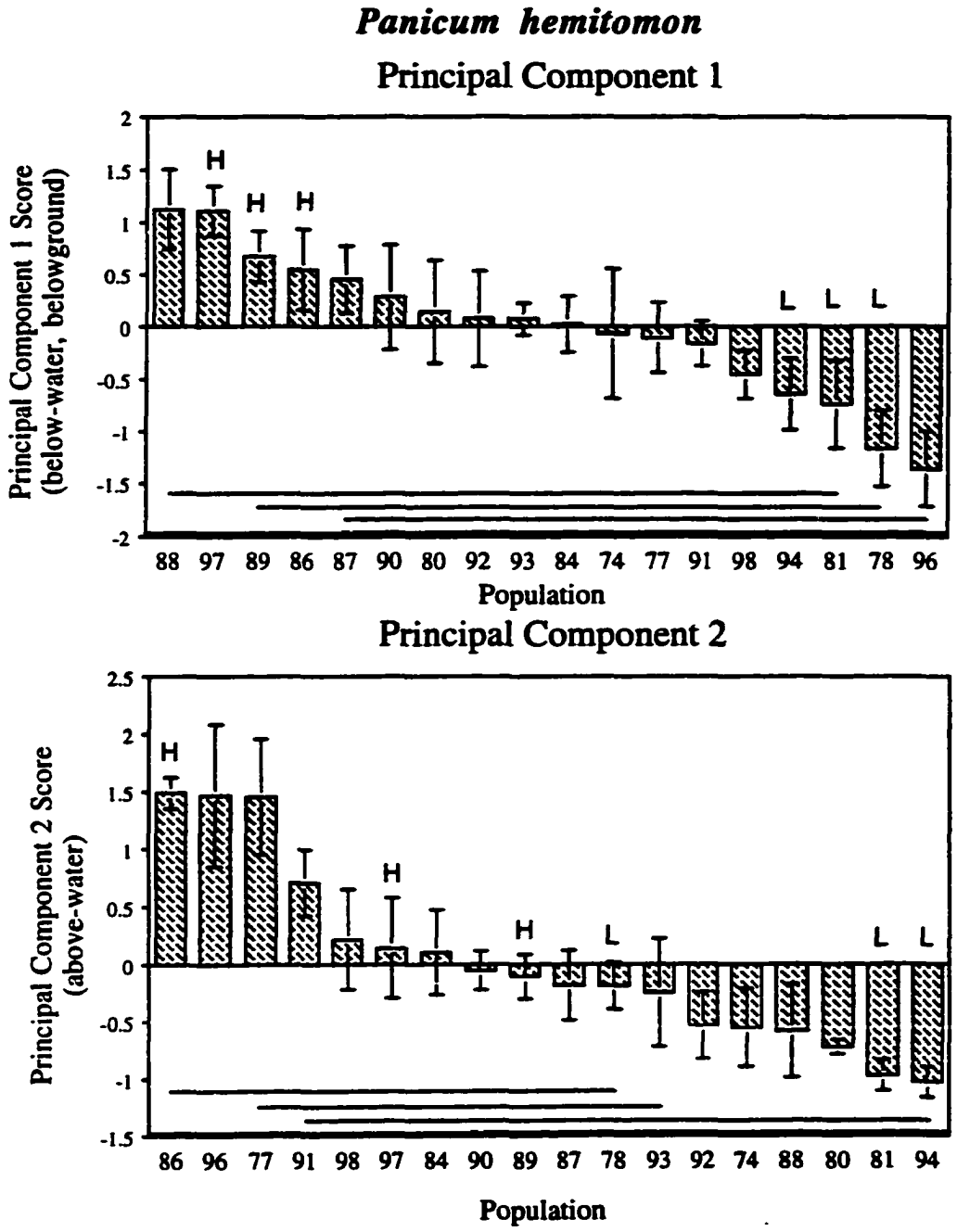


Figure 2.4 *Panicum hemitomon* population scores ranked for principal component 1 and principal component 2. The results of Tukey's Honestly Significant Difference comparison are indicated by the horizontal lines. (H) indicates populations identified as more flood tolerant; (L) indicates populations identified as less flood tolerant.

Table 2.3 Summary of biomass data for each population of *Spartina alterniflora* after the 67 day experimental period (mean±S.E.).

Population	Biomass (g d wt pot^{-1}) [a]							
	AGBIO	BGBIO	TOTBIO	AWBIO	BWBIO	ROOT	RHZM	BGST
1	5.81±0.26	3.14±0.59	9.32±0.13	0.49±0.22	5.55±0.23	1.06±0.26	0.75±0.21	1.33±0.26
L 3	0.23±0.10	0.44±0.17	1.15±0.25	0	0.18±0.09	0.10±0.05	0.21±0.16	0.14±0.07
4	2.21±0.46	1.47±0.44	4.42±0.76	0	2.21±0.46	0.42±0.06	0.43±0.24	0.62±0.22
5	3.81±1.03	1.97±0.55	6.39±1.48	0.22±0.14	3.59±0.92	0.58±0.16	0.82±0.27	0.57±0.14
6	4.09±1.86	1.81±0.71	6.55±2.39	0.85±0.63	3.24±1.26	0.41±0.26	0.71±0.21	0.69±0.26
H 7	10.56±1.33	4.83±0.92	16.30±2.14	1.30±0.38	9.25±0.98	1.76±0.32	1.41±0.50	1.66±0.62
11	3.43±1.11	0.88±0.40	4.64±1.45	0.72±0.42	2.04±0.71	0.29±0.13	0.21±0.10	0.38±0.19
18	6.82±1.19	3.06±0.48	10.44±1.66	1.10±0.44	5.72±0.87	1.15±0.26	0.64±0.21	1.27±0.22
25	7.87±2.04	2.50±0.88	10.85±2.90	1.20±0.22	6.67±1.83	0.70±0.28	0.45±0.11	1.35±0.51
29	4.09±0.89	2.33±0.75	7.61±1.41	0	4.09±0.89	0.68±0.23	0.88±0.25	0.77±0.34
L 35	2.40±1.16	0.97±0.47	4.41±1.54	0.64±0.53	1.76±0.65	0.33±0.19	0.25±0.11	0.38±0.17
39	2.09±0.56	2.07±0.63	4.81±1.06	0	2.09±0.56	0.59±0.16	0.89±0.31	0.58±0.19
40	3.77±1.26	2.51±1.15	7.40±2.33	0	3.77±1.26	0.59±0.27	1.14±0.56	0.79±0.35
43	2.97±1.06	1.42±0.31	4.98±1.19	0.24±0.18	2.73±0.93	0.53±.016	0.30±0.08	0.59±0.09
H 49	7.94±2.11	3.39±1.01	12.16±3.08	1.45±0.68	6.49±1.43	1.12±0.42	1.38±0.42	0.90±0.20
L 54	2.22±0.56	1.28±0.32	4.16±0.87	0.13±0.08	2.10±0.51	0.36±0.09	0.63±0.17	0.29±0.15
75	5.32±1.09	1.50±0.52	7.12±1.83	0.60±0.24	4.72±0.94	0.52±0.16	0.30±0.17	0.68±0.22
77	3.18±0.56	2.05±0.51	6.11±0.94	0.02±0.02	3.16±0.55	0.76±0.24	0.70±0.24	0.59±0.08
78	2.86±0.30	1.55±0.28	4.99±0.46	0.05±0.04	2.81±0.30	0.61±0.16	0.38±0.10	0.55±0.14
82	2.20±0.72	1.35±0.14	5.00±0.94	0.15±0.10	2.05±0.64	0.38±0.11	0.50±0.19	0.47±0.24
83	5.68±0.53	2.80±0.19	9.17±0.66	0.14±0.14	5.54±0.62	0.94±0.09	0.81±0.10	1.05±0.08
85	3.55±0.68	2.02±0.47	6.27±1.18	0.20±0.12	3.35±0.59	0.74±0.26	0.49±0.09	0.79±0.16
86	4.03±0.48	1.66±0.33	6.48±0.79	0.86±0.27	3.17±0.26	0.51±0.14	0.67±0.21	0.49±0.13
87	5.49±1.10	3.25±0.48	9.31±1.51	0.77±0.31	4.72±0.89	1.44±0.23	0.50±0.17	1.31±0.24
H 89	7.01±1.63	3.13±1.07	11.18±2.71	0.45±0.15	5.70±1.50	1.18±0.42	0.63±0.28	1.32±0.40

[a] AGBIO=aboveground biomass, BGBIO=belowground biomass, AWBIO=above-water biomass, BWBIO=below-water biomass, TOTBIO=total biomass, RHZM=rhizome, BGST=belowground stems. L=populations identified as less flood tolerant, H=populations selected as more flood tolerant.

Table 2.4 Summary of biomass data for each population of *Spartina patens* after the 57 day experimental period (mean±S.E.).

Population	Biomass (g d wt pot^{-1}) [a]							
	AGBIO	BGBIO	TOTBIO	AWBIO	BWBIO	ROOT	RHZM	BGST
L 1	11.59±1.16	4.61±0.37	18.36±1.50	4.41±0.78	7.18±0.48	1.65±0.13	0.63±0.18	2.33±0.11
2	17.55±1.54	3.35±0.24	23.15±1.83	9.42±0.85	8.13±0.78	1.19±0.08	0.18±0.10	1.98±0.18
3	16.46±2.20	4.35±0.47	23.35±2.58	7.98±1.31	8.48±1.11	1.77±0.17	0.55±0.09	2.04±0.36
L 4	9.15±0.68	3.73±0.30	15.95±0.82	1.81±0.20	7.34±0.60	1.31±0.15	0.14±0.09	2.28±0.17
6	13.76±0.76	6.23±0.38	23.86±1.07	3.14±0.51	10.06±0.87	2.48±0.18	0.60±0.35	3.16±0.25
7	10.32±1.75	5.23±0.76	18.22±2.69	2.25±1.05	8.07±.073	1.91±0.32	0.03±0.01	3.29±0.46
8	16.01±1.32	6.38±1.08	24.67±3.08	4.99±0.45	11.02±1.24	2.54±0.40	0.30±0.14	3.88±0.68
9	13.18±1.23	5.14±0.44	21.34±1.74	4.17±0.48	9.01±0.97	1.87±0.17	0.74±0.34	2.53±0.16
H 14	18.57±1.59	7.05±0.40	29.43±1.74	5.31±1.32	13.09±1.10	1.93±0.15	0.31±0.16	4.84±0.28
17	15.30±0.67	6.57±0.29	25.16±0.66	4.94±0.62	10.36±0.24	2.36±0.10	0.92±0.23	3.29±0.18
20	17.90±1.28	4.82±0.40	25.67±1.69	6.34±0.78	11.56±0.78	1.54±0.15	0.10±0.04	3.19±0.24
22	15.83±0.67	5.99±0.53	25.06±0.95	6.12±0.62	9.71±0.35	2.16±0.22	0.20±0.15	3.64±0.39
H 26	21.59±0.84	7.84±0.58	32.88±1.56	8.15±0.84	14.24±0.79	3.00±0.27	0.08±0.06	4.75±0.34
29	15.21±1.61	5.63±0.70	23.64±2.10	6.00±0.74	9.22±0.94	1.76±0.22	0.74±0.37	3.14±0.43
35	12.41±0.73	5.88±0.69	21.88±0.56	4.31±0.49	8.26±0.18	2.09±0.26	1.05±0.44	2.73±0.35
36	14.70±0.97	5.91±0.47	23.82±1.72	5.49±0.43	9.21±0.62	1.77±0.07	0.59±0.36	3.55±0.24
H 38	18.38±0.49	7.81±0.38	29.10±0.74	6.06±0.43	12.31±0.53	2.70±0.15	0.62±0.36	4.49±0.48
L 69	5.46±0.97	2.31±0.30	9.18±1.37	1.71±0.39	3.75±0.63	0.68±0.10	0.27±0.09	1.35±0.20
70	16.53±1.04	7.56±0.59	27.44±1.90	5.27±0.48	11.26±0.78	2.19±0.50	0.16±0.04	4.88±0.34

[a] AGBIO=aboveground biomass, BGBIO=belowground biomass, AWBIO=above-water biomass, BWBIO=below-water biomass, TOTBIO=total biomass, RHZM=rhizome, BGST=belowground stems. L=populations identified as less flood tolerant, H=populations selected as more flood tolerant.

Table 2.5 Summary of biomass data for each population of *Panicum hemitomon* after the 58 day experimental period (mean±S.E.).

Population	Biomass (g d wt pot^{-1}) [a]							
	AGBIO	BGBIO	TOTBIO	AWBIO	BWBIO	ROOT	RHZM	BGST
74	6.48±1.14	2.86±0.55	9.33±1.62	1.68±0.29	4.80±0.85	1.05±0.19	0.44±0.13	1.37±0.27
77	9.12±0.81	2.86±0.25	11.62±1.39	3.48±0.46	5.72±0.41	1.13±0.11	0.51±0.13	1.27±0.07
L 78	5.14±0.78	2.21±0.21	7.35±0.98	1.41±0.20	3.73±0.60	0.61±0.11	0.38±0.13	1.22±0.11
80	6.79±0.80	2.94±0.34	9.74±1.13	1.47±0.21	5.32±0.62	1.18±0.17	0.50±0.11	1.27±0.15
L 81	5.13±0.85	2.65±0.61	7.78±1.43	0.98±0.17	4.14±0.71	0.84±0.13	0.77±0.40	1.04±0.14
84	7.75±0.76	3.22±0.26	10.97±0.99	2.32±0.41	5.42±0.46	1.11±0.11	0.88±0.27	1.23±0.08
H 86	10.17±0.76	3.78±0.46	13.95±1.06	3.32±0.11	6.84±0.76	0.92±0.13	1.14±0.40	1.72±0.05
87	7.80±0.57	3.54±0.40	11.34±0.89	1.97±0.25	5.83±0.41	1.16±0.13	0.88±0.21	1.49±0.23
88	8.11±0.85	3.65±0.38	11.76±1.18	1.95±0.46	6.16±0.47	1.32±0.09	0.51±0.27	1.82±0.15
H 89	8.14±0.24	3.40±0.21	11.54±0.33	2.37±0.21	5.78±0.22	1.34±0.11	0.41±0.16	1.64±0.19
90	7.92±0.69	3.27±0.56	11.19±1.23	2.32±0.24	5.59±0.48	1.32±0.18	0.77±0.28	1.18±0.19
91	8.03±0.34	3.23±0.37	11.26±0.55	2.62±0.35	5.41±0.15	0.85±0.07	0.84±0.19	1.54±0.21
92	6.64±0.74	2.99±0.25	9.63±0.92	1.69±0.23	4.96±0.52	1.05±0.13	0.35±0.14	1.59±0.22
93	7.12±0.65	3.07±0.25	10.18±0.78	1.89±0.49	5.22±0.25	1.03±0.06	0.52±0.29	1.51±0.16
L 94	5.65±0.73	2.54±0.24	7.58±0.75	1.33±0.39	4.32±0.41	0.96±0.13	0.42±0.18	1.25±0.09
96	7.04±1.17	1.93±0.24	8.97±1.38	3.33±0.78	3.71±0.70	0.65±0.07	0.32±0.14	0.96±0.13
H 97	9.09±0.83	3.37±0.13	12.45±0.90	2.50±0.36	6.58±0.49	1.32±0.10	0.33±0.11	1.72±0.09
98	6.66±0.53	2.96±0.25	9.62±0.66	2.93±0.80	3.73±0.55	1.03±0.14	0.63±0.12	1.30±0.15

[a] AGBIO=aboveground biomass, BGBIO=belowground biomass, AWBIO=above-water biomass, BWBIO=below-water biomass, TOTBIO=total biomass, RHZM=rhizome, BGST=belowground stems. L=populations identified as less flood tolerant, H=populations selected as more flood tolerant.

Although leaf elongation rates for the populations of each species declined as flood duration increased (Figure 2.5), no significant population effect in either *P. hemitomon* or *S. patens* was identified. A significant population effect in *S. alterniflora* was found, but only in population 3, which had a significantly lower leaf elongation rate relative to the remaining populations during all sampling periods.

DISCUSSION

Under waterlogged conditions, the soil environment becomes deficient in oxygen due to reduced gaseous exchange rates at the soil surface with the atmosphere. This induces specific aboveground and belowground stresses in wetland vegetation, adversely affecting plant growth and survival. Anaerobic root respiration under limited soil oxygen results in a considerable loss of energy yield to the plant (Crawford 1992; Drew 1983). Decreased root conductivity affects shoot water relations, resulting in aboveground tissues receiving a limited supply of nutrients and important regulatory phytohormones and in the disruption of metabolic and transport processes (Drew 1983; Koch *et al.* 1990; Armstrong *et al.* 1994). Phytotoxins, such as sulfides, may accumulate in waterlogged soil, damaging belowground tissue directly or resulting in decreased nutrient uptake from the soil by the roots (Mendelssohn *et al.* 1982; Hook 1984; Gambrell and Patrick, Jr. 1978; Stepniewski and Przywata 1992). The overall consequence is reduced plant growth. Therefore, biomass partitioning and leaf elongation rate are useful measures of plant response to flooding.

Redox potential, a measure of the intensity of soil reduction, was relatively low in the experimental pots (<+300 mV) and confirmed that soil conditions were reducing and potentially stressful enough to decrease plant growth (Figure 2.5). *Panicum hemitomon, Spartina patens* and *S. alterniflora* clearly showed intraspecific variation with respect to biomass partitioning under flooding stress, allowing populations of each species to be identified according to their relative flood tolerance. However, determination of which biomass variables were the best measure of relative flood tolerance for identification

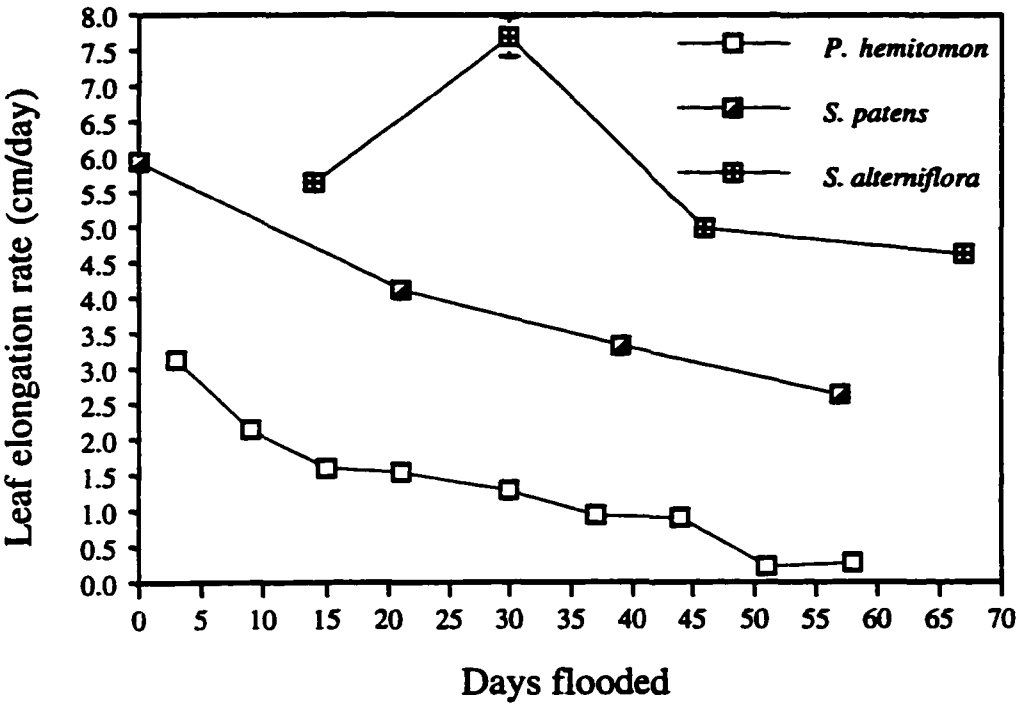


Figure 2.5 Effect of flooding on leaf elongation rates, averaged over populations, of the three dominant marsh species evaluated (n=125, 95, 90 for *Spartina alterniflora*, *Spartina patens*, and *Panicum hemitomon*, respectively). Each species study was conducted separately. Absence of bar indicates SE smaller than symbol.

proved difficult. Rapid shoot elongation to the water surface, or "depth accommodation" (Van der Sman *et al.* 1991), is an important adaptive response in flood-tolerant species (Voesenek *et al.* 1992; Armstrong *et al.* 1994). However, the maintenance of perennating organs and development of new roots and large belowground reserves have also been shown to contribute to improved flood tolerance (Laan *et al.* 1989b; Armstrong *et al.* 1994; van der Valk 1994). Consequently, both aboveground and belowground biomass variables could have been used to determine relative flood tolerance. Use of PCA allowed the determination of those biomass variables characteristic of improved population response to flooding stress (Table 2.2). A higher population score for a principal component resulted from a higher biomass for that population, indicating better maintenance of plant productivity over the flooding period. For all three wetland species, PC1 characterized improved plant response as increased total biomass production, particularly for *S. patens* and *P. hemitomon* in below-water and belowground biomass production. A secondary underlying factor was identified by PC2, above-water biomass (Table 2.2). These patterns can be seen in the original biomass data (Tables 2.3-2.5).

In general, PCA may be used as a technique not only for identifying populations for greater flood tolerance but for other desirable attributes as well. Depending on restoration needs, populations may be selected for belowground biomass production (stabilizing soil substrate), aboveground biomass (wave dampening and sedimentation), or high rhizome production (increased vegetative propagation) under a variety of stresses. In addition, identification of genotypes exhibiting a range of flood tolerance characteristics (low to high) may be useful in future studies investigating the underlying anatomical/metabolic mechanisms responsible for variation in plant flood tolerance.

Although leaf elongation rates measured for all the populations throughout the experiments showed an expected reduction by the flooding stress (Figure 2.5), a differential population response in this variable was not evident for *P. hemitomon* or *S. patens*. The significant population effect for *S. alterniflora* was due to only one out of 25

populations and thus also failed to show substantial intraspecific variation in leaf elongation. It should be noted, however, that most shoots elongated rapidly when the flooding stress was initiated, suggesting that shoot elongation, rather than leaf elongation, may be a more appropriate variable for measuring differential flood response.

Investigators have examined factors that affect transplantation success of marsh grasses and that lead ultimately to restored function and self-sustainability of the wetland (Broome *et al.* 1986; Broome *et al.* 1988; Wilsey *et al.* 1992). Several studies agree that elevation of the recipient sites is important, and at times more critical than soil nutrient content or salinity (Mendelssohn and McKee 1988; McKee and Mendelssohn 1989; Wilsey *et al.* 1992). An additional factor in the success of a marsh restoration effort is the quality of the transplant material. For these reasons, identification of more flood tolerant genotypes, as was done in this study with *Panicum hemitomon*, *Spartina patens* and *Spartina alterniflora*, is important to the continued development of restoration programs for coastal areas around the world. By selecting plant stocks that are more flood tolerant, quality of transplant material is improved, thereby enhancing flooding stress response by vegetation and overcoming the barrier to recolonization. Furthermore, planting of improved stocks while local vegetation still remains and prior to areas deteriorating fully to open water, may facilitate establishment of more tolerant transplants and maintain integrity of the soil substrate. Such early intervention, only made possible by new plant stock availabilities, could reduce the need for large scale restoration operations and minimize land losses in certain areas.

Chapter 3

A Field Evaluation of Flood- and Salt-tolerant Populations of the Brackish Marsh Species *Spartina patens*

INTRODUCTION

Land loss in coastal Louisiana comprises 80% of the coastal wetland loss nationally (Boesch *et al.* 1994). Causal factors include: 1) excessive inundation of the marsh surface resulting from a rapidly subsiding deltaic plain and man-altered hydrology; and 2) elevated salinities of fresh and brackish marshes from saltwater intrusion facilitated by sea level rise, canalization, storm surges, and altered hydrology (Mendelssohn *et al.* 1983; Deegan *et al.* 1984; Turner *et al.* 1984; Penland and Ramsey 1990; Reed 1991). Plants in these areas often fail to adjust to excessive waterlogging and increased salinity, which leads to plant death and marsh deterioration. Furthermore, subsequent recolonization can be prevented by the inundated, reduced, and more saline soil conditions (Mendelssohn and McKee 1989; Mendelssohn and McKee 1992; Boesch *et al.* 1994; Flynn *et al.* 1995; Baldwin *et al.* 1996). With increasing sea level rise worldwide, these same factors are important on a global scale.

Recovery and restoration of these deteriorated areas can often be initiated or accelerated by replanting healthy vegetation. Elevation and salinity of deteriorating recipient sites are important to long-term survival of transplants and to the restoration of function and self-sustainability (Broome *et al.* 1986; Broome *et al.* 1988; McKee *et al.* 1989; Wilsey *et al.* 1992). An additional factor influencing the success of marsh restoration is the quality of the transplant material. More stress-tolerant populations would benefit deteriorating areas by increasing productivity and stem density, enhancing sedimentation and peat formation, and thereby facilitating vertical marsh accretion. Hence, availability of more flood- and salt-tolerant genotypes of wetland vegetation is important to the successful development of restoration programs in coastal areas around the world.

Genetic differentiation among populations within a plant species is well documented (Davy *et al.* 1990; Thompson *et al.* 1990; Odasz and Savolainen 1996), with certain populations being better adapted than others to specific environmental stressors (Linhart and Baker 1973; Keeley 1979; Silander and Antonovics 1979; Davies and Singh 1983; Eleuterius 1989; Jefferies and Rudmik 1991; Hester 1995). However, the use of this genetic variation to develop and test plants of greater flood and salt tolerance for coastal restoration has received little attention. Previous research has identified populations of *S. patens* collected over a large geographical range (Texas and Louisiana Gulf Coast) that exhibit differential flood tolerance (Lessmann *et al.* 1997) and differential salt tolerance (Hester *et al.* 1996). However, the performance of these differentially stress-tolerant populations has not been tested under the combined stressor effects of flooding and salinity, as well as the performance of the more flood-tolerant populations under increased salinity or more salt-tolerant populations under increased flooding. Furthermore, researchers agree (Davy *et al.* 1990; Pezeshki and DeLaune 1993b) that evaluation of differential stress-tolerance in populations under field conditions is a critical step in applying these populations in coastal restoration. The goal of this study was to examine growth of *S. patens* populations under field conditions when subjected to increased flooding, salinity, and their interaction. Several other questions arise from this research. Is increased tolerance to either flooding or salinity more important to plant success than the other? How do nutrient use efficiencies change in response to flooding or salinity for these genotypes? What are the functional characteristics these genotypes may restore to a deteriorated system? Finally, how do the results influence the use of these populations in wetland restoration?

MATERIALS AND METHODS

Plant Material and Study Sites

Populations of *S. patens* selected for differential flood tolerance in Chapter 2 (14 and 26 as more flood-tolerant, and 4 and 69 as less flood-tolerant) were used in this

study. Previous research determined differential salt-tolerance for these same populations (Hester 1995); populations 14 and 69 were more salt-tolerant and populations 4 and 26 were less salt tolerant. This resulted in the four differentially stress-tolerant populations designated as HFHS (high flood- and high salt-tolerant), HFLS (high flood- and low salt-tolerant), LFHS (low flood- and high salt-tolerant), and LFLS (low flood- and low salt-tolerant). The populations were prepared for transplantation from greenhouse stocks. Approximately 10-15 stems of each population were potted into 7.6 liter plastic pots which had approximately twenty 2-cm diameter drainage holes drilled around the outside for throughflow of water *in-situ*. Cheesecloth lining prevented loss of potting material, which consisted of a 60:20:20 mixture of Jiffy Mix (commercial potting medium, Jiffy Mix Products, West Chicago, Illinois) and soils collected from each field site. This soil mixture, which had a salinity of 5 ppt, was intended to help acclimate the plants to field conditions and to more closely simulate field soil conditions than with a commercial potting medium alone. Additionally, this uniform soil insured that plants were exposed to the same rooting environment at both sites for comparison of results.

Each of the four populations was transplanted into two marshes of widely different salinities. The high salinity site was located at Lake Pelto on the Gulf of Mexico (29°15'N, 90°45'W). The low salinity site was located approximately 28 km north of Lake Pelto on Bayou Grand Caillou, Louisiana (29°5'N, 90°45'W). Within low-elevation deteriorating areas of each marsh, a soil core the same diameter of the pot was extracted and one pot of each population was transplanted into one of two elevations relative to the marsh surface. Pots were secured in place using wooden stakes (brackish marsh) or thick, plastic-coated wire (salt marsh) anchored into the marsh substrate. Pots of each population were placed with their soil surface either ambient to the marsh surface or elevated relative to the marsh surface. The height of the elevated pot was equal to the elevation of adjacent healthy vegetation. For the salt and brackish marshes this was 10

cm and 15 cm above the marsh surface, respectively. A total of 80 experimental units were employed for the study (8 pots/block, 40 pots/site, 20 pots/population).

Experimental Design

A split-split plot randomized block design (5 replicates) was used, with site serving as main plot, inundation and population as subplot, and sampling period as sub-subplot. The treatments were: 1) Site, a brackish marsh and a higher salinity salt marsh; 2) Inundation, two elevations denoted as ambient and elevated; 3) Population, denoted as HFHS, HFLS, LFHS, and LFLS, describing the relative flood and salt tolerances of each.

Sampling and Analyses

Prior to transplantation of populations, initial cumulative height of all tillers in each pot was measured as an index of initial plant biomass and for use as a covariate. After transplantation to marsh sites in May, 1996, populations were allowed to equilibrate with the local environment for three weeks before initiating sampling of the following variables through the growing season (June, July, September, November):

Redox Potential - Soil redox potentials at 1-cm and 10-cm depths were measured in each pot with bright platinum electrodes. The potential of a standard calomel reference electrode (+244 mV) was added to the millivolt reading to obtain Eh.

Interstitial Water - Interstitial water was withdrawn from a depth of 10 cm in the center of each pot into a 30 ml syringe using a perforated rigid plastic tube covered with nylon hosiery and cheesecloth to filter particulate material (McKee *et al.* 1988). The first 10 ml was discarded to eliminate water contaminated by air in the tube and disturbed sediment resulting from tube insertion. Salinity was then measured with a refractometer (S/Mill, ATAGO, Co., Ltd.). An Altex Model 3560 digital meter and electrode was used to measure pH. Sulfide in the interstitial water was determined using the method of McKee *et al.* (1988) (Lazar sulfide electrode, Model IS-146). Ammonium was analyzed in a 5 ml aliquot that was filtered (poresize=0.45 μ) and placed on ice in the field, then frozen upon

return to the laboratory (Technicon AutoAnalyzer II). An additional 10-ml aliquot was filtered, acidified, and stored on ice for nutrient analysis (Al, B, Ca, Cu, Fe, K, Mg, Mn, P, S, Zn) using Inductively Coupled Argon Plasma Spectrometry (Jarrel-Ash Atom Comp series 800).

The following variables were sampled at the end of the growing season (November):

Biomass - Aboveground plant material was clipped at the soil surface and each pot removed from the ground. All material was returned to the laboratory for analysis. The belowground material was rinsed of soil and sorted into roots, rhizomes, and portions of stem extending belowground. This stem material was added to the aboveground material. All plant material was dried at 65°C for one week and dry weights measured and recorded.

Tissue Nutrients - To indicate any potential differences in population uptake and translocation of nutrients to aboveground tissue or exclusion by roots, nutrient analysis was performed. Dried aboveground material was milled (60 mesh sieve, Wiley Mill) and 0.25 g milled material was digested in 3.0 ml concentrated nitric acid at 130°C for 12 hours, then brought to a final volume of 35.0 ml with deionized water (modified from Plumb 1981). The extractant was measured for macro- and micronutrients (P, K, Mg, Na, Ca, S, Fe, Mn, Zn, Cu, Al, B in mg/g d wt) using Inductively Coupled Argon Plasma Spectrometry. The milled tissue was also analyzed for percent N (Perkin Elmer CHN Elemental Analyzer). Nutrient use efficiencies of N and P were calculated as the inverse of N and P concentration (g d wt/mg nutirent), respectively.

Water level - Steven's Water Logs were installed adjacent to the experimental blocks on July 25, 1996 and water level was recorded every hour at each site for the remainder of the growing season (July 25-November 19, 1996). The number of hours drained and flooded was calculated for each inundation treatment at each site.

Statistical Analyses - All variables were analyzed using a split-split plot randomized block design (five replicates). Analysis of covariance was performed on final biomass data, with initial cumulative height as the covariate. Multivariate analysis of variance was used for interstitial and tissue nutrient variables. When main effects were significant at $p \leq 0.05$, Tukey's multiple comparisons were performed. When interactions were significant at $p \leq 0.05$, LSMEANS pairwise comparisons were made with a Bonferroni adjustment, resulting in adjusted probability values of $p \leq (0.05/n)$, where n=number of *a priori* pairwise comparisons. For redox potential, sulfide, percent N, and total elemental concentration, n=6 and $p \leq 0.0083$. For belowground biomass and Na:K, n=12 and $p \leq 0.0042$. For aboveground and total biomass, n=24 and $p \leq 0.0021$. Assumptions of normality and homogeneity of variance were tested (Shapiro-Wilk test and Bartlett test, respectively) and found to be valid. All statistics were performed using SAS (SAS Institute, Inc. 1989).

RESULTS

The brackish and salt marshes had similar tidal regimes (Figure 3.1), with the ambient treatment flooded approximately twice as long as the elevated treatment. Redox potential (Eh) was measured as an indicator of the effectiveness of the different flooding durations in creating differentially reduced conditions at the two levels of inundation (Figure 3.2). Free oxygen disappears in flooded soil below an Eh of +300 mV (Patrick and DeLaune 1977). The Eh values measured in this study ranged from +100 mV to -100 mV, indicating the absence of free oxygen in the soil. Redox potential was significantly higher in the less flooded, elevated treatment than in the more flooded, ambient treatment. However, these treatments differed significantly only for the salt marsh (significant site by inundation interaction) (Figure 3.2). Redox potential within the rooting zone (-10 cm), was more reducing than at the soil surface (Figure 3.2). There was no significant population effect for Eh.

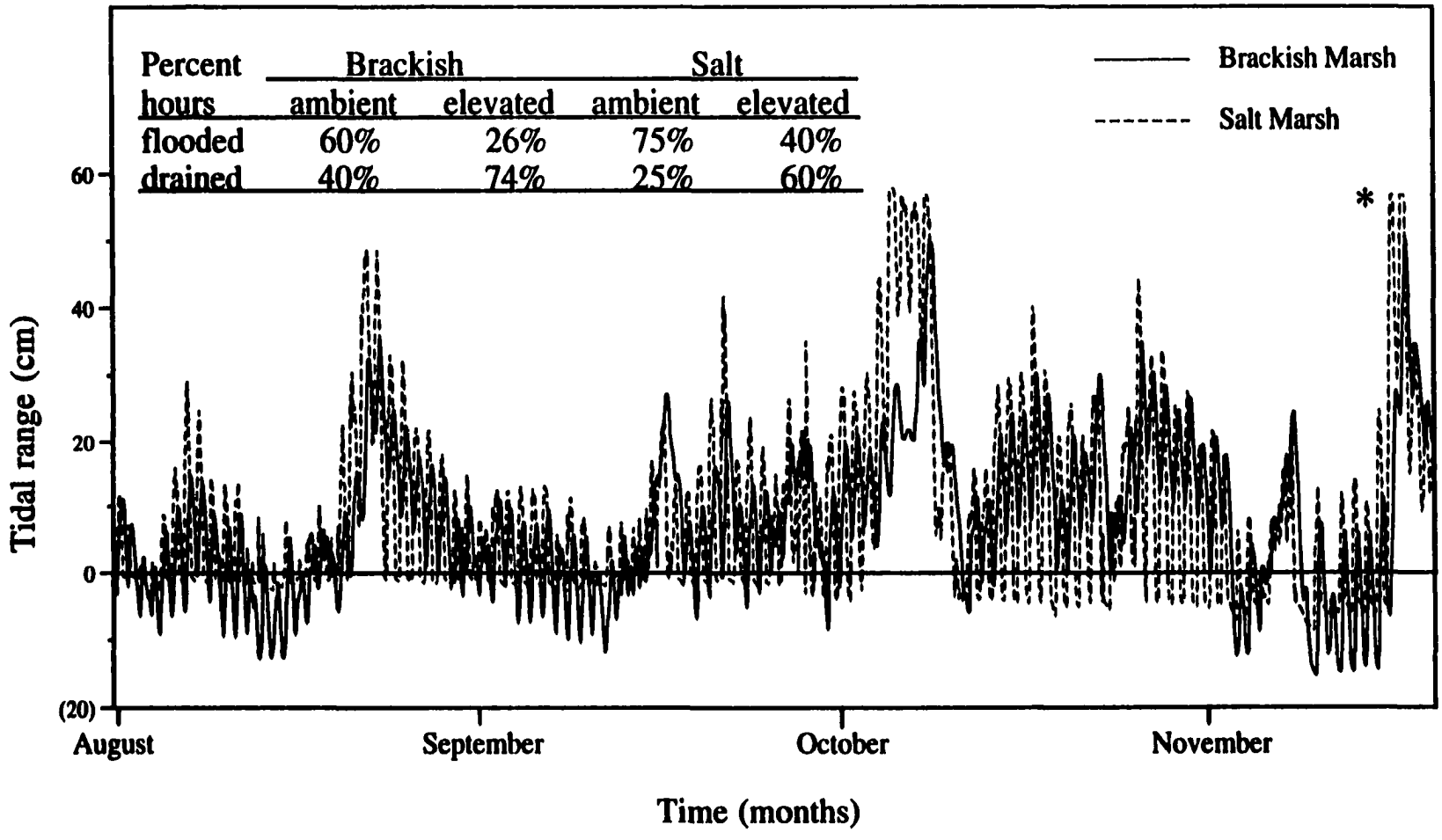


Figure 3.1 Percent of total hours each field site was flooded and drained. Values greater than zero indicate the water level is above the soil surface (flooded), values less than zero indicate the soil surface is drained. Tide data were collected at each marsh site during the growing season from the end of July to mid-November. The first day of the month is indicated by the tick mark.

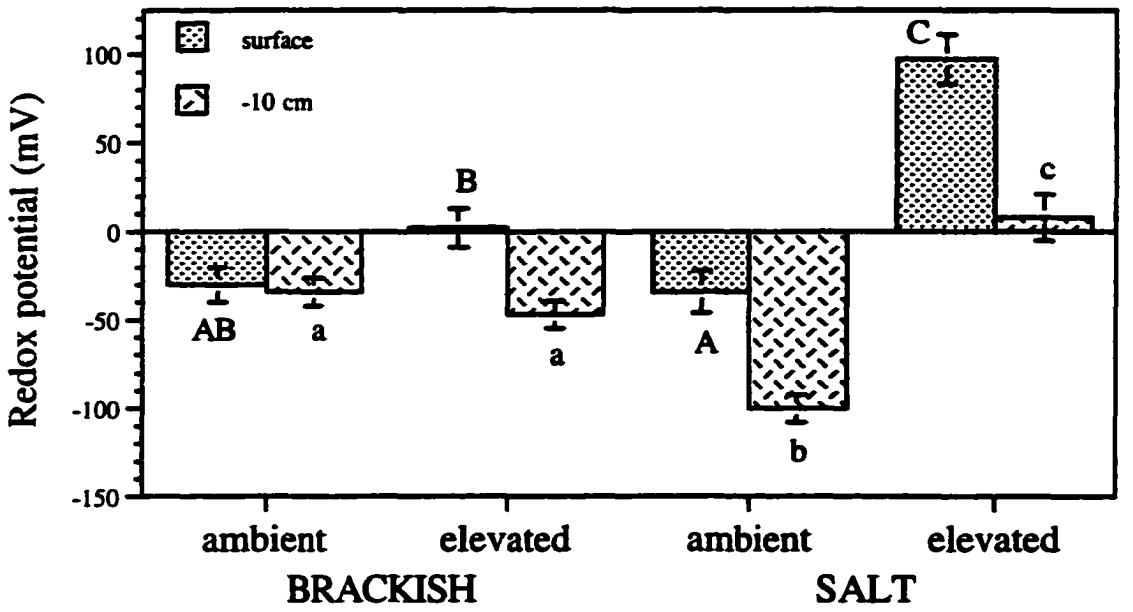


Figure 3.2 Soil redox potential for the elevated and drained treatments at the brackish and salt marsh sites. Different capital letters indicate significant differences for surface Eh; different lowercase letters indicate significant differences at a soil depth of 10 cm (no significant population effect or interaction).

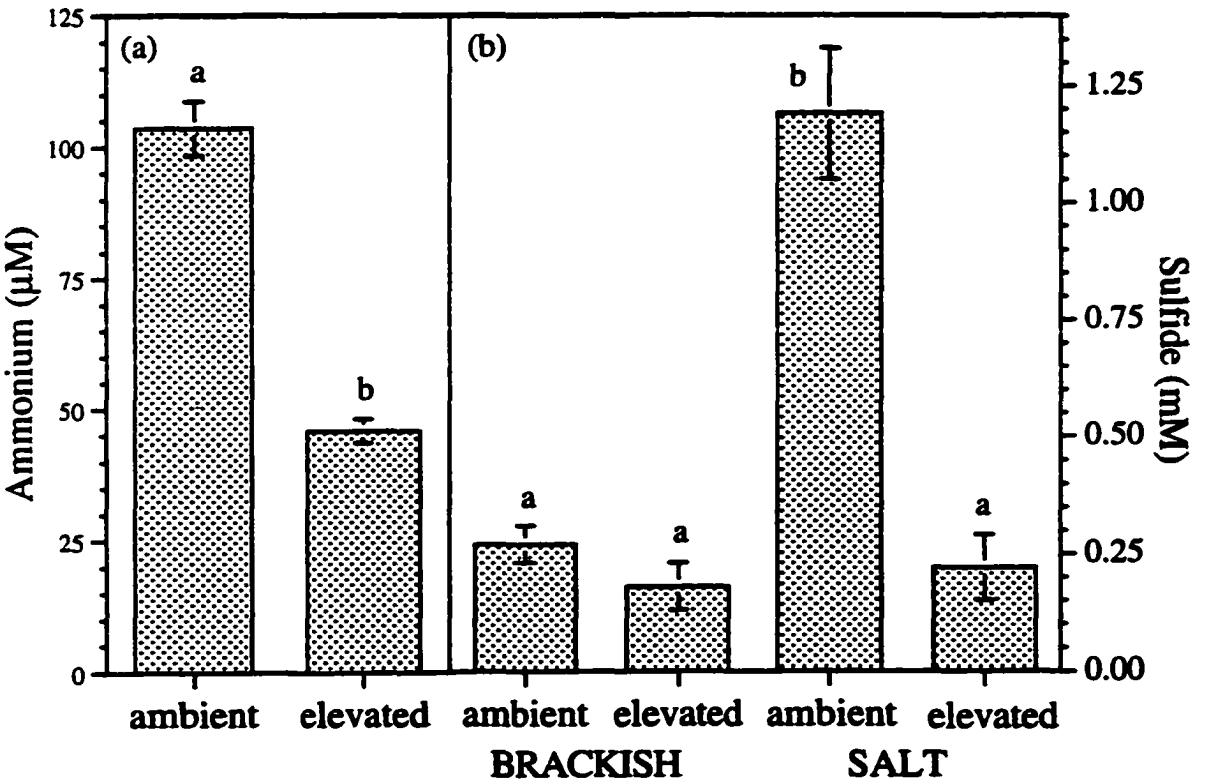


Figure 3.3 (a) Interstitial ammonium concentration for each inundation treatment (no significant marsh site or population interaction). (b) Interstitial sulfide concentration for the inundation treatment at the brackish and salt marsh sites (no significant population effect or interaction). Different letters indicate significant differences.

Corresponding to these differences in redox potential were differences in interstitial sulfide, a soil phytotoxin (Ingold and Havill 1984; Koch and Mendelssohn 1989), and ammonium, the main nitrogen source for wetland vegetation (Gambrell and Patrick 1978; Mendelssohn 1979). The more reduced ambient treatment had twice the ammonium concentration of the less reduced elevated treatment, regardless of marsh site or population (Figure 3.3a). Sulfide concentration varied with inundation by site and was significantly greater in the ambient than elevated treatment of the salt marsh (Figure 3.3b), but not in the brackish marsh. The same pattern was seen for interstitial S concentration (Table 3.1). The salinity at the salt marsh was significantly higher than at the brackish marsh. During the final sampling period, the brackish marsh salinity was significantly elevated to 18.2 ppt (Table 3.2) as a result of severe coastal flooding (Figure 3.1, indicated by the asterisk). There were no differences in pH for any of the treatments (mean=7.2±0.04). Overall, the inundation treatment and different marsh site salinities were effective in creating a potentially stressful environment for plant growth.

Spartina patens populations that were previously selected for greater flood tolerance in the greenhouse also exhibited greater flood-tolerance under specific experimental conditions in this field study. The more flood-tolerant (HF) populations had significantly greater total biomass than the less flood-tolerant (LF) populations in the elevated treatment of the brackish marsh (Figure 3.4a). Also, the aboveground biomass of HF populations was significantly higher than that of LFHS, and aboveground biomass of HFHS was significantly higher than that of LFLS in the elevated treatment of the brackish marsh (Figure 3.4b). Generally, populations had higher total and aboveground biomass in the brackish marsh compared to the salt marsh, regardless of inundation level (Figure 3.4), with the exception of LFHS and LFLS in the brackish, elevated treatment, which did not differ significantly between sites for total and aboveground biomass, and LFLS in the brackish, ambient treatment, which did not differ significantly between sites for aboveground biomass. There were no differences in total or aboveground biomass

Table 3.1 Interstitial elemental concentrations (ppm). Means (±s.e.) followed by different letters are significantly different. There are no significant elevation or population effects or their interactions for Al, Zn, Fe, Mn, K, Mg. There is no significant population effect or interaction for Mn, B, P, Ca, or S.

Ion	Brackish Marsh		Salt Marsh	
Al	0.18±0.02 a		0.27±0.05 a	
Zn	0.22±0.05 a		0.51±0.02 b	
Fe	0.75±0.10 a		0.73±0.13 a	
Mn	0.99±0.05 a		1.65±0.16 b	
K	102.8±6.2 a		232.8±3.59 b	
Mg	387.1±20.6 a		812.8±9.5 b	
	Ambient	Elevated	Ambient	Elevated
Mn	0.92±0.07 a	1.05±0.08 ab	1.92±0.25 c	1.39±0.18 b
B	1.16±0.10 a	1.01±0.10 a	2.04±0.09 b	3.24±0.24 c
P	2.58±0.35 a	2.35±0.25 a	5.80±0.65 b	0.78±0.15 c
Ca	165.8±9.2 a	169.6±9.2 a	299.8±5.1 c	264.4±5.0 b
S	294.6±37.1 a	264.4±42.1 a	1438.7±148.7 c	607.6±28.0 b

Table 3.2 Interstitial salinity (ppt) at each marsh site for each sampling period (mean±s.e.). No significant populations or inundation effect or interactions.

Sampling period	Brackish Marsh	Salt Marsh
June	5.6±0.1	30.4±0.6
July	4.5±0.1	22.4±0.7
September	7.4±0.3	22.5±0.4
November	18.2±0.3	29.4±0.2
Mean	8.9±0.5	25.8±0.4

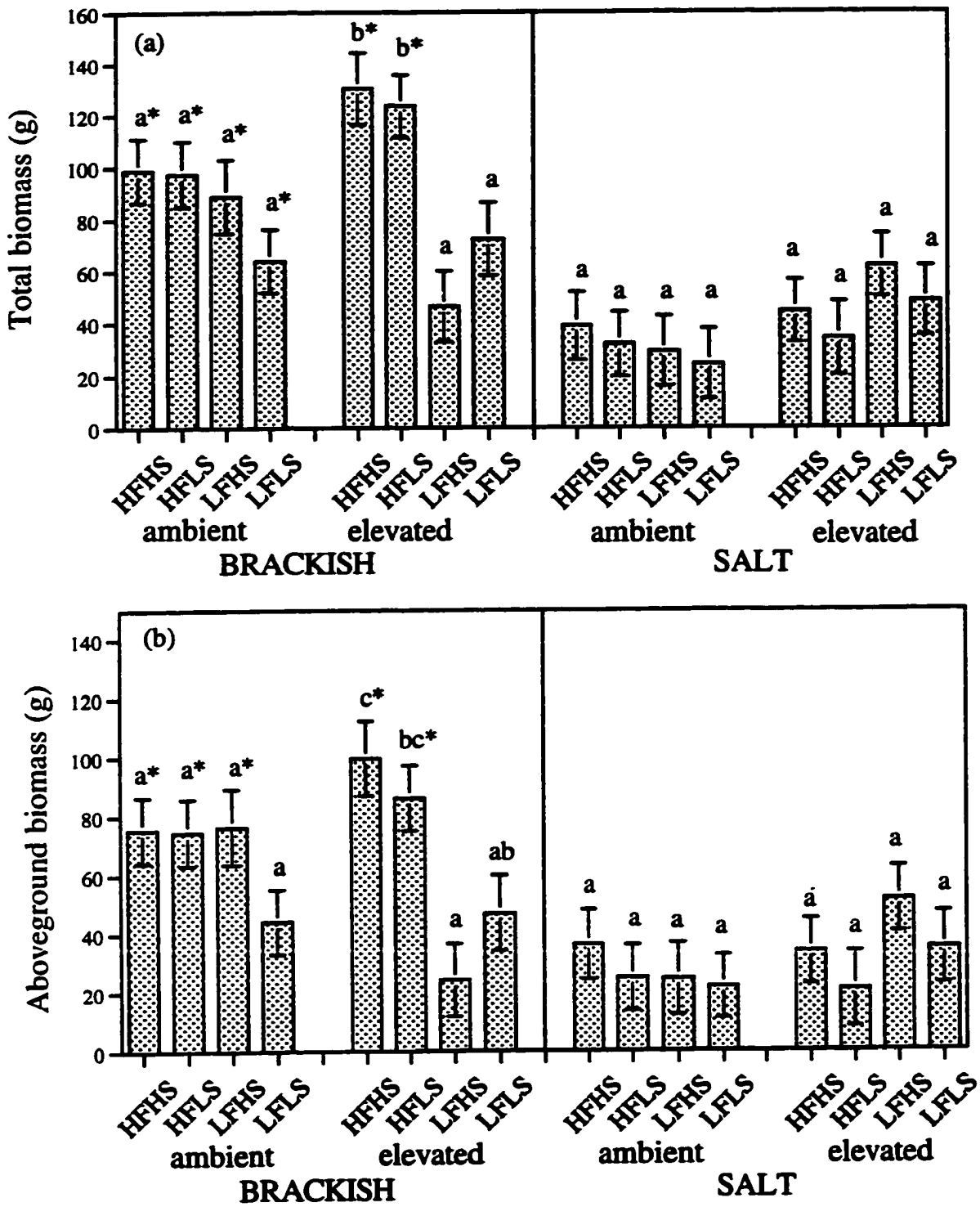


Figure 3.4 (a) Total biomass (aboveground plus belowground) and (b) aboveground biomass for *S. patens* populations in the brackish and salt marshes at each inundation level (ambient and elevated). Different letters indicate significant differences between populations within each inundation level at each site. Populations with an asterisk had significantly lower total or aboveground biomass in the salt marsh compared to the brackish marsh. LF=low flood tolerance; HF=high flood tolerance; LS=low salinity tolerance; HS=high salinity tolerance.

between the differentially salt-tolerant populations with higher salinity in the salt marsh nor between the differentially flood-tolerant populations with higher flooding in the ambient treatments.

For total belowground biomass, populations of *S. patens* varied with site (Figure 3.5a), with HF populations significantly higher than LFHS in the brackish marsh. There were no population differences at the higher salinity site. Total belowground biomass of all populations was lower in the salt marsh than in the brackish marsh (Figure 3.5a). Root, rhizome, and total belowground biomass were significantly lower in the ambient treatment than in the elevated treatment (Figure 3.5b). Root and rhizome biomass did not vary with population or site.

Populations differed in shoot concentrations of both limiting and non-limiting nutrients. Shoot nitrogen concentration (Figure 3.6a) was significantly higher in LFLS than in HF populations. LFHS did not differ in percent nitrogen from LFLS nor either HF populations. There were no significant interactions of population with salinity or inundation. Shoot tissue concentrations of P differed between populations by inundation level (Figure 3.6b). In the elevated treatment, HF populations had significantly lower P concentrations than the LF populations. However, there were no population differences in the ambient treatment. Shoot P concentrations did not vary significantly with salinity. Accumulation (concentration*total biomass) of the growth limiting nutrients, N and P, did not differ between populations (data not shown).

Nutrient use efficiency (NUE) of N was significantly higher in HFHS than in LFLS (Table 3.3). There were no significant inundation or salinity interactions with population for NUE of N. NUE of P varied by population with inundation level (Table 3.3). In the elevated treatment, HF populations had significantly higher P use efficiencies than did the LF populations. There were no population differences in NUE of P in the ambient treatment. NUE of P did not vary with salinity level.

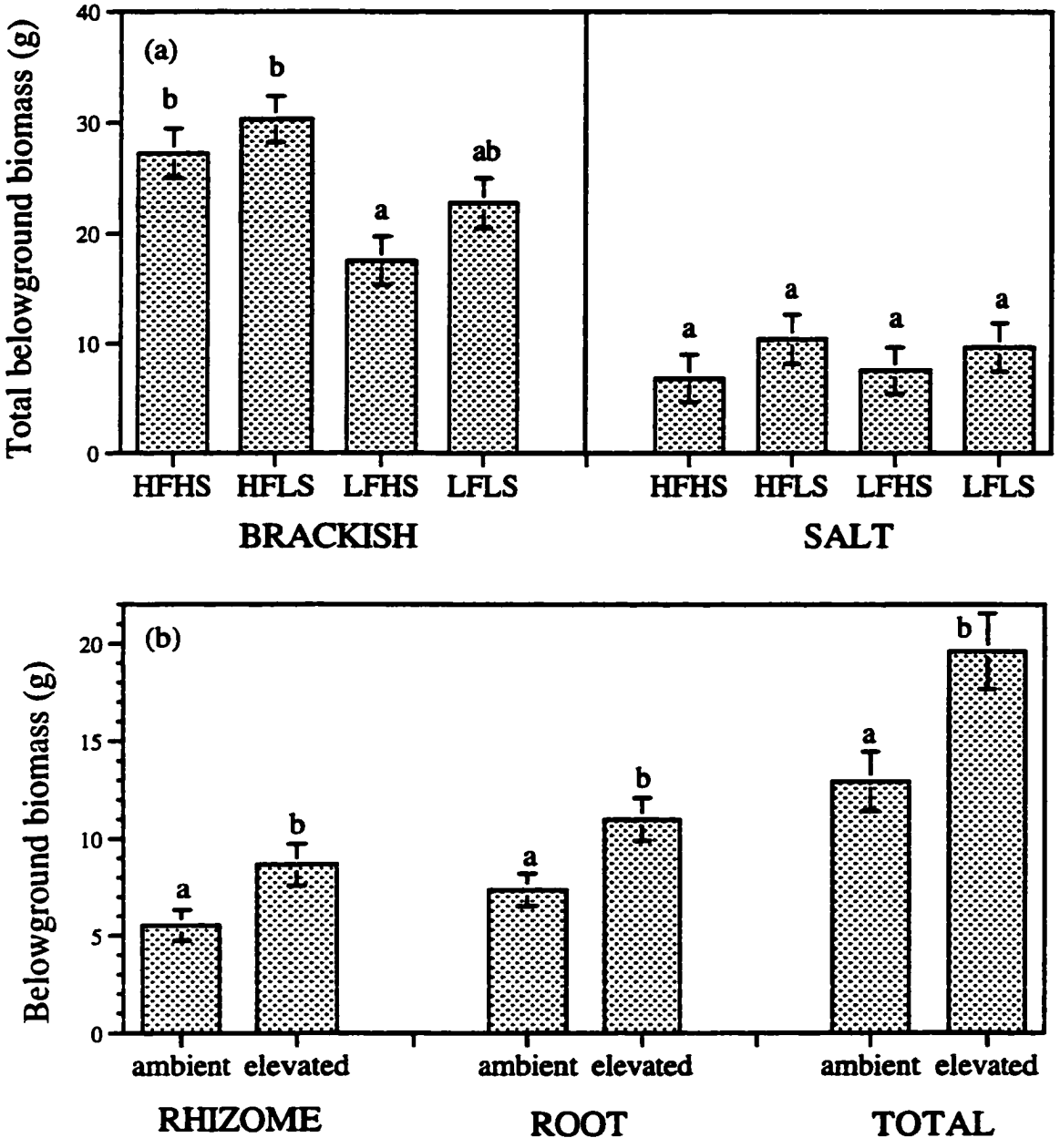


Figure 3.5 (a) Total belowground biomass (root plus rhizome) for each population at each site. Different letters indicate significant differences between populations within each site. Belowground biomass is significantly lower for all populations in the salt marsh compared to the brackish marsh. (b) Belowground biomass variables for *S. patens* at each inundation treatment. Differences between the ambient and elevated treatments for each belowground variable are indicated by different letters (no significant population effect or interaction for root or rhizome). LF=low flood tolerance; HF=high flood tolerance; LS=low salinity tolerance; HS=high salinity tolerance.

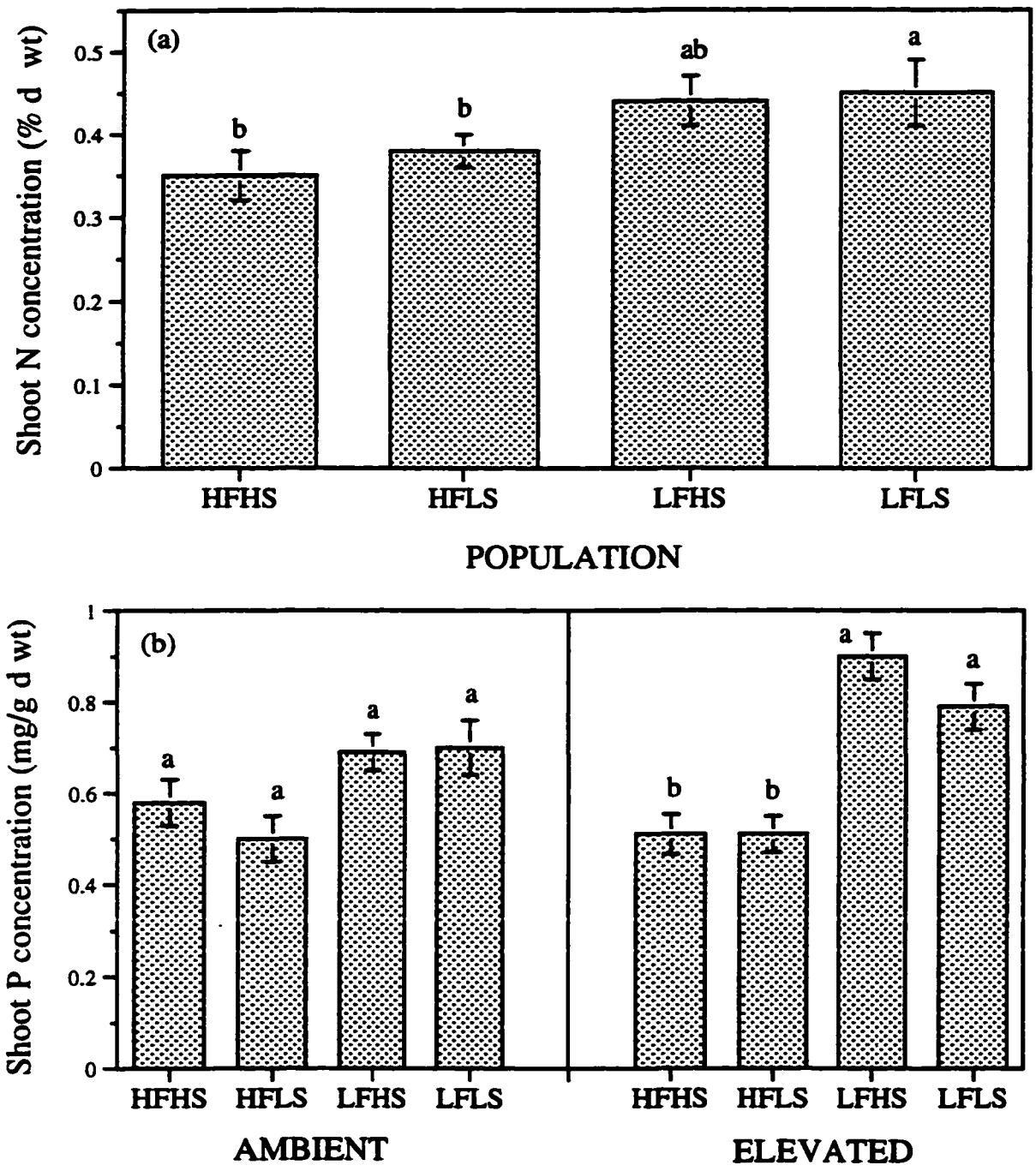


Figure 3.6 (a) Percent nitrogen in aboveground tissue for each population of *S. patens*. Different letters indicate significant differences between populations (no significant marsh site or inundation effect or interactions). (b) Phosphorus concentration in aboveground tissue for each population of *S. patens*. Different letters indicate significant differences for populations within each inundation level (no significant marsh site effect or interaction). LF=low flood tolerance; HF=high flood tolerance; LS=low salinity tolerance; HS=high salinity tolerance.

Table 3.3 Nutrient use efficiency (g d wt/mg nutrient) of nitrogen and phosphorus for each population (mean±s.e.). Different letters indicate significant differences between populations. There is no significant inundation or marsh site effect or their interactions for nitrogen. There is no significant marsh site effect or its interactions for phosphorus.

Population	NUE of Nitrogen	NUE of Phosphorus	
		Ambient	Elevated
HFHS	3.01±0.19 a	0.25±0.02 a	0.27±0.01 a
HFLS	2.78±0.21 ab	0.28±0.01 a	0.29±0.02 a
LFHS	2.61±0.13 ab	0.22±0.02 a	0.16±0.01 b
LFLS	2.29±0.17 b	0.21±0.03 a	0.18±0.01 b

Shoot Na:K differed between populations and had a significant interaction with inundation (Figure 3.7a). In the ambient treatment, LFHS was significantly higher than HFHS. There were no significant population differences in the elevated treatment nor significant differences with site salinity. Populations did not differ in Na concentration (data not shown), but they did differ by inundation in K concentration (Figure 3.7a). In the elevated treatment, LFHS had significantly higher shoot K concentration than HF populations, but did not differ from LFLS. There were no population differences for K in the ambient treatment.

Populations of *S. patens* varied with respect to the inundation treatment in shoot Mg concentration (Figure 3.8a). In the ambient treatment, LFLS had significantly higher Mg than HF populations, but did not differ from LFHS. In the elevated treatment, LFLS had significantly greater Mg concentrations than HFHS and LFHS. Populations also varied with site for shoot Mg concentration (Figure 3.8b), with the concentration in LFLS significantly higher than in the other three populations in the salt marsh. There were no population differences in Mg concentration in the brackish marsh.

Total concentrations of major cations (Ca, K, Mg, Na) in shoot tissue differed between populations (Figure 3.9), with concentrations higher in aboveground tissue of LFLS than in LFHS and HF populations. No trend was evident with respect to relative salt tolerance of populations. Total cation concentration was significantly higher at the salt marsh than at the brackish marsh, and significantly higher in the ambient treatment than in the elevated treatment (Table 3.4).

Populations varied with site in shoot Fe concentrations (Figure 3.10). LFLS was significantly higher than HFHS in the salt marsh. There were no differences between populations in shoot Fe concentration in the brackish marsh (Figure 3.10).

Accumulation of the non-growth-limiting nutrient elements in plant tissue (concentration*total aboveground biomass) followed the same pattern as total aboveground biomass (data not shown).

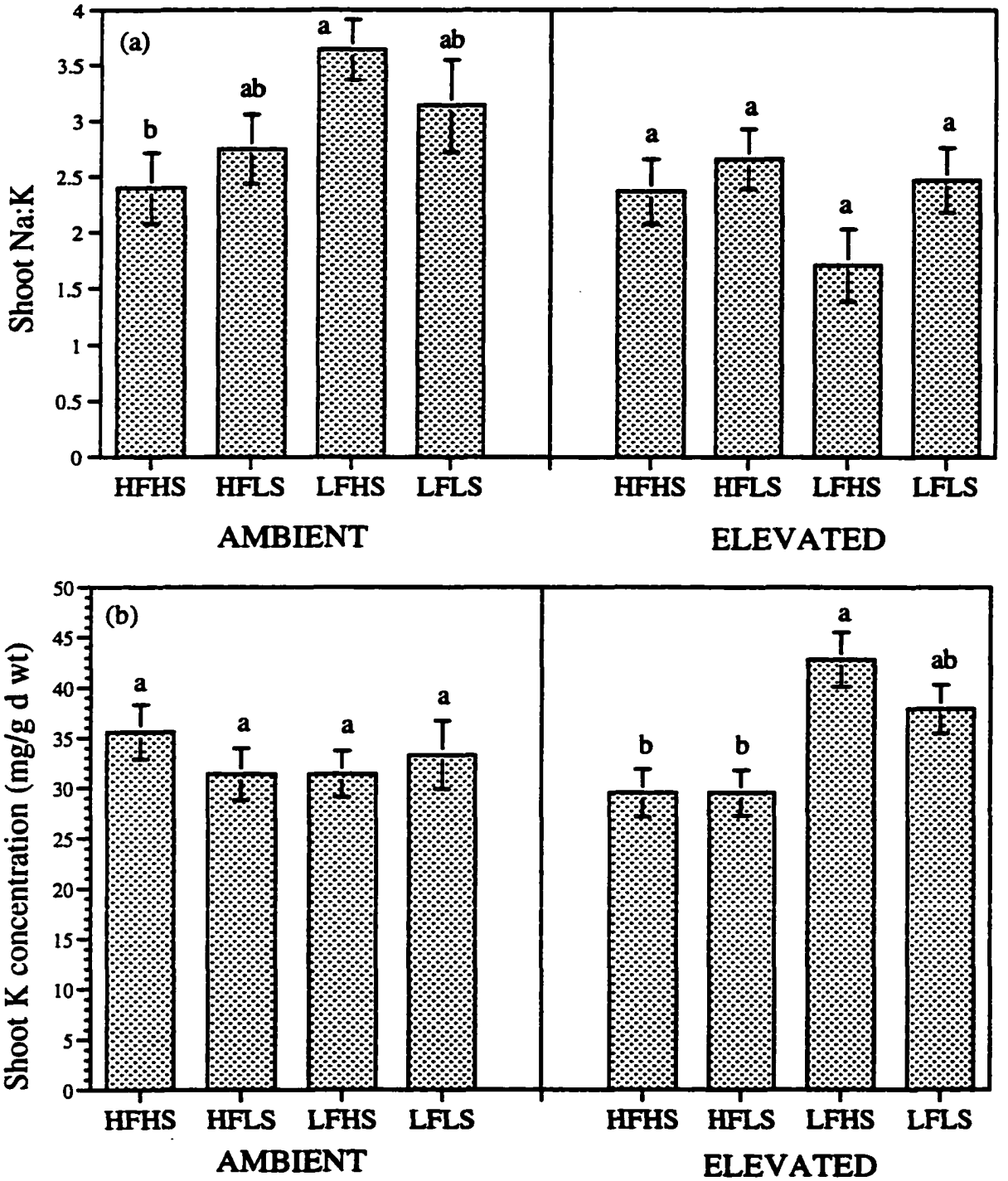


Figure 3.7 (a) Sodium to potassium ratio (Na:K) in aboveground tissue of *S. patens* populations for each inundation treatment. (b) Potassium concentration in aboveground tissue of *S. patens* populations for each inundation treatment. Different letters indicate significant differences between populations within each inundation treatment (no significant marsh site effect or interaction). Na did not have a significant population effect or significant population interaction. LF=low flood tolerance; HF=high flood tolerance; LS=low salinity tolerance; HS=high salinity tolerance.

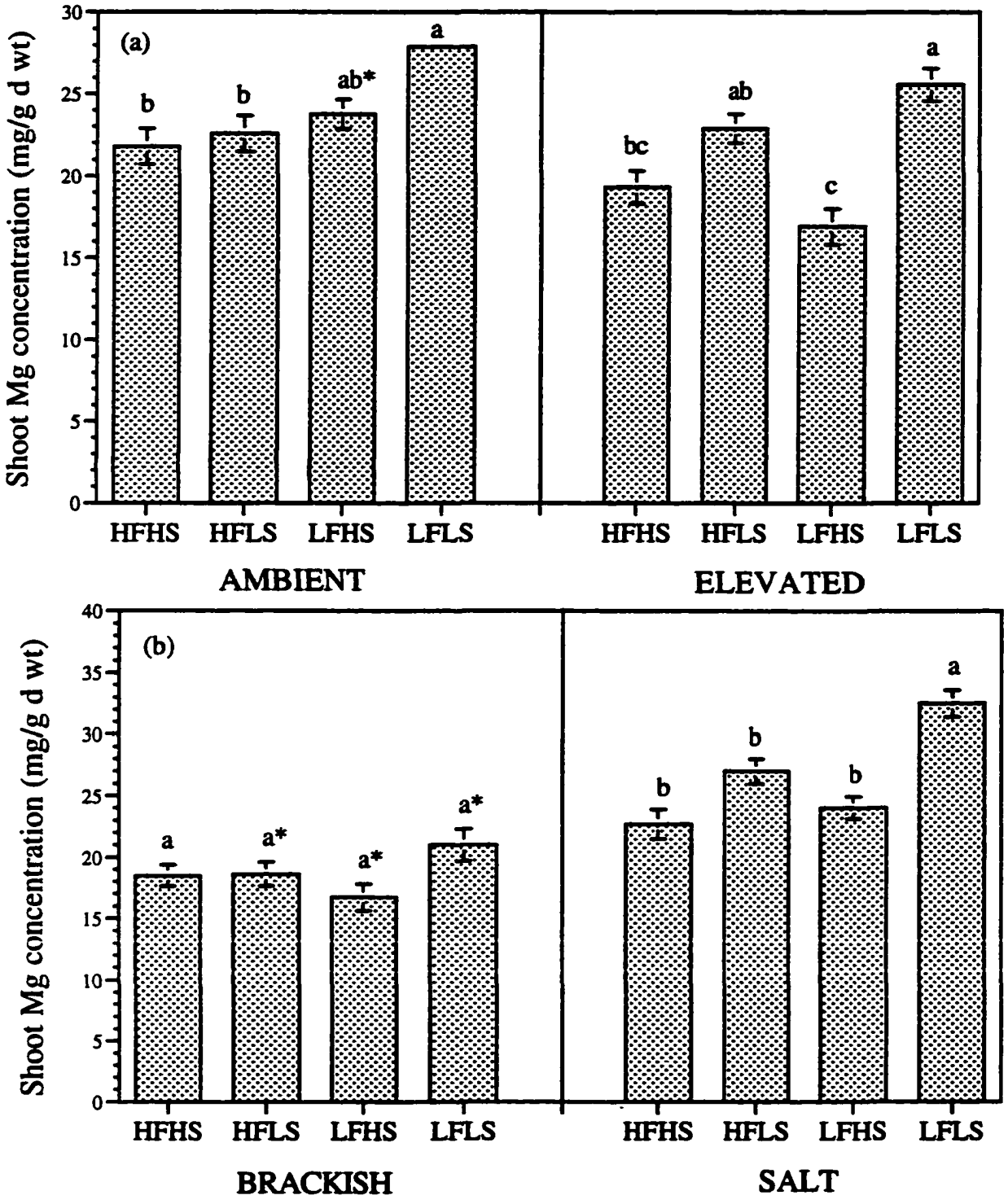


Figure 3.8 Shoot magnesium concentrations in *S. patens* populations. (a) Concentrations in tissue of each population by inundation level. Different letters indicate significant differences between populations within each inundation level. (b) Concentrations in tissue of each population by site. Different letters indicate significant differences between populations within each marsh site. An asterisk indicates a significant difference in concentration between ambient and elevated treatments (a) or brackish and salt marsh treatments (b) for each population.

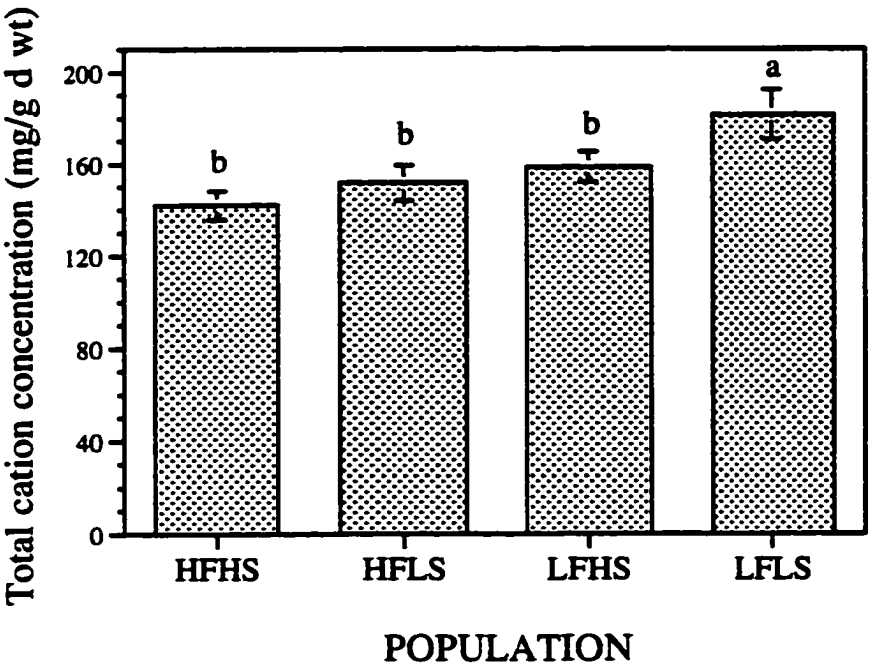


Figure 3.9 Total cation concentration (Na, Mg, K, Ca) in aboveground tissue for each population of *S. patens*. Different letters indicate significant differences between populations.

Table 3.4 Percent nitrogen and shoot elemental concentrations (mg/g d wt) in aboveground tissue of *S. patens* populations of for each site and each inundation level (mean±s.e.). Total cation is the sum of concentrations for the major cations Ca, K, Mg, and Na. Different letters indicate significant differences between means within site or within inundation level.

	SITE		INUNDATION	
Element	Brackish	Salt	Ambient	Elevated
N	0.36±0.02 a	0.46±0.03 a	0.45±0.02 a	0.36±0.01 b
P	6.6±0.4 a	6.4±0.3 a	6.3±0.3 a	6.72±0.3 a
Na	71.5±1.8 a	97.3±4.6 b	92.3±4.8 a	78.1±3.4 b
K	28.1±1.4 a	39.9±1.4 b	33.2±1.8 a	34.9±1.7 a
Mg	18.8±0.6 a	26.5±0.8 b	23.7±1.1 a	21.7±0.8 b
Ca	17.4±0.7 a	14.6±0.6 b	15.0±0.6 a	16.8±0.7 a
S	23.8±0.8 a	34.6±2.9 b	36.3±2.8 a	23.2±1.3 b
Fe	4.1±0.6 a	11.2±1.1 b	9.4±1.4 a	6.3±0.7 b
Mn	0.77±0.6 a	1.0±0.06 b	0.85±0.06 a	0.91±0.06 a
B	0.56±0.04 a	1.0±0.1 b	0.70±0.07 a	0.85±0.10 a
Zn	0.13±0.01 a	0.13±0.01 a	0.13±0.01 a	0.14±0.01 a
Total cation	135.8±2.9 a	178.1±5.8 b	164.1±46.7 a	151.4±5.1 b

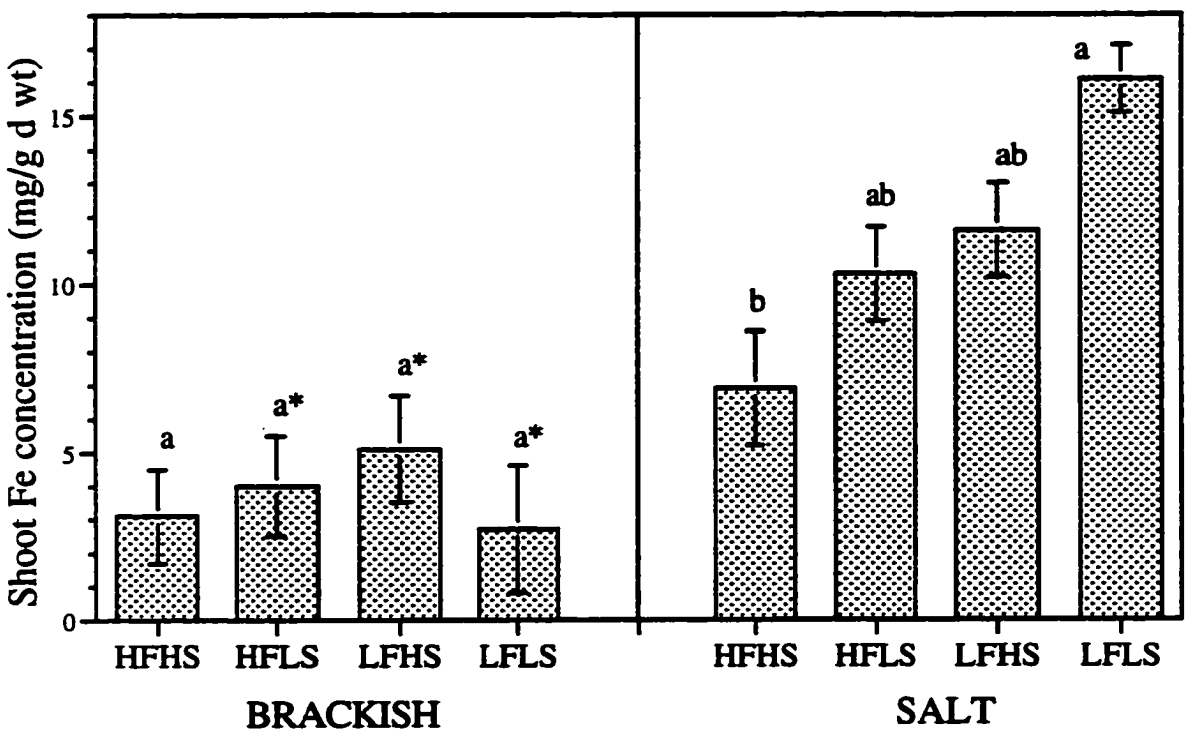


Figure 3.10 Shoot iron concentration in aboveground tissue of *S. patens* populations at each marsh site. Different letters indicate significant differences between populations within each marsh site. An asterisk indicates a significant difference in concentration between the brackish and salt marsh sites for the population.

DISCUSSION

Intraspecific variation in adaptive response to waterlogging and salinity has been examined in a number of wetland species. Major progress has been made at selecting improved genotypes for use as halophytic crops. The same genotypic potential can be utilized for applying more stress-tolerant wetland plants in coastal restoration and stabilization. An engineering approach to utilize stressful environments for plant growth is usually insufficient when implemented alone (Epstein *et al.* 1980) and supplementing efforts with plant genotypes of superior stress-tolerance would enhance planting success (Seliskar 1995). Populations of wetland plants, such as *Sporobolus virginicus*, *S. patens*, and *Agrostis stolonifera*, have been evaluated for use in coastal restoration and stabilization (Ahmad and Wainwright 1977; Gallagher 1985; Blits and Gallagher 1991; Hester *et al.* 1996). *Sporobolus virginicus* populations exhibited differential response in growth, succulence, flowering, and protein synthesis under increased salinity stress (Blits and Gallagher 1991). Differential salt tolerance in *Atriplex* populations was related primarily to differential ion accumulation (Glenn *et al.* 1992; Glenn *et al.* 1994; Glenn *et al.* 1996). *Agrostis stolonifera* ecotypes collected from salt marsh, salt spray zone, and inland habitats demonstrated greater salt tolerance through efficient ion exclusion and the synthesis of organic solutes and lesser salt tolerance through excessive ion accumulation and reduced shoot water content (Ahmad *et al.* 1981). When these same *Agrostis* ecotypes were evaluated under the combination of increased salinity and low oxygen, the pattern of reduced growth by ecotype was predictable from their respective collection source (Ahmad 1977). The growth of the inland ecotype was most depressed by salinity, root hypoxia and their interaction, while the growth of the salt marsh ecotype was least suppressed.

Although the preceeding investigations have demonstrated the ecotypic potential in wetland plants for vegetating stressful coastal habitats, relatively few studies have evaluated populations of wetland plants for performance in the field. Eleuterius (1989)

field tested intraspecific variation in response to salinity in *Juncus romerianus* through reciprocal transplant studies and determined that in the higher salinity areas the less salt tolerant ecotypes had significantly reduced growth compared to the more salt tolerant ecotypes. Low salt marsh ecotypes of *Salicornia europea* reciprocally transplanted into the high salt marsh suffered higher mortality and reduced reproductive output compared to the high salt marsh ecotypes. In an effort to restore deteriorating baldcypress swamps in southeast Louisiana suffering from saltwater intrusion, differentially salt tolerant genotypes of *Taxodium distichum* were selected under controlled conditions (Allen 1994), then evaluated in three disturbed wetland sites (Krauss 1997) ranging from freshwater to brackish conditions. Populations performing best with elevated salinities (4-6 ppt) under controlled conditions also showed improved growth and survival in the higher salinity conditions of the brackish, deteriorating swamp.

Previous studies examining intraspecific variation in *S. patens* have found population differences in response to the individual stressors of flooding and salinity, but little attention has been given to evaluating intraspecific response to the interaction of flooding and salinity. Water potential, gas exchange, growth and morphology (Silander and Antonovics 1979; Pezeshki and DeLaune 1993a; Pezeshki and DeLaune 1993b; Hester *et al.* 1996) differed in populations of *S. patens* in response to salinity, and variation in growth and biomass partitioning differed in *S. patens* populations in response to waterlogging (Chapter 2). However, Burdick and Mendelssohn (1990) found that anatomical and physiological differences relative to field inundation between dune, swale, and marsh populations of *S. patens* were not expressed in response to waterlogging under controlled laboratory conditions. Research presented in this chapter extends ecotypic studies in *S. patens* by testing under field conditions populations collected from a wide geographical area for responses to the interaction of flooding and salinity.

The experimental treatments were successful in subjecting the *S. patens* populations to differing degrees of flooding and salinity stress. Ambient and elevated

treatments differed greatly in flooding duration (Figure 3.1) and it is clear that soil conditions differed between these treatments (Figures 3.2, 3.3). However, the ambient treatment was significantly more reduced and had significantly higher sulfide concentrations than the elevated treatment only in the salt marsh. In the brackish marsh, sulfide concentrations were low and did not differ between the ambient and the elevated treatments because the redox potential was similar between the inundation levels and not low enough for appreciable sulfate reduction.

It is important to note that the elevated treatment did not eliminate all flooding stress and its associated reducing conditions, but that it was intended to be a relief from conditions present in the surrounding deteriorating marsh. The elevated treatment did experience regular flooding (Figure 3.1) and the redox potentials were well below +300 mV (Figure 3.2), the level at which free oxygen disappears from the soil. Though not as stressful to plant growth as the ambient treatment, the elevated treatment was still effective in producing potentially stressful conditions for plant growth. Environmental conditions differed between the salt and brackish marshes, but the difference in salinity exposure was the overriding stressor. Populations transplanted into the salt marsh experienced almost three times the salinity (25 ppt) as those in the brackish marsh (9 ppt). This resulted in lower growth of populations in the salt marsh compared to the brackish marsh (Figures 3.4, 3.5a) and demonstrated that the higher salinity level of the salt marsh was more stressful for population growth than that of the brackish marsh.

It was hypothesized that populations previously selected for greater flood tolerance would outperform the less flood-tolerant populations under increased flooding stress, and that the more salt-tolerant populations would outperform the less salt-tolerant populations under increased salinity stress. Furthermore, HFHS was hypothesized to perform best and LFLS perform worst with the combined stresses of increased flooding and salinity. The results show that the differentially stress-tolerant populations did vary

in response to flooding and salinity, primarily through uptake and/or translocation of essential nutrients and biomass production (Figures 3.4-3.10).

The more flood-tolerant populations had higher total and aboveground biomass in the elevated treatment of the brackish marsh (Figure 3.4) and higher belowground biomass at the brackish marsh, regardless of inundation (Figure 3.5a) Therefore, at moderate flooding and salinity levels, HF populations were more tolerant than LF populations. There were no significant differences between the differentially salt-tolerant genotypes. This suggests that improved flood tolerance confers a greater advantage to growth under these field conditions than does improved salt tolerance. With high flooding stress in the absence of increased salinity (ambient treatment of brackish marsh), the growth advantage conferred by improved flood tolerance remained evident for HF populations with higher belowground biomass regardless of inundation in the brackish marsh (Figure 3.5a). The ability of HF populations to produce higher belowground biomass with increased flooding is a priority for their use to build and maintain substrate integrity in the restoration of deteriorating marshes. The growth advantage conferred by improved flood tolerance at moderate inundation was not evident in aboveground or total biomass with high flooding.

The lack of population differences in biomass with increased salinity suggests that high salinity stress overrides the growth advantage conferred by increased flood-tolerance, even at a lower flooding level (elevated treatment of salt marsh). It is also possible that different flooding durations between the elevated treatments at each marsh site was an influencing factor (Figure 3.1). The flooding duration of the elevated treatment in the brackish marsh (26% of total hours), where population differences were found, was more moderate than the flooding duration of the elevated treatment in the salt marsh (40% of total hours), where there were no population differences. The longer flooding duration in the elevated treatment of the salt marsh may have imposed a higher flooding stress that dampened population differences in growth. However, this treatment

was in combination with salinity stress and it is unclear whether population differences in growth would have remained dampened at this flooding duration in the absence of salinity.

Unlike HF populations, the aboveground and total biomass of LF populations in the elevated treatment were not significantly higher at the lower salinity site compared to the higher salinity site (Figure 3.4). These populations may ecotypically exhibit low growth response. According to Chapin (1990), plants adapted to a low-resource environment exhibit slow growth, even when provided an optimal supply of resources. As well, they allocate fewer resources to growth because of greater allocation to functions that improve survival in stressful environments. LF populations maintained a slow growth response under lower salinity stress of the brackish marsh, and this response was unchanged with increased salinity in the salt marsh. Therefore, LF populations do not maximize their growth in moderately stressed areas whereas the HF populations do.

Rapidly growing, high-resource adapted plants maintain lower tissue nutrient concentrations and higher NUE than low-resource adapted plants under nutrient-limiting conditions (Chapin 1990). Accumulation of N and P did not differ between populations (data not shown), but there were higher concentrations of N and P in LF than in HF populations (Figure 3.6). This indicates that the pool of N and P was diluted over the larger biomass of the more rapidly growing HF populations. This was verified by calculating nutrient use efficiency. Under the same environmental conditions, HF populations had greater aboveground biomass production per unit of N and P and therefore, higher NUE at moderate stress levels, than did LF populations. Identification of higher NUE by HF populations is important for the use of these populations in restoration efforts, as the more flood-tolerant populations would have greater growth in moderately flood-stressed areas and in sites with lower nutrient availabilities. However, differences in aboveground NUE are related to mobilization of belowground resources and an understanding of plant NUE requires accurate belowground estimates of

production (Chapin 1983), which was not attempted in this study. Furthermore, mechanisms of adaptations of plants to maximumize NUE also considers allocation of nutrients to various tissues, not only nutrient concentrations. Future research should include analysis of different plant organs (i.e. leaves, stems, roots) to give more accurate estimates of NUE, as well as estimates of nutrient translocation from belowground to aboveground tissue.

There was a trend of lower Na:K in the shoot tissue of the more flood-tolerant populations in the ambient treatment (Figure 3.7a). There were no population differences in shoot Na concentration (data not shown). The differences in Na:K resulted from a decrease in K concentration in LF populations in the more flooded, ambient treatment compared to the elevated treatment, which has been shown for less flood-tolerant plants in other studies (Davies and Singh 1983; Schat 1984; Laan *et al.* 1989b; Naidoo 1994). Determinations of shoot Na and K concentrations in this study were within the range of other studies (Mendelssohn and McKee, 1987; Rozema, *et al.* 1985). Therefore, differences in population Na:K do not seem to be influenced by differential Na and K discrimination or competitive inhibition by Na, but rather by population ability to maintain K uptake with increased flooding.

The only population to alter Mg concentration with respect to inundation was LFHS, which increased in Mg in the ambient treatment compared to the elevated treatment. Under limited soil oxygen, anaerobic root respiration yields less energy to the plant (Drew 1983; Crawford 1992), resulting in decreased nutrient uptake (Drew 1983; Koch *et al.* 1990; Armstrong *et al.* 1994). However, loss of energy as a result of anaerobic respiration may reduce membrane selectivity for nutrient uptake, yielding increased flow of nutrients into the roots (Armstrong *et al.* 1994). LF may not be able to maintain needed energy levels for root discrimination of nutrients, resulting in higher concentrations Mg in shoot tissue of LF populations than of HF populations (Mendelssohn and Burdick 1988).

A trend evident with increased salinity for the populations was that LFLS had significantly higher Mg than the three other populations in the salt marsh (Figure 3.8b). Concentrations of Mg in the soil was significantly higher in the salt marsh than in the brackish marsh (Table 3.1). Apparently LFLS increased uptake of Mg with increased interstitial Mg concentrations in the salt marsh, while HFHS did not, suggesting that ion exclusion is less efficient in LFLS and more efficient in HFHS. The highest total cation concentration in shoot tissue of LFLS was due primarily to differences in Mg and K concentrations (Figures 3.7, 3.8) and appears to be a reflection of low tolerance to both flooding and salinity. Shoot concentrations of cations differed significantly with site (Table 3.4); however, concentrations in plant tissue from the brackish marsh may have been elevated due to the increase in interstitial salinity (Table 3.2) resulting from severe coastal flooding (Figure 3.1) during the final sampling period when the plant material was harvested. Accumulation of the non-growth-limiting nutrients followed the same pattern as aboveground biomass (data not shown). As shown in previous investigations (Gallagher 1985), there was higher accumulation of non-growth-limiting nutrients with higher biomass.

Interstitial soil concentrations of Fe did not differ between sites (Table 3.1), but were higher in plant tissues from the salt marsh (Figure 3.6a). Total iron concentrations are higher in salt marshes, which have higher clay content. The clay acts as an Fe reservior. Although soluble iron did not differ between sites, it is likely that greater amounts of reserved soil Fe in the salt marsh more readily replaced soluble Fe removed by plant uptake or throughflow of water. Consequently, Fe availability for plant uptake may have been greater in the salt marsh than in the brackish marsh. Alternatively, increased belowground biomass in plants growing in the brackish marsh (Figure3.5a) may have precipitated more Fe in the oxidized rhizosphere, reducing Fe uptake. Differences between HFHS and LFLS in Fe concentration at the salt marsh could also be a result of more efficient precipitation of Fe in the oxidized rhizosphere of HFHS than for

LFLS (as a result of greater rhizosphere oxidation by HFHS) or more efficient exclusion of Fe in uptake by HFHS than by LFLS.

In addition to the mechanisms proposed above, there are genotypic characteristics that influence uptake of mineral nutrients and contribute to population differences in nutrient concentration. These features include stem diameter, leaf size, and root to shoot ratio, as well as physiological parameters such as resorption, enzyme activity, and phytohormones (Saric 1983). Stem diameter and leaf size were different between populations (personal observation) and could have influenced transpiration rates and delivery of nutrients and phytohormones to aboveground tissue. However, root to shoot ratio did not differ between populations (data not shown). Though not measured in this study, physiological responses are potentially an important source of variation. The mechanisms of ion absorption and transport are membrane processes, and the maintenance of energy for uptake and metabolism requires induction of enzymes. The production of these components is genetically controlled and future research focusing on these factors may explain variability in population nutrient uptake.

The populations used in this study were previously screened for their relative salt tolerance by Hester (1995). Using weekly stepwise increases in salinity for 2-3 months, he found a lethal salinity dose for the less salt-tolerant populations at 63-66 ppt and for the more salt-tolerant populations as high as 93 ppt. This may have been a factor in identifying few differences in this field study with respect to the differentially salt-tolerant populations. Perhaps more useful to selection and more ecologically significant would have been the selection of the populations at a salinity level similar to that usually occurring in the field (15-25 ppt) over a long duration. This selection method more closely matches the salinity stress under which these genotypes will be distinguished for differential salt-tolerance in research or wetland restoration. The results from this chapter also suggest that the duration of exposure may be more important and that the level of salinity alone is not sufficient to evaluate these halophytic genotypes. The more flood-

tolerant populations had significantly lower cation concentrations and a trend of lower Na:K than the less flood-tolerant populations when flooded, indicating a possibility that given an additional growing season in the field, these populations may differentiate more clearly in their growth. It is also possible to conclude that the more flood-tolerant populations may possess longer term sustainability than the less flood-tolerant populations through maintaining appropriate nutrient balance with increased flooding and salinity. From an ecological and restoration perspective, an important aspect of the use of these populations in coastal restoration is their sustainability and function. Broome *et al.* (1986) stated that "a ten year sampling period was adequate to document the long-term persistance of the transplanted marsh...and to demonstrate that it is self-sustaining." A more complete evaluation of the level and duration of stress in determining the relative success of differentially stress-tolerant genotypes over multiple growing seasons would provide better insight into long term success of restoration projects.

We may now ask what is the range of deteriorating environments in which these populations can be used? Ideally, planting of these more stress-tolerant genotypes would require minimal preparation or modification of restoration sites and their continued maintenance. In general, the results of this study support the preferred use of the HF populations rather than the LF populations. Specifically, HFHS exhibited the greatest growth, was the least affected in nutritional status by increased flooding and salinity stress (whether separately or in combination), and had higher nutrient use efficiencies of nitrogen and phosphorus (improving success in lower nutrient areas) at more moderate stress levels. This population may therefore be considered to have the greatest potential for use in restoration of deteriorating marshes. However, the use of HF populations at higher stress levels would require modification of the environment, such as increasing the surface elevation of the marsh or fertilization. This recommendation is supported by the results of previous studies (Broome *et al.* 1975; Broome *et al.* 1986; McKee and Mendelssohn 1989; Wilsey *et al.* 1992). For example, Wilsey *et al.* (1992) found

significantly greater transplant success of *S. alterniflora* into a degraded marsh when plants were placed elevated to the marsh surface. Further, macronutrient additions were effective in stimulating growth only in the elevated plants, not in plants growing ambient to the elevation of the surrounding deteriorating marsh.

Populations identified in the greenhouse for greater flood tolerance did outperform the less flood-tolerant populations at moderate flooding and salinity stress levels. However, the more salt-tolerant populations did not demonstrate any significant improvements in growth over the less salt-tolerant populations at either low or high salinity stress. Furthermore, HFHS did perform best under the combination of flooding and salinity stress, while LFLS performed poorly under flooding and salinity.

In conclusion, higher flood tolerance conferred a greater advantage to plant growth of *S. patens* under moderate stress levels in the field than did greater salt tolerance. However, this growth advantage was overridden by higher flooding and salinity levels. In addition, the characterization of the more flood-tolerant populations as high-resource adapted suggests that these populations would maximize their growth under moderate stress levels and in areas of lower nutrient availabilities, whereas the less flood-tolerant populations, characterized as low-resource adapted, would not. Therefore, use of the more flood-tolerant populations in restoring deteriorated areas is recommended and could increase system productivity and result in greater sustainability of the marsh.

Chapter 4

Response of differentially flood- and salt-tolerant Spartina patens populations to increased flooding and salinity in the greenhouse

INTRODUCTION

An "engineering approach" in addressing the restoration of high stressed environments is usually insufficient when implemented alone (Epstein *et al.* 1980), and should be supplemented by a "biological fix," such as the use of plant genotypes selected for higher stress-tolerance. Major progress has been made in selecting improved genotypes of wetland halophytes for use as halophytic crops. Gallagher (1985) describes the evaluation of populations of *S. patens*, *Distichlis spicata*, and *Sporobolus virginicus* for differential yield under varying environmental conditions for use as forage material (Gallagher 1985). The same genotypic potential can be utilized for identifying greater stress-tolerant wetland plants for application in coastal restoration and stabilization.

Coastal wetland loss rates in Louisiana are the highest in the U.S. (Boesch *et al.* 1994). Causal factors include: 1) excessive inundation of the marsh surface resulting from a rapidly subsiding deltaic plain and man-altered hydrology; and 2) elevated salinities of fresh and brackish marshes from saltwater intrusion facilitated by sea level rise, canalization, storm surges, and altered hydrology (Mendelssohn *et al.* 1983; Deegan *et al.* 1984; Turner *et al.* 1984; Penland and Ramsey 1990; Reed 1991). Plants in these areas often fail to adjust to excessive waterlogging and increased salinity, which leads to plant die-back and marsh deterioration. Furthermore, subsequent recolonization can be prevented by the inundated, reduced, and more saline soil conditions (Mendelssohn and McKee 1988; Mendelssohn and McKee 1989; Flynn *et al.* 1995; Baldwin *et al.* 1996). Certain populations of wetland species have been demonstrated to be better adapted than others to flooding and salinity stresses (Keeley 1979; Silander and Antonovics 1979; Davies and Singh 1983; Eleuterius 1989; Hester *et al.* 1996). The use of these more

stress-tolerant populations in the restoration of deteriorating areas would be of benefit by increasing productivity and stem density, enhancing sedimentation and peat formation, and facilitating vertical marsh accretion. Hence, availability of more flood- and salt-tolerant genotypes of wetland vegetation is important to the successful development of restoration programs in coastal areas around the world.

Previous research has identified populations of *S. patens* collected over a large geographical range (Texas and Louisiana Gulf Coast) that exhibit differential flood tolerance (Lessmann *et al.* 1997) and differential salt tolerance (Hester *et al.* 1996). In the previous chapter, these differentially stress-tolerant populations were examined under field conditions, and they exhibited ecotypic variation in growth and ion relations in response to flooding and salinity. The goal of this chapter was to investigate specific adaptive mechanisms underlying the differential population stress tolerances. By comparing such responses in plants that are closely related and similar in ecological distribution, the results can contribute to a greater understanding of the relative roles of each mechanism in stress-tolerance. This would aid in better matching greater stress-tolerant populations to site conditions, as well as provide greater insight into the role of intraspecific variation in stress tolerance in predicting community responses to environmental changes.

MATERIALS AND METHODS

Plant Material

Populations of *S. patens* selected for differential flood tolerance in Chapter 2 (14 and 26 as more flood-tolerant, and 4 and 69 as less flood-tolerant) were used in this study. Previous research selected these same populations for differential salt-tolerance (Hester 1995) and identified populations 14 and 69 as more salt-tolerant and populations 4 and 26 as less salt-tolerant. This resulted in the four differentially stress-tolerant populations designated as HFHS (high flood- and high salt-tolerant), HFLS (high flood- and low salt-tolerant), LFHS (low flood- and high salt-tolerant), and LFLS (low flood-

and low salt-tolerant). The populations were prepared for experimentation from greenhouse stocks. For each population, the initial wet weight of approximately 10 stems of uniform height was measured for use as an index of initial plant biomass and as a final biomass covariate. The belowground biomass was stained using neutral red (Schumacher *et al.* 1983)to distinguish new root growth from the original root system. The stems were planted into 3.8 liter plastic pots containing a commercial potting medium (Jiffy Mix Products, West Chicago, Illinois). A 0.5 cm layer of small gravel (3-5 mm diameter) was placed over the soil surface to reduce the buoyancy of the pots while submerged.

Experimental Design

A split plot randomized block design (5 replicates) was used. Treatments were: 1) salinity, two levels at 5 ppt and 25 ppt, 2) inundation, two levels denoted as flooded and drained, and 3) four populations denoted as HFHS, HFLS, LFHS, and LFLS to describe the respective flood and salt tolerances of each. In the greenhouse, ten tanks (1 m x 0.5 m x 0.5 m) were filled with tap water to a depth of 25 cm. Using Instant Ocean Synthetic Sea Salt (Aquarium Systems Inc., Cleveland, OH), five tanks were brought to a salinity of 5 ppt and five tanks to a salinity of 25 ppt. One tank of each salinity comprised a block. Two pots of each population were placed randomly within each tank, one pot was placed on the tank bottom (flooded 5 cm above the soil surface), the other was placed on top of an inverted 3.8 liter pot to allow water to cover only the bottom 5 cm of the pot (drained). Eighty experimental units were and used for the study (8 pots/tank, 16 pots/block, 20 pots/population). All pots were maintained for 90 days under these treatments before initiating sampling, which was conducted every 25 days for 15 weeks.

Sampling and Analytical Methods

Leaf Elongation - For each sampling period, two stems from each pot were selected and the length from ligule to leaf tip of the youngest leaf was measured over three days using digital calipers. Elongation rates were calculated in mm/day.

Soil Redox Potential - Redox potentials were measured at 1-cm and 10-cm depths in each pot with bright platinum electrodes. The potential of a standard calomel reference electrode (+244 mV) was added to the millivolt reading to obtain Eh.

Leaf proline concentration - Prior to final harvest, healthy green leaves were clipped from plants in each pot and rinsed in deionized water. The leaves were freeze dried (Labconco) for five days then milled (60 mesh sieve, Wiley Mill). Proline concentration in the leaf material was determined spectrophotometrically (Ewing *et al.* 1995).

Root Specific Gravity - Immediately following harvest of the aboveground plant material, the contents of each pot was enclosed in a plastic bag (filled with air to protect the roots from being crushed) and stored in a cold room. All specific gravity measurements were taken within three days of harvesting. The belowground material was removed from cold storage and turgid, structurally intact roots were selected for analysis. Roots growing outside of the soil or pots were not used for measurements. The most distal 5 cm portion of several roots was excised and any remaining particulate matter carefully removed. The roots were gently blotted dry and laterals were removed with a stainless steel blade. Specific gravity was determined with a pycnometer (Burdick and Mendelssohn 1990; Naidoo *et al.* 1992) and root porosity was calculated with the equation [1.026-(0.969*specific gravity)] (Burdick and Mendelssohn 1987).

Biomass - The remaining aboveground and belowground biomass was sorted into live and dead material. The belowground was further partitioned into roots, rhizomes, and portions of stem extending belowground. The portions of stem material were added to the aboveground biomass. Dry weights were recorded after drying at 65°C for one week. The mass of plant material used in the previous methods was added to the final biomass.

Tissue Percent Nitrogen - Dried live shoot material was milled (60 mesh sieve, Wiley Mill) and analyzed for percent nitrogen using a Perkin Elmer 240 CHN Elemental Analyzer.

Statistical Analysis - All variables were analyzed as a split plot randomized block design. Significant treatment effects were identified with analysis of variance. All values were significant at $p \leq 0.05$, unless otherwise indicated. When main effects were significant, multiple comparisons were made using Tukey's HSD. Assumptions of normality and homogeneity of variance were tested (Shapiro-Wilk test and Bartlett's test, respectively) and found to be valid. All analyses were performed using SAS (SAS Institute, Inc. 1989).

RESULTS

Free oxygen disappears in flooded soil below a redox potential of +300 mV (Patrick and DeLaune 1977). Redox potentials resulting from the inundation treatment were below +100 mV, indicating that the rooting zone was anaerobic (Figure 4.1). The Eh of the drained treatment was significantly higher than that of the flooded treatment at both the soil surface and a depth of 10 cm. In addition, redox potential at a depth of 10 cm was more reducing than at the soil surface for the drained treatment (Figure 4.1). Population had no significant effect on soil Eh.

Leaf elongation rates in the flooded treatment (Figure 4.2) were significantly higher than in the drained treatment for the first 7 weeks, after which time the drained treatment values increased to that of the flooded treatment. The higher salinity treatment significantly reduced leaf elongation rates (Figure 4.3). Relative population performance was consistent over the sampling periods (Figure 4.4). HFLS exhibited significantly higher rates than all other populations for sampling period 1. It remained significantly higher thereafter only from LFLS during period 2 and LFHS during periods 2-5. HFHS and LFLS never differed significantly. LFHS differed from all other populations only during period 5, and from HFHS during the additional periods 1 and 4. Overall, HF populations consistently exhibited greater leaf elongation rates than LF populations (Figure 4.4). No trend was evident with respect to relative salinity tolerance of the populations.

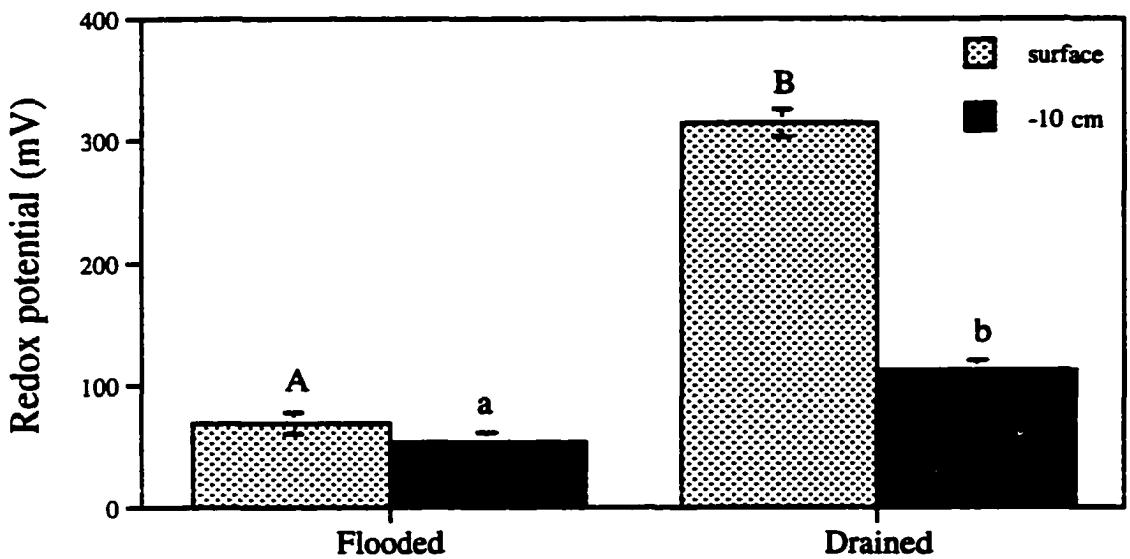


Figure 4.1 Soil redox potential for each inundation treatment. Different capital letters indicate significant differences between the flooded and drained surface Eh. Different lowercase letters indicate significant differences between the flooded and drained Eh at a depth of 10 cm.

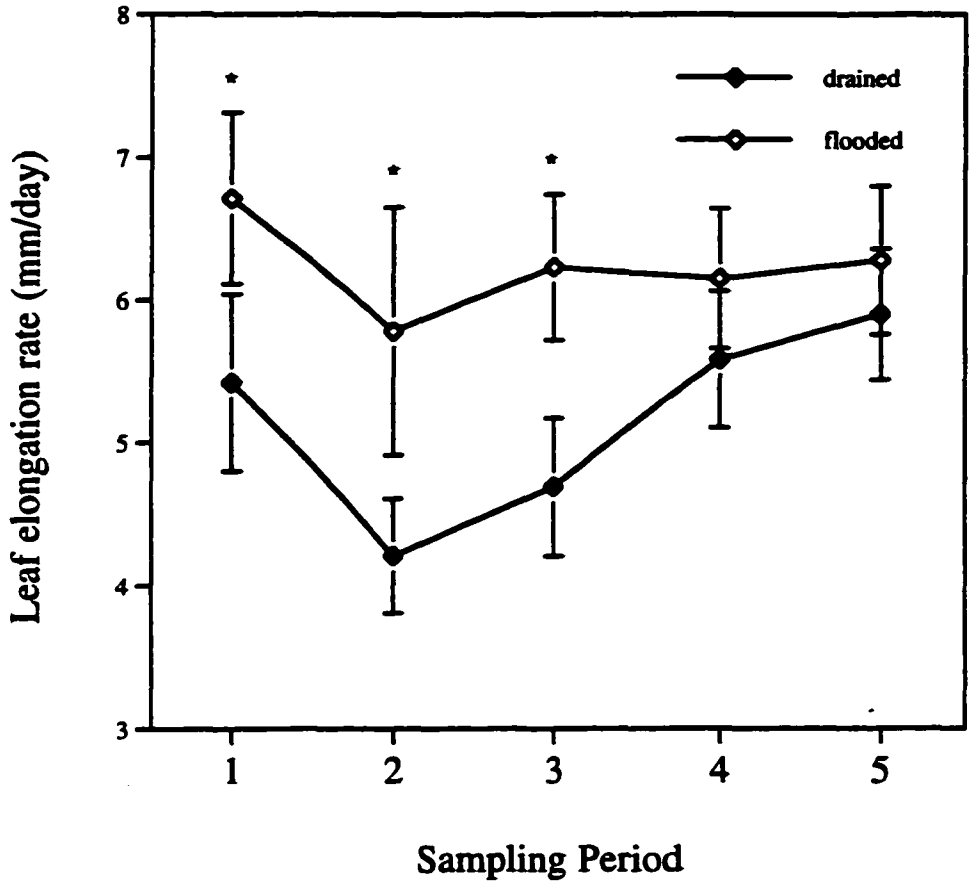


Figure 4.2 Leaf elongation rates (mm/day) over a 15 week sampling period for each inundation treatment. Populations of *S. patens* were exposed to flooding and salinity treatments for 90 days prior to initiating measurements. Sampling periods 3-5 correspond to spring growth. An asterisk denotes that the flooded and drained means for that sampling period were significantly different.

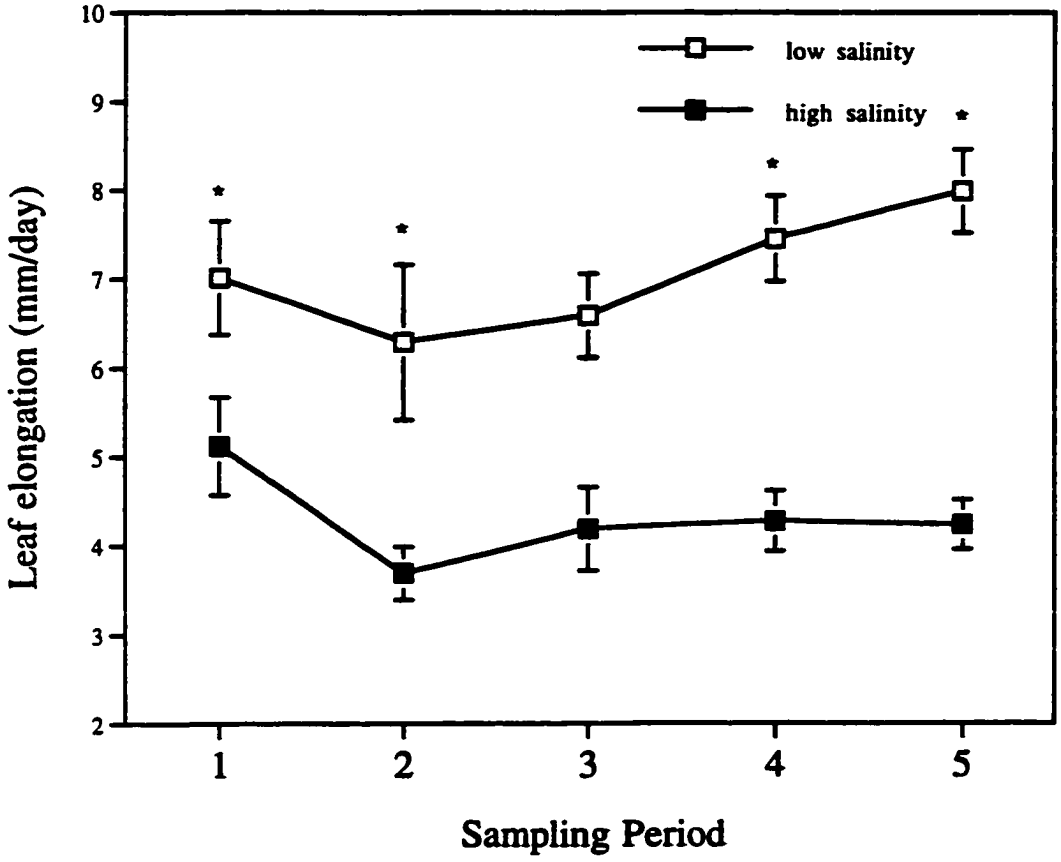


Figure 4.3 Leaf elongation rates (mm/day) over a 15 week sampling period for each salinity treatment. Populations of *S. patens* were exposed to flooding and salinity treatments for 90 days prior to initiating measurements. Sampling periods 3-5 correspond to spring growth. An asterisk denotes that the low salnity and high salinity means for that sampling period were significantly different.

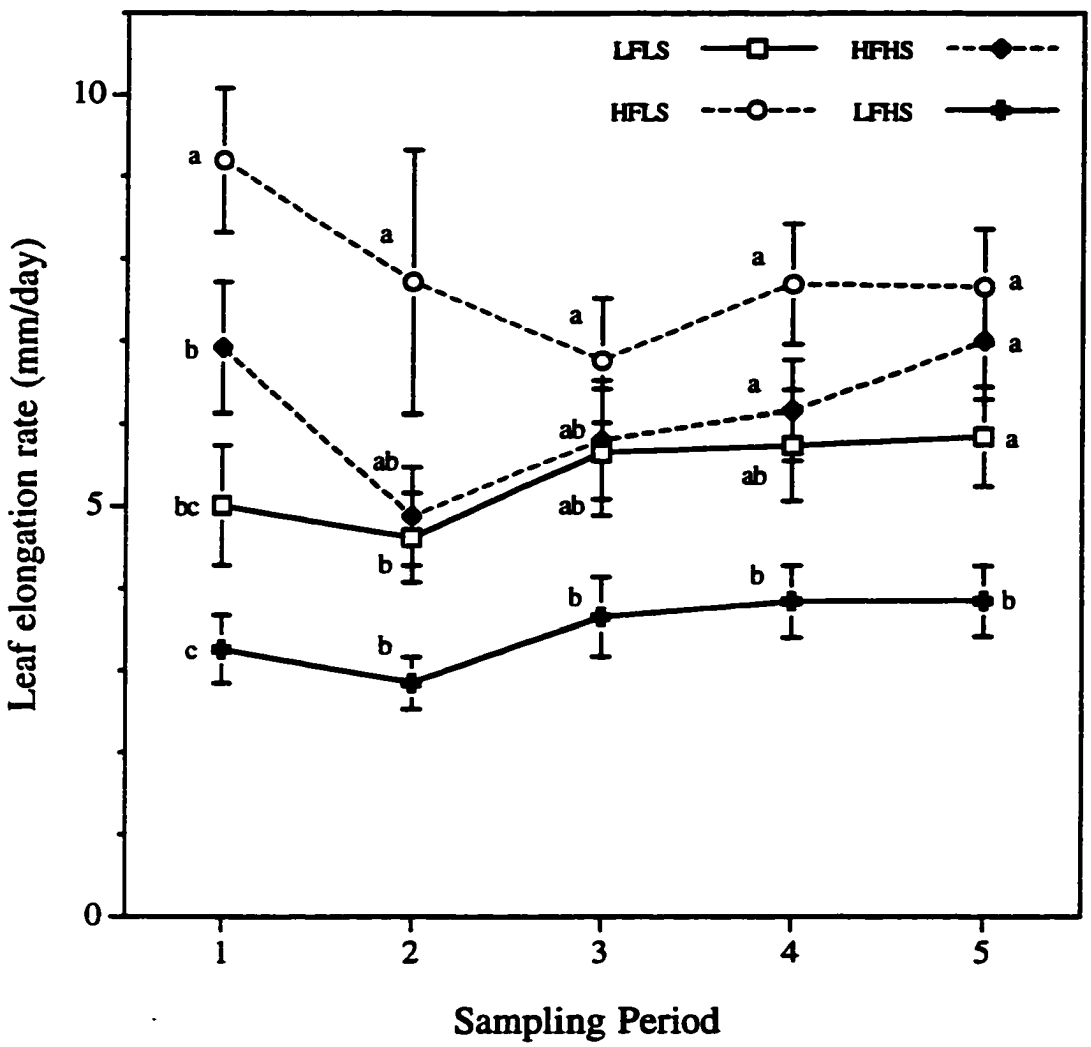


Figure 4.4 Leaf elongation rate (mm/day) over a 15 week sampling period for each population of *S. patens*. Pots were exposed to flooding and salinity treatments for 90 days prior to initiating measurements. Sampling periods 3-5 correspond to spring growth. Filled symbols represent more salt-tolerant populations. Dashed lines represent more flood-tolerant populations. Significant differences between populations within each sampling period are indicated by different letters.

For aboveground, belowground, and total biomass (Figure 4.5), HFHS was significantly higher than HFLS and LFHS, and significantly higher than LFLS for aboveground biomass. LFLS was significantly greater than LFHS for belowground and total biomass and never differed significantly from HFLS. HFLS and LFHS also did not differ significantly in biomass. Biomass results for the populations did not vary significantly with respect to the inundation or salinity treatments. As well, the lack of population interactions with inundation or salinity provided no evidence indicating that the more flood-tolerant or more salt-tolerant populations exhibited greater growth under increased flooding or salinity stress than the less flood-tolerant or less salt-tolerant populations, respectively.

Specific gravity was unchanged by the inundation treatment (Table 4.1). However, population differences revealed that HFLS was significantly higher than both LF populations for specific gravity, but did not differ from HFHS. HFHS was not significantly different from the LF populations. Root porosity was lowest in HFLS (16%) and highest in the LF populations (29%-30%).

Leaf proline concentrations were not significant for any treatment effects (mean=4.07±1.44 μmol/g d wt). Percent nitrogen, a major component of proline, was significantly higher in plant tissue grown under drained conditions (mean=0.62±0.02) than in tissue grown under flooded conditions (mean=0.58±0.02).

DISCUSSION

Intraspecific variation in response to waterlogging and salinity has been examined in several wetland species. *Veronica peregrina* populations derived from the center of a shallow pool and the pool's drained periphery differentially accumulated malate with increased waterlogging (Linhart and Baker 1973). Populations of *Nyssa sylvatica* (Keeley 1979) collected from swamp to upland habitats responded differently to increased flooding under controlled conditions. Upland populations were considerably less tolerant, exhibiting root dieback and decreased growth and survival (27%). The swamp

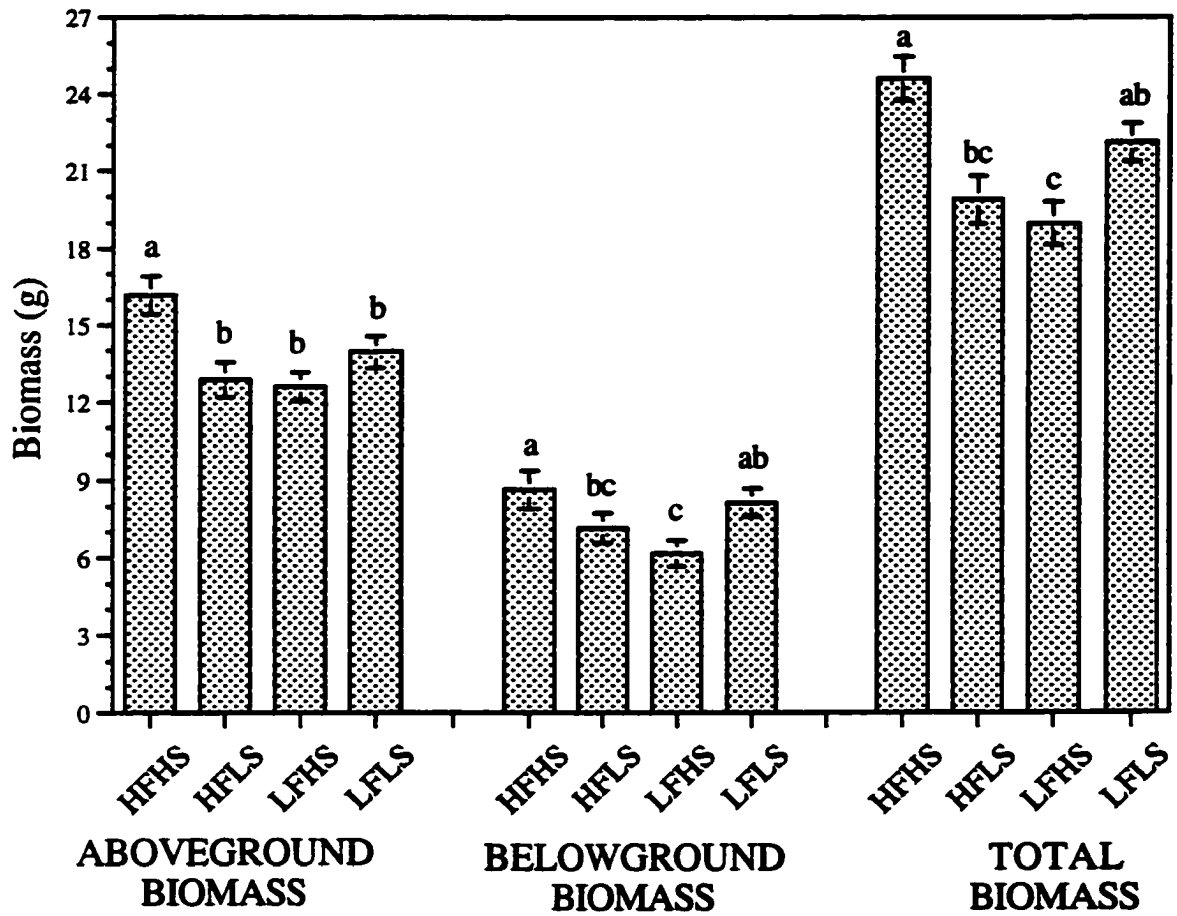


Figure 4.5 Aboveground, belowground, and total biomass for each population of *S. patens*. Different letters indicate significant differences between populations within each biomass variable (no significant inundation or salinity main effects or interactions). HFHS=high flood- and high salt-tolerant; HFLS=high flood- and low salt-tolerant; LFHS=low flood- and high salt-tolerant; LFLS=low flood- and low salt-tolerant.

Table 4.1 Specific gravity and root porosity for each population (mean±s.e.). Root porosity was determined from the relationship porosity=(1.026-0.969*specific gravity). Different letters indicate significant differences between populations. There was no significant inundation or salinity effect or interactions.

Population	Specific Gravity		Root Porosity
HFHS	0.79±0.03	ab	26%
HFLS	0.89±0.04	a	16%
LFHS	0.76±0.01	b	29%
LFLS	0.75±0.04	b	30%

population initiated a new, more porous root system and exhibited significantly greater growth and survival (98%). *Sporobolus virginicus*, a species used in shoreline stabilization, has two genetically distinct ecotypes, "marsh" and "dune" (Donovan and Gallagher 1984). In hydroponic culture, both ecotypes adapted similarly with respect to their morphology and physiology to anaerobic conditions, indicating no differences in their relative flood tolerance. However, increased salinity did induce differential response in succulence, flowering, and protein synthesis (Blits and Gallagher 1991), with no differences in ion relations or proline accumulation. These results suggest a primary role for salinity in maintaining these *S. virginicus* ecotypes.

Previous studies examining intraspecific variation in *S. patens* have found population differences in response to the individual stressors of flooding and salinity, but little attention has been given to evaluating intraspecific response to the interaction of flooding and salinity. Water potential, gas exchange, growth and morphology (Silander and Antonovics 1979; Pezeshki and DeLaune 1993a; Pezeshki and DeLaune 1993b; Hester *et al.* 1996) differed in populations of *S. patens* in response to salinity and variation in growth and biomass partitioning differed in *S. patens* populations in response to waterlogging (Lessmann *et al.* 1997). However, Burdick and Mendelssohn (1990) found no differences among populations collected from dune, swale, and marsh communities for growth, anatomy, and metabolism in response to waterlogging under controlled conditions.

Flooded soil is deficient in oxygen due to slowed gaseous exchange rates at the soil surface with the atmosphere (Patrick and DeLaune 1977). Under limited soil oxygen, plants roots respire anaerobically which results in a considerable loss of energy yield to the plant (Drew 1983; Crawford 1992). As a result of the energy loss, root conductivity decreases and shoot water relations are affected, disrupting metabolic and transport processes. Aboveground tissues receive a limited supply of nutrients and important regulatory phytohormones (Tang and Kozlowski 1982; Drew 1983; van de Werf *et al.*

1988; Else *et al.* 1995). Phytotoxins, such as sulfides, may accumulate in waterlogged soil, damaging belowground tissue or resulting in decreased nutrient uptake from the soil by the roots (Ingold and Havill 1984; Havill *et al.* 1985; Koch *et al.* 1990)

Three main stresses are associated with salinity. Osmotic stress, the most immediate, results from a lower osmotic potential in the rooting medium than in the plant tissue which inhibits water uptake by the roots and produces a "physiological drought." This results in stomatal closure, reduced photosynthesis, and reduced cell elongation (Jefferies 1981; Rozema *et al.* 1985a; Pezeshki and DeLaune 1997). Toxic ion stress results from the cellular accumulation of Na^+ and Cl^- from seawater to phytotoxic levels (Flowers *et al.* 1977; Gorham *et al.* 1985). Finally, Na^+ and Cl^- competitively inhibit the uptake of nutrients (Linthurst and Blum 1981; Morris 1984; van Diggelen *et al.* 1986; Huang and Redmann 1995) resulting in nutrient deficiencies.

Overall, HFHS maintained high leaf elongation rates and the highest aboveground, belowground, and total biomass. Population LFHS had the lowest leaf elongation rates and the lowest aboveground, belowground, and total biomass. HFLS and LFHS never differed in biomass, but distinct morphologies contributed to different leaf elongation rates of these populations (Figure 4.4). HFLS had tall thick stems of low density that exhibited faster leaf elongation rates than did the stems of LFHS, which were short and thin, with high density.

Important response mechanisms to flooding and salinity stresses were evaluated to identify potential factors determining differential flood and salt tolerance of these populations. An important anatomical response under flooded conditions to avoid anoxia in plant tissues and to buffer soil phytotoxins is the formation of aerenchyma, or interconnecting gas-filled spaces in the cortical parenchyma of the root produced by either the breakdown of cells (lysigenous) or cell separation (schizogenous). This increased porosity in plant tissues reduces both the physical resistance to diffusion and the oxygen demand of respiring tissue along the diffusion pathway (Armstrong *et al.* 1994). Greater

aerenchyma formation under low oxygen conditions allows for greater root length (Justin and Armstrong 1987), higher apical oxygen concentrations (Armstrong 1971), and development of an oxidized rhizosphere (Coutts and Armstrong 1976; Armstrong *et al.* 1994). This long term adaptation has been clearly demonstrated to be important in maintaining flood tolerance and competitive ability of wetland species in waterlogged sediments (Gleason and Zieman 1981; Schat 1984; Burdick and Mendelssohn 1987; Laan *et al.* 1989a). Flood tolerant and intolerant species can be distinguished at root porosities greater than 10% (Smirnoff and Crawford 1983; Justin and Armstrong 1987). Porosities up to 50% of total root volume have been found in *S. patens* (Burdick and Mendelssohn 1987; Naidoo *et al.* 1992).

A reliable technique to quantify aerenchyma is pycnometry (Jensen *et al.* 1969; Justin and Armstrong 1987; Burdick 1989; Laan *et al.* 1989a; van Noordwijk and Brouwer 1989), which measures both specific gravity, the amount of water displaced by a root tissue sample, and root porosity, a direct measure of tissue air space. Using pycnometry, Burdick (1987) found that the root specific gravity of *S. patens* was negatively correlated with root porosity (r=-0.99) and this relationship is described by the equation [1.026-(0.969 x specific gravity)]. Therefore, a higher specific gravity indicates a lower root porosity. This study measured specific gravity using pycnometry and root porosity was calculated from the specific gravity values.

All populations failed to increase root porosity with increased flooding. Specific gravity varied from 0.69-0.89, with a mean value of 0.80±0.11. Corresponding root porosities were 16%-30% (Table 3.1). Perhaps no differences with the inundation treatment were seen because these levels of porosity, all greater than the 10% distinguishing flood-tolerance and intolerance, were sufficient for oxygen supply in the more flooded treatments. Furthermore, the redox potential in the flooded treatment indicated that the soil in the rooting zone was not highly reduced nor significantly different from the drained treatment. This could have resulted in less induction of

aerenchyma in the roots of plants in the flooded treatment compared to the drained treatment.

Specific gravity determinations did vary with population, but yielded results contrary to that expected. The more flood-tolerant populations had higher specific gravities and lower root porosities than the less flood-tolerant populations. HFLS had the lowest root porosity (16%) and maintained the highest leaf elongation rates (Figure 4.2c). Perhaps the lower root porosity of HFLS resulted in greater tissue anoxia and higher production of ethylene, which promoted higher leaf elongation rates. Furthermore, under these experimental conditions, differences in aerenchyma formation appear not to contribute to the biomass response of these *S. patens* populations. HFLS and LFHS that did differ in specific gravity never differed significantly in biomass, while HFHS and LFHS, which did not differ in specific gravity, differed significantly in biomass (Figure 4.3).

Previous studies have determined similar values for specific gravity in *S. patens*, but have also demonstrated a decrease in specific gravity (increase in root porosity) under flooded conditions. Burdick and Mendelssohn (1990) obtained specific gravity values of approximately 0.83 for *S. patens* under drained conditions, which decreased below 0.5 after 65 days of flooding. Naidoo *et al.* (1992) obtained specific gravity values of approximately 0.72-0.75 under drained conditions and 0.55-0.62 under flooded conditions. In both studies, soil conditions were more reducing and may have been a greater inducement for aerenchyma development.

In halophytes, a response mechanism for salinity stress is the translocation and compartmentalization of ions into the vacuoles (Flowers 1985; Naidoo 1994). This maintains the appropriate water potential gradient between the soil and the root and prevents the buildup of salts to toxic levels in the cell cytoplasm (Flowers *et al.* 1977; Cavalieri and Huang 1979; van Diggelen *et al.* 1986). To prevent osmotic imbalance between the cytoplasm and the vacuole, compatible osmotic solutes are produced in the

cytoplasm. In *Spartina*, the compatible osmoticum proline accumulates. However, this accumulation has been demonstrated to have a salinity threshold level ranging from 15 to 30 ppt (Cavalieri 1983; Ewing *et al.* 1997). Proline concentrations in the leaves of these *S. patens* populations were not significant for any of the treatment effects. The higher salinity treatment (25 ppt) did not seem to meet the threshold salinity for increased proline accumulation. In previous studies of *S. patens*, proline levels under low salinity treatments were as low as 2 µmol/g d wt (Mendelssohn and McKee 1987; Naidoo *et al.* 1992; Ewing *et al.* 1995). In studies performed at elevated salinities, Naidoo *et al.* (1992) found levels as high as 46 µmol/g d wt (25 ppt). Mendelssohn and McKee (1987) determined proline levels greater than 20 µmol/g d wt at 20 ppt. Hester (1995) in previous proline analysis with these same populations obtained concentrations between 80 and 130 µmol/g d wt at 20 ppt. These values are considerably higher than those obtained in other studies of this species, perhaps due to the different technique of analysis employed (High Pressure Liquid Chromatography). Because of the high threshold level for proline accumulation in *Spartina* species, it is often concluded that proline accumulation is significant only under conditions of very high salinity, such as periods of low rainfall or in the upper marsh (Cavalieri 1983; Adams and Bates 1995). This appears to be the case for these *S. patens* populations.

An increase in proline, which is composed of 12% nitrogen, is usually accompanied by an increase in nitrogen. Therefore, proline accumulation is considered a drain on the nitrogen resources of the plant (Rozema *et al.* 1985b; Jefferies and Rudmik 1991; Naidoo 1994). As with proline in this study, percent nitrogen did not differ with salinity or population. However, unlike proline, differences in N concentration were seen with respect to the inundation treatment, in which plant tissue grown under drained conditions had significantly higher percent nitrogen than tissue grown under flooded conditions. This decrease in nitrogen concentration with increased flooding has been

demonstrated in other studies (Morris 1984; Laan *et al.* 1989a; Stepniewski and Przywara 1992) and is attributed to decreased nitrogen uptake.

The reason for the lack of increased root porosity with higher flooding stress or increased proline accumulation in leaves with higher salinity stress in not clear. Overall, the plants maintained a stressed appearance during the experimental period, exhibiting poor plant health and never producing substantial increases in growth. In addition, leaves of all populations at both inundation and salinity levels remained rolled throughout the study, preventing measurement of photosynthesis and hindering water potential measurements. Belowground production was low, failing to yield enough root material for analysis of alcohol dehydrogenase, the enzyme that mediates anaerobic respiration. The direct cause of the poor plant health is unknown.

In summary, population HFHS is concluded to have overall the best growth, with LFHS exhibiting the poorest growth. HFHS and LFLS never differed significantly for any variable measured. However, variation in population response to the individual stressor effects of increased inundation or salinity as well as to the stressor interaction was not detected. Analysis of specific gravity and proline accumulation revealed that under these experimental conditions they played no role in influencing differential population growth of these *S. patens* populations in response to flooding and salinity, respectively.

Although population differences in response to increased salinity and flooding were not detectable in this experiment, a field study (Chapter 3) with these same populations found that under moderate flooding and salinity, HF populations did perform better than the LF populations. Further research examining these populations should focus on other response mechanisms that could be important in distinguishing relative stress tolerance at an intraspecific level, as well as examining how different degrees and durations of increased flooding and salinity stress may function to distinguish genotypes. These issues are important in developing wetland plant stocks that will aid in maintaining

functional and sustainable wetlands that are at risk through both natural and anthropogenic causes. Furthermore, understanding more clearly the role of intraspecific variation in plant responses to environmental stresses is important in understanding the response of coastal plant communities at the landscape scale.

Chapter 5

Conclusions

The three species evaluated in this research demonstrated intraspecific variation in biomass partitioning in response to flooding stress and resulted in the identification of genotypes ranging in flood-tolerance. In addition, this research demonstrated that these populations can be selected for specific traits to enhance desired function, such as increased belowground biomass to stabilize soil substrates, increased aboveground biomass to enhance sedimentation or increase detrital input, or increased rhizome production for greater vegetative reproduction. In all three research chapters, higher total biomass was identified as an indicator of improved genotype performance, but allocation to belowground biomass with higher flooding stress was also important. In Chapter 2, PCA analysis identified that, in addition to total biomass, the belowground variables were important in characterizing differential population flood tolerance. In Chapter 3, the more flood-tolerant populations had higher belowground biomass than the less flood tolerant populations in the brackish marsh, regardless of flooding level. Maintenance of greater belowground biomass by these populations could provide an advantage to forage for nutrients in the soil, buffer soil phytotoxins, maintain energy reserves to support anaerobic respiration, or improve soil stability.

In the natural environment, *Spartina* species are subjected to flooding and salinity stresses concurrently, and a complete investigation of stress response in genotypes cannot evaluate one without the influence of the other. Therefore, analysis of the differentially flood-tolerant *S. patens* populations incorporating analysis of their relative salt tolerance was critical. A significant finding from this research was that increased flood tolerance conferred a greater advantage for plant growth at moderate stress levels than did increased salt tolerance. The more flood-tolerant populations demonstrated greater growth, a better ability to maintain relatively constant concentrations of nutrients with changing stress levels, and significantly higher nutrient use efficiencies for nitrogen and phosphorus. The

differentially salt-tolerant populations exhibited no differences in any measured variables, regardless of treatment. However, this greater advantage conferred by increased flood tolerance was overridden by high salinity stress, and was greatly reduced by excessive flooding stress. This leads to inquiry regarding what are the important mechanisms underlying differential tolerance to flooding and salinity in these genotypes and how does the presence of one stressor affect tolerance to the other. To address this question, Chapter 4 investigated two response mechanisms that are traditionally viewed as good indicators of plant performance under flooding and salinity stress, root porosity and leaf proline accumulation, respectively. However, the results indicated that under these experimental conditions, root porosity and proline played no role in influencing differential population growth of these *S. patens* populations in response to flooding and salinity. Other mechanisms, such as the role of salt excretion, exclusion, and compartmentalization with increased salinity, and the control of root anaerobic metabolism through alcohol dehydrogenase activity, carbohydrate consumption rates, and maintenance of adenylate energy charge with increased flooding could be the focus of future research.

Identification of the less flood-tolerant populations as low-resource adapted and the more flood-tolerant populations as high-resource adapted based on growth response and nutrient status is ecologically significant in predicting long-term population success in restored marshes as well as understanding the functions that may be restored to the marsh by the plant genotypes. As emphasized in Chapter 3, the more flood-tolerant populations demonstrated greater productivity and potential for increasing sustainability of the deteriorating marsh. However, whether the differences detected in these *S. patens* populations portray adaptive significance and increased plant fitness under higher stress is still uncertain. As well, the restoration of other marsh functions, such as nutrient retention, invasibility, and biotic interactions by these genotypes remain to be evaluated.

Another question arising from this research is at what stress level and/or duration of stress does this growth advantage begin to attenuate? What are the thresholds of tolerance to flooding or salinity and how are these thresholds altered by the presence of additional stressors? With respect to inundation, populations differed significantly in growth at lower salinity (9 ppt) when flooded only 26% of the time. However, there were no significant growth differences when the populations were flooded 60% of the time at lower salinity and when flooded 40% of the time with higher salinity (25 ppt). This suggests that a flooding duration of 40% or greater is sufficient to overcome the growth advantage of higher flood tolerance. However, this treatment was in combination with salinity stress and this flooding level could increase in the absence of salinity.

The genotypes were exposed to a relatively continuous level of salinity for a full growing season (7-9 months) in the field study (Chapter 3) and greenhouse study (Chapter 4) and this resulted in no differences in growth between the differentially salt-tolerant populations with higher salinity. One possible explanation is that the original selection of these populations utilized a high salt stress for a short term. The research presented in this dissertation utilized a more moderate salt stress for approximately the same length of time. The higher stress intensity during selection could have produced population differences more rapidly than did the lower stress intensity during the field and final greenhouse chapters. Perhaps a longer stress duration utilizing the more moderate salt stress level was required to significantly distinguish population growth. Significant differences between the populations in ion relations and nutrient use efficiencies with higher flooding and salinity levels suggest the possibility that given an additional growing season in the field, these populations may differentiate more clearly in their growth. Future studies need to be conducted to investigate how different levels of stress for varied durations determine the relative success of differentially stress-tolerant genotypes. Furthermore, identifying what factors may alter these levels for a genotype, such as the effect of stress interactions or variable nutrient levels, would be invaluable. The response

of genotypes to different levels of stress would give a clearer idea of the realized potential for applying these genotypes in a range of deteriorating wetlands and aid in better matching genotypes with site conditions for restoration. As well, it would provide a better understanding of the role of intraspecific variation in controlling community response to changing environments.

The results of this dissertation clearly demonstrated intraspecific variation in *S. patens* in response to flooding and salinity. Specifically, the more flood-tolerant populations outperformed the less flood-tolerant populations under moderate flooding and salinity stress. However, the differentially salt-tolerant populations were not distinguished under flooding or salinity stress. The use of the HF populations rather than the LF populations for transplantation is preferred, but at moderate stress levels. Using HF populations at higher stress levels would be more effective with modification of the environment, such as increasing the surface elevation of the marsh or amending the soil with nutrients. Higher nutrient use efficiencies by HF populations would possibly lend greater success to restoring areas without the addition of nutrients. This need for environmental modification to ameliorate stress levels for restoration agrees with the findings of other studies (Broome *et al.* 1975; Broome *et al.* 1986; McKee and Mendelssohn 1989; Wilsey *et al.* 1992).

Genotypic differences in stress response may be an important factor in evaluating causes of wetland deterioration. Such differences are also important in defining the role of plants in setting goals for restoring wetland function. Greater investment in studies of intraspecific variation would give invaluable insight into mechanisms determining relative stress tolerance, and into the potential role of genotypes in determining community structure and response to changing environmental conditions.

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Ms J.M. Lessmann
Wetland Biogeochemistry Institute
Louisiana State University
Baton Rouge, LA 70803
USA
Fax. 001 504 388 6820

Direct Line: (20) 4852 751
Direct Fax : (20) 4852 722

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Vita

Jeannine Lessmann was born on October 14, 1969 in Chattanooga, Tennessee. A highly mobile upbringing took her to five states before completing her bachelor of science degree in biology at University of Maryland, Baltimore County, in June 1991. The following year, she worked in coastal sciences with the National Museum of Natural History at the Smithsonian Institute in Washington, D.C. and Newfound Harbor Marine Institute in Big Pine Key, Florida. In June 1992, Jeannine began her doctoral program with Dr. Irving Mendelssohn in the Department of Oceanography and Coastal Sciences at Louisiana State University. Upon the completion of her doctoral program in January, 1998, she began a one-year Fulbright Fellowship conducting research in plant ecology with Dr. Hans Brix at the University of Aarhus in Aarhus, Denmark.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Jeannine Marie Lessmann

Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Intraspecific Variation in Three Marsh Grasses in Response to Increased Flooding and Salinity

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

12/12/97

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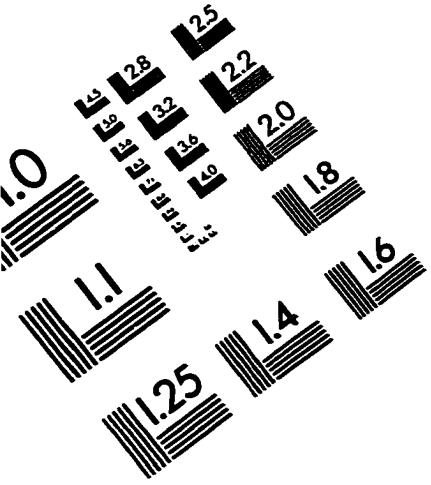

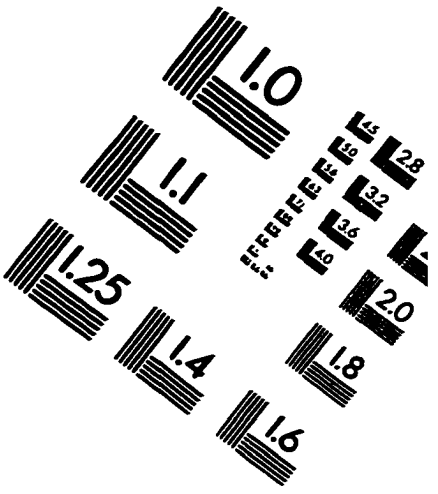

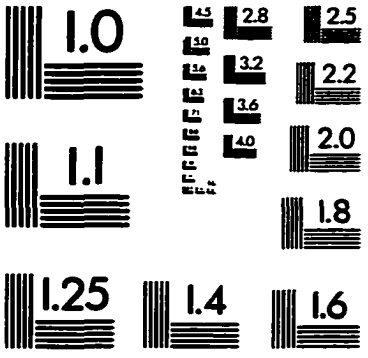

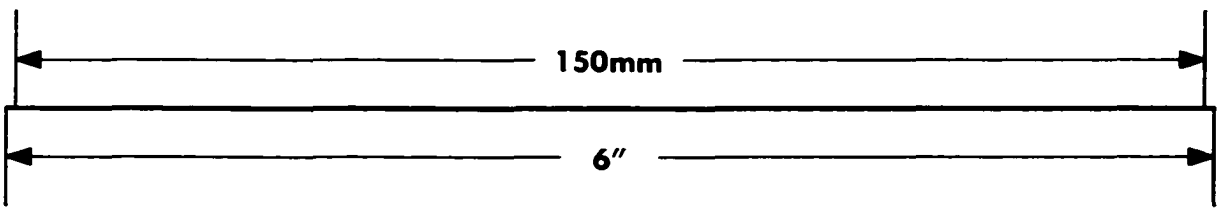

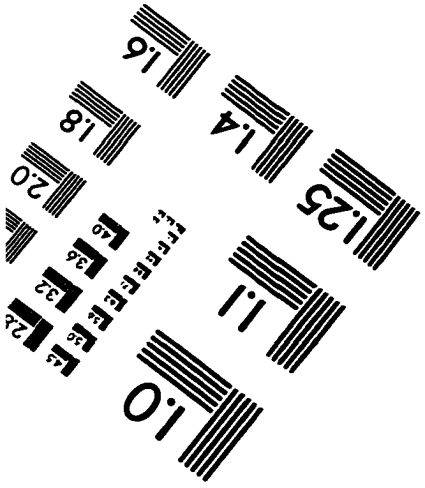

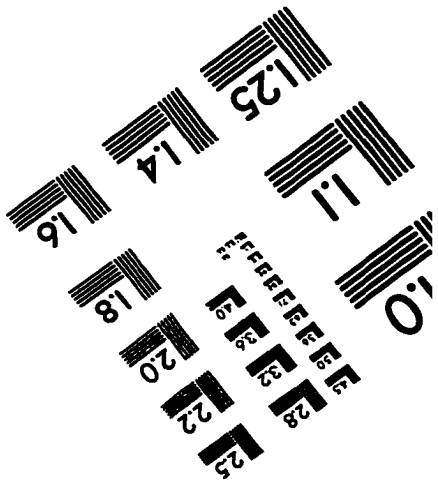

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