SOME EFFECTS OF NUTRIENT AND FLOODING STRESS MANIPULATION ON COASTAL LOUISIANA WETLAND VEGETATION

A Thesis

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James S. Ialeggio
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"Life in the delta South, where land is so dear a commodity, is inextricably tied to water. The certain rhythms of life that identify and characterize this region do not travel or translate well, for fed from the north, blown violently from the South, the passage of time in Louisiana reflects the whim and mood of water. The flooding of swollen spring washes away the sins of Mardi Gras, one year ruthlessly taking whole towns and the next benevolently granting crawfish season, followed by the true, vibrating heat to slowly lure in the hurricanes that drown suddenly from the South. Then, if allowed, we celebrate continued existence with redfish to see us through winter and round the corner, once more among the masks and brass bands, doing our best to make our Lenten seafood mean something. Continually bickering twins, land and water squabble over ownership of every square inch, and the ubiquity of this struggle colors all."

-Anna Michael

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ABSTRACT

Freshwater diversions are a relatively recently utilized tool in wetland loss mitigation that stimulate an organic accretion response in marsh vegetation, which is based in root production and thus belowground biomass. The effectiveness of freshwater diversions in slowing marsh loss probably varies across a gradient of the factors they supply: decreased salinity, increased nutrient concentrations, and increased inundation. Flooding stress is commonly thought to be the overriding factor limiting wetland vegetation growth, however its influence may vary across salinity and nutrients. Therefore, plugs of *Spartina patens* were planted in four "marsh organs" consisting each of 36 sediment-filled PVC pipes of incremental heights that created a range of growth elevation and thus flooding stress. The organs were located in mean salinities from 2.6-14.7 ppt and two of them fertilized at 22 g N m^{-2}, 11 g P m^{-2}, and 5.5 g K m^{-2}. Twice during the growing season below- and aboveground biomass was harvested, sorted, dried, weighed, and analyzed for relationships between variables. Flooding stress was found to be primary, with a secondary interactive effect between salinity and fertilization that suggests fertilization may help plants resist relatively higher salinity levels. Variations in salinity and nutrient concentrations associated with freshwater diversions, which can differ in intensity and extent, might also be linked to increased nutria herbivory. Therefore, nutria preference for fertilized versus unfertilized vegetation was also tested in a controlled setting on *Panicum hemitomon, Sagittaria lancifolia*, and *S. patens*, representative dominant plant species of fresh, fresh-brackish, and brackish marsh. Nutria showed a significant preference for fertilized vegetation within species. It is therefore possible that benefits derived from nutrient-rich fresh floodwater could potentially be negatively impacted by increased preferential herbivory.

GENERAL INTRODUCTION

Background

Coastal marshes provide valuable ecosystem services, protection from natural disasters, and vast opportunities for traditional livelihoods and recreation. Louisiana marshes experience the highest rate of land loss in the United States (CWPPRA 1994). Characterized by their impermanence (Coleman 1988), the wetlands of the Mississippi River delta are part of a dynamic system in which accretion (the creation of land, by multiple mechanisms) must actively occur in order to counter the ongoing natural subsidence of previously deposited alluvial mud (Nyman et al. 1993). Vertical accretion is maintained by two main processes. We define mineral accretion as the accumulation of matter through physical deposition of mineral sediment on the landscape and organic accretion as the accumulation of matter through the stimulation of plant growth, specifically belowground plant growth, by fresh water and associated nutrients (Nyman et al. 1990, 1993).

Defined in part by their self-regulating nature, coastal wetlands in an efficiently functioning state are eminently sustainable. Where marsh elevation is lower relative to sea-level, more frequent flooding introduces necessary limiting factors such as suspended sediments, fresh water and associated nutrients, and stimulates accretion through mineral deposition and abundant vegetation growth (Nyman et al. 1993). Where the elevation of the marsh is higher relative to sea level, flooding is less frequent, and accretion slows (Nyman et al. 1993). In the case of Mississippi River alluvial marshes, the source of necessary limiting factors for both mineral and organic accretion is the Mississippi River (Kesel et al. 1992). Although seasonal storms do provide a source of surge-driven sediment and nutrients (Reed 1989), much of Louisiana's

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wetlands are located inland, out of the direct influence of hurricanes, and are therefore reliant on the Mississippi River (Kesel et al. 1992). Traditionally, snow-melt far upriver coupled with seasonal rains caused the Mississippi to flood its banks in spring, depositing vast amounts of nutrient-rich sediments on outlying areas as well as quantities of fresh water which reduced salinity stress to vegetation growth in adjacent coastal plains (Kesel 1989).

However, over the course of the last century widespread human manipulation of historic hydrologic processes has not only increased the rate of land subsidence (Turner 2004), but also reduced the ability of the Mississippi River to meet the requirements for accretion in affected outlying wetlands (Coleman 1988). The amount of suspended sediment transported by river water is greatly reduced (Coleman 1988, Kesel 1989) and levees flanking the Mississippi River channel the remaining sediment out into the Gulf of Mexico instead of into outlying marshes (Britsch and Dunbar 1993, McFalls et al. 2010), which has subsequently reduced mineral accretion rates (Day and Templet 1989). Natural flooding has been rendered virtually nonexistent (Kesel 1988) which has reduced rates of organic accretion (Delaune et al. 2003), resulting in combined effect of widespread net land loss (Gagliano 1981). Combined with climate driven global sea-level rise, accretion is increasingly insufficient, as coastal land submergence potential subsequently grows (Rybczyk and Cahoon 2002).

Consequently, recent studies have focused on reconnecting the river to its floodplain to both actively create land and mitigate existing land loss. Given the reduced amounts of sediment contained by the Mississippi River (Kesel 1988), much research has focused on organic accretion and the possibility of maintaining surface elevation through vegetative growth (Bricker-Urso et al. 1989, Craft et al. 1990, 1993, Nyman et al. 1990, 1993, Neubauer 2008). Results suggest that accretion may benefit from increased fresh water and associated nutrients in river water

reintroductions (Nyman 1990, 1993), as reducing salinity stress may thereby stimulate vegetative growth through increased nutrient uptake and may slow wetland loss in areas where adequate mineral sediment is not available (Patrick and Delaune 1976, Foret 2001, Delaune et al 2003, 2005, Merino 2010). Belowground biomass is of particular important to organic accretion, as fibrous roots effectively trap available mineral sediment as well as increase relative elevation via vegetative growth (McCaffrey and Thomson 1980).

Although vertical accretion is also reliant on significant sediment input, evidence of organic accretion's role in maintaining wetlands is clear. In assessing certain remnant zones of deltaic formation that have not received Mississippi River sediment in hundreds of years, such as Barataria Bay, Terrebonne Bay and the Mississippi River birds-foot, it is evident that these specific wetlands have persisted in some form long since the river shifted course and localized sediment input ceased (Coleman 1988). Regardless of the sedimentary driving forces behind deltaic construction, as a river's sediment load naturally decreases over time, the disappearance of wetlands associated with that river is not necessarily instantaneous. Therefore, as the contributions of a river shift away from high loads of suspended sediments, the mechanism of marsh maintenance must also shift; even with greatly reduced sediment input, although the river/wetland system is not accreting enough via direct sediment accumulation to grow new land, it is in fact functioning to adequately counter subsidence via an organically governed system of accretion (McCaffrey and Thomson 1980, Hatton at al. 1983, Coleman 1988, Bricker-Urso et al. 1989, Nyman et al 1993).

Brackish marsh has the highest land loss rates of all marsh types in Louisiana (Foret 2001) and is consequently targeted by the most proposed hydrologic restoration projects in coastal regions (Foret 2001). It also represents the transition from fresh marsh, which is able to

regenerate vegetatively following disturbance (Van der Valk 1981), to salt marsh which lacks

that regenerative ability (Koch et al. 1990). *Spartina patens*, a dominant species in Atlantic and

Gulf coast brackish marshes, can survive a wide range of salinity (Visser et al. 2000, Merino et

al. 2010), and makes up over 50% of brackish marsh vegetation and 25% of total marsh

vegetation in Louisiana (Chabreck 1970). Its hardiness, wide salinity range, and prominence in

restoration proposals therefore make it an excellent candidate for wetland research related to

subsidence and organic accretion.

To more completely assess wetland health and viable means for restoration, we must also

examine another potential factor influencing marsh loss. Herbivory in Louisiana coastal

wetlands plays a large role in limiting marsh growth; muskrat *(Ondatra zibethicus)*, wild boar

(Sus scrofa), snow goose *(Chens caerulescens),* and swamp rabbit *(Sylvilagus sp.)* all feed on

vegetation, but the invasive nutria's *(Myocastor coypus)* ravenous diet accounts for a great bulk

of wetland plant loss (Carter et al. 1999, Baroch and Hafner 2002). The nutria negatively affects

fresh and brackish Louisiana marsh as it feeds almost entirely on vegetation, consuming 25% of

its body weight daily (Baroch and Hafner 2002). Such voracious and unchecked feeding habits

lead to loss of habitat for native species, increased subsidence due to denudation of organic

growth within the marsh, and the subsequent creation of open water (Carter et al. 1999, Baroch

and Hafner 2002, Jojola et al. 2005). Since the 1970s', the nutria numbers have ballooned within

the Gulf of Mexico region (Baroch and Hafner 2002, Jojola et al. 2005, 2006).

There is evidence that marsh herbivores such as nutria, muskrats, and snow geese prefer

lower salinity habitats over higher salinity habitats (Chabreck and Nyman 2005), although nutria

do feed on brackish marsh *S. patens* in certain circumstances (Wilsey et al. 1991). Studies also

show wetland herbivores prefer vegetation with higher nitrogen content over vegetation with

lower nitrogen content (Alisauskas et al. 1988, Wilsey et al. 1991). Thus, it is possible that

selective herbivory is likely to affect marsh similar in makeup to that which is produced by

proposed large-scale water reintroduction wetland loss mitigation projects, which effectively

provide a gradient of salinities and nutrients, converging on low salinity and high nutrient

concentrations at the diversion's source (Lane et al. 1999).

Research Objectives

 (1) To study the effect of limiting factors on plant growth, specifically to address the question:

how do flooding stress, salinity stress, and nutrient availability interact to affect the growth of

belowground biomass in *S. patens*?

(2) To test the hypothesis that nutria select vegetation of one of three representative species

(*Panicum hemitomon, Sagittaria lancifolia,* and *S. patens*) grown in higher nutrient

concentrations over vegetation within that species grown in lower nutrient concentrations.

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CHAPTER 1
EFFECTS OF FLOODING STRESS ACROSS NUTRIENT AND SALINITY LEVELS
ON BELOWGROUND BIOMASS OF *SPARTINA PATENS*

Introduction

The rate of land loss in coastal Louisiana is estimated at 42.9 km^2 yr^{-1} and is predicted to

continue (Couvillion et al 2013). The causes range widely; compaction of previously deposited

alluvial sediment leads to natural subsidence (Penland and Ramsey 1990), saltwater

encroachment reduces brackish marsh vegetation (Britsch and Dunbar 1993, Wilson 2004), and

river control through the modern levee system, first effectively employed following the flood of

1927, causes additional negative effects. For instance, the current levee system separates the

river from its floodplain and greatly diminishes the flow of river water through the surrounding

marsh (Kesel 1988, 1989, Mossa 1996). Reduced overbank flooding removes not only a historic

source of freshwater, but also associated valuable sediments and nutrients, which are instead

funneled offshore by flanking levees (Britsch and Dunbar 1993, McFalls et al 2010). Loss of

sediments and nutrients reduces vertical accretion, a process that would normally offset natural

wetland subsidence (Kennish 2001). By example, despite a controlled flow of only 30% of total

capacity, the relatively un-leveed Atchafalaya River delta is one of the few areas in coastal

Louisiana currently experiencing land growth despite constant natural subsidence (Randall and

Foote 2005, Ford and Nyman 2011).

The wetlands of the Mississippi River delta are part of a dynamic system in which

accretion must actively occur in order to counter the ongoing natural subsidence of previously

deposited alluvial mud (Nyman et al 1993). Accretion is defined as the vertical accumulation of

material on the wetland surface, measured using various defined markers (Callaway et al. 1996).

Vertical accretion is maintained by two main processes. We define mineral accretion as the accumulation of matter through physical deposition of sediment on the landscape and organic accretion as the accumulation of matter through the stimulation of plant growth, specifically belowground plant growth, by fresh water and associated nutrients (Nyman et al. 1990, 1993).

The role of mineral sediment in coastal marsh accretion has been widely studied (Baumann et al. 1984, Nyman et al. 1990, Reed 1990, Temmerman et al. 2004). Past research has described a system of vertical accretion in which mineral sediment is the primary limiting factor, and in situ restoration approaches have been proposed that identified and addressed that issue (Morris and Bowden 1986, Rybczyk and Cahoon 2002). However, research in many areas has shown that vertical accretion is more directly affected by the rate of production of organic matter by marsh plants (Hatton et al. 1983, Bricker-Urso 1989, Nyman et al. 1993, Turner 1997, Morris 2007). Turner et al. (2004) found that vegetative productivity, specifically belowground productivity, is vital to the maintenance of relative marsh elevation in Louisiana wetlands, while Nyman et al. (1993, 2006) reviewed the relative importance of organic matter and mineral matter to vertical accretion in various coastal Louisiana brackish and saline marsh sites, and found that variation in vertical accretion was significantly related to variation in organic accumulation rate. Similar data were reported in the Farm River salt marsh of Connecticut (McCaffrey and Thomson 1980), Louisiana's Barataria Bay (Hatton et al. 1983), Rhode Island's Narragansett Bay (Bricker-Urso et al. 1989), and the Bay of Fundy in Nova Scotia (Chmura and Hung 2004).

With the recent focus on the role of organic accretion in maintaining marsh elevation, there has been a subsequent emphasis on approximating and restoring some of the original connectivity between the Mississippi River and its floodplain by means of freshwater diversions (Lane et al. 1999, Lane et al. 2006, Hyfield et al. 2008). Freshwater diversions, which conduct

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water from the river into the surrounding marsh through manipulated channels, are increasingly used to slow wetland loss (Army US Corps of Engineers (USACE) and Louisiana Department of Natural Resources (LDNR) 2004). Given the highly manipulated form of the modern Mississippi river (Coleman 1988), it is assumed that freshwater diversions approximate what would be the most natural possible condition of the Mississippi River in its current state.

Freshwater diversions are increasingly employed in areas of brackish marsh (Foret 2001) where hydrological disruption has reduced freshwater inflow (Frazier 1967), increased salinity (Day et al. 1995), and shifted the brackish marsh zone further inland (Bristch and Dunbar 2004, Wilson 2004). Brackish marsh is a crucial zone in restoration, the demarcation between resilient freshwater marsh, able to re-establish vegetatively in the wake of disturbance (Van der Valk 1981), and fragile salt marsh, which is limited in that ability (Mendelssohn et al. 1981, Delaune et al. 1983, Morris and Dacey 1984). As opposed to sediment diversions, which draw from the lower part of the river's water column, introduce significant amounts of suspended sediment to affected marsh, and are intended to actively grow land (Allison and Meselhe 2010), freshwater diversions draw from the top of the water column and are designed primarily to stem saltwater intrusion and slow wetland loss (CWPPRA 2010). Therefore, the prevailing immediate effects of freshwater diversions are nutrient addition and salinity reduction (Lane 1999), both achieved through increased water flow and periodic flooding (Snedden et al. 2007).

Organic accretion, which is affected by restoring river-floodplain connectivity (Lane et al. 1999, Lane et al. 2006, Hyfield et al. 2008), is influenced by the effect of nutrient and salinity management on vegetation growth (McCaffrey and Thomson 1980, Nyman et al. 2006). Some research indicates that increasing nutrients negatively affects vegetation. For example, Swarzenski et al. (2008) found that the influx of nutrient-rich water did not increase standing

biomass, while Darby and Turner (2008) reported a decrease in the belowground biomass of 12 of 14 fertilized sites. However, Darby and Turner's (2008) experimental nutrient doses reached 372 g N m^{-2} and 425 g S m^{-2}, a much higher amount than the levels prescribed (14 g N $^{m-2}$) by agricultural rice standards (Salassi and Deliberto 2011), and S exists as the powerful phytotoxin sulfide in typically reduced wetland soils (Koch et al. 1990). The ability of high nutrient concentrations to reduce growth is also reported by Merino et al. (2010), who show that, at relatively lower salinities, biomass experienced the greatest increase at moderate fertilization levels and underwent a slight decrease from moderate to high nutrient levels.

In contrast, root proliferation within nutrient-rich environments has also been extensively documented (Valiela and Teal 1976, Drew 1979, Robinson 1994, Casper and Jackson 1997, Hodge 1998, Robinson 1999). As freshwater diversions create varying nutrient and salinity conditions in the marsh, much recent research has focused on the role of nutrients as limiting growth factors as related to salinity (Foret 2001, Delaune et al. 2003, 2005, Nyman et al. 2006, Crain 2007, Merino et al. 2010, Tobias et al. 2010). In some experiments, high salinity was the overriding factor limiting growth in the brackish marsh plant *Spartina patens*, as maximum experimental biomass occurred when plants were treated with high nutrient and low salinity concentrations (Foret 2001, Merino 2010). Foret (2001) found that vegetation that received nutrient additions in high salinity showed no significant effect, while Delaune et al. (2005) showed that the fertilizer+salinity treatment resulted in higher mean biomass than the control in *S. patens*.

Flooding stress may also be a significant limiting factor in the growth of wetland vegetation (Gleason and Zieman 1981, Gleason and Dunn 1982, Delaune 1987, Naidoo et al. 1992, Spalding and Hester 2007, Tobias et al. 2010). Webb et al. (1995) found that growth of *S.*

patens in Louisiana marshes was correlated to elevation and thus submergence, as opposed to salinity stress, while Broome et al. (1995) showed that *S. patens* was more productive in lower salinity, however at relatively higher elevations. Spalding and Hester (2007) reported that increases in flooding depth were detrimental to *S. patens.*

Thus, while salinity and nutrient have a demonstrated effect vegetation growth (Foret 2001, Delaune 2003, 2005, Merino 2010, Tobias 2010), interactions among flooding, salinity, and nutrients are unclear. Therefore, it is in the context of a freshwater diversion's influence and its purpose that we frame our research question. Do flooding stress, salinity stress, and nutrient availability interact to affect the growth of belowground biomass in *S. patens*?

Methods

We employed previously existing "marsh organs" to test six levels of flooding (Morris 2007) on the belowground biomass growth of *S. patens.* So named because they resemble a pipe organ, marsh organs are constructed by bolting together thirty-six 15.2 cm diameter PVC longitudinally for structural soundness. Pipes were cut to lengths of 122, 107, 91, 76, 61, and 46 cm. Each organ consists of six rows and six columns of pipes, such that "row one" was the tallest and least likely to be flooded, "row six" was the shortest and most likely to be flooded, and each column consisted of a set of pipes bracketing the entire range of elevations. Columns were identified "A" through "F", such that "A" was oriented toward the west and "F" to the east.

In the summer of 2007 organs were placed in four field sites, two at Marsh Island Wildlife Refuge ($29^0 34' 47$ N, $92^0 00' 40''$ W and $29^0 34' 42''$ N, $91^0 49' 29''$ W) and two at Rockefeller Wildlife Refuge ($29^0 37' 54''$ N, $92^0 38' 18''$ W and $29^0 37' 12''$ N, $92^0 34' 11''$ W),

chosen to represent a range of conditions experienced by *S. patens* in Louisiana coastal wetlands (Tobias 2010). Following Penfound and Hathaway's (1938) classification system, one organ was placed at each location in a saline area where the surrounding marsh was dominated by *Spartina alterniflora*, and the other organ was placed in an intermediate marsh where the surrounding marsh was largely dominated by *S. patens* (Tobias 2010). The two organs located at Marsh Island are within the Atchafalaya River watershed and therefore receive a high loading of riverine nutrients, while the two organs were located within Rockefeller Wildlife Refuge are out of the Atchafalaya basin and its associated influence.

Organs were set in shallow ponds and oriented such that the tallest pipes were to the north for maximum sunlight exposure to all pipes. Organs were set in soil so that the fourth row was approximately level with the surrounding marsh, resulting in the elevation of row 1 being approximately 46 cm above surrounding marsh and the elevation of row 6 being approximately 30 cm below local marsh (Tobias 2010). Travel to all field sites was accomplished by airboat.

Prior to the growing season, all pipes at each organ were cleared of any pre-existing vegetation and filled completely with sediment from the surrounding pond, such that organs pipes of different heights received different amounts of sediment. At the next visit to both the Rockefeller Refuge and Marsh Island sites (April 12, 2013 and April 19, 2013, respectively), all organ pipes were topped off with additional local mineral sediment and planted with approximate 10 culms each of *S. patens*, harvested from surrounding marsh. As past research had failed to detect a nutrient effect caused by the Atchafalaya River (Tobias 2010), all organ pipes at Marsh Island were fertilized with 2.00 grams of pulverized Forestry Suppliers (Jackson MS) 20-10-5 (total nitrogen, phosphate, potash) planting tablets, which gave concentrations of 22 g N m^{-2}, 11 g P m^{-2}, and 5.5 g K m^{-2}. Pulverized fertilizer was wrapped in nonreactive coffee

filters, sealed with a rubber band, and pushed approximately 10 cm into the sediment surface. All organ pipes at Rockefeller Refuge were treated with a control of identical amounts of Pavestone paving sand (Grapevine, TX) that had been washed in a 5% HCl/H_2O solution to remove any bound nutrients, packaged identically, and also placed at a depth of 10cm in the sediment.

Approximately halfway through the growing season, above- and belowground vegetation from half of each organ (columns A, C, and E) was harvested. The organs in Rockefeller Refuge were harvested on August 3, 2012, while the Marsh Island organs were harvested on August 7, 2012. Prior to vegetation harvest, distances from the top of each row of pipes to water level were recorded, as were distances of contained sediment to the top of the pipes. Hourly water level and salinity for the month preceding each harvest was obtained by accessing the closest Coastwide Monitoring Reference System (CRMS) sites (0523, 0530, 0608, 0610). Distances between the CRMS sites and the organs ranged from .2 - 6.9 km. Using field measurements in conjunction with data from the CRMS sites, a percentage of time flooded over the preceding month was calculated for each biomass sample retrieved. In describing water levels relative to vegetation samples, we use "flooded" to mean submerged.

As each entire core of above- and belowground biomass was removed from its pipe, the aboveground portion was removed with a sharp serrated saw and placed in a large plastic bag, and the belowground mass placed in a separate large plastic bag. After transport to Louisiana State University's School of Renewable Natural Resources, all samples were placed in a walk-in cooler to retard decomposition. This harvest process was then repeated for all remaining organ pipes (columns B, D, and F) at approximately the conclusion of the growing season; Rockefeller

Refuge organs were harvested for the second time on October 29, 2012 and the Marsh Island organs harvested on November 15, 2012.

Aboveground biomass was sorted by live or dead status. Aboveground vegetation that had any trace of living tissue, signified by green color, was termed "live aboveground" while aboveground vegetation that lacked any trace of living tissue was termed "dead aboveground". Live and dead aboveground vegetation was then sorted by species, using a dichotomous key. All categories were placed in separately labeled paper bags, dried to constant weight at 60° C, and weighed.

Belowground biomass was first washed with water to remove mud, in a 2 mm standard laboratory test sieve (Fisher Scientific no. 10). Following protocol from an earlier study, roots were considered clean when water passing through them ran clear (Tobias 2010). Once cleaned, roots were sorted into live and dead categories. "Live roots" consisted of roots that were turgid, had attached root hairs, and were light orange to white in color (Tobias 2010). "Dead roots" consisted of roots that had no root hairs, were easily crushed, and grey in color (Tobias 2010). Because of difficulty in identification, live roots were not sorted by species. Live samples were dried to a constant weight at 60^{0} C and then weighed using an Ohaus Adventurer Pro (Parsippany NJ) scale to the nearest 0.01 g. Belowground and aboveground samples of living *S. patens* were macerated and sent to the Louisiana State University Agronomy Department for elemental testing.

The data were tested for associations among elemental levels, belowground and aboveground biomass, and flooding stress as quantified by percent time flooded. Initial analysis of residuals in the raw data indicated that the biomass and elemental data were not normally

distributed, after which a log transformation was used to greatly improved normality. Using PROC GLMSELECT, full mixed effect models were reduced to the most parsimonious version through stepwise model selection, which was verified through forward and backward model selection. All analyses were conducted using the Statistical Analysis System (Cary, NC).

Results

At the Marsh Island organs, mean salinity at the relatively fresh site for the month preceding the second harvest was 11.3 ppt (1.5), while the corresponding mean salinity for the more saline site was 13.8 ppt (0.5). At the unfertilized organs in Rockefeller Refuge, the mean salinity of the relatively fresh site for the month preceding the second harvest was 2.6 ppt (0.5), while the corresponding mean salinity for the more saline site was 14.7 ppt (6.4).

Total mean belowground biomass measurements ranged from 30-52 g (Table 1). The log transformed dependent variable belowground biomass was significantly and negatively affected by percent flooding ($P<0.0001$, Fig. 1). With increasing percentage of time flooded over the preceding month, belowground biomass correspondingly decreased. This trend persisted across harvests, and did not vary between fertilization regimes. Interactions between flooding and fertilization, as well as between flooding and salinity, were not significant.

TABLE 1. Below- and aboveground biomass measurements from all organs, presented in grams followed by standard deviations

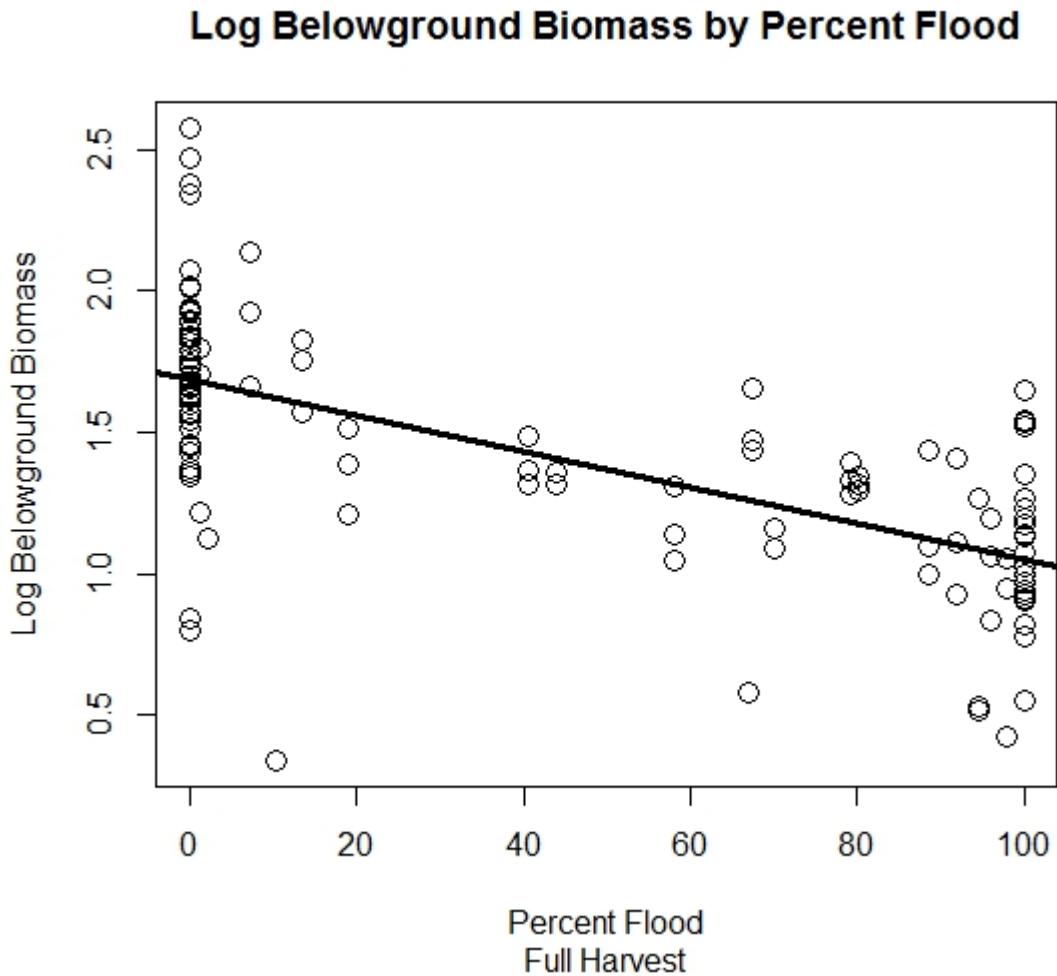| | Marsh Island | | Rockefeller Refuge | |
	Fresh	Brackish	Fresh	Brackish
Belowground	29.87 (26.29)	46.48 (72.44)	37.92 (40.73)	52.15 (60.65)
Aboveground	21.32 (16.30)*	30.37 (26.58)*	24.90 (20.10)	27.11 (25.27)

*First harvest only

FIGURE 1. Response of log transformed belowground biomass to percent flooding over the month preceding harvest; both harvests.

Belowground biomass also was significantly affected by an interaction between salinity and fertilization ($P<0.0001$, Fig. 2). Fertilized belowground biomass was greater than

unfertilized belowground biomass, with a greater biomass difference between fertilization

regimes at higher salinities. This overall trend persisted across harvests, although the effect was
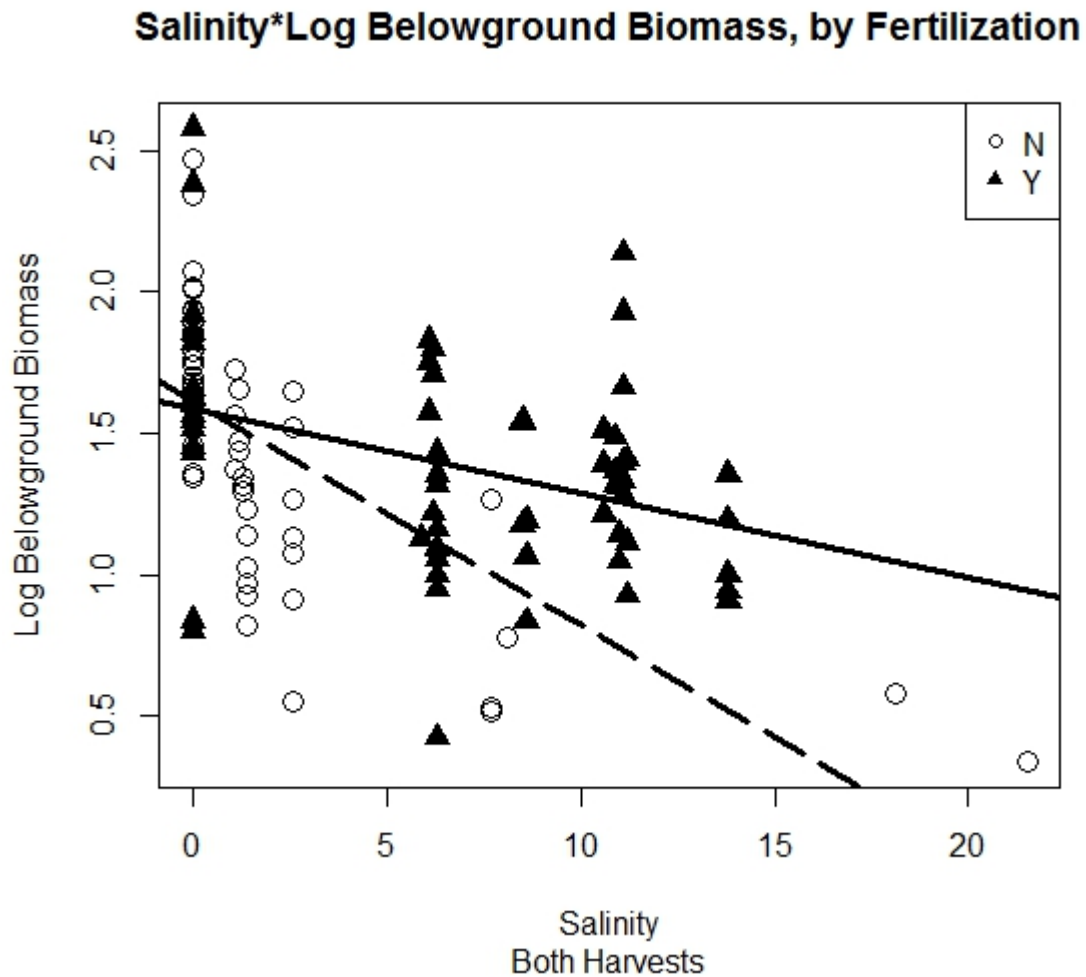
greater at the first harvest.

FIGURE 2. Response of log transformed belowground biomass to change in salinity, plotted by
fertilization regimes; both harvests.

Total mean aboveground biomass measurements ranged from 21 to 30 g (Table 1).

Aboveground biomass showed a three-way interaction between salinity, percent flooding, and

fertilization (P<0.0001). At the first harvest, aboveground biomass showed the same interaction of salinity and fertilization as belowground biomass; fertilized samples had greater biomass at higher salinities, with a greater effect between fertilization regimes shown at higher salinities (Fig. 3). By the second harvest, the aboveground vegetation of the fresher Marsh Island organ was eaten by muskrat; consequently there is no corresponding aboveground data for the second harvest at this site.
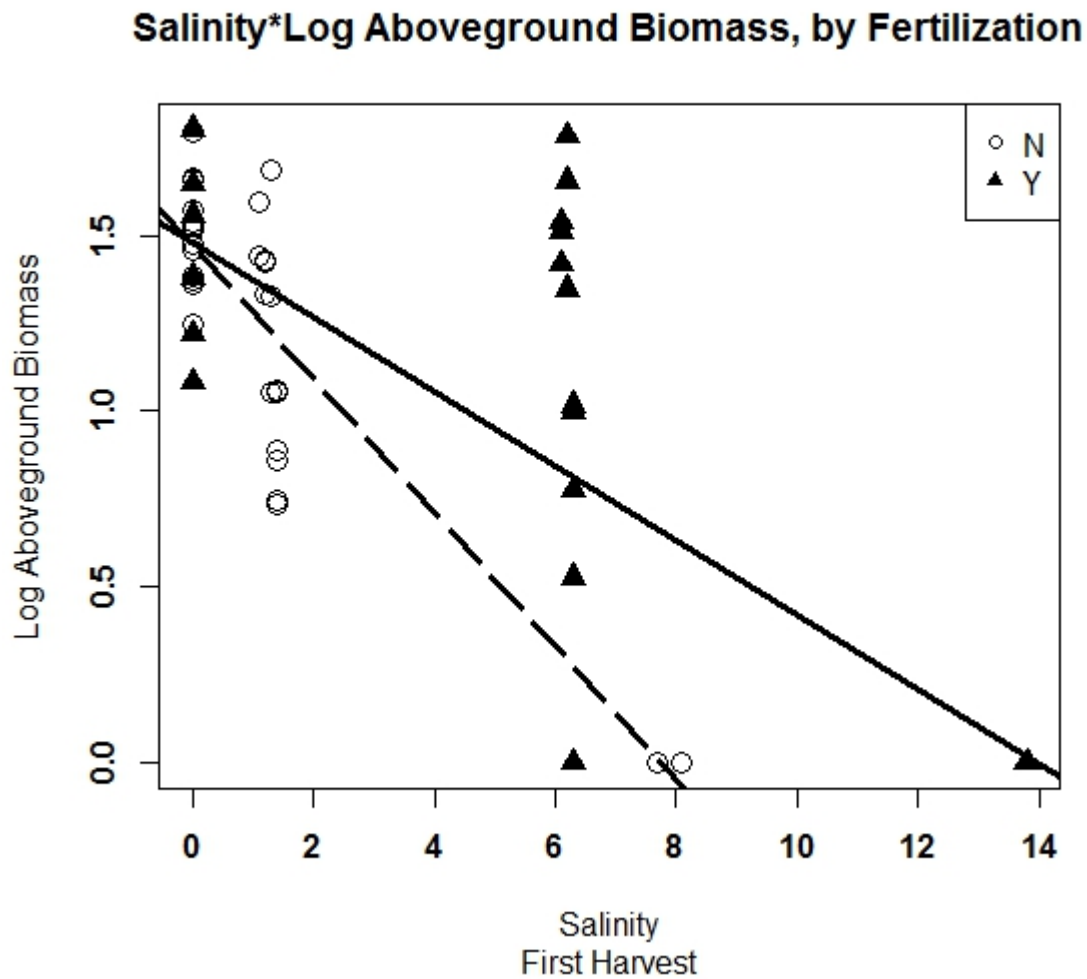

FIGURE 3. Response of log transformed aboveground biomass to change in salinity, plotted by fertilization regimes; first harvest.

Belowground C:N was reduced by fertilization (P<.0001, Fig. 4). Throughout both harvests, belowground C:N in organs at fertilized sites was significantly lower than the unfertilized sites, and was not significantly affected by salinity. Total mean C:N for fertilized belowground biomass was 44 (12.6), while total mean C:N for unfertilized belowground was 57 (13.0).
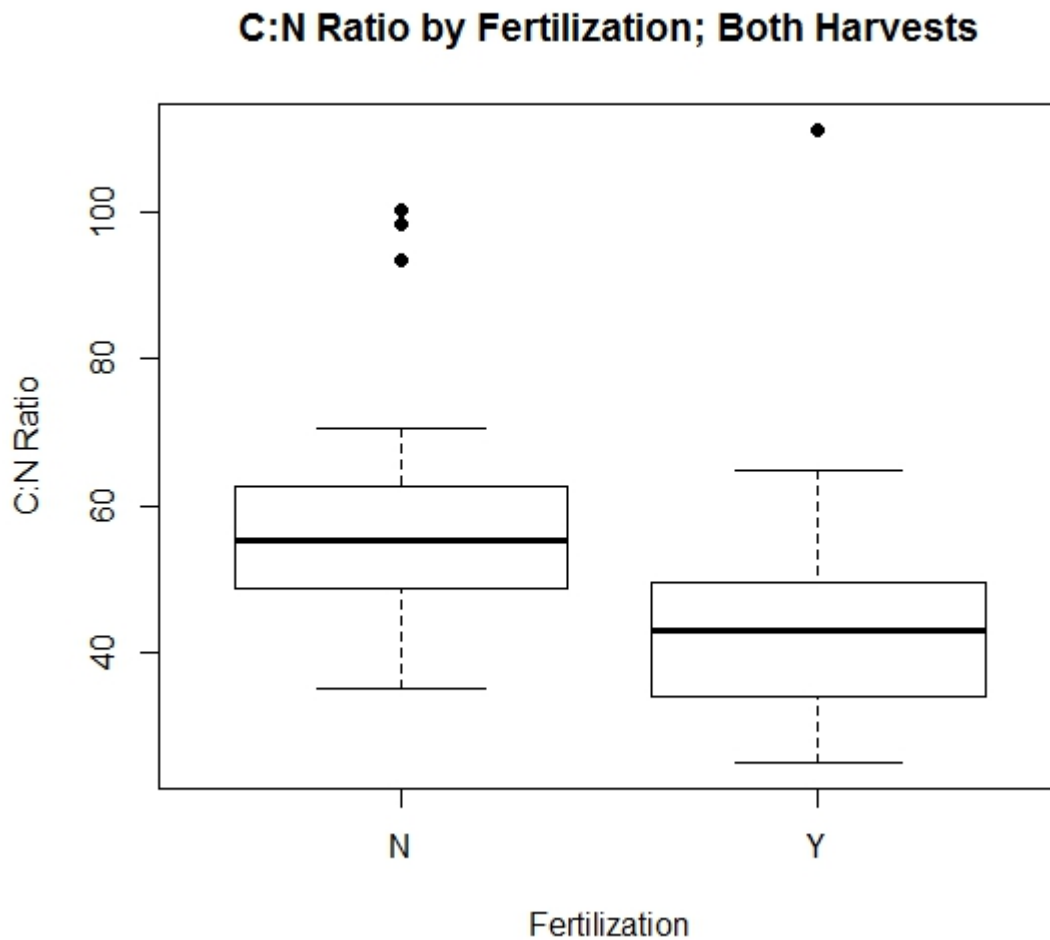

FIGURE 4. Response of belowground C:N to fertilization regimes; both harvests.

Belowground Na content showed a significant salinity by fertilization interaction (Fig. 5). Fertilized and unfertilized belowground biomass contained more Na at the more saline organs

than the more fresh organs. Fertilized belowground biomass also contained significantly higher

levels of Na (P<0.0001) than unfertilized samples, with the difference increasing with salinity.

This trend persisted across harvests, with a difference in fertilization regimes being more

pronounced in the second harvest. Total mean belowground Na (ppm) for fertilized samples was

5,086 (8,254), while total mean Na for unfertilized samples was 3,906 (6,998).
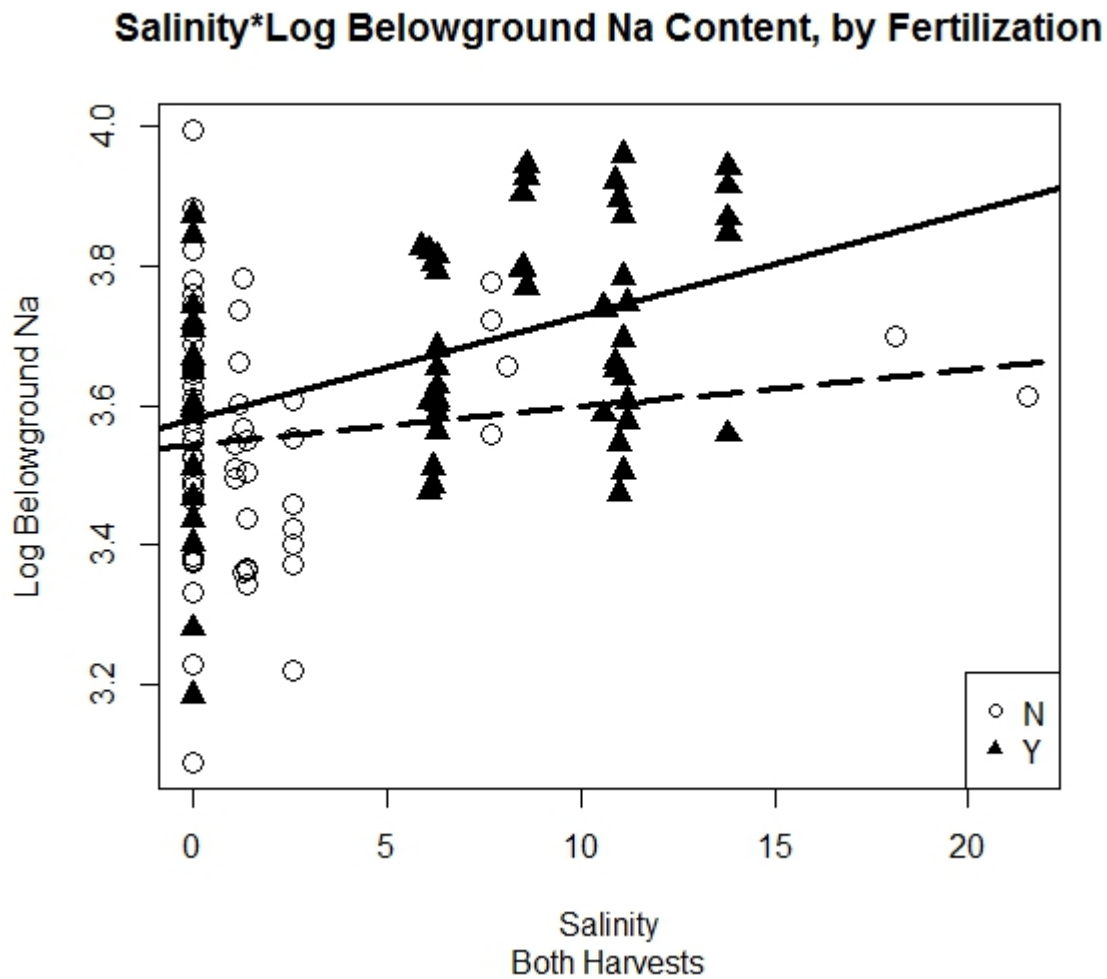

FIGURE 5. Response of log transformed belowground Na content to salinity, plotted by fertilization regimes; both harvests.

Fertilized aboveground biomass samples contained significantly higher levels of Na (P<0.0001) than unfertilized samples, with this difference increasing with a corresponding increase in salinity. Total mean aboveground Na (ppm) for fertilized samples was 11,296 (10,110), while total mean Na for unfertilized samples was 8,900 (6,578). Throughout both harvests, C:N in organs at fertilized sites was significantly lower than the unfertilized sites, and was not significantly affected by salinity. Total mean aboveground C:N for fertilized samples was 55 (24), while total mean aboveground C:N for unfertilized samples was 65 (18).

Discussion

Vegetation studies located on the Gulf of Mexico have found that the productivity of wetland vegetation decreases with increased flooding stress (Delaune 1987, Spalding and Hester 2007, Darby and Turner 2008, Tobias et al. 2010). Our results agree with such previous research, indicating a decreasing trend in belowground biomass with increasing percentage of time flooded. Other research, for example that of Morris et al. (1990), has shown that in a macro-tidal Atlantic tidal system, increased and intermittent flooding increased primary and secondary productivity, as it reduces hypersaline accumulation to oceanic levels. Our research found no interaction between percentage of time flooded with either fertilization or salinity, suggesting that within our experiment flooding influence was of primary importance, independent across all organs, and likely caused similar waterlogging stresses of vegetation as reported by Delaune et al. (1983). Tobias (2010) likewise concluded that flooding stress operated independently from salinity stress and nutrient availability.

Secondary to the primary influence of flooding stress was an interactive effect between fertilization and salinity. Fertilized belowground biomass was greater than unfertilized belowground biomass, and the difference increased as salinity increased. This interaction potentially indicates that fertilized *S. patens* belowground biomass may be affected by salinity differently than unfertilized belowground biomass (Fig. 2, Fig. 3). Previous research has also noted the interaction of salinity and fertilization. In reporting the interactive effects of nutrients and salinity on *S. patens,* Merino et al. (2010) employed 4 levels of nutrients, 4 levels of salinity (the highest of which was stressful at 36 ppt) and illustrated (Fig.1) that only the highest non-stressfull salinity (18 ppt) showed a steadily increasing trend in mean biomass from low to high nutrient concentrations, while lower salinities (2 ppt and 6 ppt) showed declining trends in mean biomass from medium to high nutrient treatments. Crain (2007) also reported that with N addition alone biomass more than doubled at higher salinities. In other words, it appeared that high nutrient levels were not harmful to vegetation already stressed by high salinity in the way they were to vegetation with greater growth potential because of lower salinity stress; fertilization appeared to affect vegetation at different salinities differently.

While past studies corroborate a positive relationship between N-addition and increased biomass (Patrick and Delaune 1976, Foret 2001, Delaune et al 2003, 2005, Merino 2010), extreme levels of fertilization appear to be deleterious to the production of biomass (Darby and Turner 2008, Merino et al 2010). While our results do show an overall slowing of production with increasing salinity, one interpretation is that increasing nutrient availability enabled vegetation to better withstand higher salinities. Correspondingly, our mean fertilized belowground biomass totals were significantly higher than our unfertilized belowground biomass

totals, both in relatively higher and lower salinities and across both harvests, with the difference between nutrient treatments increasing as salinity increases.

Theoretical support for these findings may lie in the cellular mechanisms for osmoregulation within plant tissue. The movement of water and therefore nutrients within a plant follows a system of water pressure potential (Jefferies 1981). Pure water has a potential of zero, while seawater, for example, has a lower potential of -25 bars (Jefferies 1981). Following the laws of osmosis, a substance flows from an area of high pressure to an area of lower pressure; therefore, to effect an upward flow of nutrients and water in a saline environment, vegetation must manufacture increasingly lower (i.e. more negative) water potential in each progressively higher cell. This is achieved by incorporating small amounts of dissolved salt solids into cells, where these ions contribute to lowering the water potential of the cell as a whole and maintaining the necessary overall pressure gradient within the plant (Jefferies 1981). As they would otherwise damage internal cell structure and disrupt metabolic processes, ionic compounds are sequestered within the cell vacuole (Cavalieri 1981). To counter the resulting osmotic potential within the cell, compatible osmolytes are produced in the cytoplasm (Boscaiu 2013), the most common of which is the nitrogen-based free amino acid proline (Cavalieri 1981, Hester 1994), which maintain cellular equilibrium while also serving to directly stabilize cellular organelles under dehydrated conditions (Cavalieri 1981, Jefferies 1981, Li et al. 2013).

Research has shown that with increasing salinity, the corresponding plant demand for available internal N to maintain growth also increases (Bradley and Morris 1992). The presence of lower C:N ratios in both fertilized organs indicates increased N uptake at those sites, and therefore an underlying relative N limitation across salinities. Although it is a common perception that more saline marsh conditions are N-limited (Valiela and Teal 1976, Jefferies and

Perkins 1977, Osgood and Zieman 1998, van Wijnen and Bakker 1999), this limitation may also stem from increased needs of the vegetation itself of N for maintenance of growth in increased salinity (Jefferies 1981, Crain 2007), as the necessary production of proline possibly creates an additional cellular N demand. Similarly, Crain (2007) found that *S. patens* N content declined in fresher marshes, where it should have theoretically been easier to acquire, suggesting that N tissue content may be driven more by internal N requirements than by N availability. Proline levels have been shown to be correlated positively with increasing salinity (Jampeetong and Brix 2009, Naidoo et al. 2012, Baciau et al 2013, Li et al. 2013), increasing growth (Cavalieri 1981), and Na content (Jampeetong; Fig. 3; b,f), potentially due to an increased ability of plants cells to sequester more Na ions with increased levels of proline.

While other research has detected an interactive effect of salinity and fertilization as mentioned earlier, it should be noted that those overall trends diverged from ours. For example, Merino et al. (2010) found that the difference between biomass of fertilized and unfertilized samples decreased with increasing salinity. Differences in salinity among our sites were not as anticipated; initially, relatively fresh and saline organ sites were chosen based upon Penfound and Hathaway's (1938) classification of marsh type by predominant vegetation. However, the Gulf of Mexico coast is defined in part by wind-driven and therefore sometimes extremely prolonged tides (Reid 1954), as well as relatively small land spatial variation (Childers and Day 1990), which can greatly affect the relative salinity of a specific location (Morey et al. 2003). Consequently, for the month preceding the second harvest at the fertilized Marsh Island site, the mean salinity of the relatively fresh site differed from the corresponding mean of the relatively saline site by less than 3 ppt. Therefore relatively higher salinities were potentially underrepresented in assessing the production of biomass at our unfertilized sites, while relatively

lower salinities were potentially underrepresented in assessing the production of biomass at our fertilized sites. For this reason we did not use salinity as a categorical variable and instead analyzed continuous data. Therefore, it is possible that the apparent effect of the fertilization on vegetation at higher salinities is a reflection of these underrepresentations.

As previously noted, the first harvest of aboveground biomass showed the same trend as the overall belowground trend. We believe this trend failed to continue through the second harvest because of a muskrat eat-out that included all but two pipes of aboveground biomass in our fertilized, low salinity organ. We attribute this to previous research which shows that mammalian wetland herbivores prefer fertilized, lower salinity vegetation (Ialeggio and Nyman, in press). We also contend that the correlation of production trends between above- and belowground biomass shown by Gross et al. (1991) paired with our mean first harvest aboveground biomass figures suggest that the first aboveground harvest is probably representative of a predominant trend across harvests.

We believe our results should be interpreted cautiously. We did not test levels of any osmolytes in our sample vegetation, and therefore cannot directly correlate them to our observed data. We also failed to collect accurate oxidation-reduction data, which because Eh levels can affect nitrate reduction and therefore N availability (Patrick et al 1974), as well as the solubility of substances available for uptake (Gambrell and Patrick 1978), could have greatly informed our analysis. It should also be noted that in light of the effects of freshwater diversions, it is relatively unlikely that an increase of nutrients would not be accompanied by a reduction in salinity (Lane 1999), in which case previous studies showing the effect of elevated nutrient levels on low salinity vegetation would be more informative. However, we observed the presence of fertilized *S. patens* which showed lower C:N ratios and thus less N limitation,

produced more below- and aboveground biomass at higher relative salinities as compared to

unfertilized samples, and greater plant tissue concentration of Na than unfertilized vegetation.

This leads us to conclude that we may potentially approach our observed data as part of a known

system in which fertilization resulted in greater N-limited osmolyte production, thereby lowering

cellular water potentials and allowing for improved vegetative growth in higher salinities

(Cavalieri, 1981).

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CHAPTER 2
NUTRIA GRAZING PREFERENCE AS A FUNCTION OF FERTILIZATION

Introduction

Since their introduction to Louisiana in the 1930s, nutria have significantly affected coastal

vegetation (Shaffer et al. 1992, Grace and Ford 1996, Evers et al.1998, Shaffer et al. 2009).

They are voracious grazers (Baroch and Hafner 2002) and reports of private and public land

damages are widespread (LDWF 2007). Nutria populations in coastal Louisiana are limited

neither by the seasonal droughts or extended frosts of their indigenous South American steppe

habitat (Ehrlich 1967), nor constrained by a fixed breeding season (Atwood 1950).

Consequently, the species is capable of extremely rapid population growth and by the early

1950s the statewide population estimate was 20 million animals (Carter et al. 1999, Baroch and

Hafner 2002, Jojola et al. 2005, LDWF 2007). Although a viable commercial trapping industry

historically took advantage of large nutria populations, by the 1980s pelt prices were greatly

reduced and trapping diminished (Jojola et al. 2005). Coastal land damage increased as nutria

populations exploded in the wake of weakened controlling pressures, with an aerial survey in

2001 estimating more than 83,000 acres damaged by nutria (Marx et al. 2003, Jojola et al. 2005).

In 2002, the Louisiana Department of Wildlife and Fisheries instituted the Coastwide Nutria

Control Program (CNCP) (Marx et al. 2003, Jojola et al. 2005, Dedah et al. 2010), a

geographically targeted harvest incentive program, offering US$4/nutria tail (US$5/tail

beginning in 2006) (Dedah et al. 2010). Within 5 years of the CNCP implementation, herbivory

damage decreased by 50% statewide and annual nutria harvests increased from 24,683 to

375,683 animals (Scarborough and Mouton 2007); after the 2011-2012 trapping season, 354,354

nutria were harvested and vegetation surveys found affected coastal marsh reduced to 1,129 acres (Hogue and Mouton 2012).

Robust wetland systems are vital to the sustainability of coastal human populations because they provide habitat to numerous wildlife and plant species, support an economically valuable recreation industry, and render important ecological functions such as storm surge protection and wastewater filtration (Day et al. 1995, Spalding and Hester 2007). Although the CNCP has increased control over herbivory and its associated wetland destruction, marsh loss is estimated at 42.9 km^2 yr^{-1} and predicted to continue (Couvillion et al. 2013), thus remaining an important issue in coastal Louisiana. Therefore it is of interest that certain wetland management practices may affect vegetation palatability to herbivores, thus altering risk of herbivory.

For example, there currently is an increasing emphasis on restoration methods which include re-establishing connectivity between the Mississippi River and its floodplain by diverting water from the river into the surrounding marsh (Lane et al. 1999, Lane et al. 2006, Hyfield et al. 2008). In addition to beneficial fresh water and mineral sediments, Mississippi River water also contains nutrients from such anthropogenic sources as upstream agricultural runoff of animal manure, eroded soils, pesticides and fertilizers (Goolsby et al. 1999). Although levels of suspended nutrients are relatively low, high river volume transports a correspondingly high nutrient load to affected wetlands. For example, while nitrate levels passing through the Caernarvon diversion into Breton Sound range from 1-2 mg N L^{-1}, maximum allowable flow is 280 m ^{3} s^{-1}, and yearly total N loads are approximately 10 g N m^{-2} yr^{-1} (Lane et al. 1999, 2004, Mitsch et al. 2005).

Many emergent wetlands offset local subsidence and global sea-level rise with vertical accretion via vegetative production (Nyman et al. 2006), and vegetative production in wetland systems may be positively affected by nutrient input and lowered salinity (Merino et al. 2010, Morris et al. 2013). However, there also is evidence that marsh herbivores prefer lower salinity habitats over higher salinity habitats (Nyman et al. 1993) and that wetland herbivores prefer vegetation with higher nitrogen content over vegetation with lower nitrogen content (Alisauskas et al. 1988). Thus, herbivory may have a greater effect on marshes within the low salinity, nutrient-rich end of the spectrum.

Wetlands directly northwest of Lake Cataouatche, Louisiana, have received nutrient-rich Mississippi River water via the Davis Pond diversion since 2002, and Four Mile Marsh, within the Joyce Wildlife Management Area, has been the site of secondarily treated municipal effluent from the city of Hammond, Louisiana, since fall 2006. Both sites are examples of nutrient enriched wetlands that support nutria populations which have affected surrounding marsh since nutrient inputs began (Lundberg et al. 2011, Baker, personal communication 2012). Consequently, we sought to test the hypothesis that nutria selected vegetation within a species grown in higher nutrient concentrations over vegetation within that species grown in lower nutrient concentrations. The *ex situ* implication of this research is that if the presence of high nutrient levels potentially influences the feeding habits of nutria, it is then possible that increased herbivory may in turn degrade wetlands most affected by river diversions and assimilation wetlands.

Methods

We collected *Panicum hemitomon* Schult., *Sagittaria lancifolia* L., and *Spartina patens* (Aiton) Muhl, plants that are all identified as dominant species within their marsh type, eaten by nutria in the wild and representative of nutria habitat across a salinity range. *P*. *hemitomon*, which historically made up over 25% of freshwater marsh vegetation and 9% of total Louisiana wetlands (Chabreck 1970), was grazed considerably by nutria in exclosures in Terrebonne Parish (Holm et al. 2011). We gathered it from an unnamed freshwater marsh outside Houma, LA. *S*. *lancifolia* is the most widespread fresh-brackish marsh plant in the northern Gulf of Mexico, can constitute a large part of nutria diet (Linscombe et al. 1997) and was gathered along the northern shore of Lake Cataouache, east of the Davis Pond diversion outflow and outside of its influence. *S*. *patens*, widely dominant in brackish marshes within the brackish marsh salinity limit of nutria habitat (Baroch and Hafner 2002, Jojola et al. 2005), is palatable to nutria under certain circumstances (Chabreck 1981, Wilsey and Chabreck 1991) and was gathered within Rockefeller Wildlife Refuge in Chenier, LA.

Plant samples were transported to the Louisiana State University greenhouse, where they were transplanted immediately into 15 cm, open-flow plastic pots using landscaping sand substrate. Potted plant samples were placed within 6 individual 2 m diameter plastic wading pools, such that each pool contained 30 pots, and each plant species occupied two pools. In determining appropriate nutrient fertilization rates, it was decided that the importance of maximizing difference in treatments outweighed that of approximating previously established loading rates. One pool of each plant species was then fertilized at 0.9 g N m^{-2} d^{-1} with Miracle-Gro All Purpose Plant Food (Scotts Miracle-Gro Products, Marysville, OH) for a period of 68 d with a final total loading of 61.9 g N m^{-2}, resulting in 30 fertilized and 30 non-fertilized samples

of each species. Samples were observed daily for yellowing leaves, browning of leaf margins, excessive algal blooms, or other indications that nutrient concentrations were approaching toxic levels. We temporarily suspended nutrient additions when nutrients appeared to be causing toxicity. We supplied all pools with municipally sourced fresh water, maintained at just below sediment level and constantly circulated by Mini-jet 404 submersible pumps (Marineland United Pet Group, Blacksburg, VA) to ensure homogeneity of nutrient concentrations.

From 10 June 2010 to 27 June 2012, seven adult nutria were live-trapped in the artificial ponds of the Louisiana State University aquaculture center with 3 Havahart large one-door easy set traps (Woodstream Corporation Lititz, PA) using sweet potato fragments as bait in obviously nutria-grazed areas. The nutria were housed in two 4 m x 2.5 m pens in the Louisiana State University Veterinary Medicine Center and fed a diet of sweet potatoes and commercial rodent chow by employees of the Veterinary Medicine Center. Individual nutria were identified by colored plastic neck collars and medical attention was administered on an as-needed basis.

Feeding trials were conducted nightly when nutria are believed to feed most often. Individual plant-pot samples were removed from growing pools 24 h prior to the trial and weighed 24, 12 and 0 h before the trial to account for water loss. One fertilized and non-fertilized sample of that particular trial's target species was placed in a 4 m x 2.5 m trial pen, the 15 cm pots set 0.5 m away from three walls and each other at the end of the trial pen. The 15 cm pots were set within standard concrete blocks to prevent overturning but still allow nutria to mimic natural foraging. An individual nutria was then removed from the holding pens, introduced into the trial pen and left overnight for a period of 12 h, after which the plant-pot samples were immediately weighed again. The nutria were not starved before the trials, and the location of the fertilized and unfertilized pots was alternated randomly before each trial to

minimize habitual feeding. Pre- and post-trial plant mass was used as an indicator of damage; actual material ingested was not measured because potential marsh damage could include indirect vegetative destruction as well as consumption. Pre- and post-trial plant mass loss was calculated as percent mass lost in an effort to reduce bias; for example, larger plants might lose more raw mass than smaller plants. One nutria died in captivity after only two trials, and those trials were discarded. Five remaining nutria were each offered a choice of fertilized and unfertilized samples of each plant species 3 times over 54 d, from July 27 to September 21, 2012. One remaining nutria was offered the same choice of fertilized and unfertilized *S. lancifolia* and *S. patens* over the same time period but not *P. hemitomon* because by this time, our *P. hemitomon* samples had become senescent and no longer useable.

Initial analysis of residuals in the raw data indicated that the data were not normally distributed, which we attribute to a small sample size. A log transformation greatly improved normality, and we then modeled percent plant loss as a function of the fixed effects of fertilization and plant species, measured in percent mass lost over the course of each trial, analyzed as a two-way ANOVA using nutria as a random effect in PROC MIXED (SAS Institute, Inc., Cary, NC). Results were then back-transformed and presented with the 95% lower confidence interval (LCI) and upper confidence interval (UCI) in parentheses. All research was conducted in accordance with Animal Welfare Act regulations, the United States Department of Agriculture, Animal and Plant Health Inspection Service, and the Louisiana State University Agricultural Center. The Louisiana State University Division of Laboratory Animal Medicine's Institutional Animal Care and Use Committee approved the study protocols, under which the animals were ultimately euthanized.

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Results

Fertilization had a significant effect on the percent mass of vegetation lost through nutria herbivory ($F_{1,4} = 36.8$, $P<0.0001$) in all plant species (Fig. 1). Across the three plant species, fertilized vegetation experienced an overall mean mass loss of 79.4% (LCI 63.1, UCI 100.0), as compared to 9.3% (LCI 7.4, UCI 11.7) in unfertilized vegetation. The mean carbon content of fertilized plant samples was 42.9% (0.6), the mean nitrogen content was 2.2% (0.2), and the mean C:N was 19.8. The mean carbon content of unfertilized plant samples was 44.4% (0.3), the mean nitrogen content was 0.91% (0.1), and the mean C:N was 48.8.
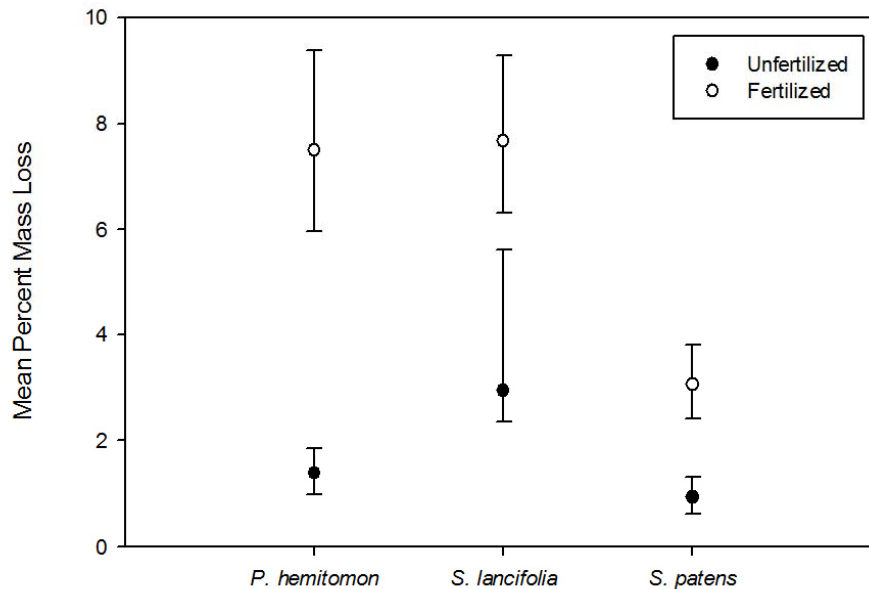

FIGURE 1.   Mean percent mass loss of fertilized and unfertilized plant samples with one standard error of the mean, plotted by species.

Species also had a significant effect on the percent mass of vegetation consumed by nutria ($F_{2,3}$ = 9.5, P = 0.0008). The mean percent mass lost by *S. patens* was significantly less than the other two species (Fig. 1). *S. lancifolia*, which had a mean C:N of 18.0, experienced the greatest consumption with a mean mass loss of 50% (LCI 39.8, UCI 63.1). This was followed by *P. hemitomon*, which had a mean C:N of 35.3 and had a mean mass loss of 31.6% (LCI 25.1, UCI 39.8). *S. patens*, which had a mean C:N of 41.1, experienced the least reduction and had a mean mass loss of 10% (LCI 7.9, UCI 12.6). In our fertilized samples, nutria did not greatly differentiate between *S. lancifolia* and *P. hemitomon*, but there was significant difference in percent mass lost between these species and *S. patens*. Nutria consumed more *S. lancifolia* and *P. hemitomon* than fertilized *S. patens*, even as fertilization lowered the mean C:N of *S. patens* to slightly below that of *P. hemitomon*. As the mean C:N was quite close in fertilized samples of *P. hemitomon* and *S. patens* (25.3 and 24.8, respectively), the distinction between the two species may lie in N:P ratios of 7.4 and 5.7, respectively (Table 1).

In the log transformed model, the species-fertilization interaction term was not significant ($F_{2,3}$ = 1.5, P = 0.24). In non-transformed raw data, fertilized *P. hemitomon* was reduced by a mean of 11.1% (3.4) and unfertilized by 0.9% (3.0). Fertilized *S. lancifolia* was reduced by a mean of 12.0% (2.8) and unfertilized was reduced by 3.0% (2.7). Fertilized *S. patens* was reduced by 5.8% (2.9) and unfertilized was reduced by 0.19% (3.1). Order of preference of plants consumed, in both fertilized and unfertilized samples, was *S. lancifolia*, *P. hemitomon*, and *S. patens*.

TABLE 1.   Mean nutrient concentrations of fertilized and unfertilized plant species reported in percent content of carbon, nitrogen, and phosphorous with respective sample standard deviations of the mean included in parentheses.

	Fertilized					Unfertilized				
Species	**C**	**N**	**P**	**C:N**	**N:P**	**C**	**N**	**P**	**C:N**	**N:P**
P. hemitomon	44.98% (1.26)	1.78% (.69)	.24% (.06)	25.27	7.42	44.7% (.87)	.75% (.17)	.09% (.03)	59.60	8.33
S. lancifolia	38.23% (1.11)	2.88% (.53)	.51% (.08)	13.27	5.65	41.9% (.06)	1.58% (.19)	.24% (.03)	26.54	6.58
S. patens	43.38% (1.20)	1.83% (.13)	.32% (.12)	24.80	5.72	46.7% (1.28)	.41% (.06)	.05% (.02)	113.90	8.2

Discussion

Animals require nitrogen and phosphorous to maintain growth, reproduce, and survive (White 1978, Mattson 1980); therefore grazing animals select vegetation containing the highest relative source of these elements within a fixed intake (Gurchinoff 1972, Westoby 1974, White 1978, Mattson 1980). Our experimental results, in which nutria caused significantly greater percent mass loss to fertilized versus unfertilized samples of *P. hemitomon*, *S. lancifolia*, and *S. patens*, are consistent with previously researched behavior. By increasing the concentration of biologically available nutrients in the tissues of fertilized plants, the ratio of carbon to nitrogen and carbon to phosphorus dropped significantly and the palatability of those plants to nutria rose.

As carbon is generally in excess in terrestrial systems (Elser et al. 2000) and N and P limit consumers bodily homeostasis (Anderson et al. 2005), these ratios may provide a means of assessing the relative desirability of varied vegetation.

As noted earlier, nutria consumed more *S. lancifolia* and *P. hemitomon* than fertilized *S. patens*, even as fertilization lowered the mean C:N of *S. patens* to slightly below that of *P. hemitomon*. Higher N:P is typical of the fresher marsh (Baroch and Hafner 2002, Ngai and Jefferies 2004) that makes up the majority of nutria habitat, and observed lack of interest in *S. patens* despite relatively high N levels may reflect a lesser relative nutritional importance of P to N in our controlled trials.

Percent mass lost in unfertilized samples followed similar C:N and C:P trends. While nutria inhabit and feed extensively in both fresh and fresh-brackish marsh (Linscombe et al. 1997, Holm et al. 2011), abundance data indicate their general preference is for fresher marsh vegetation over more saline (Kinler et al. 1998). Preference is defined as the selection of certain habitats independent of availability (Silvy 2012), and this preference is potentially due to the decreasing availability of biologically accessible N in saline environments (Baroch and Hafner 2002, Ngai and Jeffries 2004). As in fertilized samples, *S. lancifolia* had both the lowest C:N and C:P; nutria may have consumed more of it because it supplied necessary nutrients most efficiently.

Our findings are supported by previous research showing that the palatability of certain food sources to various grazing herbivores can be affected nutrient addition. In examining the interactions of plant carbon, nutrient balance, and herbivory, Bryant (1987) observed that snowshoe hares (*Lepus americanus*) prefer fertilized over unfertilized feltleaf willow (*Salix*

alaxensis). Similar results also were observed by Hartley et al. (1995), in which Orkney voles preferentially grazed Sitka spruce (*Picea sitchensis*) seedlings with increased N concentrations. Morris et al. (2002) found that *Spartina alterniflora* that had been treated with additional N was preferentially consumed by rice rats (*Oryzomys palustris*).

Shaffer et al. (2009) and McFalls et al. (2010) observed that nutria significantly reduced biomass of fertilized plots in which herbivory was allowed, and Lundberg et al. (2011) observed nutria first and in large numbers in zones nearest the nitrogen-rich effluent inflow while examining a wastewater treatment wetland. However, because Lundberg et al. (2011) did not ascertain whether nutria presence nearest the nutrient source was merely a result of increased vegetation growth and did not include an unfertilized control, their observations, while informative, are inconclusive. Similarly, Shaffer et al. (2009) and McFalls et al. (2010) noted that, with fertilization, vegetation biomass increased only in exclosures where herbivory was absent, suggesting but not isolating the effects of nutrient addition to nutria feeding preference. Wilsey and Chabreck (1991) concluded that N content affected nutria preference among plant species, while our research extends their conclusions to address nutria preference within plant species as well.

We believe the distinction that the nutria made among our samples was based on the chemical content. Because chemical content of vegetation can change with factors such as season and plant life stage, both at the stand and individual plant level, it is possible that associated herbivory also would vary. For example, research shows that while vegetation incorporates N at higher levels early in the growing season (Harper 1971, Valiela 1976, Tobias 2011), by maximum growth the C:N ratios began to increase, regardless of previous nutrient input or the ultimate size of the plant; C:N ratios of fully grown fertilized and unfertilized plants

trend toward comparable concentrations (Patrick and Delaune, 1976). Additionally, as water volume is greatly seasonally variable (Hyfield et al. 2008), elemental ratios within diversion-managed marsh vegetation are likely in constant flux. Tobias (2011) also showed that C:N, within fixed nutrient supplies, can vary with salinity. Therefore, depending on localized pre-existing background conditions, herbivory may be asymmetrically distributed within an affected area.

In practical terms, our nutrient loading fell between the rates of secondarily treated municipal wastewater (0.21 $g^{-2} d^{-1}$) entering the Joyce Wildlife Management Area in Hammond, La, (Lundberg et al. 2011) and the most nutrient-rich Santa Ana river water (5.75 g N $m^{-2} d^{-1}$) entering a treatment wetland in the Prado Basin CA, prior to groundwater recharge (Reilly et al. 2000). Therefore, our experimental nutrient levels reflect current conditions in some areas and so are useful for inferring effects in the wild.

Nutria herbivory is a widely studied problem in the Mississippi Delta, and as such, successful measures have recently been enacted to minimize it (Scarborough and Mouton 2007, Hogue and Mouton 2012). However, a correlation between increased fertilizer concentrations and preferential feeding habits has implications for both current nutria management and wetland restoration efforts. For example, the nutria harvest program currently limits the area in which hunters can take advantage of financial incentive to keep animal numbers low; an awareness of the connection between nutrient influx and increased herbivory might necessitate a re-examination of the geographical targets of the program. Additionally, a more complete assessment of the risks and benefits of treating wetlands with nutrient infused water might further inform current commentary on wetland restoration methods. For example, in assessing the efficacy of three Mississippi River diversions in post-Katrina restoration, Kearney et al.

(2011) noted that vegetation disturbance in zones closest to the diversions was noticeably greater than reference areas, however they did not include the possibility of nutrient-induced herbivory in their analysis. By identifying areas at greater risk of a nutrient-induced herbivory, such as sites of agricultural runoff, sewage treatment outflows, and river diversion sources, selective assessment and management can better address a restrictive hindrance to restoration efforts.

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GENERAL CONCLUSION

In this thesis, I have shown that fertilization may affect the growth of vegetation differently at different salinities while flooding stress is a primary limiting factor, and that nutria, an invasive marsh herbivore, may potentially select vegetation based on increased nutrient content. Although relationships between factors limiting growth have been previously researched, the importance of belowground biomass as it is affected by these three major limiting factors has been largely ignored. Similarly, while many studies peripherally implied a causality between increased nutria herbivory and increased fertilization, I was able to quantify that relationship in a controlled environment.

When considered together, the commonalities between these two experiments revolve around currently employed wetland restoration techniques, which locally manipulate hydrology and create varying levels nutrients, salinity, and flooding. As flooding appears to be the primary cause of loss of vegetative biomass, while fertilization appears to both increase growth at higher salinities and potentially increase herbivory in vegetation of a range of salinities, the implications of the relationships between these two studies may serve to inform future restoration projects.

That the introduction of nutrient-rich water to lower salinity wetland may have a threshold of positive influence on the growth of biomass, while simultaneously accruing additional risk of increased herbivory should likely factor into decisions regarding the location and utilization of wetland loss mitigation systems. Similarly, information showing that a freshwater diversion may potentially have a significant effect on saline marsh through nutrient addition as well as gradual freshening may imply that, over time, the way in which a diversion benefits affected wetlands may change. For example, it is possible that an initial beneficial response to additional nutrients in minimizing vegetation loss could give way over time to a

controlled, longer term benefit from localized salinity reduction. If nutrient levels within the introduced water source could be managed selectively, such a stepped approach to wetland loss mitigation might prove successful.

These studies suggest several paths for future study. Based on our results, research on the possible relationship between levels of so-called osmolytes and levels of nutrients, across a range of salinities, would possibly further clarify the role that fertilization plays in influencing the growth of *S. patens* in relatively higher salinities. The proposed mechanism which I have described, by which our fertilized sample biomass experienced less reduction of biomass at relatively higher salinities could also be greatly informed by studying associated oxidation-reduction potentials, as oxygen availability, which can change with degree and duration of flooding, can affect the uptake of contained beneficial matter.

Additionally, as both of these studies are closely tied to the effect of nutrient uptake by vegetation, I recommend further research into causes of variations within that uptake. For example, N utilization by vegetation, and thus C:N ratios within vegetation tissue, may change over time due to availability, cellular demand, and seasonality. If those changes could be quantified, C:N ratios' might be a more useful metric by which to gauge such dependent variables as likelihood of grazing by herbivores and potential cellular osmolyte production, both of which indirectly affect the rate of vegetation growth and consequently the mitigation of wetland loss.

VITA

James Ialeggio was born in Smithtown NY in 1981. He graduated from high school in Randolph

VT and attended Wesleyan University in Middletown CT, where he earned a Bachelor of Arts in

2003. He does not drink warm coca-cola.