2014

Effects of Saltwater Intrusion on Vegetation Dynamics and Nutrient Pools in Low-Salinity Tidal Marshes, Pamunkey River (Virginia, USA)

Lori A. Sutter
*College of William and Mary - Virginia Institute of Marine Science*

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Effects of Saltwater Intrusion on Vegetation Dynamics and Nutrient Pools in Low-Salinity Tidal Marshes, Pamunkey River (Virginia, USA)

A Dissertation
Presented to

The Faculty of the School of Marine Science
The College of William and Mary

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Lori Anne Sutter
2013
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Lori A. Sutter

Approved by the Committee July, 2013

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Abstract

This study investigated vegetation changes in a former tidal freshwater marsh (TFM) to determine the role that saltwater intrusion plays in vegetation dynamics. Field observations along a narrow salinity gradient in the Pamunkey River revealed that vegetation is shifting to fewer dominants with increasing salinity. Two remaining dominants, *Peltandra virginica* and *Zizania aquatica* (hereafter *Peltandra* and *Zizania*, respectively), had variable net CO$_2$ assimilation throughout the growing season. *Peltandra* net CO$_2$ assimilation declined both over the growing season and in marshes with higher salinity; whereas, *Zizania* generally increased over the growing season peaking in late summer. The same species' tissue nutrients tracked similarly when compared across marshes of different salinity throughout the season, suggesting that the plants have adapted to their environment. Soils, however, contained higher carbon (C) and nitrogen (N) in a TFM relative to higher salinity marshes across years and within a single season. The soil N: phosphorus (P) ratio is relatively stable and well above 16:1 in the TFM, suggesting P-limitation. The other marshes appeared to be in transition with high fluctuations throughout the season and variability within the marsh. Soils responded to changes in salinity faster than vegetation by adsorbing or releasing nutrients.

A mesocosm testing plant traits subjected to four low salinity levels found *Spartina alterniflora* (hereafter *Spartina*) unresponsive to salinity ranging from 0 to 6, although CO$_2$ assimilation decreased between treatments fresh and 6. Two TFM species, *Peltandra* and *Leersia oryzoides* (hereafter *Leersia*), responded to salinity over 2 with decreases in aboveground and belowground biomass. The same two species exhibited an improvement in biomass quality (measured by C:N and C:P) over the salinity gradient, and both held greater N and P in the vegetation pool relative to *Spartina*. The pool of nutrients held in vegetation may shift with saltwater intrusion, and the enhanced biomass quality may lead to greater herbivory due to improved palatability.

A second mesocosm study paired *Spartina*, a facultative halophyte, with each of three TFM species: *Peltandra*, *Leersia*, and *Phragmites australis* (hereafter *Phragmites*). *Spartina* outperformed *Peltandra* in all aspects measured – both aboveground and belowground. In the presence of *Leersia*, *Spartina* offered mixed responses, but *Phragmites* changed *Spartina* responses considerably. The results of this experiment suggest that *Spartina* can succeed in less benign environments in the presence of at least some species.

A field manipulation excluding insect herbivory within treatments in three marshes along a salinity gradient found that overall biomass did not respond to the removal of insect herbivory, except for *Peltandra* in Cumberland Marsh (TFM). *Peltandra* biomass in TFM exclosures was approximately double that of controls, but this result was not significant in other marshes. *Zizania* N-content was higher in exclosures, suggesting a loss of this nutrient with herbivory, perhaps from rebuilding scarred tissue and/or loss through guttation.

Given *Peltandra*'s salt intolerance and *Spartina*'s ability to outperform common species, it is possible that Sweet Hall Marsh, a previous TFM transitioning to an oligohaline marsh, will become a *Spartina-Phragmites* marsh in the future driven by bottom-up controls.
Effects of saltwater intrusion on vegetation dynamics and nutrient pools in low-salinity tidal marshes, Pamunkey River (Virginia, USA)
Tidal freshwater marshes: A primer
Introduction

Sea level is rising. Experts in the Intergovernmental Panel on Climate Change (IPCC) expressed "high confidence" that sea-level rise has increased as a result of global climate change (Bindoff et al. 2007), and others suggest that recent measurements are at the high range of those predicted (CSIRO 2012, Rahmstorf et al. 2012). Relative sea level in the lower Chesapeake Bay tributaries is estimated to be rising at 4-6.8mm yr\(^{-1}\) (Holdahl and Morrison 1974, Neubauer et al. 2002, Boon 2012) – more than twice the global estimate (Bates et al. 2008). As a consequence, intertidal habitats of Chesapeake Bay tributaries are susceptible to the increased influx of more saline water. Tidal marshes are recognized as important habitat, especially in their capacity as nursery areas and spawning grounds for anadromous fishes (Rozas and Odum 1987, Mitsch and Gosselink 2007). They are high in both plant and animal diversity (Odum et al. 1984, Perry and Atkinson 1997, Perry et al. 2009) and primary productivity – higher than most other ecosystems (Baldwin et al. 2001). Tidal marshes support estuarine food webs through carbon (C) and nutrient additions; the temporal and spatial variation of this C pulse into the estuary varies by system.

Tidal freshwater marshes (TFM) are highly productive systems occupying a small but critical position at the upper reaches of coastal estuaries. Interestingly, it is the TFM – occurring at the upstream limit of tidal influence – that may experience the greatest shifts in ecosystem structure and function as a result of sea-level rise (SLR). As sea-level rises, TFM are expected to experience increased tidal inundation, sedimentation, and salt intrusion (Morris et al. 1990, Neubauer 2008), effects that are likely to result in
physiological changes in vegetation and potentially change the ecological drivers of the system.

The prevailing paradigm for marsh succession is that as sea-level rises and the salinity and inundation fronts move upstream, salt marshes will either build their own soil to remain viable or drown to become mud flats; brackish marshes will transition to salt marshes; and freshwater marshes will ultimately become brackish marshes (Craft et al. 2009, Neubauer and Craft 2009). Even farther upstream, TFM might establish in riparian areas where woody vegetation currently occurs; however, human development adjacent to many waterways and the steep topography of the adjacent banks may limit the “accommodation space” needed for expansion. The York River, Virginia, and its tributary Pamunkey River, for example, are micro-tidal (Reay and Moore 2009), and its TFM receive only modest sediment input from low tidal range – placing them at considerable survival risk with rising sea level (Stevenson and Kearney 2009).

Elevated salinity slows germination and stresses plants (Peterson-Smith and Baldwin 2006). In contrast, regular tidal flushing could moderate the negative effects of higher salinity and allow oxygen to return to the surface of the marsh platform (Odum et al. 1983, Morris et al. 2002). Researchers in coastal Louisiana have measured community structure of oligohaline marshes in response to such stressors and suggest that increased flooding impacts community survival more than does salt water intrusion (Flynn et al. 1995, Webb and Mendelssohn 1996, Howard and Mendelssohn 2000).

At Sweet Hall Marsh, the freshest component of the Chesapeake Bay National Estuarine Research Reserve system in Virginia (CBNERRVA), the marsh landward of an intersecting thoroughfare is experiencing increasing salinity in large part because of local
conditions, including hydrodynamics. Vegetation communities have changed in response
(Perry and Hershner 1999, Davies 2004). In the fall of 2010 after dominant TFM
vegetation senescence, I observed large stands of *Spartina alterniflora* (hereafter
*Spartina*) in this section of Sweet Hall Marsh where it had not previously existed in large
expanses (Davies 2004). The marsh on the river side of the thoroughfare remains
primarily fresh (see Chapter 1, Figure 1), but early indications are that this area is also
experiencing higher salinity (Neubauer and Anderson 2003).

This work combines descriptive measures from the field along with field and
mesocosm manipulations to determine mechanisms of vegetation change such as those
seen at Sweet Hall Marsh. Taskinas Creek, a downstream component within
CBNERRVA, serves as a reference site for plants and nutrients in a saline environment.
Cumberland Marsh is a TFM above the influence of salt and serves as the tidal freshwater
reference point. Table 1 summarizes the characteristics of the different marsh sites.

Measurements of soil and plant tissue nutrient content as well as net
photosynthesis were taken at the four sites within three marshes marsh along the salinity
gradient. Two mesocosm studies investigated the role of sub-lethal salinity and
competition on plant biomass and nutrient assimilation. Finally, a field manipulation
tested the role that top down control plays with increasing salinity. I hypothesized these
stressors would change the composition of vegetative assemblages and alter the following
TFM functions.

*Primary Productivity*

TFM support a diverse variety of macrophytes with high primary productivity both above
(Odum *et al.* 1984, Perry and Atkinson 2009) and below ground (Booth 1989) that has
traditionally believed to be driven by resource availability (Teal 1962, Odum 1971). The past decade of research reveals the importance of grazing and predation in controlling primary productivity in marsh systems (Silliman and Zieman 2001, Bertness and Silliman 2008, Bertness et al. 2008, Crain 2008, Holdredge et al. 2008), but the importance of top-down controls in regulating plant growth has not been investigated in TFM. The response of herbivores to stressed vegetation is not well known, since palatability could either increase because of increased nutrients or decrease because of (1) toxic chemical by-products of stressed metabolism or (2) reduced grazing because nutritional needs are met with less consumption.

TFM support estuarine food webs through carbon (C) and nutrient additions; however, the temporal and spatial variation of this pulse into the estuary varies by marsh system. The net fate of C from marshes is variable, as TFM episodically pulse labile C into adjacent water bodies in summer and fall; whereas more refractory C is released only in the spring in salt marshes (Odum et al. 1984). The C pulses that likely serve, in part, as the base of the food web for economically important fish such as striped bass (Morone saxatilis), will change if, as predicted, the marsh systems march inland (roll-over) with SLR (Morris et al. 2002).

Elemental Cycling and Plant Productivity

It is well documented that wetlands play important roles in water quality enhancement (Jones et al. 1976, Whigham et al. 1988, Johnston et al. 1990). The improvements are the result of a variety of biogeochemical processes acting collectively to alter and improve the quality of surface and ground waters (Hemond and Benoit 1988).
Positioned at the head of estuarine systems, TFM receive freshwater sediment and nutrient inputs that ultimately are derived from upstream, terrestrial systems.

Salt marshes are generally considered to be nitrogen (N)-limited systems and freshwater systems phosphorus (P)-limited (Sharitz and Pennings 2006, Mitsch and Gosselink 2007). Recent studies, however, have found N and P co-limitation in oligohaline marshes (Crain 2007), differential limitation of plants and microbes in various marsh systems (Sundareshwar et al. 2003), and N-limitation in a TFM (Frost and Craft 2009). TFM are important nutrient sinks for the deposition and retention of P, thus preventing P from entering the main stem of the estuary. Nutrient availability and chemical speciation are affected by salinity. For example, wetland soils have higher concentrations of phosphate (PO$_4^{3-}$) than the water column (Chambers and Odum 1990); however, oxidized iron (Fe$^{3+}$) binds PO$_4^{3-}$ making it unavailable for plant uptake. Iron is common in the study sites (Chambers pers. comm.), but the presence of sulfate (SO$_4^{2-}$) in seawater and its subsequent reduction and mineral precipitation as FeS (Caraco et al. 1990, Jordan et al. 2008) may make the PO$_4^{3-}$ available. Sodium (Na) and sulfide both play a role in N-limitation in salt-controlled systems since they inhibit N (as ammonium, NH$_4^+$) uptake and plant productivity. As Na concentrations diminish with distance from the ocean source, N can be assimilated into plant tissue. Evaluating the relationship between these nutrients and plant tissue content will identify the relative availability of N and P for plant productivity.

*Nursery Habitat*

TFM are recognized as important habitat, especially in their capacity as nursery areas and spawning grounds for anadromous fishes (e.g. Rozas and Odum 1987). Graff and
Middleton (2010) have listed 33 fishes that use TFM, including species of freshwater, estuarine, anadromous and catadromous, and some estuarine-marine groups (Mitsch and Gosselink 2007). Along the east coast of the USA, striped bass (*Morone saxatilis*) use TFM widely (Odum *et al.* 1984, Graff and Middleton 2010). Historically, TFM provided spawning and nursery habitat for the federally threatened sturgeon (*Acipenser oxyrhynchus*) (Graff and Middleton 2010).

*Species Interactions*

Researchers have established that inter-specific competition plays a small role in organismal success in highly stressed environments (Menge and Sutherland 1976, 1987), but Crain *et al.* (2004) documented the importance of inter-specific competition in tidal marsh vegetation dominance, which would be expected in intermediate habitats with moderately severe physical stressors and less severe consumer pressure (Pennings and Callaway 1992, Bertness and Callaway 1994).

In New England, *Phragmites australis* has been shown to limit *Spartina*'s productivity (Burdick and Konisky 2003, Konisky and Burdick 2004), yet both co-exist at Sweet Hall Marsh and *Spartina*'s abundance has increased over three decades (Davies 2004). Higher concentrations of salt are a known stressor to *Phragmites* (Burdick *et al.* 2001); therefore it is unlikely that salt is limiting its spread. The sulfate reduction associated with salinity, however, may be contributing to the inability of *Phragmites* to limit *Spartina*'s invasion (Chambers 1997, Chambers *et al.* 2003).
TFM Response to Rising Sea Level

Salt tolerance between species has been widely studied (Wainright 1984, Munns 2002). Salinity exerts a major influence on many mechanisms in the aquatic environment – causing particulate flocculation, preventing N-uptake in plants, determining the fate of nitrate (to denitrification versus dissimilatory nitrate reduction to ammonium (DNRA)), or releasing P. These factors, in turn, all affect the vegetation – both the community composition and the physiology of those species that are present. Plants must respond not only to the differential osmotic pressure, but also the toxicity of ions (Hale and Orcutt 1987). Plant productivity is expected to decrease with increased levels of salinity and inundation, as anticipated with SLR (DeLaune et al. 1987, Neubauer 2013).

Craft et al. (2009) found TFM to accumulate N and potentially denitrify at higher rates than salt marshes downstream. If the same species remain at a given site and reflect higher N-availability by assimilating more N, they may become more palatable to herbivorous animals and shift the relative importance of consumers in these areas. If, however, the increased salinity changes the dominant species, the area would be expected to support biomass of lower quality.

Spartina’s recent expansion in Sweet Hall Marsh has prompted my investigation of its success in the presence of species that are typically considered to be stronger competitors in this environment. The gradual increase in salinity may have allowed the immigration of a native species; alternately, a sudden increase in salinity may have quickly killed the historically dominant species, opening up space for invasion by non-native or potentially invasive native plants. This dissertation seeks to unravel some of the
mechanisms that may have driven this change and to predict early consequences of the transition.

**Structure of Dissertation**

Four chapters present the original research conducted as a part of this dissertation.

Chapter 1 encompasses field observations where I asked: Do plant diversity, photosynthesis, and/or nutrient content and availability change along the salinity gradient and across two years of study? This is largely a descriptive effort to support the following three chapters. Chapter 1 also investigates the relationship of soil nutrients to vegetative assimilation in this system.

Chapter 2 presents a mesocosm study. Many researchers have evaluated the response of different species to salinity, but dosing levels are typically higher than the salinity level usually found in Sweet Hall Marsh, which is experiencing a transition from fresh toward oligohaline conditions. How will perennial plants that are common to marshes along the Pamunkey and York Rivers’ salinity gradient respond to sub-lethal levels of salinity?

Chapter 3 is another mesocosm study. Evidence has suggested that the distribution of the halophyte *Spartina* is controlled down-estuary by its physiological tolerance to salt and upstream by competitive exclusion. Its expansion into Sweet Hall Marsh is unexpected because typical salinities are still in the lower oligohaline range. Compared to salt marshes, Sweet Hall Marsh is a benign environment, so a poor competitor such as *Spartina* is an unexpected arrival. Why has the local population of *Spartina* found success in Sweet Hall Marsh? Perhaps there is a positive interaction
between the local dominant species that has allowed *Spartina*'s influx (*sensu* Bertness and Callaway 1994).

Chapter 4 is an experimental field manipulation addressing the role of insect herbivory in controlling marsh primary productivity. If C:N and C:P in TFM vegetation decrease with added salinity, will the plants become more nutritive to insects? What role, if any, will insect herbivory play in controlling primary productivity in TFM as salinity increases?

The dissertation concludes with a summary integrating the results of the individual chapters.


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Rozas LP, Odum WE (1987) Use of tidal freshwater marshes by fishes and macrofaunal crustaceans along a marsh stream-order gradient. Estuaries 10:36-43


Webb EC, Mendelssohn IA (1996) Factors affecting vegetation dieback of an oligohaline
marsh in coastal Louisiana: Field manipulation of salinity and submergence.
American Journal of Botany 83:1429-1434

Table 1 Summary of site descriptors for each of the marsh locations investigated in this study (Reay and Moore 2009, Perry and Atkinson 2009, *this study).

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</thead>
<tbody>
<tr>
<td><strong>2011 Porewater Salinity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>18.1 (0.5)</td>
<td>2.1 (0.1)</td>
<td>1.7 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>[Range]</td>
<td>[8.9-24.2]</td>
<td>[0.4-4.9]</td>
<td>[0.6-5.7]</td>
<td>[0.1-0.5]</td>
</tr>
<tr>
<td><strong>Dominant vegetation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spartina alterniflora</td>
<td>Peltandra virginica. Zizania aquatica with invading Phragmites australis nearby</td>
<td>Peltandra virginica and Zizania aquatica</td>
<td>Peltandra virginica. Zizania aquatica and Pontederia cordata*</td>
</tr>
<tr>
<td><strong>Distance from mouth of York River</strong></td>
<td>38 km</td>
<td>75 km</td>
<td>87 km</td>
<td>above confluence of Pamunkey River with Mattaponi River forming the York River</td>
</tr>
</tbody>
</table>
CHAPTER 1

Spatial variability and dynamism in plant traits and nutrient pools along a low salinity, tidal marsh gradient
Abstract

Relative sea-level rise in lower Chesapeake Bay tributaries is estimated at 4-6.8mm yr\(^{-1}\). Consequently, intertidal habitats of the Bay’s tributaries are susceptible to the increased influx of saline water. *Spartina alterniflora* has appeared and is expanding its range into part of a tidal freshwater marsh currently dominated by *Peltandra virginica* and *Zizania aquatica*. Since *Spartina* is generally considered a poor interspecies competitor, we explored environmental characteristics associated with the recent appearance of *Spartina* through measures of plant diversity, soil and vegetative nutrient content in each marsh. Nutrient pools and vegetation diversity at four sites along a salinity gradient on the Pamunkey and York Rivers (Virginia, USA) were investigated: 1) tidal freshwater (Cumberland), 2) transitional oligohaline (two sites within Sweet Hall Marsh (SHM)) and 3) mesohaline (Taskinas Creek). Percent vegetation cover in June and September 2011 captured seasonal compositional changes. Soil and plants tissues were collected monthly June through October 2011 and analyzed for carbon (C), nitrogen (N) and phosphorus (P) content. The results of a Bray-Curtis ordination showed the composition and cover of vegetation in Taskinas Creek clearly differed from the other marshes. Nutrient content of *Peltandra* and *Zizania* exhibited seasonal variation, but individual species in different marshes tracked consistently with one another through time, suggesting they have adapted in place. Soil stoichiometry of SHM was more similar to Taskinas Creek, yet the vegetation similarities were greatest between Cumberland and SHM sites. These nutrient and vegetation patterns might suggest that soils are responding to changes in salinity driven by sea-level rise, with a lag time in vegetative response.
**Introduction**

Tidal freshwater marshes (TFMs) inhabit the upper edges of the freshwater reach of estuaries under tidal influence where salinity is less than 0.5 (Odum 1988; Perry *et al.* 2009) and support many freshwater plant species. Low salinity and regular inundation contribute to the distinctive quality of these systems. In the mid-Atlantic (USA), freshwater broadleaf species such as *Peltandra virginica* dominate in early spring, die back during mid to late summer and are replaced by tall grasses such as *Zizania aquatica* (Odum 1988; Perry *et al.* 2009, Perry and Atkinson 2009; Neubauer and Craft 2009). Salinity and inundation constrain the vegetative community within TFMs (Crain *et al.* 2004), with both salinity and inundation expected to increase in TFMs subjected to sea-level rise (SLR). Along the Chesapeake Bay shoreline, sea level is rising between 4-6.8 mm yr$^{-1}$ (Holdahl and Morrison 1974, Neubauer *et al.* 2002, Boon 2012). Salt water intrusion is predicted to decrease species richness (Flynn *et al.* 1995), species diversity (Reed *et al.* 2009) and plant density and biomass accumulation (McKee and Mendelssohn 1989; Merino *et al.* 2010).

Marine vegetation typically is limited by nitrogen (N) (Schlesinger 1997); whereas, freshwater wetlands are generally considered to be phosphorus (P)-limited (Reddy and DeLaune 2008). Freshwater wetlands in some tidal areas, however, have been identified as N-limited (Ket *et al.* 2011). As rising sea level pushes the salt front up-estuary, the geochemistry of iron (Fe), sulfur (as sulfate or sulfide), and P changes the availability of P (Jordan *et al.* 2008) as reflected in the concentration of P in plant tissue (Sutter *et al.* 2013).
With the salt front moving up-estuary, salt-tolerant species such as *Spartina alterniflora* (hereafter *Spartina*) may invade transitional marshes. *Spartina* now occurs in a transitional marsh along the Pamunkey River (Virginia, USA) near the study location designated “Sweet Hall – above” (SH-a). SH-a’s transition away from a TFM has occurred within approximately the last two-three decades, as salinity was recorded within TFM range (<0.5) as recently as 1990 (Perry and Atkinson 1997). Here, we seek to identify the mechanisms behind the vegetation change: Does the presence of salt alter the nutrient allocation in plants, changing their nutrient status or potentially shifting to greater palatability encouraging top-down control, thus allowing *Spartina* to succeed?

Previous studies by Sutter and colleagues (2013) showed that *Spartina* incorporates less P in its tissues relative to some TFM species. Identifying potential changes in plant composition may become increasingly important in estuarine management in estuaries where eutrophication is a concern; shifts in storage or release of nutrients as marshes transition in response to SLR may become increasingly relevant.

We conducted a field study of vegetation and soils along a salinity gradient from freshwater through mesohaline tidal marshes. We hypothesized (1) a shift in P-limited (TFM) to N-limited (salt marsh) systems along a salinity gradient would be reflected in soil nutrient stoichiometry; (2) vegetation dissimilarity would be evident from four sites along a salinity gradient from 0 to 18, particularly in the fresh and oligohaline zones relative to mesohaline zones, and (3) in response to higher stress from increased salinity along the gradient, species common to all sites would exhibit (a) reduced CO₂ assimilation at the salty end of the gradient before species shifts occur (SH-a), and (b) decreased plant tissue carbon (C):N and C:P ratios leading to increased plant quality at
SH-a (from decreased CO$_2$ uptake and increased nutrient uptake required to manage osmotic pressure).

**Methods**

*Study Area*

We selected four sampling sites within three separate marshes in the Pamunkey-York River Estuary system (Virginia, USA). The York River Estuary is a tributary to the lower Chesapeake Bay, and the Pamunkey River is itself a tributary to the York River. Cumberland Marsh (CM) is a tidal freshwater marsh (TFM) upstream from the salt front but still influenced by daily tides. Sweet Hall Marsh, the freshest component of the Chesapeake Bay National Estuarine Research Reserve system in Virginia (CBNERRVA), is at the low salinity range of an oligohaline marsh. We investigated sites in two areas of Sweet Hall Marsh that have different salinity: Sweet Hall-above (SH-a) is slightly more saline than Sweet Hall-below (SH-b) due to hydrodynamics established by a thoroughfare cut about a century ago (Perry pers. comm.). The wetland area landward of the thoroughfare (SH-a) is experiencing increasing salinity, and the vegetation has changed in response (Perry and Hershner 1999, Davies 2004). The marsh south of the thoroughfare on the Pamunkey River edge (SH-b) is also crossing into oligohaline salinity ranges (see Neubauer & Anderson 2003), but salinity is generally lower at SH-b than SH-a (Sutter unpublished data). Taskinas Creek (TC), another component of the CBNERRVA, is a mesohaline marsh on the York River. Figure 1 shows the site locations, and Table 1 summarizes the characteristics of the different marsh sites.
**Sampling**

In early June and September 2011, we estimated vegetative cover using the Braun–Blanquet cover-abundance scale (Mueller-Dombois and Ellenberg 1974) following the North Carolina Vegetation Survey design (Peet *et al.* 1998). Taxonomy follows USDA (2013). Importance values (IVs) were calculated by summing the relative frequency, relative density and relative cover of each species identified in 1 m x 1 m plots (Mueller-Dombois and Ellenberg 1974, Perry and Hershner 1999) using the equations that follow.

\[
\text{Relative frequency} = \frac{\text{Number of plots where Species X was found}}{\text{Sum of all frequency values in the marsh}} \times 100
\]

\[
\text{Relative density} = \frac{\text{Number of stems of Species X found in the marsh}}{\text{Number of stems of all species found in the marsh}} \times 100
\]

\[
\text{Relative cover} = \frac{\text{Sum of coverage values for Species X}}{\text{Sum of coverage values for all species}} \times 100
\]

In summer 2010 and again monthly from May through October 2011, photosynthesis was measured as CO\(_2\) flux on the leaf surface (\(A_{\text{net}}\)) using a TPS-2 portable infrared gas analyzer with a leaf cuvette (PP Systems, Amesbury, MA) on randomly selected healthy leaves of dominant species along boardwalks installed at each marsh. Measurements not meeting the instrument guidelines (Bergweiler pers. comm.) were discarded.

Aboveground biomass was harvested in early July and again in September 2011 to capture seasonal variation. During each harvest, vegetation was clipped at ground
level. The early season harvest occurred adjacent to the boardwalks in the “front-left” 1m x 1m quadrat of seven randomly identified 2m X 2m plots leaving the opposite corner undisturbed for the late season harvest. TC harvest plots were reduced to 0.25m X 0.25m quadrats after verifying that each quadrant within the 1m X 1m corner plot contained equivalent percent vegetative cover. In non-harvest months (August 2010 and May, August and October 2011), three to four large leaves of seven randomly selected shoots from potentially dominant plants (*Peltandra, Zizania, Pontederia cordata* and *Bidens laevis*) were collected along each boardwalk. Leaf material was transferred to the lab on ice, rinsed briefly with deionized water to remove sediments, dried at 60 °C until they achieved constant mass, and then milled for nutrient analyses. Carbon and N content were obtained using Perkin Elmer 2400 elemental analyzer (PerkinElmer, Waltham, MA). Total P was determined using modified ashing/extraction technique and colorimetric analysis (Chambers and Fourquarean 1991).

On the same sample data that vegetation samples were collected, surface soils were collected from seven randomly selected plots along CM and SH-b boardwalks, and five plots along SH-a. Soils were removed using a 30 ml syringe with the base removed and suctioned to a depth of approximately 4 cm. Soils were transported to the lab on ice, frozen and lyophilized. Macro-organic material was removed; soils were then pulverized and homogenized. Nutrient analyses for total C, N, and P followed that of the vegetation.

**Data Analysis**

A Bray Curtis ordination of vegetation composition and abundance was completed in PC-ORD™ 6 (MJM Software Design, Gleneden Beach, OR) using Sorenson similarity index (based on relative dominance) with variance-regression to select endpoints. Differences
in 2011 soil stoichiometry and vegetative nutrient content were analyzed using SAS© 9.3 (Cary, NC) PROC MIXED correcting for longitudinal 2011 data with an autoregressive(1) correlation, with the exception of Zizania C:N, where an uncorrelated structure best defined the underlying data structure. The denominator degrees of freedom were adjusted using the methods of Kenward and Roger (1997). Where significant (p<0.05) interactions prevented main effect interpretation, slices were employed. Post hoc comparisons between treatment levels were computed using contrasts. Statistical analysis of biomass was conducted using Type I ANOVA with the base package anova function (anova()) within the R statistical software (R Core Team 2013). ANOVA assumptions were evaluated before accepting results. Post hoc comparisons between treatment levels were computed using pairwise t-tests adjusted with the False Discovery Rate to avoid Type I errors.

Results

Plant species richness was highest at CM, followed by the transitional SH-a, and then SH-b (Table 2). An ordination of vegetative composition and cover showed that TC vegetation separated clearly from the other marshes, with SH-a beginning to disaggregate from the other low salinity marshes in the direction of TC (Figure 2). From the importance values (IVs) of the species that contribute to those measures, only four species over all marshes sampled emerged at a level of IV of 10 or higher at more than one marsh: Zizania aquatica, Peltandra virginica, Pontederia cordata and Bidens laevis (hereafter Zizania, Peltandra, Pontederia and Bidens, respectively; Table 2). The presence of multiple co-dominants or sub-dominants such as Pontederia and Bidens diminished in both Sweet Hall marshes, where salinity is higher than CM. TC did not
contain any species common to the other marshes, so TC vegetation was removed from further nutrient or $A_{\text{net}}$ analyses.

Photosynthesis (CO$_2$ assimilation; $A_{\text{net}}$) decreased with increased marsh salinity in *Peltandra* and remained constant in *Zizania* early in the 2011 growing season (Figure 3). *Peltandra* in all marshes trended generally downward across the growing season; *Zizania's* trend was generally upward with an early peak displayed in July (Figure 3). The single $A_{\text{net}}$ measurement in the summer of 2010 produced results within the range of the 2011 growing season; both Sweet Hall marshes were higher in 2010, but CM was lower in 2010 (Figure 3).

Biomass was significantly different across marshes ($p<0.0001$) but not harvest periods (Table 3). When pooling across harvest periods, average biomass was significantly different among all marsh combinations ($p<0.05$) with the exception of CM compared to SH-a where only a marginal difference ($p=0.06$) was observed.

The expected variation in nutrient content in vegetation common to the different marshes was not observed; each species exhibited similar nutrient content across both marsh and time (Figures 4 and 5). Significant interactions of all vegetative nutrient content and elemental ratios limited further interpretation of differences across marshes.

Soil in CM contained significantly higher ($p<0.0001$) C, N, C:P and N:P. Cumberland Marsh P content, however, was lower than all other marshes ($p<0.0001$). The linear model testing the variation in C:N identified significant interactions across marsh and time ($p=0.008$), making an inference of the main location effect (marsh) difficult (Figure 6). The single data point in 2010 led to the determination of CM as P-
limited (N:P > 16) with the other marsh N:P values falling under 16. All sites N:P in 2011 were much higher than 16:1 across the entire growing season.

**Discussion**

Vegetation composition and abundance at SH-a are moving toward fewer co-dominant and sub-dominant species and may become less similar to SH-b and CM over time. In 1987, vegetation above the thoroughfare at Sweet Hall Marsh in the region of SH-a contained 45 species (Perry and Hershner 1999); a 1976 study had nine additional species not present in 1987 (Doumlele 1976). At the time of the current study, the highest richness measured at Sweet Hall Marsh was only seven (Table 2). The decline in the number of both co-dominants and sub-dominants, such as *Pontederia* and *Bidens* (and therefore potential *Spartina* competitors), at Sweet Hall Marsh where salinity is higher than CM suggests a continuing trend in Sweet Hall Marsh toward fewer salt-intolerant species.

The absence of other species has led to changes in the relative importance for those species that remain. As salinity increased along this narrow gradient, *Peltandra* importance increased from one of three dominant species at CM to shared co-dominance at SH-b to a full dominant at SH-a (Table 2). On the other hand, *Zizania* moved from a co-dominant of three species (CM) to a shared dominance with one other species (SH-b) to a sub-dominant (SH-a).

Early season photosynthetic activity (as measured by CO$_2$ assimilation, $A_{\text{net}}$) of *Peltandra* was highest at the freshest site, reflecting the absence of salt stress. *Zizania* $A_{\text{net}}$ was equivalent across all marshes in the same time frame (Fig 3). *Zizania*‘s low salt tolerance relative to *Peltandra*‘s salt intolerance (USDA 2013) may explain why *Zizania*
C-fixation increased at the oligohaline sites over the growing season while *Peltandra* declined. The late season decline in *Peltandra* $A_{\text{net}}$ in SH-a may be related to senescence observed to occur earlier in SH-a than at the other sites. The timing of the measurement also is past the species peak, so the reduction may be a reflection of individual plants surviving beyond their peak performance. Heavy rainfall experienced in 2011 (Table 5) may have brought heavy cloud cover and dampened photosynthetic capabilities; we tried to minimize this impact by measuring only during peak sun on non-cloudy days, but a residual effect may have occurred. High rainfall also likely reduced the salt content which could have released salt-induced stress, thus requiring less C-fixation for normal physiological processes. Alternatively, the low assimilation by *Peltandra* in peak summer may have been caused by high rainfall, so that plants minimized the need to cool themselves through evapo-transpiration (ET), potentially leading to less photosynthetic activity required to balance typical high summer ET (Cronk and Fennessy 2001). To the contrary, if high ET were occurring, lower $A_{\text{net}}$ values may reflect the balance between photosynthesis and higher ET. Recent research has examined $A_{\text{net}}$ in woody species experiencing drought stress (Letts *et al.* 2009a and 2009b), but to our knowledge, no studies of $A_{\text{net}}$ in these marsh species have been conducted. Since plants respond to salinity stress similarly to drought stress by closing stomata (Orcutt and Nilsen 2000), it could be that plants are decreasing photosynthetic activity during maximal stress (Letts *et al.* 2009a) for which those species acclimated their gas exchange to stressful conditions (Letts *et al.* 2009b).

The intermediate levels of biomass observed at SH-a relative to marshes on either side of the salinity spectrum (Table 3) may be a reflection of the spatial heterogeneity of
low salinity marshes. If these marshes eventually are converted to salt marsh, a drop in species richness would be expected with a concomitant decrease in potential habitats and different food sources. The productivity service offered by salt marsh species, however, will probably increase, potentially offsetting the anticipated vegetation pool nutrient reduction (Sutter et al. 2013).

Unlike studies in a mesocosm (Sutter et al. 2013) and during a single sampling period in the same marshes in 2012 (chapter 4, this volume), TFM plant tissues in this field-based study did not consistently experience changes in nutrient allocation due to the presence of salt. Tissue nutrient content of _Peltandra_ and _Zizania_ tracked consistently across marshes, suggesting that individual plants have adapted and may not be experiencing salinity levels as a high stressor in their respective locales. _Zizania_ and _Peltandra_ appear to exhibit a slight ability to build C content in their tissues with the addition of salt. This result concurs with Munns (2002) and suggests a metabolic cost in addressing the salt stressor at both Sweet Hall Marsh locations in low levels of salinity (approximately 2).

Nutrient processes in TFM and oligohaline marshes have not been well studied (see Perry et al. 2009). An earlier study found that _Spartina_ holds less N and P in its tissues relative to two TFM species, _Peltandra_ and _Leersia oryzoides_ (Table 4). As the salt front moves up-estuary with sea-level rise, P is expected to release from Fe-bound particles (Jordan et al. 2008) providing P to plants until the marsh transitions to N-limitation. It is unclear what will happen with nutrient processing in the marsh: _Spartina_ will hold less P per unit mass of its tissues, but there could be roughly twice as much biomass (Table 3). We did not measure _Spartina_ nutrient content in field samples, but
future investigations should test changes in plant composition and how those changes in the field will affect vegetation nutrient pools.

We do not see the same change in biomass quality in the presence of increased salinity as occurred in either a related mesocosm study (Sutter et al. 2013) where C:N and C:P decreased or a field study where the same plant trait increased (chapter 4, this volume). Thus, increased herbivory due to increased palatability hypothesized in a related study (chapter 4, this volume) may not yet be important in these systems and may vary by year and species. Similar C:N ratios suggests that surviving individuals are effective at obtaining required nutrients, but collectively the reduction in species richness and the following change in importance values suggest that fewer of the species are effective at salt adaptation.

Consistent with Craft et al. (2009), we found less C and N in salt marsh soils relative to fresher marshes. Soil N:P stoichiometry in 2010 indicated all marshes except CM were N-limited, but the same marshes (SH-b, SH-a, TC) in 2011 appeared to be solidly in the range of P-limitation where CM clearly fell. Precipitation in nearby Richmond Airport, VA was higher in all growing season months for 2011 than 2010 (Table 4), so the higher freshwater input may have driven these other systems toward environmental characteristics of their freshwater neighbors. Stochastic rainfall events such as these may offset the effects of SLR if they become more frequent (Perry et al. 2009). Contrary to popular paradigms that soil nutrient pools are more stable than vegetation pools, our data surprisingly indicate that soils in this system may be quicker than the vegetation to respond to SLR with salinity-induced nutrient changes.
A reduction in dominant species with the arrival of salt may have reduced possible interspecific competitors and may be why *Spartina* has been successful at SH-a. *Spartina* success may also be tied to life history. Nursery obtained *Peltandra* seedlings in a mesocosm study (Sutter *et al.* 2013), for example, were adversely impacted at salinity as low as 4. The same species appears to be successful in that range in the field, potentially because those individuals are more developed and have belowground resources to maintain their presence. *Peltandra* may be an unlikely pioneer in new systems where salt is introduced, but may be adapting to small increases in salinity in place.

We observed different soil nutrient content & elemental ratios across marshes of different salinity, as expected. Soil nutrient stoichiometry suggested different nutrients limit each marsh by year, suggesting soils may be responding to environmental cues more quickly than vegetation. Vegetation transitioned along the gradient, and we captured a transition in a narrow salinity gradient where dominance seems to be shifting dramatically. Finally, we did not see strong differences in CO$_2$ assimilation in dominant species, nor did we find significantly different tissue nutrient content and ratios across marshes, perhaps because these species have adapted in place for many years.


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Table 1. Site characteristics for each of the marshes investigated in this study (Reay and Moore 2009, Perry and Atkinson 2009, *this study).

<table>
<thead>
<tr>
<th>Marsh</th>
<th>Taskinas Creek</th>
<th>Sweet Hall–above (brackish section)</th>
<th>Sweet Hall–below (fresher section)</th>
<th>Cumberland</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Porewater Salinity</strong></td>
<td><strong>Mean (SE) [Range]</strong></td>
<td><strong>Mean (SE) [Range]</strong></td>
<td><strong>Mean (SE) [Range]</strong></td>
<td><strong>Mean (SE) [Range]</strong></td>
</tr>
<tr>
<td><strong>Dominant Vegetation</strong></td>
<td><strong>Spartina alterniflora</strong></td>
<td><strong>Peltandra virginica, Zizania aquatica with invading Phragmites australis nearby</strong></td>
<td><strong>Peltandra virginica and Zizania aquatica</strong></td>
<td><strong>Peltandra virginica, Zizania aquatica and Pontederia cordata</strong>*</td>
</tr>
<tr>
<td><strong>Distance from mouth of York River</strong></td>
<td><strong>38 km</strong></td>
<td><strong>75 km</strong></td>
<td><strong>87 km</strong></td>
<td><strong>above confluence of Pamunkey and Mattaponi Rivers forming the York River</strong></td>
</tr>
</tbody>
</table>
Table 2 Species richness, dominance measures and Importance Values of vegetation in three marshes along a salinity gradient across the 2011 growing season. Only species common to at least two marshes are included. [Note: Taskinas Creek is not shown as it shared no species in common with the other sites.]

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative Density (%)</th>
<th>Relative Cover (%)</th>
<th>Relative Frequency (%)</th>
<th>Importance Value (IV)</th>
<th>IV Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumberland</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zizania aquatica</td>
<td>23</td>
<td>33</td>
<td>15</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>Peltandra virginica</td>
<td>34</td>
<td>18</td>
<td>13</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>Pontederia cordata</td>
<td>24</td>
<td>22</td>
<td>11</td>
<td>57</td>
<td>3</td>
</tr>
<tr>
<td>Schoenoplectus americanus</td>
<td>1</td>
<td>7</td>
<td>11</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Bidens laevis</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Polygonum punctatum</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Leersia oryzoides</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sweet Hall (below)</strong></td>
<td>Richness: 3 (June) / 3 (Sept)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zizania aquatica</td>
<td>29</td>
<td>62</td>
<td>46</td>
<td>137</td>
<td>2</td>
</tr>
<tr>
<td>Peltandra virginica</td>
<td>70</td>
<td>38</td>
<td>46</td>
<td>154</td>
<td>1</td>
</tr>
<tr>
<td>Pontederia cordata</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Schoenoplectus americanus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bidens laevis</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Polygonum punctatum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Leersia oryzoides</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Sweet Hall (above)</strong></td>
<td>Richness: 5 (June) / 7 (Sept)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zizania aquatica</td>
<td>7</td>
<td>7</td>
<td>23</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>Peltandra virginica</td>
<td>79</td>
<td>80</td>
<td>29</td>
<td>188</td>
<td>1</td>
</tr>
<tr>
<td>Pontederia cordata</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Schoenoplectus americanus</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Bidens laevis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Polygonum punctatum</td>
<td>2</td>
<td>1</td>
<td>13</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Leersia oryzoides</td>
<td>5</td>
<td>0</td>
<td>9</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3 Mean aboveground biomass (standard error) of all species found in four marshes along a salinity gradient during two separate harvest periods.

<table>
<thead>
<tr>
<th>Increasing salinity</th>
<th>Marsh</th>
<th>Early Harvest Mean (g m⁻²)</th>
<th>Late Harvest Mean (g m⁻²)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cumberland</td>
<td>533 (40)</td>
<td>712 (71)</td>
</tr>
<tr>
<td></td>
<td>Sweet Hall - below</td>
<td>354 (30)</td>
<td>360 (44)</td>
</tr>
<tr>
<td></td>
<td>Sweet Hall - above</td>
<td>446 (48)</td>
<td>565 (82)</td>
</tr>
<tr>
<td></td>
<td>Taskinas Creek</td>
<td>1197 (55)</td>
<td>1148 (248)</td>
</tr>
</tbody>
</table>
Table 4 Percent reduction in tissue nutrient concentration relative to *Spartina* at salinity treatment of 2 (from Sutter *et al.* 2013 (chapter 2, this volume)).

<table>
<thead>
<tr>
<th></th>
<th>Aboveground (%)</th>
<th>Belowground (%)</th>
</tr>
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<tbody>
<tr>
<td><em>Peltandra</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td><em>Leersia oryoides</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>44</td>
<td>97</td>
</tr>
<tr>
<td><em>Peltandra</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>44</td>
<td>97</td>
</tr>
<tr>
<td><em>Leersia oryoides</em></td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td>44</td>
<td>97</td>
</tr>
</tbody>
</table>


Table 5 Mean monthly precipitation the Richmond Airport, VA.

<table>
<thead>
<tr>
<th>Precipitation (cm)</th>
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<th>2011</th>
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</thead>
<tbody>
<tr>
<td>May</td>
<td>6.65</td>
<td>11.05</td>
</tr>
<tr>
<td>June</td>
<td>2.08</td>
<td>7.70</td>
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<tr>
<td>July</td>
<td>3.02</td>
<td>9.68</td>
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<tr>
<td>August</td>
<td>9.96</td>
<td>18.03</td>
</tr>
<tr>
<td>September</td>
<td>16.46</td>
<td>22.73</td>
</tr>
<tr>
<td>October</td>
<td>5.44</td>
<td>7.09</td>
</tr>
</tbody>
</table>
Fig. 1 Study locations in coastal Virginia (USA).
Fig. 2 Bray-Curtis ordination results analyzing vegetative cover across four marshes along a salinity gradient using Sorenson similarity index. Endpoints were obtained using the variance-regression technique. Crosses are from the most saline marsh along the gradient (TC); solid diamonds (SH-a) appear to be moving in a direction away from CM and SH-b toward TC.
Fig. 3 Net CO₂ assimilation ($A_{\text{net}}$) of *Peltandra virginica* and *Zizania aquatica* during the 2011 growing season across three marshes along a salinity gradient. Lack of data in a given month is a result of equipment failure.
Fig. 4 Plant tissue nutrient content for *Peltandra virginica* and *Zizania aquatica* across three marshes along a salinity gradient. Lack of data in a given month reflects the absence of that species or senescence.
Fig. 5 Plant tissue nutrient ratios for *Peltandra virginica* and *Zizania aquatica* across three marshes along a salinity gradient. Lack of data in a given month reflects the absence of that species or senescence.
Fig. 6 Soil nutrient content and ratios across four marshes along a salinity gradient.

Taskinas Creek was not sampled in September.
CHAPTER 2

Tidal freshwater marsh plant responses to low level salinity increases

The citation for the chapter is as follows:

Abstract

In a tidal freshwater marsh (TFM) in lower Chesapeake Bay, salt marsh cordgrass \textit{(Spartina alterniflora)} recently has become established where TFM vegetation previously grew. To determine the potential contribution of saltwater intrusion to the observed species transition, we conducted a mesocosm study subjecting \textit{Spartina} and two TFM perennials \textit{(Peltandra virginica and Leersia oryzoides)} to sub-lethal levels of salinity (0, 2, 4 and 6). We measured plant performance as carbon dioxide flux over the leaf stomata as a proxy for net photosynthesis \textit{(A_{net})}, aboveground and belowground biomass and tissue carbon (C), nitrogen (N) and phosphorus. For \textit{Spartina}, all metrics were unchanged across the range of tested salinity. The TFM species, however, had lower \textit{A_{net}} than \textit{Spartina} and decreased with added salinity. TFM biomass was reduced up to 86% in both the aboveground and belowground compartments, and aboveground nutrient allocation was altered: N increased in \textit{Leersia} and P increased in \textit{Peltandra} with increasing salinity. Under this simulation of increased salt intrusion associated with climate change and sea-level rise, TFM species responded with decreased biomass and decreased tissue C:N. Although other factors must contribute, the abiotic salt stressor leads to plant responses consistent with the observed replacement of TFM species by \textit{Spartina}.

Keywords: tidal freshwater marsh, salinity intrusion, sea-level rise, vegetative stress response, \textit{Leersia oryzoides}, \textit{Peltandra virginica}, \textit{Spartina alterniflora}
Introduction

Relative sea level in the lower Chesapeake Bay tributaries is rising at 4-6.8 mm/yr (Holdahl and Morrison 1974, Neubauer et al. 2002, Boon 2012)). As a consequence, intertidal wetlands of bay tributaries are susceptible to the increased influx of more saline water. Among these wetlands, tidal freshwater marshes (TFM) that occupy a small but critical position at the upstream limit of tidal influence are recognized as important habitat (Simpson et al. 1983), especially in their capacity as nursery areas and spawning grounds for anadromous fishes (Rozas and Odum 1987, Mitsch and Gosselink 2007). They support high plant and animal diversity (Odum 1984, Perry and Atkinson 1997, Perry et al. 2009) and primary productivity – higher than most other ecosystems (Baldwin et al. 2001). Interestingly, these TFM near the head of tidal systems may experience the greatest shifts in ecosystem structure and function as a result of salinity intrusion with sea-level rise (SLR).

As sea-level rises, TFM are expected to experience increased tidal inundation, sedimentation, and salt intrusion (Neubauer 2008, Neubauer and Craft 2009), which may result in physiological changes to vegetation (Hester et al. 1998) and potentially change the ecological drivers of the system. Elevated salinity is known to decrease net photosynthesis (Pearcy and Ustin 1984, Hester et al. 2001, Redondo-Gomez et al. 2007), alter germination (Greenwood and MacFarlane 2006, Redondo-Gomez et al. 2008), and reduce plant growth (Hester et al. 2001, Peterson-Smith and Baldwin 2006). The regular tidal flushing of TFM, however, could moderate the negative effects of higher salinity by improving effects of other characteristics of the marsh platform, such as anoxia (Odum et al. 1983, Morris et al. 2002). Vegetation can recover from pulsing salinity influxes (e.g. 52
Flynn et al. 1995, Howard and Mendelssohn 2000), but TFM vegetation may not be able to respond to chronic, low levels of salt intrusion. Glycophytes in sub-lethal salinity have shown mixed responses to salt, often by increasing osmolytes to balance increased osmotic pressure. For example, *Panicum hemitomum* Schult. tissue nitrogen (N) and phosphorus (P) did not increase under salinity additions (McKee and Mendelssohn 1989); however, Howard and Mendelssohn (2000) found that *Sagittaria lancifolia* L. increased N and P in some saline conditions. Hester et al. (2001) also found grasses to increase N-rich amino acids with sub-lethal salinity.

The current research uses mesocosms to test the individual role of salt from a suite of possible environmental and biological factors associated with SLR. Salinity intrusion has driven vegetation changes in tidal marshes in the US and Europe (Perry and Hershner 1999, Engels and Jensen 2010, Sharpe and Baldwin 2012). At Sweet Hall Marsh (37° 34’ N, 76° 53’ W), a component of the Chesapeake Bay National Estuarine Research Reserve System in Virginia historically considered a TFM, several portions of the marsh are becoming more saline due to local hydrodynamics. Much of Sweet Hall Marsh remains primarily fresh, but early indications are that certain areas also are beginning to experience higher salinity (Neubauer and Anderson 2003, Sutter unpublished data). Two vegetation studies in the area experiencing salinity intrusion documented a change in vegetative composition since 1981 (Perry and Hershner 1999, Davies 2004). At a nearby water quality monitoring station, the water column salinity has increased, moving the marsh into oligohaline ranges, averaging 2.8 between 2009-2012, with annual means ranging from 1.4 in 2011, 2.8 in 2009 and 2012, and 4.0 in 2010 (NOAA 2012). In the fall of 2010 after dominant TFM vegetation senescence, we
observed large stands of *Spartina alterniflora* Loisel. (hereafter *Spartina*) in a section of Sweet Hall Marsh for the first time.

The generally accepted paradigm is that *Spartina*, a facultative halophyte, follows the "competition-to-stress hypothesis": *Spartina* has developed physiologic strategies to living in brackish and salt marsh environs, and as a poor competitor, only finds refuge from interspecific competition in these more harsh physical environments (Crain *et al.* 2004). Recent tests of this competition-stress hypothesis, however, have found exceptions to refute the hypothesis in tidal systems (Guo and Pennings 2012). Considering *Spartina*'s recent establishment in Sweet Hall Marsh, non-lethal amounts of salt may confer a competitive advantage to *Spartina* in TFM experiencing salinity intrusion. Although many factors may contribute to the observed species transition change, in the current experiment we isolated the vegetative response of *Spartina* and two dominant TFM species (*Peltandra virginica* (L.) Schott and *Leersia oryzoides* (L.) Sw.; hereafter *Peltandra* and *Leersia*, respectively) to the abiotic salt stressor by growing plants in mesocosms experiencing different salinity treatments from 0 to 6. *Peltandra* is the low marsh dominant throughout the historical TFM, and *Leersia* has been a historical dominant in the high marsh.

We hypothesized that, although *Spartina* typically occurs in more saline conditions, its biomass and rate of net photosynthesis would be higher in freshwater (exhibiting a physiologic release from salt stress), with concomitantly higher indications of productivity or lower stress in freshwater relative to salt water. We also expected the dominant TFM species to exhibit decreases in aboveground biomass and photosynthesis along an increasing experimental salinity gradient because TFM species do not have salt
tolerant physiology. We anticipated TFM belowground biomass to increase relative to aboveground biomass along the salinity gradient to reflect the level of salt stress experienced by these species. Finally, we expected differences in the nutrient content of the three species along the salinity gradient, as measured by the allocation and elemental ratios of carbon (C), nitrogen, and phosphorus in both shoot and root tissue.

Methods

Experimental Design

Thirty-six individuals of each of three species were obtained from Pinelands Nursery & Supply (formerly in Toano, VA). *Peltandra* (a low-marsh, C3, broad-leafed herbaceous, TFM dominant plant), *Leersia* (a high-marsh, C3 grass, TFM dominant plant) and *Spartina* (a low-marsh, C4 grass, dominant plant in brackish and salt marshes) were potted individually in a nutrient-rich (0.21-0.07-0.14 %NPK) soil mixture. Three 3.8L (1-gallon) pots, each of which contained the same species, were placed into one clear, 128.7L (34-gallon) shallow plastic tub. The tubs were arranged in a randomized block design and replicated three times; each block contained a total of 12 tubs with all unique combination of species (*Peltandra, Leersia, Spartina*) and salinity (0, 2, 4, 6) represented. Taxonomy follows the USDA (2013).

After initial transplant outdoors, plants acclimated for 2 weeks in freshwater to minimize transplant shock. Water levels were maintained in the tubs at mid-pot which was sufficient to keep the soil surface from drying. For salinity treatments, salt levels were raised to 2 and allowed to adjust for two days before increasing another 2 and again until the final group reached a salinity of 6. Salinity was measured using a YSI conductivity meter (Yellow Springs, OH) and adjusted manually approximately twice.
weekly for eight additional weeks using small volumes of 40 μm-filtered York River water (salinity approximately 23) and/or diluted with de-chlorinated freshwater. The additions of river water introduced salinity and other environmental factors that might influence plant performance (i.e., microbes and other dissolved solids besides salt); water additions also could have changed the concentrations of nutrients available to the plants. Because the initial soil mix was high in nutrients and in the absence of any studies documenting the impact of planktonic microbes on aquatic plant growth, we assume that the observed responses along the salinity gradient were due largely to differences in salt.

Plant Response Measurements

Photosynthesis, measured as net carbon dioxide (CO\textsubscript{2}) assimilation (\(A_{\text{net}}\)) was measured weekly using a TPS-2 portable infrared gas analyzer with a leaf cuvette (PP Systems, Amesbury, MA). The CO\textsubscript{2} flux was captured mid-day. On the grasses, three stems were arbitrarily selected, and \(A_{\text{net}}\) was measured every ten seconds for 30 seconds from the third fully expanded leaf on each stem. \textit{Peltandra} rarely had more than one leaf from which to select; when it did, the youngest fully expanded leaf was used. Measurements not meeting the instrument guidelines were discarded; those remaining were averaged over the sampling interval to represent the experimental unit of the tub.

At the end of the experiment (eight weeks after planting), individual plants were harvested; aboveground and belowground plant material was separated, washed, and dried at 40 °C to constant mass. Individual plant matter was milled for nutrient analysis. Total P was determined using a modified ashing/acid extraction process and colorimetric analysis (Chambers and Fourqurean 1991). Tissue C and N content was determined using a PerkinElmer 2400 elemental analyzer (PerkinElmer, Waltham, MA).
Data Analysis

Statistical analyses for biomass and nutrients were conducted using Type I ANOVA within the base package of R statistical software (R Core Team 2013). Each mean represents the response of nine plants. However, the response was averaged across the three plants within each tub to avoid issues of pseudo-replication and compared only across tubs. ANOVA assumptions were evaluated; to correct for heteroscedasticity, we modeled the inverse variance for the response variable within each tub (Pinheiro and Bates 2000). Species were each tested independently; since each species was expected to behave differently, this allowed a more direct interpretation of the salinity treatment effect. Significant interactions between treatment and design block are noted in the text.

In the case of longitudinal net photosynthesis data lacking independence, the underlying covariance structure was evaluated by selecting the lowest AIC value from maximum likelihood modeling and then treated with repeated measures using residual maximum likelihood in a fixed model ANOVA (nlme::gls()) to obtain an overall mean. Post hoc comparisons between treatment level effects employed pairwise.t.test() with non-pooled standard deviation; the False Discovery Rate adjustment minimized Type I errors without the stringent conditions found in family-wise comparisons such as Bonferroni, making this a more powerful comparison (R Core Team 2013; Benjamini and Hochberg 1995; Benjamini and Yekutieli 2001). For the case of Peltandra $A_{\text{net}}$ where block X treatment interactions were significant and a main effect seemed apparent, a linear mixed model was refit to the data with block and time nested to allow comparisons between treatments. The belowground Leersia results reflect a reduced model of only the treatment effect to
facilitate adequate degrees of freedom due to mortality at 4 and 6 combined with insignificant block or block X treatment interactions.

Results

Net Photosynthesis
For all salinity treatments, CO₂ assimilation (Aₙₑᵗ) was highest for *Spartina*, followed by *Leersia* and then *Peltandra*. Throughout the salinity gradient increasing from 0 to 6, all species maintained or decreased Aₙₑᵗ; each species, however, followed a different pattern in Aₙₑᵗ response (Fig. 1). *Spartina* Aₙₑᵗ gradually decreased 16% between 0 and 6. In contrast, photosynthetic assimilation in *Leersia* did not change significantly (a 2% decrease) across salinity treatments. Finally, *Peltandra* Aₙₑᵗ exhibited an overall decrease of 49% between 0 and 6, the majority of which occurred between 2 and 4, although a significant treatment X block interaction (p < 0.005) diluted inferences about the main effect of the salinity treatment. Further analyses using linear mixed models ascertained a statistically significant decline between the treatments 0 and 2 versus 4 and 6 (F=11.21; p<0.001).

Biomass
In the freshwater treatment, total biomass (aboveground plus belowground) of *Spartina* was approximately 1.5x heavier than *Leersia* and 4.3x heavier than *Peltandra* (Fig. 2). At 6, *Spartina* biomass was approximately 12.5x and 20x heavier than *Leersia* and *Peltandra*, respectively. Both TFM species had reduced tissue biomass aboveground and belowground relative to the freshwater treatment at salinity greater than 2 (Fig. 2). *Leersia* exhibited a significant decrease in aboveground (~82%) and belowground
(~86%) biomass with increasing salinity (Table 1). At the two lowest treatments, *Leersia* invested in more belowground biomass relative to aboveground biomass compared to those grown in salinity greater than 2. *Peltandra* also exhibited a significant decrease in aboveground (~81%) and belowground (~68%) biomass with increasing salinity up to 6, with the sharpest decline belowground between 2-4 (Fig. 2, Table 1).

**Nutrient Content**

Vegetative nutrient content varied by species (Table 2). Across salinity treatments, *Spartina* tissue C, N and P content displayed a significant difference in the ANOVA (Table 1), but pairwise comparisons indicated no difference between treatments in both aboveground and belowground compartments. *Peltandra* aboveground C content decreased significantly (p<0.001) at the same threshold between 2 and 4 as was found for $A_{\text{net}}$ and biomass. Phosphorus content in *Peltandra* aboveground tissue increased significantly (Tables 1 and 2), with the greatest increase occurring between 2 and 4 – the same salinity threshold as C – but remained stable belowground among all salinity treatments. Belowground N content in *Peltandra*, however, was significantly higher for the 4 and 6 treatment groups (Tables 1 and 2). In aboveground tissue, *Leersia* C content decreased with higher salinity (p<0.001), in contrast to $A_{\text{net}}$ which remained constant. *Leersia* tissue N content aboveground increased with salinity greater than or equal to 2, and P remained constant among salinity treatments (Tables 1 and 2). Carbon and N in belowground tissue of *Leersia* remained stable across 0, 2, and 4 treatments (only one *Leersia* plant survived salinity of 6 with enough aboveground tissue to analyze (adequate belowground tissue remained), so that sample could not be included in statistical analyses). Aboveground P in *Leersia* showed significant increases (Table 1) that were not
discernible through the pairwise comparisons; belowground P content varied within the salinity gradient but remained constant when comparing the endpoints.

Elemental ratios across salinity treatments also varied by species (Fig. 3; Table 1). Carbon:N and C:P ratios in both aboveground and belowground tissues of *Spartina* were not significantly different from 0 to 6 (Table 1). With decreasing aboveground C content in both TFM species and a coincident N content increase in *Leersia* aboveground and *Peltandra* belowground, tissue C:N declined over salinity treatments in *Leersia* aboveground and in *Peltandra* both aboveground and belowground (Table 1; Fig. 3a). Aboveground C:P decreased significantly with increasing salinity treatment in *Peltandra*, which was the only species to exhibit a significant increase in the P content of aboveground tissue (Fig. 3b). The N:P in both TFM species changed significantly but in opposing directions aboveground (*Leersia* increased (p<0.001) and *Peltandra* decreased (p=0.008)); belowground N:P increased significantly only in *Peltandra* (p<0.001). No difference in N:P ratio was observed for *Spartina* tissues across all salinity treatments.

**Discussion**

Under conditions of low salinity from 0 to 6, *Spartina* performance as measured by net CO₂ assimilation (A<sub>net</sub>) and final biomass was superior to two TFM species. In the absence of competitors or fluctuating water level, *Spartina* was successful in low saline environments in this experiment, similar to results from a field study (Crain *et al.* 2004). Our data showed that *Spartina* was capable of building equivalent biomass both aboveground and belowground across all levels of salinity treatment and irrespective of a measured decrease in CO₂ assimilation (A<sub>net</sub>) at higher salinities. In contrast, Hester *et al.* (2001) found higher investment in roots relative to shoots in populations of *Spartina*.
more tolerant of salt (but to salinity as high as 30). \textit{Spartina} tissue nutrient content and ratios also did not fluctuate with increasing salt in our study, thus \textit{Spartina} became more metabolically efficient under salinity stress by decreasing $A_{net}$ while building the same amount of biomass.

\textit{Leersia} maintained constant net CO$_2$ assimilation across treatments, but biomass declined in both aboveground and belowground compartments at salinity above 2. This high-marsh species tolerates low levels of salt but exhibits salinity stress as measured by decreased biomass at 4 and aboveground lethality at 6. We do not know the fate of the excess C that \textit{Leersia} assimilated at higher salinity to account for the observed decline in biomass production. If \textit{Leersia} lost its photosynthate via root exudates, then increasing salinity could increase the lability of soil organic matter, potentially fueling local microbial communities and enhancing methane production or exporting additional C (Marsh \textit{et al.} 2005, Stevenson and Kearney 2009). Alternatively, the excess energy may have been used in building osmolytes or in other metabolic processes to exclude salt (Munns 2002). The roots may have leaked greater photosynthate at higher salinity similar to what Turner (1978) found in \textit{Spartina} DOC leachate when the plant was subjected to increased salinity relative to freshwater controls; tests of this phenomenon, to our knowledge, have not been conducted in \textit{Leersia}. Increased photorespiration would also explain the decreased biomass at higher salinity, but the $A_{net}$ measurement incorporates this process and did not vary across salinity treatments, i.e., any C losses via photorespiration must have been matched by C gains via photosynthesis. With increasing salinity, \textit{Leersia} accumulated aboveground tissue N, thereby decreasing the C:N of stems and leaves. It is not uncommon for some plants to use N-rich compounds such as proline.
(Stewart and Lee 1974) and/or glycine betaine (Storey and Wyn Jones 1977) to balance osmotic pressure under salt stress (Munns 2002). Interestingly, the more N-rich aboveground tissue may also make the plant more palatable to herbivores (Elser et al. 2000). In the presence of nutritionally diverse food sources, TFM species with low C:N may experience greater herbivory stress, although the converse may also be true: Herbivores may need to consume greater amounts of low quality tissue to meet nutritional needs (Eskalinen et al. 2012).

*Peltandra* exhibited a concomitant decrease in both biomass and net CO$_2$ assimilation above 2 suggesting that this low-marsh species experienced salinity stress. Under conditions of stress, perennial vegetation has been shown to shunt biomass belowground to perennating organs (Geng et al. 2007, Zewdie et al. 2007) presumably for use in a less stressful environment in future years. *Peltandra*, which has a large tuber, built belowground biomass at salinity less than 4, but the species met a threshold between 2 and 4 at which point no substantial belowground biomass grew and aboveground biomass diminished. *Peltandra* also changed its allocation of nutrient resources with increased salinity. Under salt stress as low as 4, *Peltandra* increased aboveground tissue N and P and belowground tissue N, driving down the respective C:P and C:N ratios. The changing C:P and C:N ratios indicate both *Peltandra* and *Leersia* are likely responding to physiological stress by holding nutrients more tightly, increasing respiration, or leaking C belowground as photosynthate (see Neubauer et al. 2000).

Based on the average growing season (March-October) salinity at Sweet Hall Marsh between the years 2009 and 2012, our experimental range captured and book-ended the *in situ* conditions where we have seen TFM vegetation begin to transition to
Spartina. Our results suggest the recent, unexpected establishment of Spartina in Sweet Hall Marsh may be due to the inability of the TFM species to build biomass above salinity of 2. Salinity intrusion in the field will, of course, be coupled with additional physico-chemical factors. For example, sulfate will be reduced to sulfide, which can have a negative impact on the plant growth of many species. Spartina has a high sulfate requirement (Stribling 1997), which may be met as the salt front moves upstream, thus facilitating its growth in near freshwater environments; however, we did not test for this effect. The two TFM species in the current study reached a performance threshold between 2 and 4, above which A\text{net}, biomass, and/or nutrient content were affected. Our experimental results suggest that the exclusion of TFM species from more saline environments is at least in part a consequence of physiological intolerance to salt. Thus, as tidal freshwater marshes experience salinity intrusion with SLR, environmental conditions are created that are more conducive to the growth of Spartina. While Spartina was largely unaffected by salinity treatment, we found a decrease in the capacity of TFM species to build biomass, which in Peltandra could be explained, at least in part, by a decrease in its ability to assimilate CO\textsubscript{2}.

The slow transition of TFM vegetation to salt-tolerant species as sea-level rises over time may be related to the life history of the perennial vegetation found in the field. We tested young individuals in our study, but older, established plants have considerable belowground energy stored from years of growth and should be more tolerant of increases in salinity and, perhaps, tidal flooding. Aside from the direct effects of salt on the physiological tolerance of TFM plant species, however, other wetland features may change indirectly with increasing salinity. For example, a change in plant nutrient
assimilation and the associated impacts on and relationship to the soil microbial community may affect long-term vegetative stability. As well, changes in interspecific competition with an increased salinity regime likely play a role structuring wetland plant communities (Crain et al. 2004, Engels and Jensen 2010, Guo and Pennings 2012).

Nutrient allocation in TFM species changed with sub-lethal salinity, which may lead to further consequences at a broader scale. For example, the magnitude of tissue-P in Spartina (Table 2) was constant across salinity treatment and consistently lower than the TFM species – increasingly so with salinity. If Spartina replaces the TFM species without concurrent increase in stem density to offset the difference, there may be less P held in the marsh vegetation pool. If P is not bound in the vegetation pool, it could be released where it would bind to iron in freshwater (Jordan et al. 2008). Ocean-derived sulfate, however, would preferentially bind to the iron which would then free the P, where it would be released into the water column further contributing to eutrophication in the Chesapeake Bay. Finally, the decreased C:N and C:P ratios of TFM plants exposed to elevated salinity may increase the palatability of these species, leaving them not only under stress from salinity but also from increased top-down pressure via increased herbivory. Therefore, in addition to the abiotic factor of salt intrusion, biotic factors including interspecific competition and herbivory may contribute to the dynamics of species replacement currently occurring in TFM where sea-level rise has led to an influx of more saline water.
Acknowledgements

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Table 2 Means and standard errors of tissue nutrient content (% dry weight).

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Fig. 1 Mean net CO$_2$ assimilation rates (A$_{net}$) of three species in response to low salinity treatments. The height of the solid bar represents the mean rate of measurements taken on leaves in nine pots across all weeks of the study; errors bars are standard errors. Different letters above the bar represent a significant difference (p<0.05) within species at different salinity treatments. Where no letters are shown, responses did not vary statistically; n is reduced to 3 for the analysis to avoid pseudoreplication. An "*" denotes a significant block X treatment interaction, thus minimizing the ability to show statistical significance associated exclusively with the main effect. In the case of Peltandra, an additional analysis using a linear mixed effect model nesting block and time confirmed that the salinity treatments of 0 and 2 differed from 4 and 6.
Fig. 2 Aboveground and belowground biomass production of three species in response to low salinity treatments. The length of the solid bar represents the mean biomass of nine pots; errors bars are standard errors. Different letters above the bar represent a significant difference (p<0.05) within species at different salinity treatments; n is reduced to 3 for the analysis to avoid pseudoreplication. Where no letters are shown, responses did not vary statistically.
Fig. 3 Nutrient assimilation ratios (C:N in Panel a; C:P in Panel b) in aboveground and belowground tissue of three species in response to low salinity treatments. The height of the solid bar represents the mean biomass of nine pots; errors bars are standard errors.
Different letters above the bar represent a significant difference (p<0.05) within species at different salinity treatments; n is reduced to 3 for the analysis to avoid pseudoreplication. Where no letters are shown, responses did not vary statistically.
CHAPTER 3

There goes the neighborhood: Saltwater intrusion mediates species transition in low salinity, tidal marsh vegetation
Abstract

In tidal freshwater marshes (TFMs), sea-level rise has introduced both an abiotic stressor (salt) and a biotic plant competitor (*Spartina alterniflora*). Through a narrow salinity range (0-3), we examined the interplay between salt stress and competition by comparing the growth and nutrient status of *Spartina* in the presence of three TFM species (*Leersia oryzoides, Peltandra virginica* and *Phragmites australis*). We conducted a mesocosm study subjecting *Spartina* potted with itself or one of the TFM species to sub-lethal levels of salinity (0, 1.5, and 3) and measured plant traits including net photosynthesis ($A_{net}$), aboveground and belowground biomass and tissue carbon (C), nitrogen (N), and phosphorus (P). *Spartina* $A_{net}$ did not vary when paired with *Peltandra*, but was altered when paired with either grass, *Leersia* or *Phragmites*. Significant ($p<0.05$) interactions occurred between salinity and competitor for most of the traits measured. Across all competitors, *Spartina* biomass in aboveground and belowground compartments increased with added salt; however, aboveground tissue C, N, and belowground tissue N decreased with increased salinity gradient. Across all salinity treatments, *Spartina* paired with *Peltandra* only marginally changed one trait – an increase in C:N. The main effect of *Leersia* presence was to decrease *Spartina* aboveground biomass and N and P content, driving a marginally significant increase in C:N. Belowground, the presence of *Leersia* significantly decreased *Spartina* biomass, tissue C, N and C:P. Paired with *Phragmites*, *Spartina* aboveground biomass decreased, as did tissue C and C:N, while aboveground tissue N and P increased. Belowground, *Spartina* paired with *Phragmites* exhibited a decrease in biomass and an increase in tissue N and P. The predicted outcome is that *Spartina* will be able to ride higher sea-levels containing greater oceanic salt constituents.
into TFMs, where—despite the presence of plant competitors at low salinity—salt stress eventually will hasten the transition to a *Spartina*-dominated marsh.

**Introduction**

Many species of tidal marsh plants are tolerant of temporal variations in salinity (interannual, seasonal, and even tidal), but individual species also have general ranges of salinity tolerance that determine where in the estuary they are typically found. *Spartina alterniflora* Loisel. (hereafter *Spartina*), for example, is the dominant salt marsh species throughout Eastern and Gulf coasts of the U.S., forming expansive monocultures in marshes ranging in salinity from oligohaline to polyhaline (Mitsch and Gosselink 2000). At lower salinity, however, abrupt changes in plant community composition occur between salt marshes and oligohaline / tidal freshwater marshes (TFM). TFMs are home to a diverse assemblage of species adapted to low salinity (Odum *et al.* 1984, Odum 1988, Perry and Atkinson 1997). With rising sea level, this plant community transition is dynamic, as saltwater intrusion into TFMs can lead to changes in species distributions (Perry and Atkinson 1997). Focusing research on the narrow range in salinity can help identify mechanisms behind the ongoing shift in species distributions.

A number of hypotheses may be invoked to predict the outcome when saltwater (a stressor) is introduced to a freshwater plant community. For example, the competition-stress hypothesis puts forward that abiotic factors will determine plant occurrence in stressful conditions unlike benign environments where competition may rule, as espoused by the Competition-Stress-Ruderal strategy (Grime 1977). On the other hand, the Resource-Ratio Hypothesis (Tilman 1985) argues that plants respond to
stress the same across all gradients and only interspecific competition determines community inhabitants. Wiens (1977) goes so far as to suggest the reverse – that competition is most severe in harsh environments. More recent studies also emphasize the role of facilitation, a positive interaction between species, especially in stressful environments (Bruno et al. 2003, Brooker et al. 2008, Maestre et al. 2009, Holmgren and Scheffer 2010).

These hypotheses have been actively tested in recent years, with observations both supporting and refuting the competition-stress hypothesis (see, for example, Guo and Pennings (2012) in Georgia tidal marshes). Early studies on the distribution of salt marsh plants supported the competition-stress hypothesis (e.g. Barbour et al. 1978). Plants with physiological tolerance to the stress pervade in stressful environments as found in saline wetlands. In these higher salinity environments, plants use energy for maintaining osmotic balance rather than for growth (e.g. Hellings and Gallagher 1992). Crain et al. (2004) demonstrated that Spartina alterniflora accumulated more biomass in the absence of both salinity and neighbors. Plant distribution in more benign, low-salinity, environments is dictated not by physiological tolerance, but by biotic factors such as competition (Grime 1977). Pennings et al. (2005) determined that Spartina's upper estuarine range distribution (i.e., at low salinity) was established by competition. Vegetation zones often correlate well with salinity tolerances (Callaway et al. 1990, Greiner La Peyre et al. 2001). Previous work investigating the interaction between salinity and competition mostly looked at higher levels of salinity, but even low salinity treatments (0, 2, 4, 8) have been shown to influence species abundance (Greiner La Peyre et al. 2001). The same researchers found that as the species were more stressed
by salt, competition became less important. A recent study testing competition between *Spartina* and *Phragmites australis* (Cav.) Trin. Ex Steud. (hereafter *Phragmites*) found both salinity and competition negatively affected biomass of both species, although *Phragmites* had a disproportionate decline in biomass relative to *Spartina* in salinity treatments of 10 and 28 (Medeiros *et al.* 2013).

Vegetation changes in tidal marshes in the eastern U.S. and Europe have been attributed to saltwater intrusion facilitated by sea-level rise (Perry and Hershner 1999, Engels and Jensen 2010, Sharpe and Baldwin 2012). Local hydrodynamics at Sweet Hall Marsh (37° 34' N, 76° 53' W), a component of the Chesapeake Bay National Estuarine Research Reserve System in Virginia that was a TFM in recent years, have created areas of the marsh where salinity is higher than others, with pore water salinity ranging from 0.4 to 4.9 with a mean just over 2 (chapter 1, this volume). Vegetation composition in the area undergoing saltwater intrusion has been documented since 1981; historically important species include *Peltandra virginica* (L.) Schott (a C3 broad-leafed freshwater plant) and *Leersia oryzoides* (L.) Sw. (a freshwater C3 freshwater grass), hereafter *Peltandra* and *Leersia*, respectively (Perry and Hershner 1999, Davies 2004). We observed large, monospecific stands of *Spartina* in a section of Sweet Hall Marsh for the first time in the fall of 2010 after dominant vegetation senescence. Water column salinity at a nearby water-quality monitoring station averaged 2.8 between 2009-2012, with annual means ranging from 1.4 in 2011, 2.8 in 2009 and 2012, and 4.0 in 2010 (NOAA 2012).

Moving downstream through estuarine environments, a vegetative transition occurs where freshwater moves into saltwater as rivers flow to the ocean. Sea-level
induced salinity intrusion, however, has reversed that perspective as the salt front moves upstream and alters the tidal freshwater environment. Given the recent expansion of *Spartina* in Sweet Hall Marsh, a TFM transitioning to oligohaline marsh, our objective was to test in a controlled mesocosm experiment how interactions between *Spartina* (at the competitive limit to its distribution) and three TFM species (two at the physiological limit to their distribution and one cosmopolitan species that can be found in freshwater to lower polyhaline environments, including TFM) varied along a small-range, salinity-stressor gradient between fresh and oligohaline water. Our overall goal was to determine the relative influence of biotic and abiotic stressors on the observed species transition in a TFM.

**Methods**

*Experimental Design*

We obtained nursery-raised individuals of three perennial, wetland vascular plant species: *Spartina* (a C4, facultative halophytic grass), *Peltandra*, and *Leersia*. *Phragmites* (a C3, salt-tolerant grass) rhizomes were excavated from Sweet Hall Marsh in late winter, cut to approximately 5 cm across the nodes and potted until sprouts emerged. Taxonomy used throughout this manuscript follows USDA (2013). We did not test the haplotype of the specific *Phragmites* rhizomes selected, but seven samples taken in the vicinity were identified genetically as the native haplotype (L.A. Meyerson pers. comm.). Propagules of the same size of each plant were chosen to create similar baseline conditions. *Spartina* was potted in sand outdoors in a 7.5L (2 gallon) container and paired with one of the other species or with another *Spartina* sprig and fertilized with slow release fertilizer (15 mL Osmocote® 19-6-12; Scotts Sierra Co., Marysville, OH).
Three pots containing the same species combination (*Spartina vs Peltandra, Spartina vs Leersia, Spartina vs Spartina*) and one pot of *Spartina vs Phragmites* (until week 6 when a second pot was added) were placed in a 129L (34-gallon) shallow plastic tub. The tubs were randomly organized in a block and replicated three times; each block contained a total of 12 tubs with all unique combination of species and salinity represented.

After initial transplant outdoors in May 2011, plants acclimated in pots for approximately two weeks in ambient conditions as the level of de-chlorinated fresh (tap) water level was slowly raised to approximately 10 cm, thereby filling the tubs to a standard flooding level below the soil surface. Because an earlier study investigating physiological responses to low salinity treatment found *Leersia* and *Peltandra* to have a negative response by a salinity of 4 (Sutter *et al.* 2013), salinity treatments were established at 0, 1.5 and 3. After the initial two week acclimation, tubs were brought to a salinity of 1.5 with Instant Ocean® Sea Salt (Cincinnati, OH), a commercially available synthetic sea salt. The final salinity adjustment to 3 was made after an additional 4 days. We chose to use a synthetic salt to minimize variability of sulfate and nutrients in naturally-derived seawater. Salinity of both the tub (biweekly) and pore water (weekly) was measured using a YSI conductivity meter (Yellow Springs, OH) and adjusted with de-chlorinated freshwater or salt additions. The actual salinities maintained are shown in Table 1. For the remainder of the manuscript, salinity treatments of the intended 0, 1.5 and 3 will be referred to as low, medium, and high treatments, respectively.
Plant Response Measurements

Photosynthesis, measured as net carbon dioxide (CO$_2$) assimilation (A$_{net}$) was measured weekly during peak sunlight using a TPS-2 portable infrared gas analyzer with a leaf cuvette (PP Systems, Amesbury, MA). For the grasses, the third fully expanded leaf on an arbitrary stem was measured every ten seconds for 30 seconds. *Peltandra* rarely had more than one leaf from which to select; when it did, the youngest fully expanded leaf was used. Measurements not meeting the instrument guidelines (Bergweiler pers. comm.) were discarded; those remaining were averaged over the sampling interval to represent the experimental unit of the tub. Data were recorded for weeks 1-4 and 6-7.

Individual plants were harvested eight weeks after establishing the final salinity treatment levels. Aboveground and belowground plant material was separated, rinsed, and dried at 60°C to constant mass, and individual plant material was milled for nutrient analysis. Total phosphorus (P) was determined using a modified ashing/acid extraction process and colorimetric analysis (Chambers and Fourqurean 1991). Tissue carbon (C) and nitrogen (N) content was determined using a PerkinElmer 2400 elemental analyzer (PerkinElmer, Waltham, MA).

Data Analyses

Statistical analyses for biomass and nutrients were conducted using a 2-way Type I ANOVA with the base R package lm() function (R Core Team 2013). Each mean represents the response of nine plants, except *Spartina* vs. *Phragmites* where the sample size is six. For statistical comparison, however, the response was averaged across the three plants within each tub to avoid issues of pseudo-replication and compared only across tubs. Standard ANOVA assumptions were evaluated; to correct for
heteroscedasticity found in biomass and nutrient residuals, we modeled the inverse variance for the response variable within each tub and used it as a weighting factor (Pinheiro and Bates 2000). Post hoc comparisons between treatment level effects employed pairwise.t.test() with non-pooled standard deviation; the False Discovery Rate adjustment minimized Type I errors without the stringent conditions found in family-wise comparisons such as Bonferroni, making this a more powerful comparison (R Core Team; Benjamini and Hochberg 1995; Hochberg and Yekutieli 1995). Comparisons of Spartina biomass to each competitor within each salinity treatment and compartment (aboveground and belowground) employed t.test() with non-pooled standard deviation.

Results

Net Photosynthesis

Spartina $A_{\text{net}}$ was equivalent across all salinity treatments; hence we collapsed the treatments and reviewed the response of Spartina $A_{\text{net}}$ in the presence of each competitor over the study period (Figure 1). Spartina followed a similar pattern of increase over the growing season when paired with itself and with Peltandra. When paired with the other grasses, Leersia and Phragmites, Spartina exhibited a flatter pattern over all weeks and had a lower magnitude of $A_{\text{net}}$ than with itself or Peltandra.

Biomass

In general, Spartina biomass out-performed the competitor with which it was planted. At the low and medium salinity treatments, however, Leersia biomass exceeded that of Spartina ($p<0.001$ at low and $p<0.005$ at medium treatments), but Spartina biomass was equivalent to Leersia at the high treatment salinity. In pots pairing Spartina and
Phragmites, both species had lower biomass than grasses in other species combinations (Figure 2). Spartina biomass – both aboveground and belowground – presented significant interaction effects (Table 2). At all salinity levels, the aboveground, belowground, and total biomass of Spartina were lower when grown in the presence of Leersia or Phragmites than when Spartina was the only species in the pot (Figure 2). In contrast, with the exception of belowground biomass at high salinity, there were no differences in biomass between Spartina grown with Peltandra or grown alone (Figure 2).

The ratio of total biomass of Spartina relative to each species increased with increasing salinity (Figure 3). The two grasses, however, remained near a one to one ratio across the treatments; whereas, Spartina biomass consistently outpaced Peltandra more than fourfold, even in freshwater.

Nutrient Content

Plants responded significantly to salinity, competitor and their interaction on both aboveground and belowground tissue C (Table 2). Pooled across all species, C in Spartina aboveground tissues at the high salinity treatment was significantly lower than at low salinity (p<0.05). Spartina accumulated equivalent C aboveground when paired with itself, Peltandra and Leersia, but not when paired with Phragmites (Figure 4), where aboveground C content was lower (p<0.05) and more variable. We interpret the pattern of aboveground C content through salinity treatments to be the same when Spartina was paired with itself, Peltandra and Leersia, with a deviation to that pattern in the presence of Phragmites (Figure 4). Belowground tissue C was equivalent when pooled across salinity treatment and only decreased significantly (p=0.01) when paired
with *Leersia* (Figure 4). The pattern belowground is less clear, but the behavior of *Spartina* across salinity treatments was similar when paired with itself or *Peltandra*, with greater variability in the presence of the two grasses (Figure 4).

In both aboveground and belowground tissue of all species treatments, *Spartina* N-content was highest at the lowest salinity treatment and decreased with the addition of salt (Figure 5). We detected a main treatment effect of salinity (pooled across all species) of N-content in aboveground tissue (Table 2), with the low salt treatment containing greater N than the other treatments. *Spartina* aboveground N-content was not significantly different when paired with itself or *Peltandra*, but aboveground N decreased less in the presence of both grasses, *Leersia* and *Phragmites*. The pattern through salinity treatments was consistent when paired with all species except *Phragmites*, where there was a sharp increase in both N-content and variability.

Belowground, tissue N was also lower (p=0.01) for the medium and high salinity treatments (Figure 5). Belowground tissue N increased and was more variable when *Spartina* was paired with *Phragmites*. The pattern from low to high salinity followed the same trend as the aboveground tissue N.

Salinity and competitor played a significant role in both aboveground and belowground tissue P in *Spartina*, and the interaction effect of salinity and competitor was significant in aboveground P-content (Table 2). ANOVA results for the main salinity treatment effect must have been driven by the interaction, as post hoc tests did not differentiate any salinity treatment effects aboveground or belowground in tissue P. The competitors *Leersia* and *Phragmites* affected aboveground tissue P in opposing directions: *Spartina* P-content decreased when paired with *Leersia* (p<0.05) and
increased with Phragmites (p<0.05). The pattern of P content in Spartina, both aboveground and belowground through salinity levels, was similar when paired with itself, Peltandra and Leersia. In the presence of Phragmites, Spartina P increased both in magnitude and in variability in aboveground tissues (p<0.05). Spartina P content increased (p<0.05) belowground when paired with Phragmites.

We found significant effects of salinity, competitor, and their interaction on both aboveground and belowground Spartina tissue C:N (Table 2). The interaction terms were likely driven by the ANOVA results for the main salinity treatment effect, as post hoc tests did not differentiate between salinity treatments. Spartina tissue C:N was significantly lower (p<0.05) aboveground when in the presence of Phragmites. Across salinity treatments, Spartina C:N ratio did not change dramatically in the presence of Peltandra and Leersia. In the presence of Phragmites, however, the ratio dropped and again increased in variability. In belowground tissue, no discernible difference in specific salinity or competitor treatment was found.

Despite a significant, experiment-wide treatment effect (Table 2), post hoc analysis of Spartina C:P did not vary significantly by salinity treatment or when paired with itself or the other species. Tissue N:P in aboveground Spartina decreased at highest salinity, but did not change in the presence of competitors. Through the short salinity gradient, the N:P ratio in Spartina did not change dramatically when paired with itself or other species, but aboveground variability in N:P was higher in the presence of Phragmites. For belowground tissue, no discernible difference in individual salinity or competitor treatments was detected by post hoc t-tests, despite the ANOVA results indicating main effects of both salinity and competitor.
Discussion

Although considered a potentially poor competitor in low-salinity marshes, both field observations and the results of the current mesocosm study confirm that *Spartina* is capable of establishing and out-performing some tidal freshwater marsh (TFM) species. The narrow salinity range of the current study is interesting because *Peltandra* and *Leersia* are affected by subtle shifts in salinity through their physiological intolerances to salt, unlike *Spartina* and *Phragmites*. As a result, the competitive balance between species may shift due to the abiotic stressor. *Spartina* is a stronger performer at higher salinity where physiological salt intolerance of freshwater species comes into play (Sutter *et al.* 2013). Studies of *Spartina* have found that this species is a poor interspecific competitor (Crain *et al.* 2004) but competition is mediated by salt (Pennings *et al.* 2005), although most tested salt treatments have been far higher than those we applied. In this study, *Spartina* out-performed *Peltandra* at all salinity treatments by all traits measured, behaving similarly to when paired with itself (Figures 1-5). Paired with *Spartina*, *Leersia* is an intermediate performer, and *Phragmites* is an apparently strong performer. Like Guo and Pennings (2012), we find inconsistencies with the competition-stress hypothesis; in the benign range of our salinity (stress) gradient, dominant TFM species were unable to outcompete a species (*Spartina*) whose distribution has been considered limited by competition in freshwater environments.

*Spartina* interacted with each species differently. The comparable photosynthetic performance of *Spartina* performance when paired with itself or with *Peltandra* offers no evidence of declining *Spartina* brought about by the presence of a *Peltandra* neighbor (Figure 1). In fact, since the response is so similar (solid line in
Figure 1a and d), results suggest that *Spartina* is fully out-competing *Peltandra* in terms of CO$_2$ assimilation in this experiment. When paired with *Phragmites*, however, *Spartina*’s pattern of photosynthesis changed throughout the study period, indicating that *Phragmites* is impacting *Spartina* by its presence. The photosynthetic response of *Spartina* when paired with *Leersia* is less clear but appears to be intermediate between *Peltandra* and *Phragmites*. Collectively, these responses suggest that *Spartina* may perceive the grasses as competitors but not so with *Peltandra*.

The presence of either grass reduced *Spartina* biomass relative to *Spartina* grown alone, but *Peltandra* did not diminish *Spartina* biomass accumulation – supporting the interpretation that *Spartina* may perceive the grasses, but not *Peltandra*, as competitors. *Leersia*’s strong performance in freshwater in the presence of Spartina further supports this interpretation. Perhaps *Leersia* would have been able to maintain historical dominance in the Sweet Hall Marsh high marsh (Davies 2004) in the absence of saltwater intrusion, thereby preventing *Spartina* from establishing. *Leersia* is not, however, always a strong competitor in freshwater. While the current study did not test the *Leersia* vs *Phragmites* interaction, Farnsworth and Meyerson (2003) found the invasive *Phragmites* haplotype outcompeted *Leersia* in freshwater. *Phragmites* and *Spartina* biomass were virtually equivalent when potted together in low salinity. However, at the high salinity treatment *Phragmites* biomass declined relative to *Spartina* whereby *Spartina* biomass increased (Figure 2). When paired with *Phragmites*, *Spartina* biomass was far below that measured when *Spartina* was by itself – suggesting that *Phragmites* was a strong interspecific competitor in this study. This plant-plant combination appears to reveal the greatest interaction, playing out in both
aboveground and belowground compartments. Medeiros et al. (2013) found that both species were affected by salinity and competition, likely in the form of shading and belowground interactions, and noted that *Spartina* has higher salt tolerance. Others have shown that in the presence of “adequate” nutrients (as in the current study), belowground competition would not be expected (Emery et al. 2001) as there is some cue to the plant to curtail root growth to locate nutrients. We show that at low levels of salinity used here, *Spartina* biomass is not negatively impacted. In fact, *Spartina* biomass when grown with *Phragmites* in our study was lower at the lowest levels of salinity, potentially due to a high sulfate requirement of *Spartina* (Stribling 1997) that was provided with even small amounts of Instant Ocean® but missing from the freshwater treatment. Consistent with Medeiros et al. (2013), *Phragmites* in this experiment experienced limited growth relative to other grasses in the presence of *Spartina*, suggesting a greater role of competition between the two species. Although we did not grow *Phragmites* in monoculture, we have no reason to predict the low biomass found here compared to its typical large size.

No competitive reduction in aboveground C content was observed in *Spartina* when paired with *Peltandra* or *Leersia*. When paired with *Phragmites*, *Spartina*’s decline in C suggests competition between the two species in that *Spartina* was unable to build C-based structural resources in the presence of *Phragmites*. Interestingly, as *Spartina* experienced the decline in C with increasing salinity in the presence of the two grasses, its biomass increased. The increased biomass might reflect an increase in vacuole size required to respond to a higher content of no or low-C metabolites associated with salt stress. Vacuoles hold inorganic and organic salts, organic acids,
solutes, wastes and more which may contribute to biomass but not nutrient content (Taiz 1992, Marty 1999). In the presence of Phragmites regardless of salinity treatment, Spartina had an overall lower magnitude of biomass and C biomass relative to other pairs, suggesting that Phragmites prevents Spartina from making an investment in structural tissues.

Tidal freshwater marsh species tested in this study and undergoing salinity stress have been shown to increase tissue N both aboveground and belowground (Sutter et al. 2013). They do not shunt N solely to belowground tissues (Sutter et al. 2013) as might be expected with perennial vegetation (Geng et al. 2007, Zewdie et al. 2007). Acquisition of N resources here did not follow the same pattern; pairing Spartina with Leersia or Phragmites led to less N reduction in aboveground and belowground tissues than when Spartina was paired with itself or Peltandra. Nitrogen reduction occurred with any salt addition in the presence of Leersia and only at the highest salinity treatment in the presence of Phragmites. The actual N concentrations in the presence of a competitor, however, varied in different directions by species, with Spartina N lower when paired with Leersia and higher when with Phragmites. The similar N accumulation in Spartina tissue grown alone or in the presence of Spartina across the short salinity gradient suggests an absence of competition with Peltandra; whereas, the variation in the presence of Phragmites and Leersia suggests competition. An increased variability in both Spartina tissue C and N in the presence of Phragmites might reflect that Phragmites competition reduces Spartina’s carbohydrate stores and leads to more protein formation. Alternately, the propagation of Phragmites from rhizome rather than seed germination (as occurred in the nursery-obtained seedlings) may have provided
adequate N resource for *Phragmites*, leaving more soil N for *Spartina* to assimilate. This is unlikely, however, as the rhizome cuttings were small enough to minimize extensive N-stores.

*Spartina* holds little P in aboveground tissues relative to the freshwater species (Sutter et al. 2013), but the magnitude increased in the presence of *Phragmites* relative to that grown with another *Spartina* plant (Figure 5). Perhaps a signal between plants somehow stimulated *Spartina* to gather additional nutrients with this strong competitor, *Phragmites* (sensu Pearse et al. 2013). Belowground, in the presence of *Phragmites*, *Spartina* holds more P than when grown alone but the difference declines at the highest salt treatment level (to equivalent levels of *Spartina* grown without an inter-specific competitor in freshwater). If any signaling takes place to retain P, it is mediated by the physiological need to address increasing osmotic pressure at the highest salt treatment.

The equivalent C:N response in *Spartina* along the narrow salinity gradient is consistent with a related study (Sutter et al. 2013) for which C:N in *Spartina* grown alone remained constant in aboveground and belowground tissue along a salinity gradient twice the range (0-6) of the present study. The presence of another individual – whether same species or different – did not affect this trend: The decline in N was concomitant with a decrease in C when salt is introduced. The identity of the interspecific competitor in this study directed whether C:N increased or decreased when compared to *Spartina* grown in monoculture; C:N declined sharply in the presence of *Phragmites* and increased in the presence of *Leersia*. Simpson et al. (2013) found that *Avicennia germinans* leaf C:N decreased by 27% when competing with salt marsh
species. These are woody species in an acidic environment, which may lead to the differences that was found in this experiment.

Medeiros et al. (2013) recently demonstrated that salinity and competition affect both Spartina and Phragmites success through biomass reduction. From the current study at the low end of the estuarine salinity gradient, the interspecific competition is largely between Spartina and Phragmites where neither species is currently dominant. In this low salinity zone, TFM species have historically enjoyed “protection” from Spartina and Phragmites, perhaps through diversity and successful competition with these putative invaders. Now that salinity is intruding, the ability of the TFM species to compete successfully may be compromised, and Spartina and Phragmites may eventually become dominant in these marshes. Both studies confirm an interaction between Spartina and Phragmites, and we show that Spartina’s interaction with Phragmites is different than Spartina’s interaction with TFM species.

Although both Spartina and Phragmites appear to be competitive dominants in this mesocosm experiment, other factors may explain why Spartina has yet to dominate the entire marsh in the field. Keammerer and Hacker (2013), for example, found negative effects of vascular plant neighbors strongest in early life stages of germination and seedling survival, so that recently introduced Spartina may experience a lag prior to full establishment and spread. Disturbance may also contribute; Baldwin & Mendelssohn (1998) found that increased salinity and inundation only reduces species richness following disturbance created by removing aboveground vegetation. In Sweet Hall Marsh, muskrats are common herbivores that de-vegetate pockets within the marsh. If Spartina’s arrival originated at a muskrat eat-out area, Spartina may have
successfully established itself without the presence of other vegetation. Alternatively, *Spartina*’s arrival at this time and not historically may be driven by a period of higher sea level which allowed *Spartina* propagules to find placement in areas of the marsh previously occupied by dense TFM vegetation such as *Peltandra* and *Leersia* – setting up conditions that facilitated *Spartina* establishment in an area of muskrat eat-out that allowed it to outcompete younger *Peltandra* seedlings arriving by higher water.

Chambers *et al.* (2003) predicted that *Phragmites* would become the dominant in TFM exposed to salinity; however, the current study suggests that *Spartina* may also compete successfully for space in these same marshes. It is possible that the perfect storm occurred in this marsh: A disturbance from muskrats and rising sea level delivering *Spartina* propagules to an area not previously supporting the species. The sulfate in the sea water was reduced under flood conditions to meet *Spartina*’s sulfate requirement, allowing it to flourish in Sweet Hall Marsh. Instead of arriving at the predicted oligohaline marsh dominated by *Phragmites*, the TFM neighborhood is in transition, and *Spartina*’s ability to outperform common species in the area may lead it to be the new dominant in low saline marshes in the lower Chesapeake Bay.
Literature Cited


Sutter LA, Perry JE, Chambers RM. (2013) Tidal freshwater marsh plant responses to low level salinity increases. Wetlands (accepted, not yet published)


Table 1 Mean salinity achieved over the study period for each treatment in the experimental unit (tub) and within each pot containing a species mixture.

<table>
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<th>Salinity Treatment</th>
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<th>Tub SE</th>
<th>Porewater Mean</th>
<th>Porewater SE</th>
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Table 2 ANOVA p-value results comparing *Spartina* salinity, competitor and their interaction; for statistical comparisons, n=3 (see Methods section for details).

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**Fig. 1** Net carbon assimilation ($A_{\text{net}}$) of *Spartina* in the presence of another species or itself when grown as neighbors over the study period. The solid line represents *Spartina*; the dashed line represents the competitor identified over each panel.
**Fig. 2** Total biomass of *Spartina* and its competitor (as labeled in each panel) both aboveground and belowground after growing together for 8 weeks in low salinity levels. Within each panel, *Spartina* is depicted in the first three bars and the competitor in the last three bars. Large bars represent the mean and lines represent standard errors of the mean. Different lower case superscripts in the panel labels indicate statistical significance (p<0.05) of total *Spartina* biomass grown in the presence of itself or competitor. Asterisks adjacent to bars represent significant difference (p<0.05) of *Spartina* biomass in the presence of the competitor when compared to *Spartina* planted with itself. To determine means, n=9 for all except *Spartina vs Phragmites*, where n=6; for statistical comparisons, n=3 (see Methods section for details).
**Fig. 3** Response ratios of *Spartina* total biomass relative to each of the competitors' total biomass tested in this study (*Peltandra*, *Leersia* and *Phragmites*) across a short salinity gradient. Correlation coefficients: *Peltandra* = 0.8844; *Leersia* = 0.9968; *Phragmites* = 0.9846.
Fig. 4 Spartina tissue carbon of in the presence of named competitor (Peltandra, Leersia and Phragmites) or itself. The solid line in the center of the box plot represents the median (n=9 except Spartina vs Phragmites where n=6) with the first and third quartiles filling the white box, and the remaining quartiles in the whisker lines extending beyond the box. Outliers are shown by points outside of the lines.
Fig. 5 *Spartina* tissue nutrient content (% dry weight) and ratios (molar) in the presence of named competitor (*Peltandra, Leersia* and *Phragmites*) or itself after growing together for eight weeks at low (0), medium (1.5), and high (3) salinity (mean ± SE, n=9 except *Spartina vs Phragmites* where n=6).
CHAPTER 4

A test of top-down control on plant production in low-salinity tidal marshes
Abstract

Saltwater intrusion brought about sea-level rise is expected to increase the amount of nitrogen (N) in tidal freshwater marsh (TFM) plant tissues, therefore increasing tissue palatability to herbivorous insects. We tested biomass and nutrient response to insect exclusion along a narrow salinity gradient from tidal freshwater to oligohaline marshes. Overall biomass did not vary across treatments; however, *Peltandra virginica* (a salt-intolerant, C3, broad-leafed perennial) biomass in the TFM increased (p<0.05) in insect exclosures relative to controls. Tissue nitrogen (N)-content in *Zizania aquatica* (a low-salt tolerant, C3, annual grass) increased significantly (p<0.05) to release from herbivory. Increasing levels of salt may have little effect on biomass in these marshes, as *Peltandra* is replaced more salt-tolerant species, but nutrient dynamics may shift as species like *Zizania* adjust the pool of N-content in plant tissues.

Introduction

Identifying the forces driving species productivity is one of the central emphases in vegetation ecology. In tidal marshes, primary production has long been held to be driven by abiotic (or bottom-up) forces, including oxygen (Howes *et al.* 1981, Mendelssohn *et al.* 1981), sulfide (Bradley and Morris 1990), nutrients (Sullivan and Daiber 1974, Osgood and Zieman 1993), and salt (Haines and Dunn 1976, Bradley and Morris 1990). Albarracín (2005) found that bottom-up factors were more important than top-down factors in controlling herbivores in a Florida salt marsh along a salinity gradient (4-18 ppt), and Menge (1992) concluded that herbivory does not affect salt marsh plants. At the interface of terrestrial and aquatic systems with relatively weaker and stronger top-down
controls, respectively (Schmitz 2006), marshes typically were grouped with terrestrial ecosystems where abiotic forces have been shown in many cases to control primary production, but that paradigm is changing.

In recent years, however, consumers have been identified as important controllers of salt marsh productivity, whether the consumer is mammalian (feral horses (Turner 1987, Furbish and Albano 1994), rodents (Gough and Grace 1998, Crain 2008), hares (van der Wal et al. 2000) or nutria (Evans 1970, Taylor and Grace 1995)), avian (snow geese (Smith and Odum 1983, Jeffries 1997)), or invertebrate (crabs (Jackewicz 1973, Kraeuter and Wolf 1974, Pennings et al. 1998, Bortolus and Irabarne 1999) or periwinkle snails (Silliman and Zieman 2001, Silliman and Bertness 2002)). Insects also affect salt marsh biomass, at least in some cases (Bertness et al. 1987, Bertness and Shumway 1993, Daehler and Strong 1995, Daehler and Strong 1996). Insects reduced biomass in eutrophic but not pristine New England salt marshes (Bertness et al. 2008). Finke and Denno (2005) found herbivores reduced biomass in greenhouse experiments but not in the field because predators reduced the number of consumers. Pennings et al. (2009) suggest that, although variable, herbivory pressure in low latitude salt marshes is stronger than at high latitudes. Ho and Pennings (2013) present a latitudinal gradient of palatability that is the reverse of herbivory pressure.

Little work testing the importance of herbivory has occurred in mid-latitudes or in low salinity tidal marsh systems. In New England, Crain (2008) found that mammalian herbivory increased in some plant species of lower salinity marshes, but the mechanisms for which species the herbivore selects remain to be tested. Plant species most affected by herbivory may hold higher nutritive value. For example, Couture et al. (2010) found that
improved biomass quality (as indicated by decreases in carbon (C): nitrogen (N)) led to increases in consumers (and also to the consumers' nutritional quality to their predators). Low salinity marshes contain plants with high nutritive value and less structural investment (Sutter et al. 2013) relative to the common mid-latitude salt marsh dominant, *Spartina alterniflora*.

In experimental tests of some of the dominant plant species common to tidal freshwater and oligohaline marshes of the lower Chesapeake Bay experiencing sea-level induced saltwater intrusion, biomass quality of two glycophytes (as measured by C:N and C:phosphorus (P)) increased when subjected to sub-lethal salinity (Sutter et al. 2013). Previous studies of the same system (Perry and Hershner 1999, Davies 2004, chapter 1 of this volume) documented a decrease in biodiversity as salinity has intruded into tidal marshes in the lower Chesapeake Bay, although, overall, the low salinity marshes exhibit higher diversity relative to salt marshes. Because experimental results testing the relationship between plant biomass consumed to the diversity of the plants have been equivocal (Cardinale et al. 2011), the current study tests whether any changes encountered in biomass in this system could be driven by changes in biomass quality (C:N and C:P) and subsequent consumption. Bowdish and Stiling (1998) found that consumer abundance decreased with added salinity in a salt marsh, and Levine et al. (1998) found increased plant nitrogen (and presumably a decreased C:N that they did not measure) content has a positive effect on insect herbivore abundance.

We observed few vertebrates (other than muskrats) in the low-salinity wetlands along a gradient from tidal fresh to oligohaline marshes in the lower Chesapeake Bay tributaries, but we observed many insects during related sampling. Therefore, this study
tested the role of insect consumers on marsh plant productivity. With higher plant species richness and increased biomass quality in tidal freshwater marshes (TFMs) relative to salt marshes (Sutter et al. 2013), we hypothesized that consumers would impact the productivity of tidal freshwater marshes. We further hypothesized that as sea-level rises and salinity intrudes, saltwater additions may drive plants to be more nutritive, increasing pressure from top-down controls in addition to the bottom-up control of salt stress.

Methods

Study Sites

We used three marshes in the Pamunkey River, a tributary to the York River Estuary in the lower Chesapeake Bay (Virginia, USA) with similar dominant vegetation. Two of the marshes were located in the Sweet Hall Marsh component of the Chesapeake Bay National Estuarine Research Reserve system in Virginia (CBNERRVA). Both Sweet Hall sites were in the low range of an oligohaline marsh, with Sweet Hall-above (SH-a) slightly more saline than Sweet Hall-below (SH-b) due to hydrodynamics established by a thoroughfare introduced approximately one century ago. The area landward of the thoroughfare is experiencing increasing salinity, and the vegetation composition has changed in response (Perry and Hershner 1999, Davies 2004); the marsh on the river side of the thoroughfare is crossing into oligohaline ranges (Neubauer and Anderson 2003). Cumberland Marsh (CM) is a TFM above the influence of salt. Boardwalks (~37m in length) were constructed at each marsh to prevent damage to the vegetation during repeated visits.
Site locations are shown in Figure 1 and a summary of the characteristics of the different marsh sites is provided in Table 1.

**Insect Exclusion Experiment**

Each of the marshes was surveyed monthly for insect presence during the summer of 2012 using malaise traps at the end of each boardwalk, sweep nets, and randomly placed pitfall traps along each boardwalk. Pitfall traps were constructed using the specifications in Parys and Johnson (2011), modified with the addition of a funnel to prevent small mammals and herpetiles from entering the traps. Insects were preserved for identification, sorted, and identified to family taxon. Samples were then sorted according to trophic level, where 'herbivore' contained insects that consume pollen, nectar, and plant tissue. ‘Carnivore’ contained insects that are predatory, parasitic, scavengers, and blood-sucking. ‘Detritovores’ contained insects that mainly fed on decomposing tissue or other decaying organic matter.

Caged exclosures (approximately 1m X 1m X 2m) were constructed with wooden furring strips with light charcoal colored screen (~6 meshes cm\(^{-1}\)) secured to the strips. All edges were sealed with screen to prevent small insect entry into the exclosure. Caged controls were constructed with the same frame dimensions adding 40cm of screen at the base to mimic any effects of tidal influence near the soil surface in the full exclosure, and the same top as the exclosures to capture any shading effect from the control. Screens were buried vertically 10-15cm into the sediment to inhibit aquatic insect larvae from entering the cage from below.

In early May 2012, five exclosures and five caged controls were installed and five full controls marked at randomly assigned positions along existing boardwalks at each of
the three sites. Exclosures were visited every 2-3 weeks and after high-wind events to ensure they were still standing and sealed. In late July 2012, we harvested aboveground vegetation at the soil surface from 0.5m X 0.5m quadrats in the center of each treatment. Vegetation was rinsed, separated by species and dried at 60°C to constant mass. Taxonomy follows USDA (2013). Individual plant matter was milled for nutrient analysis. Tissue C and N content was determined using a PerkinElmer 2400 elemental analyzer (PerkinElmer, Waltham, MA). Total P was determined using a modified ashing/acid extraction process and colorimetric analysis (Chambers and Fourquarean 1991).

Data Analyses

Statistical analyses for biomass and nutrients were conducted using a 2-way Type I ANOVA (marsh X Treatment) with the base package anova function (anova()) within R statistical software (R Core Team 2013). ANOVA assumptions were evaluated and accepted. After determining there was no significant difference between controls and caged controls, we grouped both into a single control group to compare against the cages. The three most dominant plant species were each tested independently; since each species was expected to behave differently, this allowed a more direct interpretation of the herbivory effect. Post hoc comparisons between treatment level effects employed pairwise.t.test() with non-pooled standard deviation; the False Discovery Rate adjustment minimized Type I errors without the stringent conditions found in family-wise comparisons such as Bonferroni, making this a more powerful comparison (R Core Team 2013; Benjamini and Hochberg 1995; Benjamini and Yekutieli 2001). Nonmetric multidimensional scaling using the metaMDS() function in the R vegan package was
used to visualize the role of marsh and treatment in driving biomass response. The starting point was established randomly with the number of parameters (k) set to 2.

**Results**

Across all three marshes, 59 families of insects were identified. Of the individuals found, herbivorous families constituted 56% of those collected in CM, 63% in SH-b, and 53% in SH-a (Table 2).

*Plant biomass*

The freshwater study site (CM) had a plant species richness of 16, followed by SH-b at 3 and then SH-a (most saline) at 6. Only three species were found at all marshes: *Peltandra virginica*, *Zizania aquatica* and *Bidens laevis* (hereafter *Peltandra*, *Spartina*, and *Bidens*, respectively). An ordination of plant biomass overlayed on the convex hulls of both marsh and treatment (Figure 2) shows that neither marsh nor treatment is an obvious driver of biomass response. There was no significant increase in total vegetation biomass in the exclosures protected from insect herbivory (Figure 3). As an exploratory measure, exclosures were compared to controls at the most saline site, SH-a, as they appeared to differ graphically; however, this exploratory ANOVA test of biomass in cage versus control in SH-a indicated statistical equivalency (p>0.05). Assessing the biomass of only the three common species’ contribution to the total biomass by species and treatment, a significant interaction effect existed between marsh and species (p<0.001) but no treatment effect (Figure 4).
Nutrients

The amount of C in plant tissue pooled across species and pooled across marshes differed significantly (Figure 4; both p<0.001), but there was no effect of the insect exclosure treatment on tissue C-content. Tissue P-content also exhibited differences by marsh (p=0.02) and species (p=0.03), but we found no effect of insect exclusion on the tissue P-content (Figure 6). Tissue N-content varied by species (p<0.001) and marsh (p<0.001), and also by treatment (p<0.001; Figure 6). Pooled across all marshes and species, tissue-N was higher in tissues protected from herbivory (i.e. the exclosure treatment).

Investigating species individually, tissue N-content differed between exclosures and controls only in Zizania, where plants in exclosures were significantly higher (p<0.001) relative to controls. Zizania tissue N-content also was higher at CM (freshwater) relative to SH-a (oligohaline endpoint; p<0.05). Zizania tissue C:N responded to both treatment (p<0.001) and marsh (p=0.03), with C:N lower in Zizania protected from herbivory relative to control conditions.

Discussion

Despite strong evidence that top-down controls limit primary productivity at mesohaline and more saline marshes at locations along the U.S. East Coast (e.g. Pennings et al. 1998, Silliman and Zieman 2001, Crain 2008, Bertness et al. 2008), herbivorous consumers do not appear to be reducing primary productivity in low salinity marshes in the Pamunkey River. Our research results provide an additional line of evidence supporting the long-held paradigm that bottom-up controls more often drive vegetative productivity – at least relative to insect herbivory in low salinity marshes, although a trend may be developing in the hypothesized direction in SH-a (Figure 3). Only in freshwater (CM) did one
species, *Peltandra*, respond positively in biomass amount when released from herbivory. No difference in biomass among treatment groups was observed in the presence of even low salt levels at either Sweet Hall Marsh site for any species.

Higher plant diversity in low salinity marshes (relative to more saline marshes) may keep populations of insect grazers from exploding, thus minimizing herbivory on any given species, although experimental evidence is sparse and a consistent pattern has not emerged (Cardinale *et al.* 2011). Alternately, insect predators that are highly abundant in this system could prevent insect herbivores from over-grazing the vegetation through consumption (see Finke and Denno 2005) or trait mediated behavioral changes (in other words, insects we observed are present but not eating. Although few, if any, experiments have been conducted in low salinity marshes, predators in other systems have been shown to influence insect herbivory through reduced feeding, habitat shifts, and other cascading effects (Schmitz *et al.* 2004). Rypstra and Buddie (2013) found that the presence of spider silk alone was a mechanism for limiting herbivory. Other options include (1) the plants have a compensatory growth response to grazing pressure (e.g. Leriche *et al.* 2001) so that growth increased in controls to match standing crop biomass in the absence of grazing, or (2) resistance to herbivory using weaponry like condensed tannins (Eskelinen *et al.* 2012). If weaponry were the case in our experiment, we would have expected to see higher tissue C-content, although we did not specifically measure tannins or other specific molecules to test this possibility.

We hypothesized that plant tissues in higher salinity marshes would have higher N-content because of additional amino acids often used to balance osmotic pressure (Munns 2002) and/or lower C content from greater respiration under salt stress. The
higher N and/or lower C content would drive down C:N, thus increasing biomass quality and potentially grazing pressure through enhanced palatability. We did not observe this effect: Tissue C:N increased at SH-a relative to the other marshes in the early season dominant, *Peltandra*, and tissue C:N did not respond to herbivory. *Zizania*, the late season dominant, displayed the reverse trend in tissue C:N, increasing in C:N in more saline environments only in the controls. It is possible that the decreasing tissue N-content in controls is a response to herbivory whereby grazing pressure disrupts the ability of the plant to develop amino acids to replace tissues being grazed or loses N through gutation (Goatley and Lewis 1966). Tissue C:N remained constant in the vegetation protected from herbivory, suggesting that plants released from herbivory pressure may have to invest less energy in producing metabolites like amino acids (proline, glycine betaine, etc).

We predicted that marsh vegetation would respond to herbivory release and anticipated that, of all the species appearing in our plots, *Peltandra* would (1) have the highest nutritive content with elevated salinity and (2) be the most susceptible to herbivory thus exhibiting higher biomass in the absence of grazing. Our findings differ both from our anticipated results and by species: *Zizania* was the only species exhibiting a response – only in N-content and the resultant change in C:N. Why *Zizania* responded and not the other species requires further investigation. It may be that the fast-growing annual species had less time to incorporate N into its tissues without any enhancement from belowground reserves. Species in an un-manipulated environment (i.e. controls) varied in their biomass response in marshes along an increasing salinity gradient: *Peltandra* increased, *Bidens* decreased, and *Zizania* had highest biomass in the mid-
salinity marsh, suggesting that plants responded more to salinity stress than a release from insect herbivory.

Our work with insects as top-down controllers is just one facet contributing to a generalized hypothesis of the top-down versus bottom-up control of plant productivity. This test of a single consumer group found them to be unimportant to plant production in low-salinity marshes in the mid-Atlantic, but they may have important implications for nutrient availability to certain species of plants.
Literature Cited

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Haines BL, Dunn EL (1976) Growth and resource allocation responses of Spartina alterniflora to three levels of NH$_4$–N, Fe, and NaCl in solution culture. Botanical Gazette 137:224-230


Jackewicz JR (1973) Energy utilization in the marsh crab, Sesarma reticulatum (Say). Thesis. University of Delaware, Newark, Delaware, USA


Table 1. Summary of site descriptors for each of the marsh locations investigated in this study (Reay and Moore 2009, Perry and Atkinson 2009, *chapter 1, this volume)

<table>
<thead>
<tr>
<th></th>
<th>Sweet Hall Marsh—above</th>
<th>Sweet Hall Marsh—below</th>
<th>Cumberland Marsh</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2011 Porewater Salinity</strong></td>
<td>Mean (SE) [Range]</td>
<td>Mean (SE) [Range]</td>
<td>Mean (SE) [Range]</td>
</tr>
<tr>
<td>2011 Porewater Salinity*</td>
<td>2.1 (0.1) [0.4-4.9]</td>
<td>1.7 (0.1) [0.6-5.7]</td>
<td>0.2 (0.1) [0.1-0.5]</td>
</tr>
<tr>
<td>Dominant vegetation</td>
<td><em>Peltandra virginica, Zizania aquatica with invading Phragmites australis nearby</em></td>
<td><em>Peltandra virginica and Zizania aquatica</em></td>
<td><em>Peltandra virginica, Zizania aquatica and Pontederia cordata</em></td>
</tr>
<tr>
<td>River distance from mouth of York River</td>
<td>75 km</td>
<td>87 km</td>
<td>above confluence of Pamunkey River with Mattaponi River forming the York River</td>
</tr>
</tbody>
</table>
Table 2. Insect families found (using net sweeps, malaise traps and pitfall traps) from June - October 2012 along the Pamunkey River marsh sampling gradient. Families containing herbivorous species are marked with an “*”.

<table>
<thead>
<tr>
<th>Family</th>
<th>Marsh CM</th>
<th>SH-b</th>
<th>SH-a</th>
<th>Family</th>
<th>Marsh CM</th>
<th>SH-b</th>
<th>SH-a</th>
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<td>Nabidae</td>
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<td>1</td>
<td>Total</td>
<td>51</td>
<td>36</td>
<td>40</td>
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Fig. 1 Marsh locations along the Pamunkey River, Virginia (USA).
Fig. 2 Nonmetric Multidimensional Scaling ordination overlaid with convex hulls of both marsh and treatment using the Bray-Curtis distance with 2 axes. Stress = 0.096.
Fig. 3 Total biomass of all vegetation within 0.25m$^2$ plots by marsh and cage treatment. Height of the bar represents the mean aboveground biomass harvested after 10 weeks of growth in the exclosure or control treatments; the lines represent standard error of the mean. (n=5 for exclosure treatment; n=10 for controls). No statistical difference exists between the exclosure treatments. The non-cage treatments are shaded the same because they were collapsed into a single group to compare against the cage treatment (see text for fuller explanation).
Fig. 4 Biomass of dominant species (*Peltandra virginica*, *Zizania aquatica*, and *Bidens laevis*) contributing to biomass within 0.25m² plots by marsh and cage treatment. Height of the bar represents the mean aboveground biomass harvested after 10 weeks of growth in the exclosure treatment; the lines represent standard error of the mean (n=5 for exclosure treatment; n=10 for controls).
Fig. 5 Tissue nutrient content in aboveground dominant plant tissue at three freshwater and oligohaline sites along the Pamunkey River. The bar represents the mean C, P or N (panels a, b, or c, respectively) in vegetation harvested after 10 weeks of growth in the exclosure or control treatments; the lines represent standard error of the mean (n=5 for exclosure treatment; n=10 for controls except in *Bidens* where the individuals did not
appear in every plot). No statistical difference was found between the exclosure and control treatments for tissue C or N. *Zizania* exhibited a statistical difference in tissue P content between the exclosure and control treatments.
Fig. 6 Tissue C:N in aboveground dominant plans at three fresh and oligohaline sites in the Pamunkey River. Height of the bar represents the mean N in vegetation harvested after 10 weeks of growth in the exclosure or control treatments; the lines represent standard error of the mean. (n=5 for exclosure treatment; n=10 for controls except in Bidens where the individuals did not appear in every plot). Zizania exhibited a statistical difference between the exclosure and control treatments.
Concluding Remarks
The research in this dissertation documents that environmental changes, particularly saltwater intrusion driven by sea-level rise, will impact marsh vegetation composition in the low-salinity regions of estuaries, and plant traits will shift in response. Vegetation in Sweet Hall Marsh (Pamunkey River, Virginia) is changing toward fewer dominants. The arrival of a halophyte, Spartina, in the low-salinity marsh signals a turning point in the ecology of Sweet Hall Marsh. However, freshwater vegetation that survives and remains in the system shows a mixed response in traits responding to saltwater intrusion. There was no uniform signal that could be ascertained from this research, indicating that species should be treated individually with consideration of life history.

*Spartina* tissues held less N or P in tissue relative to tidal freshwater marsh (TFM) species tested. As sea-level rise drives the salt front up-estuary, the dynamics between sulfate and iron are likely to release P from iron-bound particulates into the water column, where P would be available for plant uptake. If the TFM species were present, they might be able to hold the additional nutrient in the vegetation pool, but this is unlikely to occur with *Spartina*. *Spartina* does provide higher productivity, so the added density will likely ameliorate the situation, but additional research is required to quantify how much P is likely to be lost from the vegetation pool.

Ecologists have long sought general hypotheses to describe observations found in nature. My dissertation provides evidence that *Spartina alterniflora*, long held to be controlled in freshwater and near-freshwater by inter-specific competition, has the capacity to outcompete the current dominant species in the lower Chesapeake Bay. *Spartina* is succeeding at the low-salt endpoint of the gradient where other species have
adequate low-salt tolerance, rather than being relegated to existence in harsher environments where few species have physiological adaptations to survive.

Top-down, herbivorous insects drivers did not control primary productivity in tidal freshwater or oligohaline marshes in the system studied. Herbivory by insects did impact one species in particular, *Zizania aquatica*, where there was lower N by dry weight in species subjected to herbivory. The amount of tissue N-content decreased with added salt only when herbivory was present, suggesting that continued saltwater intrusion may further impact the vegetation palatability.

The collective result of this research suggests that Pamunkey River marsh communities will, indeed, survive sea-level rise, although with different vegetation composition than observed at present. *Spartina’s* high capacity to trap sediment may be the factor that facilitates survival. *Spartina’s* arrival at Sweet Hall Marsh was likely the culmination of a perfect storm: (1) a devegetated area where current dominants had inadequate resources to rebound quickly post muskrat eat-out, (2) water level driven by higher sea-level containing (a) just enough sulfate to meet *Spartina’s* physiological need, and (b) greater height to deliver *Spartina’s* seeds to the interior of Sweet Hall Marsh.
Appendix
Responses for species other than *Spartina* described in Chapter 3 of this volume.

**Fig. 1** Tissue nutrient content (% dry weight) and ratios (molar) of named species (*Peltandra, Leersia* and *Phragmites*) grown with *Spartina* for eight weeks at low (0), medium (1.5), and high (3) salinity (mean ± SE, n=9 except *Phragmites* where n=6).
Vita

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