Response and recovery of low-salinity marsh plant communities to constant and pulsed saline intrusion

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A Dissertation Presented to

the Faculty of the Department of Biology and Biochemistry

University of Houston

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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By

Fan Li

May 2017

**Response and recovery of low-salinity marsh plant communities to constant and pulsed saline intrusion** 

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## ABSTRACT

The effects of climate change on environmental conditions will be manifested as both chronic changes and pulse changes of varying durations. In estuaries, future variation in sea level, freshwater withdrawal and drought will lead to saline intrusion into low-salinity tidal marshes. I sought to understand the responses to various salinization scenarios at individual, community and ecosystem levels. First, I used a mesocosm experiment to investigate the responses of individual low-salinity marsh macrophytes to different durations of saline water exposure, without interspecific interactions. Second, I used another mesocosm experiment to assess the response of plant communities to saline water pulses of three salinities for five durations, and then assessed subsequent community recovery when the saline pulses were withdrawn. Third, I used a field experiment to explore the impacts on a freshwater marsh plant community of short- and long-term changes in salinity.

I ranked the freshwater marsh plant species in order from least to most salt tolerant as follows: *L. peploides*, *P. hydropiperoides*, *P. cordata* and *Z. miliacea*, followed by *S. lancifolia* and *E. palustris* in a tie. I found that community composition was increasingly affected by the more-saline and longer-duration treatments. In the second mesocosm experiment, most but not all of the plant species were able to recover from low-salinity, short-duration saline pulses in less than one year. Because not all the species recovered, post-disturbance community structure diverged among salinization treatments. In the field experiment, both presses and pulses of saline water addition caused the loss of *L. repens*, resulting in community composition diverging away from the controls. In the press treatment, plant biomass and diversity were strongly suppressed.

In the pulse treatment, community composition did not recover to the baseline conditions in between pulses of saline water, as *L. repens* failed to fully recover from pulses of salinity. In the second mesocosm experiment, shifts in community composition prevented long-term reductions in productivity. In the field experiment, however, salt-tolerant plants could not immigrate, and salinity presses caused a significant decrease in aboveground biomass. In contrast, salinity pulses had no effect on plant production. These results show that species composition is more sensitive to environmental changes than overall ecosystem processes. However, changes in ecosystem structure due to pulse changes may not limit recovery in ecosystem function.

# CONTENTS

Chapter 1 Introduction1
Chapter 2 Responses of tidal freshwater and brackish marsh macrophytes to pulses of
saline water simulating sea level rise and reduced discharge8
2.1 Introduction
2.2 Methods11
2.3 Results13
2.4 Discussion17
2.5 Acknowledgement
Chapter 3 Response and recovery of low-salinity marsh plant communities exposed to
presses and pulses of elevated salinity
3.1 Introduction
3.2 Methods25
3.3 Results
3.4 Discussion
3.5 Acknowledgement
Chapter 4 Responses of a tidal freshwater marsh plant community to constant and pulsed
saline intrusion
4.1 Introduction
4.2 Methods
4.3 Results
4.4 Discussion
4.5 Acknowledgement72

Chapter 5 Conclusion	73
Appendix A. Chapter 2 supplementary material	79
Appendix B. Chapter 3 supplementary material	82
Appendix C. Chapter 4 supplementary material	83
References	86

## **Chapter 1 Introduction**

Climate change causes chronic alterations (presses) in climate and resources that threaten ecosystems worldwide. A major goal for ecology is to understand ongoing press changes and predict the effects of future ones. Ecosystem responses to chronic alterations can be viewed as hierarchical, with three levels of response: changes in individual biota, re-ordering of species in the community, and species loss and immigration (Figure 1.1; Smith et al. 2009). The initial response usually occurs rapidly and includes physiological and metabolic changes, and mortality. For example, elevated CO<sub>2</sub> concentrations induce stomatal closure in most grass species regardless of photosynthetic pathway (Wand et al. 1999). Drought, salinity, and low-temperature stress impose a series of metabolic changes on plants, and inhibit photosynthesis, leading to growth suppression and reduced fertility (Krasensky and Jonak 2012). As environmental changes persist, some species are favored at the expense of others. These changes could occur months to decades after environmental changes start. For example, Sharpe and Baldwin (2012) found that elevated salinities for one year caused a shift toward salt-tolerant plant communities in tidal freshwater wetlands. Collins et al. (2008) found a re-ordering of species abundance rankings over a 5 - 8 year period of adding nitrogen to a late successional old field abandoned from agriculture. With continued environmental change, some species may go locally extinct, and other species may colonize a habitat where they previously did not exist because conditions have become more favorable for them (Perry et al. 2005, Thuiller et al. 2005, Cheung et al. 2009, Guo and Pennings 2012).



Time

Figure 1.1 The hierarchical-response framework of ecological change as ecosystems are exposed to chronic alterations. The ecosystem responses are initially driven by (A) individual (physiological/metabolic, mortality) responses, followed by (B) species re-ordering as some species is favored by the changing conditions at the expense of others. Finally, (C) new species that are better suited for new environments may immigrate into the ecosystem resulting in the largest change in ecosystem response. Reproduced from Smith et al. (2009).

Climate change also increases the frequency and intensity of extreme events, leading to acute alterations (pulses) in climate and resources. Acute alterations may cause rapid individual level mortality, and changes in community structure and ecosystem function (Smith 2011). Thibault and Brown (2008) studied an extreme flooding event that occurred in 1999 in the Chihuahuan Desert near Portal, AZ and found that two species of kangaroo rats suffered > 90% mortality, and the community composition shifted to dominance by pocket mice, a structure that was not seen in the 22 years before the flood.

Ciais et al. (2005) observed a reduction of primary production and the weakening of carbon sinks after an extreme drought and heat wave in 2003 across Europe. Ecosystems may recover after a pulse change whereas a chronic change might push the community into a new state (Figure 1.2; Smith 2011). For example, Hoover et al. (2014) imposed an extreme drought and heat wave in a native tallgrass prairie in Kansas, USA and found that aboveground net primary productivity recovered completely the year after the drought.



Figure 1.2 The framework of ecosystem responses to climate extremes. Individual-level effects can cascade to higher hierarchical levels to result in significant changes in community structure (species re-ordering) and large ecosystem impacts (species loss/invasion). These alterations may be characterized by prolonged recovery, or may even lead to persistent state changes. Reproduced from Smith (2011).

Ecological responses to acute alterations depend on the magnitude, duration, and timing of the disturbances. At low levels of disturbance, competitive exclusion may reduce species richness, whereas high levels of disturbance exclude all but the most disturbance-tolerant species. Therefore, intermediate levels of disturbance promote coexistence of species that thrive at both early and late successional stages (Connell 1978). When considering disturbances and ecological responses, researchers tend to focus primarily on pure press or pulse. However, natural disturbances are often changes in their magnitude, duration and frequency over time (Donohue et al. 2016). This complexity indicates that restricting the focus on a single type of disturbance may cause us to misunderstand the impacts of abiotic change on community and ecosystem processes. In most situations, we observe a mixture of press and pulse disturbance types, which may interact to affect biota in different ways (Parkyn and Collier 2004). For example, human usage of water may interact with sea level rise and drought to create complicated patterns of salinity variation in coastal wetlands

Coastal wetlands provide valuable ecosystem services to human society, including wildlife habitat, carbon sequestration, water quality improvements, and denitrification (Costanza et al. 1998, Zedler and Kercher 2005). Coastal wetlands are sensitive to changes in marine processes and freshwater flows from upstream catchments, as salinity strongly affects plant distribution along estuaries (Odum 1988, Pennings et al. 2005, Więski et al. 2010, Guo and Pennings 2012). Situated in the upper estuary, tidal freshwater marshes support high macrophyte diversity, productivity and nutrient retention (Costanza et al. 1998, Więski et al. 2010, Van de Broek et al. 2016). Tidal freshwater plants, however, cannot move downstream because they are stressed to varying degrees by increases in salinity. Crain et al. (2004) found that in southern New England marshes, freshwater marsh plants did poorly in salt marshes with or without neighbors. When salt marsh plants were transplanted into the freshwater marsh, they did better than in their

original habitat, but only when neighbors were not present. Guo and Pennings (2012) found similar mechanisms in low-latitude Georgia marshes: freshwater and brackishmarsh plants died when transplanted into higher salinity habitats, while salt-marsh plants were suppressed by competition in freshwater and brackish marshes. Increasing salinity in tidal freshwater marshes can decrease plant growth through osmotic effects and the accumulation of toxic ions in the soil and plant tissue (Adam 1990), and drives shifts in the composition of plant communities (McKee and Mendelssohn 1989, Sharpe and Baldwin 2012, Sutter et al. 2015). With current climate change driving sea-level rise and attendant salinization of the estuary, freshwater marshes in many areas are projected to be replaced by brackish or salt marshes (Craft et al. 2009).

Both climate change and anthropogenic alterations of the hydrologic cycle are likely to alter the frequency and intensity of wetland salinization, meaning the concentrations of soluble salts increase above natural levels. Two major mechanisms of salinization in coastal wetlands are sea level rise and reduction of freshwater inflow (Herbert et al. 2015). Sea-level rise has already caused saltwater intrusion into some freshwater wetlands (Knighton et al. 1991, Wood and Harrington 2015), and the rate of sea level rise is projected to increase (Church and White 2011). Over time, sea-level rise will lead to chronic changes in salinity along an estuary. Anthropogenic activities can reduce freshwater discharge through the construction of dams and diversion of freshwater for municipal, industrial, and agricultural uses (Sklar and Browder 1998, Enright and Culberson 2009, Cloern and Jassby 2012). This could also lead to press salinization in coastal wetlands. On the other hand, altered temperature and precipitation patterns can result in acute increases of salinity (Visser et al. 2002, White and Alber 2009).

5

Although salinity changes in the estuary could occur as either presses or pulses or their combination, most experiments looking at salinity effects have used a press design. Wetland ecologists often manipulated salinity in experiments using a constant low versus a constant elevated salinity treatment to test the effects of saline intrusion (Pezeshki et al. 1987, McKee and Mendelssohn 1989, Sharpe and Baldwin 2012, Woo and Takekawa 2012). Focusing on single salinization scenario may underestimate the impacts on coastal wetlands. In contrast to the press of long-term saline intrusion, freshwater plants may be resilient to saline pulses and able to recover once the pulse is withdrawn. Howard and Mendelssohn (1999b) found that oligonaline marsh macrophytes varied in capability of recovering from saline pulses. Within any one species, recovery varied with final salinity level and duration of exposure. Flynn et al. (1995) found that revegetation could occur even if the abiotic conditions had not fully recovered to freshwater conditions because of the surviving vegetative propagules and recruitment from the seedbank. Hopfensperger et al. (2014) found no obvious changes in plant communities at a field site exposed to repeated annual exposure to low levels of salinity (2 - 7 PSU).

In this dissertation, I explored the responses of tidal freshwater marsh plants to various salinization scenarios. In Chapter 2, I studied the impact of different durations of saline water exposure on the growth of both freshwater and brackish marsh macrophytes using a mesocosm experiment. This study aimed at assessing the responses of individual species without the complications of interspecific interactions. In Chapter 3, I assessed the response of plant communities to saline water pulses of three salinities for five durations, and then assessed subsequent community recovery when the saline pulses were withdrawn. This study expanded on the previous one by including community

processes—both competition and immigration—to examine plant responses in a community context. In Chapter 4, I used a field experiment to explore the impacts of pulsed and pressed changes in salinity on a freshwater marsh plant community. Because this study was done in the field, it was more realistic than the first two mesocosm studies, but logistical constraints meant that it necessarily had a more limited set of treatment combinations. Finally, these three topics were integrated in a general conclusion in Chapter 5.

# Chapter 2 Responses of tidal freshwater and brackish marsh macrophytes to pulses of saline water simulating sea level rise and reduced discharge

## 2.1 Introduction

Global climate change is expected to affect temperature and precipitation patterns, the rate of sea level rise, and the frequency and intensity of hurricanes and tropical storms, thereby changing the delivery of fresh and saline water to coastal wetland ecosystems (Barendregt and Swarth 2013). Salinity in estuaries may increase as saline water moves upstream due to sea level rise or storm surges, increase as droughts reduce river discharge, or decrease if precipitation to the watershed increases and river discharge increases. Because the species composition and productivity of tidal marshes are sensitive to salinity (Howard and Mendelssohn 1999a), any of these scenarios will affect plant productivity and composition (Sharpe and Baldwin 2012, Neubauer 2013).

The response of estuarine biota to variation in salinity depends on the intensity and timescale of the variation (Webb and Mendelssohn 1996, Baldwin and Mendelssohn 1998, Howard and Mendelssohn 1999a). Short pulses of elevated salinities may not cause permanent changes to the ecosystem, but longer pulses could temporarily alter productivity or community structure depending on the tolerance of individual species to salinity, and interactions among plant species. Once the pulse is withdrawn, the ecosystem may recover to baseline conditions after a period of time (Smith 2011). If, however, the altered conditions become chronic, there will be a tipping point when new species better suited to the new conditions will immigrate into the ecosystem, resulting in a state change (Smith et al. 2009). Historically, wetland ecologists often manipulated salinity in experiments using a constant low versus a constant elevated salinity treatment to test the effects of saline intrusion (McKee and Mendelssohn 1989; Pezeshki et al. 1987; Sharpe and Baldwin 2012; Woo and Takekawa 2012). However, in nature, coastal wetlands typically experience fluctuating salinity conditions rather than constant press changes. Temporarily lower precipitation can lead to reductions of freshwater input into the estuaries, and therefore cause species composition shift to salt-tolerant plants (Visser et al. 2002; White and Alber 2009). In addition, human activities such as channelization, dam construction, and surface and groundwater withdrawals also alter river discharge and salinity regimes in estuaries (Sklar and Browder 1998). However, the decline of freshwater discharge caused by climate and human usage is highly variable, and the resulting saline pulses can vary in duration. Therefore, determining how coastal wetlands respond to different saline intrusion scenarios is fundamental to understanding and managing these systems.

The vegetation composition of tidal marshes (freshwater, brackish and salt marshes) along an estuary is determined by salinity (from freshwater to euryhaline), interacting with competition (Guo and Pennings 2012). Each type of marsh provides unique and important ecosystem functions (Więski et al. 2010). Situated in the upper estuary where river freshwater discharge and the tidal wave from the sea combine, tidal freshwater marshes support high macrophyte diversity, productivity and nutrient retention (Costanza et al. 1998; Więski et al. 2010). Ongoing sea level rise alters hydrologic gradients and results over time in increased saline water incursion into previously freshwater marshes. Increased salinity can decrease plant growth through osmotic effects and the accumulation of toxic ions in the soil and plant tissue (Adam 1990), and drives shifts in the composition of plant communities (Knighton et al. 1991; McKee and

Mendelssohn 1989; Sharpe and Baldwin 2012; Sutter et al. 2015). Eventually, increases in salinity will convert tidal freshwater marshes to brackish or salt marshes (Craft et al. 2009). Although these long-term changes in salinity can be thought of as "presses", on a short time scale there is constant variation in salinity. In particular, variation in the frequency and intensity of summer drought affects freshwater flow from the river, and causes pulses of saline water to penetrate further upstream. In contrast to the press of long-term saline intrusion, freshwater plants may be resilient to saline pulses and able to recover once the pulse is withdrawn (Flynn et al. 1995; Goodman et al. 2010; Howard and Mendelssohn 1999b). Moreover, pulses of saline intrusion can alter the availability of nutrients. For example, ammonium  $(NH_4^+)$  can be replaced from the cation exchange sites by the influx of sea salt cations, bio-available phosphate  $(PO_4^{3-})$  can increase due to desorption by chloride, and increases in sulfate  $(SO_4^{2-})$  concentrations can enhance rates of organic matter mineralization through sulfate reduction (Weston et al. 2006). Shifts between freshwater and saline conditions could thereby liberate repeated fluxes of nutrients from soils (Weston et al. 2011). Therefore, tidal-marsh plants might even benefit from pulses of saline water due to an increased nutrient availability (Weston et al. 2011, Ardón et al. 2013).

The objective of this study was to determine the impact of different durations of saline water exposure on the growth of both freshwater and brackish marsh macrophytes. I used a mesocosm study because it allowed us to apply more treatment combinations than we could easily impose in the field, as well as to control other biotic and abiotic variables that might confound treatment effects. I tested the hypotheses that 1) increasing duration of exposure to saline water would negatively affect tidal freshwater and brackish

marsh plants, but 2) the nature of the response would vary among species, both among and within salinity zones, and 3) some species would benefit from occasional saline pulses due to release of nutrients (Więski et al. 2010).

2.2 Methods

I collected plants from the tidal marshes of the Altamaha River estuary, Georgia, USA (31.4° N, 81.4° W). Tidal freshwater marshes in this area are dominated by *Polygonum hydropiperoides, Pontederia cordata* and *Zizaniopsis miliacea*; and brackish marshes by *Spartina cynosuroides, Schoenoplectus americanus* and *Juncus roemerianus* (Więski et al. 2010). Water-column salinities near the collection sites were recorded by the Georgia Coastal Ecosystems Long-Term Ecological Research (GCE LTER) program using moored hydrographic sondes (Sea-Bird Electronics model 37-SM MicroCATs) deployed in 2001 at the GCE7 site (approximately 800 m upstream from the site where freshwater marsh plants were collected) and the GCE8 site (approximately 750 m downstream from the site where brackish marsh plants were collected). The hydrographic data sets (GCE-LTER Hydrographic Monitoring in the Altamaha River) are available on the GCE-LTER data portal (Table A1).

I collected thirty-five individuals of each of the six species listed above (I will refer to these species by their genus names in Chapter 2) on March 9 - 11, 2013, and potted them individually in 12 L pots in sediments collected from the same site as the plants. Plants were acclimated for 2 months in freshwater to minimize transplant shock, and then were exposed to seven salinity treatments during the three-month experiment, which ran from May 26 to August 28, 2013. Most of the *Spartina* individuals, however, died during the acclimation. I collected a second set of *Spartina* plants on June 13 and

acclimated them in freshwater until June 27, 2013. The *Spartina* plants were consequently exposed to salinity treatments for only two months, rather than three; however, they grew rapidly during the experiment, and as a result we considered the two-month period as sufficient for effects of treatments to be visible.

Individual potted plants were placed inside dish pans that had drain holes at midpot height (7.5 cm) to ensure that soil wetness was similar across all replicates. Plants were grown outside in a field that was 70 m from a natural salt marsh that is ~20 km downstream from the collection point, and were therefore exposed to ambient temperature, humidity and light regimes. For salinity treatments, plants were watered once a day with saline water (5 PSU: Practical Salinity Units, created using Instant Ocean Sea Salt, Aquarium System Inc., Mentor, OH, USA) for 1, 2, 4, 8, 16, or 31 days per month, and then watered on the remaining days of the month with freshwater, except for the 31 days treatment which represented a permanent saline press. For the control treatment, plants were watered with freshwater daily. The experiment was harvested on August 28, 2013. Individual plants were separated into aboveground and belowground biomass, washed to remove soil, and dried at 60 °C to constant mass. The plant data set (Pot experiment on freshwater and brackish marsh plants responses to salinity pulses in summer 2013) is available on the GCE-LTER data portal (Table A1).

The responses of the plants to salinity appeared to vary over different ranges of salinity exposure. Therefore, to test the responses of plant production to different durations of saline water, I conducted piecewise regression on all species, with plant biomass (aboveground, belowground and total) as the dependent variables and days of saline water addition as the independent variable (Toms and Lesperance 2003). The piecewise regression was performed with SigmaPlot 11.0 software (Systat Software Inc.). 2.3 Results

The GCE7 site in the Altamaha River is located approximately 20 km upstream from the ocean. The water column salinities at the GCE7 site were very low except for periods of drought. Between August 2001 and May 2014, 26% of the days experienced a maximum salinity higher than 0.5 PSU, and only 3% of the days had peak salinities over 5 PSU (Figure 2.1). The GCE8 site is located approximately 15 km upstream from the ocean, and water column salinities at this site are mostly oligohaline to mesohaline (5 -18 PSU). Between October 2001 and May 2014, 55% of the days experienced a maximum salinity higher than 5 PSU, and 12% of the days had salinities over 18 PSU (Figure 2.2). Higher salinities at both sites happened more commonly late in the year when river discharge was lower (Blanton et al. 2001).



Figure 2.1 Daily maximum salinity from August 10, 2001 - May 17, 2014 at GCE 7. The dashed lines indicate oligohaline (0.5 - 5 PSU) and mesohaline (5 - 18 PSU) salinity levels



Figure 2.2 Daily maximum salinity from October 26, 2001 - May 17, 2014 at GCE 8. The dashed lines indicated mesohaline (5 – 18 PSU) and polyhaline (18 – 30 PSU) salinity levels

All plants in control treatments grew over the course of the experiment. All of the freshwater marsh plant species exhibited a reduced tissue biomass (both aboveground and belowground) with permanent exposure to elevated salinities (31 days per month) relative to the control, but the response to the saline pulses varied with species (Figure 2.3). *Polygonum* aboveground biomass did not respond to short saline pulses (up to 4 days in duration) but declined sharply thereafter (Figure 2.3 a). In contrast, *Polygonum* belowground biomass decreased sharply in response to short salinity pulses, but largely stabilized with pulses of 8 or more days (Figure 2.3 d). Because above- and belowground

biomass showed different trends, total biomass, which summed the two, exhibited a fairly steady decline with increasing exposure to salinity (Figure 2.3 g). *Pontederia* above-, belowground and total biomass declined steadily with increased exposure to saline water (Figure 2.3 b, e, h). *Zizaniopsis* aboveground biomass did not respond to salinity treatments (Figure 2.3 c). Both belowground and total biomass, however, increased with salinity pulses up to 16 days in length, and then declined with longer salinity pulses (Figure 2.3 f, i).



Figure 2.3 Piecewise regression of aboveground, belowground and total biomass of *Polygonum*, *Pontederia* and *Zizaniopsis* as a function of salinity treatments. Solid lines represent significant relationships (P < 0.05)

In contrast to results from the freshwater plants, the aboveground, belowground and total biomass of the three brackish-marsh species did not respond to changes in salinity exposure (Figure 2.4). The data showed considerable variation in all treatments. A more complex analysis using multiple regression, with initial size included as a predictor variable, indicated that some of the variation in final brackish plant biomass was caused by differences in initial sizes of the plantings, but did not otherwise change the general conclusions of the analysis (Table A2).



Figure 2.4 No significant relationship (all P values > 0.05) found between aboveground, belowground and total biomass of *Spartina*, *Schoenoplectus* and *Juncus* and days of saline water addition

#### 2.4 Discussion

Understanding the responses of tidal marsh plants to different saline water intrusion scenarios is critical to predicting the fate of tidal marshes. Previous studies have primarily focused on plant responses to constant elevated salinities (McKee and Mendelssohn 1989; Pezeshki et al. 1987; Sharpe and Baldwin 2012; Woo and Takekawa 2012), but salinization can occur on many time scales (Goodman et al. 2010; Visser et al. 2002; White and Alber 2009). I examined the effects of salinity duration on different tidal freshwater and brackish plant species. The results suggest that not only was *Zizaniopsis* more tolerant of salinity pulses than *Polygonum* and *Pontederia*, its belowground and total biomass may actually have increased with salinity pulses up to 16 days in length. I also found that long exposure to moderately elevated salinities (5 PSU) had negative effects on the biomass of all three freshwater species that we tested, but no effects on the three brackish marsh species that we tested.

In this study, Zizaniopsis belowground biomass showed a slight increase with salinity pulses up to 16 days. The relationship was quite variable, with relatively low  $R^2$ values (0.22) and was strongly influenced by one data point. After removing the most influential point, the hump-shaped pattern was still visually apparent but was no longer statistically significant. Thus, the finding that moderate salinity exposure benefits Zizaniopsis should be viewed with some caution. However, past work has shown that alternating between fresh and saline water can release nutrients from wetland soils (Weston et al. 2011), and such salinity-induced nutrients may benefit salt-tolerant species like Zizaniopsis (Ardón et al. 2013). Adding short duration of higher-salinity water into freshwater-marsh sediments can cause an increase of  $NH_4^+$  and  $PO_4^{3-}$  efflux from sediments (Rysgaard et al. 1999, Sundareshwar and Morris 1999). Saline water carries many ions that will compete for exchange sites with  $NH_4^+$  and  $PO_4^{3-}$  (Seitzinger et al. 1991, Weston et al. 2006). Simultaneously, the introduction of  $SO_4^{2-}$  by saline water will shift the decomposition pathway from methanogenesis to sulfate reduction, which is more efficient in organic matter mineralization (Weston et al. 2006). There is also evidence to suggest that elevated salinity can inhibit rates of nitrification, leading to the increased

release of  $NH_4^+$  (Ardón et al. 2013). Thus, there is a likely mechanism to explain the increase in *Zizaniopsis* biomass at intermediate salinity exposure, and I view the result as reasonable. However, if the plants are too stressed by salt, uptake of the nutrients will be disrupted. This is probably why longer saline pulses ceased to be beneficial to *Zizaniopsis*.

I found that belowground and total biomass of Polygonum and Pontederia steadily declined with increasing duration of salinity pulses, indicating that they were unable to benefit from increased nutrient availability even with short saline pulses. The relationship between salinity and biomass of *Polygonum* was quite strong, with relatively high  $R^2$  values (0.49 to 0.58). Salinity pulses of 8 days caused a decrease of *Polygonum* belowground biomass to nearly zero. Therefore, adding saline water for 16 and 31 days did not cause further decreases. Polygonum roots were apparently more sensitive to higher salinity than the rhizomes of *Pontederia* and *Zizaniopsis*. In tidal marshes clonal growth is favored as seeds and seedlings are vulnerable to salt stress and inundation (Pennings and Bertness 2001). In contrast, rhizomes are relatively resilient and could help with regeneration after disturbance (Flynn et al. 1995). However, Pontederia aboveground biomass declined steadily with any exposure to saline water, while aboveground biomass of *Polygonum* did not respond to short saline pulses (up to 4 days in duration). I predict, then, that when saline pulses occur, Pontederia may disappear earlier than *Polygonum*, but be faster to regrow once freshwater conditions are restored. The response to salinity of *Pontederia* was somewhat more variable than for *Polygonum*, with lower  $\mathbb{R}^2$  values (0.24 to 0.55).

This experiment focused on vegetative responses of adult plants, because all the common species at this study site are perennials. Many plants in tidal freshwater marshes,

however, are annuals. For these species, it is likely very important to also consider how the timing and extent of salinity pulses affects seed germination among species (Flynn et al. 1995, Baldwin et al. 1996).

The salinity treatments had no effect on the three brackish species. This may be because the sediments collected in the brackish marsh had adapted to the salinity fluctuations at 5 PSU. Although I did not measure nutrients in the pots, van Dijk et al. (2015) found that subjecting brackish sediments to saline pulses did not significantly alter nutrient concentrations. Therefore, I speculate that the treatments did not cause significant changes in nutrient content in the brackish sediments. As a result, plants did not benefit from saline pulses because no additional nutrients were released, and did not suffer because salinities were low compared to the range of conditions that they normally experience (Fig. 2.2).

The response of an entire plant community to saline pulses will depend not only on the characteristics of the saline pulses and the tolerances of the individual plant species, but also on intraspecific and interspecific interactions among plant species, and the composition of the seed bank (Baldwin et al. 1996; Howard and Mendelssohn 1999a; Howard and Mendelssohn 1999b). Competition, for example, plays an important role in structuring variation in wetland plant communities along the salinity gradient of an estuary (Crain et al. 2004; Engels and Jensen 2010; Guo and Pennings 2012). Therefore, I cannot extrapolate these results directly to the field without additional work, as reduced competition due to elevated salinity may benefit a given salt-intolerant species at the same time as salinity has a direct negative effect, making the net outcome hard to predict. Future work, then, needs to consider the responses of plants to salinity both with and without competition from neighbors in order to fully predict how natural communities will respond to salinity variation.

Salinity variation in estuaries is affected both by natural processes and by human alterations to hydrology. In particular, human societies utilize river water for irrigation, household use, and industrial purposes, and these withdrawals affect river discharge and hence estuarine resources (Cloern and Jassby 2012; Enright and Culberson 2009). How much water can be withdrawn without seriously harming natural resources is an important political issue in many states (Alber 2002, Montagna et al. 2002). The results from this study suggest that the pattern of freshwater withdrawals may be as important as the total amount removed: short periods of freshwater withdrawal are likely to be less disruptive to natural communities than long ones. If supported by further work, this conclusion could help resource managers develop freshwater management regimes that will minimize the negative effects of elevated salinity on coastal wetlands.

My results indicate that the responses of freshwater and brackish marsh plants to saline pulses depend on both the salt tolerance of each species and the duration of the pulses. The brackish species did not respond to the treatments, whereas *Polygonum* and *Pontederia* showed sharp decreases in biomass with increasing salinity duration. Most strikingly, I found that *Zizaniopsis* actually appeared to benefit from intermediate duration saline pulses. Although I need more information to ascertain whether the same pattern will be observed in the field, this study is unique in demonstrating that periodic saline intrusion may improve the growth of certain marsh plant species. In order to extrapolate our results to the field, further experiments that cross salinity and species interactions are needed.

# 2.5 Acknowledgement

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# Chapter 3 Response and recovery of low-salinity marsh plant communities exposed to presses and pulses of elevated salinity

## 3.1 Introduction

Climate change and human activities are affecting natural systems in many ways, ranging from shifts in plant functional groups (Saintilan et al. 2014), to cascading effects through food webs (Winder and Schindler 2004), to changes in carbon and nutrient stocks (Neubauer 2013, Zhou et al. 2016). Ecologists are interested in forecasting the consequence of various environmental changes, so that predictions can improve management and conservation practices. Climate change manifests as both chronic (press) alterations in climate and resources, and as an increasing frequency and intensity of extreme events, and acute (pulse) alterations (Karl et al. 2008, Smith et al. 2009).

Ecological responses to acute alterations depend on the magnitude, duration, and timing of the disturbances, and can range from individual mortality (Howard and Mendelssohn 1999b, Ma et al. 2015) to changes in community structure (Howard and Mendelssohn 2000, Thibault and Brown 2008, Dieleman et al. 2015) to changes in ecosystem function (Ciais et al. 2005). Pulsed disturbances may not cause permanent changes to an ecosystem, as post-disturbance recovery can bring the ecosystem back to its baseline conditions (Smith 2011). For example, Hoover et al. (2014) imposed an extreme drought and heat wave in grassland and found that aboveground net primary productivity recovered completely the year after the drought. When considering disturbances and ecological responses, researchers tend to focus primarily on pure press or pulse designs. However, natural disturbances often involve complex changes in magnitude, duration and frequency over time (Donohue et al. 2016). This complexity indicates that restricting the focus on a single type of disturbance may cause us to misunderstand the impacts of abiotic change on community and ecosystem processes.

Coastal wetlands provide valuable ecosystem services to human society, including wildlife habitat, carbon sequestration, water quality improvements, and denitrification (Costanza et al. 1998, Zedler and Kercher 2005). The vegetation composition of tidal marshes (freshwater, brackish and salt marshes) along an estuary is determined by salinity (from freshwater to euryhaline), interacting with competition (Guo and Pennings 2012). Therefore, coastal wetland ecosystems are sensitive to climate and anthropogenic changes such as sea level rise, changed precipitation patterns, and reduced freshwater inflow due to human activities (Knighton et al. 1991, Sklar and Browder 1998, Enright and Culberson 2009, Cloern and Jassby 2012, Barendregt and Swarth 2013, Wood and Harrington 2015). Low-salinity marshes support higher primary productivity and total carbon and nitrogen stocks compared to salt marshes (Więski et al. 2010). Therefore, any of the salinization scenarios mentioned above could affect both plant productivity and composition (Neubauer 2013, Sutter et al. 2015).

A number of experimental studies have contrasted a constant low versus a constant elevated salinity treatment to test the effects of saline intrusion, showing that prolonged saline presses cause mortality of salt-sensitive species and shift wetland communities towards species with greater salinity tolerance (Pezeshki et al. 1987, Sharpe and Baldwin 2012, Woo and Takekawa 2012). In contrast to the press of long-term saline intrusion, however, freshwater plants may be resilient to short-term saline pulses, and be able to recover once the pulses are withdrawn (Flynn et al. 1995, Hopfensperger et al. 2014). Howard and Mendelssohn (1999b) found that oligohaline marsh macrophytes

varied in their ability to recover from saline pulses. They found that *Scirpus americanus* was able to recover from the most extreme conditions of exposure to 12 g/L salinity for 3 month, while the recovery of three other species (*Eleocharis palustris, Panicum hemitomon*, and *Sagittaria lancifolia*) decreased with increased salinity and increased duration. Given the complexity of the pulse events and species variability, the ecological impacts of pulses of saline intrusion into low-salinity marshes are difficult to predict.

To investigate the processes that control the differential response and recovery of marsh plant communities to saline pulses, I exposed mesocosms to saline water pulses consisting of three salinities for five durations, and assessed both the immediate effects on the plant communities and their subsequent recovery. I tested the null hypothesis that saline water addition had no impacts on plant community composition and production. I expected that this hypothesis to be rejected, and predicted that increases in saline exposure would result in community shifts towards more salt-tolerant species and reduced plant production, but that the community composition and biomass would recover once the saline intrusion stopped.

#### 3.2 Methods

I established mesocosm communities using six common freshwater plant species (*Zizaniopsis miliacea*, *Pontederia cordata*, *Polygonum hydropiperoides*, *Peltandra virginica*, *Ludwigia repens*, *Sagittaria lancifolia* and *Eleocharis palustris*) that together represent >75% of the biomass of tidal freshwater plant communities along the Altamaha River estuary, Georgia, USA (authors personal observations; common names given in Table B1). All plants were collected in a single tidal freshwater marsh (31°20'16'' N, 81°27'52'' W) between April 19 and 22, 2014. I collected sediment for the mesocosms

from a freshwater pond  $(31^{\circ}23'54"N, 81^{\circ}16'47"W)$  on Sapelo Island, GA. The experimental mesocosms consisted of plastic round pools (height = 18 cm, diameter = 122 cm) filled to the top with homogenized sediment. Each mesocosm was planted with 2 individuals each of *Z. miliacea*, *P. cordata*, *P. virginica* and *S. lancifolia*, and 5 – 8 individuals of *P. hydropiperoides* and *L. repens*. *E. palustris* was present in the root balls of the other species, and so was not planted separately. Mesocosms were located outdoors in a field 70 m from a natural salt marsh that was ~20 km downstream from the collection point, and were therefore exposed to ambient temperature, humidity and light regimes. After planting, mesocosms were watered with fresh water for 30 days until the experiment began.

From May 22 to August 19, 2014, the mesocosms were watered with three salinity levels (3, 5, and 10 PSU: Practical Salinity Units) crossed with five exposure durations (5, 10, 15, 20 and 30 days per month), plus a freshwater control treatment, for 16 treatment combinations (n = 3 per treatment combination). Except for the 30-day exposure treatment, which was a permanent saline press, mesocosms were watered with freshwater in between pulses of saline water. For example, the 5-day treatment was watered with saline water for 5 days followed by fresh water for 25 days, and then the cycle was repeated. Different salinities were achieved by mixing tap water with seawater. I restricted the salinity range to 0 to 10 PSU because that is the range of salinities commonly observed in the freshwater marsh in the Altamaha River, GA, where the plants were collected (Li and Pennings in review). I simulated tidal variation during the treatment period by watering the plants every morning and then pumping out water through a 6 in (15 cm) slotted well (constructed of PVC pipe) every afternoon. I inserted

rhizon samplers (Eijkelkamp, Giesbeek, Netherlands) into soils to a depth of 5–10 cm to collect in-situ porewater samples during the treatment period (7/20/2014 and 8/10/2014). Water samples were later tested for total ammonium and phosphate content in the Stable Isotope Ecology Laboratory at University of Georgia.

From August 20, 2014 to June 30, 2015, all mesocosm communities were watered with fresh water and allowed to recover. I did not simulate tidal variation during the recovery period. Instead, I watered all treatments twice a week to maintain the water level at least 3 cm above the sediment.

I measured the percent cover of all species present, and recorded species richness within each mesocosm, at the end of the treatment (August 12, 2014) and recovery (June 30, 2015) periods. At the end of the recovery period I also measured the height of all *Z. miliacea*, *P. cordata*, *P. virginica*, *S. lancifolia*, and *P. hydropiperoides* plants. I then harvested the aboveground biomass of plants in each mesocosm (July 3 to July 31, 2015). I sorted plants to species, washed them to remove soil, and dried them at 60 °C to constant mass. Using the post-recovery data, I created polynomial relationships between biomass and plant height (the sum of all plant heights for the species in one mesocosm; n = 48) or cover (n = 48) for all species, and then used these relationships to estimate aboveground biomass during the treatment period (Table B1).

To examine the compositional dissimilarity of plant communities across the plots, I used nonmetric multidimensional scaling (NMDS), and compared dissimilarities using permutational multivariate analysis of variance (PERMANOVA). Both analyses were performed based on Bray-Curtis dissimilarity using the software package PAST ver. 3.11. To describe internal community temporal dynamics, I used rank-abundance curves based on species abundance biomass (Collins et al. 2008). To examine the treatment effects and the relationship between species richness and aboveground biomass, I conducted multiple linear-regression analysis for species richness and biomass at the end of the treatment period and the end of the recovery period, with salinity, duration, and their interactions with richness or biomass as predictor variables. I also used multiple linear-regression analysis to examine the effects of salinity, duration, biomass and their interactions on porewater ammonium and phosphate content during the treatment period. Regression analyses were conducted with JMP 10.0 statistical software (SAS Institute Inc.).

3.3 Results

With increasing salinity levels and increasing salinity exposure duration, the plant community composition diverged away from the control treatment (Fig. 3.1, PERMANOVA, salinity, p < 0.01; duration, p < 0.01; interaction, p = 0.98). The divergence of community composition was due to shifts in relative abundance of the same speices and species extinctions (Fig. 3.2). *E. palustris* was nearly always the highest-ranked species in all treatments, suggesting that it was robust to saline pulses. *S. lancifolia* and *Z. miliacea* were also relatively resistant to the saline treatments, and maintained their biomass in most of the mesocosms. In contrast, *L. repens* and *P. hydropiperoides* were strongly suppressed by the more extreme saline treatments. *L. repens* was highest in the control treatment. *L. repens* eventually disappeared in mesocosms treated with 3 PSU water for 20 or 30 days, 5 PSU water for more than 5 days, and 10 PSU water for any duration. Similarly, *P. hydropiperoides* disappeared in mesocosms exposed to 5 PSU water for 30 days, or 10 PSU water for more than 5 days. The rank abundance of *P. cordata* also dropped in mesocosms exposed to the highest
salinity treatments (5 PSU water for 20 and 30 days, and 10 PSU water for more than 5 days). *P. virginica* established poorly, was the least abundant species in almost all mesocosms, and completely disappeared in most higher-salinity and longer-duration treatments.



Fig. 3.1 Nonmetric multidimensional scaling (NMDS) plots showing the plant community composition of all plots in two-dimensional ordination space at the end of the treatments (August 12, 2014). Each symbol represents the community composition based on aboveground biomass of all species in one plot. Control plots (open circles joined with a line) are the same in every plot; filled circles represent each treatment combination. Objects that are more similar to one another are ordinated closer together. The axes are arbitrary as is the orientation of the plot.



Fig. 3.2 Rank-abundance curves of all species present in each treatment combination at the end of the treatment period (August 12, 2014). Percent abundance for each species (biomass of individual species over total biomass of one plot) was averaged across the three replicate plots of each treatment combination.

At the end of the treatment period, species richness and total aboveground biomass were negatively related to both increasing salinity and increasing exposure duration (Fig. 3.3, Table 3.1). Salinity, duration, and their interaction explained 76% of the variability of species richness (Table 3.1, simple model). By adding biomass and interactions between the predictor variables, I explained slightly more of the variability in the data set (Table 3.1, complex model). Aboveground biomass was negatively related to both salinity and duration, but not their interaction (Table 3.1).

Ammonium concentration in porewater in July was positively related to salinity, duration and the interaction of salinity and plant biomass (Fig. 3.4 a, Table 3.2). A similar pattern persisted into August, but the concentration of ammonium was lower (Fig. 3.4 b), and was affected not only by salinity, pulse duration and their interaction but also by the interaction between pulse duration and plant biomass, and their three-way interaction (Table 3.2). The concentration of phosphate in porewater was slightly higher in plots that were treated with high-salinity water, and like ammonium decreased in all plots in August (Fig. 3.4). In July, the phosphate content was affected by salinity, pulse duration (negatively), and plant biomass, while in August it was affected by salinity and the interaction between pulse duration and plant biomass (Table 3.2).

After the 10-month recovery period, mesocosms did not converge on each other or on the control treatment, but instead continued to diverge (Fig. 3.5). Different salinization patterns determined the trajectory of the mesocosms relative to the control treatment. Mesocosms that were treated with 3 PSU water for 5 and 10 days, and 5 PSU water for 5 days, followed the same general trajectory as the control mesocosms (Fig 3.5 a). With increasing treatment salinity and exposure duration, mesocosms increasingly diverged away from the controls and from each other (Fig. 3.5 b, c). Overall, during the recovery period, the dissimilarity between the treatment mesocosms and the controls increased with both salinity (PERMANOVA, p < 0.01) and duration (p < 0.01), but not their interaction (p = 0.94). Table 3.1 Best multiple linear-regression models for species richness and biomass at the end of the treatment period and the end of the recovery period. Predictor variables examined were salinity (S), duration (D), biomass (B, for species richness) and their interactions; the best model was selected based on Mallow's Cp statistic. Asterisks indicate significance (< 0.05) of individual term in the model.

		$\mathbf{R}^2$	Р
Species richness			
End of treatment (Simple model)	Species richness = $8.39 - 0.31$ S* $- 0.09$ D* $- 0.001$ (S $- 5.62$ ) × (D $- 15$ )*	0.76	< 0.01
End of treatment (complex model)	Species richness = $9.57 - 0.38 \text{ S}^* - 0.13 \text{ D}^* - 0.01 \text{ B} - 0.02 (\text{S} - 5.62) \times (\text{D} - 15)^* - 0.002 (\text{D} - 15) \times (\text{B} - 54.14)^* - 0.0002 (\text{S} - 5.62) \times (\text{D} - 15) \times (\text{B} - 54.14)^*$	0.83	< 0.01
End of recovery	Species richness = $9.27 - 0.19 \text{ S}^* - 0.08 \text{ D}^*$	0.39	< 0.01
Biomass			
End of treatment	Biomass = 94.98 – 2.70 S* – 1.71 D*	0.47	< 0.01
End of recovery	Biomass = 138.38 + 1.52 D*	0.09	0.04

32

Table 3.2 Best multiple linear-regression model for porewater ammonium and phosphate content in July and August, 2014. Predictor variables examined were salinity (S), duration (D) and plant biomass (B); the best model was selected based on Mallow's Cp statistic. Biomass was the sum of all species biomasses estimated using measurements of plant height and cover measured on July 14, 2014 or August 1, 2015, as appropriate, and was log-transformed to improve normality. Asterisks indicate significance (< 0.05) of individual term in the model.

		$\mathbf{R}^2$	Р
Ammonium			
July 2014	Ammonium = $0.23 \text{ S}^* + 0.08 \text{ D}^* - 0.62 (\text{S} - 5.62) \times (\text{B} - 1.58)^* - 0.44$	0.37	< 0.01
August 2014	$\begin{array}{l} Ammonium = 0.21 \ S^{*} + 0.10 \ D^{*} + 0.02 \ (S-5.62) \times (D-15)^{*} + 0.19 \ (D-15) \times (B-1.66)^{*} + 0.04 \ (S-5.62) \times (D-15) \times (B-1.66)^{*} - 1.37 \end{array}$	0.40	< 0.01
Phosphate			
July 2014	Phosphate = $0.01 \text{ S}^* - 0.005 \text{ D} - 0.19 \text{ B}^* + 0.55$	0.25	< 0.01
August 2014	Phosphate = $0.01 \text{ S} + 0.01 (\text{D} - 15) \times (\text{B} - 1.66) + 0.08$	0.14	0.04

33



Fig.3.3 Effects of exposure duration and salinity level on a) species richness and b) aboveground biomass of all species at the end of the treatment period (August 12, 2014).

The community composition of the mesocosms at the end of the treatment period and the end of the recovery period did not overlap in ordination space. This was largely due to five species (*Cyperus odoratus*, *Lilaeopsis chinensis*, *Juncus marginatus*, *Schoenoplectus tabernaemontani*, and *Typha latifolia*) that emerged in all the plots as volunteers (not deliberately planted) during the recovery period (Fig. 3.6). The reason that low salinization mesocosms recovered along the same general trajectory as the controls was that *L. repens* largely recovered in these mesocosms. After the recovery period, *L. repens* in mesocosms that were treated with 3 PSU water, 5 PSU water for 5 and 10 days, and 10 PSU water for 5 days had similar or even higher biomass compared to the controls. In contrast, *L. repens* remained absent in plots treated with 3 PSU for 20 and 30 days, 5 PSU water for 30 days, or 10 PSU water for more than 5 days. *E. palustris* remained the highest-ranked species in the saline mesocosms, and its abundance largely increased compared to the end of the treatment period. *P. hydropiperoides* recovered to levels similar to the control treatment in all mesocosms. *P. cordata* in mesocosms that were treated with 3 PSU water, 5 PSU water for less than 30 days, and 10 PSU water for 5, 10 and 15 days had similar or even higher biomass compared to the controls.



Fig. 3.4 Effects of exposure duration and salinity level on porewater ammonium and phosphate concentrations in July and August, 2014.



Fig. 3.5 Nonmetric multidimensional scaling (NMDS) plots showing recovery patterns of all plots in two-dimensional ordination space. Data are shown in three panels for clarity, with the control treatment shown in all three panels. Open and filled symbols represent the end-of-treatment (August 12, 2014) and end-of-recovery (June 30, 2015) community composition based on aboveground biomass. Each point is coded as "salinity\_duration". Objects that are more similar to one another are ordinated closer together. The axes are arbitrary as is the orientation of the plot.

At the end of the recovery period, plant species richness was negatively related to treatment salinity and duration, but not their interaction (Fig. 3.7 a, Table 3.1). These patterns persisted because the most salt-sensitive species that disappeared during the treatment period, such as *L. repens* and *P. cordata*, did not re-appear during the recovery period (Fig. 3.6). Aboveground biomass in the mesocosms was no longer related to salinity level, and increased slightly in mesocosms that had received a longer salinity exposure during the treatment period; however, this relationship explained less than 10% of the variation in final biomass (Fig. 3.7 b, Table 3.1). No relationship was found between biomass and species richness at the end of the recovery (Table 3.1).



Fig. 3.6 Rank-abundance curve of all species present in each treatment combination at the end of the recovery period (June 30, 2015 Percent abundance for each species (biomass of individual species over total biomass of one plot) was averaged across the three replicate plots of each treatment combination. Species listed in the key below the horizontal line were volunteers that were not deliberately planted but appeared during the recovery period.



Fig. 3.7 Effects of exposure duration and salinity level on a) species richness and b) aboveground biomass of all species at the end of the treatment period (June 30, 2015).

## 3.4 Discussion

Salinization of coastal wetlands can occur on many time scales, from a gradual and prolonged change due to the long-term trend of sea level rise (Knighton et al. 1991, Wood and Harrington 2015) to acute and periodic pulses due to drought (Visser et al. 2002, White and Alber 2009, Goodman et al. 2010). Although freshwater marshes are projected to be replaced by brackish or salt marshes given prolonged salinization (Craft et al. 2009), marsh plant communities can recover from short-term saline pulses (Flynn et al. 1995, Howard and Mendelssohn 2000, Hopfensperger et al. 2014). These results supported the hypothesis that the responses and recovery of tidal marsh vegetation were directly related to the salinity and duration of saline pulses. Higher-salinity, longerduration saline pulses caused community composition shifts towards more salt-tolerant species, as well as towards lower species richness and lower aboveground biomass. However, communities experiencing low-salinity and short-duration saline pulses recovered due to the regrowth of salt-sensitive species. Community composition of heavily salinized treatments did not recover, but aboveground biomass did, indicating that ecosystem processes may be more resilient to saline pulses than community composition.

The salinity treatments affected all the plant species, but the magnitude of the effect varied with species. L. repens was the most salt-sensitive species, followed by P. hydropiperoides. Somewhat more resistant to salinity were P. cordata, which disappeared in mesocosms with 10-PSU water for more than 10 days, and Z. miliacea, which was present in all mesocosms. These results were consistent with a previous greenhouse experiment where Li and Pennings (in review) found that Z. miliacea was more robust to low-salinity pulses than P. hydropiperoides or P. cordata. The most resistant species were S. lancifolia and E. palustris, which maintained similar abundances in control and saline mesocosms, except that they were both suppressed by continual watering with 10 PSU water. Unlike Howard and Mendelssohn (1999a) who found that S. *lancifolia* was affected earlier and for a longer extent by saline water addition than E. *palustris*, we found little difference between these two species in tolerance to saline pulses in our study. P. virginica established poorly in almost all mesocosms (perhaps the soil was too shallow for it to root effectively) and its behavior was too variable to rigorously evaluate. Overall, I ranked the species as follows, in order from least to most salt tolerant: L. repens, P. hydropiperoides, P. cordata and Z. miliacea, followed by S. *lancifolia* and *E. palustris* in a tie.

Due to different salinity tolerances of the studied species, community composition was increasingly affected by the more-saline and longer-duration treatments. The increasing suppression of salt-sensitive species resulted in species re-ordering, decreased species richness, and decreased aboveground biomass. This pattern is common across estuaries where plant species richness and productivity are typically higher in tidal freshwater marshes than in salt marshes (Odum 1988, Więski et al. 2010).

Although salinization was directly stressful to the plants that we studied, this effect may have been partially ameliorated by increased nutrient availability. Porewater ammonium and phosphate concentrations were higher in the more salinized mesocosms. One mechanism increasing ammonium concentrations was likely the replacement of ammonium from soil cation exchange sites by sea salt cations (Ardón et al. 2013). Similarly, bio-available phosphate can increase with salinization due to desorption by chloride (Herbert et al. 2015). Moreover, increases in sulfate concentrations with salinization could enhance rates of organic matter mineralization through sulfate reduction (Weston et al. 2006). Finally, the high nutrient content in the more salinized treatments could also be due to lack of uptake by plants given the lower plant biomass in these treatments. This experiment was not designed to tease these potential mechanisms apart. This experiment was also not designed to directly measure any potential benefit of increased nutrients due to salinization; however, Li and Pennings (in review) found that Z. miliacea belowground biomass increased with low-salinity pulses that lasted up to 16 days, and speculated that this was due to an increase in nutrient availability in salinized treatments. These results indicated that porewater nutrient concentrations decreased from July to August, perhaps due to utilization of nutrients by the salt-tolerant plants as they

grew to fill the mesocosms. This possibility is consistent with the idea that saline pulses may benefit the growth of salt-tolerant plants, whereas salt-sensitive plants may be too stressed to take advantage of increased nutrient availability caused by salinization.

Overall, results from the treatment period imply that prolonged periods of salinization will change species composition, reduce species richness and reduce aboveground biomass in tidal freshwater marshes. Modest saline pulses may increase porewater nutrient concentrations that will support or even increase the growth of salt-tolerant species. However, if the saline conditions persist, such benefits will be overwhelmed by the negative effects of salt stress.

Despite being strongly suppressed by the salinity treatments, most of the plant species were able to recover from low salinity, short-duration saline pulses. Although *L. repens* had the lowest salt tolerance during the treatment period, it recovered rapidly in treatments of 3 PSU water for no more than 15 days and 5-PSU water for only 5 days. *L. repens* is characterized by rapid growth, and spreads by lateral shoot growth to form dense mats. This ability to rapidly expand should facilitate the recovery of *L. repens* after a disturbance (Rejmánková 1992). *P. hydropiperoides* was able to recover from salinization to a low abundance similar to its abundance in the control treatment. *E. palustris*, the most salt-tolerant species, became the dominant species in almost all experimental treatments during the recovery period. These results were consistent with previous studies indicating that a number of freshwater marsh species have the ability to recover from short-term salinity disturbances (Flynn et al. 1995, Goodman et al. 2010). Although the aboveground biomass of several of the species disappeared during the

treatment period in our experiment, seeds and rhizomes of some species persisted and supported recovery when fresh conditions returned.

Reproduction by seed also allowed new species to colonize the mesocosms during the recovery period. Reproduction by seed is more common in freshwater than in brackish or salt marshes (Crain et al. 2008). In this study, the seed bank survived the treatment period, and volunteer species emerged during the following year during the recovery period. The volunteers were common species that could be found in nearby tidal freshwater marshes, but were not observed in the freshwater pond where I collected sediments for the mesocosms. Thus, I believe that the volunteers were present as seeds in the root balls of the species that I planted, but did not emerge until cued to do so by going through the winter.

Because of the emergence of the volunteer species, the community structure at the end of the recovery period did not overlap with the community structure at the end of the treatment period. The communities in some of the less salinized treatments (3 PSU for 5 and 10 days, 5 PSU for 5 days) followed a similar recovery trajectory as the controls, whereas the other treatments increasingly diverged from the controls. This largely because *L. repens* recovered in the mildly salinized treatments, but remained absent in the heavily salinized ones.

Because not all species recovered in the heavily salinized treatments, species richness at the end of the recovery period was still low in the treatments that were heavily salinized during the treatment period. In contrast, plant aboveground biomass fully recovered in the salinized treatments despite the low species richness, and was even slightly higher in the treatments that experienced longer saline pulses, possibly because the longer saline pulses made more nutrients available for plant growth. Because these treatments did not experience fluctuating conditions but instead permanent saline conditions, the more salt-tolerant species may have been able to grow steadily and offset the reduction in biomass of the salt-sensitive species. Because some of the plant species present in tidal freshwater marshes are resilient to saline pulses, plant productivity can recover from even a fairly strong and extended saline pulse in less than one year. Plant composition, however, may be changed by an extended saline pulse. Thus, it is likely that the species composition of tidal fresh marshes that do and do not experience periodic saline intrusion will differ, but plant biomass may not.

The mesocosm experiment enabled us to conduct a more diverse set of treatments than it would not have been easily conducted in the field. At the same time, mesocosm experiments are an imperfect replica of natural conditions. The best way to rigorously extrapolate our results to the field would be to conduct additional experiments in a natural setting, perhaps with a subset of the treatments that I studied here in order to compensate for the more complicated logistical challenges inherent in field experiments.

Ecological responses to pulsed disturbance range from individual level mortality to major effects on ecosystem structure and function (Ciais et al. 2005, Thibault and Brown 2008, Smith 2011). This study suggested that primary productivity was more resilient to saline disturbance than community composition. Although both species richness and productivity were reduced by salinization, the surviving species were able to regrow during the recovery period and restore total community production. Without such compensation, reductions in ecosystem function may have persisted. Hoover et al. (2014) also found that despite change in community composition which persisted post-drought, net primary productivity recovered completely the year after an extreme drought and heat wave in a native tallgrass prairie in a Kansas, USA grassland. Therefore, changes in ecosystem structure due to global change pulses may not preclude recovery in ecosystem function.

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# Chapter 4 Responses of a tidal freshwater marsh plant community to constant and pulsed saline intrusion

### 4.1 Introduction

Predicting ecosystem responses to future environmental changes is an important goal of ecological research. One extreme scenario of environmental change is a chronic change, or "press" (Smith et al. 2009). Many studies have explored the consequences of potential environmental changes by using press treatments of, for example, sea-level rise (Langley et al. 2013) or elevated  $CO_2$  (Pastore et al. 2016). Press disturbances should cause a sequence of events starting with physiological responses, proceeding to changes in community structure, and finally species loss and immigration of species tolerant of the new conditions (Smith et al. 2009). However, in nature, ecosystems typically experience fluctuating disturbances rather than constant press changes (Visser et al. 2002, White and Alber 2009). Such disturbances of intermittent, or "pulsed", may also cause rapid individual-level mortality, and changes in community structure and ecosystem function (Thibault and Brown 2008, Ciais et al. 2005). However, there may not be permanent changes to the ecosystem, because the system may recover following the disturbance and return to its baseline conditions (Smith 2011, Ratajczak et al. 2017).

Both press and pulse changes to environmental variables are predicted due to climate and anthropogenic factors, and they often interact to affect biota in different ways (Parkyn and Collier 2004). Climate change is expected to affect temperature and precipitation patterns; in many areas this may result in reduced freshwater delivery to coastal habitats (Nijssen et al. 2001). Anthropogenic activities may also reduce freshwater discharge through diversion of freshwater for irrigation, household use, and industrial purposes (Enright and Culberson 2009, Cloern and Jassby 2012). At the same time, mean sea level is both variable among years and expected to increase between 0.38 and 2 m during the coming century (Church and White 2011, Horton et al. 2014). Sealevel variation will force saline water upstream into lower-salinity habitats, which will lead to both pulses and a long-term average press change in salinity along estuaries (Knighton et al. 1991, Wood and Harrington 2015). Depending on how they manifest, changes in both climate and anthropogenic effects can cause either presses or pulses of seawater intrusion into estuarine freshwater habitats.

As one moves coastward within an estuarine along a salinity gradient from freshwater to euryhaline, the vegetation composition of the tidal freshwater, brackish, and salt marshes along the continuum is determined by the interaction between salinity and competition (Pennings and Bertness 2001, Crain et al. 2004, Engels and Jensen 2010, Guo and Pennings 2012). Situated in the upper estuary, tidal freshwater marshes support high macrophyte diversity, productivity and nutrient retention (Costanza et al. 1998, Craft 2007, Loomis and Craft 2010, Wieski et al. 2010, Van de Broek et al. 2016). Tidal freshwater plants, however, cannot move downstream because they are stressed to varying degrees by increases in salinity (Guo and Pennings 2012, Li and Pennings in review). Elevated salinities in a tidal freshwater marsh, such as those caused by drought or high sea levels, can decrease plant growth through osmotic effects and the accumulation of toxic ions (Adam 1990), and ultimately drive shifts in the composition of plant communities (McKee and Mendelssohn 1989, Knighton et al. 1991, Sharpe and Baldwin 2012, Sutter et al. 2015). Given long-term projections of sea-level rise, freshwater marshes are expected to be largely replaced by brackish or salt marshes (Craft

et al. 2009). If salinization is only temporary, however, as might occur due to a drought, freshwater plants may be resilient to saline pulses and able to recover once the pulse is withdrawn (Flynn et al. 1995, Howard and Mendelssohn 1999, Goodman et al. 2010).

Press and pulse salinity disturbances may also directly or indirectly through their effects on plants alter the composition and biomass of other community elements as well. As the dominant structure and source of primary productivity in freshwater marshes, macrophytes are likely to have pervasive effects on the system. These effects include influences on benthic microalgae by altering light and nutrient availability (Sullivan and Currin 2002). Plant shading reduces, while nitrogen enrichment increases, the production of marsh microalgae (Charlene et al. 1976, Whitcraft and Levin 2007, Augyte and Pickart 2014). Both vascular plants and microalgae are food for fiddler crabs, other invertebrates and fish, and in turn can be controlled by these grazers (Sullivan and Moncreiff 1990, Silliman and Bertness 2002, Holdredge et al. 2009, Bertness et al. 2014). In addition, by buffering the harsh physical conditions in the marsh, providing structural support for burrows and refuge from predators, marsh plants can increase crab densities (Bertness and Miller 1984, Nomann and Pennings 1998, Bortolus et al. 2002, Wang et al. 2015). Understanding these cascading changes in not only plants but whole communities of associated species is critical for forecasting changes in both ecological structure and function.

I used a four-year manipulative field experiment to determine the responses of a tidal freshwater marsh plant community to presses and pulses of saline water addition. I predicted that 1) both presses and pulses of saline water addition would both suppress the performance of individual plants and cause species re-ordering within communities, but 2)

the community structure in the pulse treatment would recover once the saline pulses were withdrawn. Primary by altering the structure provided by marsh plants and thus light penetration and soil properties, I further predicted that 3) changes to the plant community would cascade to affect common marsh microalgae and the dominant burrowing invertebrate in this habitat, the fiddler crab *Uca minax*.

#### 4.2 Methods

This study was conducted in a tidal freshwater marsh (31°20'16" N, 81°27'52" W) on the Altamaha River, GA. The marsh contained a diverse mix of emergent herbaceous species with four dominant species: Ludwigia repens, Polygonum hydropiperoides, Pontederia cordata, and Zizaniopsis miliacea, with Z. miliacea dominating by biomass. The site was located approximately 1.92 - 2.08 m above mean lower low water and was flooded twice daily by the tides with maximum water depths ranging from 0.4 m to 1.6 m (https://gce-lter.marsci.uga.edu/private/saltex\_data/wells/data/). A total of 30 plots were established on the marsh platform in December 2012. The plots were randomly assigned to five treatments: control, control with frame, freshwater addition, press of saline water addition, and pulse of saline water addition (detailed methods in Herbert et al. unpublished manuscript). Each plot, except for the control plots, was defined by a 2.5 m x 2.5 m ridged corrugated polycarbonate frame, inserted 20 cm into the soil with 10 cm extending above the soil surface to minimize lateral movement of added water from the plot. The frames had drain holes in the sides above the soil surface that were plugged when plots were watered but open otherwise to allow tidal exchange. The elevation of each plot was measured using high-accuracy RTK GPS (Trimble R6; NAVD88 GEOID03), and the average plot elevation was 2.01 m (range 1.92 m to 2.08 m).

Plots were monitored without imposing any treatments in 2013. Treatments started on April 14, 2014. Plots received diluted saltwater (press and pulse of saline water treatments), freshwater (freshwater addition treatment), or no water additions (control with frame and control treatments). The press plots received additions of a mixture (approximately 16 PSU) of seawater and fresh river water three to four times each week, with the goal of elevating porewater salinity to 2 - 5 PSU. Seawater (~ 25 PSU) was collected from Hudson Creek at Meridian Landing (31°27'12" N, 81°21'46" W); freshwater (< 0.5 PSU) from the Altamaha River near the study site. Pulse plots received the same saline mixture at the same rate as press plots, but only for eight weeks in September and October. For the rest of the year, pulse plots received fresh river water. The freshwater treatment plots received additions of fresh river water four times each week, and porewater salinities were monitored to ensure that they were typical of ambient conditions, typically < 0.5 PSU. Once press treatments began, porewater samples were taken from each plot (randomly chosen) at least once per week and all plots were sampled at least once per month. Salinity was then measured from the samples using a handheld refractometer, to monitor the efficacy of the treatments. Five plots (two control with frame plots, one freshwater addition plot, and two press of saline water addition plots) did not match the treatment porewater salinity goals, and therefore were dropped from this study. A more detailed description of the site and the experimental methods is provided in Herbert et al. (unpublished manuscript).

To document treatment effects on the plant community, I measured stem height, photosynthesis, and percent cover of plants, and light interception by the vegetation. I measured plant variables within a permanent  $0.75 \text{ m} \ge 0.75 \text{ m}$  subplot inside each plot,

and took the measurements three to six times during each growing season from 2013 to 2016 (Table C1). I measured the height of all stems of Z. miliacea and P. hydropiperoides, and all leaves of *P. cordata*. In each plot, I chose the tallest individual of those three species and measured their leaf level photosynthetic rates using an LCi photosynthesis System (ADC BioScientific Ltd., Hoddesdon, UK). Around the same dates, I also visually estimated the percent cover of the four most abundant plant species, L. repens, P. hydropiperoides, P. cordata, and Z. miliacea, in each entire 2.5 m x 2.5 m plot (Dethier et al. 1993). The total canopy cover can exceed 100% because the natural spread of foliage of plants overlap. I did not focus on L. repens before the treatments started due to its creeping morphology. Other plant species were rare and typically occurred in only a handful of the plots. I measured the photosynthetically active radiation (PAR) above and underneath (10 cm above the soil) the plant canopy in each plot using a Sunscan Canopy Analysis System (Delta-T Devices Ltd., Cambridge, UK) four to six times during each growing season from 2014 to 2016. On each measurement date, I calculated the proportion of light reaching underneath the canopy (PAR underneath/PAR above).

In July 2016, I collected 50 Z. *miliacea* individuals (50 cm - 235 cm), 95 P. *cordata* individuals (30 cm - 120 cm), and 54 P. *hydropiperoides* individuals (30 cm - 123 cm) from marshes immediately adjacent to the study site. The height of the stem or leaves was measured on site, and then the plant materials were dried at 60 °C to constant mass. I used these height and mass data to create polynomial relationships between biomass and plant height or cover for all species (Table C2). I assumed that the allometric relationships were the same for plants in all plots, and used these allometric relationships

to estimate aboveground biomass from non-destructive height measurements that were taken during the treatments.

To evaluate treatment effects on benthic microalgae, the biomass of green algae, diatoms and cyanobacteria were estimated using an in situ fluorimetric probe (bbe-Moldaenke BenthoTorch) in 2016 (dates in Table C1). The probe uses spectral analysis to estimate the abundance of these three microalgal groups in surface sediments (Echenique-Subiabre et al. 2016). Four readings were taken in each plot and averaged to give a single value per plot per date. The most abundant benthic macro-invertebrate at the study site was the fiddler crab *Uca minax* (Teal 1958). To determine treatment effects on fiddler crab density, adult fiddler crab burrows (> 0.5 cm in diameter) were counted in an 3750 cm<sup>2</sup> quadrat in each plot in spring and fall from 2014 to 2016 (dates in Table C1).

I used repeated-measures analysis of variance (ANOVA) to identify treatmentrelated differences in estimated aboveground biomass (individual species and total), percent cover, and proportion of light penetrating the canopy, fiddler crab burrow density, and microalgae biomass over time (2013 - 2016), with elevation as an covariate. To minimize the effects of the annual growth cycle on the results, I limited statistical analyses of the plant responses to data collected in June, July and August when aboveground biomass was at its seasonal peak. I compared differences among treatments on all sampling dates, including dates outside June, July and August (Table C1) with individual ANOVAs followed by Tukey's HSD tests. To describe the temporal dynamics of the plant community for the whole year, I used rank-abundance curves (Collins et al. 2008) based on species percent cover, as we did not have allometric relationships to estimate biomass of *L. repens* due to its creeping morphology. I used analysis of similarities (ANOSIM, Bray-Curtis dissimilarity) based on species percent cover data to examine the compositional dissimilarity of plant communities across treatments. I analyzed the abundance of the three groups of benthic microalgae in 2016 using repeatedmeasures ANOVA followed by individual ANOVAs for each date; treatments were compared with Tukey's HSD tests. To examine effects of marsh macrophytes on light penetration and fiddler crab burrows, we used linear regression to correlate these variables with estimated total plant biomass using data collected in Fall 2016 (light penetration on 11/15/2016, fiddler crab burrow counts on 9/23/2016, and plant biomass on 10/28/2016). To examine effects of light availability on benthic microalgae, we used linear regression to correlate microalgae abundance measured on 11/30/2016 with light penetration measured on 11/15/2016. For all statistical analyses, significance was set at P = 0.05. The ANOSIM analysis was conducted with PAST ver. 3.11, and all other analyses were conducted with JMP 10.0 statistical software (SAS Institute Inc.).

4.3 Results

Within a few weeks after saline water additions began, porewater salinity in the press treatment rose to oligohaline conditions (0.5 - 5 PSU), with an average of 2.08 ± 0.05 PSU over the duration of the entire experiment (Fig. 4.1 a). The porewater salinity in the pulse plots increased to oligohaline conditions during each treatment period, with an average of 1.28 ± 0.15 PSU in 2014, 1.40 ± 0.16 PSU in 2015, and 1.55 ± 0.15 PSU in 2016 (Fig. 4.1 b). The control, control with frame and freshwater addition plots had salinities typical of the tidal freshwater zone (< 0.5 PSU), with averages over the entire experiment of 0.20 ± 0.01 PSU, 0.33 ± 0.02 PSU, and 0.23 ± 0.01 PSU, respectively (Fig. 4.1 c - e).



Fig. 4.1 Porewater salinities in all plots from the beginning of the press treatments (4/14/2014) to 12/21/2016. The solid lines are the loess smoothing curves based on the actual data points (open circles). A polynomial degree of one and the nearest neighbor bandwidth method were used. No measurements were taken before April 2014.

I did not observe rapid changes in plant photosynthetic rates as expected (Fig. C1). *P. hydropiperoides* photosynthetic rate was not significantly affected by the press treatment in 2014, and it completely disappeared from the press treatment plots in 2015 and 2016, and so could not be examined further (Fig. C1 a). The photosynthetic rate of *P. cordata* did not differ among treatments throughout the study period (Fig. C1 b). *Z. miliacea* photosynthetic rate declined in the press treatment in 2014, with the strongest differences among treatments in August 2014 as indicated by a significant Tukey HSD comparison (Fig. C1 c). In later years, *Z. miliacea* was absent from some press plots, reducing the power of these comparisons.



Fig. 4.2 Estimated aboveground biomass ( $\pm$  SE) of *Polygonum hydropiperoides*, *Pontederia cordata*, and *Zizaniopsis miliacea* from 2013 to 2016. The light grey areas indicate the press treatment periods, and the dark grey areas indicate the pulse treatment periods. Asterisks indicate significant differences (P < 0.05) between treatment and control plots each month based on Tukey's HSD tests.

Aboveground biomass of *P. hydropiperoides*, *P. cordata*, and *Z. miliacea* was affected by treatment and the interaction of date and treatment (all P < 0.01; Table 4.1) with the effect of the press treatment generally strengthening through time. In July 2014 after only three months of saline water additions, *P. hydropiperoides* aboveground biomass was reduced by 75% in the press plots relative to the controls (Fig. 4.2a). *P. hydropiperoides* continued to decline in aboveground biomass in 2014, and disappeared in press plots in 2015 and 2016. The pulse treatment also reduced the aboveground biomass of *P. hydropiperoides* by 67%, 41%, and 44% relative to the controls in October

2014, 2015, and 2016, respectively, but these differences were not significant in Tukey's HSD tests due to high variation among replicates and the modest replication of the experiment. P. hydropiperoides aboveground biomass was also negatively related to elevation (P = 0.02), and was affected by the interaction of elevation and treatment (P =0.04; Table 4.1). The aboveground biomass of *P. cordata* was unaffected by treatments in 2014 but was lower in the press treatment in summers of 2015 and 2016 (Fig. 4.2b). The averaged biomass in summer (June – August) 2015 and 2016 in the press plots was reduced by 80% and 84% relative to the controls; again, however, these differences were not statistically significant based on Tukey's HSD tests on individual dates due to high variability and the modest replication of the experiment. P. cordata aboveground biomass was positively related to elevation (P < 0.01), and was also affected by the interaction of elevation and treatment (P < 0.01; Table 4.1). Aboveground biomass of Z. miliacea decreased in the press treatment starting in October 2014, with a reduction of 71% relative to the controls in 2016 (Fig. 4.2c). Z. miliacea biomass was reduced by 39% and 18% in the pulse plots in October 2014 and 2015 (not significant in Tukey's HSD tests), but was the same as the controls in October 2016. Z. miliacea aboveground biomass was also negatively related to elevation (P < 0.01), and was affected by the interaction of elevation and treatment (P < 0.01; Table 4.1).

Table 4.1 Repeated – measure Analysis of variance (ANOVA) for estimated aboveground biomass, and percent cover across June and August from 2013 to 2016. Asterisks indicate statistical significance (P < 0.05). The interaction between elevation and date was not significant for any of the variables, and therefore was dropped. For the continuous variable of elevation, + and – denote the directionality of the effect.

Variable	Effect	Polygonum hydropiperoides	Pontederia cordata	Zizaniopsis milacea	Ludwigia repens
Estimated aboveground biomass	Date	0.03*	0.30	< 0.01*	NA
	Treatment	< 0.01*	< 0.01*	< 0.01*	NA
	Date × Treatment	< 0.01*	< 0.01*	< 0.01*	NA
	Elevation	0.02*(-)	< 0.01*(+)	< 0.01*(-)	NA
	Elevation × Treatment	0.04*	< 0.01*	< 0.01*	NA
Percent cover	Date	< 0.01*	0.03*	0.18	< 0.01*
	Treatment	< 0.01*	< 0.01*	< 0.01*	< 0.01*
	Date × Treatment	< 0.01*	< 0.01*	< 0.01*	< 0.01*
	Elevation	0.93	0.66	< 0.01*(-)	0.04* (+)
	Elevation × Treatment	0.85	0.06	< 0.01*	0.10

The abundance (percent cover) of *L. repens*, *P. hydropiperoides*, *P. cordata*, and *Z. miliacea* was affected by treatment and the interaction of date and treatment (all P < 0.01; Table 4.1). *L. repens* cover was reduced by 95% in the press plots relative to the controls within one month after saline water addition, and it remained absent in the press plots afterwards except for a few small plants observed in June to August 2016 (Fig. 4.3

a). L. repens cover was reduced by 68% in the pulse plots relative to the controls in October 2014, and it remained absent in October 2015 and October 2016. However, L. repens regrew in the pulse plots over each following summer, but never attained more than 11% cover, well below its abundance in the control plots (~70% cover). L. repens was also positively related to elevation (P = 0.04), but was not affected by the interaction of elevation and treatment (P = 0.10; Table 4.1). The percent cover of *P. hydropiperoides* was reduced by 25% in press plots relative to the controls in May 2014 (Fig. 4.3 b). The percent cover continued to decrease until P. hydropiperoides disappeared in 2015 and 2016. P. hydropiperoides cover was reduced by 12% and 44% in the pulse plots relative to the controls in October 2014 and 2015, respectively (not significant in Tukey's HSD tests). In October, P. hydropiperoides cover was 33% higher in the pulse plots compared to the controls (not significant in Tukey's HSD tests). The percent cover of *P. cordata* was unaffected in 2014, but was then lower in the press treatment in summers of 2015 and 2016 (Fig. 4.3 c). The percent cover of both P. hydropiperoides and P. cordata was not affected by elevation, or the interaction of elevation and treatment (P > 0.05, Table 4.1). Percent cover of Z. miliacea was affected by treatment, the interaction of date and treatment, elevation, and the interaction of elevation and treatment (all P < 0.01; Table 4.1). Z. miliacea cover was reduced by 42% in the press plots compared to the controls in October 2014, and remained lower in the following two years (Fig. 4.3 d). The percent cover in the pulse plots was not significantly affected.



Fig. 4.3 Percent cover ( $\pm$  SE) of *Ludwigia repens*, *Polygonum hydropiperoides*, *Pontederia cordata*, and *Zizaniopsis miliacea* from 2013 to 2016. The cover of *Ludwigia repens* was not measured before May 2014. The light grey areas indicate the press treatment periods, and the dark grey areas indicate the pulse treatment periods. Asterisks indicate significant differences (P < 0.05) between treatment and control plots each month based on Tukey's HSD tests.

Plant community composition in the press and pulse plots diverged significantly from the controls once the salinity treatments started (Fig. 4.4). The rank abundance curves in the control, control with frame, and freshwater addition treatments were similar over the duration of the experiment (Fig. 4.4 a - c). In the pulse treatment, the rank abundance of *L. repens* declined sharply after the first pulse event (Oct 2014); and the community composition significantly differed from the controls from then on (Fig. 4.4 d). The press treatment diverged from the control plots one month after the treatments started due to the loss of *L. repens* and decline of *P. hydropiperoides* and *P. cordata* (Fig. 4.4 e). Although *Z. miliacea* remained the highest-ranked plant in the press treatment, it also decreased in abundance over time.

Both total aboveground biomass and the proportion of light penetrating canopy were affected by date, treatment, and their interaction (all P < 0.01; Table 4.2). As community structure shifted, total aboveground biomass in the press treatment decreased and remained low year-round (Fig. 4.5). At the beginning of each growing season all treatments had converged on similarly low biomass, and all treatments except the press exhibited rapid increase in biomass over each growing season (Fig. 4.5). Starting in 2015, the proportion of light penetrating the vegetation canopy increased in press plots (Fig. 4.5). The pulse treatment did not differ from the controls in biomass and light penetration. Elevation had a negative effect on total aboveground biomass (P < 0.01), but did not affect light penetration (P = 0.14), while the interaction of elevation and treatment affected both variables (P < 0.01; Table 4.2).

The abundance of benthic microalgae was significantly affected by treatment (all P < 0.01; Table 4.2). Green algae were less abundant than cyanobacteria and diatoms in our study site, and did not show date-specific significant differences between the press or pulse treatment and the control plots (Fig. 4.6 a). In contrast, cyanobacteria and diatoms

were significantly more abundant in the press plots than in the other treatments (Fig.4. 6 b, c). Elevation had a negative effect on cyanobacteria abundance (P < 0.01), but did not affect green algae (P = 0.73) or diatoms (P = 0.60). Both green algae and cyanobacteria were significantly affected by the interaction of elevation and treatment (both P < 0.01; Table 4.2).



Fig. 4.4 Rank-abundance curves based on percent cover data for *Ludwigia repens*, *Polygonum hydropiperoides*, *Pontederia cordata*, and *Zizaniopsis miliacea* in all plots from 2013 to 2016. The cover of *Ludwigia repens* was not measured before May 2014. The light grey areas indicate the press treatment periods, and the dark grey areas indicate the pulse treatment periods. Species percent cover was averaged across the five replicate plots of each treatment. Asterisks indicate significant differences (P < 0.05) between treatment and control plots for each month (ANOSIM).

Finally, we found that the treatment-induced changes in plant cover and composition were associated with cascading effects on physical conditions, fauna and microalgae. Specifically, light penetration through the canopy decreased and fiddler crab burrow density generally increased with increasing aboveground biomass of plants (Fig. 4.7 a – b). Crab burrow number varied significantly by date (P < 0.01), treatment (P < 0.01), the interaction of date and treatment (P = 0.01), and the interaction of elevation and treatment (P = 0.02; Table 4.2). The abundance of both cyanobacteria and diatoms were positively related with proportion of light penetrating canopy (Fig. 4.7 c – d). The abundance of green algae was not related to changes in light penetration (P = 0.78).

Table 4.2 Repeated – measure Analysis of variance (ANOVA) for total aboveground biomass, proportion of light penetrating canopy, fiddler crab burrow number, and the abundance of microalgae (green algae, cynobacteria, and diatoms) across June and August from 2013 to 2016. Asterisk indicates statistical significance (P < 0.05). The interaction between elevation and date was not significant to all variables, and therefore was dropped. For the continuous variable of elevation, + and – denote the directionality of the effect.

Effect	Total aboveground biomass	Proportion of light penetrating canopy	Green algae abundance	Cyanobacteria abundance	Diatoms abundance	Fiddler crab burrow number
Date	< 0.01*	< 0.01*	0.25	0.23	< 0.01*	< 0.01*
Treatment	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*
Date $\times$ Treatment	< 0.01*	< 0.01*	0.25	< 0.01*	< 0.01*	0.01*
Elevation	< 0.01*(-)	0.14	0.73	< 0.01*(-)	0.60	0.54
Elevation × Treatment	< 0.01*	< 0.01*	< 0.01*	< 0.01*	0.97	0.02*

62



Fig. 4.5 Estimated aboveground biomass ( $\pm$  SE, 2013 – 2016) and proportion of light penetrating canopy ( $\pm$  SE, 2014 – 2016) in all plots. The light grey areas indicate the press treatment periods, and the dark grey areas indicate the pulse treatment periods. Asterisks indicate significant differences (P < 0.05) between treatment and control plots each month based on Tukey's HSD tests.



Fig. 4.6 The abundance of green algae, cyanobacteria, and diatoms ( $\pm$  SE) in all plots from February to November, 2016. The light grey areas indicate the press treatment periods, and the dark grey areas indicate the pulse treatment periods. Asterisks indicate significant differences (P < 0.05) between treatment and control plots each month based on Tukey's HSD tests.


Fig. 4.7 Effects of plant aboveground biomass on a) proportion of light penetrating the canopy and b) fiddler crab burrow number. Effects of proportion of light penetrating the canopy on c) abundance of cyanobacteria and d) abundance of diatoms. Data were from fall (September to November) 2016. The relationship between green algae abundance and proportion of light penetrating the canopy was not significant (P = 0.78), and therefore was not shown.

### 4.4 Discussion

Climate change is predicted to cause constant and pulsed saline water intrusion into tidal freshwater marshes (White and Alber 2009, Herbert et al. 2015, Wood and Harrington 2015). The effects of salinity on freshwater marsh plants have been previously studied, but many of these studies were conducted in the greenhouse over relatively short periods of time, and typically did not contrast press and pulse treatments (Howard and Mendelssohn 2000, Spalding and Hester 2007, Sharpe and Baldwin 2012, Woo and Takekawa 2012, Sutter et al. 2015). I examined the effects of a saline press and pulse over three years in the field. The results supported my first hypothesis that both constant and pulsed salinization suppressed the performance of individual plants and caused species re-ordering. I predicted that the community structure in the pulse treatment would recover once the saline pulses were withdrawn. This prediction was not supported as the community composition in the pulse treatment remained different from the control. We found that prolonged salinization reduced plant biomass and cover over time, leading to increased light penetration, increased cyanobacterial and diatom abundance, and decreased fiddler-crab density. This supported our last hypothesis that salinization-induced changes in marsh plants would cascade to affect common marsh microalgae and the dominant burrowing invertebrate in this habitat.

The four species monitored in this study displayed distinct variation in salinity tolerance. *L. repens* largely disappeared from press plots one month after their respective salinity additions began. *P. hydropiperoides* was also sensitive to salinity and declined in cover and productivity during the first year of saline water addition. *Z. miliacea* was less sensitive. It gradually declined in the press plots since fall 2014, and remained lower than in other treatment plots in the following two years. Overall, *P. cordata* displayed the fewest significant responses to salinity increases. The aboveground biomass of *P. hydropiperoides*, *P. cordata*, and *Z. miliacea* in the non-salinized plots showed a similar seasonal pattern every year, with a peak in July and August. This pattern was similar to

previous descriptions of the phenology of *P. cordata* and *Z. miliacea* (Birch and Cooley 1982, Heisey and Antoni 1982).

The responses of L. repens, P. hydropiperoides and Z. miliacea to elevated salinity described here were consistent with those found in the greenhouse experiment described in Chapter 3, where freshwater marsh communities were exposed to a combination of salinity levels (3, 5, and 10 PSU) and durations (5, 10, 15, 20 and 30 days per month). In Chapter 3 I found that L. repens and P. hydropiperoides were strongly suppressed by the more extreme saline treatments. Z. miliacea was relatively resistant to the saline treatments, and maintained biomass in most of the mesocosms. P. cordata was only affected with high salinity treatments (5 PSU water for 20 and 30 days, and 10 PSU water for more than 5 days). In contrast, P. cordata aboveground biomass declined steadily with saline exposure when planted individually (Li and Pennings, in review). It is possible that the loss of L. repens and P. hydropiperoides reduced competition and benefited the growth of *P. cordata* in the field. Therefore, *P. cordata* growth declined in response to salinization when it was planted individually, but not in this field experiment when it benefitted from the declines of other plant species. When planted individually, P. hydropiperoides aboveground biomass did not respond to short saline pulses (up to 4 days in duration) but declined sharply thereafter, while Z. miliacea aboveground biomass did not respond to salinity (Li and Pennings, in review).

I expected hierarchical responses with relatively fast physiological changes followed by species re-ordering (Smith et al. 2009). However, I observed a concurrent combination of individual and community level changes. I did not observe any responses in plant photosynthetic rates until after the growth reduction appeared. Other studies also found that photosynthetic rate per unit leaf area remained unchanged because salinity treatments increased chloroplast density per unit leaf area (Munns and Tester 2008). I measured leaf chlorophyll content of *P. hydropiperoides*, *P. cordata*, and *Z. miliacea* every month in 2016, but did not find any difference among treatments (data not shown). Therefore, I was not able to document physiological changes preceding species reordering

I found that both presses and pulses of saline water addition caused plant community composition to shift away from the controls, but at different speeds and to different degrees. With presses of saline water, all species declined in percent cover by the end of 2016. Plant species richness and diversity are generally higher in freshwater and brackish marshes compared to salt marshes, due to salinity or sulfide-induced death of non-halophytes (Odum 1988, Adam 1990, Lamers et al. 1998, Wieski et al. 2010). Wetzel et al. (2004) found plant community shifts within 6 to 18 months of marsh exposure to elevated salinity, with more salt-sensitive species lost than salt-tolerant species gained. However, the die-off of freshwater species reduced competition and increased the chances of brackish species immigration. Brackish and salt marsh plants perform better in low-salinity environment but only if competitors are removed (Crain et al. 2004, Guo and Pennings 2012). In this experiment, brackish plants were largely absent due to dispersal limitation. If the experiment had been conducted close to the transition point from fresh to brackish vegetation, I may have observed immigration of brackish marsh plants into the press plots as the fresh marsh plants died off.

I expected the community structure in the pulse treatment to recover once the saline pulses were withdrawn. In contrast, I observed persisting dissimilarity in plant community composition between the pulse treatment and the control. L. repens disappeared in the pulse plot soon after each pulse event, and was able to partially recover in the late summer of the following year. P. hydropiperoides growth was also depressed by the pulse event, but fully recovered during the next growing season. Many tidal freshwater-marsh plants have some ability to recover after saline pulses (Flynn et al. 1995, Howard and Mendelssohn 1999, Sutter et al. 2014, Li and Pennings in review), but changes in community structure were also observed. In a mesocosm experiment conducted in Louisiana, S. lancifolia failed to recover after the community was exposed to 12 g/L (PSU) saline water for three months, resulting in a shift to a monospecific S. americanus stand (Howard and Mendelssohn 2000). In this study, the community composition in the pulse treatment remained different from the controls since the first pulse event. With no change in the growth of Z. miliacea and P. cordata and slow recovery of L. repens, communities with repeated pulses of saline water did not recover to the baseline conditions but also did not turn to a dramatically different state. These results suggest that periodic pulses of salinization may reduce species diversity and alter plant composition in tidal fresh marshes, even if the dominant plants are not strongly affected.

Although repeated saline pulses caused species re-ordering in this study site, total aboveground biomass was maintained at levels similar to those in the controls. The total aboveground biomass in the non-salinized plots peaked around August every year, and increased from 2013 to 2016. This was consistent with long-term monitoring of a *Z. miliacea*-dominated marsh located approximately 800 m upstream of our study site (Li et al. unpublished manuscript). Salinity presses, on the other hand, caused significant reduction in plant aboveground biomass since October 2014. These results implied that if immigration of brackish water vegetation is impeded, future long-term saltwater intrusion caused by climate change may significantly reduce primary production. Decreased productivity would not only reduce the carbon storage in tidal freshwater marshes, but also decrease the ability of sediment trapping (Morris et al. 2002). However, this loss in biomass may be compensated for if brackish species invade, which did not happen in our experiment.

In another long-term saltwater addition experiment in South Carolina, however, Neubauer (2013) found that adding freshwater to the *Z. miliacea* dominated marsh decreased ecosystem primary production,  $CO_2$  emissions, and net ecosystem production. However, we did not observe differences in plant performance in freshwater addition treatment relative to the control. Meanwhile, there was no difference in ecosystem carbon dioxide and methane exchange in the freshwater addition treatment within the first year of the treatments (Herbert et al. unpublished manuscript). The study site in Neubauer (2013) was located at a relatively high elevation, and did not flood on every high tide. Therefore, the plant community was sensitive to flooding stress, and responded to freshwater addition treatment.

The loss of marsh vegetation allows more sunlight to reach the sediment, and may promote the growth of benthic microalgae (Sullivan and Currin 2002, Whitcraft and Levin 2007). In addition, porewater ammonium and phosphate increased in press plots 2 -4 months after adding saline water (Herbert et al. unpublished manuscript), perhaps due to reduced uptake by plants, and this could also benefit the growth of benthic microalgae (Sin et al. 2007). I found increased light penetration and increased abundance of cyanobacteria and diatoms in the press treatment. Because benthic microalgae are a primary carbon source for estuarine food webs (Sullivan and Moncreiff 1990), the changes in microalgae production and composition will be likely to cause a cascading effect on macrofaunal community dynamics.

The presence of vegetation has significant effects on the distributions on crabs burrows. Crab burrows had particularly low density in press plots where vegetation was nearly absent. First, plant rhizomes and roots provide structural support for crab burrowing in soft sediments (Bertness 1985). The mud fiddler crab *Uca pugnax* preferentially burrows close to underground parts of *Spartina alterniflora* in low salt marsh habitats, to gain structural support for its burrows (Bertness and Miller 1984). Second, the lack of vegetation may increase the exposure of marsh crabs to predators. In salt marshes, the number of fiddler-crab burrows increased with shading that provided refuge from predators in clearings (Nomann and Pennings 1998). Theoretically it is possible that crabs and vegetation could be negatively correlated if reduced vegetation and subsequent increased light penetration increased microalgae abundance providing more food to the crabs. However, in our experiment, the positive association of crabs and vegetation suggest that structure and habitat were more important than an increase in food supply. Because the experimental plots were not particularly large, it is possible that crabs burrowed in vegetated plots but foraged in the press plots where macrophyte density was reduced and microalgal abundance increased.

Results from this study highlight the complex and nonlinear responses of tidal freshwater-marsh plants to future saltwater intrusion. Pressed salinization, such as might occur due to sea level rise or long-term freshwater withdrawal will affect both community structure and primary productivity. However, if seeds or rhizomes of salt-tolerant plants are available, their growth may compensate for the reduction in primary productivity of the fresh marsh plants (Sharpe and Baldwin 2012). In contrast, if freshwater marshes are exposed to pulsed salinization such as episodic droughts, there may be shifts in community composition without large changes in plant biomass and associated community-level primary productivity. However, the communities are not likely to recover rapidly to pre-disturbance composition after the pulse disturbance relaxes, due to the slow reinvasion of salt-sensitive species. Therefore, pulsed salinization events may not strongly affect ecosystem function, but may change the plant composition of tidal fresh marshes.

#### 4.5 Acknowledgement

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### **Chapter 5 Conclusion**

Human activities interacting with climate change are expected to threaten ecosystems worldwide and the services that they provide (Altieri et al. 2012, Cloern and Jassby 2012, Neubauer 2013, Hoover et al. 2014, Zhou et al. 2016). Pulse disturbances triggered by extreme events are expected to increase in frequency, and ongoing press disturbances are expected to continue (Karl et al. 2008, Smith et al. 2009, Smith 2011). Although the distinction between pulse and press disturbances has received much attention, natural disturbances are often combinations of both (Donohue et al. 2016). Ecologists need to understand the consequence of various environmental changes, so that they can predict the future state of ecosystems, and improve management and conservation practices.

Understanding the responses of tidal marsh plants to different saline water intrusion scenarios is critical to predicting the fate of tidal marshes. Salinization can occur on many time scales: a gradual and prolonged change like sea level rise (Knighton et al. 1991, Wood and Harrington 2015), the constant freshwater withdrawn for human usage (Sklar and Browder 1998, Enright and Culberson 2009, Cloern and Jassby 2012), or acute pulses like frequent droughts that eventually relax (Visser et al. 2002, White and Alber 2009, Goodman et al. 2010). Past studies have shown that salinity level and exposure duration can have important impacts on wetland ecosystem processes, but have been limited in terms of predicting responses to real disturbances in nature (Donohue et al. 2016). My research used a combination of greenhouse and field experiments to study the impacts of multiple salinization scenarios on tidal freshwater marsh plants.

The results of the studies in this dissertation suggested that common freshwatermarsh plants in coastal Georgia varied in their tolerance to saline water addition (Table 5.1). In all three studies, *Zizaniopsis miliacea* exhibited higher tolerance to saline pulses than Polygonum hydropiperoides and Pontederia cordata. In Chapter 2, I showed that Z. *miliacea* belowground and total biomass appeared to increase with salinity pulses up to 16 days in length, although this relationship was quite variable. Alternating between fresh and saline water can release nutrients from wetland soils (Weston et al. 2011), and such salinity-induced nutrient availability may benefit salt-tolerant species. However, if the plants are too stressed by salt, uptake of the nutrients will be disrupted. Therefore, the belowground and total biomass of P. hydropiperoides and P. cordata steadily declined with increasing duration of salinity pulses. In Chapter 3, I discuss an experiment in which I grew several freshwater species together, and ranked the salinity tolerance of the species as follows, from least to most salt tolerant: L. peploides, P. hydropiperoides, P. cordata, and Z. miliacea, followed by S. lancifolia and E. palustris in a tie. In this study, I found porewater ammonium and phosphate concentrations were higher in the more salinized mesocosms, and nutrient concentrations decreased from July to August, suggesting utilization of nutrients by the more salt-tolerant plants as they grew to fill the mesocosms. This is consistent with the idea that saline pulses may benefit the growth of salt-tolerant plants, whereas salt-sensitive plants may be too stressed to take advantage of an increase in nutrient supply. In Chapter 4, I showed that L. repens and P. hydropiperoides were the first species to disappear with presses of saline water addition. However, P. cordata displayed few significant responses to salinity increases, in contrast to the greenhouse experiments in which it was fairly sensitive to salinity. It is possible that the loss of L.

*repens* and *P. hydropiperoides* from the field plots reduced overall levels of competition and thereby benefited the growth of *P. cordata*.

Species	Chapter 2	Chapter 3	Chapter 4
Zizaniopsis miliacea	Tolerant	Tolerant	Tolerant
Pontederia cordata	Sensitive	Sensitive	Tolerance
Polygonum hydropiperoides	Highly sensitive	Sensitive	Sensitive
Ludwigia peploides	N/A	Highly sensitive	Highly sensitive
Sagittaria lancifolia	N/A	Tolerant	N/A
Eleocharis palustris	N/A	Tolerant	N/A
Peltandra virginica	N/A	Poorly	N/A
		established	

Table 5.1 The tolerance to saline water addition of species studied in this dissertation.

I found that saline pulses caused the plant community composition to shift away from the controls, both in mesocosms (Chapter 3) and in the field (Chapter 4). Highersalinity, longer-duration saline pulses caused community composition shifts towards more salt-tolerant species in the mesocosm experiment where seven species were planted together. By the end of the treatment period, *E. palustris* dominated in almost all treated mesocosms. The dominance of *E. palustris* lasted through the recovery period, which saw the emergence of several volunteer species (*Cyperus odoratus, Lilaeopsis chinensis, Juncus marginatus, Schoenoplectus tabernaemontani*, and *Typha latifolia*) in all mesocosms. Therefore, neither the treated mesocosms nor the controls recovered to match the pre-treatment community composition. However, due to the regrowth of *L. repens*, mesocosms receiving mild salinity pulses recovered along the same general trajectory as the controls. In the field experiment, both presses and pulses of saline water addition caused the loss of salt-sensitive species, i.e., *L. repens* and *P. hydropiperoides*. With presses of saline water, *Z. miliacea* and *P. cordata* also declined in percent cover by the end of 2016. With repeated pulses of saline water, community composition did not recover to the baseline conditions due to the slow recovery of *L. repens* and *P. hydropiperoides*, *hydropiperoides*, even though there was no change in the growth of *Z. miliacea* and *P. cordata*.

In the mesocosm experiment (Chapter 3), shifts in community composition prevented long-term reductions in productivity. Plant aboveground biomass fully recovered in the salinized treatments despite reduced species richness, and was even slightly higher in the treatments that experienced longer saline pulses, possibly because the longer saline pulses made more nutrients available for plant growth. In the field experiment (Chapter 4), productivity was maintained despite pulses of saline water addition that removed some plant species from the community. Salinity presses, on the other hand, caused a significant reduction in plant aboveground biomass. This was not compensated for because the nature of the field experiment precluded immigration of more salt-tolerant species from downstream; however, species immigration would probably occur following a severe salinization event in nature, and would probably compensate at least in part for the loss of biomass of salt-sensitive species.

In the field experiment, the loss of vegetation increased light penetration and increased abundance of benthic cyanobacteria and diatoms. As a primary carbon source for estuarine food webs, these changes in microalgae production and composition will be likely to cause a cascading effect on macrofaunal community dynamics. Meanwhile, decreased aboveground biomass in the press treatments also decreased the number of fiddler crab burrows, through either due to loss of structural support for burrows or increased exposure of crabs to predators.

Results from these studies highlight the complex and nonlinear responses of tidal freshwater marsh plants to future saltwater intrusion. If salinization proceeds as sea level rise or long-term freshwater withdrawal (a press), I predict a strong suppression growth of salt-sensitive plants. If seeds or rhizomes of salt-tolerant plants are available, shifts in species composition may prevent large reductions in biomass. In contrast, if salinization proceeds as an episodic drought (a pulse), I predict that there will be shifts in community composition without large changes in plant biomass. However, the communities are not likely to rapidly recover to pre-disturbance composition after the pulse disturbance relaxes, either because salt-sensitive species may take time to reinvade, or because new species may have taken advantage of the new conditions to immigrate. In general, species composition of most habitats should be more sensitive to both pressed and pulsed environmental changes than overall ecosystem processes to environmental change. However, changes in ecosystem structure due to global change pulses may not preclude recovery in ecosystem function (Fig. 5.1).



Fig. 5.1 Ecosystem structure and productivity response to pressed and pulsed environmental changes. Both pressed and pulsed environmental changes can cause individual-level effects and changes in community structure (species re-ordering). Pressed changes lead to large ecosystem impacts (species loss/invasion), and may cause state change. Community structure is not likely to rapidly recover to pre-disturbance composition after the pulse disturbance. However, changes in ecosystem structure pulses may not preclude recovery in ecosystem productivity. Reproduced from Smith (2011).

### Appendix A. Chapter 2 supplementary material

Site	Study period	DOI
GCE 7	10/08/2001 – 12/31/2001	10.6073/pasta/b14d5352e9085042a7fb906bc0847cda
GCE 7	01/01/2002 – 12/31/2002	10.6073/pasta/3f44fd887ded4dac916a0d88fc184c10
GCE 7	01/01/2003 – 12/31/2003	10.6073/pasta/6d71e7e7337b6b1b9993435b31fe3fa7
GCE 7	01/01/2004 – 12/31/2004	10.6073/pasta/2844b05a4cfd2818fc56c53f6c40b4d2
GCE 7	01/01/2005 – 12/31/2005	10.6073/pasta/1dc323631ea225c63e0ab15b7b975b88
GCE 7	01/01/2006 <b>-</b> 12/31/2006	10.6073/pasta/6414c7622116f87259175f4c61a5e13b
GCE 7	01/01/2007 - 12/31/2007	10.6073/pasta/5819a62f66c30a7315778febe9fc098c
GCE 7	01/01/2008 - 12/31/2008	10.6073/pasta/090d45948b5d0cc44490dd9ef22c905c
GCE 7	01/01/2009 - 12/31/2009	10.6073/pasta/6e9e6d8ee7192fb5ac551faf54ce424c
GCE 7	01/01/2010 - 12/31/2010	10.6073/pasta/1ae312058900f6b8df63cbd401891a17
GCE 7	01/01/2011 - 12/31/2011	10.6073/pasta/8d1d18d92ddc9000a4b1096dfadb0a38
GCE 7	01/01/2012 - 12/31/2012	10.6073/pasta/85e8f0f6e2bb80232c2a1fa485adcb21
GCE 7	01/01/2013 - 12/31/2013	10.6073/pasta/a2ac2439a1025817a5c48e45e6bbcefe
GCE 7	01/01/2014 - 12/31/2014	10.6073/pasta/c606ce39a016e352bc92ec89b6fcd69d

Table A1. Sources of salinity data from GCE 7 and GCE 8, and plant data set

Table A1 continued

GCE 8	10/26/2001 – 12/31/2001	10.6073/pasta/e2464efb7e227513cef612911accc4ed
GCE 8	01/01/2002 – 12/31/2002	10.6073/pasta/8b4792a6cca8ccebba2421b5295b9c06
GCE 8	01/01/2003 – 12/31/2003	10.6073/pasta/3b82fc8e14fc7a59f2dd69fccddbf3b4
GCE 8	01/01/2004 – 12/31/2004	10.6073/pasta/dc0abcd67ff002ae550be9e3fa55e3b9
GCE 8	01/01/2005 – 12/31/2005	10.6073/pasta/d8f0c8f98ea2ff0ca58ced8318cdd9b9
GCE 8	01/01/2006 – 12/31/2006	10.6073/pasta/a51ca2b303f089d82babb5aad8543a28
GCE 8	01/01/2007 - 11/07/2007	10.6073/pasta/4d8b00517cc7993f82f9284df0b2414e
GCE 8	01/01/2008 - 12/31/2008	10.6073/pasta/d6c859390b16b35cf74d37a12322bbb0
GCE 8	01/01/2009 - 12/31/2009	10.6073/pasta/35cda0623e4b7dbaed388942f8146d7b
GCE 8	01/01/2010 - 12/31/2010	10.6073/pasta/2deda2ed289c50c2305e5dd0769fd7c9
GCE 8	01/01/2011 - 12/31/2011	10.6073/pasta/9959bd47200bd004d663732ea8d907a4
GCE 8	01/01/2012 - 12/31/2012	10.6073/pasta/addc3b20bdee2e5f69eaca775e86ab52
GCE 8	01/01/2013 - 12/31/2013	10.6073/pasta/c852a4783c02526d767e94d0bef90378
GCE 8	01/01/2014 - 12/31/2014	10.6073/pasta/ffe61edd9c217752c67186938ba2545e
Plant	05/26/2013 - 08/28/2013	10.6073/pasta/690678e3e59c48b975e65a354d798a76

		Dave of calino	(Dave of calina	Days of saline water x Initial
Variables	Initial size	water	(Days of same water) <sup>2</sup>	size
Polygonum				
AG	0.18	0.03	0.36	0.74
BG	0.18	< 0.01	0.01	0.48
Total	0.09	< 0.01	0.26	0.79
Pontederia				
AG	0.21	< 0.01	0.31	0.94
BG	< 0.01	< 0.01	0.40	0.23
Total	< 0.01	< 0.01	0.27	0.24
Zizaniopsis				
AG	0.39	0.91	0.45	0.46
BG	0.08	0.06	0.01	0.99
Total	0.08	0.07	0.01	0.90
Spartina				
ÂG	< 0.01	0.20	0.15	0.02
BG	< 0.01	0.73	0.81	0.90
Total	< 0.01	0.58	0.63	0.59
Schoenoplectus				
AG	< 0.01	0.59	0.58	0.28
BG	0.53	0.42	0.88	0.64
Total	0.42	0.45	0.85	0.59
Juncus				
AG	< 0.01	0.41	0.81	0.38
BG	0.09	0.13	0.12	0.63
Total	0.04	0.15	0.13	0.60

Table A2. P values of the model coefficients in multiple regression analysis. Variable abbreviations are: AG = aboveground biomass, BG = belowground biomass, Total = total biomass. Initial sizes were calculated as the total stem height of each pot measured pre-treatment (May, 2013). Bold values were significant at the 0.05 level.

# Appendix B. Chapter 3 supplementary material

Table B1. Polynomial relationships between biomass and plant height (the sum of all plant heights for the species in one mesocosm; $n = 48$ ) or
cover $(n = 48)$ measured at the end of the recovery period (June 30, 2015).

Species	Common name	Equation	$\mathbf{R}^2$
Zizaniopsis miliacea	Giant cutgrass	log (Biomass) = $0.3458 \times \log(\text{Height})^2 - 0.4273 \times \log(\text{Height})$ - 0.003	0.67
Pontederia cordata	Pickerelweed	$Biomass = 0.0097 \times Height + 0.1764$	0.82
Peltandra virginica	Arrow arum	$Biomass = -13.533 \times (Coverage)^2 + 5.328 \times Coverage - 0.0526$	0.21
Sagittaria lancifolia	Bulltongue arrowhead	$Biomass = 25.229 \times (Coverage)^2 + 20.19 \times Coverage + 4.256$	0.15
Polygonum hydropiperoides	Smartweed	$log (Biomass) = 0.236 \times log(Height)^2 - 0.3274 \times log(Height) + 0.0857$	0.64
Ludwigia repens	Creeping primrose- willow	$log (Biomass) = -6.063 \times log(Cover)^2 + 4.1615 \times log(Cover) + 0.027$	0.50
Eleocharis palustris	Spikerush	Biomass = $156.7 \times (\text{Height})^2 - 87.153 \times \text{Height} + 53.427$	0.35

# Appendix C. Chapter 4 supplementary material

Table C1 Sampling dates for plant measurements.

Measurements	2013		2014		2015	2	016
	6/1/2013,	3/24/2014,	7/7/2014,	3/16/2015,	7/20/2015,	3/15/2016,	8/2/2016,
Stem height	7/15/2013,	5/20/2014,	8/1/2014,	5/23/2015,	8/3/2015,	6/27/2016,	10/28/2016
	8/11/2013	6/5/2014,	10/18/2014	6/29/2015,	10/19/2015	7/13/2016,	
	6/18/2013,	3/26/2014,	7/8/2014,	3/15/2015,	10/21/2015	3/16/2016,	
Photosynthesis	7/19/2013,	5/12/2014,	8/1/2014,	5/22/2015,		7/14/2016	
	8/9/2013	6/3/2014,	10/16/2014	6/25/2015,			
	6/1/2013,	3/24/2014,	7/31/2014,	3/16/2015,	7/20/2015,	3/16/2016,	8/2/2016,
Percent cover	7/15/2013,	5/29/2014,	10/14/2014	5/23/2015,	8/3/2015,	6/28/2016,	10/28/2016
	8/11/2013	6/24/2014,		6/25/2015,	10/19/2015	7/13/2016,	
	NA	3/26/2014,	7/7/2014,	3/16/2015,	10/19/2015	3/15/2016,	8/2/2016,
PAR		5/20/2014,	8/1/2014,	5/22/2015,		6/27/2016,	11/15/2016
		6/5/2014,	10/14/2014	6/29/2015,		7/13/2016,	

# Appendix Table 1 Continued

Carl barrage	NA	3/19/2014,	4/9/2015,	4/13/2016,	
Crab burrows		9/5/2014	9/4/2015	9/23/2016	
	NA	NA	NA	2/20/2016	7/9/2016
				3/31/2016	8/14/2016
Benthic				4/23/2016	9/24/2016
Microalgae				5/23/2016	10/31/2016
				0,20,2010	10/01/2010
				6/8/2016	11/30/2016

84

Table C2 Polynomial relationships between biomass and plant height for *Polygonum hydropiperoides*, *Pontederia cordata*, and *Zizaniopsis miliacea*.

Species	Equation	<b>n</b> =	$\mathbf{R}^2$
Polygonum hydropiperoides	$Biomass = 0.0004 \times (Height)^2 - 0.0178 \times Height + 0.5496$	54	0.79
Pontederia cordata	Biomass = $0.0002 \times (\text{Height})^2 - 0.0143 \times \text{Height} + 0.5312$	95	0.72
Zizaniopsis miliacea	$Log_{10}$ (Biomass) = $1.424 \times log_{10}$ (Height) <sup>2</sup> - $3.3354 \times log_{10}$ (Height) + $1.5173$	50	0.88

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