ABSTRACT

Title of Thesis:	SALINITY AND INUNDATION TOLERANCE OF *PHRAGMITES AUSTRALIS* SSP. *AMERICANUS*: A GREENHOUSE EXPERIMENT AND FIELD STUDY ON A TRIBUTARY OF THE CHESAPEAKE BAY
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Natural vegetation communities of tidal and non-tidal wetlands are threatened by invasive species, e.g. *Phragmites australis* (Cav.) Trin. Ex Steud., resulting in diversity losses and declines in wetland services. The native lineage of *Phragmites, Phragmites australis* ssp. *americanus* Saltonstall, P. M. & Soreng could be a valuable addition to species currently used in restoration projects aimed at increasing wetland services. However, tolerances of native *Phragmites* to environmental conditions are uncertain. Salinity and water level tolerances were investigated by monitoring growth of adult plants, established from rhizomes, under varying water and salinity levels in a greenhouse experiment and an observational study. Results show salinity levels above 5 ppt significantly limited growth of native *Phragmites* regardless of water level indicating appropriate restoration use across the marsh platform of fresh and

oligohaline systems. Educational materials and demonstration sites were created to

improve field identification of native *Phragmites.*

SALINITY AND INUNDATION TOLERANCE OF *PHRAGMITES AUSTRALIS*
SSP. *AMERICANUS*: A GREENHOUSE EXPERIMENT AND FIELD STUDY ON
A TRIBUTARY OF THE CHESAPEAKE BAY

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2017

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Acknowledgements

I would like to thank my major advisor, Dr. Andrew Baldwin, for providing endless support, guidance, and reassurance throughout my graduate school experience. Your passion started me on this journey – thank you for sharing. I would also like to thank my co-advisors, Dr. Stephanie Yarwood and Dr. Robert Tjaden, for offering their wisdom and support whenever needed. To my mentor and friend, Dr. Patricia Delgado, thank you for believing in me and providing invaluable opportunities and thanks to all at Jug Bay Wetland Sanctuary for providing access to all of your resources. I would also like to thank all of my wonderful colleagues whose support and hard work made the completion of my graduate degree enjoyable and possible: Eni Baballari, Zack Bernstein, Zach Berry, Kyle Derby, Josh Gaimaro, Amr Keshta, Jessica King, Amy Kuritsky, Christine Maietta, Martina Gonzalez Mateau, and Lindsay Wood. To Arati and Elaine thank you for cheering me on and providing respite care as needed. And lastly, I would like to give a big shout out to the most amazing support group ever - my husband and children. I can't thank you enough for your unflappable love and encouragement and for all of the sacrifices you've made these last 3 years.

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Chapter 1: Introduction

The European lineage of *Phragmites australis* (Cav.) Trin. Ex Steud. ssp. *australis* (European common reed) (herein after referred to as non-native) has spread throughout the Atlantic coastal region since its introduction to North America in the mid-19th century (Saltonstall 2002) and is now found along 14.6% of Maryland's estuarine shoreline (Chambers et al. 2008). Analysis of aerial imagery suggests invasion rates have slowed in tidal fresh areas, however, brackish marshes are experiencing population increases (Packett and Chambers 2006) (Rice et al 2000; Packett & Chambers 2006). Human development, rising sea level, and warming temperatures cause bare soils and increased nutrient levels, creating environmental conditions conducive to the establishment and spread of *Phragmites australis* ssp. *australis* (Hellings and Gallagher 1992a; Silliman and Bertness 2004; King et al. 2007; Chambers et al. 2008). *Phragmites* invasion has been shown to displace native wetland plants (Chambers et al. 1999), decrease species diversity and change marsh hydrology (Silliman and Bertness 2004), thereby diminishing valuable ecosystem services.

A native lineage, *Phragmites australis* ssp. *americanus,*(herein after referred to as native) has been confirmed (Saltonstall et al. 2004) but little is known about its ecology. This lineage has been in North America for thousands of years. Native Americans used it more often than most herbaceous plants (Kiviat & Hamilton 2001) and its reestablishment through restoration efforts has the potential to improve

ecosystem function. However, use in restoration practices requires an understanding of growth characteristics and factors influencing growth.

Unlike the non-native form, native does not grow in dense monotypic stands and its use in restoration efforts may lead to increased diversity (Meadows and Saltonstall 2007; Price et al. 2014) thereby increasing marsh resiliency (Folke et al. 2004). Additionally, native outperforms the non-native and other wetland plants in assimilating inorganic nitrogen and has high rates of organic nitrogen uptake (Mozdzer and Zieman 2010) making it an ideal candidate in restoration of areas with high nutrient levels. The non-native lineage is considered more aggressive and is thought to have displaced the native in many wetlands (Saltonstall 2002; League et al. 2006). Ironically, eradication efforts aimed at the non-native (primarily herbicides), may inadvertently kill the native form (Rinella et al. 2009)(Baldwin, personal observation). Thus, it is important for managers to distinguish between the two lineages; determining habitat requirements of the native will contribute to that understanding.

Wetlands are defined by the temporary or continuous flooding of soils with fresh or salt waters. Plant establishment, growth, and productivity are, in part, determined by these environmental factors (e.g. salinity and inundation). All plants are sensitive to salt, including wetland plants. Saline solutions alter water potential and ion distribution inhibiting growth at the cellular and whole plant level. Initially, nutrient and water uptake slow as energy expenditures shift from photosynthesis to maintaining osmotic potential (Reddy et al. 1992; Munns 2002; Parida et al. 2004). Stomata conductance slows to minimize water loss decreasing transpiration rates and

reducing carbon dioxide uptake available for photosynthesis (Brugnoli and Lauteri 1991; Rahnama et al. 2010). Transpiration does not stop but continues and ions accumulate in transpiring leaves eventually reaching toxic levels (Parida and Das 2005). Numerous studies have shown that salinity suppresses growth in all plants but the rate of reduction varies among species (Munns and Termaat 1986; Ball 1988; McKee and Mendelssohn 1989; Katerji et al. 1996; Mauchamp and Mésleard 2001a; Shaoliang Chen et al. 2003; Wang et al. 2006a; Gorai et al. 2010; Glenn et al. 2012; James et al. 2012) and genotype (Rahnama et al. 2010; Achenbach and Brix 2014).

Increased salinity has been shown to limit seed germination and growth of non-native *Phragmites* (Wijte and Gallagher 1996; Buchsbaum et al. 2006a; Greenwood and MacFarlane 2006; Wang et al. 2006a) with severity dependent on growth stage (Lissner and Schierup 1997; Bart and Marie Hartman 2002). Optimal growth of non-native *Phragmites* occurs in salinities less than 20 ppt, but it can persist in areas of 30 ppt (Chambers et al. 2003). Salinity tolerance of distinct clones varies widely (Achenbach et al. 2013) with some evidence suggesting that native *Phragmites* has a lower salinity tolerance than non-native (Vasquez et al. 2005) but tolerance levels are uncertain. While optimal growth of monocotyledonous halophytes generally occurs in the absence of, or at low concentrations of salt (Flowers and Colmer 2008a), results of the few native studies previously conducted are conflicting. On the Rappahannock River, native grew best in areas of salinity ≤1 ppt while non-native stands occurred over a broader salinity range of 0-11 ppt (Packett and Chambers 2006). Yet in Chicago, the non-native was found in areas of lower salinities as compared to the native (Price et al. 2013). Native has been

identified on Maryland's Choptank River in salinity levels up to 6.7 ppt (preliminary

data Baldwin) and in a Rhode Island tidal marsh where salinity reached 27 ppt

(Lambert and Casagrande 2006). A greenhouse study (Vasquez et al. 2005) found that

native did not grow in salinities greater than 6 ppt and the non-native was limited at

24 ppt.

In addition to salinity, increased duration and frequency of inundation, as is

likely under sea level rise, is expected to alter the composition and distribution of

plant communities (Baldwin et al. 2001) and reduce productivity due to decreased

seedling recruitment and diminished growth of some wetland plants (McKee and

Mendelssohn 1989; Baldwin et al. 1996; Lessmann et al. 1997; Warren et al. 2001;

Peterson and Baldwin 2004; Galatowitsch et al. 2016). Inundation slows the diffusion

of oxygen into the root zone inhibiting growth and establishment although,

physiological adaptations provide mechanisms such that high levels of productivity

can occur within an optimal range of inundation (Mauchamp et al. 2001; Morris

2007; Kirwan and Guntenspergen 2012; Byun et al. 2017a) for a given species

(Bockelmann et al. 2002; Long et al. 2017). Species dominant in the low marsh, e.g.

Spartina alterniflora and *Zizania latifolia,* respond positively to high water levels

(Byun et al. 2017a) but surface inundation of non-native *Phragmites* suppressed bud

emergence; increases in submergence were found to decrease height and culm density

(Hellings and Gallagher 1992a; Vretare et al. 2001; Zhao et al. 2013). Optimal

performance of the non-native occurs in areas with low flooding frequency but the

tolerance range of the native is uncertain. Meadows and Saltonstall (2007) observed

that native *Phragmites* on Maryland's eastern shore extended across the marsh

platform suggesting tolerance to a range of flooding frequencies but in Canada Taddeo and de Blois (2012) observed native mostly in low lying areas. A literature review yielded no experimental results concerning the effects of inundation on native *Phragmites* or on the tolerance levels of the different haplotypes.

With rising sea levels, wetlands are likely to experience the effects of salinity and inundation simultaneously. It is unclear how vegetation will respond but understanding the environmental thresholds of native *Phragmites* will improve our ability to restore and create wetlands with high plant biodiversity, improve land management practices in regard to eradication practices of invasive species, and help predict future loss of a native species due to rising sea levels and increased salinity.

This study examines the response of native *Phragmites* to environmental stressors of salinity and inundation. The objective was to evaluate the effect of increased salinity under varying inundation conditions both alone and in combination on morphological and physiological characteristics of native *Phragmites* to determine tolerance levels. I hypothesize that salinity and inundation levels each, and in combination, will be negatively correlated with the growth of native *Phragmites*. Hypothesis testing was conducted in a greenhouse experiment and then in a field study to determine if greenhouse results could be replicated in a natural setting. Understanding salinity and inundation tolerance of a native species will improve current management and restoration practices.

Chapter 2: Response of Native *Phragmites* to Varying Salinity and Water Level Treatments: A Greenhouse Experiment

Abstract

Salinity and flooding regimes are key environmental determinants of wetland plant communities. Human activity, sea level rise, and invasive species often alter wetland environmental conditions thereby modifying natural assemblages of plants. Populations of the native *Phragmites australis* ssp. *americanus* Saltonstall, P. M. & Soreng are in decline as the non-native lineage has replaced the North American native throughout much of its range. Determining the environmental thresholds of native *Phragmites* will improve wetland management and restoration practices and aid in the protection of a native species. This study provides a quantitative assessment of the growth of native *Phragmites* under three hydrological regimes (water levels at 10 cm below, 10 cm above, and at the soil surface) at eight salinity levels (0, 2, 5, 9, 14, 20, 27, and 35 ppt). Biomass yield reduction, stem-root anatomical changes, and photosynthetic rates were used to evaluate the effect of stress. A greenhouse experiment was conducted at the University of Maryland, College Park, Maryland. In general, all measures of growth responded to salinity regardless of water level. Results of this experiment combined with observations from a field study conducted on the Patuxent River in Maryland (see chapter 3) find growth of native *Phragmites* to be inhibited at salinity levels above 5 ppt but able to tolerate a range of water levels. I recommend the addition of native *Phragmites* to species currently used in wetland restoration with installation appropriate across the marsh platform of fresh

and oligohaline systems. This study improves our ability to predict the location of a native wetland plant and provides useful information for the development of wetland management and restoration strategies.

Introduction

Wetlands are among the most productive ecosystems in the world providing many valuable services such as flood control, sequestration of carbon, shoreline stabilization, nutrient cycling, and wildlife habitat (Mitsch and Gosselink 2007). The provision of services is limited by threats from invasive species and sea level rise as biodiversity shrinks and hydrology and salinity levels change (Zedler and Kercher 2004; Craft et al. 2009; Więski et al. 2009).

Invasive species modify wetland ecosystems through structural changes to the landscape or by altering community composition (Zedler and Kercher 2004). *Phragmites australis* (Cav.) Trin. ex. Steud., hereafter referred to as non-native, is considered invasive due to its rapid spread, abundance, and impact on the landscape. Slow decomposition rates of litter from non-native *Phragmites* may lead to a higher marsh platform thereby altering marsh hydrology (Chambers et al. 1999) and its tendency to grow in large monotypic stands reduces both plant and animal diversity (Benoit and Askins 1999; Chambers et al. 1999; Keller 2000; Bertness et al. 2002). The invasive behavior of non-native *Phragmites* has led to control and eradication efforts across the United States, with the US spending $4 million annually (Blossey and Casagrande 2016). Management tools include prescribed burns, mowing, and application of herbicides. Currently, the primary method of control is broadcasting of herbicides by plane or truck. Unfortunately, this method can have the unintended

consequence of eliminating non-target native species including the native lineage of *Phragmites, Phragmites, australis* ssp. *americanus* Saltonstall, P. M. & Soreng (Saltonstall et al. 2004). The northeastern US native populations are believed to be in decline (Saltonstall 2002) and current management efforts may eradicate remaining native stands as it can be found growing in close proximity to the non-native form. Increased awareness as to its existence and knowledge of habitat requirements would help to sustain current populations.

The combined effects of flooding and salinity typically decrease growth and survival more than does either stress alone (Marcar 1993; Conner et al. 1997; Kozlowski 1997; Isla et al. 2014). However, hydrology is considered to be a dominant factor determining the structure of wetlands as it dictates species composition and constrains productivity levels (Tiner 2005; Mitsch and Gosselink 2007; Batzer and Baldwin 2012). Water significantly restricts the diffusion of oxygen into the soil (Armstrong et al. 1994), reducing or eliminating the amount of oxygen available in the rhizosphere for aerobic respiration (Mendelssohn et al. 2014). Oxygen deficits cause reductions in growth, photosynthetic processes, and, eventually, plant death (Baldwin et al. 2001; Jackson and Colmer 2005; Voesenek et al. 2006; Colmer and Flowers 2008). The presence of water may also reduce light available to submerged tissues for photosynthesis limiting production of energy. Adaptations that alleviate oxygen deficiencies, such as aerenchyma tissue or rapid stem elongation, and energy deficiencies, i.e. anaerobic glycolysis, facilitate growth in flooded environments (Mitsch and Gosselink 2007).

Interspecific variation in response to flooding has been reported (Justin and

Armstrong 1987; Pezeshki and Anderson 1996; Kozlowski 1997; Kercher and Zedler

2004; Byun et al. 2017b) as has intraspecific variation (Voesenek et al. 2006; Ismail

et al. 2009). Germination and survival of emergent species is reduced under

submergence (McKee and Mendelssohn 1989; Kozlowski 1997; Baldwin et al. 2001;

Buchsbaum et al. 2006b; Baldwin et al. 2010). In non-native *Phragmites*, productivity

declines in response to submergence (Osland et al. 2011) and is often most severe in

young plants (Chambers et al. 2003) while established plants tolerate flooding and,

during short periods of submersion, an increase in stem density and height may occur

(Lessmann et al. 1997; Mauchamp et al. 2001; Vretare et al. 2001). However, long

term submergence prohibits stem production in rhizome grown plants (Hellings and

Gallagher 1992a) but low water levels appear to facilitate growth (Cross and Fleming

1989; Burdick et al. 2001; Warren et al. 2001; Whyte et al. 2008). I am unaware of

studies evaluating native *Phragmites* tolerance to varying water levels.

Although hydrology plays a dominant role in the structure of wetlands,

salinity is a key determinant of the structure and function of wetlands as well. Salinity

differentiates systems from one another, for example, freshwater marsh from salt

marsh, and differential tolerances to saline conditions influences the distribution and

productivity of the vegetation found in each system. Salt stress inhibits plant growth

in the short term due to osmotic stress, and in the long term, by the accumulation of

toxic ions in transpiring leaves and impaired nutrient uptake (Munns and Termaat

1986). Halophytes are able to complete their life cycle in saline conditions due to

various adaptations that enable the plant to avoid or tolerate salts (Flowers et al. 1986;

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Munns 2002; Flowers and Colmer 2008b). Despite these adaptations, research has shown salinity induces injury, inhibits vegetative and reproductive growth, and alters plant morphology and physiology; however, the degree to which growth is limited varies among species (Ball 1988; Ashraf and Harris 2004; Flowers and Colmer 2008b; Da Cruz et al. 2013; Xianzhao et al. 2013). Numerous studies have shown a negative response of morphological and physiological features such as stem height, leaf area, biomass, and photosynthetic rate to increased saline conditions (T J Flowers et al. 1977; Greenway and Munns 1980; Munns and Termaat 1986; Parida and Das 2005; Colmer and Flowers 2008; Parihar et al. 2015). In the woody species *Acacia ampliceps* and *Rhizophoria apiculata*, significant reductions in stem height and leaf area in response to increases in salinity were found (Ball 1988; Ashraf and Harris 2004). Non-native *Phragmites* tolerates a range of salinity levels (Chambers et al. 1999; Burdick et al. 2001), but Lissner and Schierup (1997) found growth to be negatively related to salinity with tolerance differing between plants with those grown from seed having a lower threshold than those grown from rhizomes. Additional studies found decreases in height, density, and biomass in response to increases in salinity, above approximately 20 ppt, in plants grown from rhizomes (Hellings and Gallagher 1992a; Bart and Marie Hartman 2002). However, some salt tolerant species have shown a stimulation to growth and then, once salinity goes beyond the threshold level, growth is inhibited (Mendelssohn et al. 2014).

Intraspecific differences have been identified as well for a variety of species including *Phragmites* (Gao et al. 2012; Lieth and Masoom 2012; Achenbach and Brix 2014; Sandhu et al. 2017). Numerous studies have assessed the salt tolerance of non-

native *Phragmites* (Burdick et al. 2001; Mauchamp and Mésleard 2001b; Vasquez et al. 2006), but few have evaluated the native lineage. Plants grown above approximately 7 ppt (reported as 0.1M NaCl) failed to survive in a greenhouse experiment (Vasquez et al. 2005). Field observations on the Delaware Peninsula appear to support those findings as native populations were only found in fresh and oligohaline waters (Meadows and Saltonstall 2007). However, native stands do exist in the high salinity waters of Block Island in Rhode Island (Lambert and Casagrande 2006).

I investigated the effect of salinity and water level on the growth of a native species, *Phragmites australis* ssp. *americanus.* The aim of the study was to understand native *Phragmites* growth in response to eight salinity levels (0, 2, 5, 9, 14, 20, 27, and 35 ppt) at three water levels (10 cm below substrate surface, 10 cm above substrate surface, and at the substrate surface). The objective was to evaluate the effect of salinity increases at varying water levels, separately and in combination, on morphological and physiological characteristics of native *Phragmites*. I hypothesized that (a) salinity would be negatively correlated with vegetative growth as evidenced by reductions in stem height, diameter, biomass, and photosynthetic activity; (b) water level would be negatively related to biomass but positively related to stem height; and (c) the combined effect of salinity and water level would be negatively related to growth.

Materials and Methods

Materials

Rhizomes were collected from a stand of native *Phragmites* on the Patuxent River, Maryland, USA (N38° 42' 8", W76° 41' 48", map datum: WGS 84) on March 18, 2015 (Figure B.2.1). This stand was previously confirmed as native following the methodology described in Saltonstall 2003. Rhizomes were excavated using a shovel, rinsed with river water, placed in 19-liter buckets, and transported to the University of Maryland in College Park and placed in cold storage (4 °C). Firm white rhizomes, with at least two nodes, were planted in 2:1 mixture of potting soil and washed sand and grown in the University of Maryland greenhouse — one rhizome per pot. Rhizomes were watered regularly to maintain moisture. Temperature was kept between at 32 °C during the day and dropped to 7 °C at night to mimic natural conditions. After eight weeks, plantlets were moved to 6.033-liter circular pots (Classic 600, Nursery Supply Inc.) with a surface area of 2280.18 cm^2 containing well drained soils (2:1 peat and washed sand). To prevent substrate loss, each pot was placed in a second 6.033-liter pot such that drainage holes overlapped. All pots had similar numbers of stems of similar size. Potted plants were allowed to acclimate for two weeks in the greenhouse.

Experimental Design

A randomized complete block design (RCBD) with a factorial arrangement of water levels and salinity (three water levels x eight salinity levels) was established at the University of Maryland greenhouse in June 2015 (refer to Appendix B, Figure
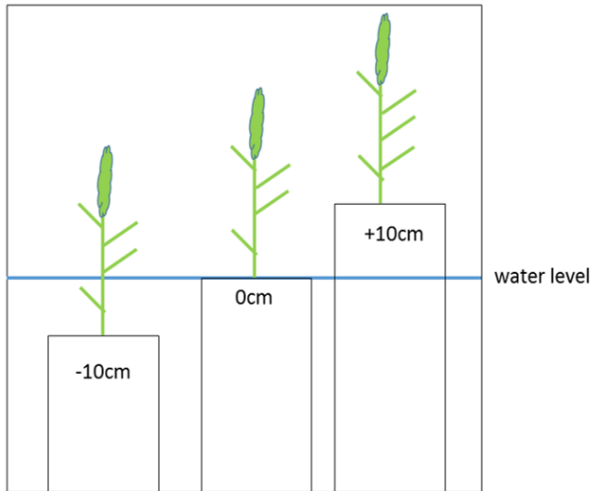
Fig. 2.1. Schematic showing experimental water levels relative to soil surface.

B.2.2 for graphic of experimental layout and images of greenhouse setup). Plants were randomly assigned to one of three water level treatments (submerged conditions defined as substrate surface -10 cm below water level, surface conditions defined as water level at substrate surface 0 cm, or exposed conditions defined as substrate surface +10 cm above water level) (Figure 2.1) and one of eight salinity treatments (0, 2, 5, 9, 14, 20, 25, 35 ppt). Plants were randomly organized and replicated four times (n = 4) in blocks. Blocking was used because of possible humidity and temperature gradients in the greenhouse. Greenhouse temperature was maintained above 26 °C and supplemental lighting simulated a 16-hour day.

Treatment Application

Application of treatments began on June 26 with all plants receiving 0 ppt and assigned water level. Salinity levels were progressively increased twice weekly until final treatment levels were reached on July 17, 2015 (Appendix A, 2 Table A.2.3). Salinity solutions were mixed immediately prior to application by adding the appropriate amount of Instant Ocean to a fixed amount of water in a 19-L bucket and applied by: (1) lifting potted plant from water and flushing with old solution (to flush any precipitated solids) and then allowed to completely drain; (2) the pot was placed

back in the bucket, iron sulfate solution was poured onto the substrate surface; and (3) 5-L of the new treatment solution was slowly poured onto the substrate surface, the remaining saline solution was poured into the outer bucket to a pre-marked level on the outer bucket. A six-week experimental period followed during which treatment water was changed weekly with the appropriate salinity following the above procedure. Random salinity checks were performed daily and adjustments made as needed.

PVC pipe was cut to one of three lengths, holes were drilled into the sides to allow for circulation of treatment water. The PVC was used as a riser to attain the assigned water level treatment (Appendix B, Figure B.2.3, image (a) PVC lengths). Each potted plant was placed on top of a riser which had been placed in a 19-liter bucket. Water levels were maintained at: (1) 10 cm below the soil surface (submerged), (2) the soil surface 0 cm (surface), or (3) 10 cm above soil surface (exposed) (Appendix B, Figure 2.3 (b), potted plants at experimental levels). Reservoir water was monitored daily and adjusted as needed with de-chlorinated water.

Previous attempts to grow native *Phragmites* under greenhouse conditions were unsuccessful due to chlorosis. To prevent chlorosis, a 100 mL solution of iron sulfate ($FeSO_4$) and deionized water was prepared the morning of water change. One mL was poured onto the substrate surface of each plant prior to application of the new salinity treatment. $FeSO_4$ was added at a rate of 0.1462 grams/pot/week based on Eller et al. 2013 (Appendix A, Table A.2.4, $FeSO_4$ loading calculation). A slow release fertilizer (Osmocote® Scotts Sierra Co, Maryville, OH, 19-6-12) was

broadcast on top of the growth media once at the beginning of the experiment at the recommended application rate (approximately 26.2 g per pot) to prevent nutrient limitation.

Data Collection

Salinity, temperature, and pH of reservoir and drainage water were measured before each water change using handheld meters (YSI, Yellow Springs Ohio). Non-destructive measurements of growth were taken twice weekly during the treatment period and then weekly during the experimental period. For each pot, stems and leaves were counted and stem height and diameter were measured. All stems and fully developed leaves were counted. Each stem was measured from the sediment surface to the uppermost collared (flat) leaf to determine height. Diameter measurements were taken approximately 4 cm from the soil surface with a 100 mm pocket caliper.

To quantify physiological response to stress, photosynthetic rates were determined by measuring the yield and maximum leaf chlorophyll fluorescence (Fv/Fm ratio) of two leaves per pot twice during the experimental period using a Walz PAM-2100 Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany) (Maxwell and Johnson 2000, Maricle et. al., 2007). Yield readings were taken in the morning, starting approximately at 0900 hours, and Fv/Fm were taken at night, starting at approximately 2200 hours.

After 5 weeks of treatment, final height, basal diameter, stem count, and leaf count measurements were taken and plant leaves, stems, and roots were harvested. Leaves on every stem in each pot were stripped, starting from the lowest leaf on the

stem to the top in order to keep the sheath attached to the blade. The leaves from each pot were weighed, counted, and their total projected area measured using an LI-3100C Area Meter (LI-COR, Lincoln, Nebraska, USA). Stems were clipped at the soil surface and weighed. The rhizomes and lateral roots in each pot were removed from the growth media by rinsing with tap water over a 5-mm mesh sieve. Lateral roots were then stripped from the rhizomes, counted, and weighed. The total length and average diameter of rhizomes in each pot were measured and weighed before drying. Dead material was separated from live material and weighed. All plant fractions were weighed wet then dried to a constant mass at 70 °C in a ventilated oven (Appendix A, Table A.2.5). Dried fractions were then weighed to the nearest 0.01g to determine final above- and belowground dry biomass.

The specific leaf area (SLA) was calculated as the ratio of the sum of the leaf area to the dry mass of the leaves per pot. SLA serves as an index of the thickness of leaves, and thus their photosynthetic capability per leaf unit area (Evans and Poorter 2001). The sum of the projected areas of the leaves in each pot were divided by the total dry mass of all above- and below ground plant material to determine the leaf area ratio (LAR), an index correlated with relative growth rate (Poorter and Remkes 2001). The total belowground dry mass (lateral roots + rhizomes) was divided by the total aboveground dry mass (stems + leaves) in the pot to determine root:shoot ratio which reflects the resources allocated for nutrient uptake to belowground as opposed to aboveground growth.

Data Analysis

The greenhouse study was a randomized block design. All growth measurements, below and aboveground biomass estimates, and fluorescence data were analyzed to determine significant main effects of salinity and water level as well as significant interactions. Data were analyzed using two-way ANOVA for the dependent variables measured over the course of the experiment and for those measured at the conclusion of the experiment. Data were checked for normality and homogeneity. Results were considered significant at the $\alpha = 0.05$ level. Post-hoc multiple comparisons of means were performed using the Tukey procedure. Analysis was performed using SAS, SAS University Edition, SAS studio, version 3.5 (SAS Institute Inc., Cary, NC, USA).

Results

Morphological Variables

Salinity generally had a significant negative effect on growth (Table 2.1) as reflected in repeatedly measured variables - cumulative height, stem height, stem count, live and dead leaf count, Fv/Fm, and yield. Salinity inhibited most variables at treatment levels above 5 ppt, Fv/Fm was inhibited at 27 ppt, although plants continued to persist at 35 ppt (Appendix B, Figures B.2.4-B.2.6a). Significant differences between salinity treatments were also seen for stem diameter, however, a linear relationship was not found (Appendix B, Figure B.2.6b). The negative influence of salinity escalated over time and varied by water level (salinity x water level x day of experiment, Table 2.1; Appendix B, Figs. B.2.7-B.2.9). For example, at low salinity, stem count was similar for all water levels on day 26; but, by day 61 stem count was highest for the submerged treatment (11 stems). For plants receiving

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35 ppt, stem count was similar on day 26, but by day 61, stem count had not changed significantly and was highest for the exposed treatment (1.75 stems). Significant three way interactions were also found for cumulative height and Fv/Fm.

The negative effect of salinity was generally observed between weeks `3 and 4 with significant differences seen in the means of all variables except yield (salinity x day of experiment interaction; Table 2.1; Figs. 2.2 a-d; Appendix B, Figs. B.2.10 and B.2.11). For example, significant differences in cumulative height were not found initially but by day 26 significant differences between plants receiving treatments of $\leq$ 5 ppt and those receiving $\geq$ 9 ppt were found. Plants receiving 5 ppt had reached 134.8 cm which was 58% greater than that of plants receiving 9 ppt (85.3 cm) and more than double the cumulative height at 14 ppt (66.3 cm). On day 61, cumulative height was 521.3 cm for plants at 5 ppt which was two times that of those at 9 ppt (255.6 cm) and more than four times those at 14 ppt (121.0 cm). Generally, the means for the 5 ppt and 9 ppt treatments were not statistically different from one another but 9 differed from treatments < 5 ppt and 5 ppt differed from treatments > 9ppt. The effect of salinity and water level on stem diameter was additive, however, a clear trend was not observed (Table 2.1; Appendix B, Figure B.2.12).

Submergence tended to result in increased growth, although the effect varied with time (Table 2.1; Appendix B, Figure B.2.13) as seen in cumulative stem height, stem height, stem diameter, and dead leaf count (water level x day of experiment interaction). Stem height was highest for plants under submerged conditions but on day 46, exposed plants were taller than submerged and by day 61 the trend reversed again with submerged taller than exposed plants. A main effect of water level was

found for stem height and stem diameter with the largest means occurring in submerged plants with significant differences between submerged and exposed plants but neither were significantly different from plants with water level at the substrate surface (Appendix B, Figure B.2.14).

By the conclusion of the experiment, ANOVA results confirmed the trends found during the experiment with significant differences between salinity treatments for most morphological variables (Table 2.2; Appendix B, Figures B.2.15 and B.2.16 a-c), stem and rhizome diameter were the exceptions. Pair wise comparisons of salinity levels on the data collected at the conclusion of the experiment found 5 ppt to be the threshold beyond which decreases in growth were observed. No interactions were found.

Biomass Fractions

Analysis of data collected at the conclusion of the experiment confirmed that growth of native *Phragmites* was significantly inhibited by salinity (Table 2.2; Figure 2.3). All biomass fractions had a significant negative response to salinity at $\alpha < 0.05$ except rhizome biomass where a significant positive response was found at $\alpha < 0.1$ (P=0.0876, Table 2.2). However, water level treatments did not produce a significant response in any of the biomass fractions and neither treatment amplified the effect of the other (Table 2.2).

Resource Capture and Allocation

A significant difference between salinity treatments was found for the root:shoot ratio (P=0.0016, Table 2.2, Appendix B, Figure B.2.16 d) which increased as salinity increased but no significant differences were seen in either leaf area ratio

(LAR) or specific leaf area (SLA) (P=0.2774 and P=0.3923, respectively; Table 2.2).

No responses to water level treatments were found nor were any interactions

identified.

Table 2.1. Results of two-way ANOVA of *Phragmites australis* ssp. *americanus* response to salinity and water level treatments based on repeatedly measured data collected weekly throughout the experimental period. Bolded values represent a significant treatment effect (p<0.05).

Variable	Effect	Ndf	Ddf	F	PValue
Stem Height (cm)	Salinity (S)	7	160	17.73	**<0.0001**
	Water level (W)	2	172	3.15	**0.0293**
	S x W	14	181	0.81	0.5800
	Day of Experiment (DOE)	9	515	55.12	**<0.0001**
	S x DOE	63	471	4.77	**<0.0001**
	W x DOE	18	531	2.92	**<0.0043**
	S x W x DOE	126	424	1.30	0.2296
Cumulative Stem Height (cm)	S	7	190	24.94	**<0.0001**
	W	2	235	1.69	0.1868
	S x W	14	239	1.09	0.3654
	DOE	9	553	82.71	**<0.0001**
	S x DOE	63	460	11.82	**<0.0001**
	W x DOE	18	525	2.91	**<0.0001**
	S x W x DOE	126	446	1.96	**<0.0001**
Stem Count	S	7	173	20.08	**<0.0001**
	W	2	194	0.43	0.6520
	S x W	14	198	1.20	0.2767
	DOE	9	520	36.27	**<0.0001**
	S x DOE	63	417	6.48	**<0.0001**
	W x DOE	18	487	8.54	**<0.0001**
	S x W x DOE	126	401	3.57	**<0.0001**
Stem Diameter	S	7	148	2.87	**0.0076**
	W	2	213	3.03	**0.0503**
	S x W	14	214	2.90	**0.0005**
	DOE	9	466	3.08	**0.0013**
	S x DOE	63	448	1.37	**0.0376**
	W x DOE	18	463	1.10	0.3538
	S x W x DOE	126	471	0.87	0.8196
Leaf Count, Live	S	7	163	10.49	**<0.0001**
	W	2	187	0.20	0.8215
	S x W	14	198	0.94	0.5196
	DOE	9	526	5.79	**<0.0001**
	S x DOE	63	497	2.35	**<0.0001**
	W x DOE	18	525	1.47	0.0949
	S x W x DOE	126	494	0.92	0.7070
Leaf Count, Dead	S	7	190	19.29	**<0.0001**
	W	2	209	1.14	0.3221
	S x W	14	220	1.21	0.2710
	DOE	9	537	8.17	**<0.0001**
	S x DOE	63	511	2.70	**<0.0001**
	W x DOE	18	536	1.74	**0.0294**
	S x W x DOE	126	521	1.05	0.3432
Fv/Fm	S	7	136	30.44	**<0.0001**

Variable	Effect	Ndf	Ddf	F	PValue
Fv/Fm(cont.)	W	2	136	0.71	0.4934
	S x W	14	137	1.27	0.2315
	DOE	1	174	2.42	0.1218
	S x DOE	7	174	5.25	**<0.0001**
	W x DOE	2	174	2.40	0.0938
	S x W x DOE	14	173	2.63	**0.0017**
Yield	S	7	127	19.10	**<0.0001**
	W	2	126	0.96	0.3867
	S x W	14	128	0.68	0.7858
	DOE	1	186	11.85	**0.0007**
	S x DOE	7	186	0.75	0.6340
	W x DOE	2	186	1.40	0.2485
	S x W x DOE	14	185	0.89	0.5726
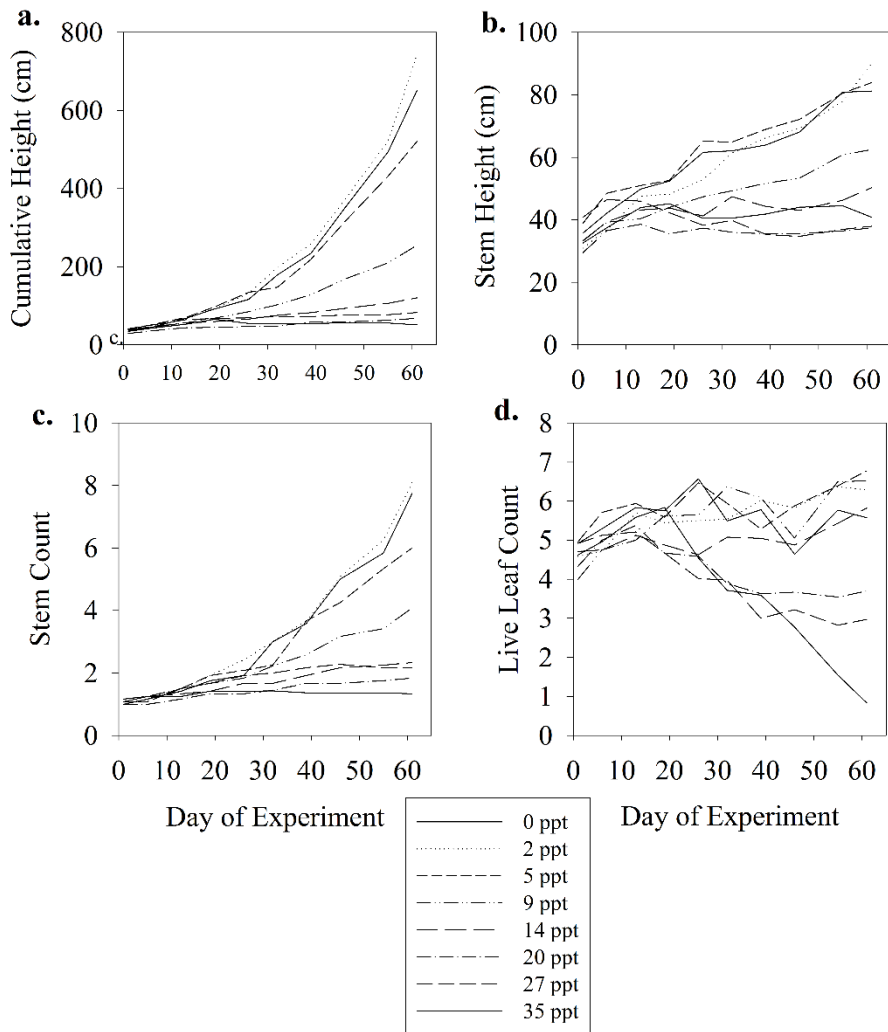
Figure 2.2. Variation of *Phragmites australis* ssp. *americanus* (a) cumulative stem height, (b) stem height, (c) stem count, and (d) live leaf count in response to salinity treatments. Plotted values are arithmetic means of weekly measurements and plotted using a straight line curve. By the end of the experiment, salinity levels >5ppt had inhibited growth.

Table 2.2. Results of two-way ANOVA showing the effects of salinity and water level treatments and interactions on final measurements of dry biomass, morphological, and resource capture and allocation parameters of *Phragmites australis* ssp. *americanus*. Bolded values represent a significant treatment effect (p<0.05).

Variable	Source of Variation								
	Salinity			Water level			Salinity*Water level		
	ndf, ddf	F	P	ndf, ddf	F	P	ndf, ddf	F	P
Morphological Variables									
Stem Height (cm)	7, 72	6.90	**<0.0001**	2, 72	1.86	0.1634	14, 72	1.25	0.2624
Cumulative Height (cm)	7, 72	8.08	**<0.0001**	2, 72	0.28	0.7529	14, 72	0.91	0.5544
Stem Count	7, 72	3.27	**<0.0045**	2, 72	0.71	0.4951	14, 72	0.97	0.4902
Stem Basal Diameter	7, 72	0.49	0.8358	2, 72	1.75	0.1816	14, 72	1.17	0.3186
Live Leaf Count	7, 72	7.50	**<0.0001**	2, 72	0.88	0.4209	14, 72	0.86	0.6082
Leaf Area	7, 72	7.83	**<0.0001**	2, 72	0.48	0.6221	14, 72	0.65	0.8163
Lateral Root Count	7, 69	10.95	**<0.0001**	2, 72	0.63	0.5347	14, 72	0.67	0.7922
Rhizome Length	7, 69	9.27	**<0.0001**	2, 69	0.01	0.9868	14, 69	1.00	0.4629
Rhizome Diameter	7, 72	1.30	0.2611	2, 72	0.83	0.4395	14, 72	1.79	0.5666
Biomass Fractions									
Aboveground Biomass	7, 69	10.99	**<0.0001**	2, 69	0.60	0.5537	14, 69	0.46	0.9447
Leaf Biomass	7, 69	11.23	**<0.0001**	2, 69	0.41	0.6630	14, 72	0.51	0.9174
Stem Biomass	7, 69	10.85	**<0.0001**	2, 69	1.30	0.2788	14, 69	0.53	0.9053
Dead Above Biomass	7, 72	1.98	**0.0496**	2, 72	0.43	0.6523	14, 72	0.63	0.8275
Belowground Biomass	7, 72	3.29	**0.0043**	2, 72	0.54	0.5878	14, 72	0.60	0.8598
Lateral Root Biomass	7, 69	6.48	**<0.0001**	2, 69	0.39	0.6814	14, 69	0.51	0.9185
Rhizome Biomass	7, 72	1.87	0.0876	2, 72	0.50	0.6109	14, 72	0.64	0.8183
Total Dry Biomass	7, 69	9.76	**<0.0001**	2, 69	0.60	0.5491	14, 69	0.47	0.9422
Resource Allocation									
Root:Stem Ratio	7, 72	3.76	**0.0016**	2, 72	0.34	0.7160	14, 72	0.88	0.5840
Leaf Area Ratio	7, 72	1.27	0.2774	2, 72	0.92	0.4016	14, 72	1.02	0.4409
Specific Leaf Area	7, 69	1.07	0.3923	2, 69	1.08	0.3454	14, 72	1.02	0.4421
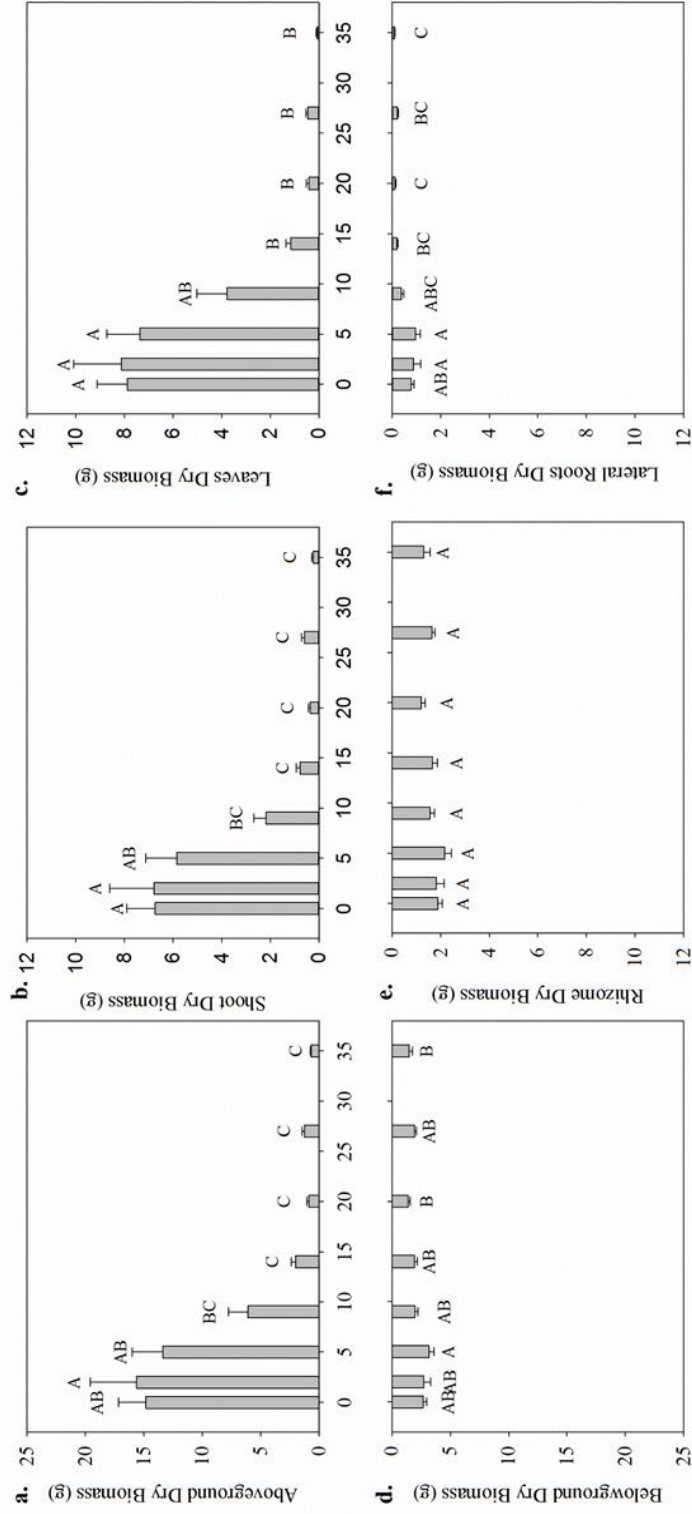
Figure 2.3. Effect of increasing salinity levels on biomass of *Phragmites australis* ssp. *americanus*. Plotted values are arithmetic means +1 SE of above and belowground biomass (panels a and d), stem and rhizome growth (panels b and e), and leaf and lateral root fraction biomass (panels c and f) on day of harvest at the conclusion of the experiment. Within each panel, different letters indicate significant differences.

Discussion

Growth of native *Phragmites* was negatively related to salinity as hypothesized. Specifically, salinity levels greater than 5 ppt significantly inhibited growth within 4 weeks of treatment. The growth response measured here was similar to the response found by Vasquez et al. (2005). In that greenhouse study, plants grown from rhizomes showed a significant decrease in stem height, density, and above and below ground biomass in response to increasing salinity levels with complete mortality at levels above 6 ppt.

Salinity has been shown to have a significant negative effect on non-native *Phragmites* although its maximum tolerance is much higher than the natives. In greenhouse studies of non-native *Phragmites*, Vasquez (2006) saw 50% reduction in growth above 24 ppt however, growth was sustained at 30 ppt (Achenbach and Brix, 2014) while complete mortality occurred at 32 ppt (Lissner and Schierup 1997; Achenbach et al. 2013). In North America, the non-native form has been observed in a range of conditions from freshwater to polyhaline tidal wetlands (Hellings and Gallagher 1992b; Chambers et al. 1999; Rice et al. 2000; Burdick et al. 2001; Packett and Chambers 2006). These results, and those of other investigators (Hellings and Gallagher 1992a; Lissner and Schierup 1997; Lissner et al. 1999; Vasquez et al. 2005; Achenbach et al. 2013; Achenbach and Brix 2014), indicate intraspecific-variation within this species. The degree to which plants are able to tolerate saline conditions is known to vary within species. For example, *Spartina alterniflora,* which is similar to *Phragmites* in its wide ranging distribution, shows a differential response to salinity that is dependent upon location of the population (Mateos-Naranjo and Redondo-

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Gómez 2016). Several studies of non-native *Phragmites australis* have shown salinity

tolerance to vary widely and is dependent upon the genotype (Hanganu et al. 1999;

Gao et al. 2012; Achenbach et al. 2013). The North American native *Phragmites*

grows along the Atlantic seaboard and gulf coast under a variety of saline conditions

(Meyerson et al. 2000; Saltonstall 2011; Achenbach and Brix 2014). However, the

known stands of native *Phragmites* in the Mid-Atlantic region are located in fresh to

oligohaline waters suggesting a limited range of tolerance to salinity (Vasquez et al.

2005; Packett and Chambers 2006; Meadows and Saltonstall 2007).

Growth has been shown to vary with water level (Wang et al. 2006b) and the

combined effect of salinity and water level decreases growth more than either stress

alone (Baldwin and Mendelssohn 1998). I was unable to confirm those results or

prove my hypotheses in this study. Although, stem height varied with water level

during the experiment, at its conclusion neither water levels nor the combined effect

of water and salinity showed a significant influence on growth. Much work has been

devoted to understanding the role of inundation on plant growth for a wide variety of

halophytes and non-halophytes. Submergence has been shown to stimulate the

production of ethylene but the presence of water inhibits its diffusion such that it

accumulates in plant tissue triggering rapid stem elongation restoring gaseous

exchange and resumption of aerobic respiration (Armstrong et al. 1994; Voesenek et

al. 2004; Voesenek et al. 2006; Colmer and Voesenek 2009). Coops et al. (1996)

found an increase in stem height but a decrease in overall growth. As was the case in

this study, stem height responded to water level during the experiment with stems

significantly taller under the submerged (i.e., -10 cm) treatment as compared to either

the surface (0 cm) or exposed (+10 cm) treatments but there was an overall decrease in growth. Non-native *Phragmites* response to prolonged submergence has produced mixed results with both significant reductions (Hellings and Gallagher 1992a; Mauchamp et al. 2001) and increases (Vretare et al. 2001; Wang et al. 2006b) in biomass and height. However, the results of this study confirm those of Coops et al (1996) which did not find a significant effect of flooding on biomass, cumulative height, density, or basal diameter.

Physiological adaptations in wetland plants provide an escape from oxygen deprivation; however, effectiveness is dependent upon duration and growth stage. For example, non-native *Phragmites* seedling emergence is limited under flooded conditions (Baldwin et al. 2010), while mature plants appear to tolerate flooding (Armstrong et al. 1999). The results of this study combined with those of the field study provide evidence that mature native *Phragmites* plants can tolerate a wide range of flooding conditions. It is possible, however, had we started with seedlings, our results may have been different.

Salinity and flooding regimes are known to be a primary influence on wetland plant community composition and distribution. Understanding a species tolerance to physical stress is important for predicting natural community dynamics and for practical applications. This is particularly useful in facilitating the conservation and restoration of native species under threat from non-native species, rising sea levels, and anthropogenic activities that destroy or modify wetland hydrology. The results from this greenhouse experiment were confirmed in a natural setting (see chapter 3) where growth a negative response to the salinity gradient of the Patuxent River

occurred and no response to flooding was observed. While some populations of native *Phragmites* exhibit a higher tolerance to salt concentrations, this study suggests that the Chesapeake Bay population has a limited tolerance similar to populations of the larger Mid-Atlantic region. If salinity levels do not rise above 5 ppt in response to changing climatic conditions, native *Phragmites* may be able to retain current populations even as water levels rise. The findings provide evidence that while native *Phragmites* is limited by salinity, water level does not influence growth indicating its usefulness in restoration efforts of fresh and oligohaline wetlands that experience a range of hydrologic conditions in the Chesapeake Bay and Mid-Atlantic regions.

Chapter 3: Growth of Native *Phragmites* on the Patuxent River: Assessing Salinity and Flooding Tolerance

Introduction

Wetlands provide a variety of services including stabilizing shorelines, protecting against storm surges, and providing habitat for a diversity of plant (and biotic) life found nowhere else (Mitsch and Gosselink 2000). However, wetlands are vulnerable to changes due to natural environmental processes (e.g., storms and subsidence), anthropogenic modifications (e.g., land development), or unintended consequences resulting from both natural and anthropogenic modifications (e.g., sea level rise and invasive species). Sea level rise threatens to alter hydrology while invasive alter species diversity. Current restoration efforts are aimed at creating habitats for native plant species but will need to consider the effects of increased inundation and salinization due to sea level rise.

The non-native *Phragmites australis* (Cav.) Trin. ex Steud., hereafter referred to as non-native, is an invasive plant shown to decrease biodiversity (Meyerson et al. 2000; Lathrop et al. 2003) and alter the hydrology of North American wetlands (Lathrop et al. 2003) which can diminish ecosystem function. The significant impact of non-native *Phragmites* has prompted management efforts to decrease its current population and control its spread into new environments. The US spends $4 million annually on control efforts (Martin and Blossey 2013); herbicides are the primary method of control and while effective, the potential to damage non-target species exists (Rinella et al. 2009; Skurski et al. 2013). Sometimes found growing in close

proximity to non-native *Phragmites* (Blossey and Casagrande 2016) is the recently

identified native form, *Phragmites australis* ssp. *americanus* Saltonstall, Peterson and

Soreng, hereafter referred to as native. Given that the native and non-native forms are

congeners, the native may easily be mistaken for the non-native and unintentionally

treated during control efforts. As a result, native stands along the Choptank River on

Maryland's eastern shore have been eradicated (Baldwin personal communication).

Environmental changes due to sea level rise, rising temperatures, and current

land use practices are likely to cause increased salinization of water and soil (Kaushal

et al. 2005; Jeppesen et al. 2015). Saline conditions limit plant growth as it can inhibit

the uptake of nutrients and water and, at levels beyond tolerance, cause tissue damage

and, over time, death. The degree to which growth is limited depends on species and

genotype (Lessmann et al. 1997; Inan et al. 2004; Glenn et al. 2012; Da Cruz et al.

2013). Many wetland plants are successful in saline conditions due to physiological

and morphological adaptations which provide mechanisms that exclude, excrete, or

adjust ion concentration levels.

While halophytes are adapted to saline conditions, maximums do exist. Non-

native *Phragmites* is capable of tolerating a range of salinities but is generally found

in fresh and brackish marshes (<18 ppt) (Chambers et al. 1999; Burdick et al. 2001)

with decreases in biomass, height, and density at levels above 0.1M NaCl

(approximately 7 ppt) (Vasquez et al. 2006) and complete mortality above 15 ppt

(Lissner and Schierup 1997). Response to salinity is dependent upon growth stage

with decreases in germination rates occurring at 10ppt, decreases in growth of

seedlings occurring at 7.5 ppt, and decreases in survival occurring at 15-20 ppt

(Mauchamp and Mésleard 2001b). Although the maximum reported salinity for non-native seedlings varies among studies, seedlings appear to have higher tolerance than seeds while rhizome grown plants have a higher tolerance than seedlings. Lissner and Schierup (1997) found 75% of rhizome grown plants survived 22.5 ppt while only 12% of seedlings survived that level. Mature plants appear to be most tolerant with established stands in Delaware thriving at conditions where soil salinity reaches approximately 50 ppt (Mills and Gallagher unpublished). *Phragmites* tolerance also varies among genotypes. In a study of Eurasian and Asian types, Achenbach et al (2013) found survival rates varied among types and identified different maximums based on growth and survival rates. Few studies on the tolerance of native *Phragmites* have been conducted. A study of natives from the Mississippi delta found that growth was negatively related to salinity but the response varied by genotype with the least sensitive experiencing growth reductions at 20 ppt (Achenbach and Brix 2014) but an earlier experiment which included natives of the Mid-Atlantic region found growth significantly decreased in saline conditions greater than 0.1 M NaCl (approximately 7 ppt)(Vasquez et al. 2005). Field observations have identified native stands in freshwater and oligohaline waters (League et al. 2006; Packett and Chambers 2006; Meadows and Saltonstall 2007) as well as in mesohaline conditions (Lambert and Casagrande 2006).

While salinity plays a role in determining plant success or distribution, hydrology also determines wetland structure (Baldwin et al. 2001; Mitsch and Gosselink 2007; Batzer and Baldwin 2012) as its influence on chemical and physical processes dictate species composition, primary productivity, organic material

accumulation, and nutrient availability. Flooding is known to decrease species

richness, limit seedling germination and survival, and inhibit productivity (McKee

and Mendelssohn 1989; Baldwin et al. 1996; Lessmann et al. 1997; Baldwin et al.

2001; Peterson and Baldwin 2004). The stress imposed by flooding drives adaptive

evolution allowing for growth and reproductive success in conditions that would be

highly damaging to most plant species. But, for those adapted to saturated conditions,

the degree to which flooding and the resulting anoxic conditions are detrimental,

varies with age and duration of stress (McKee and Mendelssohn 1989; Baldwin et al.

2001; Peterson and Baldwin 2004). For example, when under complete submergence,

non-native *Phragmites* seedlings have reductions in germination (Baldwin et al.

2010), rhizomes fail to emerge (Hellings and Gallagher 1992b; Bart and Hartman

2003), and productivity decreases (Buchsbaum et al. 2006b; Wang et al. 2006b) with

the most severe productivity losses in young plants (Armstrong et al. 1999;

Mauchamp et al. 2001). However, established plants are able to tolerate flooding

(Warren et al. 2001; Chambers et al. 2002) and may even experience an increase in

stem density and height under submergence (Vretare et al. 2001; Voesenek et al.

2004). I am aware of no studies evaluating the flood tolerance of native *Phragmites*.

In recent decades, scientific understanding of wetland functions has increased,

as has the desire to protect and restore native species and their habitats. Created or

restored wetlands are specifically designed to support native species and a primary

objective of land managers is to restore the native flora (Martin and Blossey 2013).

However, the success of these efforts depends on our knowledge of species tolerance

to environmental constraints. Understanding plant tolerance to environmental conditions is crucial to successful establishment of native species in restoration.

An experiment to identify the tolerance to physical stressors across a range of natural conditions would fill a considerable gap in our knowledge of native *Phragmites*. This study, in conjunction with a greenhouse experiment (Chapter 2), was designed to determine the tolerance of native *Phragmites* to two environmental stressors: salinity and inundation both alone and in combination. My objective was to examine the growth of native *Phragmites* planted at three sites along the salinity and flooding gradient of Maryland's Patuxent River. Because of the limits imposed by flooding and salinity, I hypothesized that the growth response of native *Phragmites* would differ across various flooding regimes and salinity levels. Specifically, growth would be negatively correlated to salinity and inundation frequency. Growth is measured by culm height, basal diameter, and culm density.

Methodology

Study Area

Originating in the Piedmont physiographic province of western Maryland, USA, the Patuxent River flows through urban and suburban areas and then through more rural areas before emptying into the Chesapeake Bay. The 2,393-km^2 drainage basin is located between Washington, DC, and Baltimore, MD. Current land use patterns in the watershed are as follows: forests 38%, residential 32%, agriculture 19%, other developed lands 10%, and wetlands 1% (Patuxent River Commission 2014). The average annual temperature near the study sites is 33°C with average low of 1.1°C in January and average high of 24°C in July and an average annual

precipitation of 45.8 inches (NOAA, National Climatic Data Center, Monthly Normals 1981-2010 for Mechanicsville 5 NE, MD US GHCND:USC00185865).

The 170 km river is divided into non-tidal and tidal; the lower 95 km section of the river is tidal. Observational field studies were conducted along the salinity gradient of the Patuxent River at three tidal marshes dominated by dense stands of non-native *Phragmites*: 1) Jug Bay Wetland Sanctuary (Lothian, MD; N38°46'53, W76°42'23"); 2) God's Grace Point (Prince Frederick, MD; N38°32'20", W76°40'3"); and 3) Jefferson Patterson Park and Museum (St. Leonard, MD; N38°23'23", W76°30'26") (Figure 3.1).

Site selection was based on salinity reports from Eyes on the Bay http://mddnr.chesapeakebay.net/eyesonthebay) and field salinity measurements to obtain three distinct salinity regions — low, tidal fresh (Jug Bay), middle, oligohaline (God's Grace), and high mesohaline (Jefferson Patterson Park and Museum). Two of the sites are public lands; the third is privately held and adjacent to agricultural land. Permission to access the property was granted by the land owner.

Figure 3.1: Map of field site locations. Sites are located on the Patuxent River in Maryland. Source of map: ESRI.

In April of 2015, ten 1-m^2 plots were randomly positioned along perceived elevation gradients at each site (10 plots per site x 3 sites = 30 plots). A monitoring well outfitted with conductivity and water level continuous data loggers (Odyssey, New Zealand) was installed at the lowest point within each site; one additional un-instrumented monitoring well used to make manual water level measurements was also installed in each plot. Dense stands of non-native *Phragmites* exists at all three sites. Resource managers broadcast herbicides at Jug Bay and Jefferson Patterson Park in the fall 2014 and for at least two consecutive years prior. At God's Grace, the

plots and a 3 foot perimeter were treated with herbicide directly 5 weeks prior to plant installation. Within each plot, all vegetation was clipped to the marsh surface prior to installing native *Phragmites* plants to minimize competition. Weeding of non-native *Phragmites* within each plot was done weekly as needed throughout the duration of the observation period. In May of 2015, five plants were installed into each plots, 25cm on center (5 plants per plot x 10 plots per site x 3 sites = 150 plants). A 0.5-m cleared border around the perimeter of each plot was maintained to minimize the influence of shading.

Plant Material

Rhizomes were collected for the field planting study from a confirmed stand of native *Phragmites* on the Patuxent River, Maryland, USA (N38° 42' 8", W76° 41' 48", map datum: WGS 84) on March 18, 2015. Stands were identified first using morphological characteristics (Saltonstall et al. 2004; Blossey) and then confirmed genetically following methodology described by Saltonstall (2003) which uses a restriction fragment length polymorphism assay to distinguish native from non-native. Rhizomes were excavated using a shovel, rinsed clean with river water, placed in 19-liter buckets, transported to the University of Maryland in College Park and placed in cold storage (4°C).

Firm white rhizomes with at least two nodes were planted in a 2:1 by volume mixture of potting soil and washed sand in small pots. Rhizomes were watered regularly to maintain moisture. To mimic natural conditions, greenhouse room temperature was controlled at 32°C during the day 7°C at night. After ten weeks, plants were installed at study sites. All pots had one shoot of similar size, with an

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average height of 39.1 cm (± 1.6) at Jug Bay, 38.8 cm (± 0.8) at God's Grace, and

34.4 cm (± 2.2) at Jefferson Patterson at the time of installation.

Variable Measurements

Growth measurements (culm height, diameter, and culm density) and

environmental measurements (pore water salinity, temperature, and pH, and water

levels) were collected every other week. Measurements began on May 29 and

concluded October 9 of 2015. During the 2016 season, initial measurements were

taken on June 6 and final measurements were taken on August 9, 62 days after the

first observation was made. Native *Phragmites* were identified morphologically

(Saltonstall et al. 2004; Blossey) and genetically following Saltonstall (2003)

methodology. The height of each culm was measured from the soil surface to the

tallest collared (flat) leaf. Basal diameter was measured using calipers at

approximately 4 cm above soil surface. Salinity, temperature, and pH were measured

using portable meters (YSI, Yellow Springs, Ohio) with the probe placed in the

monitoring wells at approximately 10-20 cm beneath the marsh surface.

Standing water levels were monitored manually by measuring the distance

from the top of the well to the water level and to the marsh surface with a steel tape at

three marked positions on the well. When water was absent from the marsh surface,

water level was determined by inserting a steel tape into the well to the point of

contact with the water surface, determined visually, repeated three times at each well.

Time of measurement was recorded. Relative elevation of plots was determined using

water as a leveling device (Evgenidou and Valiela 2002). An average marsh surface

level, based on observed measurements at the logger, was calculated. Calibrated

logger values were paired to their corresponding observed measurements by date and time. The calibrated water level was subtracted from the marsh surface value to determine water level relative to the marsh surface. An equation of the line was constructed describing the relationship between the logger data and observed data in order to predict the water levels throughout the experiment at each plot. The resulting water levels were then used to determine frequency of inundation at each plot for Jug Bay and God's Grace. A graphical examination of Jefferson Patterson's hydrology data suggested that water flow was restricted and not tidally influenced. This is likely due to a sand berm along the sites perimeter bordering the river. Therefore, inundation frequency at Jefferson Patterson is based on observed water levels for each plot. See Appendix B, Figure B.3.1 for hydrographs of study sites.

Final culm counts, culm height, basal, and leaf count measurements were taken on August 9, 2016. Species count and cover estimates were not done in 2015. Cover was estimated visually following the cover classes of Peet et al. (1998) before clipping and bagging all aboveground vegetation at the soil surface. Plant material was transported to University of Maryland stored in a black trash bag at 4 °C until processed. Vegetation was separated into two categories, native *Phragmites* and all other species, weighed and dried to a constant mass at 70°C to the nearest 0.01 g.

Three soil cores (10 cm diameter, 50 cm depth) were collected haphazardly from each study site across elevation levels using a McCauley peat corer to calculate moisture content, bulk density, and organic matter content. Wet soils were weighed then dried at 70°C to a constant mass and weighed again. Water content was calculated as the percentage of water mass of the wet sample. Bulk density was

determined as the mass of dried soil per volume of the sample collected. Organic matter content was calculated following methodology for loss on ignition by Klute (1986): (1) each dried soil sample was crushed into fine particles, mixed thoroughly, and 1-3 grams were placed in a muffle furnace for 16 hours to burn off all combustible organic matter; (2) washed samples were allowed to cool to room temperature in a desiccator and then reweighed; and (3) the percent change in sample weight was calculated (%OM).

Results

Site Characteristics

While the latter part of 2016 was a wetter than normal year, precipitation during the study period, May 2015 – October 2016, was normal (Appendix B, Figure B.3.2). Soils in the upper 50 cm at Jug Bay are primarily composed of organic matter while God's Grace is predominantly clay loam and Jefferson Patterson predominantly is a sandy clay loam. Sites were similar in soil pH, organic matter, and bulk density with very little variation. Salinity was as expected providing low, medium, and high salinity sites (Table 3.1).

Table 3.1. Field study site characteristics for three tidal wetlands located along a salinity gradient along the Patuxent River, Maryland, USA.

Variable	Site		
	Jug Bay $\bar{x}$ ±se	God's Grace $\bar{x}$ ±se	Jefferson Patterson $\bar{x}$ ±se
Salinity (ppt)	0.7 ±0.09	8.3 ±0.09	12.8 ±0.24
pH	6.6 ± 0.12	6.6 ± 0.11	6.5 ± 0.14
Soil organic matter (%)	38.0 ±1.9	27.6 ±3.9	37.1 ±9.2
Bulk Density (g/cm^3)	0.55 ±0.00	0.47 ±0.02	0.48 ±0.01
Soil horizon depth (cm) with field texture	peat 0-38cm, mucky silt loam, 38-50cm	clay loam 0-40cm, sandy loam 40-43cm, sandy clay loam 43-50cm	sandy loam 0-29cm, sandy clay loam 29-50cm
Soil Series	Nanticoke and Mannington	Mispillion and Transquaking	Mispillion and Transquaking

Salinity Effect

Growth as measured by culm density, height, and basal diameter decreased along the salinity gradient of the river (Figure 3.1). Initially, during the 2015 observation period, the number of culms per m^2 plot increased at similar rates at all sites; however, by the middle of July (around day 50), additions to culm count at Jug Bay increased at a faster rate than God's Grace and Jefferson Patterson. God's Grace and Jefferson Patterson saw a dramatic decline in culm production reaching a maximum of 10 culms at the end of August while at that same time, Jug Bay had an average of 17 culms and it wasn't until October 9th that a maximum of 20 stems was reached, for an increase of 222%. At the conclusion of 2015, Jug Bay had the most culms, with God's Grace and Jefferson Patterson both having lower counts than Jug Bay but similar counts to each other (Figure 3.2a).

Throughout the 2015 growing season, cumulative height (Figure 3.2c) increased at Jug Bay and continued at a positive rate reaching 1281.5 cm on August 29 (day 95). Initially, God's Grace and Jefferson Patterson showed positive growth in cumulative height reaching a maximum of 332 cm and 257 cm, respectively, on July 15 (day 50) after which, growth became negative as individual culms died and above ground growth decreased. Culm height at Jug Bay increased 75% from the initial observation (39 cm, ±1.6) to its maximum height (68.4 cm, ±5.6) on August 29 (day 95). During that same period, God's Grace and Jefferson Patterson saw negative patterns in average height, consistently declining after the first measurement. Basal diameter (Appendix B, Figure B.3.3) showed similar patterns.

In spring 2016, native *Phragmites* resprouted at the freshwater site, Jug Bay, only; no regrowth occurred at the more saline sites. One plot at Jug Bay was destroyed during the 2015 season, likely during sampling, and did not re-establish. Initial measurements, on June 8, 2016, found an average of 7 culms (± 1.4 culms) and by August 9, 2016, final measurement, the average number of culms had increased to 13 (± 3.2 culms). Although, culm count at Jug Bay was lower in 2016 than in 2015, culms were taller initially and remained taller throughout 2016 as compared to 2015 (Figure 3.2). Cumulative height was also greater in 2016, even though culm count was lower, until harvest date at which point annual cumulative heights were similar. During the 2016 harvest, culms were found growing outside of plots (not included in analysis) whereas none were found growing outside of the plots in 2015.
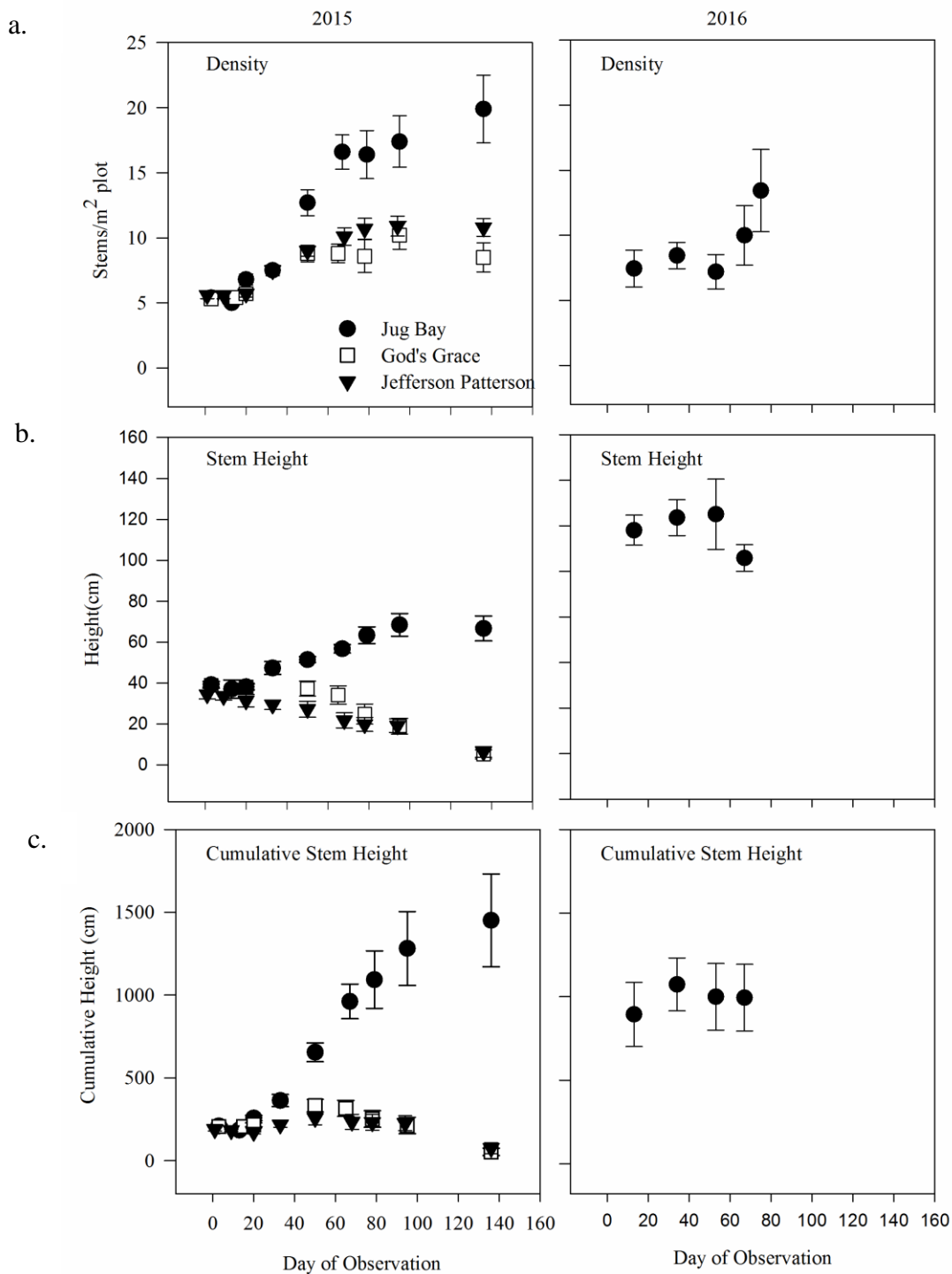
Figure 3.2: Mean density (a), stem height (b), and cumulative stem height (c) of *Phragmites australis* ssp. *americanus* found at each study site during 2015 and 2016 observation periods (mean values ±SE).

Inundation Effect

The plots at each site experienced a range of flooding frequencies (Figure 3.3). Plots at Jug Bay experienced flooding 35-90% of the time. Jefferson Patterson had a similar range of elevations based on flooding frequency (7-73%). God's Grace tended to be drier and did not have the large range of flooding frequency seen at Jug Bay or Jefferson Patterson, however, variation in flooding frequency did occur with plots flooding 0-41% of the time. The average cumulative height for each plot was greatest at Jug Bay across all flooding frequencies when compared to either God's Grace or Jefferson Patterson. Jug Bay culm counts were generally greater than the counts at any of the plots at God's Grace and Jefferson Patterson. Despite the range of flooding frequencies, inundation did not show a clear effect on growth.
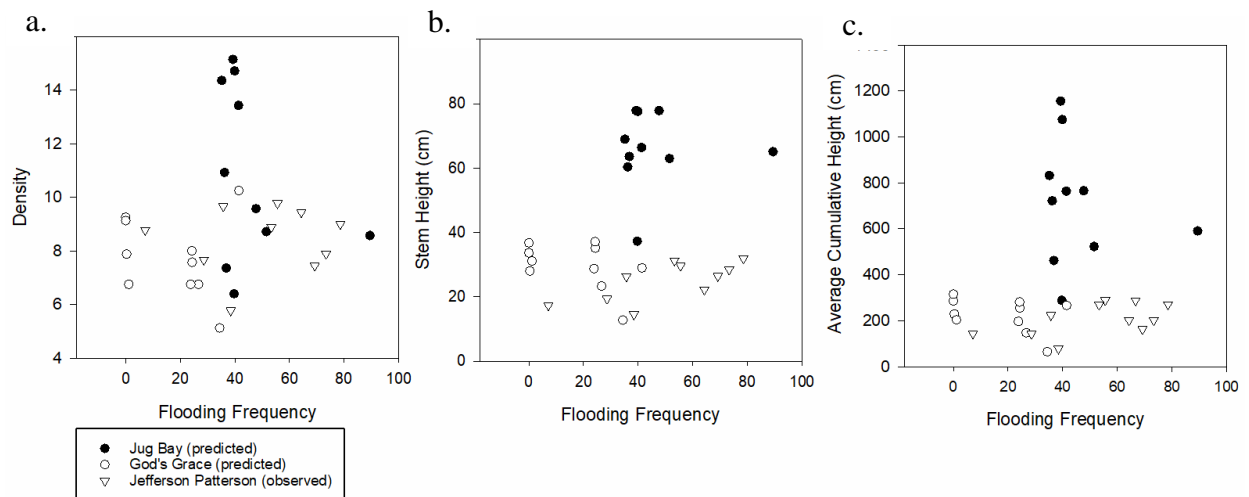
Figure 3.3. Mean density (a) stem height (b) and cumulative culm height (c) of *Phragmites australis* ssp. *americanus* found in each plot at study sites in response to different flooding regimes. Flooding frequency is based on 2015 and 2016 water level readings. Density and cumulative culm height are based on 2015 plant measurements.

Aboveground Biomass and Community Composition

Jefferson Patterson had the highest aboveground biomass and God's Grace

had the lowest (Figure 3.4). Plant community composition differed across the salinity
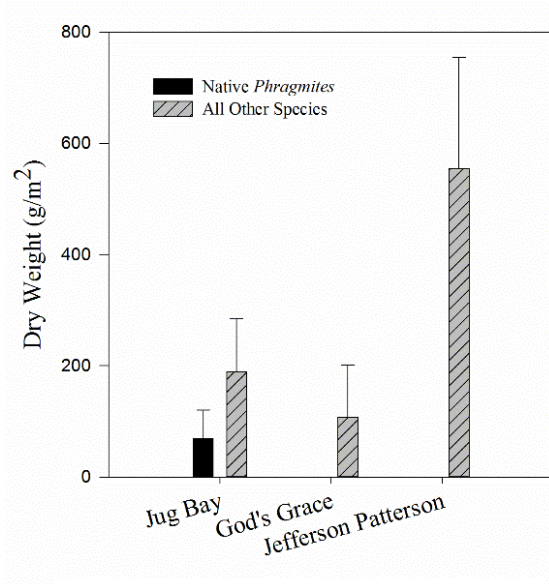
Figure 3.4. Aboveground biomass of plants harvested on August 9, 2016 (mean values, +1SE).
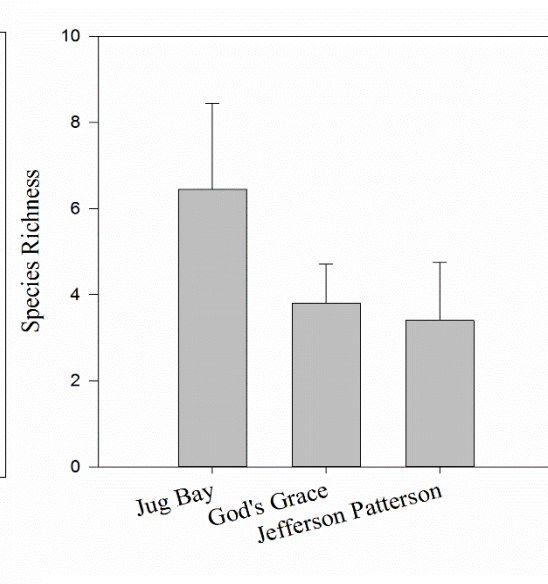
Figure 3.5. Species richness found at Patuxent River field sites in 2016 (mean values, +1SE).

gradient (Appendix A, Table 3.2) as did species richness, which decreased as salinity

increased (Figure 3.5). In 2016, a combined total of 26 species were identified: 14 at

Jug Bay, 7 at God's Grace, and 10 at Jefferson Patterson. Jug Bay had the highest

species richness, while God's Grace and Jefferson Patterson had similar richness.

Native *Phragmites* was only found at Jug Bay in 2016.

The non-native lineage was found at Jug Bay and God's Grace in 2016. While

not found in the Jefferson Patterson plots, large swaths of non-native grow along the

perimeter of the study site. The absence of the non-native in the study area is likely

due to prior eradication efforts at the site.

In 2016, Jug Bay was dominated by *Murdannia keisak* and areas of no vegetation; God's Grace had very little plant cover, as plots were primarily unvegetated; and Jefferson Patterson was dominated by *S. patens, S. alterniflora,* and *D. picata* with few areas of no vegetation (Figure 3.6).
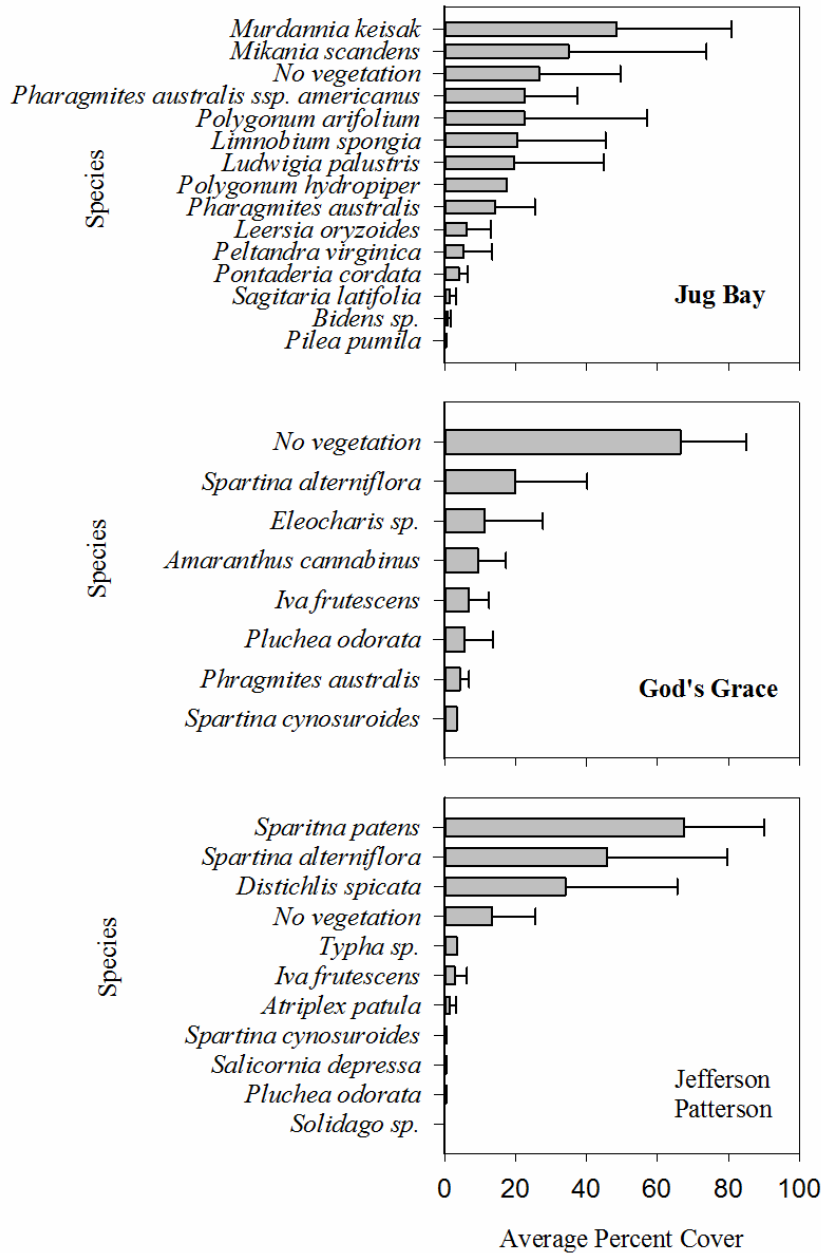
Figure 3.6. Cover (%) of standing vegetation identified during 2016 harvest at Patuxent River study sites. Nomenclature is in accordance with the USDA Plants Database (plants.usda.gov, accessed September 2016).

Discussion

      This research measured the growth of native *Phragmites* under varying

salinity and inundation levels. As hypothesized, productivity reductions were

observed along the salinity gradient. Additionally, at levels of 8 ppt and above,

growth was not only reduced but a complete cessation of growth was observed at the

conclusion of the first growing season. Inundation differences within sites appeared

not to contribute to plant stress. The field planting results combined with results of

the greenhouse study (Chapter 2) suggests native *Phragmites* has a limited tolerance

to salinity, possible maximum of 5 ppt as demonstrated in the greenhouse study, but

can tolerate a wide range of flooding conditions.

      I observed decreases in all morphological parameters measured (culm count,

average height, culm diameter) as salinity levels increased across the estuary.

Contrary to Lambert and Casagrande (2006) observations of native stands in high

salinity conditions, 27 ppt, my native plantings failed to grow at levels above 8 ppt.

Complete dieback at God's Grace and Jefferson Patterson during 2015 as well as the

lack of regrowth in 2016, suggests that native *Phragmites* maximum tolerance is less

than 8 ppt (2015 average salinity at God's Grace). These findings support results of

this greenhouse experiment (chapter 2) and those of Vasquez et al. (2005) who found

reduced height and density at salinity levels greater than approximately 7 ppt. Several

haplotypes of native *Phragmites* have been identified and appear to have

geographical ranges (Achenbach and Brix 2014; Saltonstall 2016) suggesting that

tolerance differences are due to physiological differences of haplotypes. In fact,

studies have found that salinity tolerances not only vary by genotype (Achenbach et

al. 2013) but haplotype. In Mississippi, Achenbach and Brix (2014) found decreased growth of *Phragmites* in response to salinity varied by haplotype. In fact, one of the four types examined was capable of survival at 40 ppt. The native *Phragmites* haplotype F, which was used in the greenhouse study by Vasquez et al. (2005), is primarily found in the mid-Atlantic region although, haplotype F is also found in New England along with haplotypes E and AB where growth is documented at higher salinity levels (Lambert and Casagrande 2006; Meadows and Saltonstall 2007). Interestingly, haplotype AB, was found in low salinity section of Maryland's Choptank River (Meadows and Saltonstall 2007) further documenting variance of haplotypes. Additional research is needed to determine the role of genetics in salinity tolerance of the various haplotypes to further understand possible responses to environmental changes and use in restoration.

This study did not find a clear effect of inundation on growth in contrast to my hypothesis. Instead, the response of native *Phragmites* to inundation was not uniform; in the fresh water conditions of Jug Bay, the native was successful under extreme flooding (90%) yet, under oligohaline conditions at God's Grace and Jefferson Patterson, it was unable to survive even under minimal flooding (10%). Warren et al. (2001) found that non-native *Phragmites* occupied areas with a mean flooding frequency of 40% and concluded that it was hydro period, not salinity that limited growth. However, given that growth was inhibited at God's Grace where flooding frequencies were less than 50%, I believe it is salinity that limits growth of native *Phragmites*. Culm height and count was greatest at Jug Bay for all inundation levels which coincides with results from (Voesenek et al. 2004; Jackson and Colmer 2005)

who found, under submergence, shoot elongation increased in an effort to increase gas exchange. However, this study did not produce those results at the high and mid salinity sites suggesting the negative growth pattern and failure to reestablish at God's Grace and Jefferson Patterson was due to the higher salinity of those sites and not due to differences of flooding frequency. These results suggest an ability to tolerate flooding, which aligns with a spatial distribution study in Canada that found the native to be more prolific at lower elevations while the non-native occupied drier land (Taddeo and Blois 2012). Additionally, observations of native *Phragmites* stands on Maryland's eastern shore, which extend across the marsh profile (Meadows and Saltonstall 2007), are able to tolerate varied flooding conditions. However, my results contradict these studies showing a negative growth response to flooding frequency. Previous studies used seedlings or young plants while those in this study may have been old enough to tolerate flooding.

Cover estimates identify *S. patens* and *S. alterniflora* as the dominant species at Jefferson Patterson, While, God's Grace was almost void of vegetation, and Jug Bay was dominated by an invasive low growing herbaceous perennial (*Murdannia keisak*). Unexpectedly, aboveground biomass was highest at the high salinity site. I expected Jug Bay to have higher levels since freshwater tidal marshes have been shown to be more productive than mesohaline marshes (Barendregt et al. 2009; Craft et al. 2009) however, Wieski (2001) also found higher above ground biomass at brackish sites compared to fresh sites. The low biomass may have resulted from my efforts to limit competition with native *Phragmites* at Jug Bay - all plants other than native *Phragmites* plants were clipped to the surface at Jug Bay during the

observation period. Clipping did not occur at God's Grace because plots were mostly barren nor did it occur at Jefferson Patterson once it was clear that native *Phragmites* had not re-established. To eliminate competitive pressure, clipping of the non-native *Phragmites* continued during the 2016 season at Jug Bay. *M. keisak* with its prostrate sprawling growth habit likely avoided removal because of its low stature and its propensity to form dense mats may explain why it had the greatest cover. The low biomass at God's Grace was due to several un-vegetated plots as compared to Jug Bay which only had one and none were found at Jefferson Patterson. Establishment is dependent upon several factors with limited light availability, seed bank limitations, and land disturbance all known to decrease vegetation and species richness in flooded saline conditions (Baldwin and Mendelssohn 1998; Ailstock et al. 2001; Baldwin et al. 2010) Prior to site establishment, God's Grace was very densely populated by non-native *Phragmites* and scattered *Iva frutescens* which may have contributed to the low number of species found at God's Grace as viable seeds or limited light conditions may have prevented the establishment of additional species once non-native *Phragmites* had been cleared from the plots. Although, given the low flooding frequency at God's Grace, I would have expected to see higher species richness as was found by Baldwin et al where richness increased by 42% under dry conditions as compared to submerged conditions. Jug Bay had the largest number of species found which was to be expected since tidal freshwater marshes are more diverse than both oligohaline and mesohaline (Crain et al. 2004; Sharpe and Baldwin 2009; Więski et al. 2009; Batzer, Darold P. and Sharitz, Rebecca R. 2014). Even though Jug Bay is

more diverse, it is likely that the number of species was underestimated due to efforts to minimize the effect of competition.

Invasive species have had a significant impact on our natural systems requiring intensive management and restoration efforts to improve ecosystem function. Plant selection is essential to restoring ecological integrity and is the primary focus of many restoration projects — restore or enhance natural vegetation communities, increase biodiversity, improve ecosystem function — all of which require an understanding of native plant tolerance to environmental conditions. Restoration and enhancement projects are deemed successful when plant diversity and vegetative cover expectations are met (USDA NRCS 2003). However, maintaining the desired plant community is dependent on the physical and chemical processes present. This study was conducted along a natural salinity gradient and varying inundation frequencies in order to identify tolerances to those stressors based on the physical response of native *Phragmites*. The results of this study suggest that plantings of adult native *Phragmites* would be successful in areas both frequently and infrequently flooded but where salinity levels are lower than 8 ppt and possibly no higher than 5 ppt.

Chapter 4: Final Conclusions

Wetlands are recognized as highly important ecosystems providing services valued in the trillions of dollars (Zedler 2000) such as habitat, erosion control, containment of flood waters, and pollution abatement. Yet, from 1998-2008 coastal wetland losses increased from 60,000 -80,000 acres per year (Stutz 2014 Jul 28). Losses are not only quantitative but qualitative. Degradation in the form of low native biodiversity due to the spread of invasive species or from increased flooding due to either a reduction in the ability of the ecosystem to regulate water flow or the threat of sea level rise limit the ability of wetlands to provide valuable services.

Increased public awareness and recognition by policy makers regarding the value of wetlands delivered $4.2 billion to the restoration of wetlands in recent decades (Hansen et al. 2015). Successful restoration is understood to be the return of a wetland and its functions to a close approximation of its original condition as it existed prior to disturbance. In addition to restoring hydrology and soil conditions, the restoration of native vegetation is necessary to restore wetland diversity, value, and function. The value of restoring native species is recognized by the federal government. In fact, executive orders task federal agencies and partners to restore native species and habitat conditions in ecosystems that have been invaded and to develop guidance on the use and maintenance of native species (PROTECT 2008).

I investigated the tolerance levels of native *Phragmites* to environmental stressors – salinity and inundation. I examined native *Phragmites* plants grown from

rhizomes that were collected from the Patuxent River in Maryland. As hypothesized, the results of the greenhouse experiment indicate that salinity inhibits growth; additionally, the results indicate that the salinity tolerance is 5 ppt, beyond which, growth is inhibited. Similar results were found in field observations conducted along the salinity gradient of the Patuxent River. Where salinity levels averaged 8 ppt, growth was inhibited during the first field season and at higher salinity levels of 12 ppt I observed 100% mortality by the end of the first observation season. I also observed a complete lack of regrowth in the second season at those same locations. I hypothesized that growth would vary with water level; however, both the greenhouse results and field observations suggest that water levels do not influence growth.

According to the US EPA, one priority of wetland restoration is to re-establish ecological integrity of degraded ecosystems, specifically, the composition and natural processes of its biotic communities by simulating the native communities and diversity found in the region (US EPA OW 2015 Jun 30). Effective restoration designs incorporate the natural communities that have sustained native ecosystems through time. Restoration success is measured by the establishment of vegetation (USDA NRCS 2003); however, establishment is dependent upon species ability to tolerate existing environmental conditions. This research demonstrates that native *Phragmites* would be appropriate in the restoration and re-vegetation of natural communities found in the fresh or oligohaline marshes of the Mid-Atlantic region. Further, the ability of native *Phragmites* to tolerate various water levels broadens its scope of use to include restoration efforts aimed at re-establishing hydrologic regimes, particularly in cases where flooding or runoff is expected as sea level rises

or land development occurs, and in projects aimed at limiting erosion, such as living

shorelines. I recommend that native *Phragmites* be added to plant identification

guides currently used by professionals involved in restoration projects. In addition to

increasing biodiversity, the use of native *Phragmites* may also improve water quality

as the native is able to assimilate inorganic nitrogen and has high rates of organic

nitrogen uptake.

The presence of non-native *Phragmites* along the Atlantic coast has been a

nuisance to resource managers for decades. Control efforts are vital in restraining the

invasive and in protecting the native vegetation communities. Knowing where and in

what conditions native *Phragmites* is found is imperative to the protection of this

native species. Current investigations in near infrared spectroscopy may prove useful

in remote identification of unidentified populations while mapping the currently

known locations and incorporating those locations into the decision making process

could help stave off inadvertent eradication of this native species.

Protecting native *Phragmites* not only requires understanding its tolerance to

environmental conditions and where it can be found but, also the ability to identify it

in the natural settings. In addition to this research, I provided training, developed

outreach materials, and established demonstration sites (Appendix C). Surveys I

conducted after educational seminars showed most attendees were unaware of the

native lineage and were unable to distinguish between the non-native and the native.

Additional educational programs, outreach materials such as fact sheets and YouTube

videos, and demonstration sites aimed at increasing awareness of resource managers,

restoration practitioners, and technicians would result in more effective techniques for the management of native wetland vegetation.

Future research into the relationship of native *Phragmites* with other plant and/or animal species may uncover relationships currently unknown, potentially identifying indicator species. Further studies examining salinity tolerance of native *Phragmites* using plant material from other regions has the potential to identify populations with greater tolerance thereby expanding its range of use in restoration.

In summary this research concludes that planting native *Phragmites* in fresh and oligohaline marshes of the Mid-Atlantic region at varying water levels is appropriate for restoration and management control efforts. I suggest incorporating installation of native *Phragmites* into management and restoration projects which identify as a primary goal:

- increasing biodiversity;

- restoring natural vegetation communities;

- removing invasive species, e.g. non-native *Phragmites;*

- restoring site hydrology;

- controlling shoreline erosion (when used as shoreline vegetation, i.e. living shorelines); or

- improving water quality.

The loss of ecological integrity accompanies the decline and degradation of wetlands, however incorporating native *Phragmites* into management and restoration practices is likely to improve biodiversity and increase ecosystem services locally and at the landscape scale.

Appendices

Appendix A. Supplemental Tables

Table A.2.1. Schedule of incremental salinity increases and the amount of Instant Ocean added to approximately 5 gallon of water (18.9 L) in order to create appropriate salinity treatment.

Salinity treatment (ppt)	Instant Ocean (g) added to water	Date and Number of experimental units receiving treatment							
		6/23/2015	6/26/2015	6/29/2015	7/2/2015	7/6/2015	7/9/2015	7/13/2015	7/17/2015
0	0.0	96	12	12	12	12	12	12	12
2	35.0	0	84	12	12	12	12	12	12
5	95.0	0	0	72	12	12	12	12	12
9	173.0	0	0	0	60	12	12	12	12
14	268.0	0	0	0	0	48	12	12	12
20	393.0	0	0	0	0	0	36	12	12
27	530.0	0	0	0	0	0	0	24	12
35	700.0	0	0	0	0	0	0	0	12

Table A.2.2. Calculation of iron sulfate additions.

Amount	Units
0.0006	M $FeSO_4$ (based on Eller et al., 2014)
0.0003	0.5L added weekly
151.9076	$FeSO_4$ molecular weight (g/mole)
0.0464	g $FeSO_4$ added weekly
0.0132	g/L of soil using 3.5L pot
0.0077	g needed for 6.03L pot
278.0146	g $FeSO_4$ $7H_2O$ molecular weight
151.9076	g $FeSO_4$ molecular weight
1.8302	Amount of $FeSO_4$ in $FeSO_4$ $7H_2O$
0.0141	g $FeSO_4$ needed/pot/week

Table A.2.3. Above- and belowground parts dried at 70 °C until constant mass was reached. Random samples of each fraction were chosen and weighed on 3 dates unitl no change was recorded.

Plant ID	Fraction	9/11/2015		9/15/2015		9/17/2015	
		Wet Weight (mg)	Dry Weight (g)	Dry Weight (g)	Change in Dry Weight (g)	Dry Weight (g)	Change in Dry Weight (g)
71	Stem	19581	13.72	13.34	0.38	13.34	0.00
66	Rhizome	8681	11.15	10.57	0.58	10.56	-0.01
23	Lateral	16444	10.59	10.37	0.22	10.36	-0.01
96	Leaves	25371	16.40	16.00	0.40	15.99	-0.01
31	Leaves	54875	63.91	62.39	1.52	62.37	-0.02
83	Stems	5344	51.52	49.91	1.61	49.90	-0.01
86	Rhizome	5849	10.15	9.86	0.29	9.87	0.01
44	Stem	60001	19.52	18.72	0.80	18.68	-0.04
91	Leaves	16560	13.43	13.09	0.34	13.09	0.00
65	Rhizome	11732	9.94	11.22	-1.28	11.23	0.01

Table A.3.2. Species identified at each study location in 2016. Nomenclature is in accordance with the USDA Plants Database (plants.usda.gov, accessed September 2016).

Species	Jug Bay Wetland Sanctuary	God's Grace	Jefferson Patterson Park and Museum
		Location	
Amaranthus cannabinus		X	
Atriplex patula			X
Bidens sp.	X		
Distichlis spicata			X
Eleocharis sp.		X	
Iva frutescens		X	X
Leersia oryzoides	X		
Limnobium spongia	X		
Ludwigia palustris	X		
Mikania scandens	X		
Murdannia keisak	X		
Peltandra virginica	X		
Phragmites australis	X	X	
Phragmites australis ssp. *americanus*	X		
Pilea pumila	X		
Pluchea odorata		X	X
Polygonum arifolium	X		
Polygonum hydropiper	X		
Pontaderia cordata	X		
Sagitaria latifolia	X		
Salicornia depressa			X
Solidago sp.			X
Spartina alterniflora		X	X
Spartina cynosuroides		X	X
Sparitna patens			X
Typha sp.			X
Total Species Count	14	6	10

Appendix B. Supplemental Figures

Figure B.2.1. Photographs of (a) *Phragmites australis* ssp. *americanus* rhizomes collected from the Patuxent River on March 18, 2015, (b) potted rhizomes in the greenhouse on March 30, 2015, and (c) rhizome growth in the greenhouse on April 29, 2015.

Figure B.2.2. Greenhouse layout of a random complete block design evaluating response of Phragmites australis ssp. americanus to water level and salinity treatment. Blocks were used to account for greenhouse gradients. Each block contained 24 buckets in two rows. Numbers above or below bucket are plant id which was used to randomly assign treatments. Numbers within circles identify salinity treatment followed by the water level treatment.
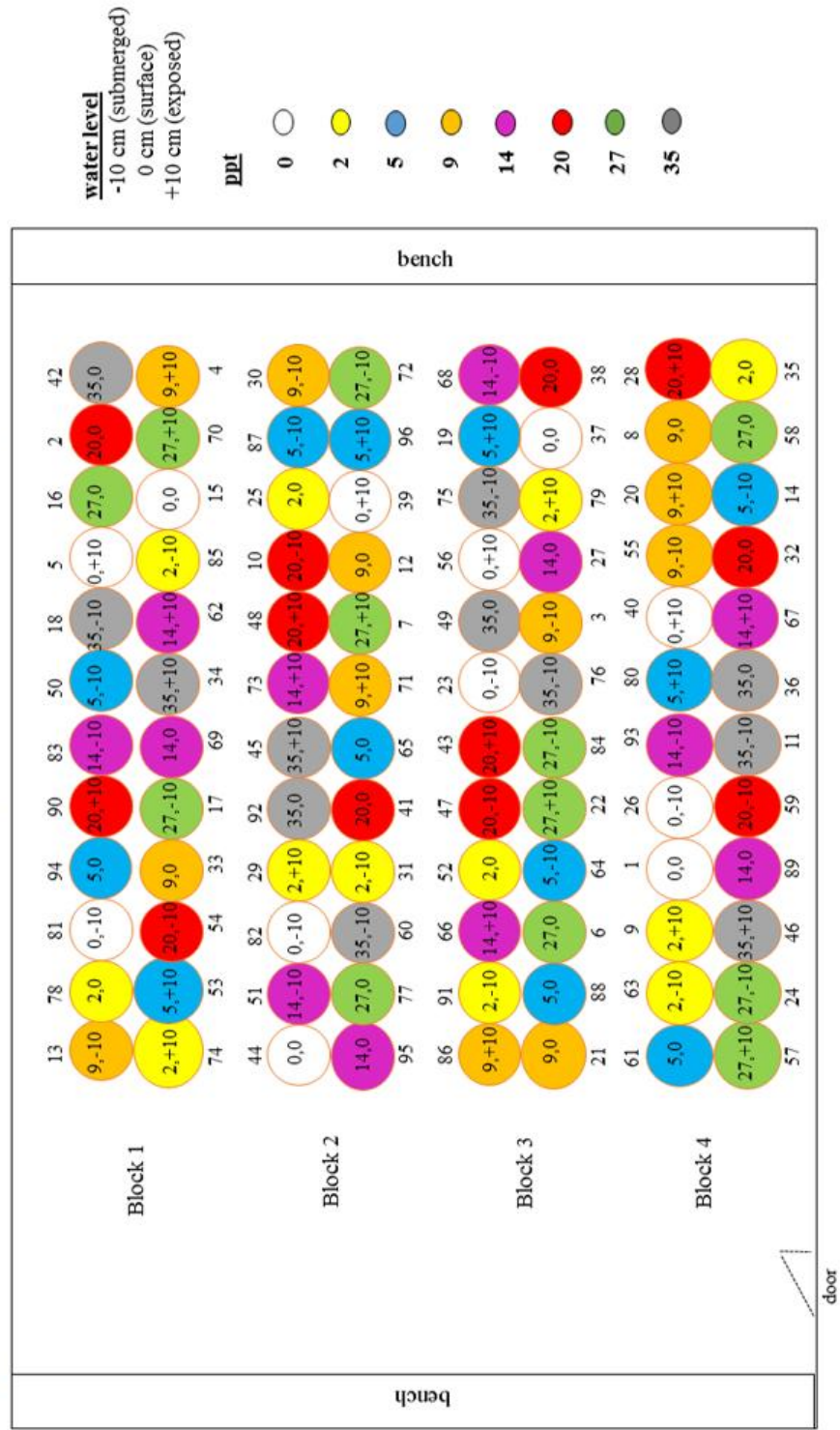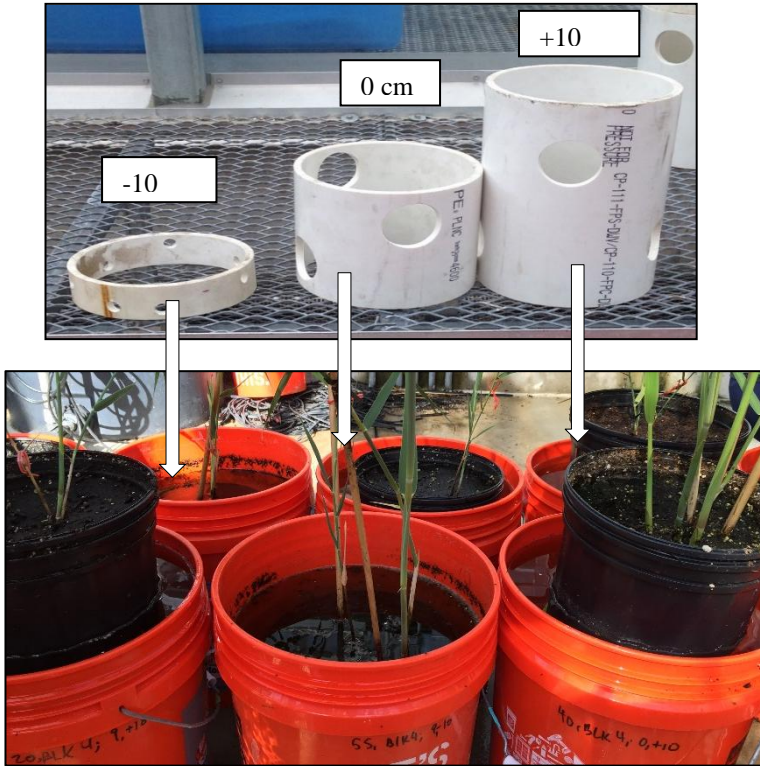
Figure B.2.3. Photograph of (a) PVC risers used to elevate potted plants to assigned water level treatments, and (b) experimental set up on harvest day showing growth of native *Phragmites* at the conclusion of experiment.

a.

b.

Figure B.2.4. Main effect of salinity on growth of *Phragmites australis* ssp. *americanus*. Plotted values are arithmetic means +1 SE of weekly measurements of (a) cumulative stem height, (b) stem height, (c) stem count, and (d) live leaf count. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, P<0.05).
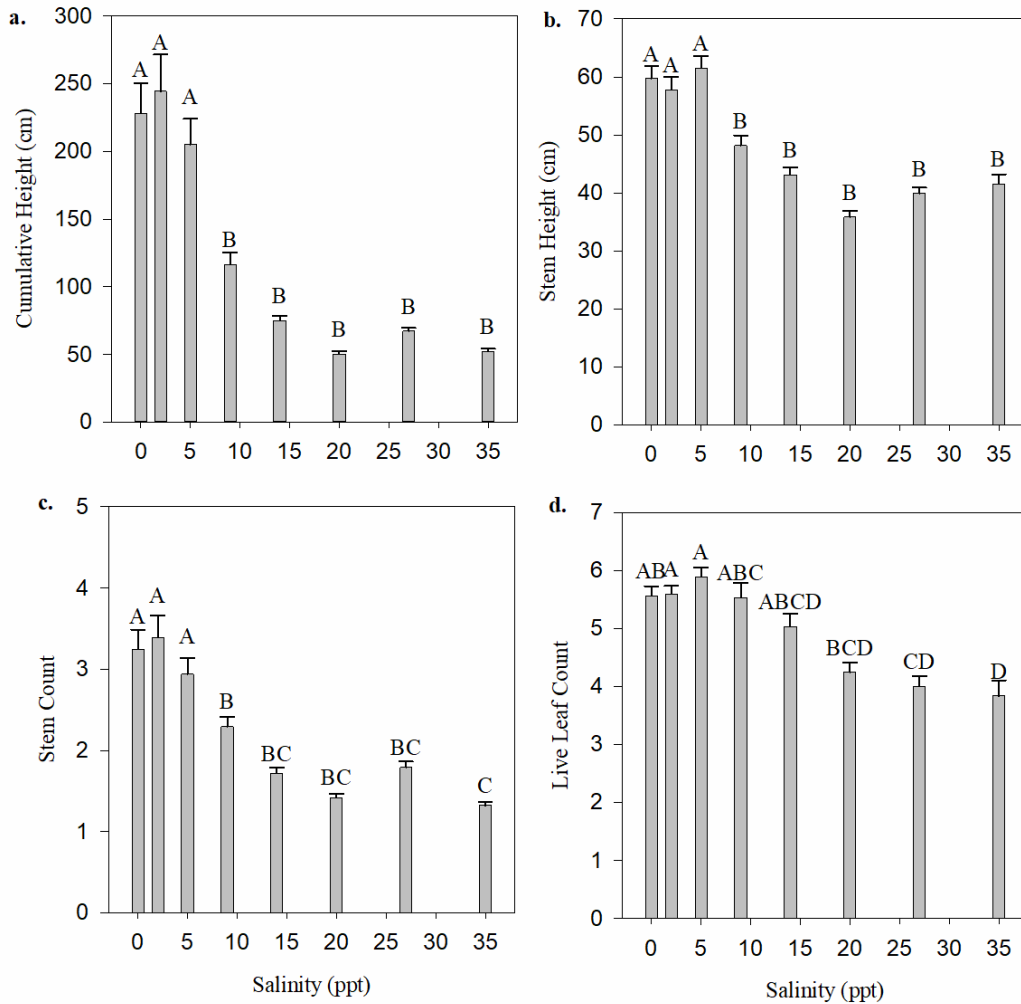
Figure B.2.5. Effect of salinity on fluorescence as measured by (a) Fv/Fm and (b) yield. Plotted are arithmetic means of measurements, + 1 SE, taken on day 50 and day 57 of the experiment. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, P<0.05).

Figure B.2.6. Effect of salinity on growth as measured by (a) dead leaf count and (b) stem diameter. Plotted values are arithmetic means +1 SE of values measured repeatedly during experimental period. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, $P<0.05$).

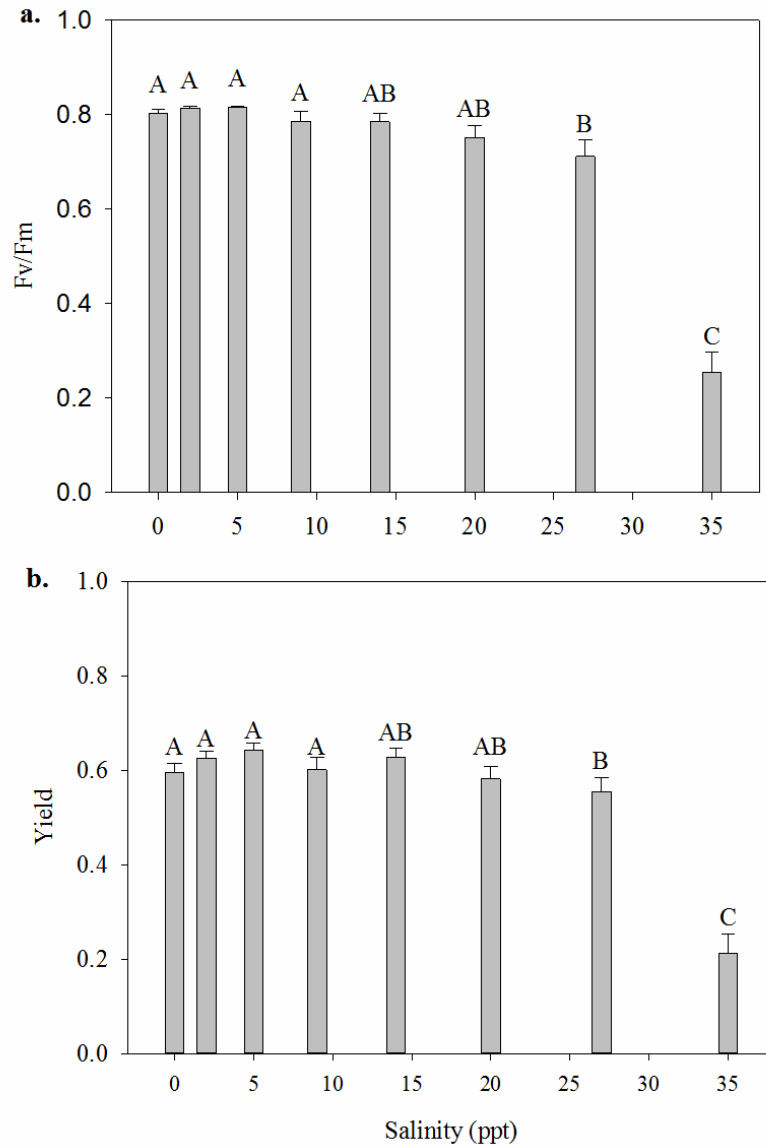Figure B.2.7. Variation of *Phragmites australis* ssp. *americanus* cumulative height in response to water level over the course of the experiment for selected salinity treatments. Plotted values are arithmetic means of weekly measurements and plotted using a straight line curve.

Figure B.2.8. Variation of *Phragmites australis* ssp. *americanus* stem count in response to water level over the course of the experiment for selected salinity treatments. Plotted values are arithmetic means of weekly measurements and plotted using a straight line curve.
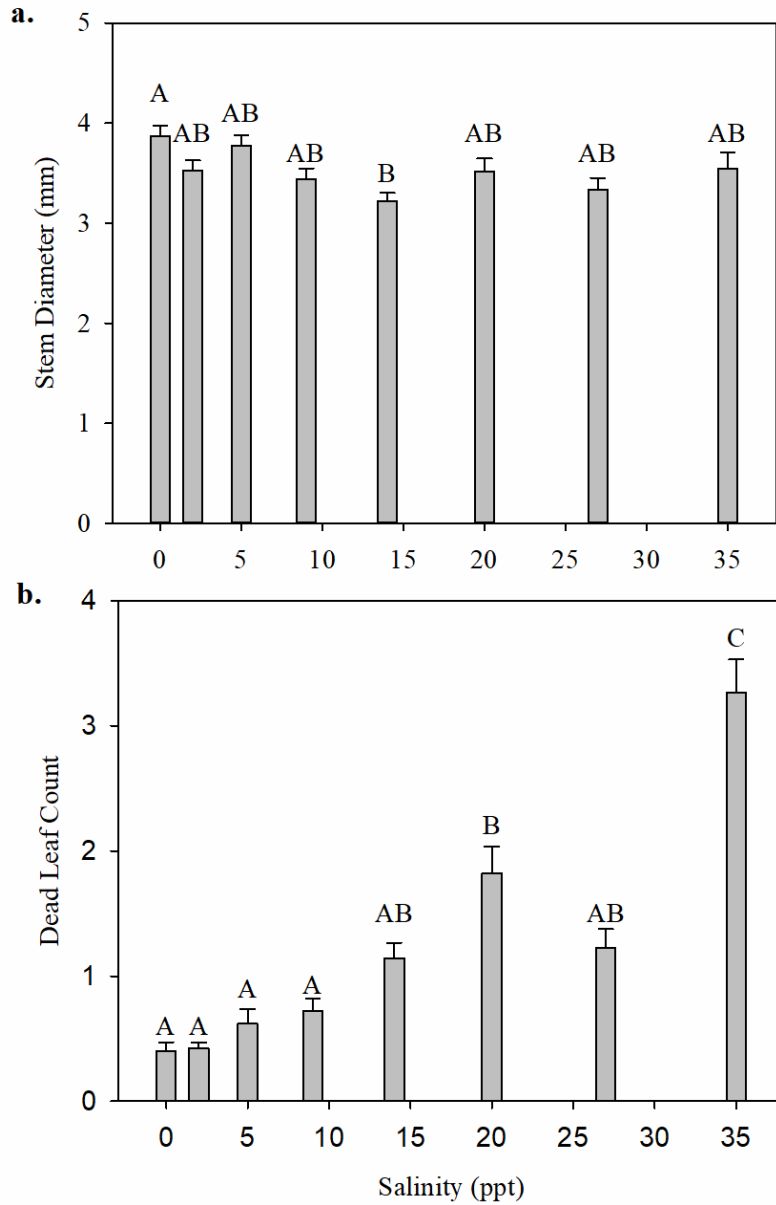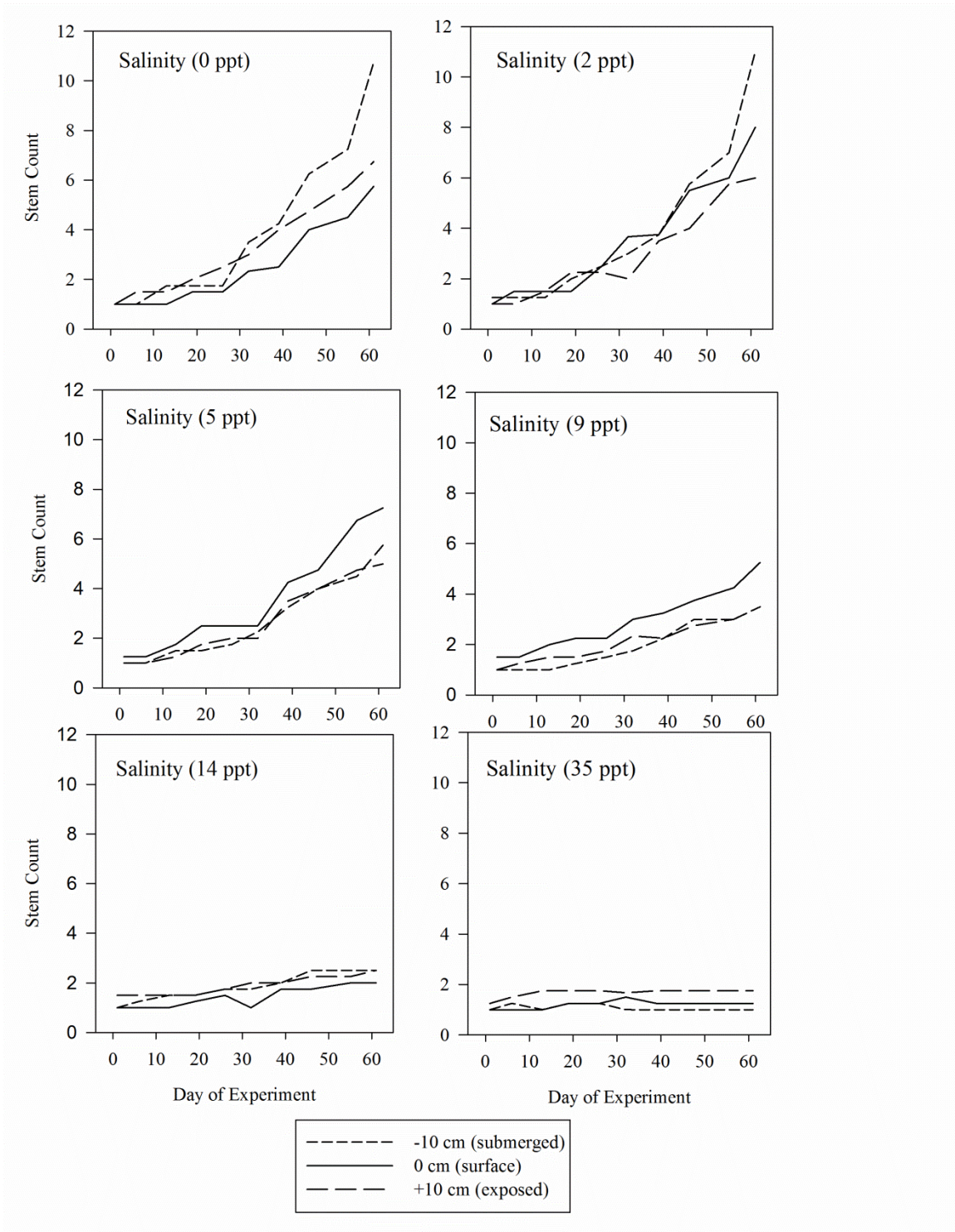
Figure B.2.9 Variation of *Phragmites australis* ssp. *americanus* Fv/Fm in response to water level over the course of the experiment at each salinity treatment. Plotted values are arithmetic means of measurements on Day 50 and Day 57 and plotted using a straight line curve.
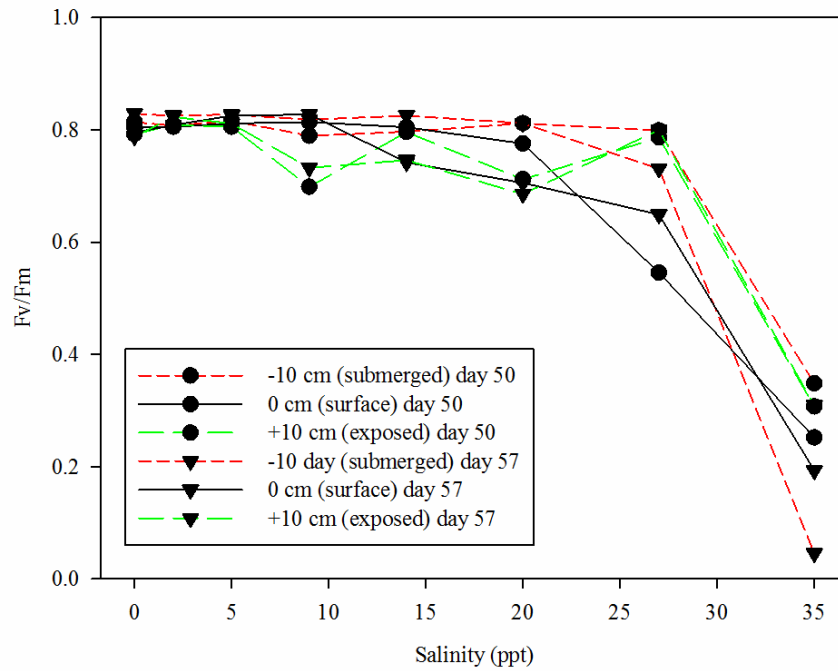
Figure B.2.10. Variation of *Phragmites australis* ssp. *americanus* (a) dead leaf count and (b) stem diameter in response to salinity treatments. Plotted values are arithmetic means of weekly measurements and plotted using a straight line curve.
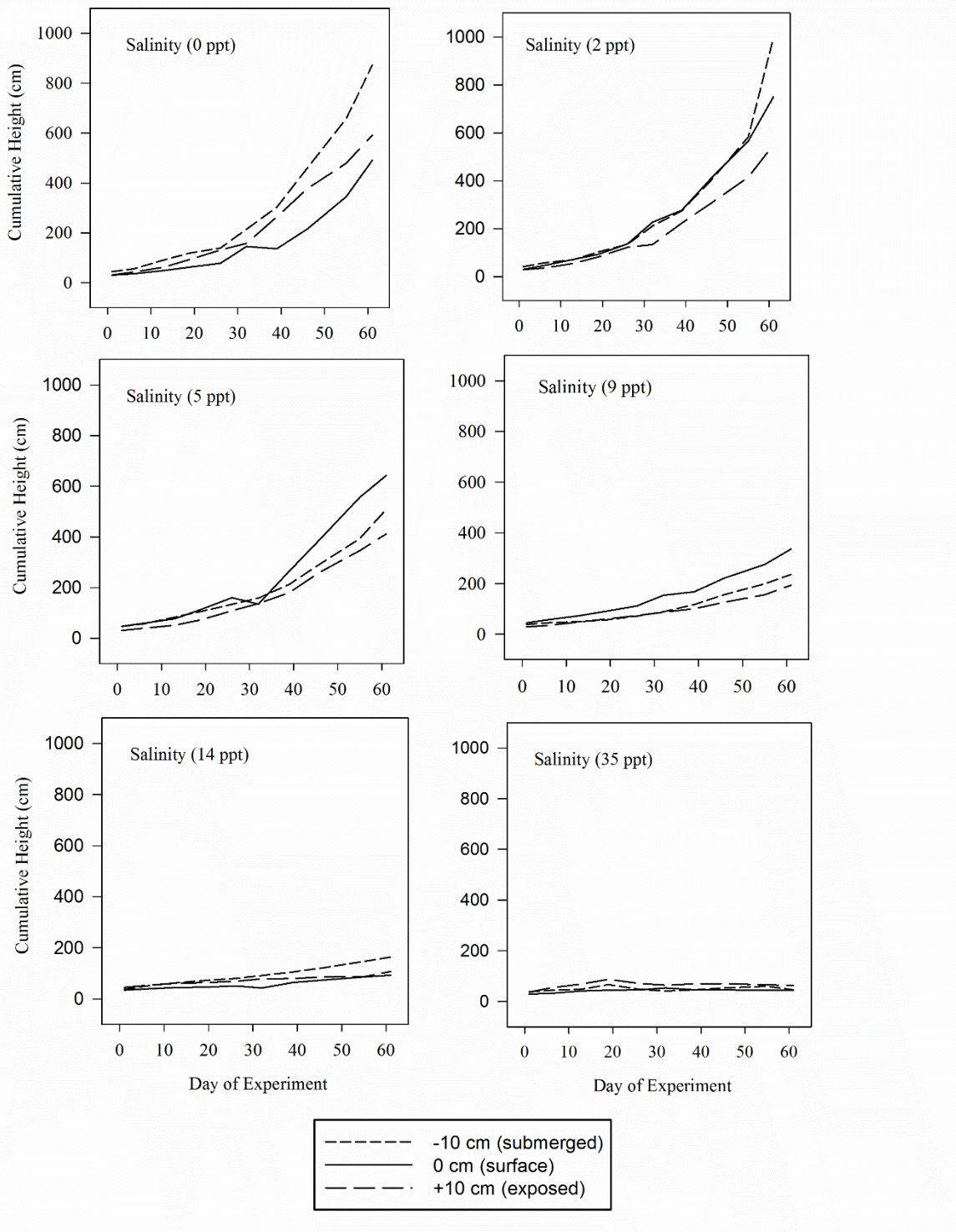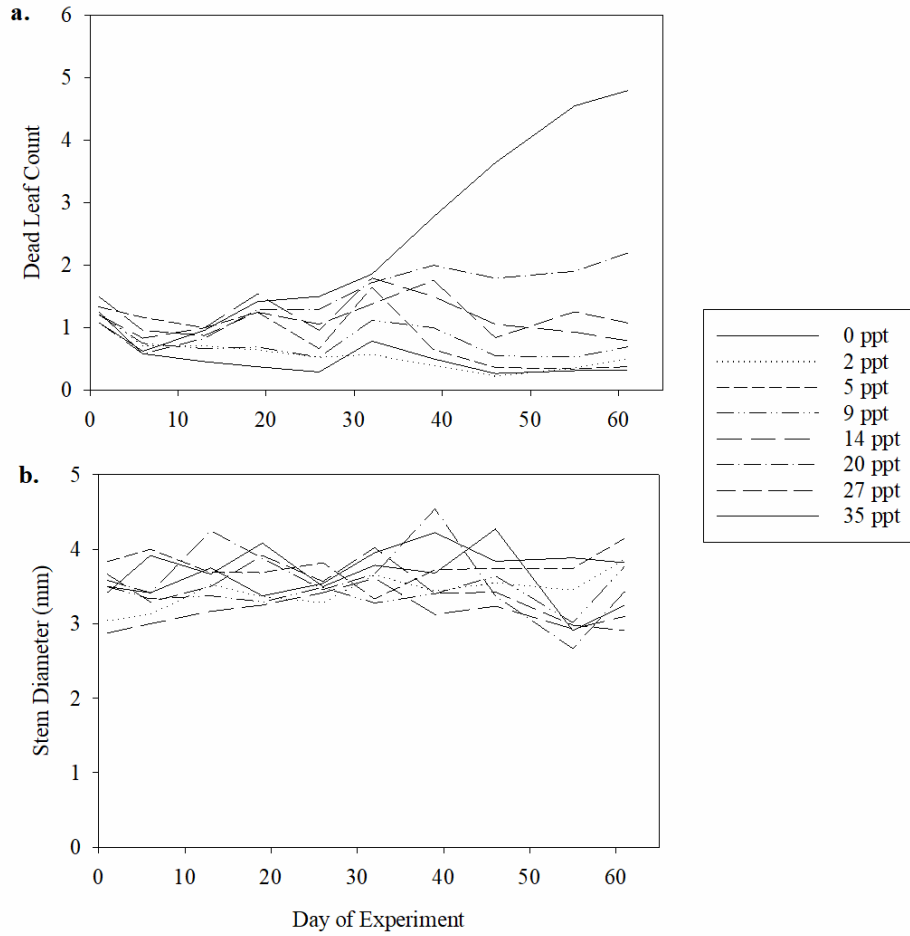
Figure B.2.11. Variation in Fv/Fm of *Phragmites australis* ssp. *americanus* due to salinity treatment on (a) day 50 and (b) day 57. Plotted values are arithmetic means +1 SE. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, P<0.05).
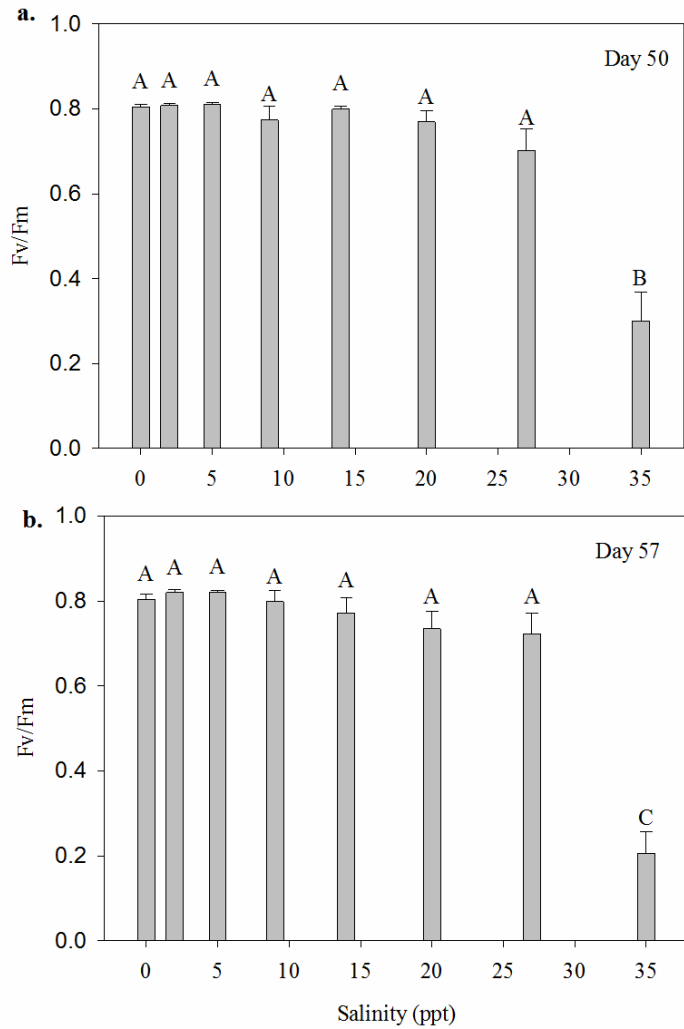
Figure B.2.12 Variation of *Phragmites australis* ssp. *americanus* stem diameter in response to salinity treatments at three water levels. Plotted values are arithmetic means +1 SE of weekly measurements of diameter at one of three water treatments -10 cm (submerged) below water level, 0 cm (surface), or (c) +10 cm (exposed) above water level.
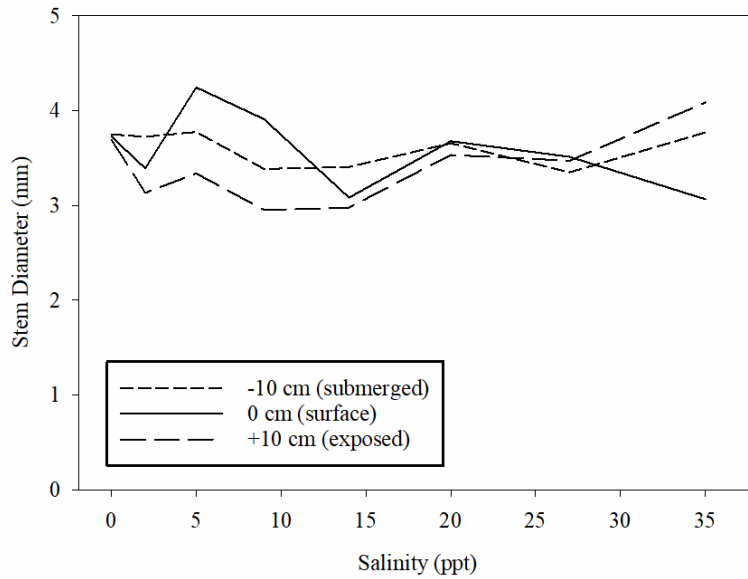
Figure B.2.13. Variation of *Phragmites australis* ssp. *americanus* (a) cumulative stem height, (b) stem height, (c) stem count, and (d) dead leaf count in response to water level treatments. Plotted values are arithmetic means of weekly measurements plotted using a straight line curve.
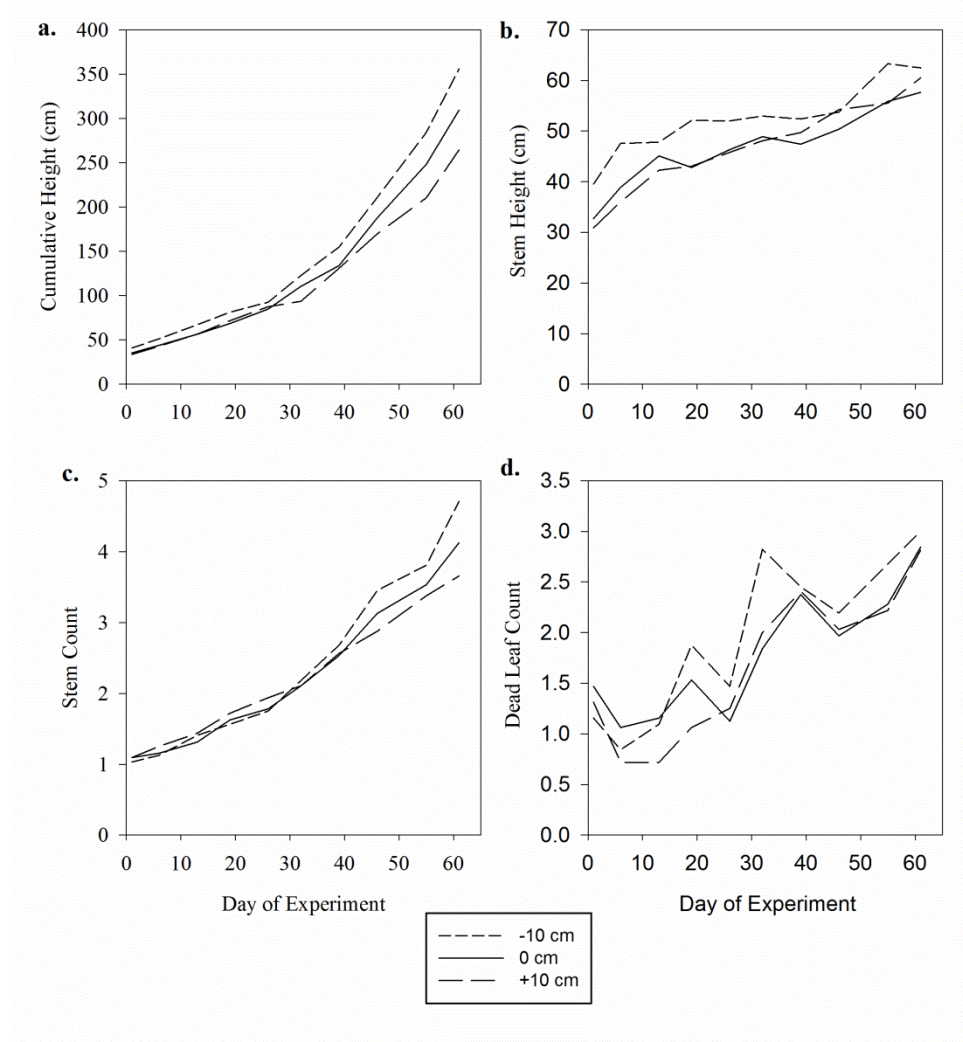
Figure B.2.14. Main effect of water level on growth of *Phragmites australis* ssp. *americanus.* Plotted values are arithmetic means +1 SE of (a) stem height, and (b) stem diameter measured weekly. Within each panel, different letters indicate significant differences of means (Tukey-Kramer test, $p < 0.05$ for stem height and $p < 0.10$ for stem diameter).
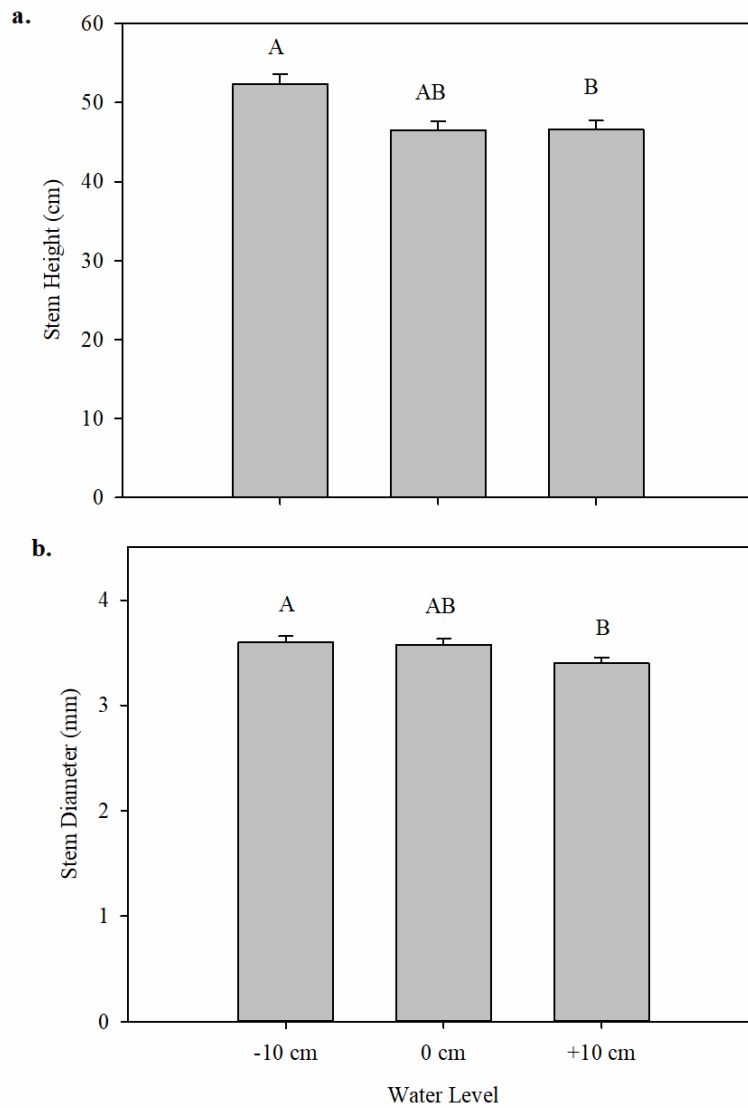
Figure B.2.15. Main effect of salinity on (a) cumulative height, (b) stem height, (c) stem count, and (d) live leaf count. Arithmetic means of measurements at conclusion of experiment + 1 SE are plotted. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, P<0.05).
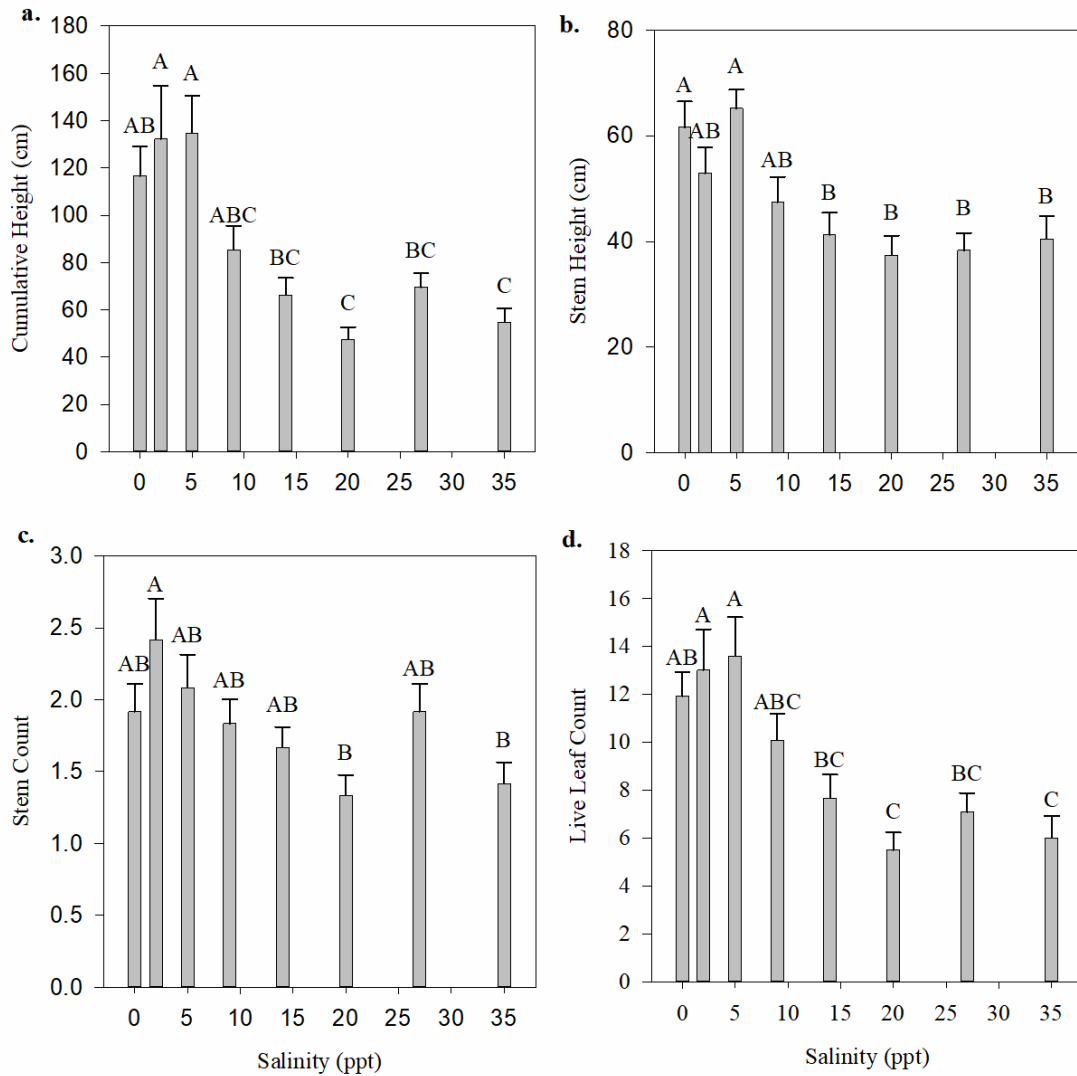
Figure B.2.16. Main effect of salinity on (a) lateral root count, (b) leaf area, (c) rhizome length, and (d) root:shoot ratio. Arithmetic means of measurements at conclusion of experiment + 1 SE are plotted. Within each panel, means with the same letter are not significantly different from each other (Tukey–Kramer test, P<0.05).
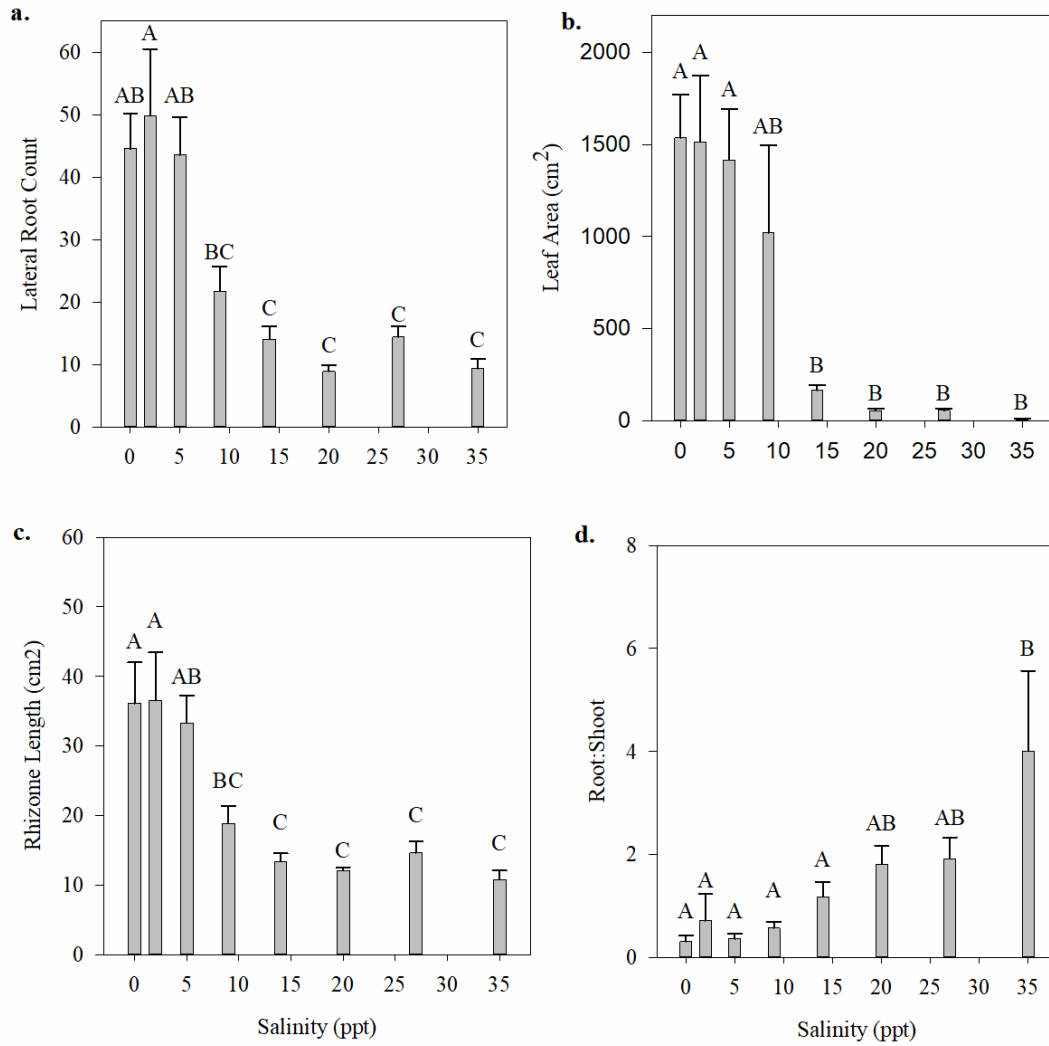
Figure B.2.17. Experimental salinity treatment level and measured salinity level in drainage water and reservoir water. Plotted are the salinity means (±1 SE) of reservoir water and drainage water measured before water treatment was applied.
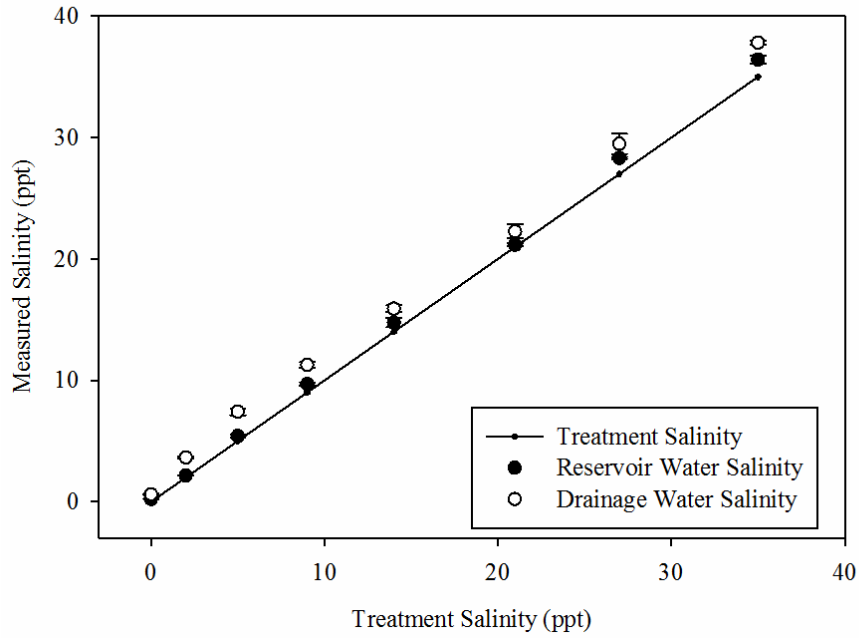
Figure B.2.18. Amy Kuritsky taking stem height measurements.

Figure B.2.19. Photographs of (a) Amy Kuritsky taking basal measurements and (b) 100 mm pocket caliper and YSI hand held meter.

Figure B.2.20. Photograph showing salinity reading of reservoir water.

Figure B.2.21. Photograph of root washing. Growth media was washed away from belowground parts using a garden hose and a 5 mm mesh screen.

Figure B.2.22. Example of harvested belowground material (rhizome and lateral roots) after being rinsed.

Figure B.3.1. Hydrographs of Patuxent River study sites: (a) Jug Bay, (b) God's Grace, and (c) Jefferson Patterson Park and Museum for June and July of 2015.
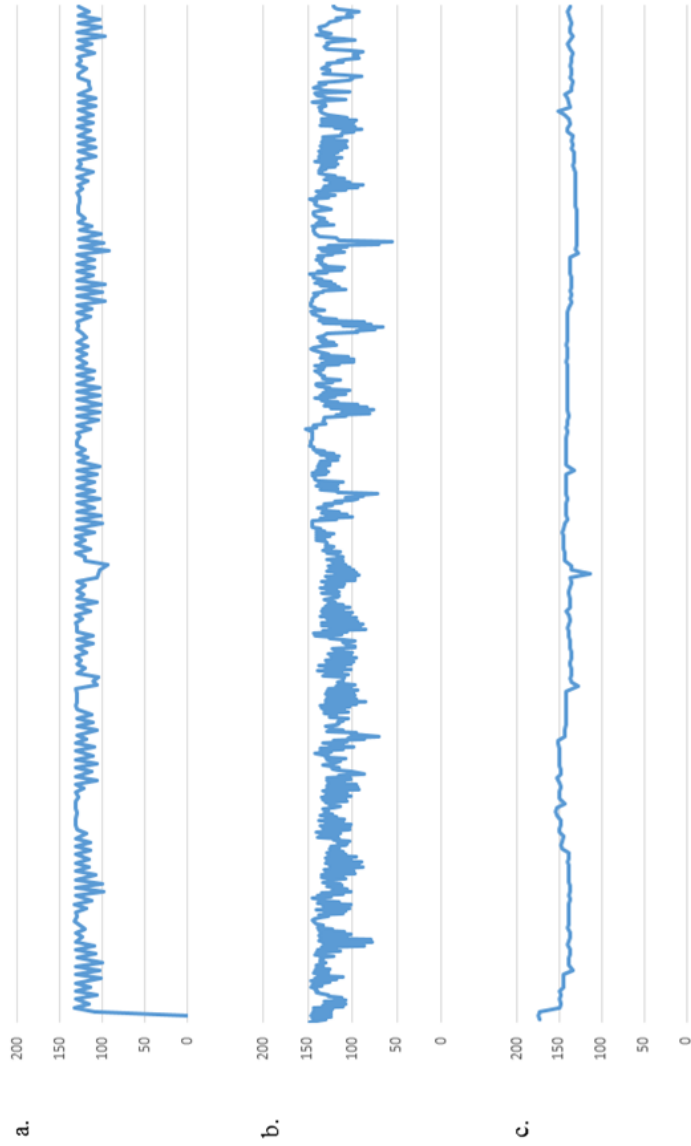
Figure B.3.2. Normal range of annual rainfall with three month running average of actual rainfall during 2015 and 2016. Annual rainfall range from NRCS WETS precipitation trend data for Mechanicsville, MD; results based on data from 1927-2016; extracted 8/30/2016 from http://agacis.rcc-acis.org/24037/wets. Running average data from NOAA Online Weather Data, NOW, monthly rainfall for Mechanicsville, MD (http://w2.weather.gov/climate/xmacis.php?wfo=lwx).
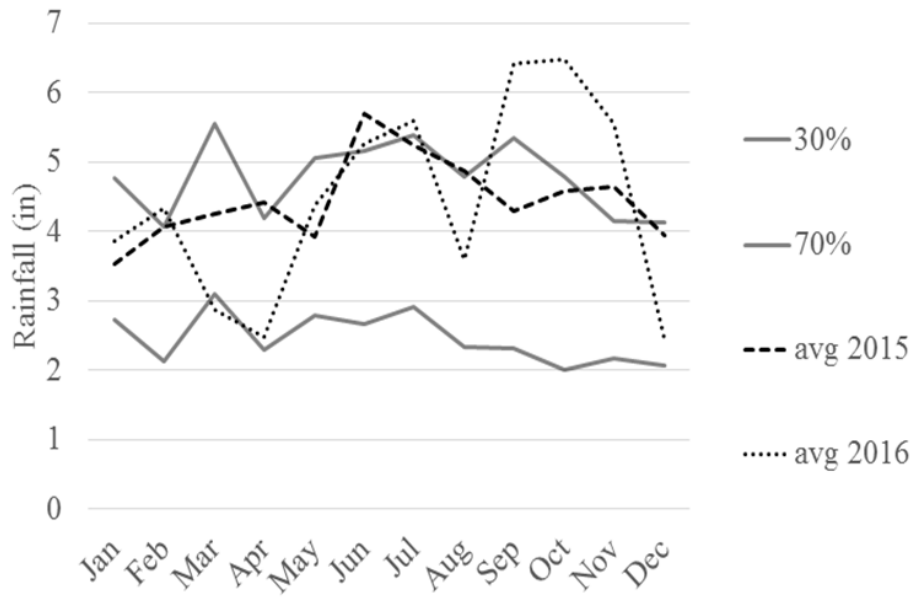
Figure B.3.3. Basal diameter of culms at study sites in 2015. Plotted values are arithmetic means ±1 SE of weekly measurements made during the 2015 observation period.
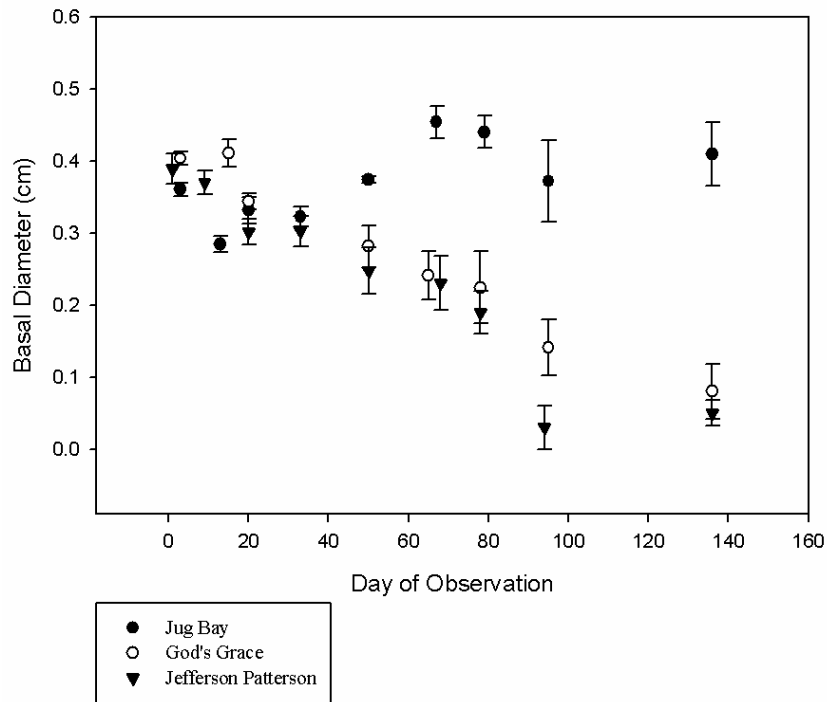
Figure B.3.4. Example of plot layout, photo taken at God's Grace one week after plant installation.

Figure B.3.5. Image of continuous data logger well, installed at God's Grace.

Figure B.3.6. Zack Bernstein installing native *Phragmites* at Jug Bay, 2015.

Figure B.3.7. Data recording at Jug Bay.
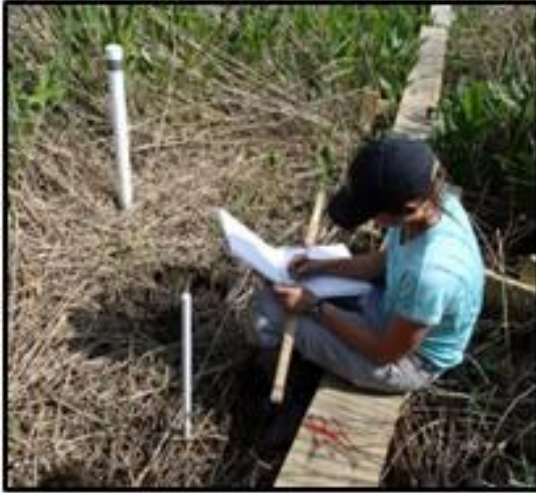
Figure B.3.8. 2016 aboveground biomass harvest at (a) Jug Bay and (b) Jefferson Patterson Park.

a.

b.

Figure B.3.9. Example of soil core samples from (a) Jug Bay, (b) God's Grace, and (c) Jefferson Patterson Park
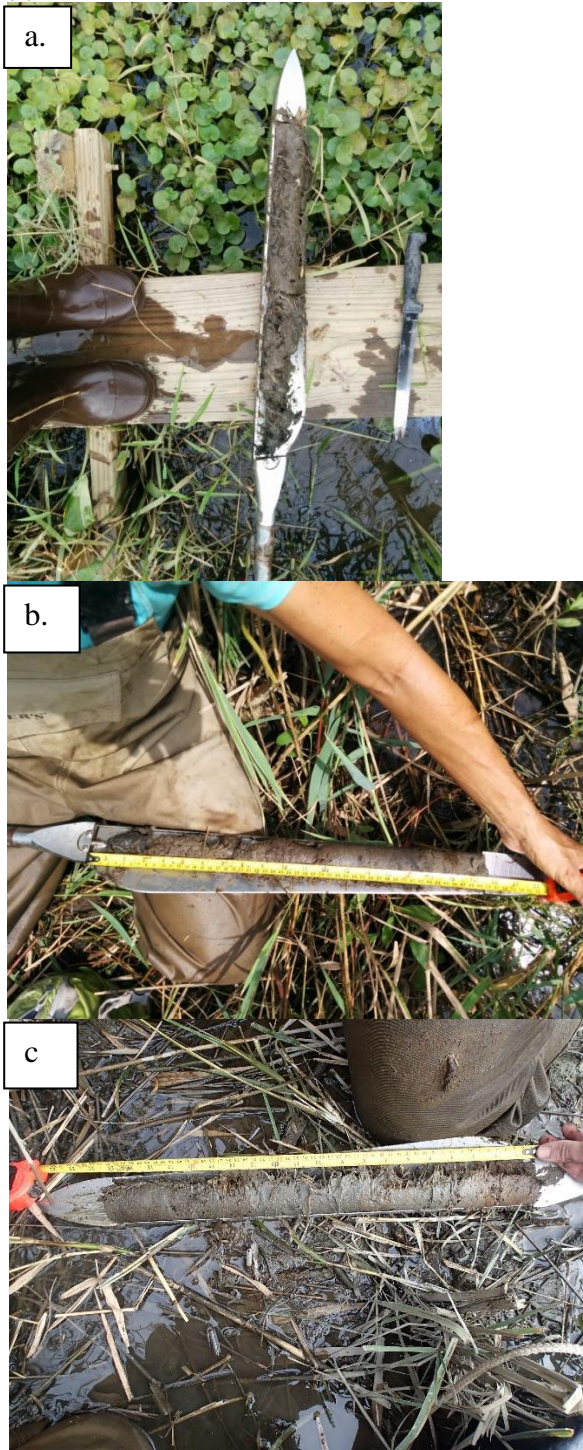
Appendix C. Demonstration and Outreach

In 2016, two demonstration sites were established on the Patuxent River in tidal fresh marsh areas. One at Jug Bay Wetland Sanctuary (approximate center point of demonstration area 38°46'53.8"N 76°42'25.3"W) (Figure C.4.1) adjacent to our field study site (see Chapter 3) and the other at Wooton's Landing Wetland Park, a restored area (approximate center point of demonstration area 38°51'22.0"N 76°41'26.4"W)(Figure C.4.2). Eurasian *P. australis* is dominant at each site enabling us to situate the native plants adjacent to the Eurasian. Each location was treated with glyphosate prior to installation. One hundred and fifty plants were installed in an area approximately 6m^2, 25 plants per m^2. Seeds for the plants were collected from a genetically confirmed native *Phragmites* stand on the Choptank River, Maryland (38°50'25.4"N 75°51'52.4"W). Seed heads were placed in cold storage at the University of Maryland greenhouse until ready for processing. Florets were hand stripped from inflorescence on January 6, 2015 and mailed to Environmental Concern the following day. Environmental Concern established plants from the seeds. Rhizomes were divided in the fall of 2015 and again in the winter of 2016. Plants were delivered to Jug Bay on April 28, 2017 and installed at the Jug Bay demonstration site on May 3, 2016 and at the Wooton's Landing site on June 9, 2016. Plants grew during the 2016 season however, in July, plants at Wooton's had not grown as vigorously as those at Jug Bay which may have been due to heavy shade (Fig. C.4.1.c and Fig.C.4.2.b). In November of 2017, no live plants were found at Wooton's Landing while plants at Jug Bay appeared healthy (Figs. C.4.1.d and C.4.2.c).

Distinguishing between the native and Eurasian forms is difficult however, several morphological features can be used to positively identify native *Phragmites* (Saltonstall et al. 2004). Specimen boxes were created using the inflorescence, stems, leaves, and ligules to illustrate some of the morphological differences between the two forms (Fig.C.4.3). Additional materials, such as identification cards, fact sheets, and maps of currently known locations, should be developed to ensure that native *Phragmites* is considered during land management efforts. Awareness about the existence of this native species and educational materials that elucidate the differences between the invasive form and the native would serve to protect a native species and improve our land management practices.

Figure C.4.1. Jug Bay Wetland Sanctuary demonstation site (approximate center point of demonstration site 38°46'53.8"N 76°42'25.3"W): (a) native *Phragmites* plants from Envrionmental Concern, (b) installation of native *Phragmites* on May 3, 2016 (pictured from left are Dr. Andrew Baldwin, Josh Gaimaro, Martina Gonzalez Mateau, and Zach Berry), (c) native *Phragmites* on June 13, 2016, and (d) native *Phragmites* on November 7, 2017.

Figure C.4.2.Wooton's Landing Wetland Park demonstation area (approximate center point of demonstration area 38°51'22.0"N 76°41'26.4"W): (a) installation of native *Phragmites* on June 9, 2016 (pictured from left are Lindsay Wood, Josh Gaimaro, and Zach Berry), (b) plantings in July 2016, and (c) native *Phragmites* demonstration site on November 7, 2017.

a.

b.

c.

Figure C.4.3. Photographs of specimen boxes used to illustrate morphological differences between the native and non-native *Phragmites australis* as seen in the (a) stems, (b) inflorescence, and (c) leaves. Specimen boxes used during extension programs.

Appendix D. Sample of SAS code used to analyze data collected on harvest day at the conclusion of the greenhouse experiment.

```
PROC MIXED data=ghharvestdatatran;
CLASS uniqid salinity elevation block;
MODEL col1 = salinity elevation salinity*elevation / ddfm=satterth outp=resids;
RANDOM block;
LSMEANS salinity elevation salinity*elevation / adjust=tukey diff=all cl;
BY variable;
ods listing;
ods output lsmeans=lsmean1;
ods listing exclude diffs; ods output diffs=diff1;
ods output tests3=stat2;
RUN;
%include '/folders/myfolders/PDMix800.sas';
%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);
QUIT;
/* check anova assumptions */
PROC SORT DATA=resids;
BY variable;
RUN;
PROC PLOT data=resids vpercent=50;
PLOT resid*pred/vref=0;
BY variable;
QUIT;
data resids;
set resids;
aresid=ABS(resid);
RUN;
PROC CORR SPEARMAN data=resids;
VAR aresid pred;
QUIT;
PROC PLOT data=resids vpercent=50;
PLOT resid*pred/vref=0;
BY variable;
RUN;
PROC UNIVARIATE data=resids plot normal;
VAR resid;
BY variable;
QUIT;
RUN;
PROC PRINT data=stat2;
QUIT;
ods graphics off;
quit;
```

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