Response of Baldcypress (*Taxodium distichum*) at Different Life Stages to Flooding and Salinity

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Baldcypress are dominant trees in forested coastal wetlands. As sea levels rise, it is important to increase our understanding of how increased salinity and flooding will affect forested coastal wetlands. This study combined field observations and a greenhouse experiment to examine the effects of salinity and flooding on baldcypress growth at different life stages: seedlings, young trees, and mature trees. 1) I conducted a greenhouse experiment with flooded and drought conditions and different salinity treatments looking at the growth of seedlings. 2) To assess the response of young trees I examined the diameter at breast height (DBH) and height of >9 year old trees that were planted in a restored wetland across salinity and flooding gradients. 3) I also conducted a regional survey of adult baldcypress growth using increment tree ring cores along a salinity gradient in the Albemarle Sound, North Carolina.

In the greenhouse experiment, I found that drought, saltwater, and sulfate had significant negative effects on height over an exposure period of 26 weeks, while drought and saltwater had a significant negative effect on root collar growth. Overall, I found that the presence of saltwater decreased height of baldcypress seedlings by 15% and decreased the diameter at root collar by 21% compared to the control, regardless of hydrology. Seedlings watered with saltwater showed a decrease in biomass of leaves (55%), stems (50%), and roots (71%) compared to seedlings in the control. For the young trees in a wetland restoration site, DBH and tree height were not significantly correlated with water depth. However, DBH and height were negatively correlated with chloride concentrations in soil solution. Height was also negatively correlated with ammonium and total dissolved nitrogen concentration in soil solution, which are known to increase due to increases with salinity. The increment tree ring cores showed that the average tree age for five sites ranged between 94 to 118 years old. I found a trend towards declining growth of adult trees with increasing salinity, but the lack of long-term environmental data and multiple possible stressors on adults trees (droughts, hurricanes, and fires to name a few) make it difficult to isolate the effects of salinity and flooding on adults tree growth. My results suggest that baldcypress trees in this region of North Carolina are more sensitive to increased salinity than to increase flooding. I found lines of evidence for decreased growth of baldcypress trees in response to salinity at the seedling, young adult, and adult stage. I did not find such support for the effects of flooding on growth at the young adult and adult stage. My results suggest that increases in saltwater incursion and sea level rise could lead to decreased growth and/or death of baldcypress trees, which are foundational species in forested coastal wetlands.

Response of Baldcypress (*Taxodium distichum*) at Different Life Stages to Flooding and Salinity

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RESPONSE OF BALDCYPRESS (*TAXODIUM DISTICHUM*) AT DIFFERENT LIFE STAGES TO FLOODING AND SALINITY

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TABLE OF CONTENTS

List of Tables…………………………………………………………………….. III

List of Figures……………………………………………………………………. IV

Chapter I: Introduction……………………………………………………….. 1

Figure…………………………………………………………………….. 5

References………………………………………………………………... 6

Chapter II: Effects of Hydrology, Salinity, and Sulfate on the Growth of

Baldcypress Seedlings……………………………………………… 9

Abstract………………………………………………………………….. 9

Introduction………………………………………………………………. 9

Methods…………………………………………………………………... 11

Statistical Analysis………………………………………………. 13

Results……………………………………………………………………. 13

Discussion………………………………………………………………... 15

Figures……………………………………………………………………. 18

Tables…………………………………………………………………….. 20

References……………………………………………………………….. 25

Chapter III: The Growth of Young Baldcypress across Flooding and Salinity

Gradients at a Wetland Restoration Site…………………………… 27

Abstract…………………………………………………………………... 27

Introduction………………………………………………………………. 28

Methods…………………………………………………………………... 31

Soil Solution Water Chemistry…………………………………. 32

Statistical Analysis………………………………………………. 33

Results……………………………………………………………… 33

Discussion………………………………………………………….. 34

Figures……………………………………………………………… 38

Tables………………………………………………………………. 45

References………………………………………………………….. 48

Chapter IV: Does Salinity Affect the Growth of Baldcypress Trees in a Large Brackish Estuary? A Survey of Adult Trees Along the Albemarle Sound, North Carolina………………………………………………………… 52

Abstract…………………………………………………………….. 52

Introduction………………………………………………………… 52

Methods…………………………………………………………….. 53

Statistical Analysis………………………………………………. 55

Results……………………………………………………………… 55

Discussion………………………………………………………….. 56

Figures……………………………………………………………… 59

Tables………………………………………………………………. 68

References………………………………………………………….. 70

Chapter V: Conclusion…………………………………………………. 73

Figure………………………………………………………………. 75

LIST OFTABLES

Chapter II

2.1 Recipe for artificial saltwater………………………………………………… 20

2.2 Mean water quality measurements………………………………………… 21

2.3 Summary of the mean diameter at root collar, mean height for the treatments for the start and the end of the experiment, and the percent growth. 22

2.4 Summary of repeated measures MANOVA for diameter at root collar and height of baldcypress seedlings…………………………………………… 23

2.5 Summary of ANOVA results of leaf, stem, and root biomass of baldcypress seedlings………………………………………………………………… 24

Chapter III

3.1 Summary of the sample size, minimum, mean, and maximum for diameter at breast height, height and water depth, stem density, total basal area and carbon accumulation (n = 253)……………………………………………….. 45

3.2 Summary of trees not large enough to measure (DBH < 1cm) (n = 14)……… 46

3.3 Summary of regression results for diameter at breast height and tree height of all tagged baldcypress trees (n = 253)………………………………………… 47

Chapter IV

4.1 Summary of the month in which the cores were collected and the mean water quality measurements taken at the site……………………………………. 68

4.2 For each site, the minimum, mean, maximum for core age of the 20 cores that were collected per site and the mean DBH, and total basal area for the 10 trees per site……………………………………………………………….. 69

LIST OF FIGURES

Chapter I

1.1 Diagram depicting the objectives………………………………………………. 5

Chapter II

2.1 Mean diameter at root collar (cm) for drought and saturated and mean
height (cm) for drought and saturated of the seedlings over 26 weeks……… 18

2.2 Mean biomass across all treatments for total biomass (leaves, stems, plus roots),
leaves, stems and roots…………………………………………………... 19

Chapter III

3.1 Map of Timberlake Observatory Wetland Restoration Site (TOWeR)……… 38

3.2 Height difference between baldcypress in flooded areas experiencing high
salinity compared to less frequently flooded areas that have not experienced
high salinity……………………………………………………………….. 40

3.3 Mean water depth, diameter at breast height, basal area, height, chloride,
nitrate, ammonium, and total dissolved nitrogen across sites………………… 42

3.4 Linear relationship between mean diameter at breast height and height versus
mean water depth from 2011-2013…………………………………………… 43

3.5 Linear relationship between mean diameter at breast height and height versus
mean chloride…………………………………………………………… 44

Chapter IV

4.1 Map of Albemarle Sound, North Carolina…………………………………… 59

4.2 Images of each coring site…………………………………….……….. 61

4.3 Mean diameter at breast height (DBH), water depth, core age and conductivity across the sites along the Albemarle Sound from west to east… 62

4.4 Coefficient of variance for mean core age across the sites along the Albemarle Sound from west to east……………………………………………………… 63

4.5 Total growth (mm/yr) for each decade for all five sites and the Palmer Drought Severity Index for the region……………………………………..… 64

4.6 Linear regression between mean salinity (ppt) and mean growth (mm/yr) per decade for each site…………………………………………………………….. 66

4.7 Linear regression between mean water depth (cm) and mean growth (mm/yr) for each tree………………………………………………………………….. 67

Chapter V

5.1 Diagram depicting the objectives…………………………………………… 75

Chapter I

Introduction

In the southeastern United States, swamp forests are important and unique ecosystems (Conner and Toliver 1990). Swamps and other wetlands provide crucial habitat for wildlife and plants (Balcombe et al. 2005, Yeager 1938, Zedler and Kercher 2005). Despite the recognition that wetlands are functionally and economically valuable (e.g. flood control, lessen effects of storms, source of food and recreation) the United States has lost about 50% of its wetlands since European settlement (Mitsch and Gosselink 2007). More recently, between 2004 and 2009, the US lost 256,320 ha of forested wetlands, 41% of this loss occurred in the southeastern states of North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Arkansas (Dahl et al. 2011).

While land-use change is a major driver of wetland losses, the combination of land-use change and sea-level rise will likely accelerate losses of forested wetlands in the low lying coastal areas of the southeast US. As sea levels rise, it is becoming increasingly important to understand how saltwater intrusion and increased flooding will affect forested wetlands along the Gulf of Mexico and Atlantic coasts (Chabreck 1972, Conner and Brody 1989, Conner and Toliver 1990, Dicke and Toliver 1990, Effler and Goyer 2006, Hoeppner et al. 2008, Krauss et al. 2007, Krauss et al. 2009, Kraus and Duberstein 2010, Pezeshki et al. 1990). An increase in salinity will have adverse effects on plant productivity and growth in forested wetlands (DeLaune et al. 1987, Doyle et al. 2007, McKee and Mendelssohn 1989, Pezeshki et al. 1990). If there is no recharge of freshwater, saltwater intrusion can lead to the death of tree species and the dieback of forests (Doyle et al. 2007). Prolonged flooding may also be harmful to many tree species, often leading to decreased productivity (Wallace et al. 1996, Effler and Goyer 2006).

One study at Caddoo Lake on the border of Texas and Louisiana, found that after 18 years of flooding, survival of mature trees was reduced by up to 50% (Keeland and Young 1997). The effects of flooding on trees depend on duration and timing; for examples during the growing seasons short-term flooding may have little impact on mature trees (Conner 1994). If flooding is prolonged, as is expected due to sea-level rise, the resulting death of tree species in forested wetlands can cause the transition from a forested wetland to a marsh or even open water (Krauss et al. 2007).

Droughts, storm surges, and strong weather fronts can increase salinity in swamp forest for short durations (Conner and Askew 1992, Krauss et al. 2007). Tree species have to tolerate this increase in salinity until rainfall or freshwater recharge. Species located in these areas may have higher tolerance to salinity, such that their growth and physiological processes decrease temporarily until freshwater returns (Krauss et al. 2007). Mature trees may be able to obtain fresh water with deeper roots or reduce the overall need for water transport through deeper sapwood, therefore allowing them to lessen stress due to salt (Krauss et al. 2009, Krauss and Duberstein 2010).

Tidal freshwater swamps have always dealt with periodic salinity; however, in recent years the extent and frequency of salinity in these systems has increased because of human activities such as dredging, dam construction, and other water diversions (Krauss et al. 2007). In freshwater swamps, sinking of the land (subsidence) has increased due to decreased delivery of freshwater, sediments, and nutrients (Effler and Goyer 2006). Canal dredging, has provided direct linear routes for saltwater to move into freshwater swamps (Effler and Goyer 2006). As sea levels rise, plants will be forced to migrate further inland (Moorhead and Brinson 1995);

however, they may not be able to move inland because of development, termed called coastal squeeze.

Baldcypress (*Taxodium distichum* (L.) Rich.) trees are foundational species, meaning that they have an impact on community dynamics, population, and ecosystem processes, native to the coastal plains in the southeastern United States (Ellison et al. 2005). Baldcypress trees are found from Delaware to Florida, along the Atlantic Coast, westward along the Gulf of Mexico from Florida to Texas, and north up the Mississippi River floodplain to the southern parts of Illinois and Indiana (Mattoon 1915, Shaler 1887). The majority of baldcypress (>90%) are located at elevations of 30.5m or less above sea level (Mattoon 1915). Baldcypress are known to be slow-growing and long-lived (Doyle et al. 2007, Mattoon 1915, Stahle et al. 1985). For example, baldcypress trees found along the Black River in North Carolina were found to be 1700 years old (Stahle et al. 1988). Many of the baldcypress stands along the southeastern coastal plain were harvested for timber and developed for agriculture in the 1700's (Richardson 1983). Up until the 1900's, baldcypress lumber was a stable commodity because of the workability and durability of the wood (Mattoon 1915). Some uses for the lumber were construction of houses, tanks (water storage), coffins and shingles (Mattoon 1915).

Because of their ecological and economic significance, there have been many studies on baldcypress and their high tolerance to salinity compared to other native species in the floodplains (Conner and Askew 1992, Conner 1994, Conner et al. 1997, DeLaune et al. 1987, Pezeshki et al. 1990). Along the Gulf Coast of Louisiana, saltwater intrusion is responsible for the extensive destruction of baldcypress in coastal swamp forests (Akumu et al. 2011, Pezeshki et al. 1990). Baldcypress has the greatest tolerance for flooding and salinity compared to other tree species in coastal regions in the southeast (Krauss et al. 2007). Studies have found that baldcypress growth was reduced when salinity concentrations were above 2 ppt, but tolerance of individual genotypes beyond normally stressful salinities exceeding 2 ppt has been described (Allen et al. 1994, Krauss et al. 2009).

Despite the large amount of research that has been conducted along the Gulf Coast on baldcypress trees, much less attention has been given to the trees in the estuarine coast of North Carolina. Because the estuarine coast of North Carolina is protected from the Atlantic Ocean by the Outer Banks, it does not experience lunar tides. The fact that baldcypress trees have developed for at least the last 300 years without experiencing regular flooding and salinity (Corbett et al. 2007) suggests that these trees might have different tolerances to flooding and salinity.

This study combined field observations and a greenhouse experiment to examine the effects of increased salinity and flooding on baldcypress growth at different life stages (seedlings, young trees, and mature trees) (Figure 1.1). To examine the effects of water level and salinity on seedlings, I conducted a greenhouse experiment with saturated and drought conditions and different salinity treatments for 26 weeks. To assess the response of young trees, I examined the diameter at breast height (DBH) and height of >9 year old trees that were planted in a restored wetland across flooding and salinity gradients. To examine the effects of salinity on mature trees, I also conducted a regional survey of baldcypress growth using increment tree ring cores along a salinity gradient in the Albemarle Sound, North Carolina. I expect adult trees at the eastern most sites to have lower mean growth than the trees at the western most sites, which experience less frequent saltwater incursion events.

Figure:

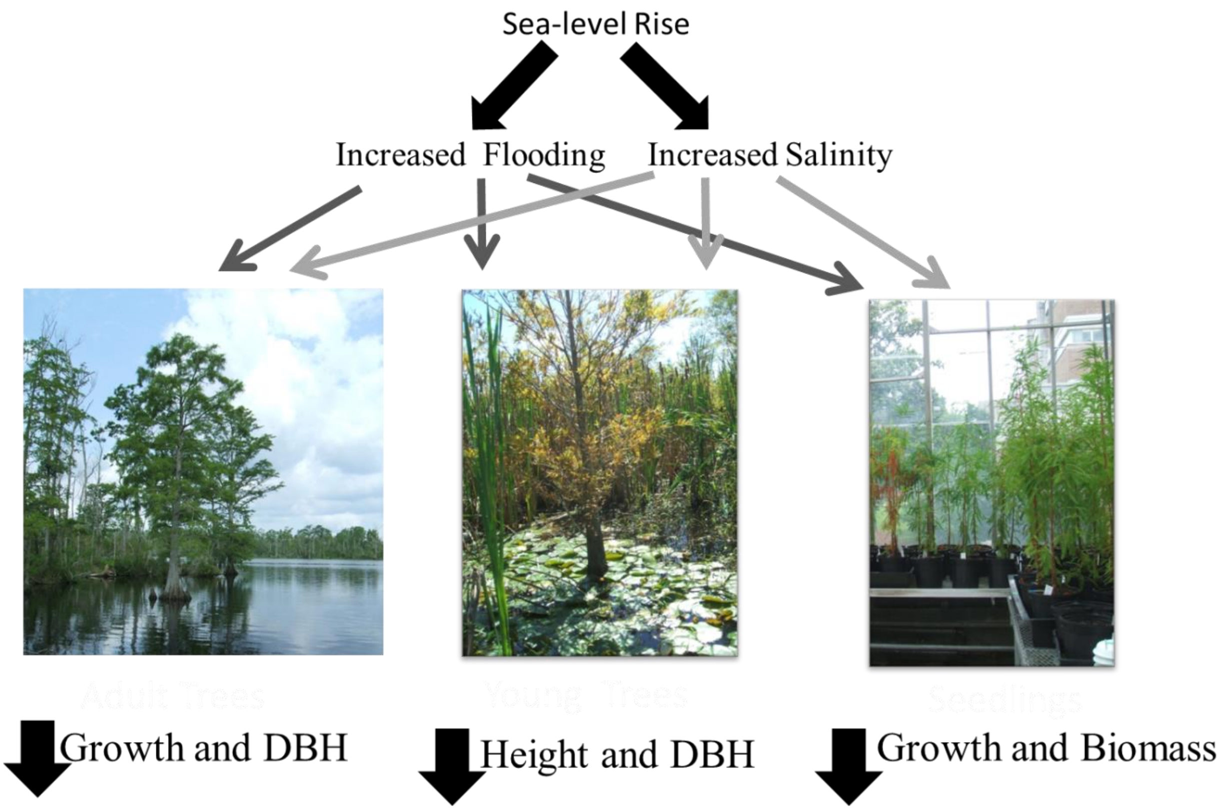

Figure 1.1: Looking at sea level rise, I was looking at increase in flooding as well as increase in salinity. I believe that increase flooding and salinity will decrease the growth and DBH (diameter at breast height) of adult trees. For young trees I believe that increase in flooding and salinity would decrease height and DBH. Finally, for seedlings I believe that flooding and salinity would decrease growth and biomass.

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Chapter II

Effects of Hydrology, Salinity, and Sulfate on the Growth of Baldcypress Seedlings

Abstract:

In many swamps of the southeastern United States baldcypress are dominant trees. Their growth has been shown to be affected by water and salinity. Both flooding and salinity decrease growth and survival of baldcypress seedlings, but the magnitude depends on the depth and duration of flooding and the level of salinity. The goal of this project was to examine the effects of elevated salinity and water saturation on the growth of baldcypress seedlings using a greenhouse experiment. I was also interested in isolating the effects of sulfate from other salt ions on baldcypress growth. One year old baldcypress seedlings were watered with freshwater and artificial saltwater (~5 parts per thousand, with and without sulfate) over 26 weeks, and I measured diameter at root collar and height weekly. At the end of the 26 weeks the seedlings were harvested for biomass. Overall, seedlings in the saturated control had a 25% greater diameter at root collar and height compared to the seedlings in the drought control. The presence of saltwater led to a 21% decline in diameter at root collar and 15% decline in height, regardless of hydrology. The presence of sulfate did not have a significant effect on diameter at root collar, but did cause a 6% decline in height. Salinity led to a 63% decline in total biomass, regardless of hydrology. Overall my results show that salinity and water availability are important determinants of seedling growth.

Introduction:

Baldcypress (*Taxodium distichum*) trees are dominant trees in the swamps of the Southeastern United States (Mattoon 1915). They have been seen as a symbol of these swamps and have been studied for many years (Brown and Montz 1986). Because baldcypress trees grow near rivers and other bodies of water, their growth and survival is known to be affected by water level

(Brown and Montz 1986). Baldcypress are often the dominant trees in coastal wetlands, in proximity to salt water bodies (Mattoon 1915, Shaler 1887); thus salinity has also been shown to affect their growth. Both climate change and sea level rise will alter water level and salinity (Conner et al. 1997); such that it is important to understand their individual and combined effects. Considerable research has focused on the effects of flooding and salinity on baldcypress, but less research has been conducted on their combination, especially related to effects on baldcypress growth and survival (Allen et al. 1996).

Baldcypress seedlings have been known to tolerate moderate flooding, but their growth is decreased with deep or prolonged flooding (Conner and Brody 1989). Baldcypress seedlings are able to withstand flooding early by slowing down their growth, but resume growth once flooding recedes. An example, for several weeks no measureable growth occurred while baldcypress seedlings were living under water in pots in an outdoor pool; however, upon emerging from the pool growth continued (Welch 1931). It has been proposed that baldcypress survive the effects of initial flooding due to aerenchyma, which forms rapidly during early life stages (Kludze et al. 1993). Thus, experiencing flooding at early stages may help baldcypress tolerate flooding later as older saplings and trees (Kludze et al. 1993). Species adapted for flood conditions were able to tolerate lower levels of salinity stress better than species less tolerant of flooding (Allen et al. 1996, Krauss and Duberstein 2010).

Similar to flooding, salinity stress can cause decreases in growth and survival (Chapin 1991). In greenhouse experiments and in the field, baldcypress can survive with salinity of up to 3 – 4 ppt, but exhibit decreased growth (Allen et al. 1994, Krauss et al. 2007). In a greenhouse experiment, seedlings were germinated from seeds collected from southern Louisiana and Mobile Bay, Alabama; as salinity levels increased, growth of seedlings decreased along with overall survival (Allen et al. 1994). In another experiment in Louisiana, seedlings were watered with salt solution in a greenhouse experiment which led to decreased growth rates late in the growing season (Stiller 2009).

Much of the research on the effects of increased salinity on baldcypress growth and survival has focused on osmotic stress caused by increased salinity. Increased salinity can also cause increases in hydrogen sulfide (from the microbial use of sulfate in saltwater) which is known to be toxic to plant roots (Lamers et al. 2013). Plants, such as *Festuca rubra* and *Puccinellia maritime*, have reduced growth due to soluble sulfide (Ingold and Havill 1984). Sulfide combining with transition metal ions can lead to a reduction of essential elements (such as manganese, copper, iron, and zinc) in root cells (Havill et al. 1985). It is unclear how sulfide toxicity affects baldcypress in wetlands.

The goal of this project was to examine the effects of elevated salinity and saturation on the growth of baldcypress seedlings using a greenhouse experiment. Furthermore, I was interested in examining the role of sulfate versus other salt ions on baldcypress growth. I predicted that: 1) seedlings in saturated conditions would have a higher growth rate than seedlings in drought conditions; 2) seedlings not treated with salinity and/or sulfate would have the highest growth rate and biomass; 3) the combination of drought and increased salinity would produce the lowest biomass and growth rate; 4) seedlings watered with sulfate would have a lower root biomass compared to the control because of the toxicity of sulfate to plant roots.

Methods:

One-year-old, dormant, bare root seedlings (North Carolina Forestry Service, New Bern) were potted using Fafard 3B (Agawam, MA) soil in early December 2012 and kept at the East Carolina University Department of Biology greenhouse for the duration of the experiment

(35°36´18.7236, 77°21´49.6182). One seedling was planted per pot (600 cm, 1 ½ gallon) and watered with 1 L of fresh water twice a week until February 25th, 2013. During the experiment the lowest average monthly temperature in the greenhouse was 23°C (February) and the highest was 27°C (July).

Leaf-out began on December 31st, 2012, treatments began on February 25th, 2013 and collection for biomass began on August 22nd, 2013, for a total of 26 weeks of treatments and measurements. There were two hydrological treatments, drought and saturated, with 32 seedlings in each treatment. Seedlings in the drought treatment were watered twice a week with 500 mL of the appropriate water chemistry treatment. The seedlings in the saturated treatment were placed in trays that were filled with approximately 7.5 cm of freshwater with all eight pots in a treatment being in the same tray; the water level was maintained throughout the experiment. Within the hydrological treatments there were four different water chemistry treatments: 1) freshwater as a control, 2) only sulfate (SO_4^{2-}), 3) artificial saltwater without sulfate (SO_4^{2-}), and 4) artificial saltwater (target 5 ppt) (as in Ardón et al. 2013), with eight seedlings in each water chemistry treatment. All seedlings were watered from the top twice a week, once with 500 mL of the appropriate treatment and once with 500 mL of freshwater, for the first seven weeks. Starting at week eight, seedlings were watered twice a week, once with 500 mL of freshwater and with 500 mL of the appropriate treatment or 500 mL of freshwater on alternating weeks. Controls were watered twice a week with 500 mL of freshwater, for all 26 weeks. Artificial saltwater was made with a modified version from Kester et al. (1967) (Table 2.1). For every one liter of full-strength saltwater (~30 ppt), six liters of deionized water were added in order to get the target concentration (or 5 ppt). Water conductivity for the treatments was measured prior to watering (YSI Model 556, Yellow Spring, OH, USA; Table 2.2). Height, diameter at root collar (here-after “diameter”), and general health of the seedlings were recorded once a week. At the end of the experiment, final heights and root collar measurements were taken and seedlings were divided into leaves, stems and roots which were dried at 70° C for 48 hours in order to collect biomass data.

Statistical Analyses:

Root collar and height data, which were log transformed, were analyzed using repeated measures multivariate analysis of variance (MANOVA), and the difference between means was considered significant when p-value < 0.05 (version 10, JMP Software, Cary, NC, USA). Factors in the MANOVA were: date (26 weeks), hydrology (saturated and drought), sulfate (present and absent), salt (present and absent) and all interactions. Data was transformed to test for normality and equal variance.

Biomass data, which were log transformed, were analyzed using ANOVA (JMP 10). Factors in the ANOVA were: date (26 weeks), hydrology (saturated and drought), sulfate (present and absent) salt (present and absent) and their interactions. Tukey-Kramer comparisons of means tests were calculated to obtain pair-wise comparisons for any mean biomass that differed across treatments and all interactions (JMP 10).

Results:

Overall, seedlings in the drought treatments had a lower diameter (mean = 1.36 ± 0.21 SD cm) and height (mean = 86.85 ± 14.43 SD cm) compared to the seedlings in the saturated treatments (diameter mean = 1.58 ± 0.34 SD cm and height 105.84 ± 15.26 SD cm). For diameter, seedlings in the drought and saltwater present treatments had the lowest percent growth on average (39.39% and 40.43%, respectively, Table 2.3). Seedlings in the saturated and saltwater absent treatments had the highest percent growth for the average diameter (69.89% and 77.17%, respectively, Table 2.3). For average height, seedlings in the drought and saltwater present treatments had the lowest percent growth (20.59% and 29.93%, respectively, Table 2.3). Seedlings in the saltwater absent and saturated treatments had the highest average height percent growth (43.88% and 53.84%, respectively, Table 2.3). By week seven seedlings that were watered with saltwater without sulfate and saltwater started dying. By week nine, the majority of their leaves were dying and some of the seedlings started putting on new growth and continued to put on new growth for the duration of the experiment.

The saturated treatment had a significant positive effect on diameter ($p = 0.0043$, Figure 2.1B) and height ($p < 0.0001$, Figure 2.1D). The presence of saltwater had a significant negative effect on diameter ($p = 0.0014$, Figure 2.1A and 2.1B) and height ($p = 0.0412$, Figure 2.1C and 2.1D), regardless of hydrology. The presence of sulfate did not have a significant effect on diameter ($p = 0.6489$, Figure 2.1A and 2.1B). However, the presence of sulfate did have a significant negative effect on height ($p = 0.0360$, Figure 2.1C and 2.1D). For diameter, hydrology ($p= 0.0043$), saltwater ($p= 0.0014$), date ($p < 0.0001$) date × hydrology ($p = 0.0007$), date × saltwater ($p < 0.0001$), date × hydrology × saltwater ($p = 0.0346$) were all significant (Table 2.4). When looking at height, hydrology ($p < 0.0001$), saltwater ($p = 0.0412$), sulfate ($p = 0.0360$), date ($p = 0.0026$), date × hydrology ($p < 0.0091$) were all significant (Table 2.4).

For the biomass of the seedlings, saltwater ($p = 0.0415$), hydrology × saltwater ($p = 0.0007$), and hydrology × saltwater × sulfate ($p = 0.0204$) had a significant on leaf biomass (Table 2.5). Hydrology ($p < 0.0001$), saltwater ($p = 0.0450$), hydrology × saltwater ($p < 0.0001$), hydrology × sulfate ($p = 0.0010$), and hydrology × saltwater × sulfate ($p = 0.0042$) had a significant effect on stem biomass (Table 2.5). Root biomass was reduced by saltwater ($p = 0.0021$, Table 2.5).

Regardless of hydrology (saturated or drought), seedlings treated with saltwater had an average lower total biomass (63%, Figure 2.2A), biomass of leaves (55%, Figures 2.2B), stems (50%, Figure 2.2C), and roots (71%, Figure 2.2D), compared to seedlings in the control treatment. Saturated control was significantly different from all treatments for total biomass (Figure 2.2A), leaf (Figure 2.2B), and stem (Figure 2.2C). For root biomass saturated control was significantly similar to saturated sulfate (Figure 2.2D). By the end of the experiment, 8 of the 64 seedlings died, all from saltwater treatments: drought saltwater (2), drought saltwater without SO_4^{2-} (1), saturated saltwater (3), and saturated saltwater without SO_4^{2-} (2).

Discussion:

Flooding affected both the diameter and height. Trees in saturated condition had a larger diameter and greater height than seedlings in drought treatment. Seedlings in the drought control and drought sulfate only treatments had lower leaf biomass by 41% and 19%, respectively, compared to those growing in saturated conditions. Similar to the present experiment, baldcypress seedlings were found to have a greater diameter in the flooded treatments than that of the unflooded treatments (Conner 1994, Shanklin and Kozloski 1985). After 8 weeks Mattoon (1916) found that baldcypress seedlings growing in saturated conditions grow twice as much as than seedlings under drought conditions. I found that seedlings in the saturated treatments had a greater root biomass than seedlings in the drought treatments. Unlike my results, Kludze et al. (1993) found that dry root weight for plants in drought treatment were 1.5 times more than roots from flooded plants.

Saltwater had a negative effect on diameter and height. Compared to seedlings in the control treatment, seedlings watered with saltwater had on average a 21% smaller root collar and 15% shorter height. Seedlings watered with saltwater compared to seedlings watered with sulfate had on average a 13% smaller root collar and an 8% shorter height. Allen et al. (1994) found that, seedlings flooding with salinity had a reduced height compared to seedlings flooded with freshwater. I found that saltwater (target of concentration of ~5 ppt) had a negative effect on the diameter, height, and biomass of the leaves, stems, and roots of 1 year old seedlings. Conner et al. (1997) found that for both treatments (flooded and watered) baldcypress had a significant reduction in height when watered with 2 ppt saltwater and 10 ppt saltwater. Conner et al. (1997) also found that baldcypress was not significantly affected when watered with or flooded with 2 ppt saltwater, but had a significant reduction in diameter when watered with or flooded with 10 ppt saltwater.

Seedlings watered with saltwater had a significantly lower total biomass compared with the other treatments, due to the majority of their leaves senescing and having smaller diameters at root collar and heights. Conner (1994) found a slight decline in biomass values for roots and shoots as salinity levels increased. I found that saturated seedlings had three times the average total biomass compared to plants that were saturated and watered with saltwater. In another study, plants that were flooded with addition of salt, had an 8% reduction in biomass when compared with flooding alone (Pezeshki 1992).

The saturated baldcypress control seedlings in saltwater had about a 36% growth increase for diameter and height over seedlings in the drought saltwater treatment. These results were similar to Stiller (2009), who found that control plants had about twice the growth rate when compared to salt and drought treatments. In drought conditions, a hormonal signal from roots causes leaf growth to reduce (Chapin 1991). Moderate drought affected dry mass of stem and leaves in baldcypress while severe drought significantly reduced stem and leaf dry mass (Nash and Graves 1993).

Sulfate affected the height but not the diameter. Saturated sulfate seedlings had the second highest height and diameter, behind saturated control seedlings. I found that watering with a treatment of sulfate solution had a significant negative affect on height of baldcypress seedlings, but was not toxic to the seedlings. Contrary to my results, seedlings fumigated with sulfate had no change in rate of growth in height (Shanklin and Kozlowski 1985). The difference between fumigation and watering directly with sulfate solution may account for the effect on height. However, more research should be conducted to see at what levels sulfate maybe harmful for the growth and/or survival of baldcypress seedlings. Sulfate did not significantly affect the biomass of the leaves, stems, or roots.

I found that, once the majority of the leaves had died, the seedlings in the saltwater treatments would put on new growth multiple times before the experiment was terminated. Similar to the present study, baldcypress seedlings have been found to respond to saltwater flooding by dieback and then re-sprouting (Conner and Askew 1992). Van der Moezel et al. (1988) found in plant species more tolerant of salinity, the older leaves experienced necrosis and senescence before the younger leaves. This was true for the current study as well; older leaves showed signs of necrosis and senescence prior to younger leaves.

With the possible increase in saltwater incursion into freshwater swamps, baldcypress seedlings will most likely start dying off and those that continue to grow will be smaller than baldcypress trees growing in fresh water. This information will be important to land managers and areas that are being considered for restored wetlands. Baldcypress are moderately tolerant to salinity; however, they should not be planted in areas that experience brackish water (Brown and Montz 1986). Depending on the saturation, saltwater and sulfate levels will determine on if baldcypress seedlings will be able to grow and survive in these areas long term.

Figures:

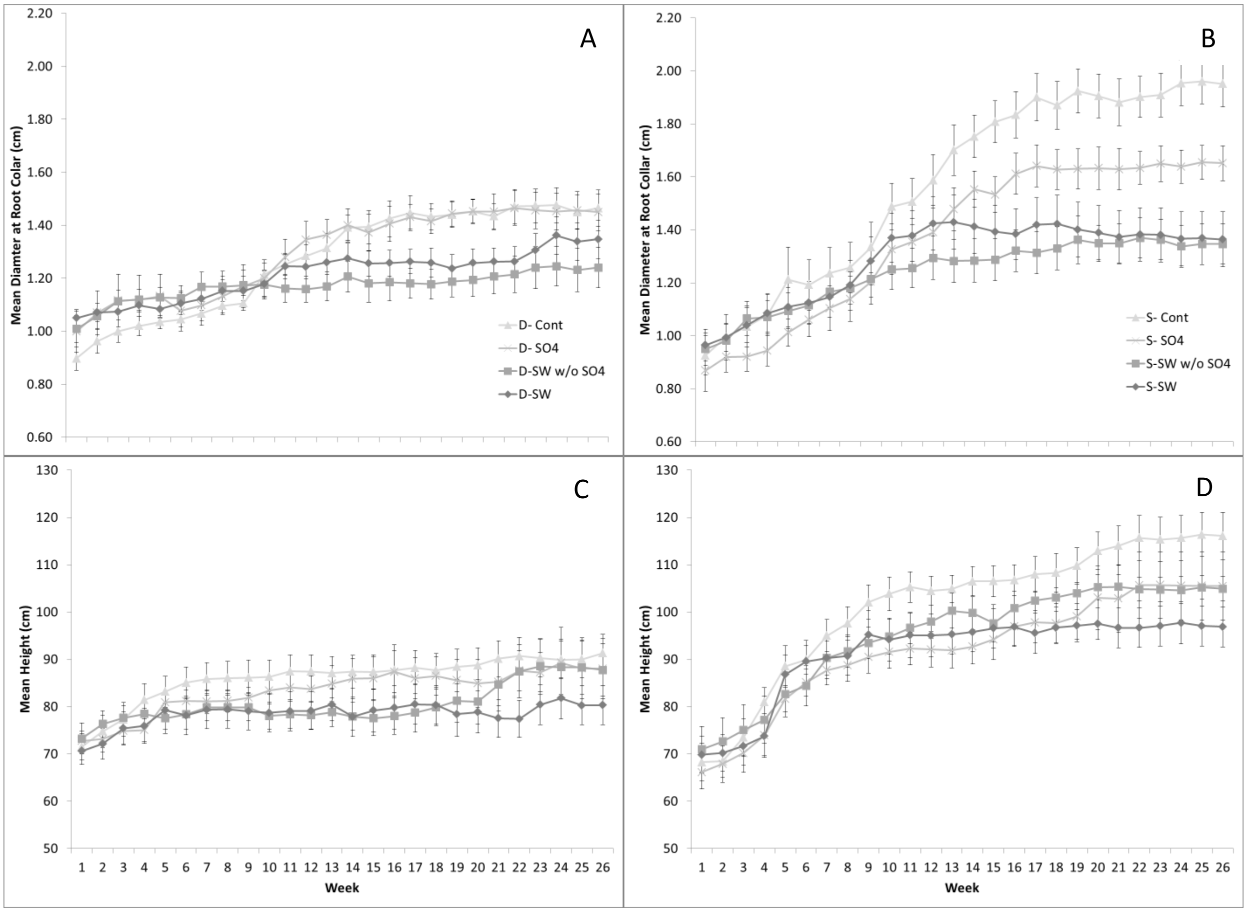

Figure 2.1: Mean diameter at root collar (cm) for drought (A) and saturated (B) and mean height (cm) for drought (C) and saturated (D) of the seedlings over 26 weeks. The lines represent the different treatments (control, sulfate only, saltwater without sulfate, and saltwater) (n = 8, error bars = ± 1 standard error).

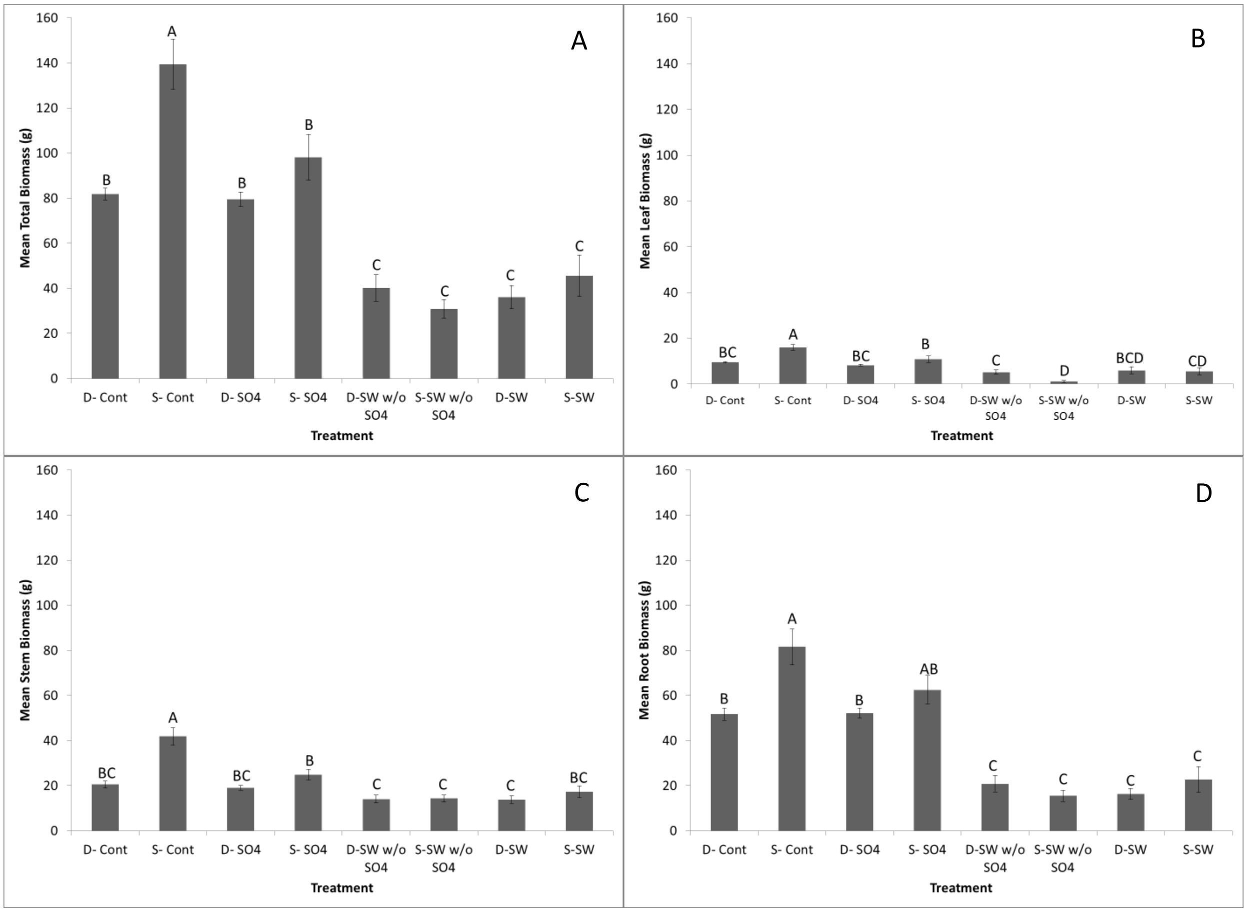

Figure 2.2: Mean biomass across all treatments for total biomass (leaves, stems, plus roots) (A), leaves (B), stems (C) and roots (D). Treatments that share the same letter were not significantly different from each other. D – Drought, S – Saturated, Cont. – Control, SO_4 – Sulfate, SW w/o SO_4 – Saltwater without sulfate, and SW - Saltwater (n = 8, error bars = ± 1 standard error).

Tables:

Table 2.1: Recipe for artificial saltwater for a 1 L solution.

	Unit of Measurement	Artificial Seawater	Artificial Seawater without Sulfate	Sulfate Only
NaCl	g	23.944	21.254	0
K_2SO_4	g	4.011	0	4.011
KCl	g	0.679	4.111	0
$NaHCO_3$	g	0.198	4.065	0
KBr	g	0.010	0.010	0
H_3BO_3	g	0.028	0.028	0
NaF	g	0.003	0.003	0
$MgCl_2$-$6H_2O$**	mL	57.053	57.053	0
$CaCl_2$-$2H_2O$**	mL	11.208	11.208	0
$SrCl_2$-$6H_2O$**	mL	0.912	0.912	0

**Recipe to make

	Deionized Water	Amount (g)
$MgCl_2$-$6H_2O$	250 mL	50.83
$CaCl_2$-$2H_2O$	100 mL	14.7
$SrCl_2$-$6H_2O$	25 mL	0.667

Table 2.2: Mean water quality measurements for the treatments (n=10).

	Control	Sulfate	Saltwater without Sulfate	Saltwater
Temperature (°C)	22.1	21.2	21.1	21.0
Conductivity (mS/cm)	0.2	1.0	9.1	9.4
% Dissolved Oxygen	69.8	68.9	66.7	67.1
Dissolved Oxygen	6.0	6.1	12.5	5.8
pH	8.1	8.0	8.1	7.8
ORP	229.5	124.3	122.8	139.4

Table 2.3: Summary of the mean diameter at root collar (cm) and mean height for the treatments for the start (week 1) and the end (week 26) of the experiment (n=8). % growth represents the % change in the diameter at root collar and height for each treatment from the start of the experiment to the end.

		Drought	Saturated	SW Present	SW Absent	SO_4 Present	SO_4 Absent
Diameter at Root Collar (cm)	Start	0.99	0.93	0.94	0.92	0.97	0.95
	End	1.38	1.58	1.32	1.63	1.45	1.50
	% Growth	39.39	69.89	40.43	77.17	49.48	57.89
Height (cm)	Start	72.02	68.80	71.17	69.66	69.83	69.78
	End	86.85	105.84	92.47	100.23	92.68	100.16
	% Growth	20.59	53.84	29.93	43.88	32.72	43.54

Table 2.4: Summary of repeated measures MANOVA for log transformed diameter at root collar and height of 64 baldcypress seedlings over 26 weeks looking at the different treatments. Factors were hydrology (drought or saturated), saltwater (present or absent), and sulfate (present or absent).

	Diameter at Root Collar (cm)				Height (cm)			
	NumDF	DenDF	F	P-Value	NumDF	DenDF	F	P-Value
Between Subjects								
Hydrology	1	56	8.88	**0.0043**	1	56	29.87	**<0.0001**
Saltwater	1	56	11.36	**0.0014**	1	56	4.36	**0.0412**
Sulfate	1	56	0.21	0.6489	1	56	4.62	**0.0360**
Hydrology * Saltwater	1	56	1.71	0.1958	1	56	0.32	0.5711
Hydrology * Sulfate	1	56	1.51	0.2249	1	56	0.49	0.4853
Saltwater * Sulfate	1	56	2.19	0.1450	1	56	0.82	0.3699
Hydrology * Saltwater * Sulfate	1	56	1.75	0.1914	1	56	0.12	0.7318
Within Subjects								
Date	25	32	9.10	**<0.0001**	25	32	2.89	**0.0026**
Date * Hydrology	25	32	3.35	**0.0007**	25	32	2.44	**0.0091**
Date * Saltwater	25	32	4.92	**<0.0001**	25	32	1.49	0.1413
Date * Sulfate	25	32	1.46	0.1560	25	32	1.36	0.2032
Date * Hydrology * Saltwater	25	32	1.98	**0.0348**	25	32	0.83	0.6835
Date * Hydrology * Sulfate	25	32	0.60	0.9071	25	32	0.70	0.8181
Date * Saltwater * Sulfate	25	32	1.29	0.2442	25	32	0.78	0.7412
Date * Hydrology * Saltwater * Sulfate	25	32	0.64	0.8694	25	32	0.73	0.7833

Table 2.5: Summary of ANOVA results of leaf, stem, and root biomass of 64 baldcypress seedlings. Factors were hydrology (drought or saturated), saltwater (present or absent), and sulfate (present or absent).

	Leaves				Stems				Roots			
	DF	Sum of Squares	F Ratio	P-Value	DF	Sum of Squares	F Ratio	P-Value	DF	Sum of Squares	F Ratio	P-Value
Hydrology	1	1.00	0.91	0.3440	1	1810.63	45.76	**<0.0001**	1	0.74	2.71	0.1051
Saltwater	1	4.78	4.36	**0.0415**	1	166.40	4.21	**0.0450**	1	4.62	16.91	**0.0021**
Sulfate	1	0.09	0.08	0.7770	1	9.04	0.23	0.6346	1	0.00	0.00	0.9636
Hydrology * Saltwater	1	14.15	12.88	**0.0007**	1	887.30	22.42	**<0.0001**	1	0.88	3.21	0.0784
Hydrology * Sulfate	1	0.14	0.13	0.7032	1	481.08	12.16	**0.0010**	1	0.15	0.55	0.6136
Saltwater * Sulfate	1	0.16	0.15	0.7245	1	2.62	0.07	0.7950	1	0.07	0.26	0.4626
Hydrology * Saltwater * Sulfate	1	6.25	5.69	**0.0204**	1	353.35	8.93	**0.0042**	1	0.40	1.47	0.2311

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Chapter III

The Growth of Young Baldcypress across Flooding and Salinity Gradients at a Wetland Restoration Site

Abstract:

Wetland restoration allows for the recovery of important ecosystem services, such as habitat for wetland species, flood protection, and carbon sequestration. Baldcypress trees, *Taxodium distichum*, are considered foundational species in southeastern coastal plain swamps. Many factors influence how baldcypress grows and survives including the depth and duration of flooding and salinity. At a wetland restoration site in eastern North Carolina I examined the growth of 9 year old baldcypress trees by measuring tree height and diameter at breast height (DBH) along salinity, nutrient, and flooding gradients. This site receives nutrient runoff from an upstream farm on one end and experiences saltwater incursion during drought years. I examined how flooding, nutrients, and/or salinity affect the growth of baldcypress trees since being planted in 2005. Thirteen plots were established and 253 trees were measured. Across the plots, DBH (1.00 to 14.35 cm), tree height (1.6 to > 5.03 m) and average water depth (0 to 78 cm) varied. I found that chloride concentrations in soil solution had a significant negative effect on DBH and height; water depth did not have an effect on DBH or height. Because ammonium and total dissolved nitrogen increase with salinity, these nutrients had a significant negative effect on height, but not on DBH. My results show that restoration practitioners should consider the possibility of saltwater incursion when planning forested wetland restoration sites in the coastal plain. Furthermore, this study provides a baseline to allow the collection of long term data on the growth of baldcypress trees at a wetland restoration site.

Introduction:

Wetland restoration is an important mechanism for the recovery of ecosystem services lost when wetlands are degraded, such as habitat for water-dependent fauna, flood protection, and carbon sequestration. Much of the success of restoration depends on the establishment of wetland vegetation (Middleton 2003), which has fairly exacting requirements associated with elevation, hydrologic and salinity regimes. Thus, changes in hydrology due to climate change (incl. sea-level rise) and human activities will affect the establishment of vegetation in restored wetlands.

Baldcypress trees, *Taxodium distichum*, are foundational, they have an impact on community dynamics, population, and ecosystem processes species in southeastern coastal plain swamps (Ellison et al. 2005), and are often used in wetland restoration projects there. Despite much research on the effects of flooding, salinity, and nutrients on growth of baldcypress trees in the Gulf coast states, not much work has been conducted in other places or along gradients within field settings. In the Gulf coast states baldcypress trees growing close to the coast experience lunar tides, thus they are exposed to regular changes in flooding and salinity (Chabreck 1972, Conner and Brody 1989, Conner and Toliver 1990, Dicke and Toliver 1990, Effler and Goyer 2006, Hoeppner et al. 2008, Pezeshki et al. 1990). In other locations like the estuarine coast of North Carolina, baldcypress trees might not experience regular flooding or increases in salinity, due to the lack of lunar tides (Corbett et al. 2007). Both human activities and sea-level rise are likely to alter the frequency and duration of flooding and exposure to salinity in the North Carolina estuarine shoreline. It is unclear how baldcypress trees might respond to seasonal or episodic increased flooding or salinity. It is unlikely that wetland restoration sites will develop into mature baldcypress swamps if important components such as hydrology and nutrient availability are not properly recovered (Middleton 2003).

The depth and duration of flooding can determine how well mature baldcypress trees grow and survive (McKnight et al. 1980). Some symptoms of flooding stress in baldcypress include branch dieback, decreased respiration, lack of fruiting, decreased shoot growth, and vulnerability to predators and pathogens (Broadfoot and Williston 1973). Stress and mortality of mature trees increased with increasing water depth in the Oklawaha River Valley (north central Florida, Lugo and Brown 1984) and the lower Mississippi River Valley (Broadfoot and Williston 1973). At Lake Oklawaha in north central Florida, there was little to no effect on baldcypress tree growth and survival in water that was less than 0.6 m deep (Harms et al. 1980). However, there was an increase in mortality when water was >1 m deep (Lugo and Brown 1984). Studies in Tennessee and Arkansas suggested that water depths of 1.5-2 m were optimal for the growth and survival of baldcypress (Demaree 1932).

Swamp forest species may be exposed to periods of high salinity due to droughts, storm surges, and weather fronts (Krauss et al. 2007). In the southeastern United States, baldcypress has the greatest flood and salinity tolerance compared to other tidal swamp tree species (Krauss et al. 2007). Under controlled settings, baldcypress can tolerate being inundated with salinity more than 50% of the year if salinity is < 2-3 ppt (Brown and Montz 1986). If salinity levels are higher than 2-3 ppt for 50% of the year, baldcypress will be severely stressed, but can survive for a period of time until eventual death or hydrological remediation (Brown and Montz 1986, Wicker et al. 1981). In coastal Louisiana, South Carolina and Georgia, stand height and basal area decreased as salinity increased (Krauss et al. 2009). Furthermore, sites harboring mean porewater salinity concentrations at > 2 ppt are actively converting to marsh along multiple coasts in the Southeast (Krauss et al 2009), including North Carolina (Hackney et al. 2007).

Flooding of the soil can cause anoxic conditions and limit root respiration (Bowers 1981, Hosner 1960). During periods of no rainfall, oxygen becomes depleted where water is impounded, but spring and early summer rain showers can completely recharge shallow-water impoundments with oxygen (Broadfoot 1967). Manganese, nitrites, ferrous iron, ammonium, and sulfides can accumulate to toxic concentrations in submerged soils (Broadfoot and Williston 1973, Lugo and Brown 1984, Robinson 1930). Submerged soils can start to have a foul order and hydrogen sulfide accumulates after 24 hours of flooding (Robinson 1930). High concentrations of hydrogen sulfide are poisonous to plants (Lamers et al. 2013). In a tidal freshwater swamp in the Cape Fear River, North Carolina, USA, sulfate concentrations were higher during the summer and lower during the winter; this may have important consequences for plants that have a lower tolerance for sulfide (Hackney et al. 2007). Increased flooding with high salinity water can lead to the accumulation of sulfide, which can be toxic to plants. It is unclear how drought-induced saltwater incursion could affect the growth of baldcypress trees on wetland restoration sites.

My objective was to examine the growth of 9-year old baldcypress trees planted in a restored wetland in eastern North Carolina along a salinity, nutrient, and flooding gradient. This site receives high nutrient runoff from an upstream farm and has experienced saltwater incursion during drought years (Ardón et al. 2010, 2013). Because trees were all planted in 2005, this site provides an opportunity to examine how flooding, nutrients, and/or salinity affect the growth of baldcypress trees. This study also aimed to establish a baseline to facilitate the monitoring of trees for long-term research.

Methods:

The Timberlake Observatory for Wetland Restoration (TOWeR) site is located in Tyrrell County, NC,USA about five miles east of Columbia (35°54´32.835″, 76°9´36.054″) (Figure 3.1). This area was formerly pocosins (freshwater wetlands) until it was logged extensively in the mid-1900s, then later cleared for agriculture production in the 1970s and 1980s (Carter 1975, Richardson 1983). When the land was converted to corn and soybean agricultural fields, it was drained in order to be suitable for the crops, leaving many drainage ditches and canals throughout the area (Carter 1975). In 2004 restoration at the TOWeR site began with land movement; and filling ditches to restore pre-agriculture state (Ardón et al. 2010). In 2005, 750,000 seedlings from eight different tree species were planted throughout the site, of which about 60% were baldcypress seedling. Currently, agricultural fields to the south of the site drain into the restored wetland, providing seasonal inputs of water containing high nitrogen levels (Ardón et al. 2010).

Since the seedlings were planted, the site has been subjected to flooding and saltwater incursion, creating salinity and flooding gradients (Ardón et al. 2013). Water flow at the site depends on precipitation and wind direction and can flow downstream or upstream (Ardón et al. 2010). Water level across the site has increased since the initial flooding in 2007 (Ardón et al. 2013). Water flows from the south side of the site to the north side. During periods of drought, saltwater has entered the site from the north, causing salinity to increase from 0 to a maximum of 6 ppt (Ardón et al. 2013). Within sites water depth varied from 3.5 to 31.7 cm from 2011-2012.

I conducted an inventory of baldcypress trees in 13 plots across five transects in January 2013 (Figure 3.1B). Figure 3.2 shows the height differences between two representative baldcypress trees that were planted at the same time. The tree on the left was located in an area that was permanently flooded and experienced salinity while the other was periodically flooded with lower water levels and has not experienced salinity. Each plot contained a central sampling point. Using the sampling point as the center, circular plots with a diameter of 20 m were established (area = 314 m^2; 0.03 ha). Within the circular plots, all baldcypress trees that had a diameter at breast height (DBH) greater than 1 cm at 1.3 m were tagged and measured (height and DBH). Plots averaged 18.9 trees (± 6.4 SD), with a range of 10-27 trees among the 13 plots. Trees that had a DBH larger than 5 cm had a nail and tag placed at 1.4 m, on the south side of the tree. A height of 1.4 m was selected to mark the DBH assessment point 10 cm lower without obscuring the measurement. All other trees (DBH greater than 1 cm, but less than 5 cm) have a tag tied around the trunk with green grafting tape. For baldcypress that were smaller than 1 cm at 1.3 m, only height was recorded. For trees that were greater than 5.03 m, true height was recorded as greater than 5.03 m. However, by the end of the study, only 33 trees out of a total of 253 measured exceeded this height, thus limiting underestimation of tree heights by plot. Standing water depth was also measured at each tree in January 2013. In addition, I used multi-year records (2011-2013) of water depth that had been recorded every 15 minutes (Solinist Levelogger Gold, Canada) for general site-level hydrological descriptions of surface water patterns with greater temporal resolution.

Soil solution water chemistry

Between 2008-2012 soil solution was sampled from 15 cm piezometers at bimonthly to quarterly frequency. Samples were collected in HDPE plastic bottles, filtered in the field and frozen until analyses (Whatman GF/F, 0.7 mm). Collection methods and analyses have been described in detail in Ardón et al. (2010).

Statistical Analysis

Data were analyzed using regression analyses looking at the relationships between water depth and water chemistry with DBH and height (version 10, JMP Software, Cary, NC, USA). Tukey-Kramer comparisons of means tests were used to obtain pair-wise comparisons across the plots for water depth, DBH, basal area, height, chloride, nitrate, ammonium, and total dissolved nitrogen. Total and mean basal area was calculated per plot. Dry weight was calculated using an allometric equation for baldcypress: $y=-0.97+2.34 \text{ Logl0}(x)$, where y was aboveground woody biomass (kg) and x was the DBH (cm) (Scott et al. 1985). Once the woody biomass was obtained, I assumed that 50% of the woody biomass is carbon.

Results:

In total, 253 trees were measured and tagged. Mean diameter at breast height (DBH) varied across the sites from 2.93 (plot 105B) to 9.65 cm (plot MP3), mean tree height from 2.24 (plot 105B) to 4.87 m (plot MP3) and water depth from 0 (plots 209 and 603) to 61 cm (plot 101) (Table 3.1). Mean stem density ranged from 300 (plot 107) to 900/ha (plots 1A02 and 602), mean basal from 0.32 (plot 107) to 5.04 m^2/ha (plot 209), and mean C sequestration from <0.00 (plot 105B) to 0.48 g/ha (plot 602) (Table 3.1). There were 14 trees across the different sites that were not large enough to measure DBH, but were measured for height (Table 3.2).

There were significant differences across the site in regards to mean water depth (Figure 3.3A). However, water depth was not related to DBH or height ($p = 0.4208$, Figure 3.4A and $p = 0.0947$, Figure 3.4B, respectively). Each of the sampling transects (1, 1A, MP and 6) had been designed to span the flooding gradient, as is observed in the declining water depth within each transect (Figure 3.3A). Plot 209 was the only plot sampled on transect 2, and is a higher elevation, and thus dry plot (Figure 3.3A). Diameter at breast height, tree basal area, and tree height tended to be higher in the Midpoint and transect 6 (Figure 3.3B, C and D).

Chloride (Cl^-) concentrations in soil solution were negatively related to diameter at breast height (DBH) ($p = 0.0270$, Figure 3.5A) and height ($p = 0.0232$, Figure 3.5B). Ammonium concentrations (NH_4-N, $r^2 = 0.34$, $p = 0.0359$) and total dissolved nitrogen (TDN, $r^2 = 0.32$, $p = 0.0453$) also were negatively related to height, but not to DBH (Table 3.3). Cl^- concentrations (Figure 3.3E), NH_4-N (Figure 3.3G), and TDN (Figure 3.3H) were higher near the outflow due to saltwater incursion. Nitrate concentrations (NO_3^--N) (Figure 3.3F) was higher near the inflow because of runoff from the upstream farm.

Discussion:

I found significant variation in baldcypress DBH and height across the site (Table 3.1). On average, DBH and height were 69% lower in plots that had a higher chloride, ammonium, and total dissolved nitrogen concentrations than sites with low concentrations. I estimate that increased salinity led to an 89% decrease in C sequestration by baldcypress trees (Table 3.1). Overall my results suggest that while baldcypress trees might be able to withstand moderate increased flooding due to sea-level rise, changes in salinity will cause declines in their growth and likely long-term survival.

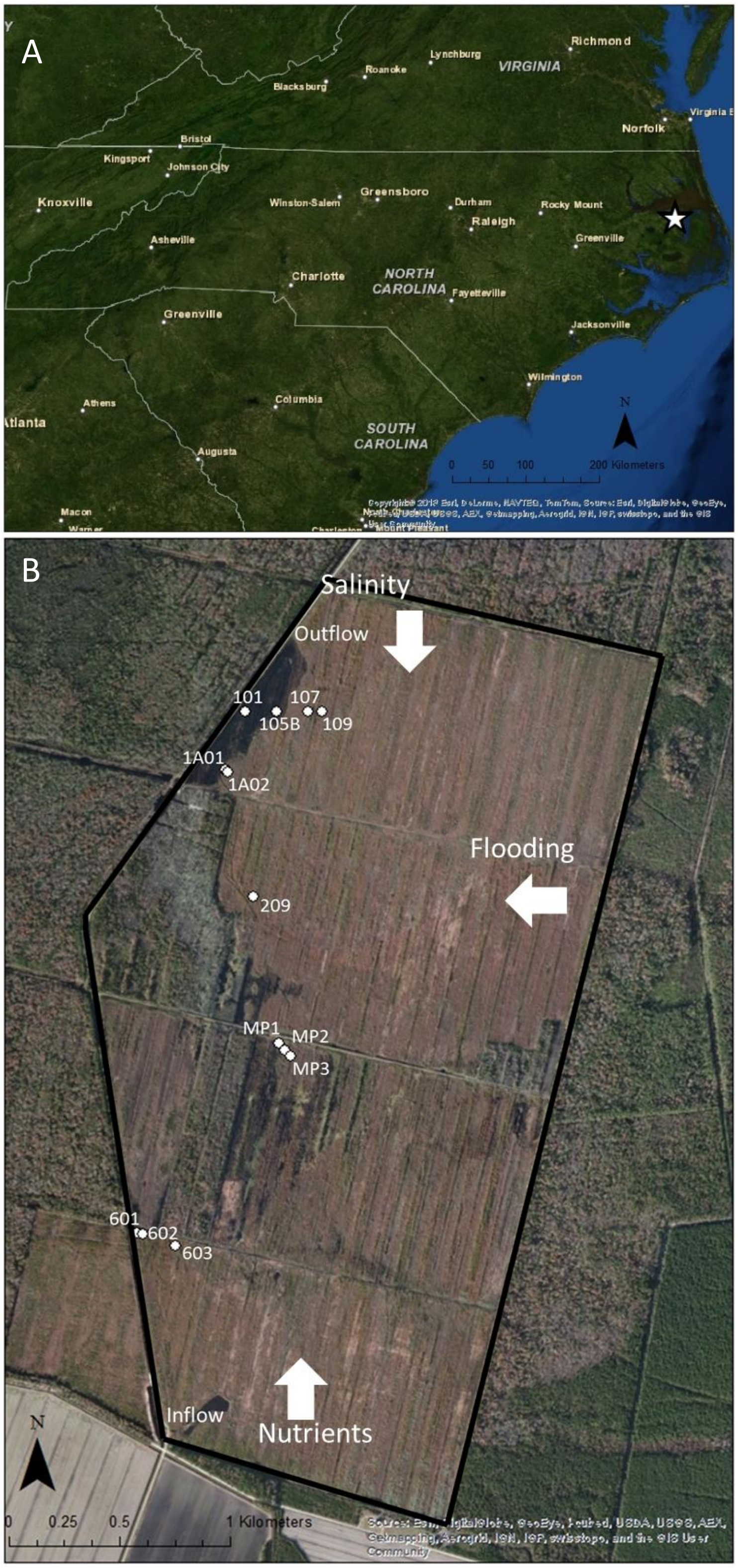
Chloride (Figure 3.3B), ammonium (Figure 3.3F), and total dissolved nitrogen (Figure 3.3G) were higher closest to the outflow. These levels may be explained from the wind driven tides that bring salinity into the site from the Alligator River. Ammonium has been shown to increase with increasing salinity, and that is likely also driving the increase in total dissolved nitrogen (Ardón et al. 2013). The lower growth rates of baldcypress with higher concentrations of ammonium and total dissolved nitrogen is counterintuitive; I would expect higher nitrogen concentrations to lead to higher growth rates. The decreased growth in this case is likely due to the negative effect of chloride on growth being stronger than the positive effect of extra nitrogen. Nitrate concentrations were higher closest to the inflow (Figure 3.3E), which is what I would expect given that runoff from agricultural fields to the south of the site persists. Interestingly, higher nitrate concentrations did not appear to lead to higher growth of baldcypress trees (Figure 3.3).

Water depth did not have a significant correlation with DBH or height (Figure 3.4). Iwanaga et al. (2009) found no significant difference in diameter and height between young baldcypress grown in flooded and unflooded pots outdoors. I may have not seen tree mortality or impacts on survival due to the water depth being less than 1 m deep. Even though the trees at the site were flooded, they may be able to survive due to the flow of water throughout the site which provides oxygen to the roots. Baldcypress in areas with flowing water can withstand flooding longer than trees in areas with standing water due to the higher concentrations of oxygen associated with water flow (Brown and Montz 1986). The lack of a significant negative relationship between water depth and DBH and height of baldcypress trees across the site could also be because water level has been increasing since the initial flooding. Because of this increase in water level, the variance around the mean water level within each site is very large compared to how much water level varies across the site (Figure 3.3A and B). If water levels continue to rise, I suspect that trees in the sites with deeper water will start showing signs of stress.

Several factors are known to affect baldcypress success in restoration plantings. When planting baldcypress on a restoration site, land managers should take note of any possible paths in which saltwater incursion could enter the site, as well as monitoring water levels. Research in other areas has shown that it is better to plant older baldcypress seedlings, at least a year old, than young seedlings which tend to die after 2 to 3 days in flood waters (Brown and Montz 1986). Surrounding vegetation can also impact the growth and survival of seedlings. Transplanted seedlings had a significant greater growth and survival in cleared areas versus areas where vegetation was not removed in the Corkscrew Swamp Sanctuary, Florida (Gunderson 1984). At the Corkscrew Swamp Sanctuary Gunderson also found that it is better to plant seedlings rather than seeds. On average only 2.1% of the seeds that were sown germinated across the 5 sites in FL, 95% of the germinated seedlings died within the first year after being planted, and only one survived to the third year (Gunderson 1984). Whereas, after 21 years 41% of the baldcypress seedlings planted in 0 – 0.6 m of water in a plantation survived in Washington County, Mississippi (Krinard and Johnson 1976). At TOWeR 1 year-old seedlings were planted on cleared land, which may have contributed to the success of their growth and survival. Overall more than 95% of the baldcypress seedlings at TOWeR have survived since being planted in 2005.

There is also a need for long term data on baldcypress management (Brown and Montz 1986). Information on the growth and functioning of baldcypress seedlings in response to flooding in a natural setting, such as wetland ecosystems, are scarce (Flynn 1986, Pezeshki 2001). Measuring and tagging young trees allows continued measurements of baldcypress growth at a wetland restoration site in both flooded and non-flooded areas, as well as areas that are being affected by saltwater incursion. This allows a unique opportunity to study the long-term growth of the baldcypress trees, as well as environmental factors at the site. The information that will be collected from the long-term data set will help land managers know how year old baldcypress seedlings that were planted across flooding and salinity gradients grow and survive as restoration of the site continues.

Figures: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Raleigh
Greenville
Asheville
Charlotte
NORTH CAROLINA
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Jacksonville
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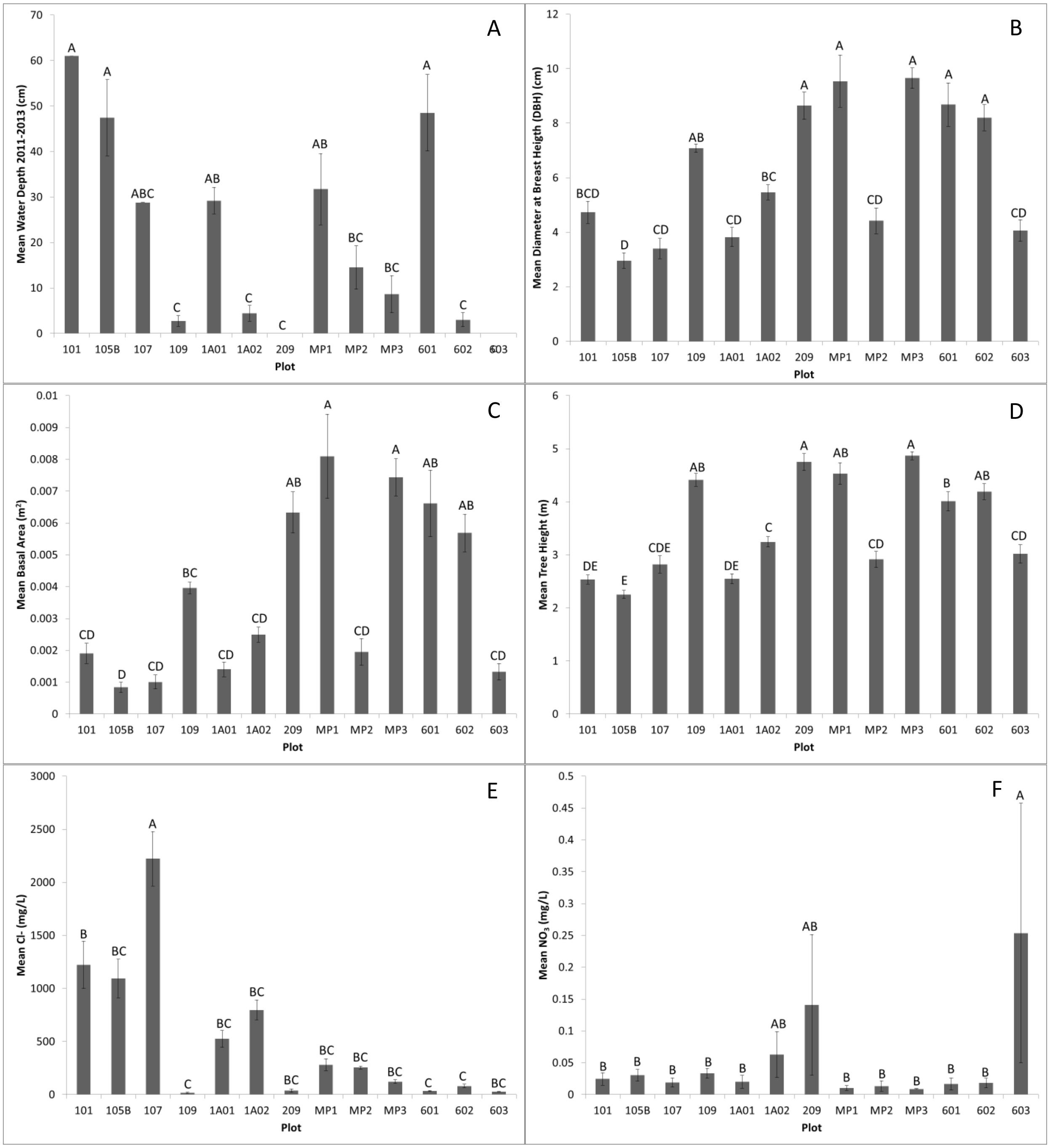
Figure 3.1: Map of Timberlake Observatory Wetland Restoration Site (TOWeR) A) The star shows the location of Timberlake Observatory Wetland Restoration Site (TOWeR). B) The black pentagon outline, shows the area of TOWeR that was converted from an agricultural field to a wetland restoration site; the circles represent the 13 plots that were inventoried. Arrows indicate that nutrients are higher at the inflow and decrease across the site, salinity is higher at the outflow during drought years and declines across the site, and flooding increases towards the west of the site.

Figure 3.2: The height difference between baldcypress in flooded areas experiencing high salinity (left) compared to less frequently flooded areas that have not experienced high salinity (right).



A
Mean Water Depth 2011-2013 (cm)
A A ABC C AB C C AB BC BC A C
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot
B
Mean Diameter at Breast Heigth (DBH) (cm)
BCD D CD AB CD BC A A CD A A A CD
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot
C
Mean Basal Area (m2)
CD D CD BC CD CD AB A CD A AB AB CD
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot
D
Mean Tree Hieght (m)
DE E CDE AB DE C A AB CD A B AB CD
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot
E
Mean Cl- (mg/L)
B BC A C BC BC BC BC BC BC C C BC
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot
F
Mean NO3 (mg/L)
B B B B B AB AB B B B B B A
101 105B 107 109 1A01 1A02 209 MP1 MP2 MP3 601 602 603
Plot

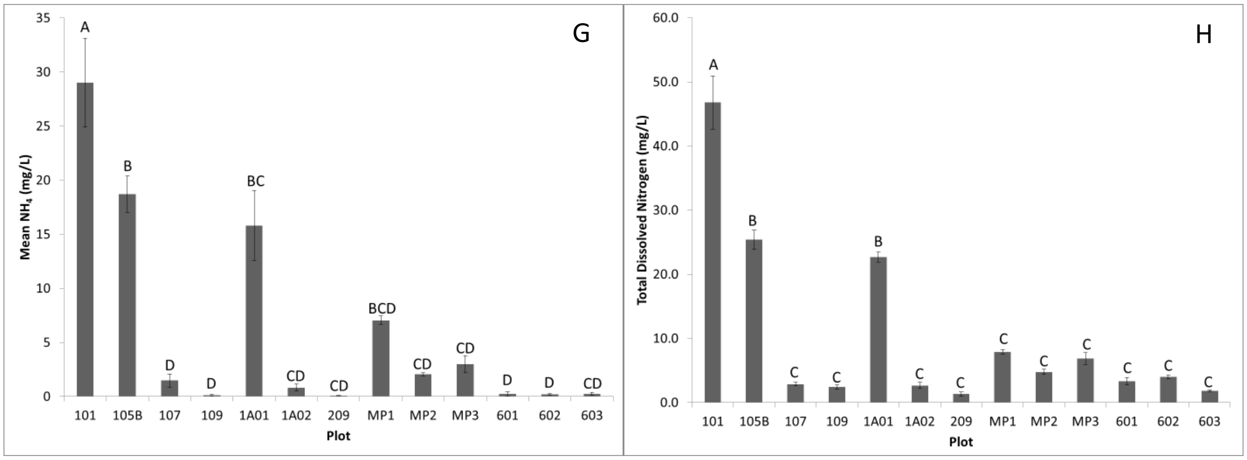
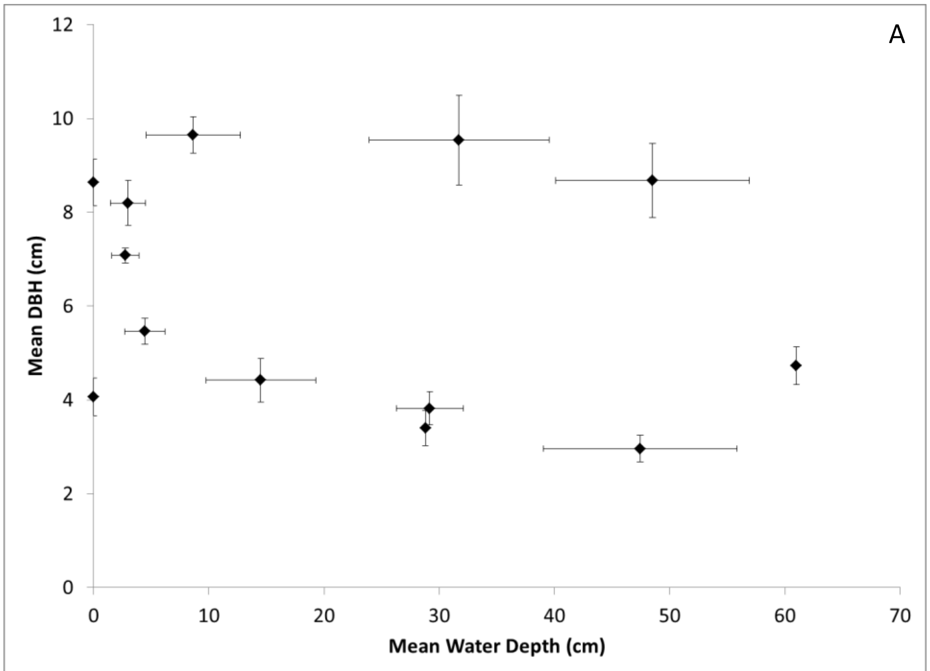


Figure 3.3: Mean water depth (A), diameter at breast height (B), basal area (C), height (D), chloride (E), nitrate (F), ammonium (G), and total dissolved nitrogen (H) across TOWeR at the sampling plots. Plots that share the same letter were not significantly different from each other. Error bars = standard error.



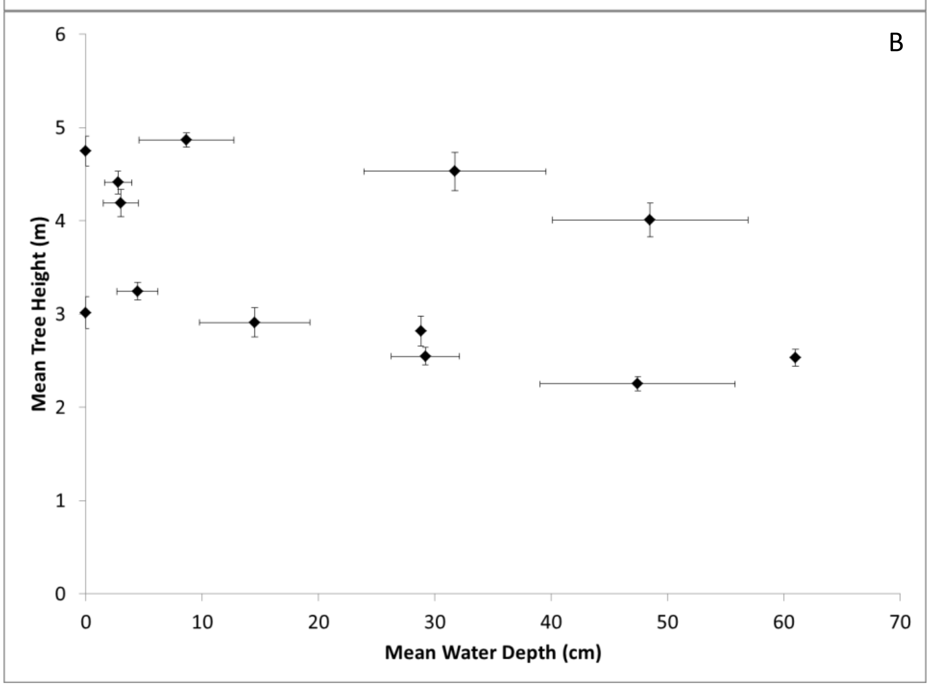


Figure 3.4: A) Linear relationship between mean diameter at breast height and mean water depth from 2011-2013 ($p = 0.4208$, $r^2 = 0.06$, n=13). B) Linear relationship between mean tree height and mean water depth from 2011-2013 ($p = 0.0947$, $r^2 = 0.23$, n = 13) Error bars = standard error.

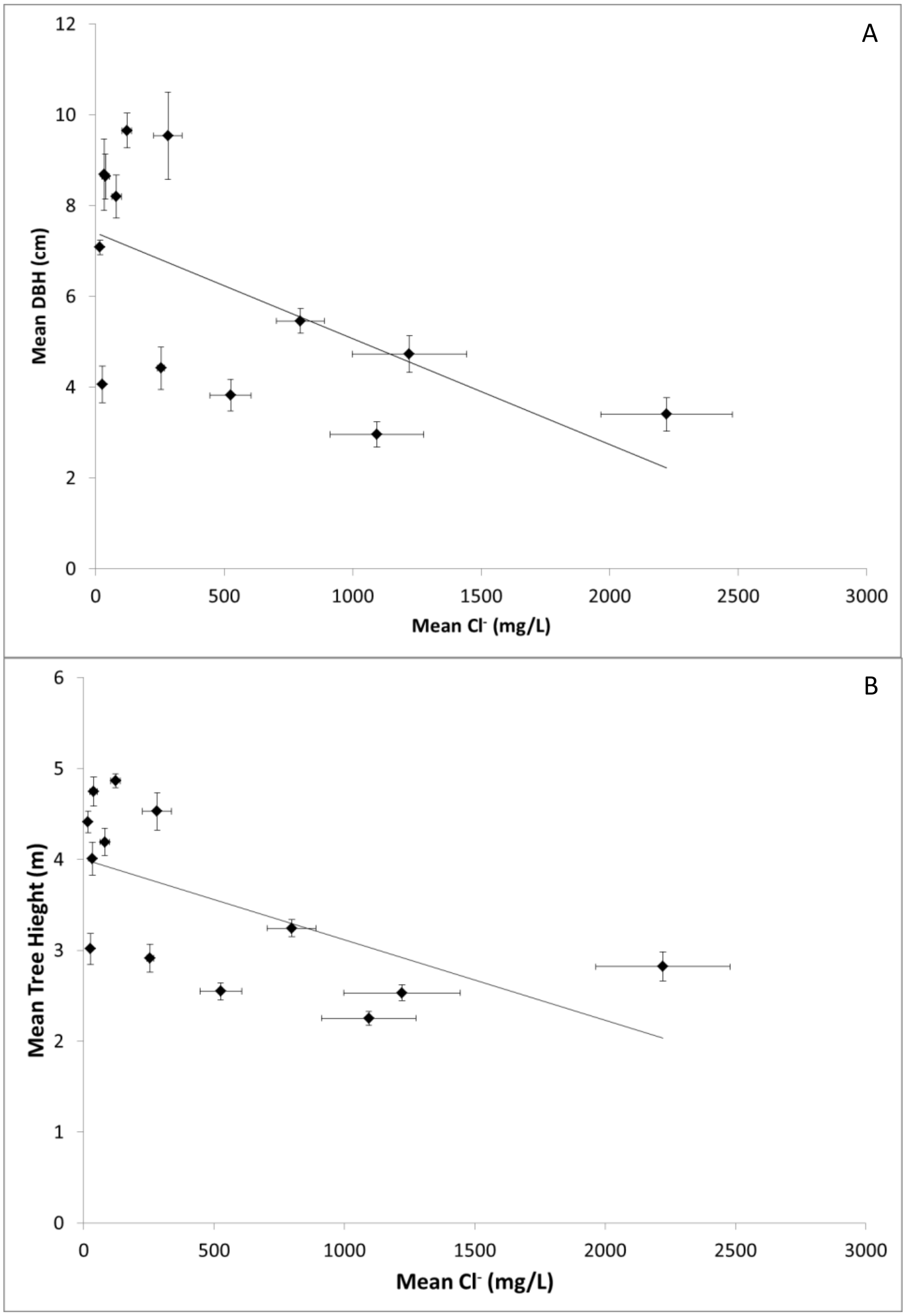


Figure 3.5: A) Linear relationship between mean diameter at breast height and mean chloride ($p = 0.0270$, $r^2 = 0.37$, $n = 13$). B) Linear relationship between mean tree height and mean chloride ($p = 0.0232$ $r^2 = 0.39$, $n = 13$) Error bars = standard error.

Tables:

Table 3.1: Summarizes the sample size (n) stem density, basal area and carbon accumulation for each plot. Also summarizes the minimum (min.), mean, maximum (max.) for diameter at breast height (DBH), height and water depth (2011-2013 (n = 253).

Plot	n	DBH (cm)			Height (m)			Water Depth (cm)			Stem Density (m^2)	Basal Area (m^2/ha)	C (g/m^2)
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max			
101	17	2.19	4.67	8.27	2.00	2.53	3.40	46.00	61.00	72.00	0.05	1.03	14.50
105B	25	1.00	2.93	5.95	1.60	2.24	3.02	35.00	47.44	78.00	0.08	0.67	0.03
107	10	1.65	3.40	5.73	2.10	2.82	3.96	21.00	28.80	37.00	0.03	0.32	3.46
109	11	6.06	7.08	8.07	3.82	4.41	5.03	0.00	2.76	11.00	0.04	1.39	17.80
1A01	30	1.46	3.91	7.28	1.85	2.52	3.32	21.00	29.18	60.00	0.08	1.07	11.94
1A02	27	2.46	5.46	8.37	2.19	3.24	3.89	2.00	4.44	25.00	0.09	2.15	30.80
209	25	2.63	8.64	2.63	2.80	4.75	2.80	0.00	0.00	0.00	0.08	5.04	46.48
MP1	14	3.11	9.54	14.35	3.15	4.53	5.03	14.35	31.71	18.00	0.05	4.38	33.17
MP2	25	1.16	4.42	11.28	1.89	2.91	4.91	11.28	14.50	41.00	0.08	1.55	15.48
MP3	12	7.60	9.65	11.93	4.35	4.87	5.03	11.93	8.65	29.00	0.04	2.84	25.29
601	16	3.96	8.68	13.61	2.74	4.01	5.03	15.00	48.50	30.00	0.05	3.37	29.39
602	27	3.25	8.18	11.83	2.61	4.19	5.03	0.00	3.00	21.00	0.09	4.89	47.95
603	17	1.54	3.77	7.06	1.98	3.02	4.58	0.00	0.00	0.00	0.05	0.72	7.31

Table 3.2 Summary of trees not large enough to measure (DBH < 1 cm) (n = 14).

Plot	Number of Trees	Average Height (m)	Average Water Depth (m)
101	5	1.42	0.35
105b	8	1.35	0.64
1A01	1	1.62	0.69

Table 3.3: Summary of regression results for diameter at breast height (DBH) and tree height of all tagged baldcypress trees (n = 253) versus water chemistry (ammonium, phosphate, total dissolved nitrogen, chloride, sulfate, and nitrate) and water depth (m). DF = degrees of freedom. All relationships were negative.

	DBH (cm)			Height (m)		
	DF	r^2	P-Value	DF	r^2	P-Value
NH_4-N (mg/L)	12	0.17	0.1653	12	0.34	**0.0359**
PO_4-P (ug/L)	12	0.04	0.5132	12	0.12	0.2569
Total Dissolved N (mg/L)	12	0.15	0.1965	12	0.32	**0.0453**
Cl- (mg/L)	12	0.37	**0.0270**	12	0.39	**0.0232**
SO_4^{2-} (mg/L)	12	0.27	0.0712	12	0.23	0.1001
NO_3^- (mg/L)	12	0.03	0.5598	12	0.00	0.9604
Water Depth (2011-2013)	12	0.06	0.4208	12	0.23	0.0947

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Chapter IV

Does salinity and flooding affect the growth of baldcypress trees in a large brackish estuary? A survey of adult trees along the Albemarle Sound, North Carolina.

Abstract:

Baldcypress trees are known to be moderately tolerant to flooding and salinity. However, research has shown that increases in salinity can cause baldcypress to decrease growth and productivity, ultimately causing the trees to die. Baldcypress are slow growing and long-lived, which makes them ideal for dendrochronology. In order to look at how salinity and flooding affect the growth of adult baldcypress trees in the Albemarle Sound, North Carolina; increment cores were collected to analyze growth rates of trees from five sites spanning salinity and flooding gradients. Conductivity increased from 0.21 mS/cm (salinity 0 ppt) at the western site to 7.78 mS/cm (salinity 4 ppt) at the eastern site. Flooding depth across all sites ranged from more than 100 cm to less than 5 cm. I found that the mean age of the trees was 105 years old across all sites, which matches expectations given that most of this site was logged at the turn of the century. Salinity did not have a significant effect on mean growth. I found no correlation between measured flooding depth and mean growth in the last decade. My ability to isolate the effects of flooding and salinity on adult trees was constrained by the lack of long-term data and the multiple stressors that can affect tree growth over decadal time scales (such as storms, droughts, and fires). However, my results suggest that increased salinity due to sea level rise might decrease the growth of adult trees along the Albemarle Sound.

Introduction:

Baldcypress swamps are freshwater ecosystems common throughout the southeastern United States (Mattoon 1915). In studies in Georgia and South Carolina, swamp forests that experience salinities above 1.8 parts per thousand (ppt) were dominated by baldcypress (*Taxodium* distichum (L.) Rich) (Krauss et al. 2007). For the majority of the year these swamps are inundated with freshwater (salinity less than 0.5 ppt); therefore, hydrologic regime and water quality directly affect the growth of the dominant trees (Duever and McCollom 1987). Drought and hurricanes can increase salinity to tidal freshwater swamps, leading to a decrease in baldcypress growth (Doyle et al. 2007). However, hurricanes can also bring major flooding and freshwater due to heavy rains and surge in coastal systems (Conner et al. 1997, Doyle et al. 2007, Krauss et al. 2007). As sea levels increase, flooding and salinity will increase, resulting in tree mortality in forested areas (Pezeshki et al. 1990).

Both duration of flooding and salinity can affect the growth and survival of baldcypress trees. Growth of baldcypress trees in Corkscrew Swamp Sanctuary in Florida were adversely affected by year-round inundation (Duever and McCollom 1987). In South Carolina, short-term flooding led to increases in radial growth; however, radial growth declined when flooding persisted (Young et al. 1995). In addition to the duration of flooding, salinity of the water can be an important determinant of growth. In Louisiana, there was a significant negative linear relationship between the number of baldcypress stems per acre versus soil pore salinities; with soil salinity greater than 1.0 ppt having the greatest effect (Wicker et al. 1981). Salt stress has a negative effect on plant productivity and growth (Effler and Goyer 2006, Pezeshki et al. 1990, Wallace et al. 1996, Wicker et al. 1981).

Baldcypress are slow growing and long lived, making baldcypress trees ideal for dendrochronology (Doyle et al. 2007, Mattoon 1915, Stahle et al. 1985). Along the Black River, North Carolina trees as old as 1700 years have been found (Stahle et al. 1988). Tree ring widths vary annually due to changes in water availability and salinity (Speer 2010). In a study conducted in Florida, trees had consistently smaller rings due to increased saltwater into the area after the 1960s (Duever and McCollom 1987). During years when the trees are stressed, there may be locally absent rings due to insufficient growth hormone production (Speer 2010). Many studies have found a positive correlation between tree ring width and precipitation; drought years produce narrower rings and rainy years lead to wider rings (Brown and Montz 1986, Davidson et al. 2006, Doyle et al. 2007, Stokes and Smiley 1968). Much of this research has been conducted in tidal systems along the Gulf Coast and the coasts of Georgia and South Carolina. In the Cape Fear Estuary in North Carolina, elemental analyses of baldcypress rings found some trees could continue to survive for up to 100 years after experiencing low levels of salinity intrusion (Yanosky et al. 1995). Much less is known from microtidal systems along the Albemarle and Pamlico Sounds in North Carolina where salinities are much lower, and there is no influence of lunar tides.

Albemarle Sound, North Carolina is a freshwater lagoon which receives freshwater from the Chowan and Roanoke Rivers at an average of 380 m^3/sec (Folger 1972, Riggs et al. 2003). Freshwater also enters the system by precipitation, about 1.3 m/year (Riggs et al. 1993). Tidal influence on the sound is limited due to the connected, barrier island system associated with the Outer Banks which have only narrow inlets for tidal waters to pass (Folger 1972, Riggs 1996). Currently there are no inlets that allow a direct flow from the Atlantic Ocean to the Albemarle Sound, thus, unlike the Gulf coast, the Sound experiences irregular wind driven tides (Copeland et al. 1983, Riggs et al. 1993). Salinity in the Sound averages 0 - 7 ppt from west to east (Bowden and Hobbie 1977). Throughout history, hurricanes have opened and closed inlets along the Outer Banks. The closing of most inlets has caused the salinity to decrease in the last 200 years (Fisher 1962).

My objective was to examine the effects of flooding and salinity on mature baldcypress trees. I conducted a regional survey of baldcypress growth using increment tree ring cores in five sites spanning a salinity gradient in the Albemarle Sound, North Carolina. Within each site I sampled trees at different flooding depths.

Methods:

Increment cores of adult baldcypress trees were collected along the Albemarle Sound, North Carolina from five sites: Eden House Bridge, 4-H Center, Pocosin Lakes National Wildlife Refuge, Palmetto Peartree Preserve, and Alligator River National Wildlife Refuge (Figure 4.1).The sites differed in the extent of flooding and the overall health of the trees (Figure 4.2). Upon arriving at the sites, ten dominant trees were chosen. Cores were taken at 1.3 m, diameter at breast height (DBH) unless there were buttresses, in which case cores were taken above the buttress. Two cores were taken from each tree. I also measured DBH for all trees and water temp (°C), conductivity (mS/cm), dissolved oxygen (%, and mg/L), pH, oxidation reduction potential (ORP), and depth nearby each tree (YSI Model 556, Yellow Spring, Ohio, USA; Table 4.1). I assigned a water depth of 15 cm for trees located in the tidal zone, based on average tidal variations for the Sound.

I used an increment borer to extract cores from baldcypress trees (Stokes and Smiley 1968). Cores were transported in straws and then air dried. Once dried, cores were mounted into wooden blocks (Billy Jacks Woodworking, MS.) and then sanded using a belt sander (180 and 220 grit) and then by hand (320, 400, and 600 grit). Skeleton plots were constructed to compare narrow rings among tree cores (Stokes and Smiley 1968, Speer 2010). When crossdating, each core was analyzed for narrow and/or wide rings and if these rings occur for the same years for multiple cores (Bowers 1981). Cores were read using a stage micrometer and software program Measure J2 (VoorTech Consulting, New Hampshire, USA).

<u>Statistical Analysis</u>

To remove individual tree variability and examine stand level signals, I averaged growth between the two cores taken from each individual tree and then averaged growth between the 10 trees on each site (Bowers 1981, Speer 2010). Tukey-Kramer comparisons of mean test were used to look at mean DBH, mean water depth, mean conductivity, and mean age of cores across the sites (version 10, JMP Software, Cary, NC, USA). I examined the relationships between decadal growth rates and environmental factors from each site, such as salinity, precipitation, and Palmer Drought Severity Index from the North Carolina State Climatology Office (Raleigh, NC). I performed a linear regression on the mean growth (mm/yr) versus mean salinity measured by the North Carolina Division of Water Quality and mean tree age versus diameter at breast height (DBH) (JMP 10).

<u>Results:</u>

There was a significant difference in water depth (Figure 4.3B) and conductivity (mS/cm) (Figure 4.3C) among sites. Water depth varied from 8 to 116 cm across the sites, with Eden House Bridge having trees growing in the deepest waters. Conductivity varied from 0.21 – 7.78 mS/cm (salinity from 0 to 4 ppt), with the eastern most sites (Palmetto Peartree and Alligator River) having significantly higher conductivity than the other three sites (Figure 4.3C).

I did not find significant differences across sites in DBH (Figure 4.3A) or mean tree age (Figure 4.3C). I did find that the coefficient of variation (%) of core age increased from west to east along the salinity gradient (Figure 4.4). Alligator River Wildlife Refuge contained both the youngest and oldest trees. The youngest cores were 35 years (Palmetto Peartree and Alligator

River), the mean core age for all the sites was 105 years, and the oldest core was 302 years (Alligator River) (Table 4.2). Some the trees were rotten in the center; therefore, annual ring count was underestimated. Mean diameter at breast height (DBH) ranged from 34.72 to 42.56 cm (Eden House Bridge, Pocosin Wildlife Refuge, respectively, Table 4.2). Total basal area varied from 0.97 at Eden House Bridge to 1.53 m^2 at Pocosin Wildlife Refuge (Table 4.2).

Total growth per decade for the different sites suggests that there has been declining growth after the1960s at four of the sites (Figure 4.5A). Total growth per decade of these four sites showed similar temporal variations as the mean Palmer Drought Severity Index for the region (Figure 4.5B). Because long-term salinity data was not available, I was only able to examine the relationship of mean growth and salinity for one decade for each of the sites (for all sites I used 2000-2009, except for Eden House Bridge for which I used 1990-1999). A linear regression of mean growth (mm/yr) on mean salinity (ppt) per decade was not significant (r^2 = 0.71, p = 0.0728), but there was a negative trend (Figure 4.6). There was no relationship between mean water depth measured at the time of core collection and mean growth (mm/yr) for the most recent decade (Figure 4.7)

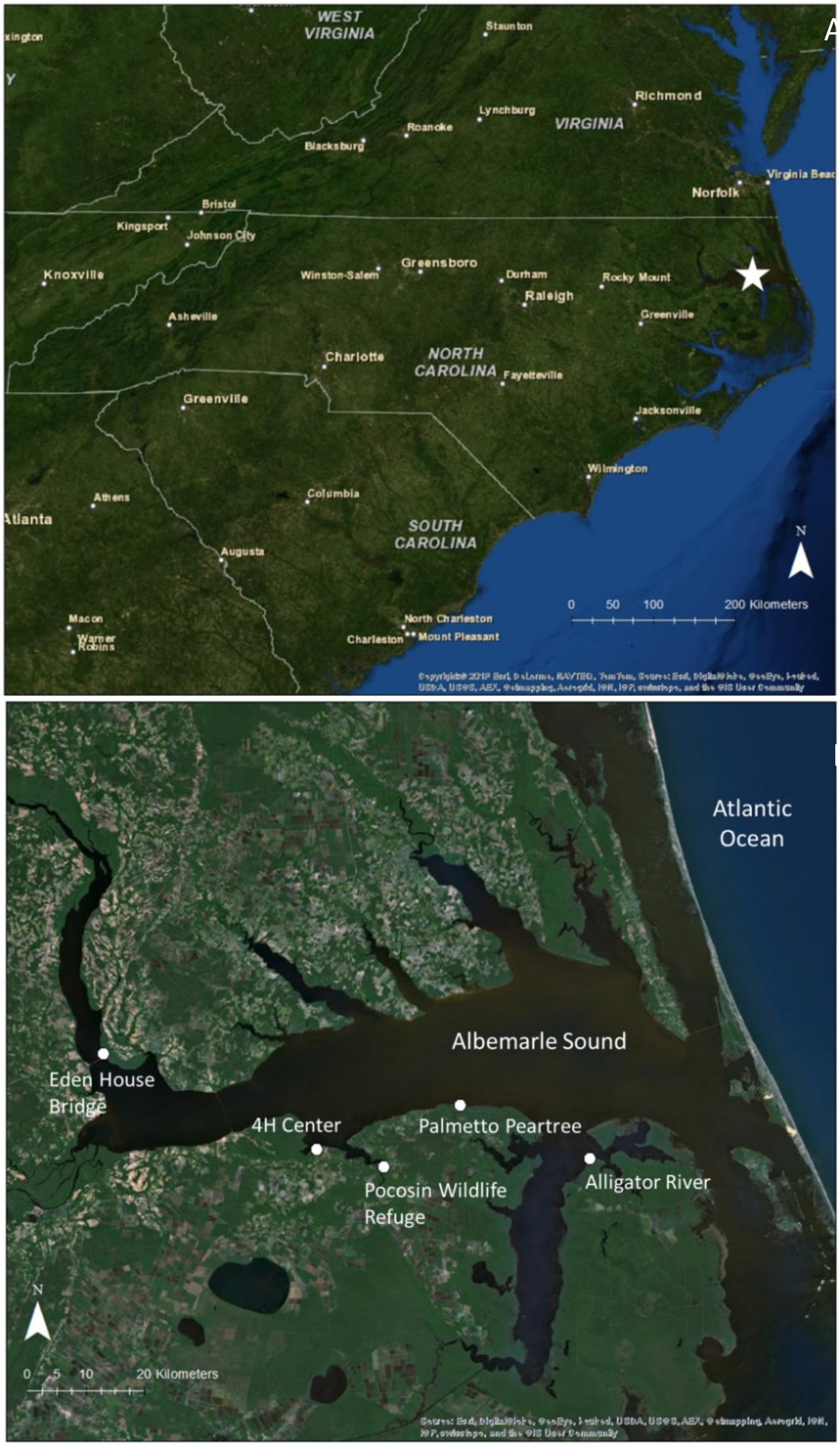
Discussion:

Combining historical salinity data with decadal growth, my results suggest that salinity led to a 45% decline in tree growth rate. However, due to the limited long-term salinity data, this relationship must be interpreted with caution. Disentangling which environmental factors, flooding or salinity, affected the growth of baldcypress along the Sound is compounded by the ever changing conditions of the Sound. I found no differences in DBH and mean core age across the five sites. This is likely a result of extensive logging that occurred in this area after the Civil War (Sawyer 2010). The total growth (mm) per decade shows that four sites (Eden House Bridge, 4-H Center, Pocosin Wildlife Refuge, and Alligator River) behaved similarly, with consistent periods of faster growth during the 1960-70s. Palmetto Peartree Preserve, on the other hand, exhibited declining growth since 1910, then maintaining a consistent growth rate from 1950-1990. Other studies in Palmetto Peartree Preserve have found that parts of the forested swamp are being converted to marshes or open water due to salinity intrusion (Poulter et al. 2009).

Other evidence suggest salinity has negative effects on baldcypress grown along the Albemarle Sound, NC. Eden House Bridge was the only site that had all ten trees inundated with more than 100 cm of water, experience less frequent saltwater incursion events, and was the site with the least amount of mortality. For Palmetto Peartree Preserve and Alligator River Wildlife Refuge, water depth is very low compared to the other sites. This was because all the trees located in standing water were dead or there were only stumps left, the only live trees at these sites were on shore. The trees at Eden House Bridge were surviving in water that was about 1 m deep; in contrast, the dead trees and stumps at Palmetto Peartree and Alligator River were in less than 1 m of water. This suggests that salinity is affecting the survival of baldcypress along the Albemarle Sound. Erosion in the sound is a natural ongoing process and may have contributed to the increased water depth where the dead trees were located (Riggs et al. 2003).

Due to the nature of the storms that affect the Outer Banks, inlets are continually migrating, opening, and closing (Riggs et al. 2003). These changes will continue to alter physical and chemical conditions as well as the regional biota (Riggs et al. 2003). However, due to the Outer Banks the sounds are buffered from the worst of the storms (Riggs et al. 2011). Baldcypress will ultimately die as sea level rises and dieback of forests will occur, if there is no recharge of freshwater (Doyle et al. 2007, Riggs et al. 2003). In the future, sea level rise may continue at an accelerating rate and the coast may be impacted by storms more regularly (Riggs et al. 2011). With more than 90% of baldcypress located within 30.5 m above sea level, rising sea levels could have a great impact on baldcypress trees (Mattoon 1915). For future research, increment cores should be collected along the Pamlico Sound, which experiences greater salinity than the Albemarle Sound due to direct flow through adjacent inlets. I suspect that there would be greater mortality along the Pamlico Sound and living trees would have a reduced growth rates compared to the Albemarle Sound.

Figures:



A
WEST VIRGINIA
Staunton
Richmond
Lynchburg
Roanoke
VIRGINIA
Blacksburg
Virginia Beach
Norfolk
Bristol
Kingsport
Johnson City
Knoxville
Winston-Salem
Greensboro
Durham
Rocky Mount
Raleigh
Greenville
Asheville
Charlotte
NORTH CAROLINA
Fayetteville
Greenville
Jacksonville
Wilmington
Athens
Columbia
Atlanta
SOUTH CAROLINA
Augusta
N
0 50 100 200 Kilometers
Macon
Warner Robins
North Charleston
Charleston
Mount Pleasant
Atlantic Ocean
Albemarle Sound
Eden House Bridge
4H Center
Palmetto Peartree
Alligator River
Pocosin Wildlife Refuge
N
0 5 10 20 Kilometers

Figure 4.1: A) The location of the Albemarle Sound, North Carolina. B) The location of the study sites along the Albemarle Sound.

Figure 4.2: Images of the baldcypress trees at each sampling site. A) Eden House Bridge, B) 4H Center, C) Pocosin National Lakes Wildlife Refuge, D) Palmetto Peartree Preserve, and E) Alligator River.

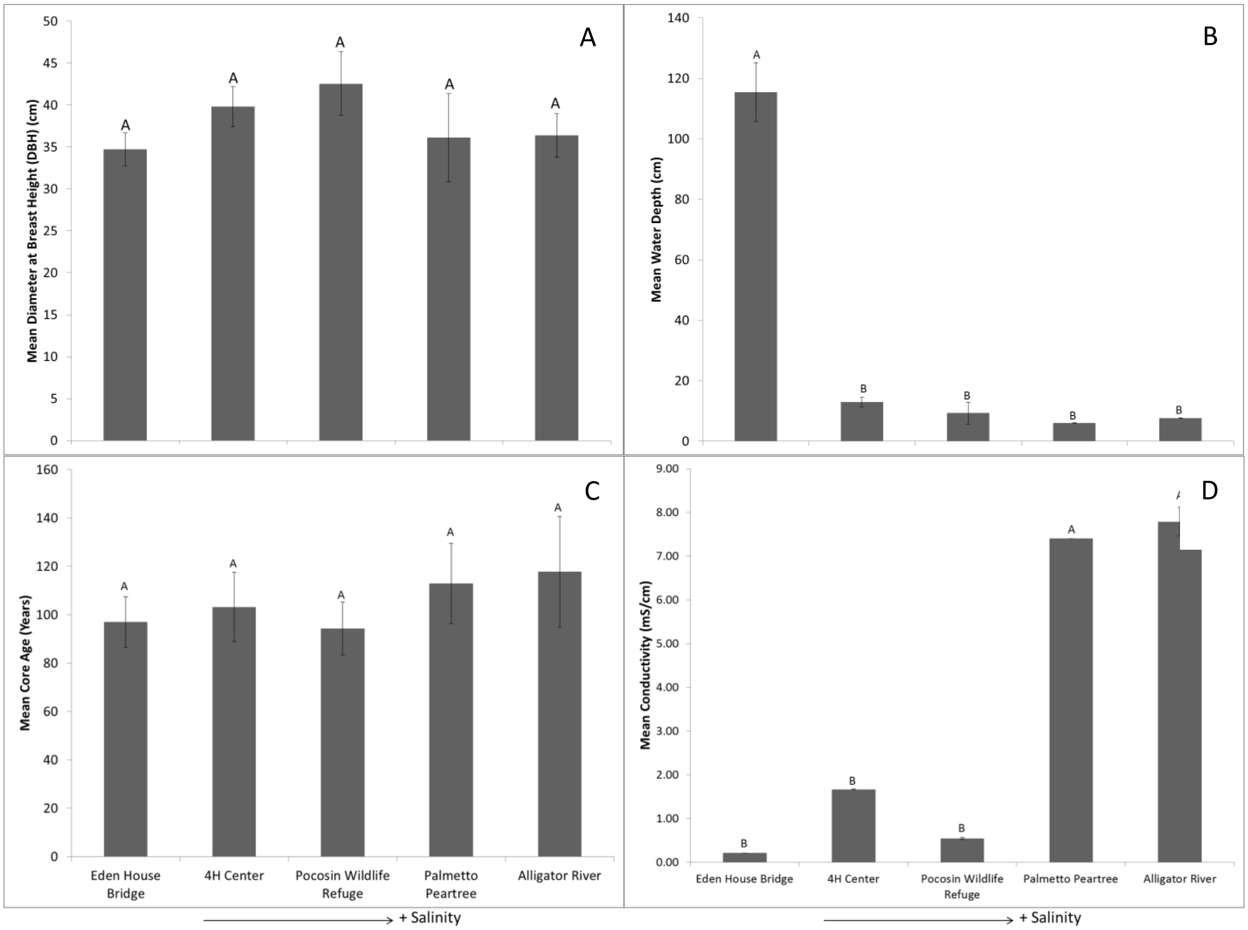


Figure 4.3: A) Mean diameter at breast height (DBH) across the sites along the Albemarle Sound from west to east (n=10). B) Mean water depth (cm) across the sites (n=10). C) Mean core age in years across the sites (n=20). D) Mean conductivity across the sites (n=2). Sites that share the same letter were not significantly different from each other. Error bars = standard error.

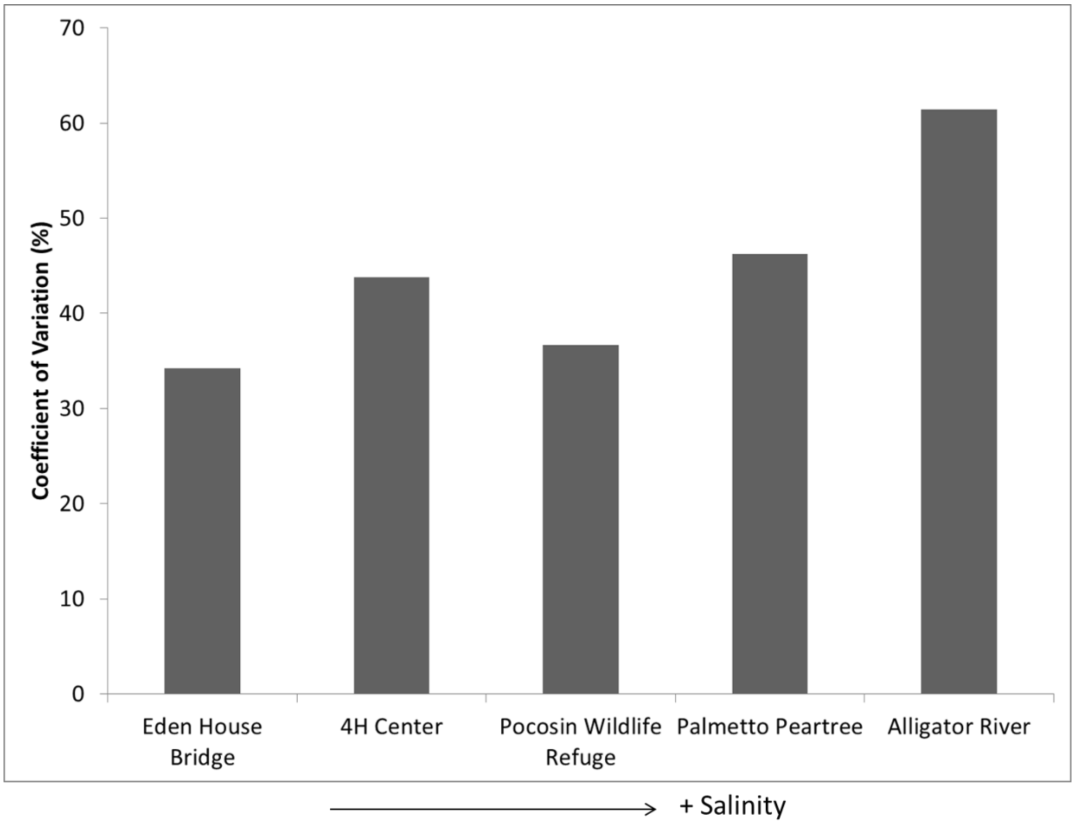


Figure 4.4: The coefficient of variance for mean core age across the sites along the Albemarle Sound from west to east.

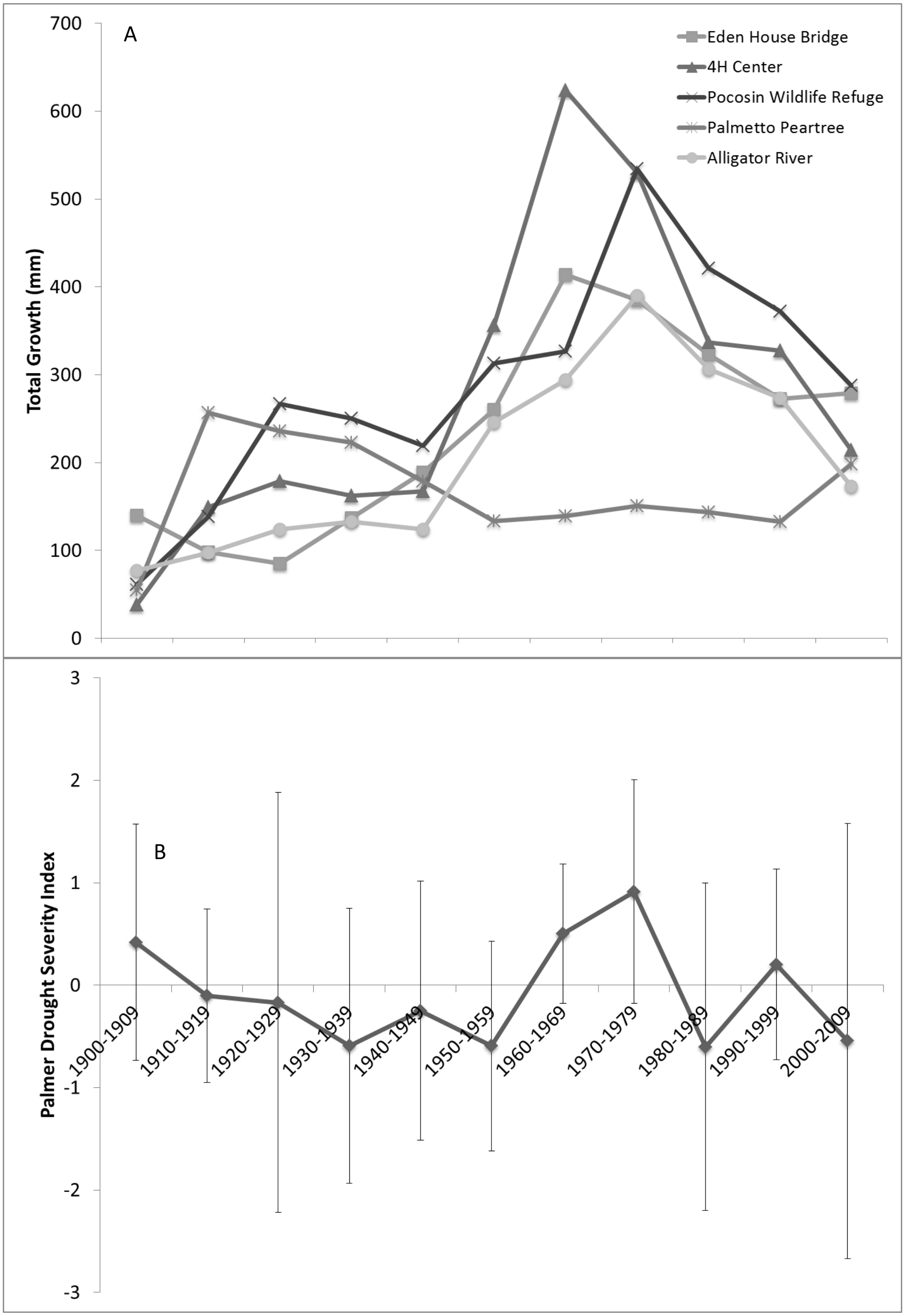

A
Eden House Bridge
4H Center
Pocosin Wildlife Refuge
Palmetto Peartree
Alligator River
Total Growth (mm)
700
600
500
400
300
200
100
0
B
Palmer Drought Severity Index
3
2
1
0
-1
-2
-3
1900-1909
1910-1919
1920-1929
1930-1939
1940-1949
1950-1959
1960-1969
1970-1979
1980-1989
1990-1999
2000-2009

Figure 4.5: A) The total growth (mm) per decade for all five sites (n=20). B) The mean Palmer Drought Severity Index for the region per decade.

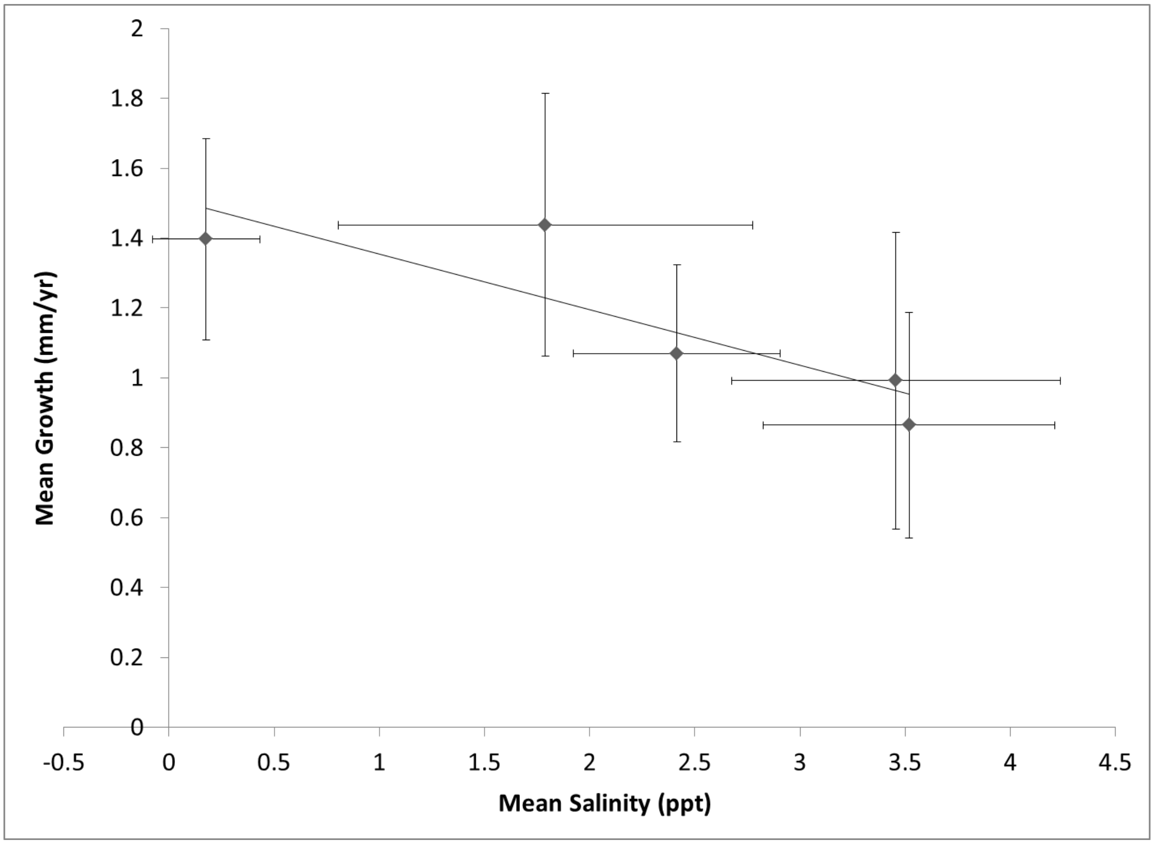


Figure 4.6: The linear regression between mean salinity (ppt) and mean growth (mm/yr) per decade for each site ($r^2 = 0.71$, $p = 0.0728$, $n = 5$).

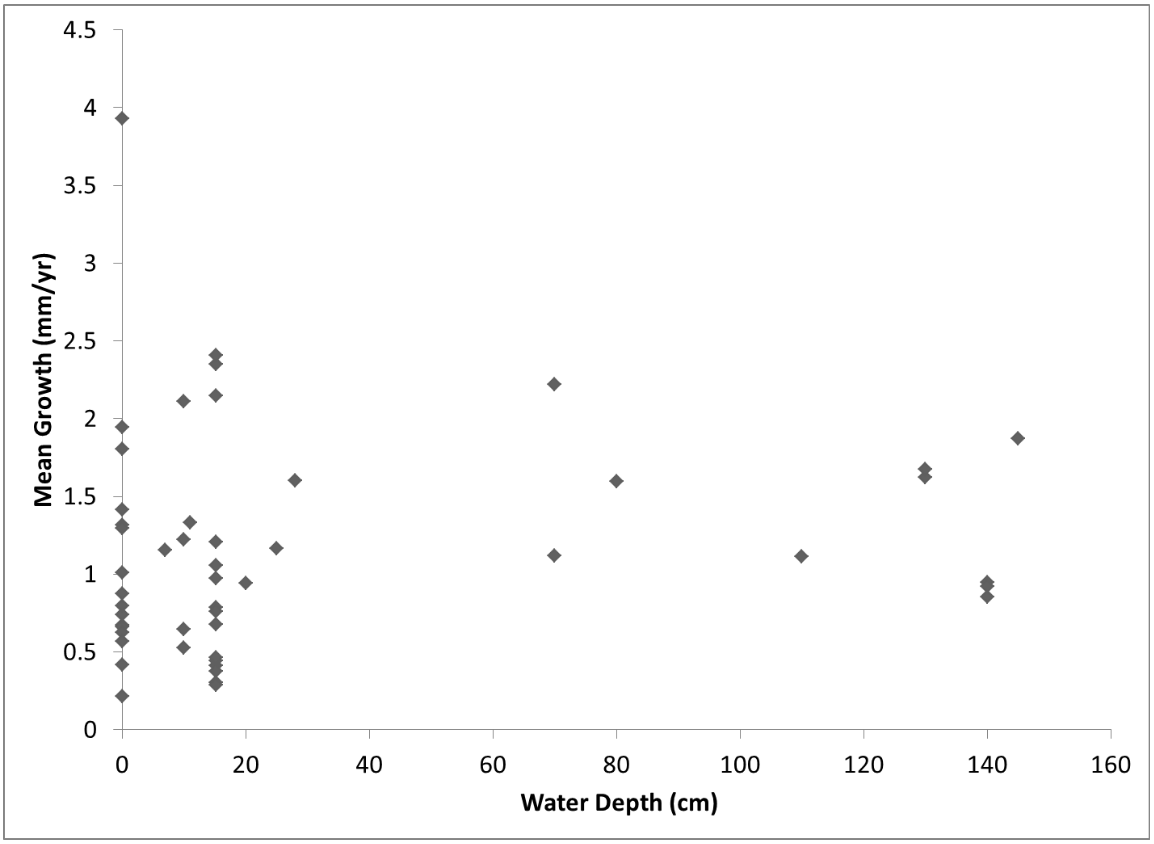


Figure 4.7: The linear regression between mean water depth (cm) and mean growth (mm/yr) for each tree (r^2 = 0.01, p = 0.3655, n = 50).

Tables:

Table 4.1: Summary of the month in which the cores were collected and the mean water quality measurements taken at the site.

Site	Date Collected	Water Temp (°C)	Conductivity (mS/cm)	%DO	DO (mg/L)	pH	ORP
Eden House Bridge	July 2013	29.70	0.21	75.5	5.74	7.51	38.7
4H Center	March 2013	10.67	1.66	103.25	11.44	8.6	63.25
Pocosin Wildlife Refuge	January 2013	8.63	0.55	54.63	6.33	6.45	97.25
Palmetto Peartree	January 2013	8.92	7.40	84.4	9.53	7.72	61.00
Alligator River	October 2013	18.47	7.78	82.27	7.65	8.31	15.23

Table 4.2: For each site, the minimum (min), mean, maximum (max) for core age of the 20 cores that were collected per site and the mean diameter at breast height (DBH) and total basal area for the 10 trees per site.

Site	Min Core Age	Mean Core Age	Max Core Age	Mean DBH (cm)	Total Basal Area m^2
Eden House Bridge	51	97	148	34.72	0.97
4H Center	53	103	197	39.81	1.28
Pocosin Wildlife Refuge	44	94	186	42.56	1.53
Palmetto Peartree	35	113	223	36.12	1.22
Alligator River	35	118	302	36.37	1.09

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Chapter V

Conclusion

Overall, I found that saltwater affected the growth of baldcypress seedlings, young trees and mature trees more than did flooding. In a greenhouse experiment, seedlings in salinity treatments had a 21% reduced diameter at the root collar and 15% reduced height compared to seedlings in the control treatment. At a wetland restoration site that is experiencing saltwater incursion, increased chloride concentrations due to saltwater incursion led to a 69% decline in DBH and tree height. Along the Albemarle Sound, NC, it appears that salinity may be causing a 45% decline in the growth of mature baldcypress trees (Figure 5.1).

Flooding did not appear to affect baldcypress growth, but saturation of the seedlings did have an effect on growth. Seedlings in saturated treatments had a significant greater diameter at root collar and height compared to seedlings in drought conditions regardless of salinity treatments. Water depth did not have a significant effect on young trees at a wetland restoration site. However, at the wetland restoration site, water has been increasing since restoration began, thus further monitoring is needed. For the Albemarle Sound, it is harder to say how water depth affected the growth. In one of the sites (Eden House), adult trees were healthy in 100 cm of freshwater. On the other hand in my two most saline sites all the trees in the water were dead, leaving only trees on the shore or inland to be cored.

These studies allowed me to see how growth and survival of seedlings, young trees, and mature baldcypress trees were affected by saltwater and flooding. Continued monitoring at the wetland restoration site will provide a unique opportunity to monitor growth and survival of baldcypress across salinity and flooding gradients for years to come. My greenhouse experiment, provided insight on how seedlings will grow and survive in areas that are experiencing salinity, sulfate, and flooding. This information is important for wetland restoration and can help direct and inform land managers where baldcypress should be planted. By looking at mature trees along the Albemarle Sound, I also provide a baseline to examine the future health and growth of baldcypress trees. While saltwater incursion mostly happens with wind driven tides and droughts, all it would take is a hurricane to open or close an inlet to change the water chemistry of the Albemarle Sound, with potentially negative consequences on the growth and survival of baldcypress trees.

Figure:

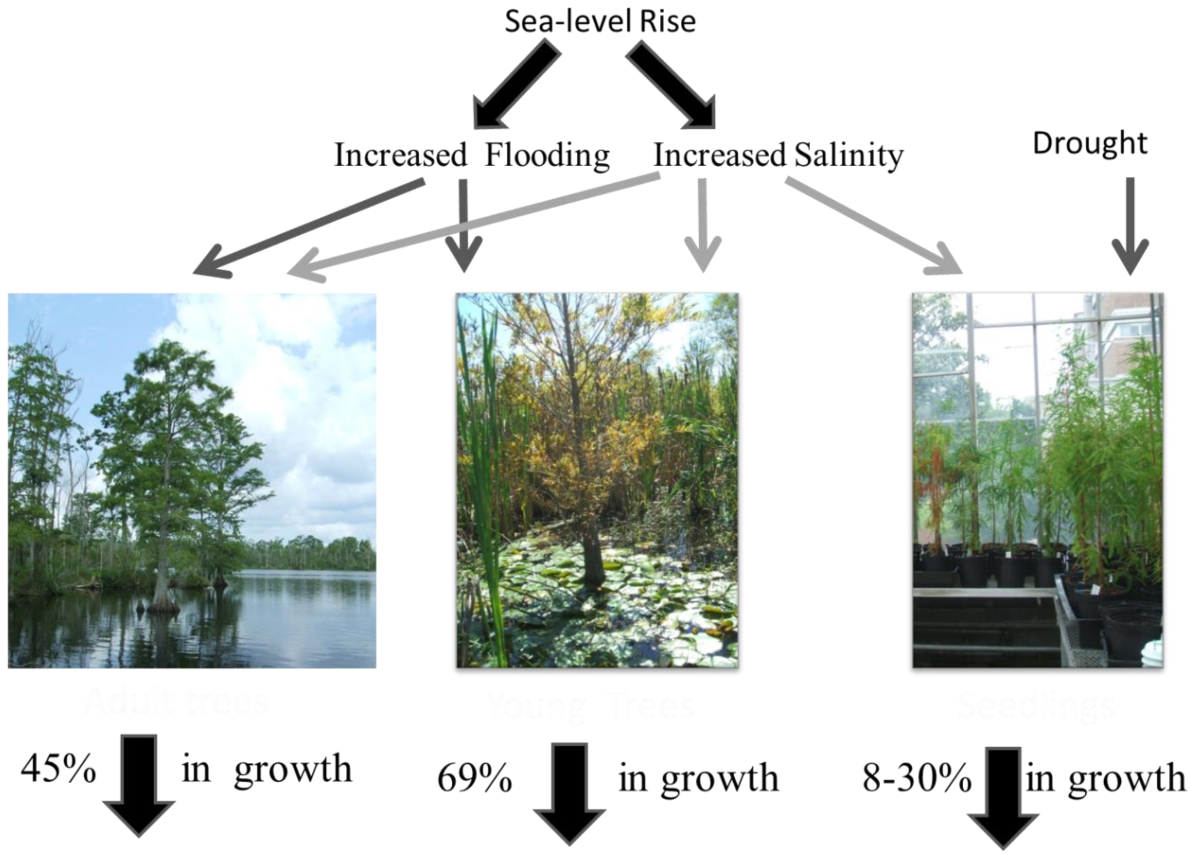

Figure 5.1: Looking at sea level rise and the increase in flooding and salinity. I found that flooding did not appear to have a significant effect on the adult trees and young trees. I found that drought had a significant effect on the diameter at root collar and height of seedlings. Increase in salinity affected baldcypress at all life stages. Salinity caused a 45% decline in growth of adult trees growing along the Albemarle Sound. A 69% decrease in growth of young trees at a wetland restoration site. There was a decrease in diameter at root collar, 8% (drought seedlings) and 30% (saturated seedlings), and height, 12% (drought seedlings) and 16% (saturated seedlings).