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# Effect of Increased Salinity Exposure on Photosynthetic Indicators and Water Status of Bald Cypress (*Taxodium distichum*) Seedlings

## **Cover Page Footnote**

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## 1. Introduction

In the United States, the southeastern Atlantic coastal ecosystems are vulnerable to change due to rising seas levels and increased storm frequency and intensity as a result of climate change, potentially altering inland water chemistry (Allen et al. 1997). For example, saltwater incursion is threatening plant species near or in freshwater and brackish wetlands, due to the sensitivity of these species to salinity change (Zhou et al. 2010). Increased salinity is known to alter growth, development, and photosynthesis within certain species (Munns 2002). Bald cypress [*Taxodium distichum* (L.) Rich] is a dominant tree species found along the southeastern coast and is considered a keystone, foundational species in freshwater forest wetlands of this region (Allen et al. 1996). Some specific, negative physiological effects of salinity exposure in Bald cypress, even for relatively brief exposures have included metabolic changes due in association with water stress (Munns 2002, Krauss et al. 1999). However, seedlings have also shown a physiological tolerance to salt water stress, with relatively slight declines in growth, photosynthetic capacity, or stomatal conductance when exposed to increased salinity (Allen et al. 1996). It was suggested that, under more severe salt stress, a significant decline in photosynthesis may occur due to inhibition of root growth and function (Allen et al. 1996).

The objective of the present study was to evaluate the impacts of experimentally increased salinity exposure on leaf physiological properties that implicate specific effects on photosynthetic capacity, e.g. photosynthetic pigment concentrations, chlorophyll fluorescence, and plant water status. We hypothesized that a reduction in these indicators of photosynthetic performance would occur with exposure to experimental treatments of increased salinity values representing natural field conditions that occur following ocean over-wash episodes. Only one of these three measured responses showed some degree of physiological tolerance to the salinity treatments.

## 2. Methods and Materials

Over a four week period, a greenhouse study was conducted using *T. distichum* seedlings grown from seed and exposed to increased salinity. Treatment salinity

levels were chosen based on field measurements taken immediately after natural over-wash events (unpublished data). Effects of salinity were evaluated by comparing a control group (freshwater) to a group exposed to brackish water with 5 ppt NaCl, plus other seawater salts including potassium chloride, potassium bromide, sodium chloride, sodium fluoride. Changes in photosynthetic potential were evaluated from measurements of leaf pigment content, fluorescence of photosystem II, and whole-plant water status.

Seed stock for growing greenhouse plants was obtained from the Timberlake Observatory for Wetland Restoration (TOWeR) in Tyrrell County, NC, on October, 12, 2013. Seedlings were scarified using a dilute solution of sulfuric acid and allowed to germinate in either potting soil, soil obtained from the field site, or vermiculite with fertilizing solution. Seedlings began sprouting approximately one to two weeks after being planted. Two groups of ten healthy appearing seedlings were selected for the experimental group and the control group. At the onset of treatment, seedlings were allowed to grow for seven months. During the subsequent four week study period, the control group was treated with artificial freshwater (dissolved salts = 0 ppt NaCl) while the experimental group was watered with slightly brackish water (ca. 5 ppt). Throughout the study, photosynthetic capacity was estimated from a combination of leaf pigment extractions, fluorimetry (indicator of the health of photosystem II), and by monitoring plant water status using xylem water potential measurements (pressure chamber).

#### *Photosynthetic pigment extractions*

Leaf pigment extractions were completed weekly to determine the concentrations of chlorophyll *a/b* and carotenoids within sampled leaves. The extraction protocol was adapted from a previous study on similar plant pigments (Hughes 2011). An 80% acetone dilution was used to extract the pigments from leaves at a ratio of 0.0092g of leaf tissue per ml of extraction solution. The solutions were analyzed with a spectrophotometer for absorbance at 470 nm, 646 nm, 647 nm and 663 nm and chlorophyll *a/b* concentrations calculated using the equations provided in Porra (2002) where

$$\text{Anthocyanin} = A_{529} - (0.228 A_{650})$$

$$\text{Chl a (nmol ml}^{-1}\text{)} = 0.01373 A_{663} - 0.000897 A_{537} - 0.003046 A_{647}$$

$$\text{Chl b (nmol ml}^{-1}\text{)} = 0.02405 A_{647} - 0.004305 A_{537} - 0.005507 A_{663}$$

$$\text{Carotenoids (nmol ml}^{-1}\text{)} = (A_{470} - (17.1 \times (\text{Chl a} + \text{Chl b}) - 9.479 \times \text{anthocyanin}))/119.26.$$

and A is the absorbance measured at the indicated wavelengths (subscripts). Chlorophyll a/b concentrations were determined due to their importance to photosynthetic light harvesting (Genty et al. 1989).

#### *Photosystem II status*

Fluorimeter measurements indicate the relative health of photosystem II by comparing the ratio of the dark-adapted minimum (F<sub>v</sub>) to maximum (F<sub>m</sub>) chlorophyll fluorescence (Schreiber et al. 1986). Weekly fluorimeter measurements were taken during the early morning hours on leaves near the mid-section of the seedling using a Hansatech Fluorescence Monitoring system (model 1090). For standardization, leaves were dark-adapted prior to taking fluorimeter measurements. To create consistent and comparable measurements, leaves chosen for measurement were fully expanded and healthy in appearance.

#### *Plant water status*

Xylem water potential measurements ( $\Psi$ ) were used as an indicator of whole-plant water status (Boyer 1967) and taken weekly during predawn hours (0600-0700 h) to reflect the maximum hydration level for the day (Running 1976). Measurement frequency was limited to two leaves per individual (n = 6 individuals) to avoid excessive defoliation that could influence plant water status and growth, and subsequent predawn values. All measurements were taken using a Scholander-type pressure chamber, with individual leaves sampled from a consistent 6-7 cm height (mid-crown), on the north-facing side of the plant.

#### *Leaf tissue sectioning*

At the initiation and completion of the project (August 31<sup>st</sup> to September 28<sup>th</sup>), live leaf tissue sections were collected for a five individuals in both the control and salt treatment group to qualitatively assess overall leaf health and pigment presence

(green coloration) and distribution within the leaf mesophyll. Fresh tissue samples were placed in a block of a 2.5% agar solution and sliced at a 200  $\mu\text{m}$  thickness using a Pelco 1000 vibratome sectioning system. Sections were then wet-mounted and viewed under a compound light microscope at 10x magnification. Leaf sections were qualitatively observed for chlorophyll presence and distribution across the mesophyll thickness.

### *Statistics*

Data from florescence, water status measurements and pigment extractions, obtained from the four week period were analyzed using a combination of a one tailed t-test and Mann Rank sum test. The Mann-Whitney Rank sum test were used when a data set failed a normality test for a Gaussian distribution.

## **3. Results**

### *Pigment concentrations*

*Chlorophyll a* concentrations were not significantly different for the control and treatment groups following the initial two weeks of treatment ( $p > 0.057$ ), but were significantly lower in the subsequent two weeks for the saltwater treatment group ( $p < 0.03$ ) (Figure 1A). Similarly, *Chlorophyll b* levels were not significantly different for the first 3 weeks of observation ( $p > 0.064$ ), but were significantly lower in the treatment group for the last week of observation ( $p = 0.011$ ) (Figure 1B). Carotenoid levels were not significantly different throughout the observation period ( $p > 0.85$ ) (Figure 1C). Negative values for Chl b are most likely underestimates due to the inaccuracy of the regression estimates (Porra 2002), but they are still valuable for comparing relative differences between treatments over time.

### *Fluorescence*

Chlorophyll fluorescence values ( $F_v/F_m$ ) were not significantly different between the control versus salt treatment groups over the duration of the experiment ( $p > 0.85$  and  $p > 0.558$ , respectively) (Figure 2). Mean  $F_v/F_m$  values ranged from 0.81 to 0.83 for all measurements.

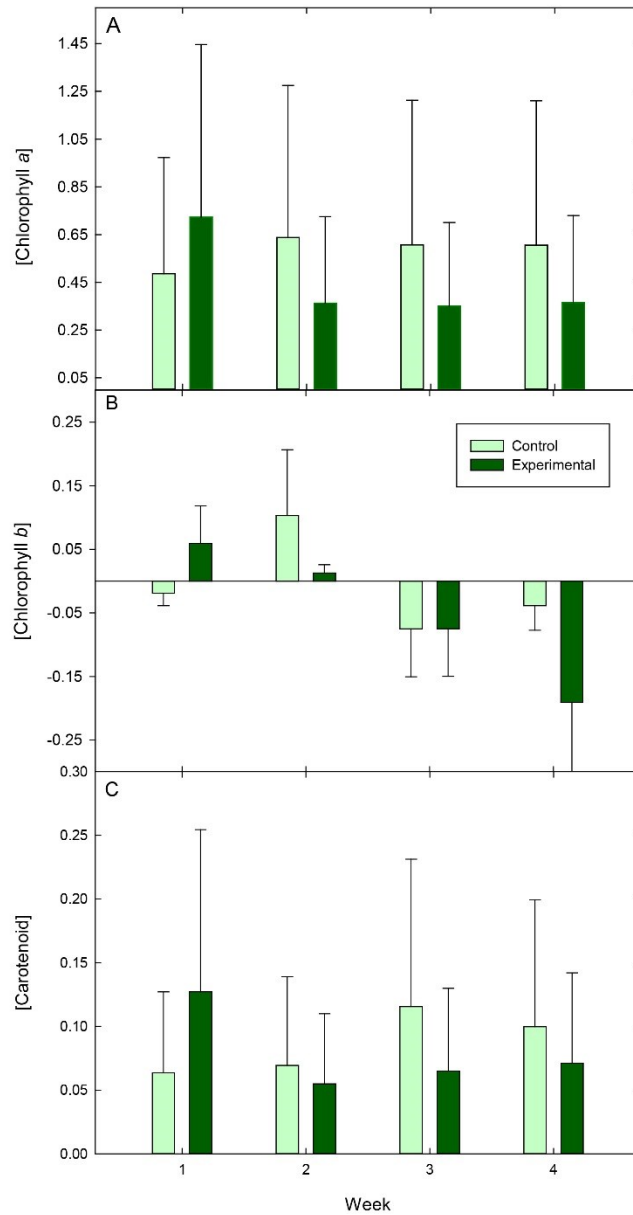


Figure 1: Mean concentration of chlorophyll *a/b* and carotenoids (nmol/ml photosynthetic pigments) present in *Taxodium distichum* leaves for the control and treatment groups (error bars show  $\pm$  standard deviation).

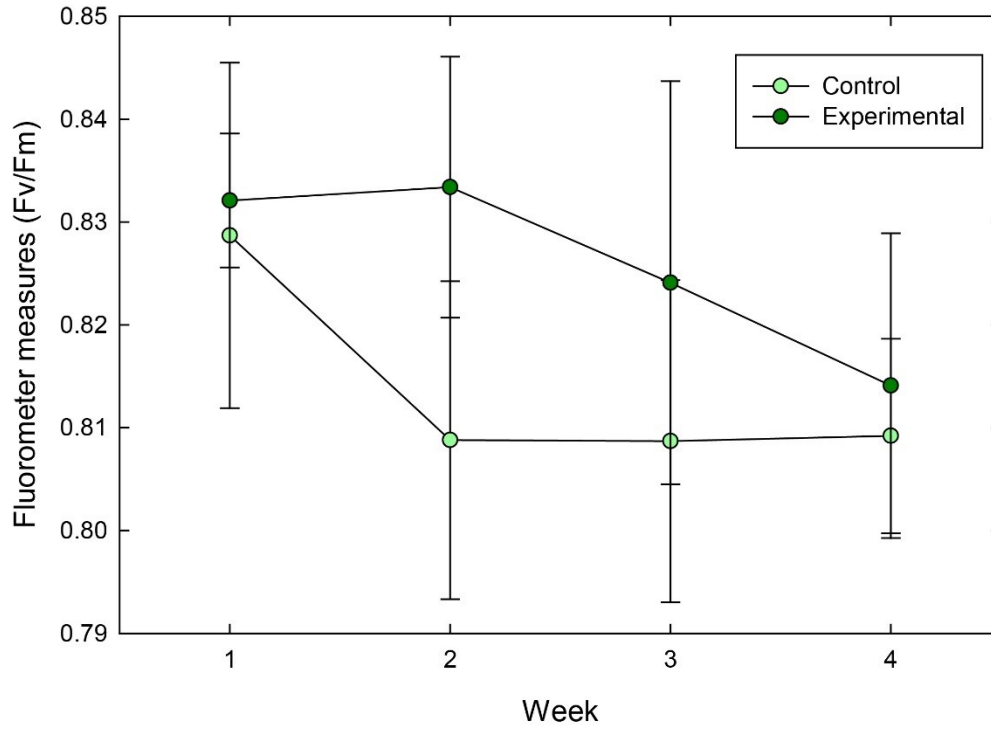


Figure 2: Mean fluorometer readings (Fv/Fm ratio) for the control and treatment groups (error bars show  $\pm$  standard deviation). Higher values near 1.0 indicate a healthier photosystem for generating the high energy compounds needed for the carboxylation reactions of photosynthesis.



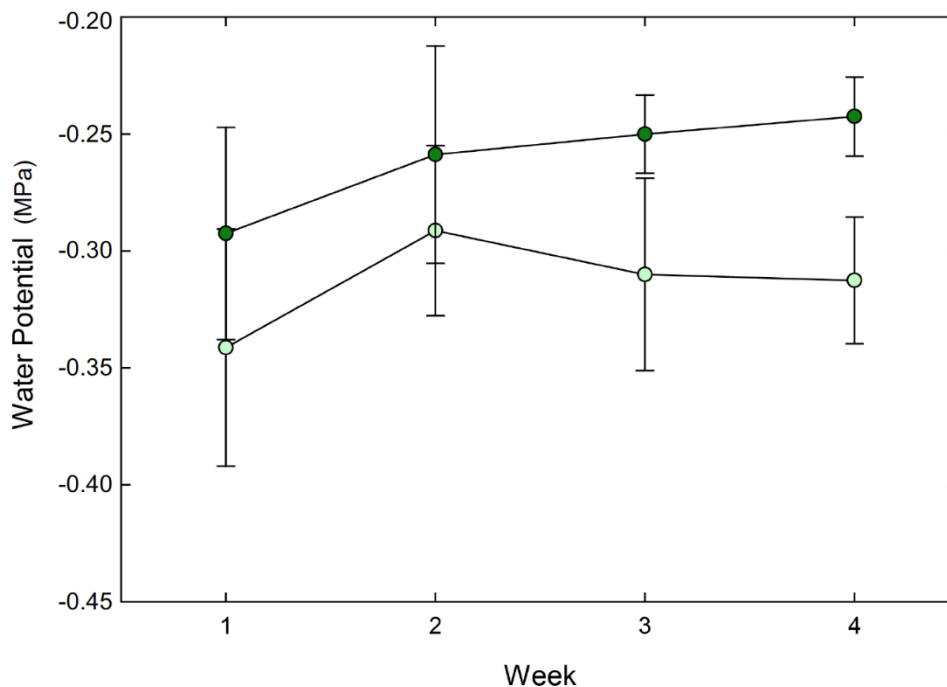


Figure 3: Mean predawn leaf water potentials for the control (dark symbols) and treatment (light symbols) groups (error bars show are  $\pm$  one standard deviation). Predawn values indicate the maximum hydration level at the start of the daylight period and before stomatal opening initiates the typical drop in water status that continues throughout the day.

#### *Predawn water status*

Predawn water potential values ( $\psi$ ) for the treatment group were significantly lower ( $p < 0.036$ ) than the control group over the entire 4-week duration of the experiment (Figure 3).

#### **4. Discussion**

In our experiments on *T. distichum*, salinity exposure had a significant effect on the treatment group compared to the control group in significantly lowering plant water status. Declines in primary photosynthetic pigments (chl a and b) also occurred.

Yet, there was no change in the health of the light reaction system of photosynthesis, as indicated by unchanged fluorescence ( $F_v/F_m$ ) values (a direct measure of relative photosynthetic health). Notably, this unchanged  $F_v/F_m$  was despite the declines in the photosynthetic pigments that occurred in the higher salinity treatment.

Plant responses to salinity exposure have been reported for a large number of species in a variety of physiological and growth processes (Parida et al. 2005). The specific responses of *T. distichum* trees to salinity exposure has included inhibition of such factors as growth, photosynthetic capacity, and root function (Allen et al. 1996, Allen et al. 1994, Pezeshki 1991, Zhou et al. 2010). However, plants that tolerate salinity exposure especially well have been shown to develop a method of excluding excess sodium and calcium ions from leaf tissue by compartmentalization in the leaf cell vacuoles (Allen 1996). In another experiment, individuals were given a treatment of increased salt water to examine salt water tolerance among several plant species, and examined the possible increase in salt tolerance of forests overall by examining individual resistances (Allen et al. 1996). An interesting response documented in Bald cypress is that seedlings appeared to tolerate fairly well the impacts of salinity exposure following over-wash flooding during especially high tides and storm surges (Pezeshki 1990). This report also showed that compounding stress factors can elicit a much different response when occurring together (synergistically) rather than independently, e.g. salt concentration and flooding intensity.

In terms of photosynthetic performance, specifically, it has been hypothesized that a decline in photosynthesis with salinity exposure could also be due to the inhibition of root function (Allen 1995). However, prolonged salinity exposure can also be harmful to leaf nutrient contents in response to differences in salinity exposure across two *Taxodium* genotypes and one hybrid (Zhou et al. 2010). Salinity treatments were applied in weekly pulses and the observation period was several months long. This treatment protocol was detrimental to growth and photosynthetic capacity that was also associated with increases in concentrations of foliar NA ion. The data from this experiment appear to support our results, whereby the xylem water potential measurements in the experimental group declined significantly over

the entire observation period. Also, the reduction in photosynthetic pigment production (chlorophyll a and b), indicated by the chlorophyll extraction data, would also support these findings. However, the measured fluorescence values did not change with the elevated salinity exposure, indicating a certain amount of salinity tolerance in the light reactions of photosynthesis, despite measured reductions in primary photosynthetic pigments (Rosenthal and Camm 1996) and lowered water status. It appears from our study that Bald cypress photosynthesis may be able to tolerate a certain level of transient salinity exposure due to over-wash events, but more research is needed to predict the ultimate impacts on photosynthesis. Moreover, such factors as increased sea level rise and a greater frequency and intensity of salt water over-wash events in a climate-change future also need to be evaluated.

Climate change is a phenomenon that is affecting a wide variety of species and ecosystems around the globe, including the eastern seaboard of the southeastern USA. Two results of these changes include rising sea levels and increased storm activity, both of which will lead to greater over-wash events with higher salinity levels within the wetland ecosystems found along oceanic coastlines. Bald cypress is an endemic foundational species of these freshwater wetland ecosystems that generate a host of critical ecosystem services. For example, these coastal wetland ecosystems are fundamentally important for combining fresh water coming from terrestrial sources and salt water from the ocean, facilitating a number of critical reproductive life stages for both freshwater and saltwater species that depend on brackish water.

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