LINKING PHYSIOLOGICAL RESPONSES, CHLOROPHYLL FLUORESCENCE AND HYPERSPECTRAL IMAGERY TO DETECT ENVIRONMENTAL STRESS IN COASTAL PLANTS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science.

- Albert Einstein

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ABSTRACT

THE RELATIONSHIP BETWEEN LEAF OPTICAL PROPERTIES AND PHYSIOLOGICAL RESPONSE FOR STRESS DETECTION IN COASTAL PLANT SPECIES

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Environmental stresses may temporarily affect the photosynthetic apparatus, especially photosystem II (PSII) before irreversible morphological damage is apparent. Stress detection prior to visible damage could permit quick and accurate assessment of the physiological response to environmental stress at the landscape level, revealing spatial variation in the physiological status of plants and the environment. Non-invasive remote sensing techniques, such as chlorophyll fluorescence and plant reflectance, have been developed to monitor plant stress and photosynthetic status, and to detect and predict changes in the natural environment. The objective of this research was to investigate the effects of multiple environmental stressors on plant physiology using remote sensing detection in both laboratory and field experiments. Laboratory studies examining the effects of salinity and drought on physiology and chlorophyll in two coastal plants, *Myrica cerifera* and *Phragmites australis* revealed that xanthophyll-cycle dependent

energy dissipation may be the underlying mechanism in protecting photosystem II from excess energy in these plants. Freshwater and saltwater flooding experiments showed that *M. cerifera* is able to survive short-term saltwater flooding by gradually closing stomata and dissipating excess light energy via the xanthophyll cycle without damage to PSII. The physiological reflectance index (PRI) was effective at tracking changes in fluorescence at the leaf-level scale in salinity flooded plants. Field experiments revealed that under conditions of drought, *M. cerifera* is able to effectively dissipate excess light and drought stress is not detectable via fluorescence or reflectance. However, salinity appeared to be a factor responsible for patterns of stress across the landscape, and was detectable using PRI from airborne hyperspectral imagery in the dominant woody vegetation on a Virginia barrier island. These findings have implications for monitoring the effects of climate change in coastal systems and suggest that PRI may be used for early identification of salt stress that may lead to changes in plant distributions at the landscape level as a result of rising sea-level and increased storm intensity.

CHAPTER 1

STRESS DETECTION IN COASTAL PLANTS

Introduction

Physical disturbances occur over spatial and temporal scales, and are important in the consideration of ecosystem structure and function. Species distributions and community composition also affect ecosystem function, and are in turn influenced by biotic and abiotic processes. As global climate continues to change and as ecological communities are exposed to anthropogenic factors, the ability of species to respond to changes in the environment will ultimately determine survival in a particular habitat (Helmuth et al., 2005, Harley et al, 2006). Understanding the physiological mechanisms that impact community structure are critical in predicting how disturbances will influence future community patterns and distributions. In order to make predictions about ecosystem response, scaling up from individual physiological responses to the landscape is essential (Bazzaz, 1993, Caveder-Bares and Bazzaz, 2004, Helmuth et al., 2005).

Stress detection in plants before visible symptoms are apparent is required to prevent severe damage and unnecessary mortality (Lichtenthaler et al., 2000, Caveder-Bares and Bazzaz, 2004). Stress may be apparent in morphological and physiological characteristics, which represent integrated responses to multiple environmental factors. Optically, these characteristics can be measured independently using reflectance or fluorescence remote sensing. Experiments in the laboratory setting examine the effects of specific stressors on the physiology of a plant and compare that to control or non-

stressed conditions, while interactions among stressors are often ignored. Reflectance has been traditionally used as a method of remotely detecting stress in plants; however, plant response is the same regardless of the type of stress (e.g. changes in water and chlorophyll content). Fluorescence appears promising at detecting specific stressors, but there are many challenges to using it in the natural environment, where multiple stressors occur. Research examining interacting stressors, methods to sort potential stressors in the field, and identifying the fluorescence signal in reflectance data is key to using fluorescence remote sensing on a large scale (Caveder-Bares and Bazzaz, 2004, Weng et al., 2006). Experiments conducted at the leaf level can relate physiological responses to fluorescence for specific types of stress. Scaling up to landscape level can be achieved by linking fluorescence to reflectance data. **I hypothesize that specific environmental stress can be detected using a combination of remote sensing methods, and that information obtained at the leaf level can be used to explain spatial and temporal variations in the landscape.**

Background

Remote sensing methods and indices have long been used to characterize the type, amount, and spatial distribution of vegetation, but have had mixed success with estimating canopy level photosynthesis and productivity (Dobrowski et al., 2005). Vegetation indices have been developed based on changes in plant reflectance, which is sensitive to changes in biomass, chlorophyll content and plant water status. However, most of these indices have no real direct link to photosynthetic function. By combining

remote sensing methods, large-scale detection of plant physiology and response to environmental stress is possible.

Because many plants are exposed to excess light energy than is used in photochemistry, numerous mechanisms that safely dissipate excess light have evolved to prevent photodamage (Flexas and Medrano, 2002). In healthy plants, the excited chlorophyll molecule is subjected to various competing de-excitation reactions including photosynthesis, heat loss, and fluorescence, which is the production of red and far red light in photosynthetic tissues upon excitation with light in the visible spectrum. Under conditions of stress, these mechanisms for disposing of excess energy do not work efficiently, thus causing changes in the competing reactions.

Photosynthetic chemistry causes the reaction centers of each photosystem to become fully reduced (or closed), and subsequent photosynthesis cannot take place until the reactions centers are oxidized (or open). When the reaction centers of photosystem II (PSII) are closed, fluorescence yield increases. Any physiological process that affects the function of PSII and associated de-excitation pathways will have an effect on chlorophyll fluorescence, since the fluorescence signal is assumed to originate primarily from PSII (Krause and Weis, 1991). Changes in chlorophyll function take place before changes in chlorophyll content, therefore alterations in the fluorescence signal occur before any visible signs are apparent. The percentage of absorbed light used in photosynthesis or dissipated as heat can be estimated by chlorophyll fluorescence parameters, and is directly related to plant physiological processes (Flexas and Medrano, 2002).

There is extensive literature on stress tolerance in plants, and a great deal of effort has been dedicated to monitoring stress using non-invasive remote sensing methods (Dobrowski et al., 2005). Traditionally, reflectance has been used to characterize stress associated with the visible and near infrared region of the optical spectrum. Plant spectral reflectance is sensitive to stress at 535 – 640nm and 685 – 700 nm visible light wavelengths, due to changes in chlorophyll reflectance and absorption (Carter, 1993). Changes in the near infrared region (700-1200 nm) are due to biomass, while leaf turgor pressure are seen in the middle infrared (1500 – 1800 and 2100 - 2300 nm; Jensen, 2000). Thus, reflectance indirectly indicates changes in plant physiology due to environmental stress. However, plants are generally considered as stress integrators (Chapin 1991) because they respond in the same manner regardless of the environmental stress (e.g. temperature, nutrients, light, etc.).

Many vegetation indices have been developed from remotely sensed reflectance data to detect changes in canopy structure and pigmentation, but most have no direct link to photosynthetic functioning (Dobrowski et al., 2005). One exception is the photochemical reflectance index (PRI), also known as the physiological reflectance index, which is linked to the xanthophyll cycle, a non-photochemical de-excitation pathway (Gamon et al., 1990). Peñuelas et al. (1997) found a significant linear, inverse relationship between PRI and the fluorescence yield of PSII in a light adapted leaf under progressive water stress. Significant correlation between PRI and nonphotochemical quenching have also been observed in drought stressed plants (Evain et al., 2004). Studies of the PRI have been primarily focused on changes in light, nutrient level, and

water status (Gamon et al. 1997, Strachan et al 2002, Dobrowski at al. 2005, Weng et al., 2006). Investigation into the application of PRI to other environmental stressors may be useful for linking leaf level measurements to the landscape.

There are several approaches that may be used to evaluate chlorophyll fluorescence emission of plants under stress. By far, the most applied method is pulse amplitude modulated (PAM) fluorescence in which a saturating pulse of light is used to monitor photosynthetic activity of plants under various stressors (Agati, 1998, Baker and Rosenqvist, 2004). Numerous parameters can be estimated from these saturating pulses including dark-adapted maximum quantum efficiency (F_v/F_m), and light adapted values including energy harvesting efficiency of PSII (F_v/F'_m), fraction of absorbed photons used in photochemistry ($\Delta F/F'_m$), steady-state fluorescence (F_s), electron transport rate (ETR), photochemical quenching (q_P) and non-photochemical quenching (q_N).

The dark-adapted parameter for maximum quantum efficiency has been widely used to detect changes in the photosynthetic apparatus due to stress (Baker and Rosenqvist 2004), but some studies have shown that this parameter may not be effective in detecting drought and salinity stress before visible signs are apparent in plants from high light environments. Laser and flash light induced fluorescence imaging can detect spatial differences in the fluorescence signals across a leaf (Buschman et al. 2000, Barbagallo et al. 2003); however, laser based fluorescence for practical remote sensing applications, especially in field studies, remains to be developed (Schuerger et al. 2003). Because chlorophyll fluorescence cannot be monitored from a distance due to a weak signal (less than 2% of total reflected visible light), finding the fluorescence signal in

reflectance data will enable rapid large-scale detection of plant physiological status. It is important that research is focused into light-adapted measurements of fluorescence since dark-adaptation is not currently feasible at scales beyond the leaf level.

Steady-state fluorescence (F_s) and other light-adapted parameters are easily applied to field situations and are possible to use at the canopy scale. Zarco-Tejada et al. (2003) and Dobrowski et al. (2005) demonstrated the superposition of the dual band emission spectra of F_s on canopy level reflectance measurements using the difference reflectance spectra between heat stressed and unstressed canopies. As a result, two ratio-based indices have been found to quantify the Fs contribution to canopy level reflectance, R_{690}/R_{600} and R_{740}/R_{800} (Dobrowski et al., 2005). Steady-state fluorescence has also been linked to photosynthetic function of water stressed plants (Flexas et al., 2000; Carter et al., 1990). Further exploration into the link between F_s and physiological status of the plant is an area of active research, as well as identifying the F_s signal in reflectance data (Dobrowski et al., 2005).

Fluorescence and reflectance measurements are ideal because they are rapid, potentially stressor specific, and can be made with portable instrumentation. These techniques when supported with physiological measurements should permit quick and accurate assessment of the physiological response to environmental stress. Identified responses can be extrapolated to the landscape level, revealing spatial variation in the physiological status of plants and the environment. There are ongoing research efforts to utilize remote sensing methods for photosynthesis and stress monitoring that has been applied to both agricultural systems and natural systems. Remote sensing detection

enables rapid assessment of plant health in response to climate change, sea level rise, and other physical and biotic disturbances. The ability to detect plant stress on a large scale before visible signs are apparent will enable land managers to respond and take appropriate actions to prevent catastrophic loss.

My research objective was to investigate the effects of multiple environmental stressors on plant physiology using remote sensing detection in both laboratory and field experiments. Specific goals were as follows:

- **For coastal species that form monospecific canopies (*Myrica cerifera* and *Phragmites australis*) link leaf fluorescence patterns, reflectance, and physiological responses with different kinds and degrees of environmental stress.**

- **Extend laboratory results to field situations to link field measurements of leaf fluorescence and reflectance to natural environmental stress (drought, salinity, flooding) and to use responses to sort and identify potential stressors.**

The following chapters examine these objectives in detail and are written in the style of the manuscript in which each was published or submitted. Chapter 2 is a laboratory study on the effect of salinity and drought stress to the physiology and fluorescence of *Myrica cerifera* and *Phragmites australis*. It is published in *Physiologia Plantarum* (2007), 131: 422-433. Chapter 3 examines the effect of freshwater and saltwater flooding stress on the physiology, fluorescence and reflectance of *Myrica cerifera*. It is published in

Environmental and Experimental Botany (2008), 63: 402-409. Chapter 4 is a spatiotemporal field study focused on the physiology, fluorescence, and reflectance of *Myrica cerifera*. It has been accepted by *Remote Sensing of Environment.* Chapter 5 is a landscape level study that examines spatial variations in stress in *Myrica cerifera* and *Iva frutescens* during a severe drought and compared to an extremely wet year. It is in review for *Plant Ecology*.

CHAPTER 2

LINKING LEAF OPTICAL PROPERTIES TO PHYSIOLOGICAL RESPONSES FOR STRESS DETECTION IN COASTAL PLANT SPECIES

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Abstract

Effects of salinity and drought on physiology and chlorophyll fluorescence were used to evaluate stress in two coastal plants, *Myrica cerifera* (L.) and *Phragmites australis* (Cav.) Trin. ex Steud. Drought and salinity stress were induced and measurements of stomatal conductance, photosynthesis, xylem pressure potential, and fluorescence were conducted following treatment. The onset of stress began at 2 g L^{-1} for *M. cerifera*, and 5 g L^{-1} for *P. australis*, as seen by significant decreases in physiological measurements. Despite the physiological effects of salinity, there was no significant difference in dark-adapted fluorescence (F_v/F_m) for either species at any salinity level. Significant decreases in the light-adapted measurement $\Delta F/ F'_m$ occurred at 10 g L^{-1} in *M. cerifera* and *P. australis*, days before visible stress was evident. NPQ increased with decreasing $\Delta F/ F'_m$. Drought studies showed similar results, with significant decreases in physiological measurements occurring by day 2 in *M. cerifera* and day 4 in *P. australis*. Differences in $\Delta F/ F'_m$ were seen by day 5 for both species, while F_v/F_m showed no indication of stress, despite apparent visible signs. Xanthophyll-cycle dependent energy dissipation may be the underlying mechanism in protecting photosystem II from excess energy in salinity and drought treated plants.

Abbreviations – PSII, photosystem II; PPFD, photosynthetic photon flux density; g_{wv}, stomatal conductance; A_{Net}, net photosynthetic rate; ψ, xylem pressure potential; Fo, minimal fluorescence in dark-adapted leaves; F_m, maximal fluorescence in dark-adapted leaves; F_v/F_m, maximum quantum use efficiency of PSII in the dark-adapted state; F'_o, minimal fluorescence in light-adapted leaves; F'_m, maximal fluorescence in light-adapted leaves; F_s, steady-state fluorescence $\Delta F/ F'_m$, fraction of absorbed photons that are used for photochemistry in a light-adapted leaf; F'_v/F'_m, effective quantum use efficiency of PSII in the light-adapted state; H-F, Huynh-Feldt; PRI, physiological reflectance index.

Introduction

Plants are usually exposed to more radiant energy than is needed for photosynthesis, and have evolved numerous mechanisms that safely dissipate excess light to avoid photoinhibition and photooxidation (Flexas and Medrano 2002). Under conditions of stress, these mechanisms for disposing of excess energy do not work efficiently, thus causing changes in the competing reactions of photochemistry, heat loss, and fluorescence. Any process that affects the function of photosystem II (PSII) and associated de-excitation pathways will have an effect on chlorophyll fluorescence, since the fluorescence signal is assumed to originate primarily from PSII (Krause and Weis 1991). Changes in chlorophyll function take place before changes in chlorophyll content, and therefore alterations in the fluorescence signal occur before any visible signs are apparent (Krause and Weis 1991). The percentage of absorbed light used in photosynthesis or dissipated as heat can be estimated by chlorophyll fluorescence

parameters, and is directly related to plant physiological processes (Flexas and Medrano 2002). Understanding the physiological mechanisms of various environmental stressors is critical in predicting how disturbances will influence future plant community patterns and distributions.

The physiological condition of plants is indicative of plant productivity, adaptability to stress, and a general indication of the environment in which they grow (Zarco-Tejada et al. 2002). Plant growth depends on photosynthesis, which is affected by environmental factors such as salinity, drought, temperature, and light. Stress may be apparent in morphological and physiological characteristics, which represent integrated responses to multiple environmental factors. Early detection of stress could identify plant physiological condition at larger spatial and temporal scales before visible effects are apparent (Zarco-Tejada et al. 2002). The dark-adapted parameter for maximum quantum use efficiency (F_v/F_m) has been widely used to detect changes in the photosynthetic apparatus due to stress (Baker and Rosenqvist 2004). This parameter is not efficient for large scale use in the field since it is problematic to dark-adapt an entire canopy. Research into light-adapted measurements of fluorescence is imperative as dark-adaptation is not currently practical at scales beyond the leaf level. Steady-state fluorescence and other light-adapted parameters are easily applied to field situations, as dark-adaptation is not required, and light-adapted measurements are possible to use at the canopy scale (Gamon et al. 1990, Zarco-Tejada et al. 2003, Evain et al. 2004).

Application of leaf level measurements to the landscape becomes increasingly difficult as species and structural diversity are increased. Not surprisingly, research has

focused on fluorescence and canopy level remote sensing for agricultural species, which usually form monospecific canopies (Gamon et al. 1990, Flexas et al. 2000, Zarco-Tejada et al. 2003, Baker and Rosenqvist 2004). Fluorescence appears promising at detecting specific stressors, but there are many challenges for use in natural environments with multiple stressors. Experiments conducted in controlled environments at the leaf level can relate physiological responses to fluorescence for known stressors, which can then be applied to field situations to identify specific types of stress. Scaling up to the landscape will be easiest if using species that form monospecific canopies naturally in the ecosystem.

The objective of our study was to evaluate the effects of drought and salinity on plant physiological responses and functionality of the photosynthetic apparatus, measured by chlorophyll fluorescence. We used two focal species that form monospecific canopies (*Myrica cerifera* and *Phragmites australis*) to link leaf fluorescence patterns and physiological responses with different kinds and degrees of environmental stress. Comparisons of light and dark-adapted measurements of fluorescence were made to determine the ability to detect stress before any visible signs were apparent.

Materials and methods

Plant materials

Myrica cerifera L., Myricaceae, (wax myrtle) is a relatively salt intolerant, evergreen shrub that forms extensive, dense thickets and is the dominant woody species on most barrier islands of the southern United States (Ehrenfeld 1990, Young et al. 1994). *Phragmites australis* (Cav.) Trin. ex Steud., Poaceae, (common reed) is an

invasive perennial grass that has formed numerous large colonies, fringing freshwater and saltwater marshes of the North American Atlantic Coast (Chambers et al. 1999). Due to limited numbers and variations in size, seedlings of *M. cerifera* were obtained from a nursery using local seed stock. Seedlings were transplanted into 2L plastic pots and acclimated for at least 4 weeks prior to experimentation. Rhizomes of *P. australis* were collected from a brackish water population at Oyster, Virginia (37° 17'N; 75° 56'W), which is located on the Eastern Shore of Virginia. Rhizomes were transplanted into 2L plastic pots and grown for 3 months in the environmental chamber before experimentation.

Induction of stress

Plants were grown in a Convrion environmental chamber (CMP 3244, Controlled Environments Limited, Asheville, NC) under a photosynthetic photon flux density (PPFD) of approximately 700 μmol m^{-2} s^{-1}, 48% relative humidity, a photoperiod of 14 h, and a day/night temperature of 30/25 °C. Exposure to soil salinity was increased weekly with concentrations of 0, 2, 5, 10, and 15 g L^{-1} for *M. cerifera* and up to 20 g L^{-1} for *P. australis*, using dilutions of a commercial mixture that approximates total ocean salts (Instant Ocean, Aquarium Systems, Mentor, OH). Major cations present in the mixture are Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} (5.1, 0.18, 0.19, 0.62 g L^{-1}, respectively) and the major anion is Cl^{-} (8.9 g L^{-1}) (Atkinson and Bingman 1997). Salinity concentrations were monitored with a conductivity meter (model 33, YSI, Yellow Springs, OH; Tolliver et al. 1997). Drought stress was induced by not watering plants and responses were compared to well-watered control plants.

Measurements of gas exchange and fluorescence

Plant response to drought and salinity treatments were quantified by measuring stomatal conductance to water vapor (g_{wv}), leaf net photosynthesis (A_{Net}), mid-day leaf xylem pressure potentials (ψ), and leaf fluorescence (n =10 per treatment). Measurements were conducted mid-day (1000 – 1400 h) on days 1, 3, and 5 following salinity treatments and days 1, 2, 4, 5, and 7 for drought experiments. Rates of stomatal conductance and leaf net photosynthesis were measured using a portable infrared gas analyzer at a light intensity of 700 μmol m^{-2} s^{-1}, 48% relative humidity, and 28 °C (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE). Mid-day leaf xylem pressure potentials were quantified with a Scholander pressure chamber (Model 650, PMS, Corvallis, OR).

Light-adapted and dark-adapted measurements of chlorophyll fluorescence were conducted on the fourth or fifth fully expanded leaf of each plant using a pulse amplitude modulated leaf chamber fluorometer (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE). Minimal fluorescence values in the dark-adapted state (F_o) were obtained by application of a low intensity far-red measuring light source, while maximal fluorescence values (F_m) were measured after applying a saturating light pulse of 8000 μmol m^{-2} s^{-1}. Minimum (F'_o) and maximum (F'_m) values of fluorescence in the light-adapted state at 700 mol m^{-2} s^{-1} were also obtained in this manner. Using these parameters, the following ratios were calculated: effective quantum use efficiency of PSII in the light-adapted state, $F'_v/F'_m = (F'_m - F'_o)/ F'_m$, fraction of absorbed photons used for photochemistry, $\Delta F/F'_m = (F'_m - F_s)/F'_m$, non-photochemical quenching, $NPQ = (F_s/ F'_m) – (F_s/F_m)$ (Hendrickson et al. 2004), and maximum quantum use efficiency of PSII in the dark-adapted state, $F_v/F_m =$

$(F_m - F_o)/F_m$. Leaves were dark-adapted for 30 min using dark-adapting leaf clips (LI-COR Biosciences, Inc., Lincoln, NE) for F_v/F_m measurements.

Statistical Analysis

Variations in photosynthetic characteristics, stomatal conductance, leaf xylem pressure potential, and fluorescence relative to control plants over time were analyzed with repeated measures analysis of variance for each stress experiment (Zar 1999). Day was specified as the repeated factor (within-subject), and treatment as fixed effect (between-subjects). The validity of a within- subjects test depends on sphericity of the data (Von Ende 1993). A measure of deviation that addresses this assumption, the Huynh-Feldt (H-F) correction (Huynh and Feldt 1976), was calculated and adjusted *P* values (H-F *P*) were reported. Significant differences in treatment means for individual days were identified with Tukey tests ($\alpha = 0.05$). In addition, differences in fluorescence were identified and evaluated in response to the specific stressor.

Results

Physiological responses to salinity

Both species showed physiological responses to salinity treatments at low salinity levels. There was a significant day x treatment interaction for stomatal conductance ($F = 10.80$, $P < 0.0001$), photosynthesis ($F = 10.37$, $P < 0.0001$) and leaf xylem pressure potential ($F = 5.83$, $P < 0.0001$) in *M. cerifera*. Tukey multiple comparisons for individual days revealed that control and treatment plants differed significantly by day 3 of 2 g L^{-1} salinity for both stomatal conductance and photosynthesis (Fig. 2.1), and remained significantly different throughout the experiment. This was followed by a

significant decrease in leaf xylem pressure potential from -0.46 ± 0.04 on day 0 of salt-stressed plants to -0.67 ± 0.07 on day 5 of 2 g L^{-1} salinity ($F = 9.30$, $P < 0.0001$; Fig. 2.1). Visible signs of stress (i.e. browning and fallen leaves) were not observed in treatment plants until day 5 of 10 g L^{-1}.

Phragmites australis also exhibited significant day x treatment interactions for stomatal conductance ($F = 17.96$, $P < 0.0001$), photosynthesis ($F = 18.35$, $P < 0.0001$), and xylem pressure potential ($F = 2.56$, $P = 0.0199$). Significant differences in stomatal conductance occurred on day 1 of 5 g L^{-1} salinity for *P. australis* between control and treatment plants, followed by a significant decline in leaf xylem pressure potential by day 3 of 5 g L^{-1} (Fig. 2.1). Photosynthesis in salinity treated plants remained significantly lower than control plants by day 3 of 10 g L^{-1} salinity (Fig. 2.1), and continued to decrease throughout the experiment. Visible signs of stress were apparent by day 3 of 15 g L^{-1} in salinity treated plants of *P. australis*.

Chlorophyll fluorescence responses to salinity

A significant day x treatment interaction occurred in the light-adapted measurements $\Delta F/F'_m$ ($F = 3.14$, $P = 0.0005$) and F'_v/F'_m ($F = 14.49$, $P = 0.0019$) in *M. cerifera*. NPQ ($F = 3.46$, $P = 0.0005$) increased in salinity treated plants. Significant differences in $\Delta F/F'_m$, F'_v/F'_m and NPQ occurred by day 1 of 10 g L^{-1} salinity (Fig. 2.2), 4 days before signs of visible stress. Control plants had an average $\Delta F/F'_m$ value of 0.55 ± 0.01 and F'_v/F'_m value of 0.60 ± 0.00. By day 1 of 10 g L^{-1}, $\Delta F/F'_m$ of salinity treated plants was 0.37 ± 0.04, which continued to decline to 0.29 ± 0.02 at the end of the experiment. Average NPQ for control plants was 0.26 ± 0.01. NPQ increased to 0.50 ±

0.01 by the last day in salinity treated plants. There were no significant differences in F_v/F_m between control and treatment plants over the course of the experiment ($F = 2.48$, $P = 0.0741$; Fig. 2.2). The average F_v/F_m value was 0.81 ± 0.01 for both control and salinity treated plants over the entire experiment. On the last day of measurements, the average value of salinity treated plants decreased to 0.79 ± 0.01, but was not significant.

A significant day x treatment interaction was also seen in $\Delta F/ F'_m$ ($F = 5.15$, $P < 0.0001$), F'_v/F'_m ($F = 5.08$, $P < 0.0001$), and NPQ ($F = 9.33$, $P < 0.0001$) in ***P. australis***. These significant reduction in PSII efficiency occurred by day 5 of 10 g L^{-1} salinity, 3 days prior to apparent visible stress (Fig. 2.2). *Phragmites australis* control plants had a higher $\Delta F/F'_m$ of 0.61 ± 0.01 and F'_v/F'_m value of 0.67 ± 0.00 compared to control plants of ***M. cerifera***. By day 5 of 10 g L^{-1}, F'_v/F'_m of salinity treated plants had decreased to 0.57 ± 0.02 and to 0.54 ± 0.03 by day 5 of 15 g L^{-1}. $\Delta F/F'_m$ declined to 0.32 ± 0.03 by the end of the experiment, while NPQ increased from 0.16 ± 0.01 to 0.44 ± 0.02. As in ***M. cerifera***, there were no significant changes in F_v/F_m for ***P. australis*** between control and salinity treated plants throughout the experiment ($F = 0.63$, $P = 0.6902$) despite declines in physiological responses (Fig. 2.2). By the end of the experiment, F_v/F_m values of salinity treated ***P. australis*** plants had dropped slightly to 0.78 ± 0.01 compared to control plants (0.80 ± 0.00), but this change was not significant.

Significant positive linear relationships (i.e. slope significantly different from 0) occurred between F'_v/F'_m and stomatal conductance ($r^2 = 0.40$, $F = 139.85$, $P < 0.0001$), photosynthesis ($r^2 = 0.40$, $F = 135.75$, $P < 0.0001$), and leaf xylem pressure potential ($r^2 = 0.49$, $F = 91.25$, $P < 0.0001$) for ***M. cerifera*** (Fig. 2.3). Significant positive linear

relationships were also seen between F'_v/F'_m and stomatal conductance ($r^2 = 0.25$, F = 73.98, P < 0.0001), photosynthesis ($r^2 = 0.32$, F = 106.25, $P < 0.0001$), and leaf xylem pressure potential ($r^2 = 0.25$, F = 71.98, $P < 0.0001$) for *P. australis*, but with much less predictive power (Fig. 2.3). Linear relationships were also observed between $\Delta F/F'_m$ and measurements of gas exchange for both species (data not shown).

Physiological responses to drought

Significant reductions in physiological parameters of drought treated plants were observed early in both species. *Myrica cerifera* showed significant day x treatment interactions in stomatal conductance (F = 42.55, $P < 0.0001$), photosynthesis (F = 28.42, $P < 0.0001$), and leaf xylem pressure potential (F = 12.37, $P < 0.0001$) in response to drought. Control and treatment plants differed significantly by day 2 for all three physiological parameters (Fig. 2.4). Visible signs of stress were observed on day 6 of drought treatment in some *M. cerifera* plants. By day 7, all plants showed signs of drought stress as evidenced by wilted leaves. *Phragmites australis* exhibited similar physiological responses to drought, with significant day x treatment interactions in stomatal conductance (F = 23.60, $P < 0.0001$), photosynthesis (F = 28.06, $P < 0.0001$), and leaf xylem pressure potential (F = 17.90, $P < 0.0001$). Leaf xylem pressure potential was significantly lower in drought plants by day 2 of the experiment, followed by a significant reduction in both stomatal conductance and photosynthesis by day 4 (Fig. 2.4). Drought stressed plants had rolled and wilted leaves by day 5 of the experiment.

Chlorophyll fluorescence responses to drought

A significant day x treatment interaction for $\Delta F/F'_m$ ($F = 13.39$, $P < 0.0001$), F'_v/F'_m ($F = 14.87$, $P < 0.0001$), and NPQ ($F = 11.35$, $P = 0.0001$) was seen in *M. cerifera*. Drought stressed plants had significantly lower $\Delta F/F'_m$ values by day 2 of the experiment (0.44 ± 0.01) compared to control plants (0.58 ± 0.01), which occurred 4 days prior to visible signs of stress (Fig. 2.5). $\Delta F/F'_m$ values continued to decrease until the end of the experiment, when values were down to 0.17 ± 0.01. Decreasing $\Delta F/F'_m$ values were accompanied by increasing NPQ values. By day 7 NPQ increased to 0.56 ± 0.01, compared to control plants which had an average NPQ of 0.25 ± 0.01. There were no significant changes in F_v/F_m throughout the experiment between control and treatment plants ($F = 2.42$, $P = 0.1211$), even when visible signs of stress were obvious. *Phragmites australis* also exhibited significant interactions between day and treatment for $\Delta F/F'_m$ ($F = 12.66$, $P < 0.0001$), F'_v/F'_m ($F = 15.02$, $P < 0.0001$) and NPQ ($F = 13.22$, $P < 0.0001$). Significant decreases in $\Delta F/F'_m$, F'_v/F'_m, and NPQ of drought stressed plants were also observed by day 4 of treatment (Fig. 2.5). Again, despite physiological responses and visible signs of stress, there was no significant difference in F_v/F_m between well-watered and drought stressed plants ($F = 1.61$, $P = 0.2072$).

F'_v/F'_m exhibited a significant positive linear relationship with stomatal conductance ($r^2 = 0.37$, $F = 68.34$, $P < 0.0001$), photosynthesis ($r^2 = 0.36$, $F = 66.42$, $P < 0.0001$), and leaf xylem pressure potential ($r^2 = 0.50$, $F = 119.90$, $P < 0.0001$) for *M. cerifera* (Fig. 2.6). Significant positive linear relationships were also seen between F'_v/F'_m and stomatal conductance ($r^2 = 0.45$, $F = 94.93$, $P < 0.0001$), photosynthesis ($r^2 =$

0.51, F = 122.18, $P < 0.0001$), and leaf xylem pressure potential ($r^2 = 0.43$, F = 92.38, $P < 0.0001$) for *P. australis* (Fig. 2.6) under drought stress. Again, significant linear relationships were observed between $\Delta F/ F'_m$ and gas exchange measurements for both species (data not shown).

Discussion

Myrica cerifera is salt sensitive (Young 1992) while *P. australis* is able to grow in brackish and saltwater marshes (Amesberry et al. 2000). However, both species occur in coastal environments and must withstand periods of drought and episodic flooding with brackish water. This was evident in the physiological responses to both salinity and drought treatments. As seen in the rapid decreases of stomatal conductance, photosynthesis, and leaf xylem water potential compared to *P. australis* (5 g L^{-1}), *M. cerifera* was stressed at a lower concentration of salinity (2 g L^{-1}). Stomatal closure appears to be responsible for the reductions in photosynthesis due to salinity stress in both species. This rapid physiological response by *M. cerifera* enables survival of short-term episodes of salinity by keeping tissue chloride concentrations low within the leaves (Tolliver et al. 1997). The response in *P. australis* was much slower compared to *M. cerifera*, with maintained significant photosynthetic stress not apparent until 10 g L^{-1}. Visible signs of stress were obvious earlier in *M. cerifera*, with browning and wilting leaves occurring at 10 g L^{-1}, while visible effects of salinity were not seen in *P. australis* until 15 g L^{-1}.

There is no change in the maximum quantum use efficiency of open PSII centers (F_v/F_m) in the early stages of salinity stress, indicating that salinity does not induce

sustained photodamage (Baker and Rosenqvist, 2004, Morales et al. 2005). However, some studies have shown effects on F_v/F_m after 30 minutes of dark-adaptation due to salinity stress (Belkhodja et al. 1994, Lee et al. 2004, Castillo et al. 2005), and perennial plants may be more sensitive to salinity compared to annuals (Morales et al. 2005). Other studies have shown little to no effect of salinity on F_v/F_m at low to mid salinity levels (Morant-Manceau et al. 2004, Netondo et al. 2004, Redondo-Gomez et al. 2006) even when leaf growth and gas exchange were reduced. This is because salinity initially decreases stomatal conductance, which reduces photosynthesis, leaving PSII unaffected in the early stages of stress (Baker and Rosenqvist 2004).

In our study, F_v/F_m was not affected by salinity in *M. cerifera* and *P. australis* for the duration of the experiment suggesting that there was no damage to PSII, even at mid-range salinity levels. Conversely, significant changes in the efficiency of PSII ($\Delta F/Fm'$ and F'_v/F'_m) were observed at 10 g L^{-1} in both species, prior to visible signs of salt stress. Decreases in these light-adapted fluorescence parameters were coupled with increases in NPQ in both species. Values of NPQ were higher by the end of the experiment in *M. cerifera*. Changes in fluorescence were detectable sooner in *M. cerifera* than in *P. australis*, but significant decreases in $\Delta F/F'_m$ occurred within 8-9 days of physiological stress for both species. Reductions in fluorescence measurements in *M. cerifera* were likely due to chloride toxicities. Tolliver et al. (1997) found significantly higher tissue chlorides in *M. cerifera* plants over time at 10 g L^{-1} salinity, the concentration at which changes in chlorophyll fluorescence were observed. In comparison, *Phragmites australis* is considered a salt excluder by its ability to maintain a relatively constant Na^+

concentration within the shoot tissues at different salinity levels (Vasquez et al. 2006). Therefore, osmotic stress and not salt toxicities was most likely the cause of reductions in fluorescence measurements in *P. australis*.

Decreases in $\Delta F/F'_m$ and increases in NPQ with no subsequent decline in F_v/F_m for both species suggests an enhancement in thermal dissipation in PSII in such a way as to match the decrease in photosynthesis in order to avoid photodamage (Qiu et al. 2003). Xanthophyll-cycle dependent energy dissipation may be the underlying mechanism in protecting the photosynthetic apparatus from excess energy in the salinity treated plants (Demmig-Adams and Adams 1996). Stępień and Kłbus (2006) also reported that Φ_{PS2} ($\Delta F/Fm'$) was sensitive to salt stress in *Cucumis sativus* leaves while there was no change in F_v/F_m at any salinity level. F_v/F_m began to decline in both species by the end of the salinity experiments, but this decline occurred well after visible signs of stress were apparent. Declining F_v/F_m values could indicate the possibility of photoinhibition had the experiment continued (Krause and Weis 1991). Alternatively, lower F_v/F_m could be due to sustained thermal energy dissipation after dark-adaptation associated with the xanthophyll cycle (Adams and Demmig-Adams 1995), as evidenced by high values of NPQ in stressed plants. Variations in $\Delta F/F'_m$ are more sensitive to salinity stress compared to changes in F_v/F_m. Thus, light adapted fluorescence measurements are better indicators for detecting salinity stress before severe damage occurs in these species.

Myrica cerifera and *P. australis* had similar responses to drought. All three physiological parameters were significantly lower in drought treated plants of *M. cerifera* by day 2 of the experiment. Leaf xylem pressure potential dropped by day 2 of drought

for *P. australis*, but stomatal conductance and photosynthesis were not affected until day 4. Visible signs of stress were evident earlier in *P. australis*, with rolling leaves seen by day 5 of drought as opposed to day 6 in *M. cerifera*. Again, stomatal closure was likely the mechanism responsible for reductions in photosynthesis in both species (Cornic 1994, Medrano et al. 2002). Stomatal effects on photosynthesis due to drought do not initially affect the efficiency of PSII, as measured by F_v/F_m (Flexas and Medrano 2002, Baker and Rosenqvist 2004). In our study, drought did not affect F_v/F_m for either *M. cerifera* or *P. australis*. de Mattos et al. (1997) found that coastal plants maintained high F_v/F_m values throughout the day, even at high leaf to air vapor deficit values. Saltmarsh et al. (2006) found no significant change in afternoon F_v/F_m values for *P. australis* subjected to drought over the first 7 days. F_v/F_m values in our experiment did slightly decrease by day 7, but this was not significant, and visible signs of stress were apparent in all plants by the end of the experiment. Some studies have reported significant declines in afternoon F_v/F_m as water stress increases (Souza et al. 2004, Liberato et al. 2006), while other authors have reported no significant change in F_v/F_m during drought stress or only after severe water stress (Marques da Silva and Arrabaca 2004, Miyashita et al. 2005, Subrahmanyam et al. 2006). However, these experiments did not address whether or not visible damage was evident.

Our results indicate that water stress did not cause damage to PSII by the end of the experiment, or before any visible damage to the leaves occurred, as expected. Significant decreases in $\Delta F/F'_m$ and F'_v/F'_m were evident by day 4 in both species, which occurred 1-2 days before visible signs of stress. Subrahmanyam et al. (2006) also found

significant changes in F'_v/F'_m in water stressed plants relative to control plants. A reduction in $\Delta F/F'_m$ with increasing NPQ, in the absence of change in F_v/F_m suggests an increase in thermal energy dissipation, likely due to the xanthophyll-cycle (Demmig-Adams and Adams 1996).

The possibility of xanthophyll-cycle dependent energy dissipation in both species under drought and salinity treatments may enable rapid stress detection at the canopy level. The physiological reflectance index (PRI) is sensitive to changes in de-epoxidation state of the xanthophyll cycle and subsequent accumulation of zeaxanthin (Gamon et al. 1990). $\Delta F/F'_m$ and NPQ have been correlated to PRI under drought conditions (Peñuelas et al. 1997, Evain et al. 2004). Because $\Delta F/F'_m$ and NPQ changed progressively as stress was increased in our experiment, investigation into the links between PRI and fluorescence may prove powerful for linking leaf level measurements to the landscape. Changes in fluorescence were observed prior to signs of visible stress, which may be an important consideration, especially for drought, when linking fluorescence to PRI at the canopy level (Gamon et al. 1992, Evain et al. 2004). Further research into identifying the fluorescence signal in reflectance data is key to using fluorescence remote sensing on a large scale.

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Figure Legends

Figure 2.1. Effect of salinity on stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera* and *Phragmites australis* over time. Closed symbols and open symbols represent means with standard errors for control and treatment plants, respectively.

Figure 2.2. Changes in the fluorescence measurements F'_v/F'_m, F_v/F_m, $\Delta F/ F'_m$, and NPQ for *Myrica cerifera* and *Phragmites australis* with incremental increases in salinity. Closed symbols and open symbols represent means with standard errors for control and treatment plants, respectively.

Figure 2.3. Relationships between light-adapted fluorescence (F'_v/F'_m) and stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera* and *Phragmites australis* salinity treated plants.

Figure 2.4. Effect of drought on stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera* and *Phragmites australis* over time. Closed symbols and open symbols represent means with standard errors for control and treatment plants, respectively.

Figure 2.5. Changes in the fluorescence measurements F'_v/F'_m, F_v/F_m, $\Delta F/ F'_m$, and NPQ for *Myrica cerifera* and *Phragmites australis* as drought increases over time. Closed symbols and open symbols represent means with standard errors for control and treatment plants, respectively.

Figure 2.6. Relationships between light-adapted fluorescence (F'_v/F'_m) and stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera* and *Phragmites australis* drought treated plants.

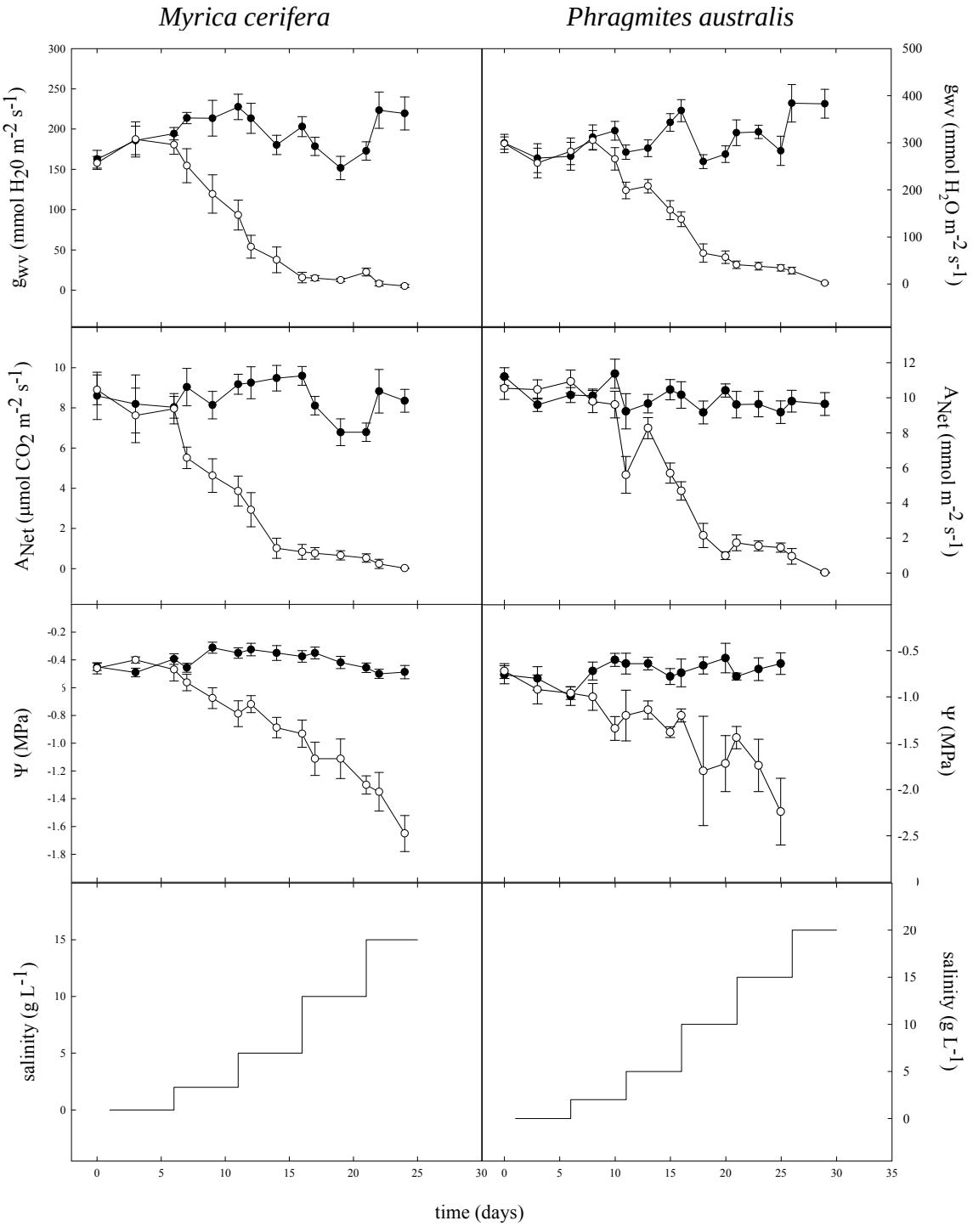
Myrica cerifera
Phragmites australis
gwv (mmol H20 m-2 s-1)
gwv (mmol H2O m-2 s-1)
ANet (µmol CO2 m-2 s-1)
ANet (mmol m-2 s-1)
Ψ (MPa)
Ψ (MPa)
salinity (g L-1)
salinity (g L-1)
time (days)

Figure 2.1

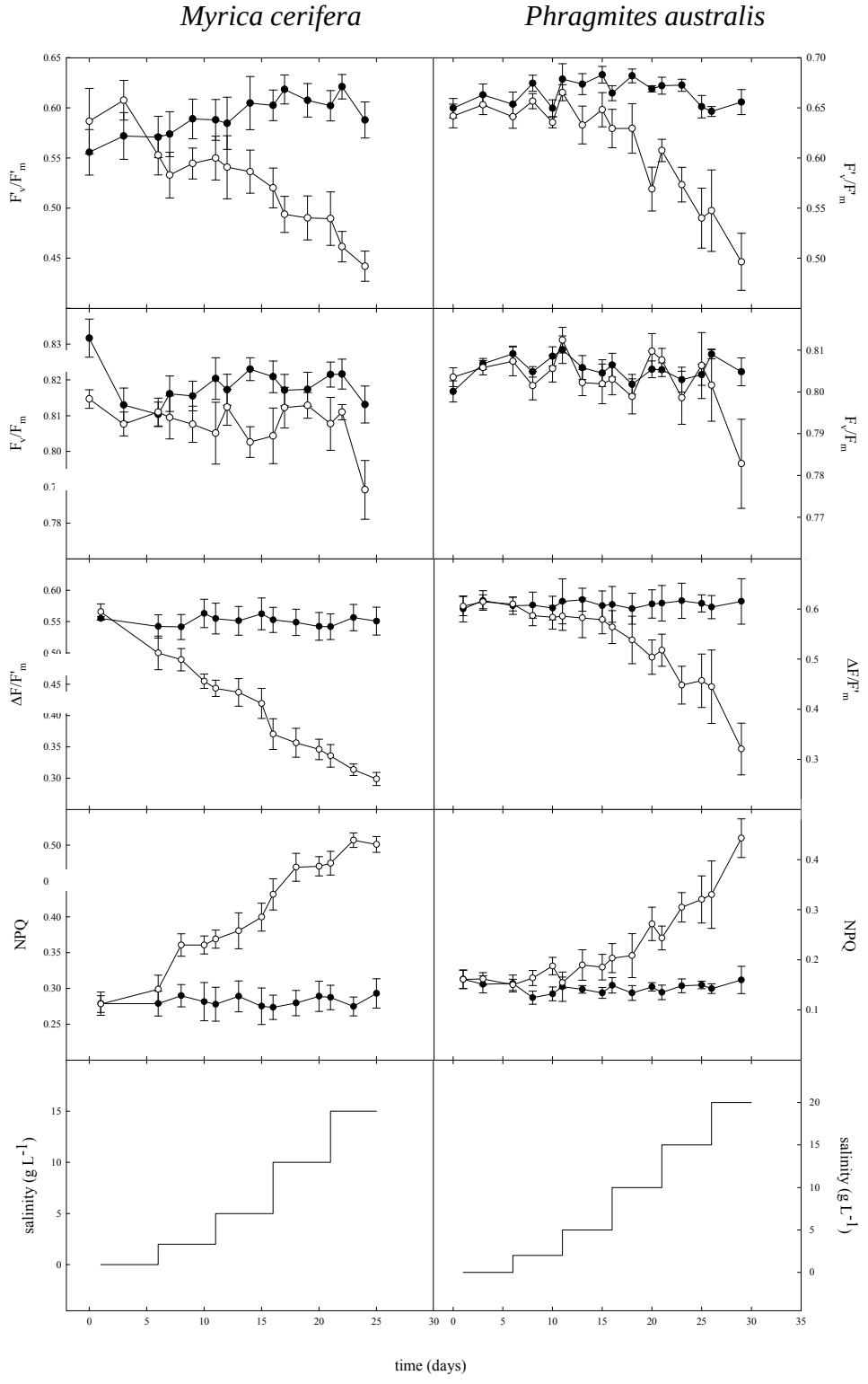
Myrica cerifera
Phragmites australis
F'v/F'm
Fv/Fm
ΔF/F'm
NPQ
salinity (g L-1)
time (days)

Figure 2.2

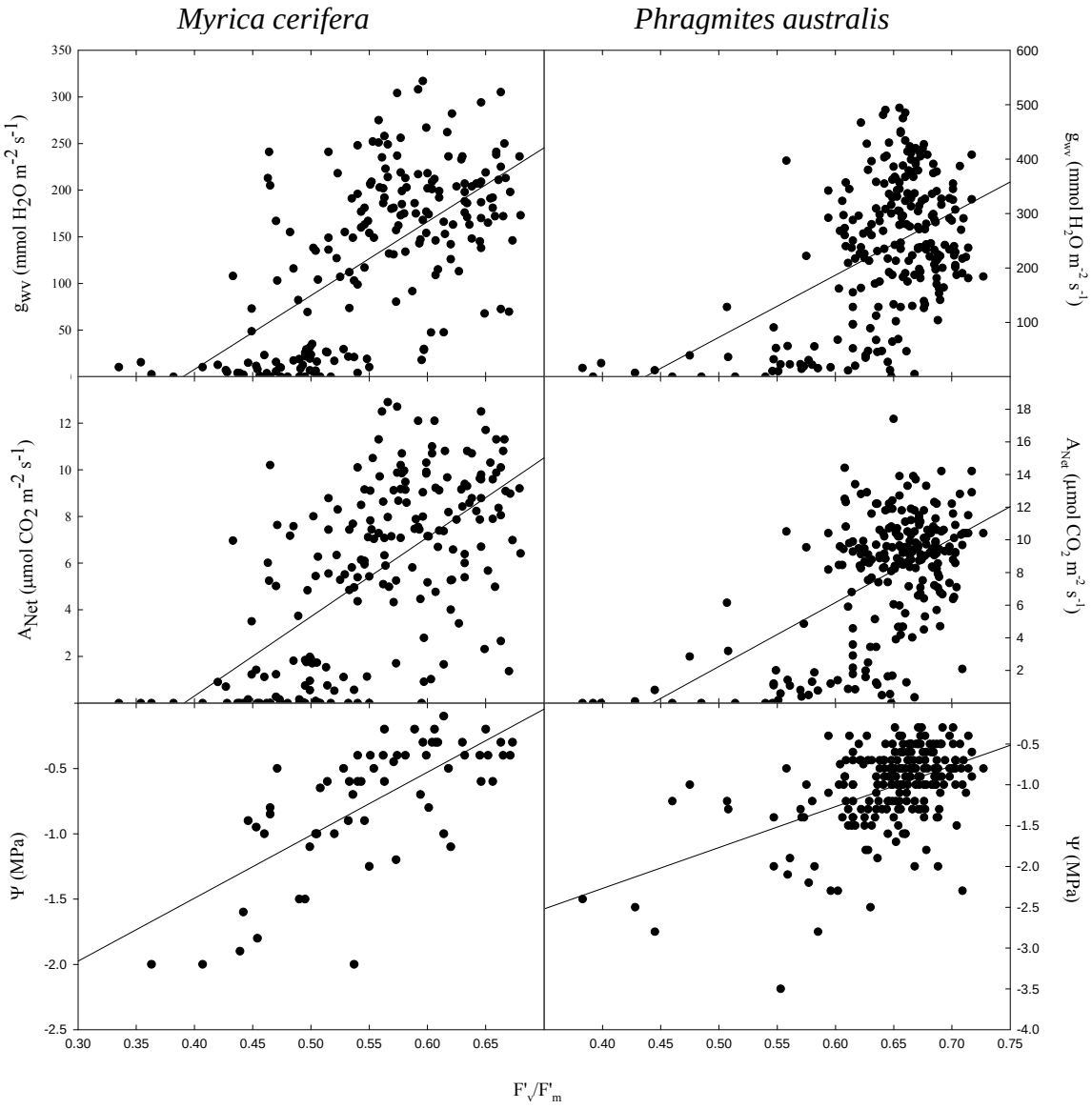
Myrica cerifera
Phragmites australis
gwv (mmol H2O m-2 s-1)
ANet (µmol CO2 m-2 s-1)
Ψ (MPa)
F'v/F'm

Figure 2.3

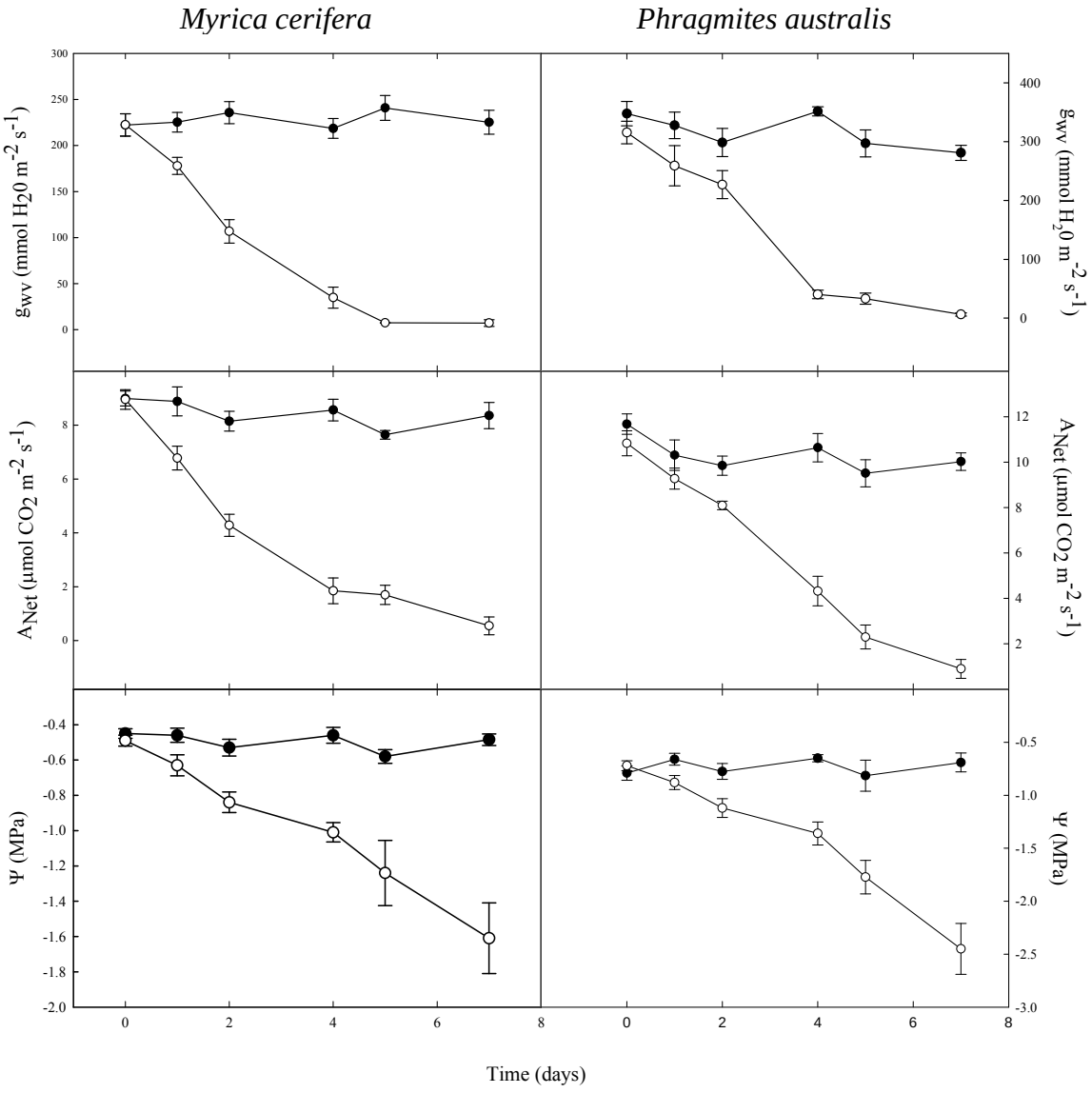
Myrica cerifera
Phragmites australis
gwv (mmol H20 m-2 s-1)
ANet (µmol CO2 m-2 s-1)
Ψ (MPa)
Time (days)

Figure 2.4

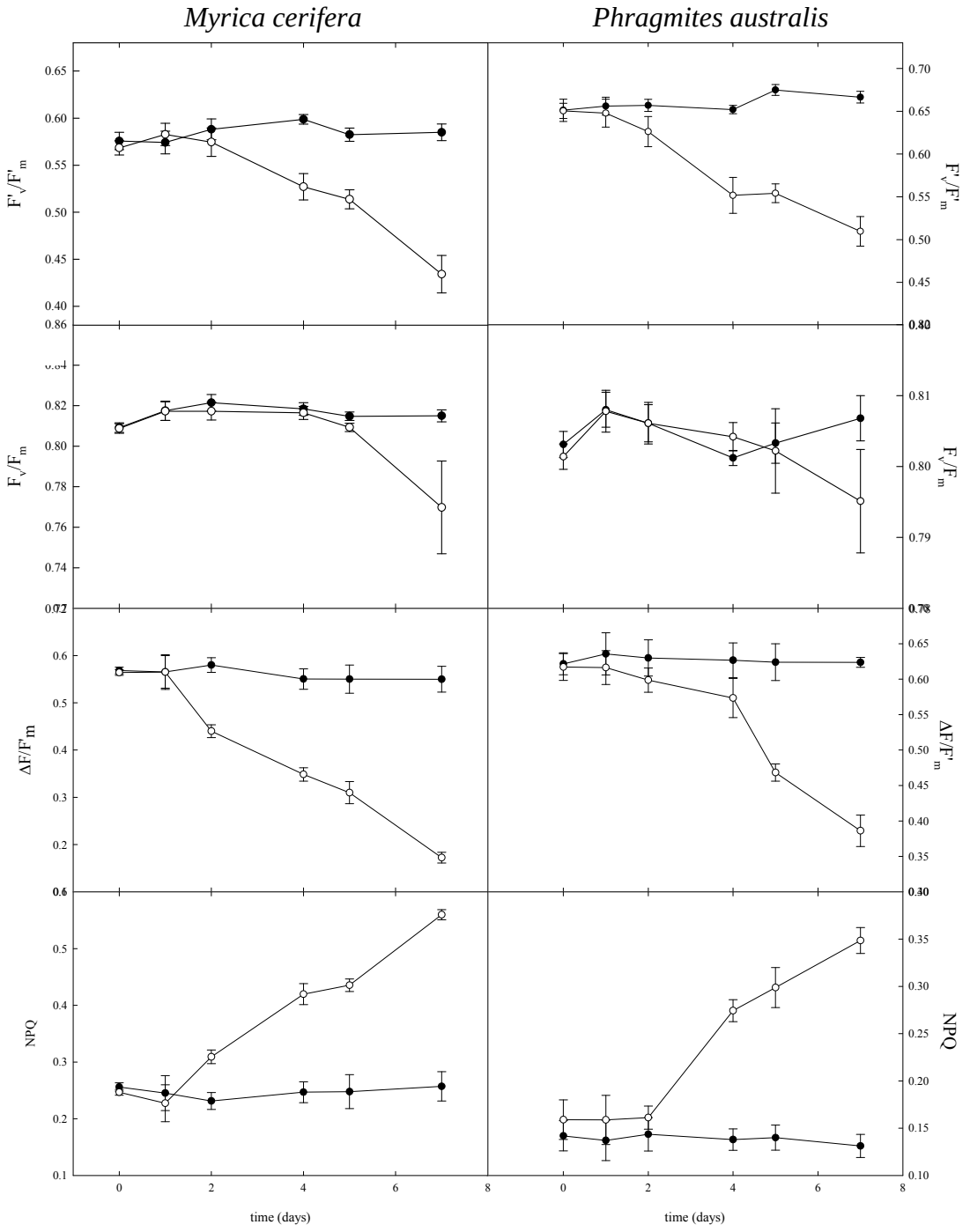
Myrica cerifera
Phragmites australis
F'v/F'm
Fv/Fm
ΔF/F'm
NPQ
time (days)
time (days)

Figure 2.5

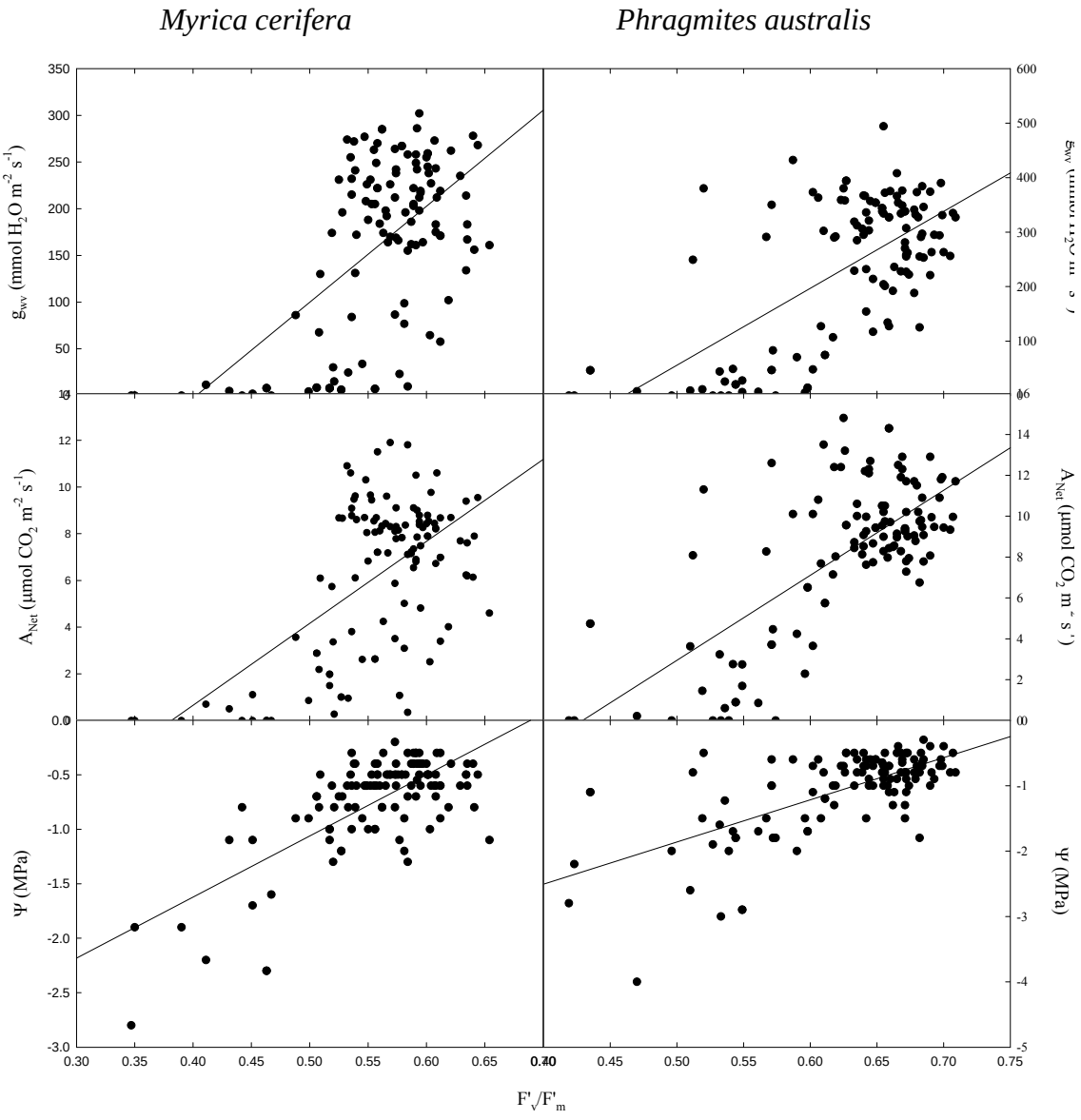
Myrica cerifera
Phragmites australis
g_{wv} (mmol H_2O m^{-2} s^{-1})
A_{Net} (µmol CO_2 m^{-2} s^{-1})
Ψ (MPa)
F'_v/F'_m

Figure 2.6

CHAPTER 3

LEAF CHLOROPHYLL FLUORESCENCE, REFLECTANCE, AND PHYSIOLOGICAL RESPONSE TO FRESHWATER AND SALTWATER FLOODING IN THE EVERGREEN SHRUB, *MYRICA CERIFERA*

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Abstract

Photosynthesis, water relations, chlorophyll fluorescence, and leaf reflectance were used to evaluate stress due to freshwater and saltwater flooding in the evergreen coastal shrub, *Myrica cerifera*, under controlled conditions. *Myrica cerifera* forms large monospecific thickets that facilitate scaling up from leaf-level measurements to the landscape. Based on physiological responses, stress began by day 3 in flooded plants treated with 5, 10, and 15 g L^{-1} salinity, as seen by significant decreases in stomatal conductance and net photosynthesis relative to control plants. Decreases in physiological measurements occurred by day 9 in freshwater flooded plants. Visible signs of stress occurred by day 5 for plants treated with 15 g L^{-1}, day 8 for flooded plants exposed to 10 g L^{-1}, and day 10 for those treated with 5 g L^{-1} salinity. Significant differences in light-adapted fluorescence yield ($\Delta F/F'_m$) were observed by day 3 in plants flooded with 5, 10, and 15 g L^{-1} salinity and day 6 in freshwater flooded plants. Nonphotochemical quenching (Φ_{NPQ}) increased with decreasing $\Delta F/ F'_m$. In comparison, statistical differences in dark-adapted fluorescence yield (F_v/F_m) were observed by day 12 in plants flooded with 5, 10, and 15 g L^{-1} salinity, well after visible signs of stress were apparent. Fluorescence parameters were successful at detecting and distinguishing both freshwater and saltwater flooding stress. A positive, linear correlation ($r^2 = 0.80$) was observed between $\Delta F/F'_m$ and the physiological reflectance index (PRI). Xanthophyll-cycle dependent energy dissipation appears to be the underlying mechanism in protecting photosystem II from excess energy in saltwater flooded plants. $\Delta F/F'_m$ was useful in detecting stress induced changes in the photosystem before any visible signs of damage

were evident at the leaf-level. This parameter may be linked to hyperspectral reflectance data for rapid detection of stress at the canopy-level.

Introduction

The photosynthetic apparatus, especially photosystem II (PSII), may be temporarily affected by environmental stresses before irreversible morphological damage is observed. This early detection of stress could identify the physiological condition of plants at larger spatial and temporal scales before visible effects are apparent (Zarco-Tejada et al., 2002). Non-invasive remote sensing techniques, such as chlorophyll fluorescence and plant reflectance, are being developed to monitor plant stress and photosynthetic status, and to detect and predict changes in the natural environment (Kerr and Ostrovsky, 2003; Baker and Rosenqvist, 2004; Dobrowski et al., 2005). Stressed plants use less radiant energy for photosynthesis, and have evolved numerous mechanisms that safely dissipate excess light to avoid photoinhibition and photooxidation (Flexas and Medrano, 2002). The excited chlorophyll molecule is subjected to various competing de-excitation reactions including photosynthesis, heat loss, and chlorophyll fluorescence, which has been successfully used as a non-invasive method to detect plant stress (Baker and Rosenqvist, 2004). Any physiological process that affects the function of PSII and associated de-excitation pathways will have an effect on chlorophyll fluorescence, because the fluorescence signal is assumed to originate primarily from PSII (Krause and Weis, 1991).

Many vegetation indices have been developed based on changes in plant reflectance, which is sensitive to changes in biomass, chlorophyll content and leaf water

status. However, most of these indices have no direct link to photosynthetic function (Dobrowski et al., 2005). One exception is the physiological reflectance index (PRI, the reflectance at 531 nm relative to reflectance at a reference wavelength of 570 nm), which has been linked to the xanthophyll cycle (Gamon et al., 1990; Gamon et al., 1997; Peñuelas et al., 1995; Peñuelas et al., 1997; Evain et al., 2004). Carotenoid pigments of the xanthophyll cycle are tied to photochemical efficiency of PSII by dissipating light not used in photosynthesis (Demmig-Adams and Adams, 1996). Changes in the de-epoxidation state of the xanthophyll cycle and subsequent accumulation of zeaxanthin are reflected by absorbance changes centered near 531–535 nm (Bilger et al., 1989; Gamon et al., 1990; Ruban et al., 1993). PRI has also been linked to changes in chlorophyll fluorescence (Gamon et al., 1997; Peñuelas et al., 1997; Méthy, 2000). The amount of zeaxanthin formed is correlated with the rate of dissipation of excess energy as heat (Demmig-Adams et al., 1989). Relationships between PRI and measurements of fluorescence have been established in plants under water stress at both the leaf-level and canopy-level (Flexas et al., 2002; Winkel et al., 2002; Evain et al., 2004). Thus, pigments of the xanthophyll cycle are useful indicators of the efficiency of photosynthetic energy conversion (Gamon et al., 1990) and may provide information on the photosynthetic status of a leaf, plant or canopy using remotely sensed reflectance measurements (Gamon et al., 1997).

As species and structural diversity increase, the application of leaf level measurements to the landscape becomes increasingly difficult. Agricultural species, which typically form monotypic canopies, have been the focus of many studies linking

fluorescence and canopy level remote sensing (Gamon et al., 1990; Flexas et al., 2000; Zarco-Tejado et al., 2003; Baker and Rosenqvist 2004). Thus, species that form monotypic canopies naturally facilitate scaling up to the landscape level. *Myrica cerifera* is the dominant woody species on mid-Atlantic barrier islands (Ehrenfeld, 1990), and forms extensive, monospecific thickets, making it a model species for scaling up in natural ecosystems. *Myrica cerifera* is a salt sensitive species, and is, thus, limited to mesic swale sites on barrier islands (Young, 1992; Tolliver et al., 1997). Periodic, short-term freshwater flooding occurs naturally in these areas during summer and early autumn (Young et al., 1995), while storm overwash can lead to saltwater flooding (Ehrenfeld, 1990; Young et al., 1995).

Salinity and flooding are two important environmental factors inhibiting *Myrica cerifera* growth in coastal environments. This study explores the link between leaf-level reflectance, chlorophyll fluorescence, and plant physiological status for ***M. cerifera*** under freshwater and saltwater flooding conditions. Specific goals were to (1) detect flooding induced stress (as induced by changes in stomatal conductance and photosynthesis) via chlorophyll fluorescence before visible structural damage occurred, (2) provide evidence for a link between chlorophyll fluorescence and plant stomatal control and photosynthetic status, (3) and to relate leaf-level reflectance to chlorophyll fluorescence using PRI. Many studies have examined the use of PRI at leaf and canopy levels in drought stressed plants (Gamon et al., 1997; Dobrowski at al., 2005), but very few have applied the use of PRI to flooding or salinity stress. These links are essential to establish at the leaf-level in order to scale up to canopy-level variations in the landscape.

Materials and methods

Plant materials and growth conditions

Seedlings of *Myrica cerifera* L. (Myricaceae) were obtained from a nursery using local seed stock and transplanted into 2L plastic pots, due to limited numbers and variations in size of plants in the field. Seedlings (~ 20 cm tall) were acclimated to the environmental chamber for at least 4 weeks prior to experimental treatment. Plants were grown in a Convrion environmental chamber (CMP 3244, Controlled Environments Limited, Asheville, NC) under a photosynthetic photon flux density (PPFD) of approximately 700 μmol m^{-2} s^{-1}, 48% relative humidity, a photoperiod of 14 h, and a day/night temperature of 30/25 °C.

Induction of flooding stress

Salinity treatments of 0, 5, 10, and 15 g L^{-1} were prepared using dilutions of a commercial mixture that approximates total ocean salts (Instant Ocean, Aquarium Systems, Mentor, OH; Tolliver et al. 1997). Major cations present in the mixture are Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} (5.1, 0.18, 0.19, 0.62 g L^{-1}, respectively) and the major anion is Cl^{-} (8.9 g L^{-1}; Atkinson and Bingman, 1997). A previous experiment showed 100 % mortality for *M. cerifera* plants flooded with 20 g L^{-1} (Tolliver et al., 1997). Saltwater flooding treatments were kept below this concentration to prevent mortality during the experiment. Flooding was induced by setting potted plants in plastic tubs filled with the treatment solution (n = 10). Salinity concentrations were monitored with a conductivity meter (model 33, YSI, Yellow Springs, OH) and water levels were maintained at the soil surface. Water and salinity levels were monitored daily, and adjusted as needed.

Measurements of physiology and fluorescence

Plant response to flooding treatments were quantified by measuring stomatal conductance, leaf net photosynthesis, leaf fluorescence, and mid-day xylem pressure potentials, and compared to non-flooded, well-watered control plants (n = 10). Measurements were conducted on days 3, 6, 9, and 12 following flooding treatment at mid-day (1000 – 1400 h). Stomatal conductance (g_{wv}) and leaf net photosynthetic rate (A_{Net}) were measured using a portable infrared gas analyzer at a PPFD level of 700 μmol m^{-2} s^{-1} (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE). Mid-day xylem pressure potentials (ψ) were quantified with a Scholander pressure chamber (PMS 650, Corvallis, OR).

Dark-adapted and light-adapted measurements of chlorophyll fluorescence were conducted on the fourth or fifth fully expanded leaf of each plant using a pulse amplitude modulated leaf chamber fluorometer (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE). Maximum quantum efficiency of photosystem II (PSII) was calculated as: $F_v/F_m = (F_m - F_o)/F_m$ by measuring the fluorescence signal from a dark-adapted leaf when all reaction centers are open using a low intensity pulsed measuring light source, (F_o), and during a pulse of saturating light when all reaction centers are closed (F_m). Leaves were dark-adapted for 30 min using dark-adapting leaf clips (LI-COR) for F_v/F_m measurements. The energy harvesting efficiency of PSII in a light –adapted leaf is calculated as: $F'_v/F'_m = (F'_m - F'_o)/ F'_m$, where F'_o is the minimal fluorescence in the light-adapted state, obtained by turning off the light temporarily to drain the electrons from PSII, and F'_m is the maximal value when all reaction centers are closed after a pulse of saturating light.

Similarly the fraction of absorbed photons used for photochemistry can be calculated as: $\Delta F/F'_m = (F'_m - F_s)/ F'_m$ using the steady-state parameter (Fs). Non-photochemical quenching was also calculated as: $\Phi_{NPQ} = (F_s/ F'_m) – (F_s/F_m)$ (Hendrickson et al. 2004).

Chlorophyll concentrations

Leaf samples were collected by punching forty 0.32 cm^2 disks from each plant (n = 10). Chlorophyll concentrations were determined based on methods recommended by Šesták (1971) by extracting chlorophyll using a 100 % acetone solution. Samples were then ground with a mortar and pestle, filtered, and analyzed using a Spectronic 21 spectrophotometer. Chlorophyll concentrations were calculated using equations given by Holm (1954).

Reflectance measurements

In a separate experiment, measurements of $\Delta F/F'_m$ and reflectance were made on leaves with no visible damage (n=6). Measurements were made on day 7 to assure that $\Delta F/F'_m$ in saltwater flooded plants had declined significantly compared to controls, which was determined in the first experiment. An ASD FieldSpec Pro reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO) was used to measure the spectral reflectance of leaves between 350 – 2500 nm. The ASD spectral resolution is approximately 1 to 3 nm from the visible to the short-wave infrared. Laboratory leaf measurements were collected orthogonally using a 3200° K lamp as an illumination source. The fore-optic of the radiometer was held nadir at a nominal distance of 0.25 meter using an 8° field-of-view. Data were reduced from binary using the manufacturer's program. Reflectance spectra were calculated by dividing the spectral radiance of the

axial surface of a leaf by a NIST Spectralon reflectance standard. This standard provides a near 100 % lambertian reflectance surface for calibration. Using the resulting reflectance values the physiological reflectance index was derived as: PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$, where R_{531} is the reflectance at 531 nm and R_{570} is the reflectance at a 570 nm reference wavelength (Gamon et al., 1992).

Statistical Analysis

Repeated measures analysis of variance was used to test for variations in photosynthetic characteristics, stomatal conductance, xylem pressure potential, and fluorescence due to flooding relative to control plants over time (Zar, 1999). Day was specified as the repeated factor (within-subject), and flooding treatment as fixed effect (between-subjects). The validity of a within- subjects test depends on sphericity of the data (Von Ende, 1993). The Huynh-Feldt correction (H-F; Huynh and Feldt, 1976) addresses this assumption and adjusted *P* values (H-F *P*) were reported. Dunnett's tests ($\alpha = 0.05$) were used to identify significant differences in treatment means from controls for individual days. One-way analysis of variance was used to test for differences in chlorophyll concentrations among flooding treatments. Tukey's multiple comparisons ($\alpha = 0.05$) identified significant differences in chlorophyll content among treatments. Variations in photosynthesis, stomatal conductance, xylem pressure potential, and PRI were related to variations in fluorescence using linear regression.

Results

Gas exchange measurements

Saltwater flooding induced physiological stress in plants of *Myrica cerifera.* There was a significant day x treatment interaction for all three physiological measurements: g_{wv} ($F = 25.27$, $P < 0.0001$), A_{Net} ($F = 33.50$, $P < 0.0001$) and Ψ ($F = 4.80$, $P < 0.0001$). Dunnett's multiple comparisons for individual days revealed that stomatal conductance was significantly lower in saltwater flooded plants by day 3, and in freshwater plants by day 9 compared to control plants (Figure 3.1). Complete stomatal closure occurred by day 9 for plants at mid-range salinity levels (10 and 15 g L^{-1}). Measurements of A_{Net} followed a similar pattern. By day 3, saltwater flooded plants had significantly lower A_{Net} compared to control plants. Freshwater flooded plants displayed lower A_{Net} by day 9, compared to non-flooded controls (Figure 3.1). Plants flooded with 10 and 15 g L^{-1} had ψ by day 3. Mid-day ψ were < -1.0 MPa by day 6 of the experiment in plants treated with 15 g L^{-1} saltwater flooding, and by day 9 in plants exposed to 10 g L^{-1} saltwater flooding (Figure 3.1). Plants flooded with 5 g L^{-1} saltwater had ψ on days 3 and 9, but were not significantly different from control plants on the other days. There were no differences in ψ between freshwater flooded and control plants. A significant positive linear relationship was observed between A_{Net} and g_{wv} ($r^2 = 0.86$, $F = 1566.29$, $P < 0.0001$; Figure 3.2).

Fluorescence measurements

Both light- and dark-adapted measurements were quantified to assess stress due to flooding. Dark-adapted fluorescence (F_v/F_m) exhibited a significant day x treatment

interaction in *Myrica cerifera* ($F = 2.77$, $P = 0.0036$). Multiple comparisons for individual days revealed that plants flooded with 10 and 15 g L^{-1} saltwater had lower F_v/F_m by day 12 (0.67 ± 0.05, 0.62 ±0.07, respectively) compared to control plants (0.81 ± 0.00; Figure 3.3). However, these significant differences occurred well after visible signs of stress occurred in the saltwater flooded plants. Plants flooded with freshwater and 5 g L^{-1} saltwater did not exhibit significantly lower F_v/F_m from the control over the course of the experiment (Figure 3.3).

A significant day x treatment interaction was also observed in the light-adapted fluorescence parameters, F'_v/F'_m, ($F = 8.88$, $P < 0.0001$), $\Delta F/F'_m$ ($F = 20.28$, $P < 0.0001$), Φ_{NPQ} ($F = 6.37$, $P = <0.0001$). F'_v/F'_m was significantly lower in plants flooded with 10 and 15 g L^{-1} saltwater compared to controls by day 3 (Figure 3.3), well before observable signs of stress were apparent. Average F'_v/F'_m for control plants was 0.58 ± 0.00 over the course of the experiment. By day 3, average F'_v/F'_m for plants treated with 10 and 15 g L^{-1} saltwater was 0.49 ± 0.15 and 0.50 ± 0.15, respectively. Plants treated with 5 g L^{-1} saltwater exhibited low F'_v/F'_m values of 0.43 ± 0.13 by day 6 (Figure 3.3). Visible signs of stress in at least one plant per treatment (i.e. mottled and dead leaves) did not occur until day 5 at 15 g L^{-1}, day 8 at 10 g L^{-1}, and day 10 at 5 g L^{-1} saltwater flooding, after stress was detected via light-adapted fluorescence. There were no significant differences between control and freshwater flooded plants throughout the experiment (Figure 3.3), despite significant decreases in physiological measurements (Figure 3.1). $\Delta F/F'_m$ was significantly lower in all saltwater treated plants by day 3, with a subsequent increase in Φ_{NPQ}, also by day 3. By day 6, plants treated with freshwater exhibited significantly

lower $\Delta F/F'_m$, 0.44 ± 0.01, compared to controls, 0.55 ± 0.00. $\Delta F/F'_m$ values were very low in all saltwater flooded plants by the end of the experiment. Average Φ_{NPQ} for control plants was 0.26 ± 0.00. Φ_{NPQ} reached 0.61 ± 0.03 on day 12 in plants treated with 5 g L^{-1}. Plants flooded with 15 g L^{-1} saltwater reached maximum Φ_{NPQ} on day 9 (0.58 ± 0.02), which slightly declined by day 12 (0.54 ± 0.03).

Significant relationships existed between light-adapted fluorescence parameters and gas exchange measurements. There was a positive, linear relationship between $\Delta F/F'_m$ and g_{wv} ($r^2 = 0.67$, F = 507.39, $P < 0.0001$; Figure 3.4), A_{Net} ($r^2 = 0.71$, F = 598.49, $P < 0.0001$; Figure 3.4), and Ψ ($r^2 = 0.41$, F = 175.09, $P < 0.0001$; Figure 3.4). Significant linear relationships also were seen between F'_v/F'_m and g_{wv} ($r^2 = 0.64$, F = 395.09, $P < 0.0001$) and A_{Net} ($r^2 = 0.69$, F = 488.04, $P < 0.0001$). A weak, but positive relationship was evident between F'_v/F'_m and Ψ ($r^2 = 0.28$, F = 97.36, $P < 0.0001$).

3.3 Chlorophyll content

Chlorophyll *a* concentrations decreased with increasing stress, ranging from 351 ± 38 to 267 ± 19 mg m^{-2}, but there were no significant differences among freshwater and saltwater treatments (F = 1.99, $P = 0.135$; Table 3.1). Chlorophyll *b* concentrations ranged from 198 ± 8 to 245 ± 25 mg m^{-2} without significant differences among treatments (F = 0.97, $P = 0.447$). There were no significant differences among freshwater or saltwater treatments for total chlorophyll (F = 1.40, $P = 0.270$). The chlorophyll *a:b* ratio was significantly lower for plants flooded with 10 and 15 g L^{-1} saltwater (1.25 and 1.20, respectively) than control plants (1.43; F = 6.33, $P = 0.001$;

Table 3.1). There were no differences in plants flooded with freshwater or 5 g L^{-1} saltwater compared to controls.

Reflectance

PRI was significantly lower for plants flooded with 10 and 15 g L^{-1} saltwater compared to control plants ($F = 15.40$, $P < 0.0001$). There was a positive, linear correlation with $\Delta F/F'_m$ ($r^2 = 0.80$, $P < 0.0001$; Figure 3.5), with values of PRI decreasing from 0.03 in control plants to -0.06 in plants flooded with 15 g L^{-1} saltwater. Freshwater flooded plants and plants treated with 5 g L^{-1} had lower PRI values compared to control plants, but these were not significant.

Discussion

Stomatal closure and reduced photosynthesis are common responses to soil oxygen deficiency caused by flooding (Kozlowski and Pallardy, 1979; Pezeshki, 1993). Both stomatal and non-stomatal limitations are responsible for decreases in photosynthesis after salinity stress (Kozlowski, 1997). The positive relationship between stomatal conductance and net photosynthesis indicate that the primary limiting factor for net photosynthesis after flooding may be stomatal closure, which can be further limited by non-optimal metabolic conditions. *Myrica cerifera* occurs primarily in coastal environments and must withstand periods of episodic flooding with brackish water due to storm overwash and freshwater flooding due to precipitation. Reduced root hydraulic conductivity induced by freshwater flooding increases internal water stress, causing reductions in leaf water potential and stomatal conductance (Else et al., 2001). Freshwater flooding caused decreases in stomatal conductance, but did not reduce leaf

xylem pressure potential in *M. cerifera*, which has been reported in other woody plants (Dreyer et al., 1991; Fernández, 2006). Partial stomatal closure observed in freshwater flooded plants allows for the influx of CO_2 to maintain photosynthesis. Previous work showed the onset of saltwater flooding stress for *M. cerifera* by day 3 as indicated by decreases in stomatal conductance (Tolliver et al., 1997). Our results also indicated stress by day 3 for all saltwater flooded plants, as measured by decreases in stomatal conductance and photosynthesis. Again, stomata closed gradually, enabling low levels of photosynthesis for a few days after the induction of stress. Initial reductions in stomatal conductance of saltwater flooded plants were likely the result of salinity exposure, rather than flooding (Naumann et al., 2007). The rapid physiological response, seen by decreases in stomatal conductance, enables the survival of short-term salinity flooding by keeping tissue chloride concentrations low within the leaves (Tolliver et al., 1997).

Chlorophyll fluorescence is a sensitive, non-invasive tool to study stress-induced changes in PSII. Both light and dark-adapted measurements can be used to determine whether or not photodamage has occurred in leaves. F_v/F_m has been used extensively as a method of early stress detection (Baker and Rosenqvist, 2004). Some researchers have found reduced F_v/F_m due to flooding stress (Smethurst and Shabala, 2003; Ahmed et al., 2006), while others have not, despite physiological and morphological signs of stress (Smith and Moss, 1998). There is usually no change in F_v/F_m in the early stages of salinity stress (Baker and Rosenqvist, 2004). In our study F_v/F_m declined significantly in plants flooded with 10 and 15 g L^{-1}, but only after noticeable damage to leaves had occurred. Low F_v/F_m at the end of the experiment, especially in 15 g L^{-1} saltwater

flooded plants indicated possible photodamage; observed values were below the ranges proposed for plants that are not chronically photoinhibited (Adams et al., 1990). Freshwater and 5 g L^{-1} saltwater flooded plants did not experience low values of F_v/F_m that would indicate damage to PSII by the end of the experiment.

Stress was effectively detected through $\Delta F/F'_m$ prior to visible signs for both freshwater and saltwater flooding. In all saltwater flooded plants, physiological responses and changes in $\Delta F/F'_m$ occurred by day 3, while changes in $\Delta F/F'_m$ occurred before physiological decreases in stomatal conductance and photosynthesis in freshwater flooded plants. This change in $\Delta F/F'_m$ with no change in CO_2 assimilation could be used to differentiate between freshwater and saltwater flooding during the initial phases of flooding stress. Decreases in $\Delta F/F'_m$ were accompanied by increases in Φ_{NPQ} in both freshwater and saltwater flooded plants. These changes along with no subsequent decreases in F_v/F_m suggests increased photoprotection through the xanthophyll cycle to match reductions in photosynthesis in order to avoid photodamage in plants flooded with freshwater and 5 g L^{-1} saltwater (Qiu et al., 2003). Plants flooded with 10 g L^{-1} exhibited low F_v/F_m values that were accompanied by increasing Φ_{NPQ}. These high values of Φ_{NPQ} are evidence that thermal energy dissipation was sustained after dark-adaptation due to the retention of zeaxanthin (Adams and Demmig-Adams, 1995). Photodamage is the likely explanation for plants flooded with 15 g L^{-1} salinity. Values of F_v/F_m were low at the end of the experiment, and Φ_{NPQ} leveled off by day 6. Severe damage to leaves was observed by the end of the experiment.

One of the early symptoms of flooding stress is the decline in chlorophyll concentration of leaves resulting in chlorosis (Webb and Fletcher, 1996). Chlorosis also develops from chloride injuries (Kozlowski, 1997). The effect of flooding on chlorophyll content has been mixed, with some species demonstrating reductions after a few days of flooding (Smethurst and Shabala, 2003), whereas other species have not been affected (Pezeshki et al., 1996). In our study, there were no differences in chlorophyll concentrations among treatments. Thus, short-term freshwater and saltwater flooding did not induce changes in *M. cerifera* chlorophyll content.

Changes in chlorophyll content is an indicator of stress that can be detected by reflectance, and most reflectance indices are used to detect changes in vegetation biomass or chlorophyll content (Carter, 1993; Peñuelas and Filella, 1997), but these changes often occur after visible signs of stress. PRI is indirectly linked to the photosynthetic status of a plant by measuring changes in xanthophyll de-epoxidation status, which is linked to heat dissipation and the complementary changes in photosynthesis and fluorescence (Demmig-Adams et al., 1989; Peñuelas et al., 1997). Significant relationships have been found between PRI and $\Delta F/F'_m$ across species and functional types (Gamon et al., 1997), under water stress (Peñuelas et al., 1997; Evain et al., 2004), and over a wide range of irradiances (Méthy, 2000). Our results showed correlation at the leaf-level between $\Delta F/F'_m$ and PRI under saltwater flooding stress, with PRI and $\Delta F/F'_m$ decreasing as salinity was increased. This is further support for xanthophyll cycle dependent energy dissipation as the underlying mechanism in protecting PSII, particularly in salinity flooded plants. PRI appears to be a quick and accurate assessment of the physiological

response to salinity stress. Stylinski et al. (2002) found a strong correlation between leaf and canopy PRI, concluding that leaf-level PRI values could be scaled to the canopy level for evergreen shrubs. Therefore, PRI may be used to detect stress in *M. cerifera* thickets due to saltwater flooding at large spatial scales.

In summary, *M. cerifera* is able to survive short-term saltwater flooding by gradually closing stomata and dissipating excess light energy via the xanthophyll cycle without damage to PSII. Partial stomatal closure enabled the plant to maintain low levels of photosynthesis during the first few days of stress. Measurements of light-adapted chlorophyll fluorescence were successful at detecting flooding induced stress before and visible signs of damage had occurred to the leaves. $\Delta F/F'_m$ was related to plant physiological status, as seen in the relationships with stomatal conductance and photosynthesis. $\Delta F/F'_m$ was a good indicator of stress due to freshwater and saltwater flooding and could be used to differentiate between the two based on the physiological status of the plant. PRI was effective at tracking changes in $\Delta F/F'_m$ at the leaf-level scale in salinity flooded plants. These findings applied at the canopy level could help identify salinity stress in plants prior to visible signs of damage and may be used to prevent severe damage and unnecessary mortality due to saltwater flooding.

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Table 3.1. One-way analysis of variance of chlorophyll concentrations among flooding treatments. Values are means ± 1 SE. Different letters denotes statistically significant differences based on Tukey's multiple post hoc comparisons among the treatments.

	Salinity (g L^{-1})				
	control	0	5	10	15
Chl *a* (mg m^{-2})	351 ± 38[a]	276 ± 6[a]	283 ± 23[a]	280 ± 22[a]	267 ± 20[a]
Chl *b* (mg m^{-2})	245 ± 25[a]	198 ± 9[a]	211 ± 19[a]	222 ± 15[a]	223 ± 16[a]
Total chlorophyll (mg m^{-2})	596 ± 63[a]	473 ± 14[a]	494 ± 42[a]	502 ± 36[a]	490 ± 35[a]
Chlorophyll *a:b* (mg m^{-2})	1.4 ± 0.0[a]	1.4 ± 0.1[a]	1.3 ± 0.0[a]	1.2 ± 0.0[b]	1.1 ± 0.0[b]

Figure Legends

Figure 3.1. Effects of freshwater and saltwater flooding on stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera.* Symbols represent means with standard errors for control and treatment plants.

Figure 3.2. Relationship between stomatal conductance (g_{wv}) and net photosynthesis (A_{Net}) for *Myrica cerifera* under flooding treatment.

Figure 3.3. Changes in the fluorescence measurements F'_v/F'_m, F_v/F_m, $\Delta F/F'_m$, and Φ_{NPQ} for *Myrica cerifera* under freshwater and saltwater flooding treatments over time. Symbols represent means with standard errors for control and treatment plants.

Figure 3.4. Relationships between light-adapted fluorescence ($\Delta F/F'_m$) and stomatal conductance (g_{wv}), net photosynthesis (A_{Net}), and water potential (Ψ) for *Myrica cerifera* freshwater and saltwater flooded plants.

Figure 3.5. Relationship between leaf-level PRI and $\Delta F/F'_m$ on day 7 in plants flooded with freshwater and saltwater.

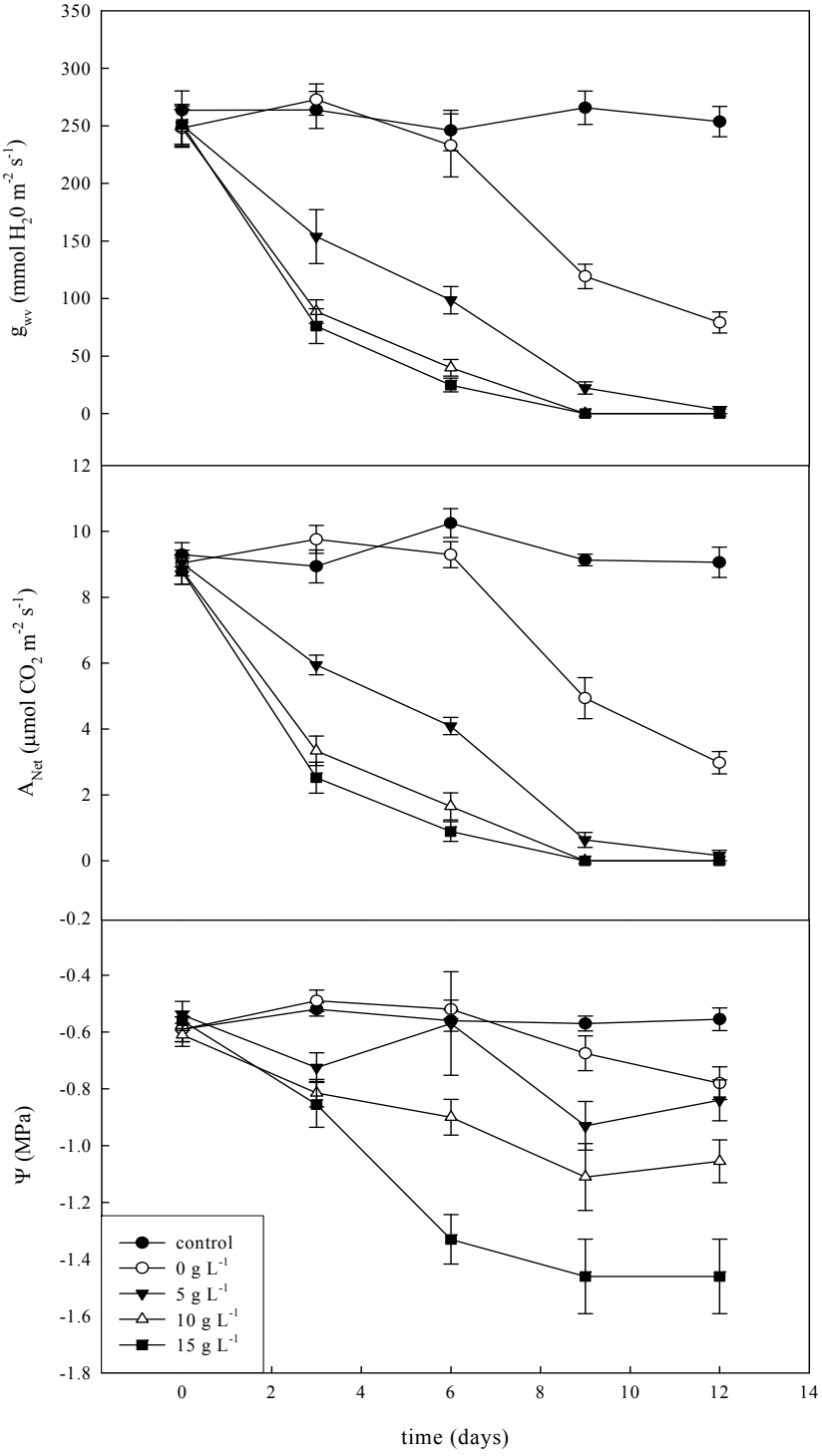
g_{wv} (mmol H_20 m^{-2} s^{-1})
A_{Net} (µmol CO_2 m^{-2} s^{-1})
Ψ (MPa)
time (days)
control
0 g L^{-1}
5 g L^{-1}
10 g L^{-1}
15 g L^{-1}

Figure 3.1

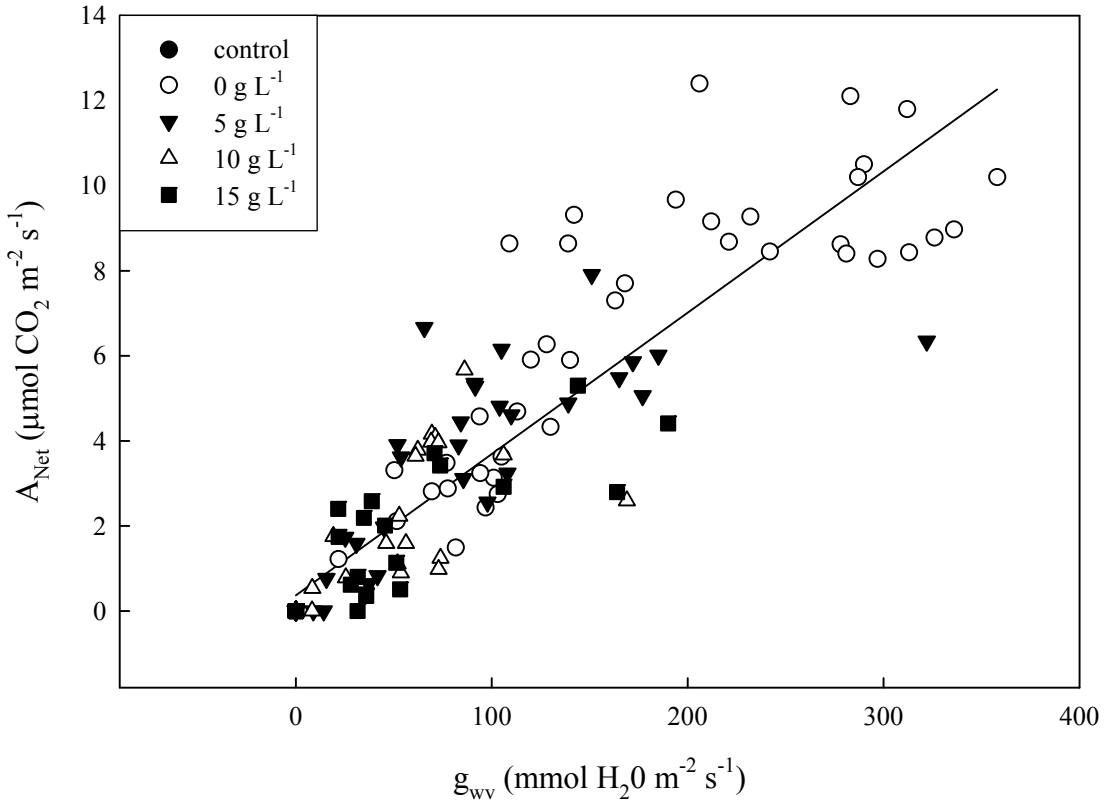
control
0 g L-1
5 g L-1
10 g L-1
15 g L-1
ANet (µmol CO2 m-2 s-1)
gwv (mmol H20 m-2 s-1)
0
2
4
6
8
10
12
14
0
100
200
300
400

Figure 3.2

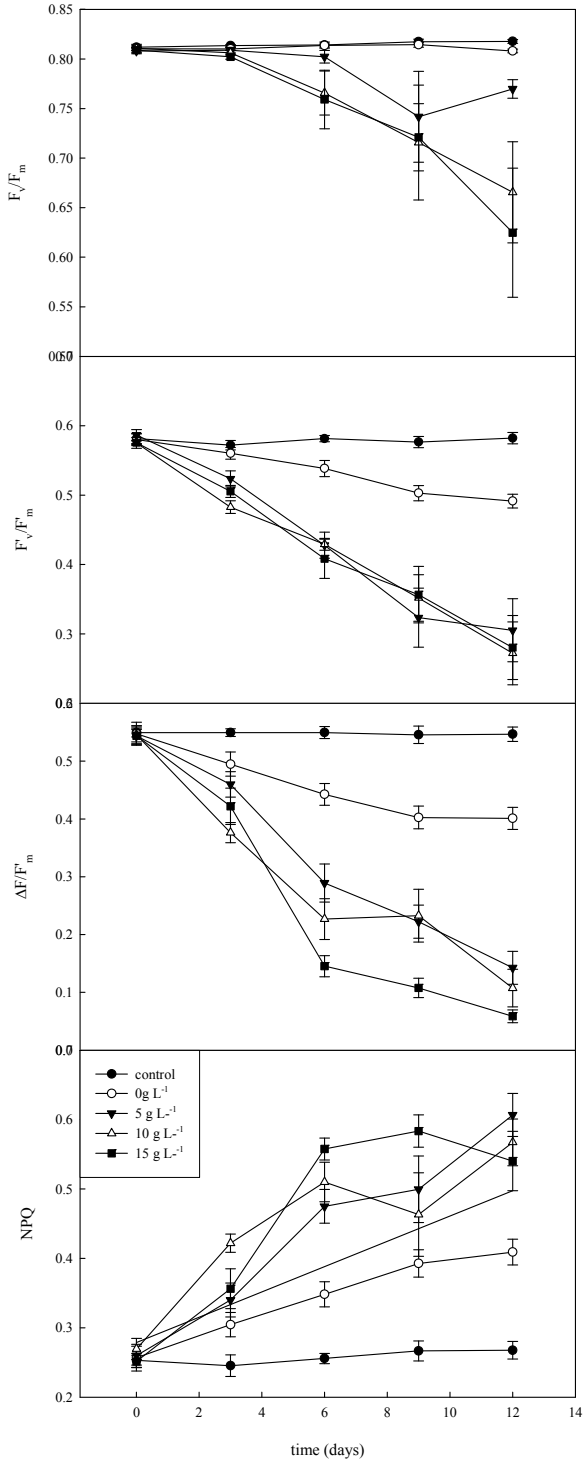

Figure 3.3

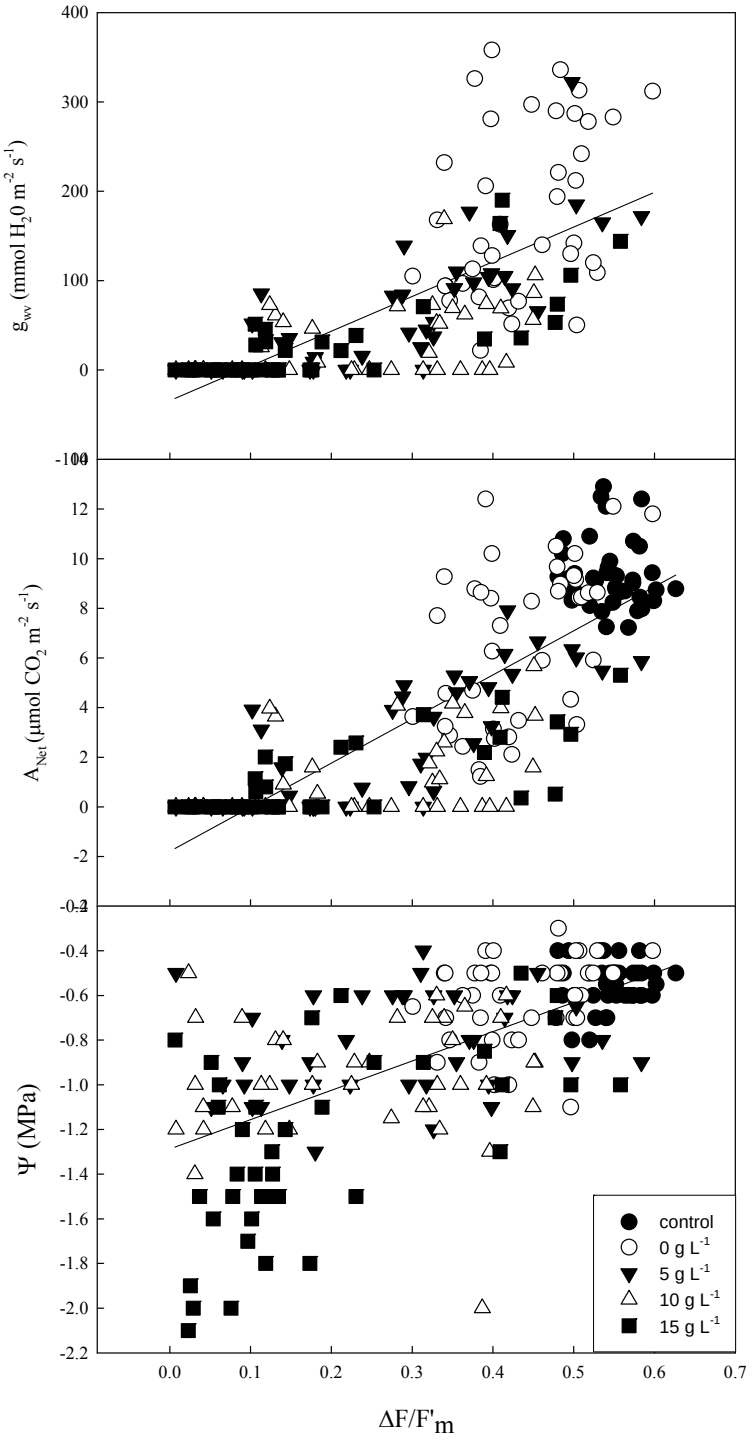
g_{wv} (mmol H_20 m^{-2} s^{-1})
A_{Net} (µmol CO_2 m^{-2} s^{-1})
Ψ (MPa)
ΔF/F'm
control
0 g L^{-1}
5 g L^{-1}
10 g L^{-1}
15 g L^{-1}

Figure 3.4

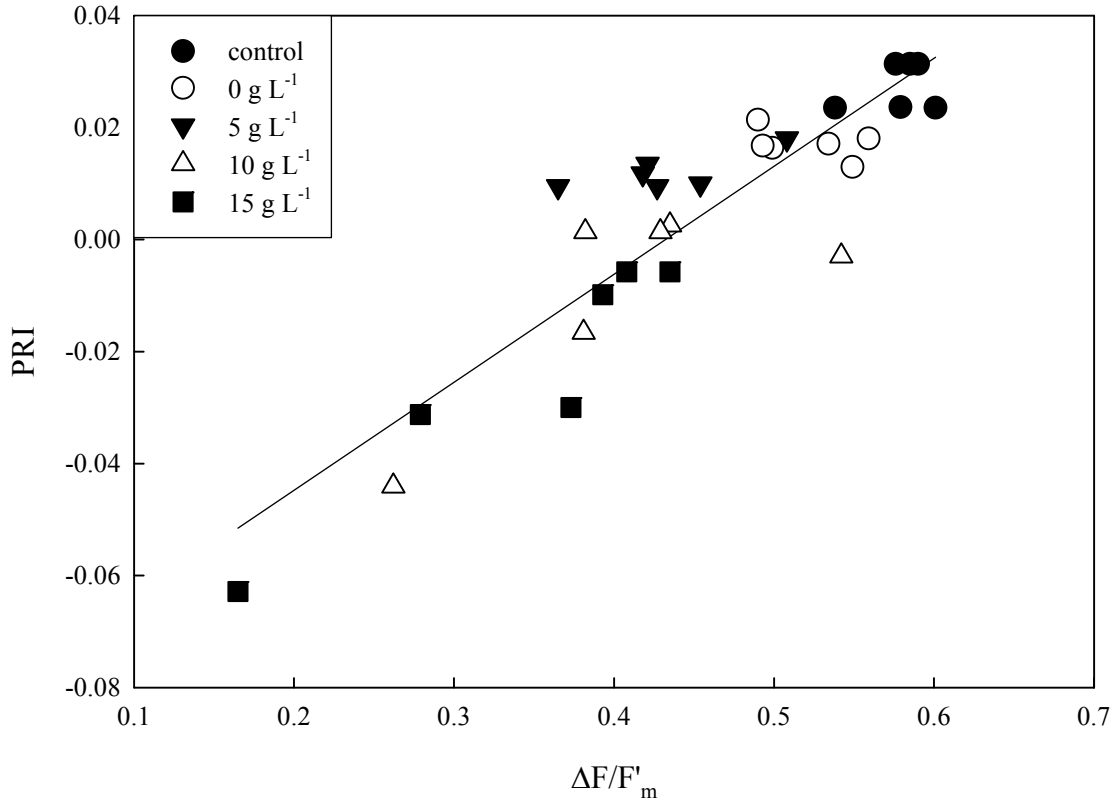
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
PRI
0.1
0.2
0.3
0.4
0.5
0.6
0.7
ΔF/F'm
control
0 g L-1
5 g L-1
10 g L-1
15 g L-1

Figure 3.5

CHAPTER 4

LINKING PHYSIOLOGICAL RESPONSES, CHLOROPHYLL FLUORESCENCE AND HYPERSPECTRAL IMAGERY TO DETECT SALINITY STRESS USING THE PHYSIOLOGICAL REFLECTANCE INDEX IN THE COASTAL SHRUB, *MYRICA CERIFERA*

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Abstract

Photosynthesis, chlorophyll fluorescence, canopy-level hyperspectral reflectance and landscape-level reflectance imagery were used to evaluate stress due to salinity and drought in the evergreen coastal shrub, *Myrica cerifera*. Using the physiological reflectance index (PRI), we were able to detect in salinity stress at both the canopy and landscape level. *Myrica* thickets have leaf area index (LAI) values that exceed most temperate woody communities, above the values at which PRI becomes insensitive to the background effect of the soil, reducing the confounding effects of canopy structure. Monthly variations in stomatal conductance, photosynthesis, and relative water content indicated a strong summer drought response that was not seen by variations in chlorophyll fluorescence. There were statistically significant differences between physiological measurements and tissue chlorides between the two sites used in the study, indicating salinity stress. This was reflected in measurements of PRI. There was a significant, positive relationship between PRI measured at the canopy-level and $\Delta F/F'_m$ ($r^2 = 0.69$, $F = 13.74$, $P = 0.0100$). PRI was significantly lower on the oceanside ($F = 6.31$. $P = 0.0457$) of the *Myrica cerifera* thicket. PRI was not significantly related to the normalized difference vegetation index (NDVI) ($r^2 = 0.01$, $F = 0.06$, $P = 0.8098$) at the canopy-level, and only weakly related ($r^2 = 0.04$, $F = 9.29$, $P = 0.0026$) at the landscape-level, suggesting that the indices are spatially independent. Frequency histograms of pixels sampled from airborne hyperspectral imagery revealed that the distribution of PRI was shifted to the right on the backside of the thicket relative to the oceanside, and there was a significant difference between sites ($F = 114.22$, $P < 0.0001$). These results

suggest that PRI may be used for early identification of salt-stressed areas and to predict changes in community structure across the landscape due to sea-level rise.

Introduction

Salinity, drought, high irradiance and high temperatures are among many factors that influence the ecophysiology of plants in coastal ecosystems and place severe limits on plant growth (Ehrenfeld, 1990). On barrier islands, salinity is considered to be the primary environmental factor influencing community patterns (Oosting & Billings, 1942; Ehrenfeld, 1990; Stalter & Odum, 1993). With predicted changes due to climate change, such as rising sea level and increases in storm intensity and frequency (Gregory & Oerlemans, 1998; Zhang et al., 2000), plant distribution on barrier islands is likely to be affected. Coastal communities are particularly sensitive to periodic, short-term flooding due to storms and hurricanes (Young et al. 1995). Aspect and distance from the shoreline strongly influence the effect of salinity on plants and storm overwash can lead to saltwater flooding (Ehrenfeld, 1990; Young et al., 1995). Early identification of stressed areas will allow for predictions of changes in community structure across the landscape.

Characteristics of plant stress can be measured independently using reflectance or fluorescence remote sensing which provides rapid and non-destructive measurements (Anderson & Perry, 1996). Remote sensing appears useful in predicting changes in the structure and function of various ecosystems. Many studies have applied various spectrally derived indices to monitor changes in biomass (via changes in NDVI), pigment composition (e.g. chlorophylls and carotenoids), photosynthetic efficiency (determined by xanthophyll pigments), and water status (Gamon et al., 1992; Peñuelas et al., 1995; Evain et al., 2004; Filella et al., 2004; Suárez et al., 2007). There is a need to examine the usefulness of different reflectance indices in every community for landscape-level application (Filella et al., 2004). These indices may allow for the monitoring and

evaluation of the health of plant communities in response to global environmental change (Filella et al., 2004).

Under conditions of stress, plants are often exposed to more radiant energy than is needed for photosynthesis. The mechanisms for disposing of excess energy are limited, manifesting changes within the photosystem as a function of fluorescence and heat dissipation. Reversible declines in photosynthesis are generally accompanied by an increase in non-radiative energy dissipation mediated by the xanthophyll cycle, which protects the photosystem against permanent damage (Demmig-Adams, 1996). Changes in the epoxidation state of the xanthophyll cycle pigments and the accumulation of zeaxanthin are reflected by absorbance changes in the green region around 531–535 nm (Bilger et al., 1989; Gamon et al., 1990; Ruban et al., 1993). The physiological reflectance index (PRI, the reflectance at 531 nm relative to a reference wavelength) is linked to the xanthophyll cycle and may provide a non-destructive tool for the optical study of photosynthetic function (Gamon et al., 1992; Peñuelas et al., 1995). The amount of zeaxanthin formed is correlated with the rate of dissipation of excess energy as heat (Demmig-Adams et al., 1989). This dissipation energy can be estimated using chlorophyll fluorescence.

Relationships between chlorophyll fluorescence and PRI have been demonstrated under conditions of salinity stress (Naumann et al., in press) and water stress at the leaf-level (Winkel et al., 2002; Evain et al., 2004; Dobrowski et al., 2005) and canopy level (Evain et al., 2004; Suárez et al., 2007). Despite these successful applications of PRI, applications at spatial scales larger than the leaf require attention to the confounding effects of canopy structure (Peñuelas et al., 1995). More recently, algorithmic products

representing fluorescence, derived from hyperspectral reflectance, may have advantages to such investigations (Zarco-Tejada, et al., 2002). Dark-adapted fluorescence (F_v/F_m) has been extensively used to detect changes in the quantum use efficiency of PSII due to stress (Baker & Rosenqvist 2004); however, this is not efficient for canopy-level use in the field. Light-adapted measurements of fluorescence promote rapid detection of stress and can be easily applied beyond the leaf-level. Early detection of stress remote sensing could identify plant stress at larger spatial and temporal scales, before visible effects are apparent (Zarco-Tejada, et al., 2002, Cavender-Bares & Bazzaz, 2004, Helmuth et al., 2005).

Species that form monotypic canopies naturally facilitate scaling up beyond the leaf level, and have been used in many agricultural studies (Gamon et al., 1990; Flexas et al., 2000; Zarco-Tejada et al., 2003). *Myrica cerifera* is the dominant species of woody vegetation on many Atlantic barrier islands and forms dense, monospecific thickets (Ehrenfeld, 1990). *Myrica* thickets have LAI values that exceed most temperate woody communities (Brantley & Young, 2007), above the values at which PRI becomes insensitive to the background effect of the soil (Barton & North, 2001), reducing the confounding effects of canopy structure. Thus, *M. cerifera* may be a model species for scaling up in natural ecosystems. Laboratory studies of *M. cerifera* showed decreases in light-adapted chlorophyll fluorescence and subsequent increases in non-photochemical quenching under salinity and drought stress, indicating the possibility of xanthophyll-cycle dependent energy dissipation, and thus may enable rapid stress detection at the canopy level (Naumann et al., 2007).

We evaluated the effect of natural salinity and drought stress on plant physiological status, chlorophyll fluorescence, and canopy-level fluorescence derived from hyperspectral reflectance of *Myrica cerifera* located on a barrier island of the Atlantic Coast. Specific objectives were to (1) determine whether chlorophyll fluorescence could be used to detect salinity and drought stress in the field, (2) determine if chlorophyll fluorescence is related to plant physiological status, (3) relate canopy-level hyperspectral reflectance to chlorophyll fluorescence, and (4) use airborne remote sensing reflectance data to algorithmically identify stress at the landscape-level. Through monitoring areas of stress and understanding specific physiological responses, we may be able to predict changes in plant dominance and community structure (Filella et al., 1998). Sea-level rise and storm-induced floods can result in saltwater flooding and saltwater intrusion on barrier islands, causing shrub mortality. Thus *M. cerifera* shrub thickets may serve as sentinels for climate change (Young et al., 2007).

Methods

Study site

The study was conducted on the North end of Hog Island (37° 40'N; 75° 40'W), a barrier island located on the Eastern Shore of Virginia, from June to October 2007 (Fig. 4.1). On Hog Island, four existing thickets represent a range of successional stages. The oceanside, northern end of the island has been accreting approximately 5 m/year for 140 years (Hayden et al., 1991), resulting in a parallel series of dunes and swales. The mesic swales are dominated by *M. cerifera*, an evergreen, nitrogen-fixing, salt sensitive shrub (Ehrenfeld, 1990; Young, 1992). We conducted our study in two experimental sites of the easternmost thicket, which lies 200 m from the shoreline, and contains patches of

young shrubs (~10 years old). The thicket is ~ 32 m in width and 197 m long. Measurements were taken from the oceanside of the thicket, which is exposed to sea spray, and from the protected, leeside of the thicket.

Physiological Measurements

Daily variations in air temperature and precipitation were obtained from a meteorological station on Hog Island (Krovetz et al., 2007). Measurements of stomatal conductance, leaf net photosynthesis, leaf fluorescence, relative water content, and tissue chlorides were collected monthly at mid-day (1000 – 1400 h) on the fourth or fifth fully expanded sunlit leaf of each plant. New leaf growth begins in early May and continues until mid-September. We randomly selected five individuals, and made 2 measurements per individual (n=10) for each sampling date. The same leaves were for each measurement on a given day, except for tissue chlorides since leaves were altered during measurements of relative water content. Stomatal conductance (g_{wv}) and leaf net photosynthesis (A_{Net}) were measured using a portable infrared gas analyzer (LI- 6200, LI-COR, Inc., Lincoln, NE).

Light-adapted measurements of chlorophyll fluorescence were conducted using a pulse amplitude modulated leaf fluorometer (PAM-2000, Walz, Effeltrich, Germany). The relationship between maximal fluorescence in a light-adapted leaf after a saturating pulse of light (F'_m) and steady-state fluorescence prior to any saturating pulse (F_s) was used to estimate the effective quantum yield of photosystem II:

$\Delta F/F'_m = [F'_m - F_s]/F'_m$

After gas exchange and fluorescence measurements, leaves were clipped at the stem and kept at 100% humidity. Relative water content was measured as:

RWC (%) = (FW – DW)/ (SFW – DW) *100

where FW is fresh weight, DW is dry weight, and SFW is saturated fresh weight of the leaves after re-hydrating samples for 24 h (Turner, 1981). Tissue chlorides were quantified for leaves collected adjacent to those used for physiological measurements (n =10). Leaf samples were oven-dried at 80°C for 72 h and then ground in a fine mesh mill. For each sample, 0.5 g of material was placed in a tube with 40-mL of deionized water. Samples were placed in a boiling water bath for 2 h, cooled, and filtered into 100-mL volumetric flasks. To each sample, 2-mL of 5 M $NaNO_3$ was added as an ionic equalizer, and then samples were brought to volume with deionized water (Young et al., 1994). Chloride levels were determined using a chloride electrode (model 9617b, Orion, Boston, MA).

Canopy Hyperspectral Measurements

Canopy spectral reflectance (350 – 2500 nm) was measured at eight sites using an ASD FieldSpec Pro Full Range reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO). The ASD spectral resolution is approximately 1 to 3 nm from the visible to the short-wave infrared. The fore-optic of the radiometer was held from a tall pole in a nadir position at a distance approximately 1 m above the canopy using an 8° field-of-view on a cloudless day around solar noon. To acquire a representative value, multiple spectra were collected and averaged for each canopy. Data were reduced from binary using the manufacturer's software. Reflectance spectra were calculated by dividing the spectral radiance of the canopy by a NIST Spectralon reflectance standard. A reference measurement from the standard was taken before each canopy measurement. This standard provides a near 100% lambertian reflectance surface for calibration. Using the

resulting reflectance values, several canopy reflectance indices were calculated as follows:

Physiological Reflectance Index (Gamon et al., 1992)

$$PRI = (\rho_{531} - \rho_{570})/ (\rho_{531} + \rho_{570})$$

Normalized Difference Vegetation Index (Rouse et al., 1974)

$$NDVI = (\rho_{800} - \rho_{670})/ (\rho_{800} + \rho_{670})$$

Chlorophyll Index (Gitelson & Merzlyak 1994; Gitelson et al. 1996)

$$CI = (\rho_{750} - \rho_{705})/ (\rho_{750} + \rho_{705})$$

Water Band Index (Peñuelas et al., 1993)

$$WBI = \rho_{970}/\rho_{900.}$$

Concurrent measurements of $\Delta F/F'_m$ were made on 50 leaves at each site with a pulse amplitude modulated leaf fluorometer (PAM-2000, Walz, Effeltrich, Germany) to represent canopy fluorescence.

Airborne Image Acquisition

An airborne hyperspectral mission was flown concurrent with the physiological and canopy reflectance measurements at Hog Island on September 13, 2007. Hyperspectral data (3 nm resolution) were provided by the SpectIR using the ProSpecTIR VIS hyperspectral imaging spectrometer (SpectIR Corp.). Hyperspectral imagery covering 450 nm to 2450 nm was collected under cloud-free conditions at 1700 m (AGL) providing a data set representing 2 m/pixel on the ground and a final spectral cube 356 bands deep. These data products were post-processed to correct for geometric and radiometric (e.g., bi-directional) effects. Ground reflectance radiometry was used to calibrate the data based on target endmembers collected in-scene with the ASD

reflectance radiometer. This effectively placed the scene into reflectance units and helped to negate any atmospheric effects. Calibration was performed using the empirical line calibration method within ENVI (RSI, Inc.). One hundred points/pixels were selected from both the backside and oceanside sites sampled and the corresponding spectra extracted. The extraction of pixels with 2 m resolution enabled the calculation of indices without any shadowing effects. Landscape-level reflectance indices were calculated as follows:

PRI = $(\rho_{30} - \rho_{39})/ (\rho_{30} + \rho_{39})$ where ρ_{30} is the reflectance band centered at 529 nm and ρ_{39} is reflectance centered at 572 nm

NDVI = $(\rho_{87} - \rho_{60})/ (\rho_{87} + \rho_{60})$ where ρ_{87} is the reflectance centered at 802 nm and ρ_{60} is the reflectance centered at 672 nm

CI = $(\rho_{76} - \rho_{67})/ (\rho_{750} + \rho_{705})$ where ρ_{76} is the reflectance centered at 749 nm and ρ_{67} is the reflectance centered at 705 nm

WBI = ρ_{120}/ρ_{107} where ρ_{120} is the reflectance centered at 970 nm and ρ_{107} is the reflectance centered at 900 nm

Statistical Analyses

Two-way analysis of variance was used to test for variations in month and site for the following measurements: stomatal conductance, photosynthesis, chlorophyll fluorescence, relative water content and tissue chlorides (Zar, 1999). Significant differences among months were identified with Tukey tests ($\alpha = 0.05$). Differences in PRI, NDVI, CI, and WBI between sites were tested using the t-test. Variations in reflectance indices were related to variations in chlorophyll fluorescence using linear regressions.

Results

Leaf-level physiological measurements

The summer of 2007 was unusually dry and characterized by a persistent drought (Fig.4.2). Temperatures were average and did not go above those reported for optimal photosynthesis in *M. cerifera*, except for August, 8 (Young, 1992). Precipitation was concentrated in June and early July. Only 2 rain events > 5 mm occurred after July 11 and no precipitation occurred after mid-August (Fig. 4.2).

Stomatal conductance and net photosynthetic rates were highest on the backside of the thicket throughout the summer ($F = 24.43$, $P < 0.0001$; $F = 45.83$, $P < 0.0001$, respectively; Fig. 4.3). There were significant differences between months for stomatal conductance ($F = 24.43$, $P < 0.0001$) and photosynthesis ($F = 45.83$, $P < 0.0001$), as well as significant interactions between site and month ($F = 4.39$, $P = 0.0011$ for stomatal conductance; $F = 10.48$, $P < 0.0001$ for photosynthesis). The highest rates of stomatal conductance occurred in August for both the backside and oceanside sites (236 ± 17 and 227 ± 16 mmol H_2O m^{-2} s^{-1}, respectively; Fig. 4.3). The highest rates of net photosynthesis occurred in June (13.3 ± 0.9 μmol CO_2 m^{-2} s^{-1}) on the oceanside of the thicket, with rates declining each month thereafter. Net photosynthesis was highest in July on the backside of the thicket (22.5 ± 1.5 μmol CO_2 m^{-2} s^{-1}). Despite high values of stomatal conductance, rates of photosynthesis decreased in August. The lowest values of stomatal conductance and photosynthesis were reached in October for both the backside (54 ± 11 mmol H_2O m^{-2} s^{-1}, 2 ± 1 μmol CO_2 m^{-2} s^{-1}) and oceanside (61 ± 12 mmol H_2O m^{-2} s^{-1}, 2 ± 1 μmol CO_2 m^{-2} s^{-1}).

Seasonal patterns of relative water content and stomatal conductance reflected the late summer drought (Fig. 4.3). Relative water content was significantly different over time ($F = 14.27$, $P < 0.001$) but did not differ significantly between sites ($F = 3.21$, $P = 0.0758$), and there was no interaction between sites and month ($F = 1.33$, $P = 0.2559$). The lowest values of relative water content occurred in August, when stomatal conductance was high for both the backside (76 ± 2%) and the oceanside (74 ± 2%) sites (Fig. 4.3). Both the oceanside and backside of the thicket experienced partial stomatal closure after August, allowing relative water contents to increase (Fig. 4.3). By October, relative water content had reached over 90% for both sites (96 ± 1% backside, 91 ± 2% oceanside). Total chlorides present in leaves were higher on the oceanside (10193 ± 403 $\mu g\ g^{-1}$) compared to the backside (4329 ± 200 $\mu g\ g^{-1}$; $F = 394.62$, $P < 0.0001$; Fig. 4.3). There was no significant difference among months ($F = 0.69$, $P = 0.6319$) and there was no significant interaction between month and site ($F = 0.47$, $P = 0.7989$).

Despite the unusually dry summer, there was no significant change in $\Delta F/F'_m$ from one month to the next ($F = 0.28$, $P = 0.9208$). $\Delta F/F'_m$ did differ by site, and was significantly lower on the oceanside site compared to the backside throughout the summer ($F = 51.36$, $P < 0.001$; Fig. 4.4). Values averaged around 0.64 on the backside and 0.58 on the oceanside.

Canopy-level reflectance

Spectral reflectance data were collected under sunny and cloud-free conditions. Air temperature was 31 ºC, with relative humidity of 44% and 1944 $\mu mol\ m^{-2}\ s^{-1}$ PPFD at solar noon. PRI was positively related to $\Delta F/F'_m$ ($r^2 = 0.69$, $F = 13.74$, $P = 0.0100$). PRI decreased from -0.07 to -0.10, while $\Delta F/F'_m$ decreased from 0.67 to 0.49, with higher

$\Delta F/F'_m$ occurring on the backside of the thicket (Fig. 5). PRI was significantly lower on the oceanside (F = 6.31. *P* = 0.0457). NDVI was not related with $\Delta F/F'_m$ (r^2 = 0.01, F = 0.06, *P* = 0.8131; Fig 4.5) or Fs (r^2 = 0.05, F = 0.33, *P* = 0.5841; Fig 4.6). NDVI values were very high ranging from 0.90 to 0.93 and did not differ between sites (F = 0.18, *P* = 0.6834). There were no significant relationships between CI and $\Delta F/F'_m$ (r^2 = 0.01, F = 0.06, *P* = 0.8206). WBI was not related to $\Delta F/F'_m$ (r^2 = 0.00, F = 0.01, *P* = 0.9407*;* Fig. 4.5). WBI ranged from 0.88 to 0.93, and there was no significant difference between sites (F = 0.05, *P* = 0.8329). PRI was not significantly related to NDVI (r^2 = 0.01, F = 0.06, *P* = 0.8098; Fig. 4.6), suggesting that the indices are spatially independent and that PRI is not tracking changes in NDVI.

Landscape-level airborne reflectance

On the day of the flight, air temperature was 29 ºC, with relative humidity of 45% and 2076 μmol m^{-2} s^{-1} PPFD at solar noon. Oceanside and backside study sites used in physiological measurements were employed for landscape-level determination of PRI (Fig. 4.7). At the landscape-level, PRI was higher on the backside sites relative to the oceanside. Frequency histograms of pixels in different size classes showed that the distribution of PRI was shifted to the right on the backside of the thicket relative to the oceanside, although there was some overlap between sites (Fig. 4.8). Average values of PRI were 0.009 on the backside and -0.003 on the oceanside and there was a significant difference between sites (F = 114.22, *P* < 0.0001). 69 % of the sites on the oceanside had values of PRI lower than 0, compared to only 15 % of the sites on the backside of the thicket. NDVI did not differ between the two sites and averaged 0.73 (F = 0.71, *P* = 0.4011; Fig. 4.9). CI was not significantly different between the two sites (F = 2.96, P =

0.0870; Fig. 4.10). There was a significant difference in WBI between sites (F = 10.90, *P* = 0.0011), however frequency histograms of pixels in different size classes revealed that both sites approximated normal distributions and values of WBI spanned the same range (Fig. 4.11). There was a significant, but weak relationship between PRI and NDVI (r^2 = 0.04, F = 9.29, *P* = 0.0026; Fig. 4.12), again suggesting that the indices are spatially independent.

Discussion

Because *M. cerifera* occurs in coastal environments, it must withstand periods of drought and episodic flooding with saltwater in the presence of high irradiances. The summer of 2007 was extremely dry with little rainfall occurring after mid-July. Young (1992) showed that *M. cerifera* is sensitive to moisture stress, with partial stomatal closure occurring at a leaf water potential of -0.8 MPa. This sensitivity to water stress was observed in the field where partial stomatal closure was observed after relative water content reached very low values. Values of stomatal conductance during the summer were lower than those reported by Young (1992; approximately 400 mmol H_2O m^{-2} s^{-1}) probably due to drought conditions. Rates of photosynthesis were also slightly lower compared to those in an unusually wet July (approximately 32 μmol CO_2 m^{-2} s^{-1}; Young, 1992). Drought induced stress was apparent in CO_2 assimilation and relative water content by August. Stomatal conductance and photosynthetic rates continued to remain very low through November.

Depressions in CO_2 assimilation due to stress are generally associated with stomatal closure, which also reduces water loss (Pereira & Chaves, 1993). Partial stomatal closure was observed by September, and this likely accounted for low

photosynthetic rates at both sites. Under conditions of mild water stress, photosynthesis may be inhibited through ATP limitation not CO_2 diffusion (Tezara et al., 1999). Leaf relative water content was very low during August, and may have contributed to declining rates of photosynthesis, even though stomatal conductance values were at the highest rates recorded during the study. Temperatures during the study were near optimum for photosynthesis (~ 30 ºC) and likely did not inhibit CO_2 assimilation (Young, 1992).

Drought did not induce changes in $\Delta F/F'_m$ in the field. This was unexpected considering the low values of CO_2 assimilation and that declines in $\Delta F/F'_m$ occurred in *M. cerifera* after 2 days of drought stress in lab studies (Naumann et al., 2007). Differences in chlorophyll fluorescence between the two sites were likely due to salinity effects. Values were consistently lower on the exposed oceanside site, which also exhibited higher leaf tissue chlorides. Salinity may also be responsible for lower values of stomatal conductance, photosynthesis, and relative water content on the oceanside site.

Photorespiration increases with stomatal closure and subsequent reduction in photosynthesis. This serves as one of numerous mechanisms to safely dissipate excess light to avoid photoinhibition and photooxidation (Flexas & Medrano, 2002). Another such mechanism involves changes in pigments of the xanthophyll cycle. The rate of dissipation of excess energy as heat is correlated with the amount of zeaxanthin present (Demmig-Adams et al., 1989). In some species, PRI has been demonstrated to be a reliable indicator of plant stress, more so than chlorophyll-based indices (Richardson et al., 2001; Thorhaug et al., 2006) because of rapid changes in xanthophyll cycle pigments; however, this is not true in all species (Carter, 1998; Estep and Carter, 2005), making it

necessary to examine the efficacy of reflectance indices in different canopies (Filella et al., 2004). $\Delta F/F'_{m\,is}$ an indicator of the actual PSII efficiency in light (Ball, 1994). Significant relationships have been identified between PRI and $\Delta F/F'_m$ under conditions of drought stress (Evain et al., 2004), saltwater flooding (Naumann et al., in press), and over a range of irradiance levels (Méthy, 2000). Our results showed a positive, linear relationship at the canopy-level between $\Delta F/F'_m$ and PRI, providing evidence for xanthophyll cycle dependent energy dissipation as the underlying mechanism in protecting PSII.

Spatial variations in canopy-level PRI may be an indication of variations in xanthophyll cycle pigments between plants with different capacities for photosynthetic efficiency (Nichol et al., 2006). Higher PRI values on the backside of the thicket indicated a higher xanthophyll epoxidation state and may be a reflection of the increased photosynthetic rates seen on the backside relative to the oceanside site. Changes in PRI between the two sites at both the canopy-level and landscape-level were likely due to salinity stress more so than water stress. Leaf tissue chlorides were much higher on the oceanside site, with corresponding lower PRI values, while relative water content and WBI did not differ significantly between the two sites. Values for WBI were far from those corresponding to low water content and, thus, an indication that plants were not experiencing extreme water stress (Peñuelas et al., 1993). These results suggest that PRI is able to track spectral changes in salinity stress as evidenced by differences in chlorophyll fluorescence and tissue chlorides. Thorhaug et al. (2006) demonstrated changes due to salinity in spectral reflectance indices in seagrass, especially PRI, that

were consistent with stress responses in terrestrial plants. Our results support the possibility of using PRI at larger spatial scales.

Soil background and canopy LAI must be accounted for when using PRI to detect water stress (Suárez et al., 2007). In our study, LAI of the shrub thicket was estimated to be 10, with very little to no variation among sites ranging from the backside to the oceanside sites (Brantley & Young, 2007). Values of LAI above 6 are insensitive to the background effect of the soil, eliminating soil background as a confounding factor (Barton & North, 2001). The lack of relationship between PRI and NDVI at both the canopy and landscape level suggests that issues related to structure and viewing geometry were not a factor affecting the PRI signal. PRI can be scaled from upper canopy leaves to the whole-plant canopy (Stylinski et al., 2002). Due to the aforementioned factors, *M. cerifera* thickets are ideal for stress detection and scale-up to the landscape level.

Conclusions

Drought stress was evident in late summer, as seen in low photosynthetic rates and stomatal closure. Despite the pronounced drought, values for chlorophyll fluorescence did not change, indicating that *M. cerifera* is able to effectively dissipate excess light even in times of stress. While drought effects were not measurable via fluorescence, salinity stress did cause lower values of $\Delta F/F'_m$ on the oceanside sites. Rates of CO_2 assimilation and stomatal conductance were also lower on the oceanside, also attributed to salinity stress. Few studies have examined the suitability of different reflectance indices for plants growing naturally in the field (Filella et al., 2004). In this study, ground-based canopy reflectance showed good correlation between PRI and chlorophyll fluorescence. Changes in both PRI and fluorescence were attributed to

salinity stress. Airborne landscape-level reflectance measurements also showed lower PRI values in areas of higher salinity (i.e. oceanside sites). *Myrica cerifera* thickets have been expanding in many locations on the Virginia barrier islands, but this expansion has been confounded by the effects of sea-level rise and storm intensity (Young et al., 2007). Our results suggest that PRI may be used for early identification of salt-stressed areas due to storm overwash or groundwater intrusion and to predict changes in community structure across the landscape due to sea-level rise.

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Figure Legends

Fig. 4.1 Location map for study sites at Hog Island, Virginia.

Fig. 4.2. Monthly variations in maximum (solid line) and minimum temperature (dotted line) as measured from a meteorological station on Hog Island between June 1 and November 30, 2007 (a). Monthly variation in precipitation on Hog Island (b).

Fig. 4.3 Monthly variations in stomatal conductance (a), net photosynthesis (b), relative water content (c) and tissue chlorides (d) on the backside (filled symbols) and oceanside (open symbols) of the *Myrica cerifera* thicket. Values represent means ± 1 standard error.

Fig. 4.4. Monthly variations in $\Delta F/F'_m$ on the backside (filled symbols) and oceanside (open symbols) of the *Myrica cerifera* thicket. Values represent means ± 1 standard error.

Fig. 4.5. Relationships obtained between PRI (a), NDVI (b), CI (c), and WBI (d) with $\Delta F/F'_m$ on the backside (filled symbols) and oceanside (open symbols) of the *Myrica cerifera* thicket. Indices were obtained from canopy-level reflectance data.

Fig. 4.6. Relationship between PRI and NDVI on the backside (filled symbols) and oceanside (open symbols) of the *Myrica cerifera* thicket. Indices were obtained from canopy-level reflectance data.

Fig. 4.7 SpectIR hyperspectral images of Hog Island and the oceanside (o) and backside (b) study sites used. False color composite uses bands 802 nm, 672 nm, and 529 nm (RGB).

Fig. 4.8. Frequency histograms of PRI values obtained from hyperspectral image data on the backside (a) and oceanside (b) of the *Myrica cerifera* thicket and computed PRI resultant images for SpectIR hyperspectral data using bands 30 (529 nm) and 39 (572 nm) (c).

Fig. 4.9. Frequency histograms of NDVI values obtained from airborne hyperspectral image data on the backside (a) and oceanside (b) of the *Myrica cerifera* thicket and computed NDVI resultant images for SpectIR hyperspectral data using bands 87 (802 nm) and 60 (672 nm) (c).

Fig. 4.10. Frequency histograms of CI values obtained from airborne hyperspectral image data on the backside (a) and oceanside (b) of the *Myrica cerifera* thicket.

Fig. 4.11. Frequency histograms of WBI values obtained from airborne hyperspectral image data on the backside (a) and oceanside (b) of the *Myrica cerifera* thicket.

Fig. 4.12. Relationship between PRI and NDVI on the backside (filled symbols) and oceanside (open symbols) of the *Myrica cerifera* thicket. Indices were obtained from airborne hyperspectral image data.

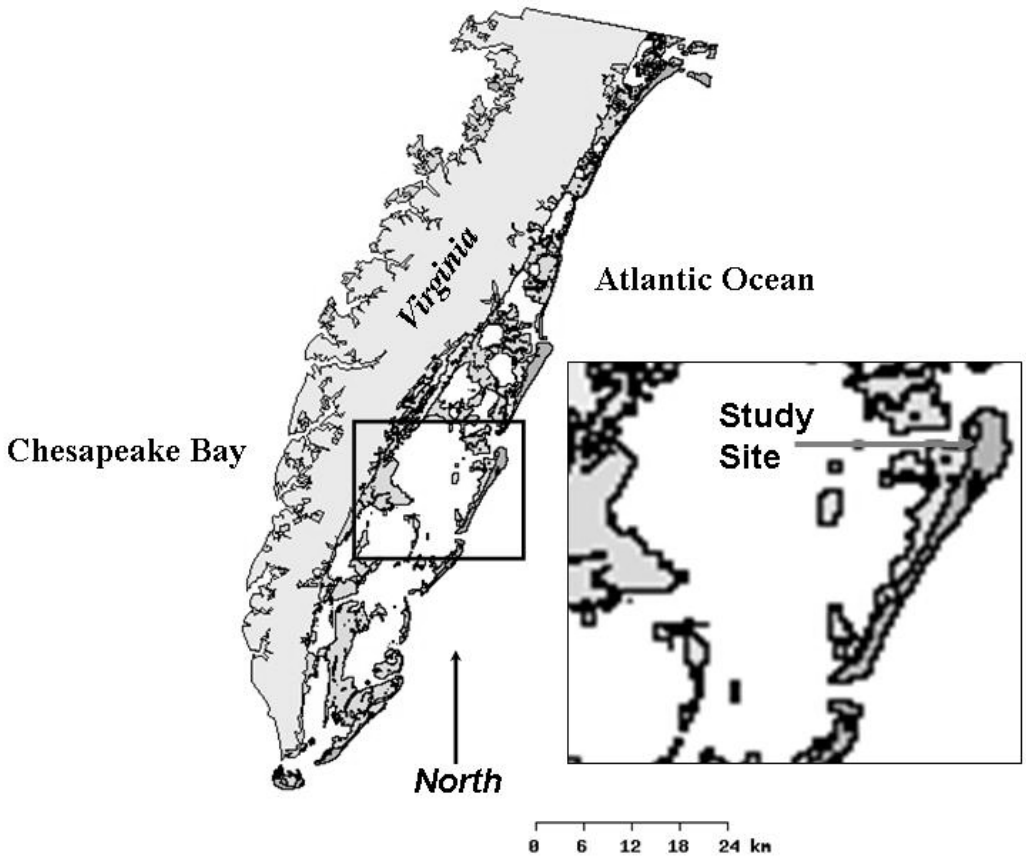

Virginia
Atlantic Ocean
Chesapeake Bay
Study
Site
North
0 6 12 18 24 km

Figure 4.1

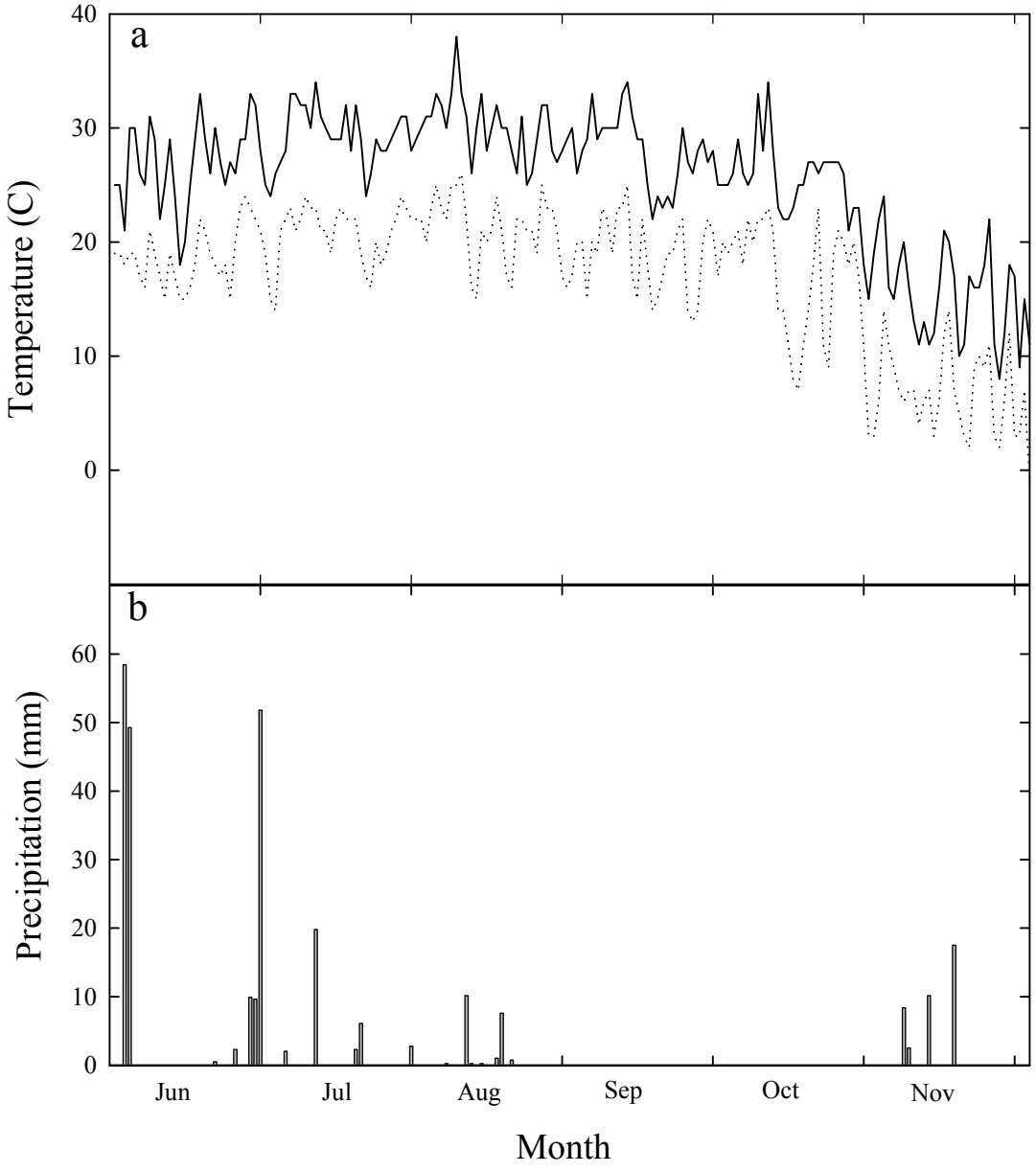

a
b
Temperature (C)
Precipitation (mm)
40
30
20
10
0
60
50
40
30
20
10
0
Jun
Jul
Aug
Sep
Oct
Nov
Month

Figure 4.2

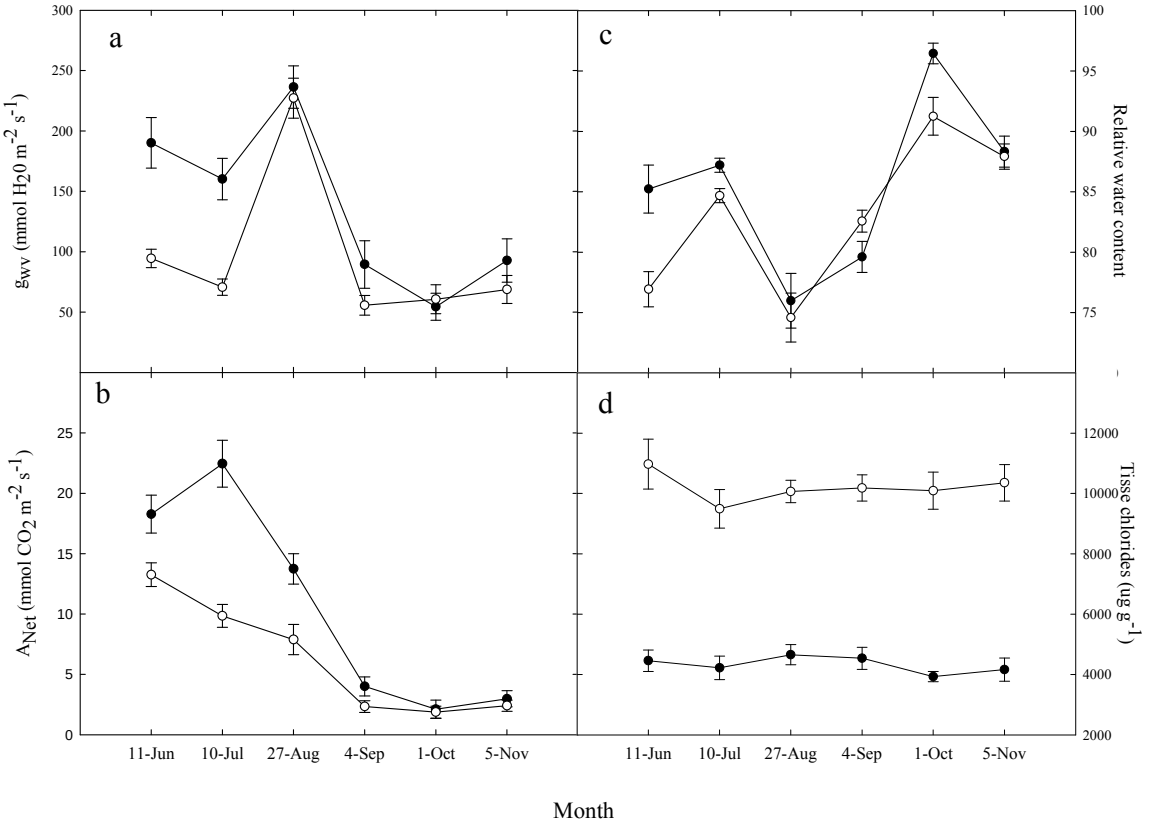

a
b
c
d
g_{wv} (mmol H_20 m^{-2} s^{-1})
A_{Net} (mmol CO_2 m^{-2} s^{-1})
Relative water content
Tissue chlorides (ug g^{-1})
11-Jun
10-Jul
27-Aug
4-Sep
1-Oct
5-Nov
Month

Figure 4.3

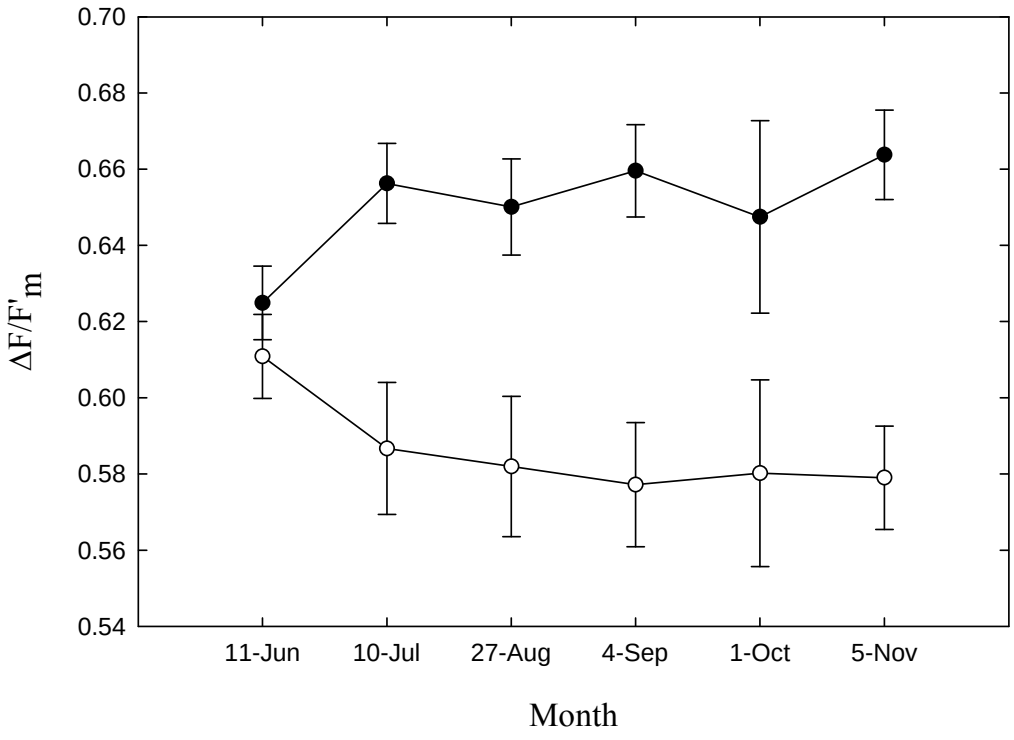

0.70
0.68
0.66
0.64
0.62
0.60
0.58
0.56
0.54
ΔF/F'm
11-Jun
10-Jul
27-Aug
4-Sep
1-Oct
5-Nov
Month

Figure 4.4

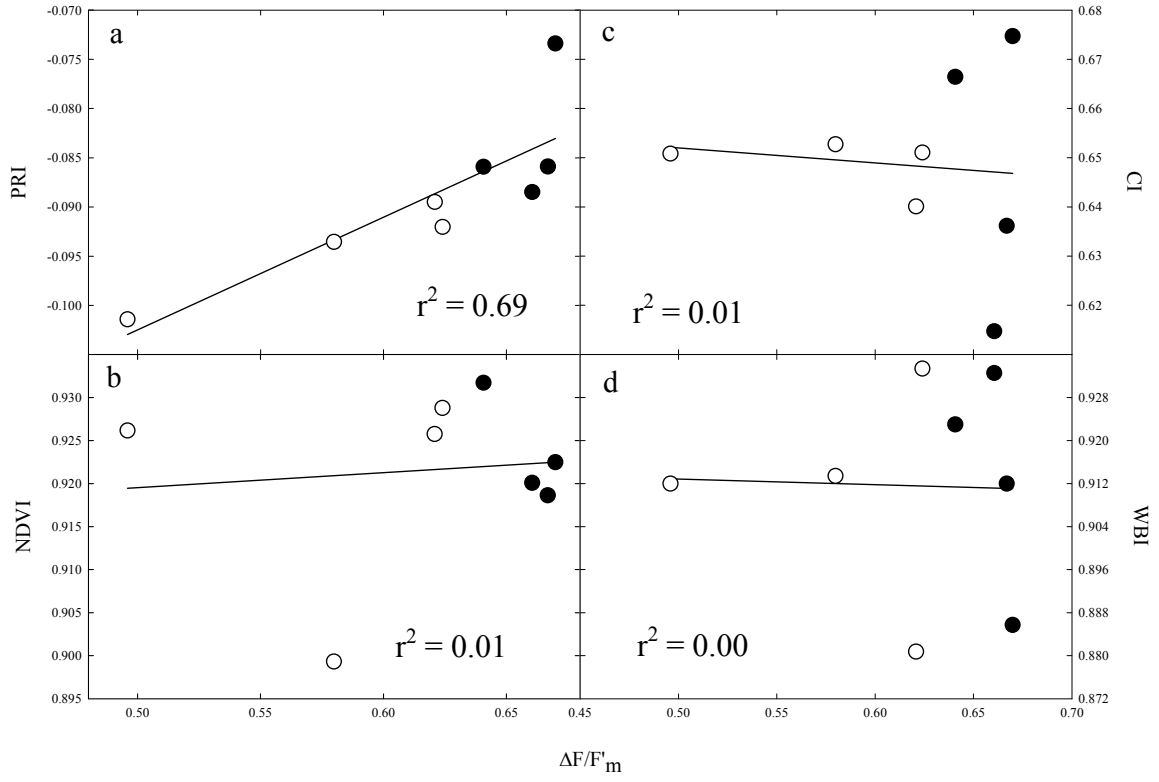


Figure 4.5

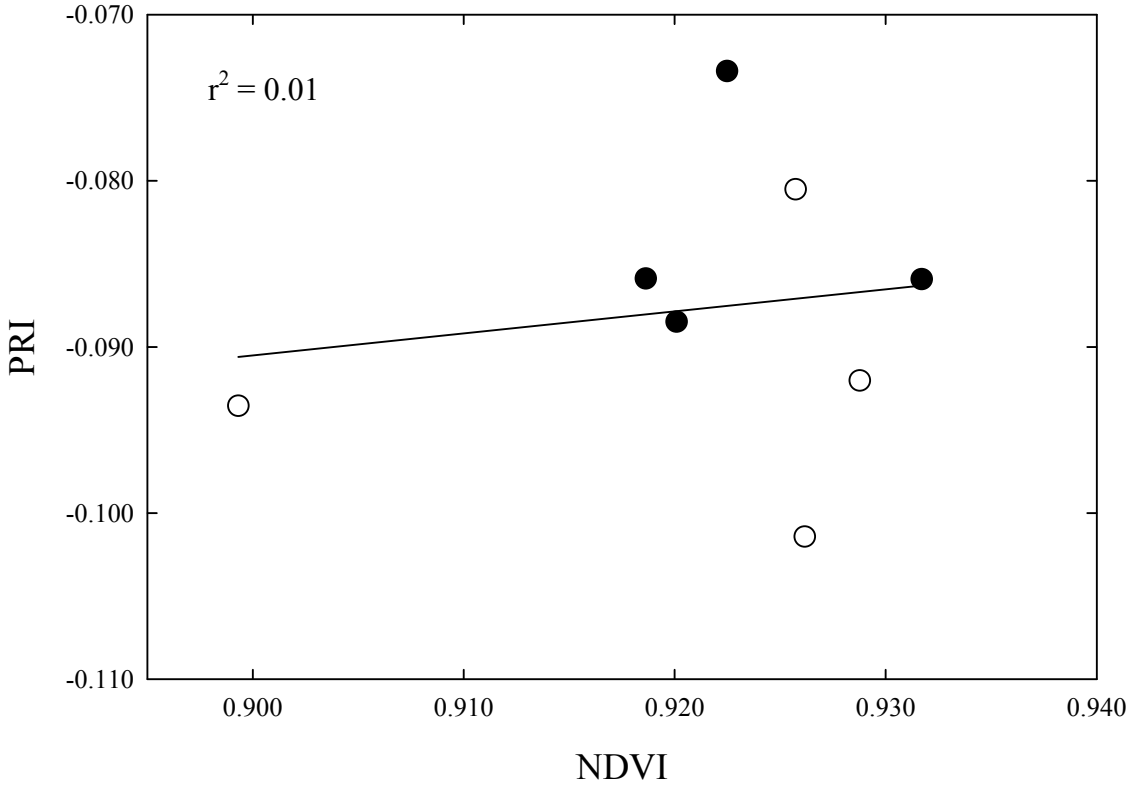
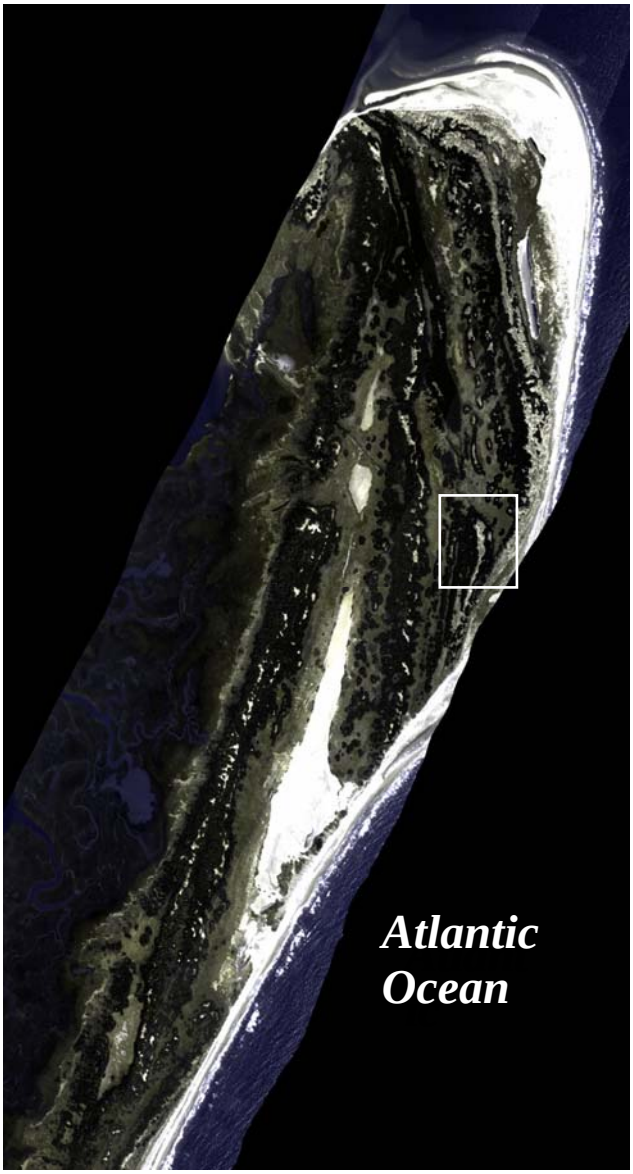

-0.070
-0.080
-0.090
-0.100
-0.110
PRI
r2 = 0.01
0.900
0.910
0.920
0.930
0.940
NDVI

Figure 4.6



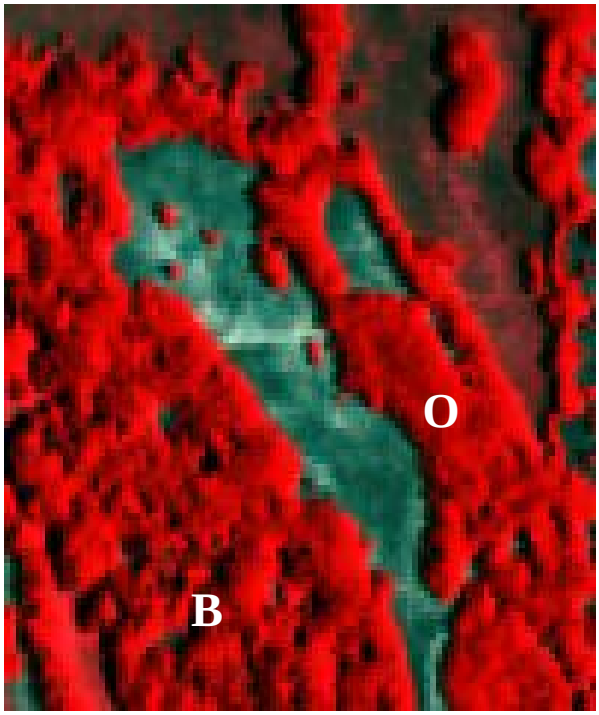
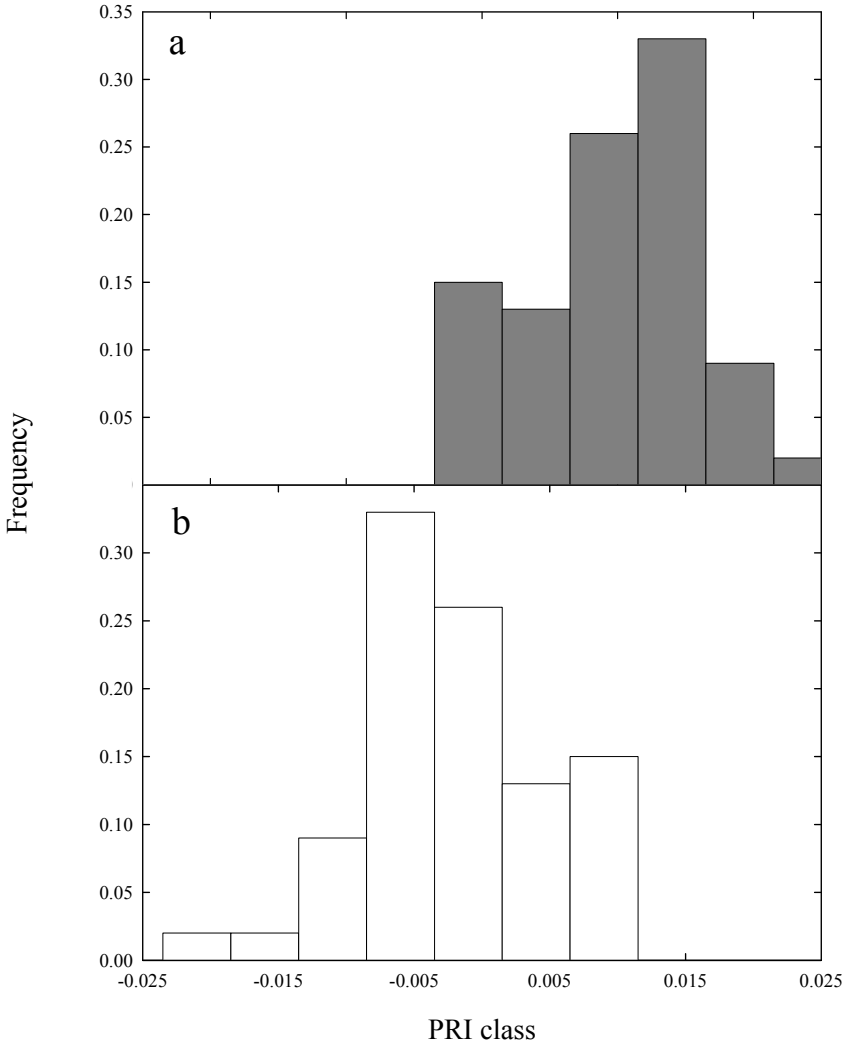


Figure 4.7


a
b
Frequency
PRI class
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.025
-0.015
-0.005
0.005
0.015
0.025

c

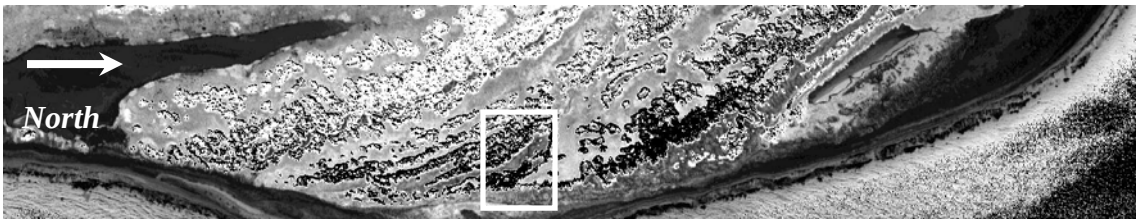
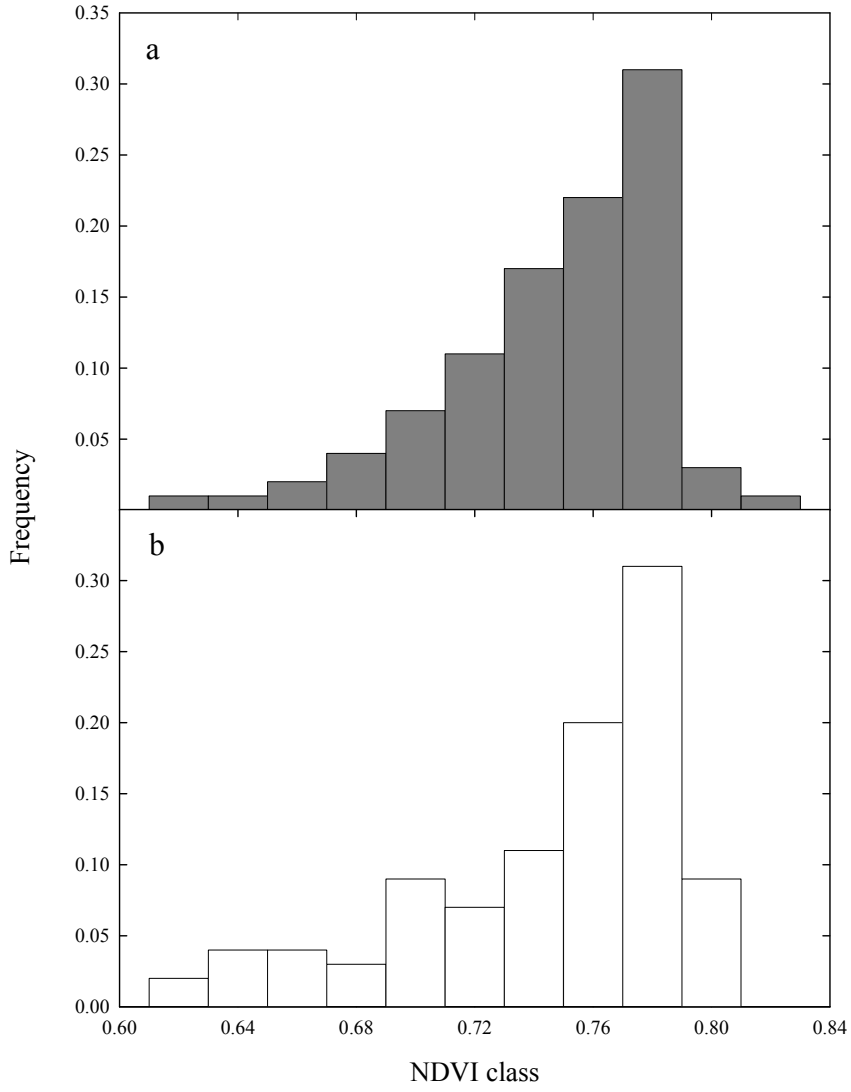

North

Figure 4.8


a
b
Frequency
NDVI class
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.60
0.64
0.68
0.72
0.76
0.80
0.84

c

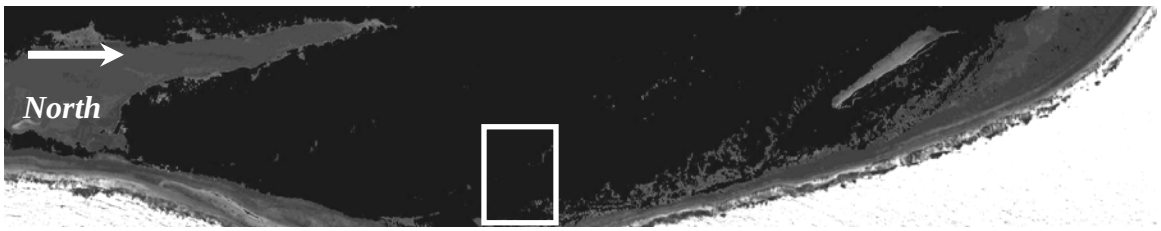

North

Figure 4.9

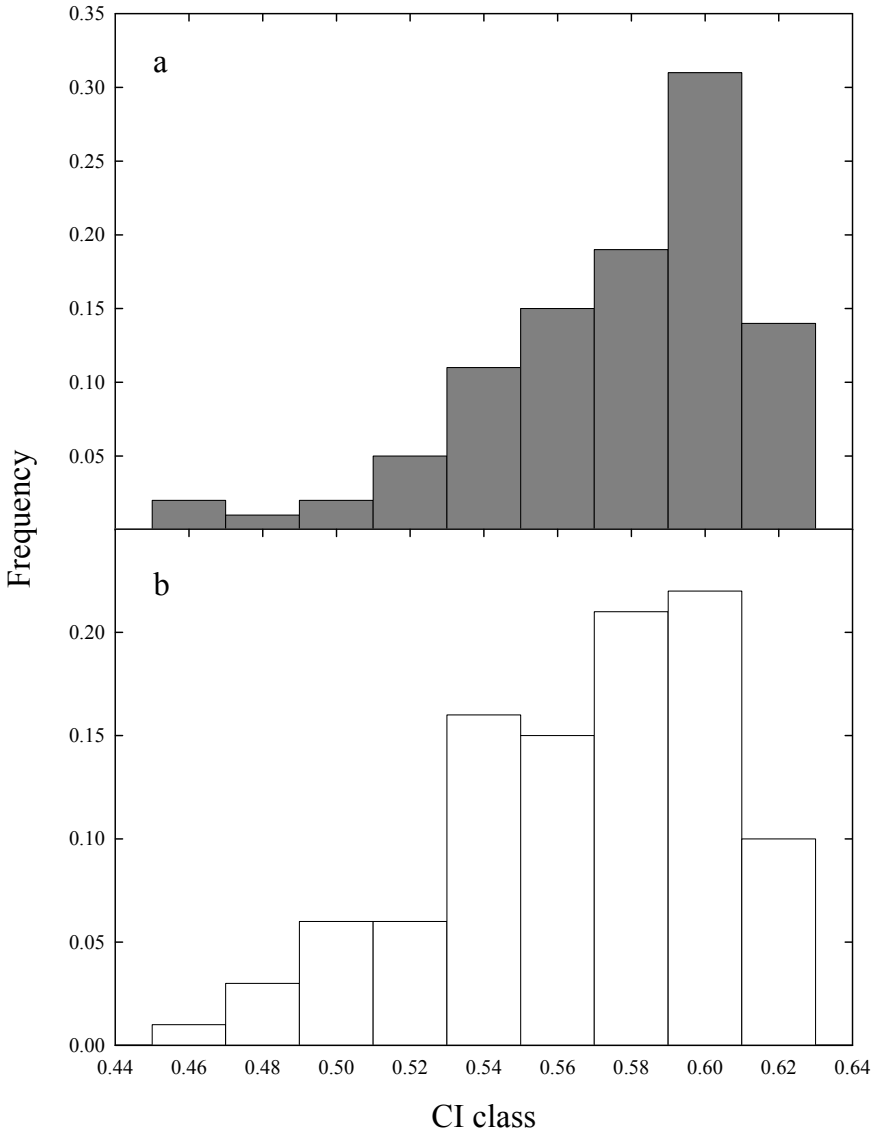

a
b
Frequency
CI class
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.44
0.46
0.48
0.50
0.52
0.54
0.56
0.58
0.60
0.62
0.64

Figure 4.10

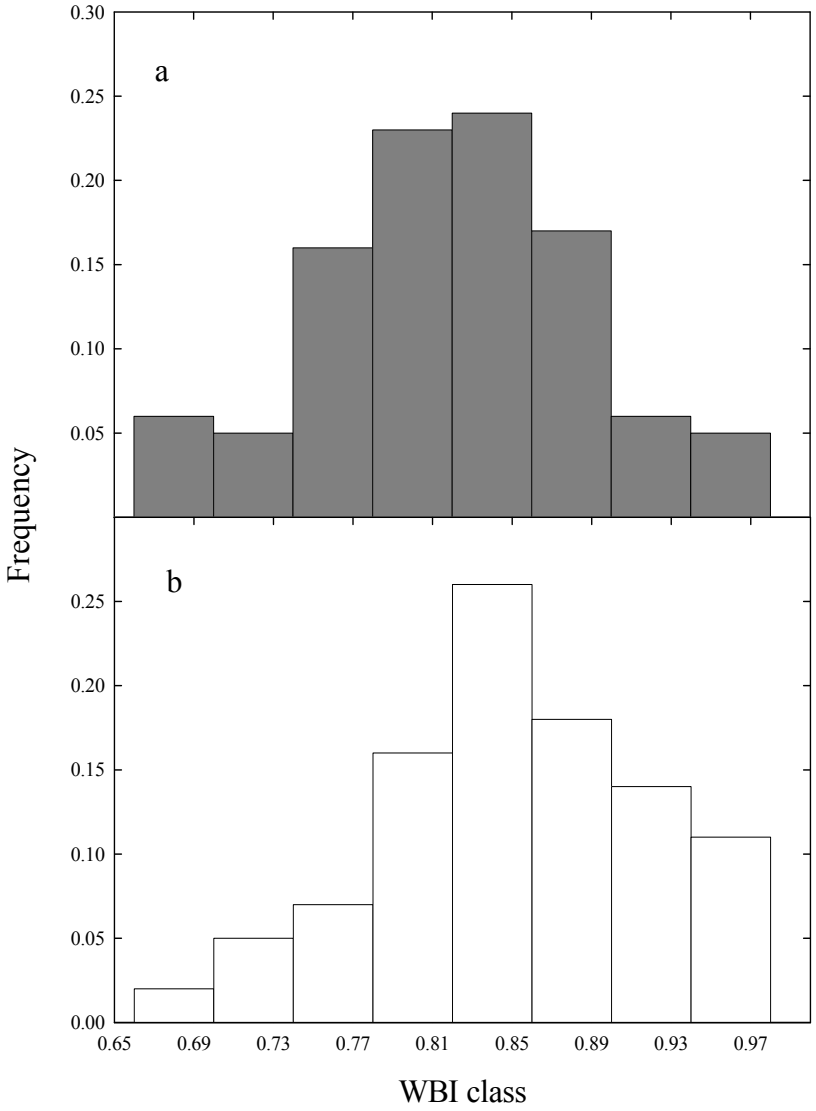

a
b
Frequency
WBI class
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.65
0.69
0.73
0.77
0.81
0.85
0.89
0.93
0.97

Figure 4.11

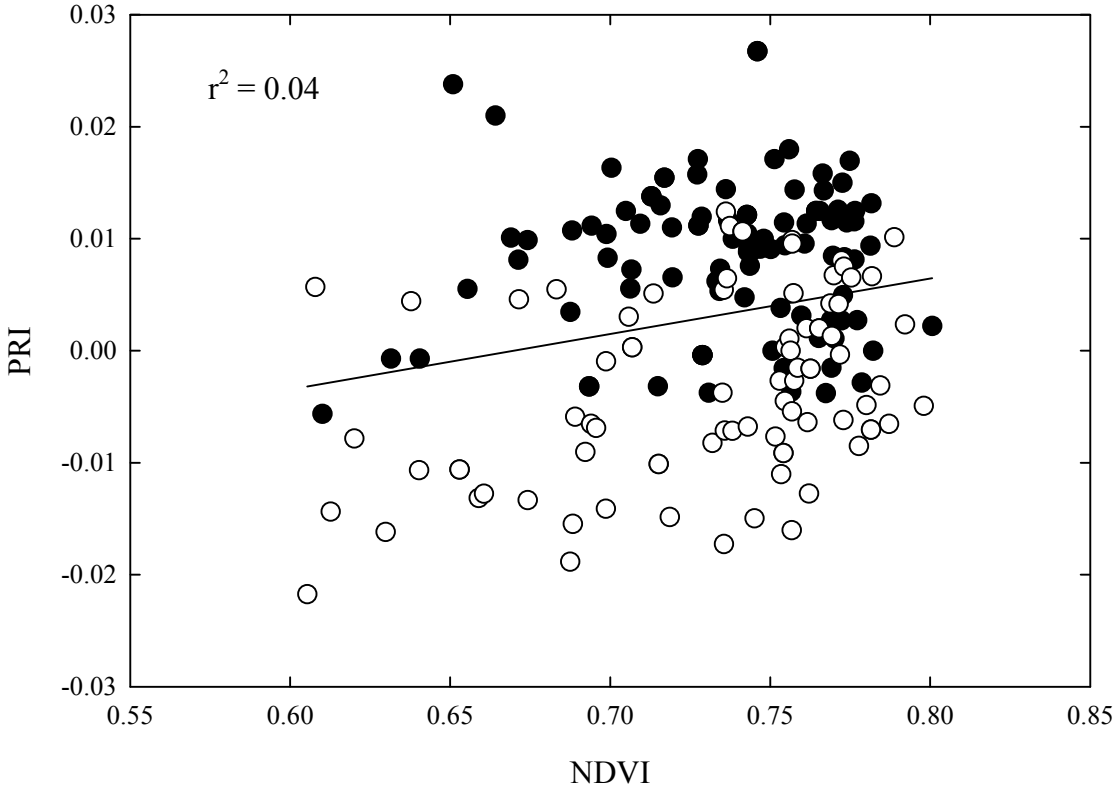

0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
PRI
r2 = 0.04
0.55
0.60
0.65
0.70
0.75
0.80
0.85
NDVI

Figure 4.12

CHAPTER 5

SPATIAL VARIATIONS IN SALINITY STRESS ACROSS A COASTAL LANDSCAPE USING VEGETATION INDICES DERIVED FROM HYPERSPECTRAL IMAGERY

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Abstract

Chlorophyll fluorescence and landscape-level reflectance imagery were used to evaluate spatial variations in stress in *Myrica cerifera* and *Iva frutescens* during a severe drought and compared to an extremely wet year. Measurements of relative water content and the water band index (WBI_{970}) indicated that water stress did not vary across the island. In contrast, there were significant differences in tissue chlorides across sites for both species. Using the physiological reflectance index (PRI), we were able to detect salinity stress across the landscape. For *M. cerifera* PRI did not differ between wet and dry years, while there were differences in PRI during the two years for *I. frutescens*, possibly related to flooding during the wet year. There was a positive relationship between PRI and $\Delta F/F'_m$ for *M. cerifera* ($r^2 = 0.79$) and *I. frutescens* ($r^2 = 0.72$). The normalized difference vegetation index (NDVI), the chlorophyll index (CI) and WBI_{970} were higher during the wet summer for *M. cerifera*, but varied little across the island. CI and WBI_{970} were higher during 2004 for *I. frutescens,* while there were no differences in NDVI during the two years. PRI was not significantly related to NDVI, suggesting that the indices are spatially independent. These results suggest that PRI may be used for early identification of salt stress that may lead to changes in plant distributions at the landscape level as a result of rising sea-level. Comparsions between the two species indicate that variations in PRI and other indices may be species specific.

Introduction

Salinity is considered to be the primary environmental factor influencing community patterns in coastal ecosystems (Oosting and Billings 1942; Ehrenfeld 1990; Stalter and Odum 1993). Drought, high irradiance and high temperatures are among many other factors that limit plant growth in these environments (Ehrenfeld 1990). These physical forces create distinct zones of vegetation across the coastal landscape relative to distance from the ocean. Environmental boundaries are not as discrete as the zonation of coastal plant communities, yet there is a relationship between changes in species composition and gradient for abiotic stressors (Crawford 1989). Young et al. (1994) demonstrated that spatial and seasonal variations in groundwater salinity and soil chlorides are partly responsible for the spatial distribution of woody vegetation on barrier islands. Shifts in plant distributions due to salinity in coastal areas are likely to occur with the predicted effects of climate change, most notably sea-level rise and increased storm intensity and frequency (Gregory and Oerlemans 1998; Zhang et al. 2000).

Much effort has been devoted to identifying stress in plants before visible signs are observed. Changes in visible reflectance, shifts in the reflectance curve red edge and differences in various indices have correlated strongly with plant stress (Carter 1993; Carter and Young 1993; Blackburn 2007). In recent years, attention has been focused on measurements of chlorophyll fluorescence as a means of early stress detection. However, research into light-adapted measurements of fluorescence is imperative as dark-adaptation is not currently feasible at scales beyond the leaf level. Chlorophyll fluorescence measurements are ideal because they are non-destructive and linked to physiological functioning of plants (Zarco-Tejada et al. 2000). One drawback to

fluorescence is that most instruments are not capable of making measurements from a distance because of the weak signal (less than 2 % of total reflected visible light). Thus, finding the fluorescence signal in reflectance data has been a focus of more recent research efforts and would enable rapid large-scale detection of plant physiological status (Zarco-Tejada et al. 2003; Evain et al. 2004; Dobrowski et al. 2005).

All environmental stresses that lower the photosynthetic rate of a plant will increase the need for energy dissipation of excess, absorbed light (Demmig-Adams and Adams 1992). One mechanism for the dissipation of excess energy involves changes in xanthophyll cycle pigments. Under excess light, violaxanthin is converted to zeaxanthin. Increases in zeaxanthin levels are correlated to increases in energy dissipation, which can be measured by chlorophyll fluorescence (Demmig-Adams and Adams 1996). The increase levels of zeaxanthin can also be monitored by changes in reflectance at 531 nm using the physiological reflectance index (PRI). PRI has been successfully used to indicate physiological changes from both acute (Evain et al. 2004; Dobrowski et al. 2005; Naumann et al. 2008) and chronic stress (Filella and Peñuelas 1999; Asner et al. 2004; Filella et al. 2004). It is also a useful index for remote sensing of water stress in agricultural and natural systems (Peñuelas et al. 1998; Suàrez et al. 2008), yet investigations into the application of PRI for detection of salinity stress are limited (Thorhaug et al. 2006; Naumann et al. 2008).

Applicability of PRI at the landscape and larger scales are complicated due to the heterogeneous composition of the landscape and other errors (Peñuelas et al. 1997). Recently, researchers have demonstrated the success of using airborne imagery for calculating PRI in various sytems (Asner et al. 2004; Fuentes et al. 2006; Suárez et al.

2008; Naumann et al. in review). The objective of our study was to identify spatial variations in plant stress in the dominant woody vegetation on a Virginia barrier island using a combination of field measurements and hyperspectral, airborne reflectance. Specific goals were to (1) identify areas of stress using field measurements of chlorophyll fluorescence, (2) determine the cause of stress, (3) link field measurements to hyperspectral imagery and (4) use hyperspectral imagery and indices to identify stress across the landscape.

Materials and methods

Study sites

The field study was conducted on the North end of Hog Island (37° 40'N; 75° 40'W), a barrier island located on the Eastern Shore of Virginia on September 13, 2007 The oceanside, northern end of the island has been accreting approximately 5 m/year for 140 years (Hayden et al. 1991), resulting in a parallel series of dunes and swales. We conducted our study across the northern end of the island, focusing on two shrubs: *Myrica cerifera* L. (Myricaceae), an evergreen, nitrogen-fixing, salt sensitive shrub which dominates the mesic swales and *Iva frutescens* L. (Asteraceae), a salt-succulent shrub most common along the edge of salt marsh (Ehrenfeld 1990; Young et al. 1994). Five ***M. cerifera*** and two ***I. frutescens*** sites were used in our study with five sampling locations at each site (Fig. 1). Study sites were chosen based on exposure to salinity. The ***M. cerifera*** Oceanside site is ~ 200 m from the Atlantic Ocean and the most exposed to salt spray. The Backside site is the leeward, protected side of the same thicket. The Young site is ~ 30 m from the Backside site, and is ocean facing. The Dune site is located in the middle of the island adjacent to a dune and samples were collected from the ocean facing side.

The Mid-island site is in the middle of the island adjacent to a freshwater pond, and is generally the most freshwater flooded site. Samples taken from the Mid-island site faced away from the ocean. The two *I. frutescens* sites are located on the bayside of the island. The Bayside site is located at the edge of the saltwater marsh, while the Path site occurs at a higher elevation along a dry path and is adjacent to a hypersaline saltpan (Fig. 5.1).

Field Measurements

Light-adapted chlorophyll fluorescence was measured at each site (n = 50) using a pulse amplitude modulated leaf fluorometer (PAM-2000, Walz, Effeltrich, Germany). The relationship between maximal fluorescence in a light-adapted leaf after a saturating pulse of light (F'_m) and steady-state fluorescence prior to any saturating pulse (F_s) was used to estimate the effective quantum yield of photosystem II:

$\Delta F/F'_m = [F'_m - F_s]/F'_m$

Five leaves from each sampling location were clipped at the stem and kept at 100% humidity. Relative water content was measured as:

RWC (%) = (FW – DW)/ (SFW – DW) *100

where FW is fresh weight, DW is dry weight, and SFW is saturated fresh weight of the leaves after re-hydrating samples for 24 h (Turner 1981). Tissue chlorides were quantified at each sampling location (n =5). Leaf samples were oven-dried at 80°C for 72 h and then ground in a fine mesh mill. For each sample, 0.5 g of material was placed in a tube with 40-mL of deionized water. Samples were placed in a boiling water bath for 2 h, cooled, and filtered into 100-mL volumetric flasks. To each sample, 2-mL of 5 M $NaNO_3$ was added as an ionic equalizer, and then samples were brought to volume with

deionized water (Young et al. 1994). Chloride levels were determined using a chloride electrode (model 9617b, Orion, Boston, MA).

Airborne Image Acquisition

The airborne hyperspectral mission was flown concurrent with the field measurements at Hog Island on September 13, 2007. On the day of the flight, air temperature was 29 ºC, with relative humidity of 45% and 2076 μmol m^{-2} s^{-1} PPFD at solar noon. Hyperspectral data (3 nm resolution) were provided by the SpectIR using the ProSpecTIR VIS hyperspectral imaging spectrometer (SpectIR Corp.). Hyperspectral imagery covering 450 nm to 2450 nm was collected under cloud-free conditions at 1700 m (AGL) providing a data set representing 2 m / pixel on the ground and a final spectral cube 356 bands deep. These data products were post-processed to correct for geometric and radiometric (e.g., bi-directional) effects. Ground reflectance radiometry was used to calibrate the data based on target endmembers collected in-scene with the ASD FieldSpec Pro Full Range reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO). This effectively placed the scene into reflectance units and helped to negate any atmospheric effects. Calibration was performed using the empirical line calibration method within ENVI (RSI, Inc). Points were selected from each sampling location based on GPS measurements and the corresponding spectra extracted. The extraction of pixels with 2 m resolution enabled the calculation of indices without any shadowing effects. Numerous reflectance indices were calculated to elucidate spatial variation across the island. Only the relevant indices are included in this paper and are listed in Table 1.

The summer of 2007 was very dry. To understand relationships in spectral indices across the island, we also acquired imagery of Hog Island during an unusually

wet year. The airborne hyperspectral mission was flown on August 24, 2004 using the Portable Hyperspectral Imager for Low-Light Spectroscopy (PHILLS) (Davis et al. 2002). On the day of the flight, air temperature was 29 ºC, with relative humidity of 71% and 2018 μmol m^{-2} s^{-1} PPFD at solar noon. Hyperspectral imagery covering 384 nm to 1000 nm was collected at a spatial resolution of 3 m / pixel. These data products were post-processed to correct for geometric and radiometric (e.g., bi-directional) effects.

Statistical Analyses

Analysis of variance (ANOVA) was used to test for variations in sites of the each species for the following measurements: chlorophyll fluorescence, RWC, and tissue chlorides (Zar, 1999). Significant differences among sites were identified with Tukey tests ($\alpha = 0.05$). Two-ANOVA was used to test for significant interactions between site and year for reflectance indices. In cases where a significant interaction occurred, one-way ANOVAs were used to test for variations among sites within a year, and to test for variations between years at a specific site. Variations in reflectance indices were related to variations in chlorophyll fluorescence, RWC and tissue chlorides using linear regressions.

Results

Precipitation for June through September of 2004 was 52% above the 30 year average (388 mm). In comparison, 2007 was unusually dry and characterized by a persistent drought. Precipitation for June through September was 39% below the 30 year average (Fig. 5.2). In 2004, the longest period without rainfall was 11 days in June, whereas in 2007 only 2 rain events > 5 mm occurred after July 11 and no precipitation occurred after mid-August (Fig. 5.2).

In 2007, relative water content for *M. cerifera* averaged 96 ± 1% at the Mid-island site and was significantly higher compared to other island sites ($F = 16.76$, $P < 0.001$). Average RWC ranged from 85 ± 1% to 78 ± 0% at the other *M. cerifera* sites (Fig. 5.3). RWC did not significantly differ between *I. frutescens* sites ($F = 0.26$, $P = 0.621$) and averaged 73 ± 2% (Fig. 5.3). Total chlorides present in leaves were higher for the salt succulent plant, *I. frutescens* compared to *M. cerifera* (Fig. 5.3). Among *M. cerifera* sites, the Oceanside thicket had the highest chlorides and was significantly different from all other sites ($F = 261.72$, $P < 0.001$). The Young and Dune site were similar in tissue chloride concentrations as were the Mid-island and Backside sites (Fig 5.3). Tissue chlorides were significantly lower for *I. frutescens* at the Bayside site compared to the Path site ($F = 42.43$, $P < 0.001$; Fig 5.3).

$\Delta F/F'_m$ significantly differed across the island in *M. cerifera* thickets ($F = 19.29$, $P < 0.001$). The Dune site had the lowest $\Delta F/F'_m$ values (0.48 ± 0.03) and was significantly lower than all other sites (Fig 5.4). Highest $\Delta F/F'_m$ were found at the Backside (0.70 ± 0.03) and Mid-island sites (0.68 ± 0.02). There was a significant difference in $\Delta F/F'_m$ of *I. frutescens* between the Bayside (0.73 ± 0.01) and Path sites (0.70 ± 0.02; $F = 9.89$, $P = 0.014$; Fig 5.4).

There was no significant interaction in PRI between *M. cerifera* sites and year ($F = 0.73$, $P = 0.575$). PRI was significantly different among *M. cerifera* sites ($F = 20.86$, $P < 0.001$) and varied from -0.02 to 0.03. *Post-hoc* comparisons revealed that the Dune site had the lowest values of PRI. The Young and Oceanside sites had midline values of PRI, and were significantly lower than the Backside and Mid-island sites (Fig. 5.5). There was no significant difference between the two years ($F = 0.26$, $P = 0.613$). Values of PRI

were higher in *I. frutescens* compared to *M. cerifera*, ranging from 0.01 to 0.02. There was a significant interaction between site and year for *I. frutescens* ($F = 55,84$, $P < 0$ .01). In 2007, PRI was significantly higher at the Bayside site ($F = 9.89$, $P = 0.014$), whereas PRI was significantly lower at the Bayside site in 2004 ($F = 30.40$, $P < 0.001$; Fig. 5.4). There was a significant difference between the two years at the Bayside site ($F = 122.24$, $P < 0.01$), but not at the Path site ($F = 4.33$, $P = 0.070$).

There was a significant interaction in NDVI between *M. cerifera* sites and year ($F = 2.77$, $P = 0.040$; Fig. 5.4). There were a significant differences among sites with the highest values of NDVI at the Backside site ($F = 5.46$, $P = 0.002$), and there was a significant difference between years ($F = 227.24$, $P < 0.001$). In 2007, NDVI values ranged from 0.65 to 0.77 across sites, while values were much higher in 2004, ranging from 0.79 to 0.94. NDVI values at the *I. frutescens* sites were much lower compared to *M. cerifera* and ranged from 0.46 to 0.57. There was no significant interaction between year and site for *I. frutescens* ($F = 0.94$, $P = 0.346$; Fig. 5.4) and there no significant differences between years ($F = 2.75$, $P = 0.117$) or sites ($F = 3.14$, $P = 0.095$).
PRI was positively related to $\Delta F/F'_m$ ($r^2 = 0.79$, $P < 0.001$; Fig. 5.5) at *M. cerifera* sites. PRI decreased from 0.014 to -0.019, while $\Delta F/F'_m$ decreased from 0.78 to 0.40 with highest $\Delta F/F'_m$ recorded at the Backside site. For *I. frutescens,* PRI was positively related to $\Delta F/F'_m$ ($r^2 = 0.72$, $P = 0.002$; Fig. 5.5).

PRI was not related to RWC in *M. cerifera* ($r^2 = 0.09$, $P = 0.135$; Fig. 5.6) or *I. frutescens* ($r^2 = 0.00$, $P = 0.994$; Fig. 5.6). There were no significant relationships between $\Delta F/F'_m$ and RWC in *M. cerifera* or *I. frutescens* ($r^2 = 0.09$, $P = 0.151$; $r^2 = 0.02$, $P = 0.701$, respectively). There was a weak but significant negative relationship between

PRI and tissue chlorides in *M. cerifera* ($r^2 = 0.27$, $P = 0.008$; Fig. 5.7). The Oceanside site had extremely high tissue chlorides compared to the other sites, which is likely influenced by salt spray on the leaves. When this site was removed from the regression, a much stronger relationship between PRI and tissue chlorides emerged ($r^2 = 0.81$, $P < 0.001$; Fig. 5.7). There was also a negative relationship between PRI and tissue chlorides for *I. frutescens* ($r^2 = 0.71$, $P = 0.002$; Fig. 5.7). A similar pattern was seen between $\Delta F/F'_m$ and tissue chlorides. With all *M. cerifera* sites included, there was no significant relationship ($r^2 = 0.11$, $P = 0.100$), but once the Oceanside site was removed, a strong relationship was seen ($r^2 = 0.72$, $P < 0.001$). For *I. frutescens* a significant relationship was also seen between $\Delta F/F'_m$ and tissue chlorides ($r^2 = 0.45$, $P = 0.033$).

In 2007, PRI was not significantly related to NDVI at the *M. cerifera* sites ($r^2 = 0.04$, $P = 0.637$; Fig. 5.8), suggesting that PRI is not tracking changes in NDVI and the indices are spatially independent. For *I. frutescens*, PRI was related to NDVI, but this relationship was not considered significant at the $\alpha = 0.05$ level ($r^2 = 0.38$, $P = 0.059$; Fig. 5.8). Similar trends were seen in 2004 for PRI and NDVI among *M. cerifera* sites ($r^2 = 0.00$, $P = 0.707$) and *I. frutescens* sites ($r^2 = 0.00$, $P = 0.938$; Fig. 5.8).

There were no significant relationships between CI and $\Delta F/F'_m$ among *M. cerifera* sites ($r^2 = 0.05$, $P = 0.292$). There was a significant interaction in CI between site and year for *M. cerifera* ($F = 4.31$, $P = 0.005$). CI was significantly higher in 2004 ($F = 92.80$, $P < 0.001$). There were no differences among sites in 2004 ($F = 0.75$, $P = 0.568$). In 2007, CI was significantly lower at the Mid-Island site ($F = 8.97$, $P < 0.001$). For *I. frutescens*, CI exhibited a relationship with $\Delta F/F'_m$ but was not significant ($r^2 = 0.35$, $P = 0.071$). There was no interaction between site and year for *I. frutescens* ($F = 0.39$, $P =$

0.539). CI was significantly higher in 2004 ($F = 64.18$, $P < 0.001$), but there were no differences between sites ($F = 0.45$, $P = 0.514$). WBI_{970} was not related to $\Delta F/F'_m$ at the *M. cerifera* sites or the *I. frutescens* sites ($r^2 = 0.08$, $P = 0.183$; $r^2 = 0.00$, $P = 0.906$, respectively) in 2007. Among *M. cerifera* sites, there was no interaction between site and year ($F = 1.95$, $P = 0.121$). WBI_{970} was significantly higher during 2004 ($F = 41.32$, $P < 0.001$) and the Mid-island site was significantly higher than other sites ($F = 5.43$, $P < 0.001$). There was a marginally significant interaction between year and site for *I. frutescens* ($F = 4.26$, $P = 0.056$). WBI_{970} was higher in 2004 ($F = 277.55$, $P < 0.001$) and was higher at the Bayside ($F = 35.64$, $P = <0.001$). For *M. cerifera*, WBI_{970} was significantly related to RWC in 2007 ($r^2 = 0.69$, $P < 0.001$; Fig. 5.9) but there was no relationship between WBI_{970} and RWC for *I. frutescens* ($r^2 = 0.00$, $P = 0.910$; Fig. 5.9).

Discussion

The results of our field study show a strong link between $\Delta F/F'_m$ and PRI for both *M. cerifera* and *I. frutescens* on a Virginia barrier island. These findings are similar to studies focused on water stress which have shown positive relationships between PRI and fluorescence (Peñuelas et al. 1998; Winkel et al. 2002). During the same 2007 field season, Naumann et al. (in review) showed that *M. cerifera* experienced a drought response as seen in decreases in stomatal conductance, photosynthesis and RWC relative to earlier in the season. Chlorophyll fluorescence did not respond to drought, but rather the differences in salinity. In this study the cause of stress is attributed to variations in salinity rather than drought based on tissue chlorides and RWC, as well as a comparison in reflectance data from the dry summer of 2007 with a relatively wet summer in 2004. Salinity affects plant water status and produces a suite of effects similar to those caused

by drought in newly developed, transpiring leaves (Munns 2002). Previous research has shown declines in chlorophyll fluorescence and PRI to be indicators of salinity stress in *Myrica cerifera* prior to visible signs of stress in both laboratory (Naumann et al. 2007) and field experiments (Naumann et al. in review).

PRI has been correlated to plant water status under drought conditions (Suárez et al. 2008). In our study, spatial variations in PRI and $\Delta F/F'_m$ were not linked to variations in water content during the summer drought of 2007, but did relate to tissue chlorides across the island. This is further supported by the similar pattern of spatial variation in PRI for *M. cerifera* during the unusually wet summer of 2004. Across the coastal landscape, *M. cerifera* is restricted to well-defined mesic swales due to sensitivity to moisture stress (Young 1992). However, distance from the shoreline and distance to the water table affect soil moisture content at a given landscape position such that every thicket differs considerably in soil water availability (Shao et al. 1995). Thus, in an extremely wet summer, we would expect to see less variation in PRI across the landscape if the spatial variations seen in 2007 were due to microsite differences in drought stress. NDVI values were higher in *M. cerifera* thickets during the wet summer of 2004 relative to 2007, in agreement with values of annual shoot growth across the island from these years (Donald R. Young unpublished data).

CI and WBI_{970} were also higher in 2004, consistent with expectations for a wet year. In 2007, CI was lower at the Mid-island site, but aside from this, there were no differences across sites, suggesting that differences in chlorophyll content are not responsible for changes in $\Delta F/F'_m$ or PRI across the island. Although there were no significant differences in WBI_{970} across the island, it did exhibit a good relationship with

RWC for *M. cerifera*, suggesting that WBI_{970} is a good index for monitoring the water status in this species. There was no relationship between WBI_{970} and RWC for *I. frutescens*, but WBI_{970} was higher at the Bayside site. Because this site is frequently flooded, these plants are likely to have access to more water than the Path site, resulting in a higher WBI_{970}, while microsite differences in salinity may influence the RWC of each plant (Hacker and Bertness 1995).

Differences in PRI, $\Delta F/F'_m$ and tissue chlorides from the Oceanside and Backside of the same thicket, which are separated by a distance of no more than 50 m, are of interest. The Backside site did not differ significantly from the Mid-island site (~ 900 m inland) in terms of PRI, fluorescence and tissue chlorides. Aspect and distance from the ocean are very important in determining the effect of salinity on plants and creating spatial variation in salinity stress across the landscape (Ehrenfeld 1990; Young et al. 1995). The Backside site, while only 250 m away from the shoreline, is the protected, leeside of the thicket and thus does not receive as much sea spray as sites that are ocean facing. The Oceanside site had extremely high values for tissue chlorides relative to other sites, yet the Dune site had the lowest values of PRI and $\Delta F/F'_m$. The high values of chlorides at the Oceanside site may be influenced not only by those in the tissues, but by chlorides from sea spray impacted to the surface of the leaf, which is likely to be pronounced in an extremely dry season. The Dune site, while further inland, is a very dry site and may be influenced by other factors such as depth to the water table, which could cause the effects of salinity to be greater. More study is needed to assess the exact cause of stress at this site. The Mid-island site, which is the most protected, appeared to be the least stressed site from field measurements and reflectance data.

For the halophyte *I. frutescens*, tissue chlorides were much higher and RWC values were considerably lower compared to the salt and moisture sensitive plant *M. cerifera.* Measurements of $\Delta F/F'_m$ and PRI were also higher in *I. frutescens* during 2007. We did not expect to see such low values of PRI in 2004. The higher 2007 PRI values could be due to low salinity effects from increased freshwater input during the wet summer, it could be a function of flooding at the site or a combination of both. Thorhaug et al. (2006) showed that decreased salinity reduced PRI in halophytic seagrass. However, *Iva frutescens* generally only occurs at elevations where the roots are not subject to prolonged water table flooding (Bertness et al. 1992). The Bayside site is at the edge of the marsh at a lower elevation than the Path site. Mean elevation of the water table at the Bayside site in 2004 was 0.5 m higher than mean elevation in drier years (Brinson 2007). Despite the inconsistency at the Bayside site in 2004, differences in PRI during 2007 can be explained by variations in salinity across the island.

In 2007, PRI was lower at the Path site and tissue chlorides were much higher compared to the Bayside site. This is due to periodic flooding during extreme high tides at the Path site and subsequent evaporation resulting in higher levels of soil salinity (Hayden et al. 1995). Approximately 15 m from the Path site lays a hypersaline saltpan (Fig. 5.1). In comparison, the Bayside site lies in a tidal area and is flooded daily. Thus, soil salinity levels are not as likely to build up due to the constant input of water (Hayden et al. 1995). NDVI values did not differ between years for *I. frutescens* and were much lower compared to *M. cerifera*. Because there was no significant relationship between PRI and NDVI in either species suggests that factors such as LAI and leaf angle were not affecting the PRI signal and that PRI can be used at the landscape level in these species.

Hyperspectral measurements over homogenous ecosystems are lacking (Inoue et al. 2008). Barrier islands are model systems for remote sensing because of the homogeneous community composition. Our study demontrates the usefulness of PRI for remote detection of salinity stress in *M. cerifera* thickets. These thickets are ideal becasue they form dense, monotypic canopies with very high LAI, reducing the confounding effects of canopy structure and heteronegenous composition for applying PRI at the landscape scale (Brantley and Young 2007). Caution should be taken in assessing stress in *I. frutescens* using PRI. Knowledge of the system is important for correct interpretation of PRI values and the cause of stress. Regardless, PRI successfully identified areas of stress across the landscape.

Conclusion

Spatial variations in stress were detected on the barrier island using chlorophyll fluorescence, which were related to variations in tissue chlorides for both *M. cerifera* and *I. frutescens.* Salinity appeared to be a factor responsible for patterns of stress across the landscape, and was detectable using PRI from airborne hyperspectral imagery. Variations in PRI remained constant during a wet and dry year for *M. cerifera*, while NDVI, CI and WBI_{970} were higher during the wet summer, but varied little across the island. Thus, PRI was the most useful index for stress detection in *M. cerifera*. For *I. frutescens,* PRI was related to chloride concentrations during the dry year, but a different pattern in PRI emerged during a wet year, suggesting that this index is useful in detecting stress, but the cause may not always be obvious. These findings, especially for *M. cerifera* have implications for monitoring the effects of climate change in coastal systems. Our results suggest that PRI may be used for early identification of salt stress

that may lead to changes in plant distributions at the landscape level as a result of rising sea-level and increased storm intensity.

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Table 5.1

Vegetation indices used in our statistical analyses

Reflectance Index	Formula	Reference
Physiological Reflectance Index (PRI)	$(R_{531} - R_{570})/(R_{531} + R_{570})$	(Gamon et al. 1992)
Normalized Difference Vegetation Index (NDVI)	$(R_{801} - R_{670})/(R_{801} + R_{670})$	(Daughtry et al. 2000)
Chlorophyll Index (CI)	$(R_{750} - R_{705})/(R_{750} + R_{705})$	(Gitelson et al. 1996)
Water Band Index (WBI_{970})	R_{970} / R_{900}	(Peñuelas et al. 1993)

Figure Legends

Fig. 5.1. SpectIR hyperspectral image of sites at Hog Island, Virginia. The following letters denote *M. cerifera* thickets used in the study: O = Oceanside, B = Backside, Y = Young, D = Dune, M = Mid-island. The *I. frutescens* sites in the study are: IB = Bayside, IP = Path.

Fig. 5.2. Monthly variations in precipitation as measured from a meteorological station on Hog Island between June 1 and September 30 for 2004 and 2007. The dotted lines represent the flight dates during each year.

Fig. 5.3. Variations in relative water content (RWC) (a) and tissue chlorides (b) across the island for both *M. cerifera* and *I. frutescens* sites. Values represent means ± 1 standard error.

Fig. 5 4. Variations in $\Delta F/F'_m$ (a), PRI (b), and NDVI (c) across the island during 2007 for both *M. cerifera* and *I. frutescens* sites. Variations in PRI (d) and NDVI (e) during 2004 are also presented. Values represent means ± 1 standard error.

Fig. 5.5. Relationship between PRI and $\Delta F/F'_m$ for *M. cerifera* sites (a) and *I. frutescens* sites (b), where ◆ = . Oceanside, ○ = Backside, ▼ = Young, △= Dune, and ■ = Mid-island *M. cerifera* sites; ⊞= Bayside and ▽= Path *I. frutescens* sites.

Fig. 5.6. Relationship between PRI and RWC during 2007 for *M. cerifera* site (a) and *I. frutescens* (b). Symbols are defined in Fig. 5.

Fig. 5.7. Relationship between PRI and tissue chlorides during 2007 for all *M. cerifera* sites (a), for *M. cerifera* sites with the Oceanside site removed (b), and for *I. frutescens* sites (c). Symbols are defined in Fig. 5.

Fig. 5.8. Relationship between PRI and NDVI during 2007 for *M. cerifera* sites (a) and *I. frutescens* sites (b) and during 2004 for *M. cerifera* sites (c) and *I. frutescens* sites (d). Symbols are defined in Fig. 5.

Fig. 5.9. Relationship between WBI_{970} and RWC during 2007 for *M. cerifera* (a) and *I. frutescens* (b). Symbols are defined in Fig. 5.

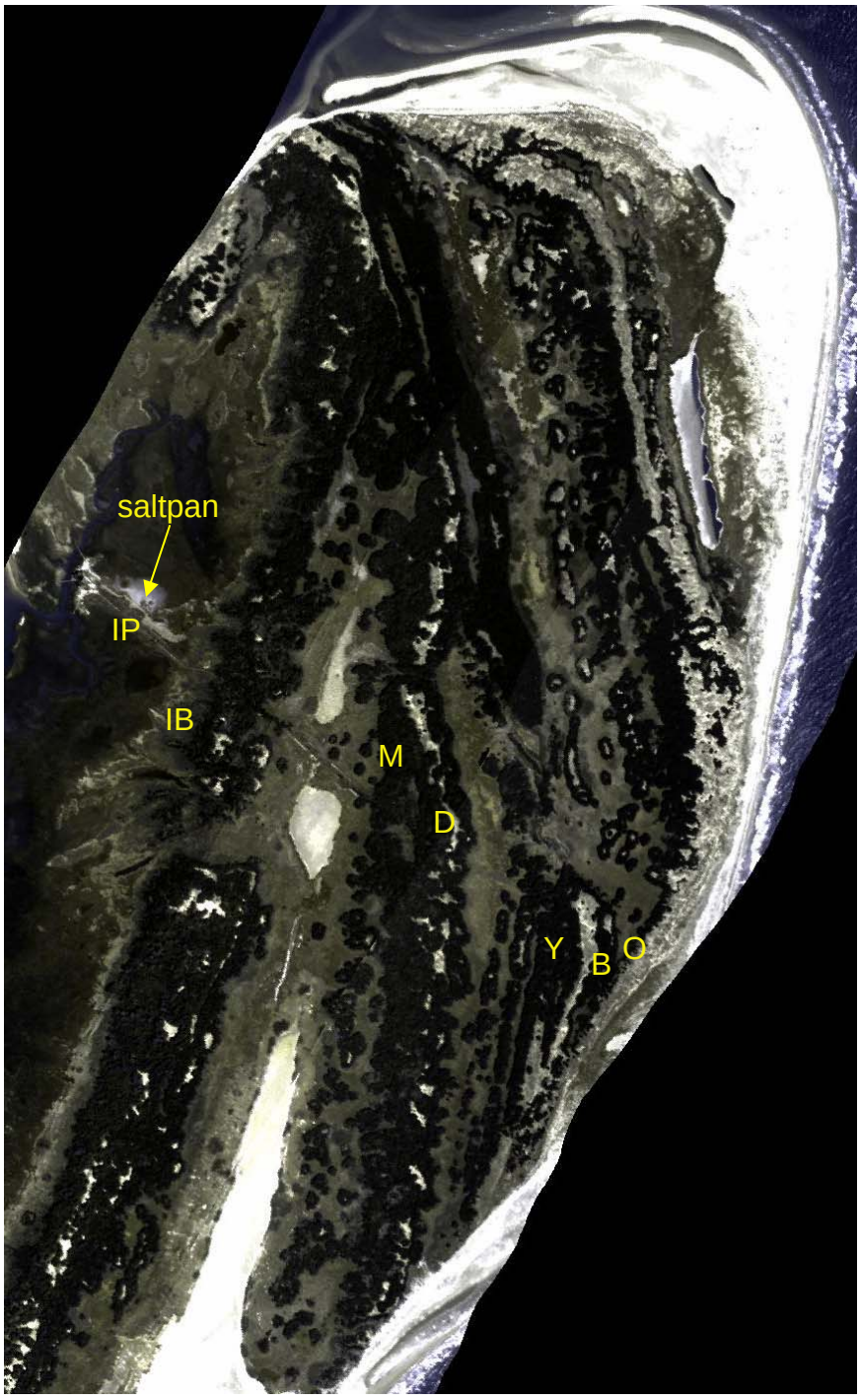

Figure 5.1

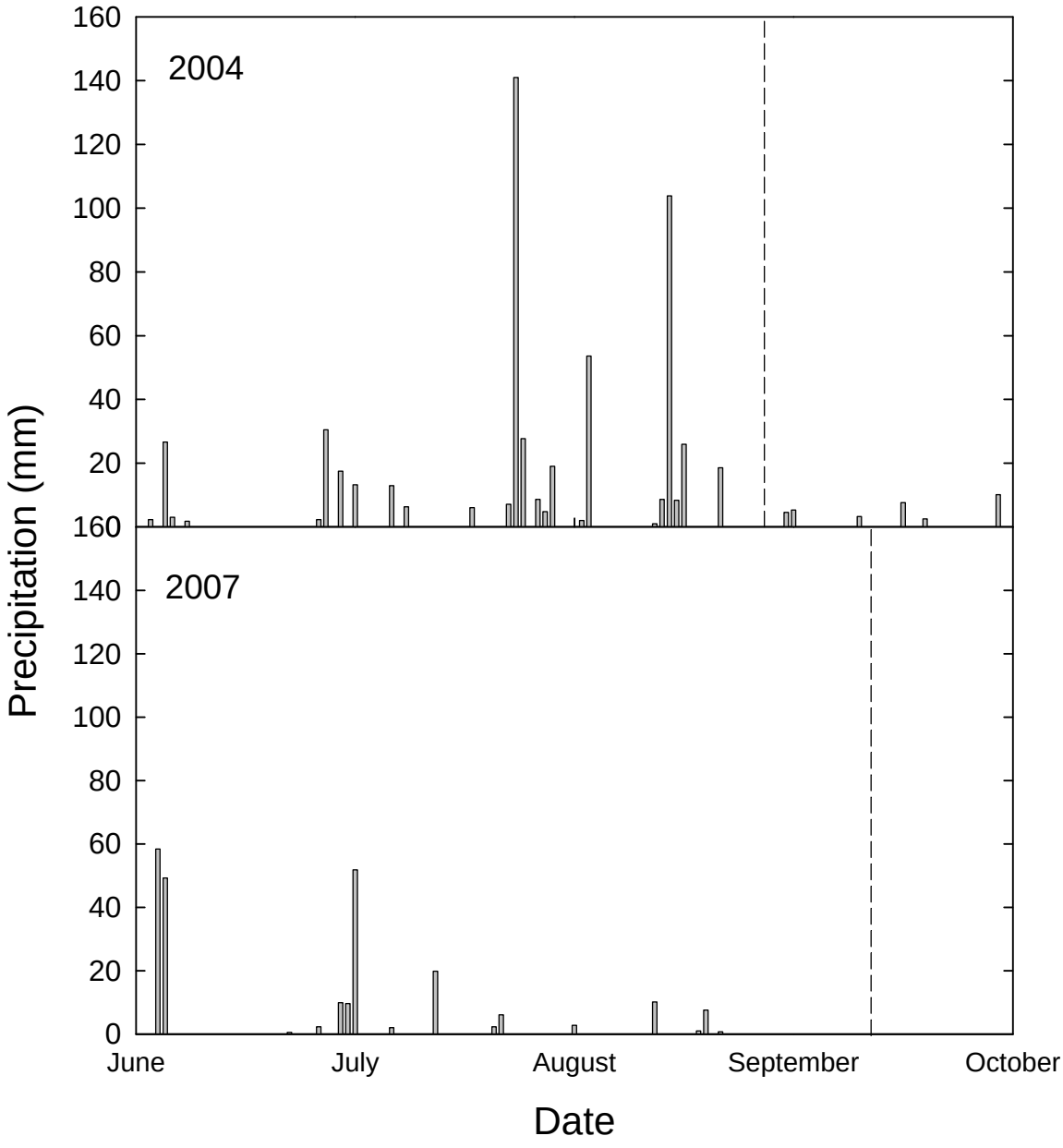
2004
2007
Precipitation (mm)
160
140
120
100
80
60
40
20
0
June
July
August
September
October
Date

Figure 5.2

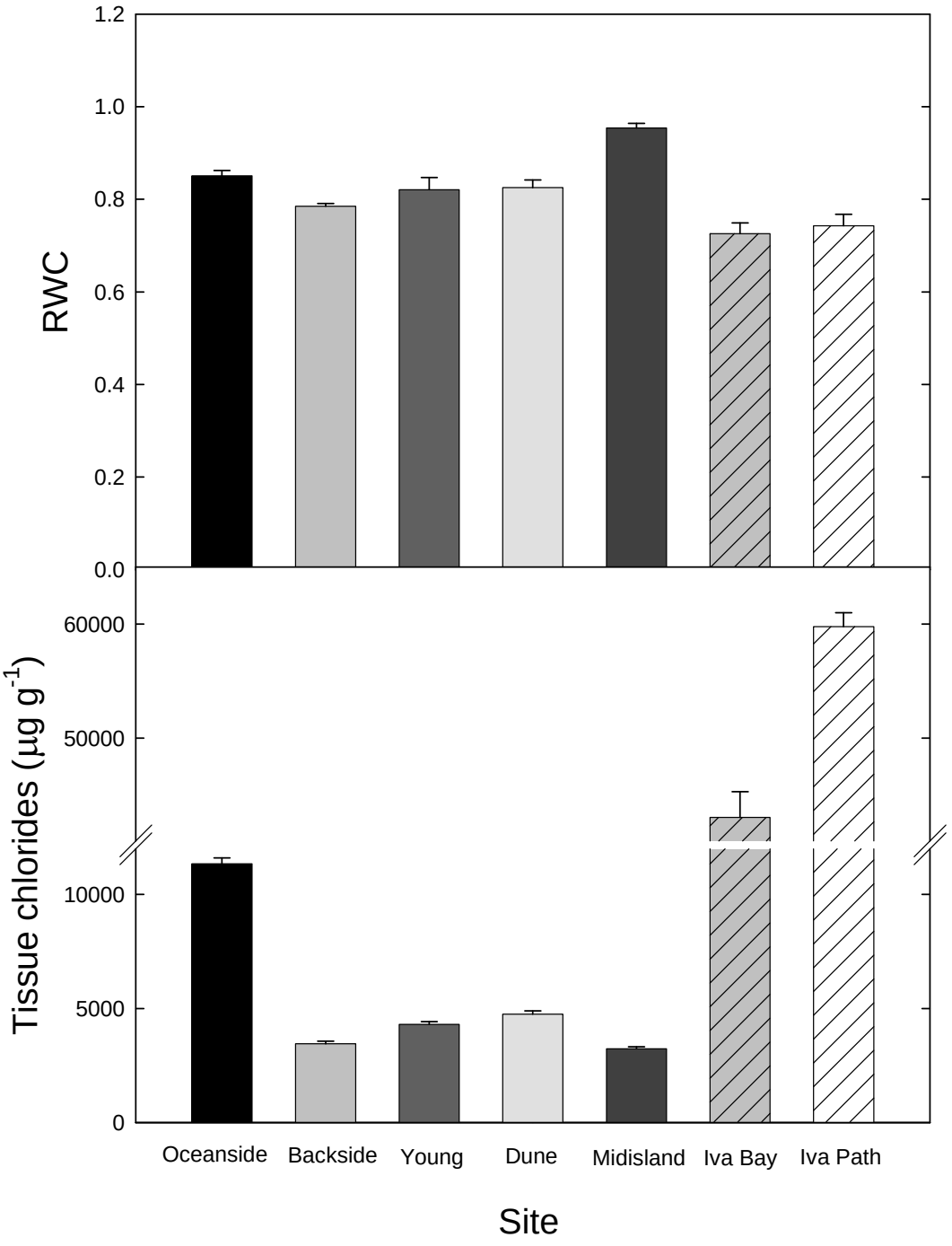
RWC
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Tissue chlorides (µg g-1)
60000
50000
10000
5000
0
Oceanside
Backside
Young
Dune
Midisland
Iva Bay
Iva Path
Site

Figure 5.3

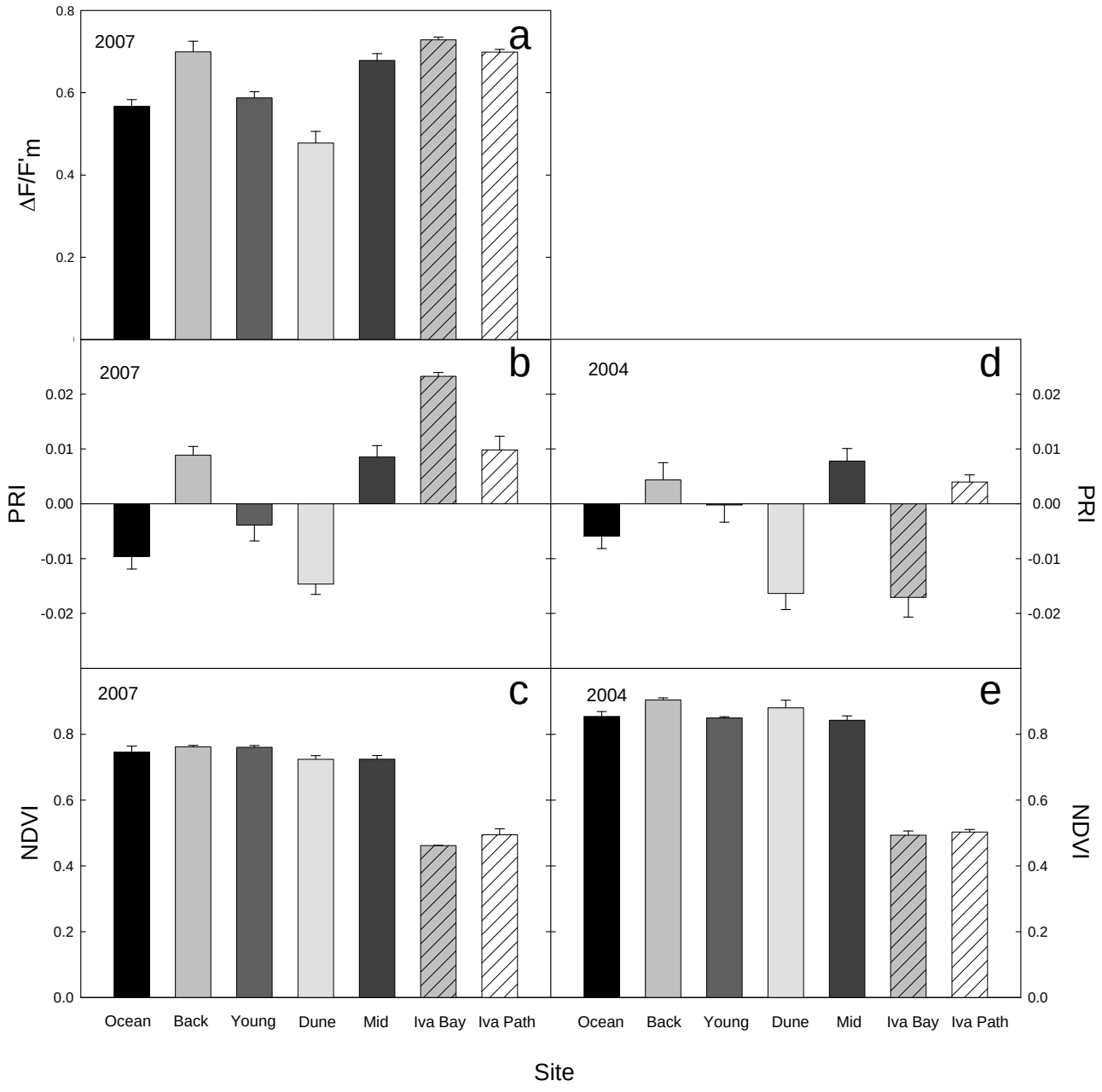
2007
a
ΔF/F'm
2007
b
PRI
2004
d
PRI
2007
c
NDVI
2004
e
NDVI
Ocean
Back
Young
Dune
Mid
Iva Bay
Iva Path
Site

Figure 5.4

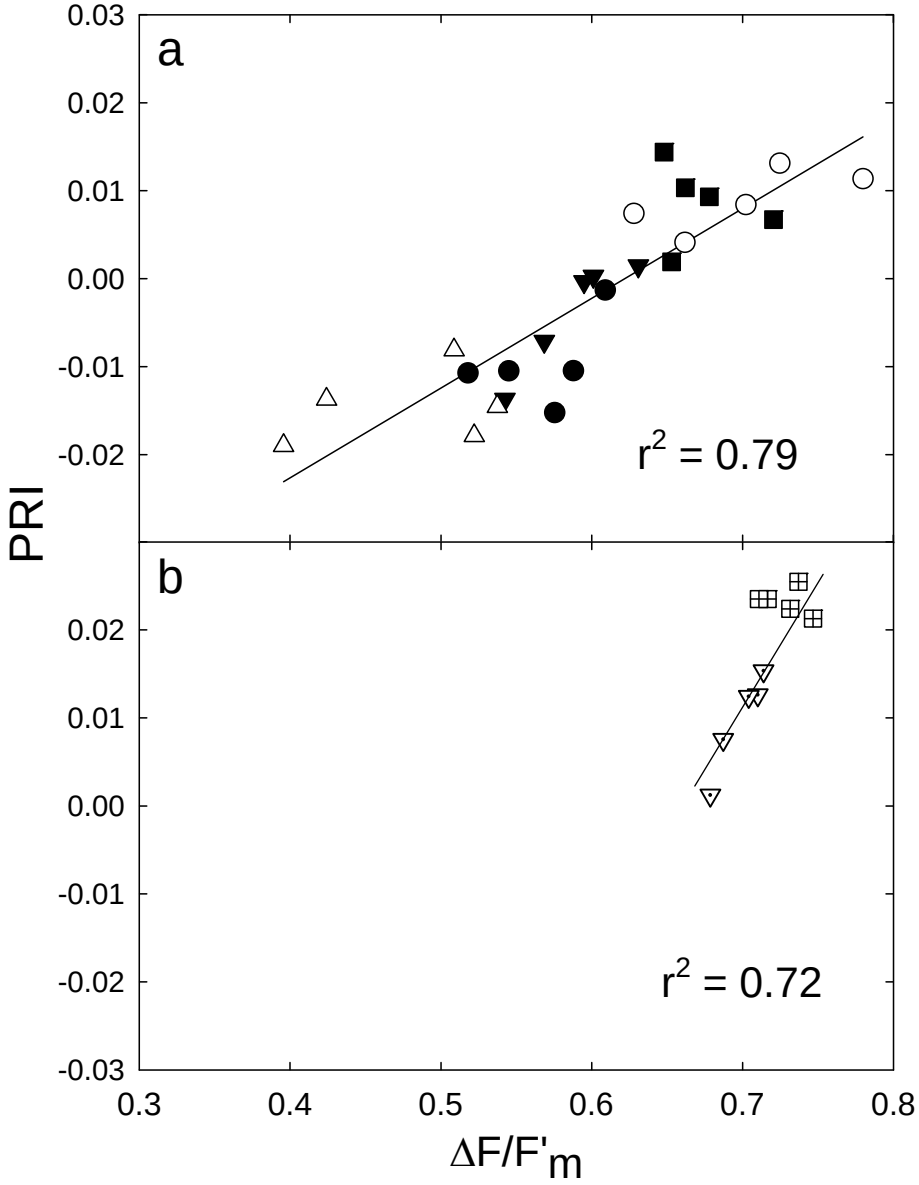
a
b
r^2 = 0.79
r^2 = 0.72
PRI
ΔF/F'm
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
0.3
0.4
0.5
0.6
0.7
0.8

Figure 5.5

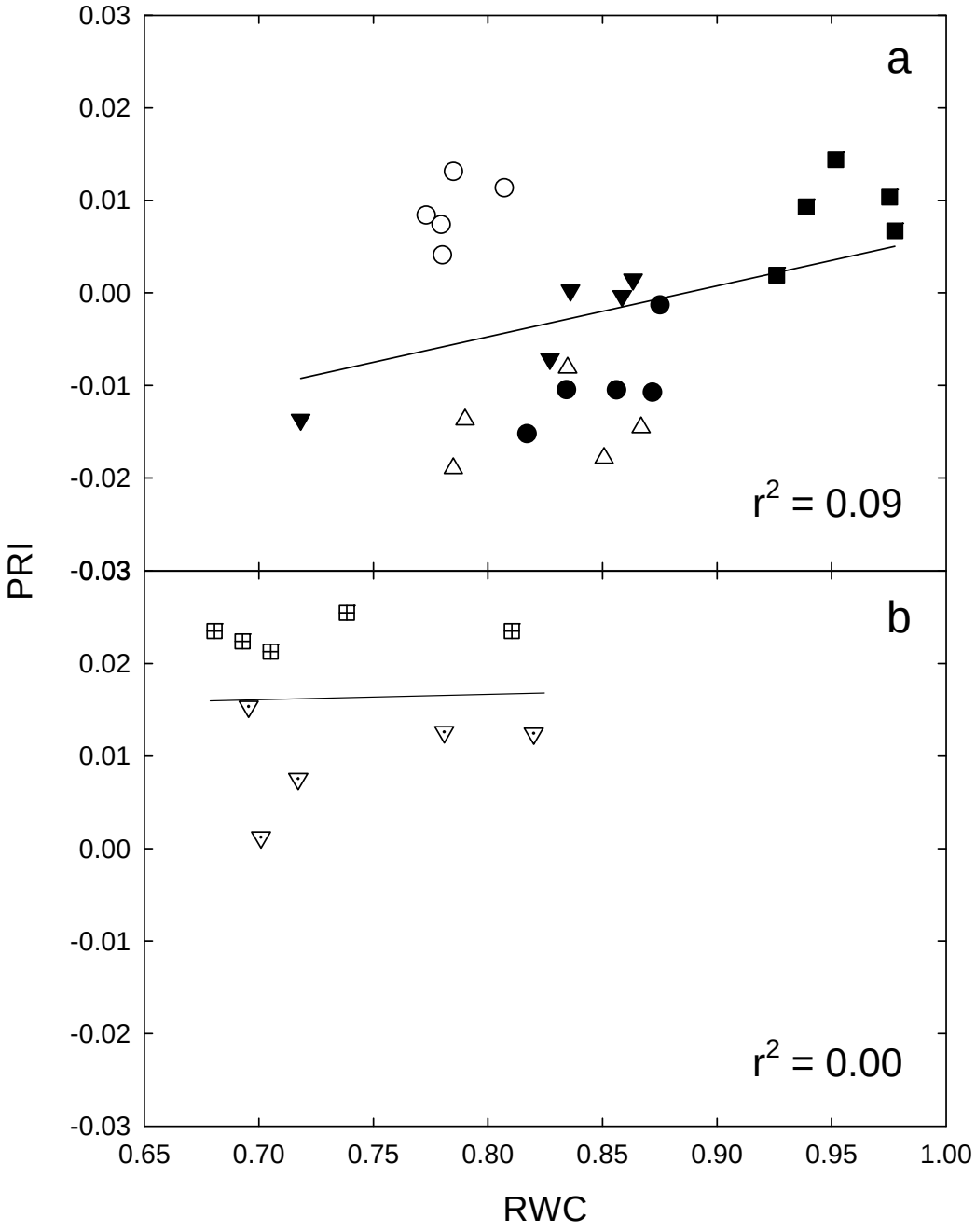
a
b
r^2 = 0.09
r^2 = 0.00
PRI
RWC
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00

Figure 5.6

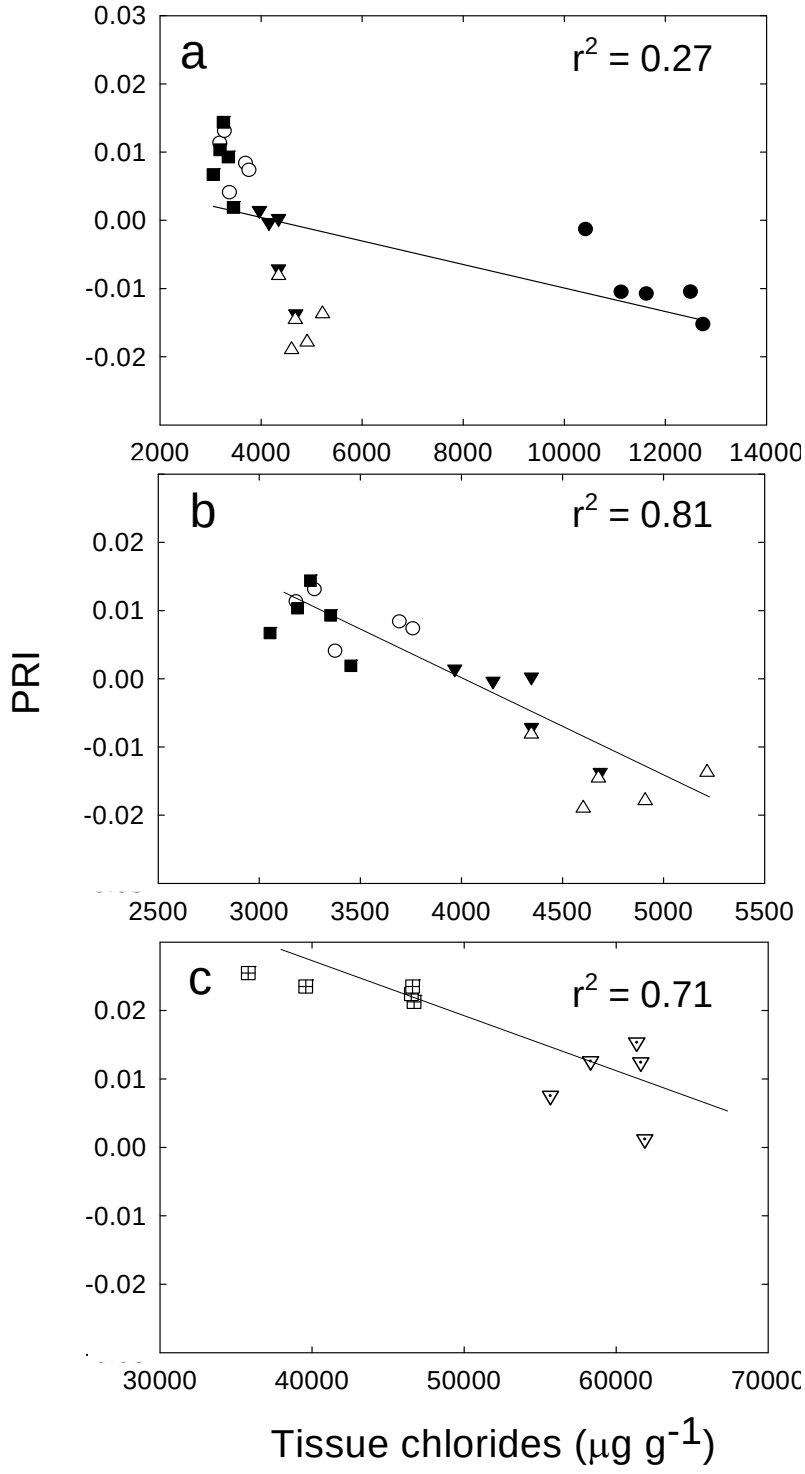

Figure 5.7

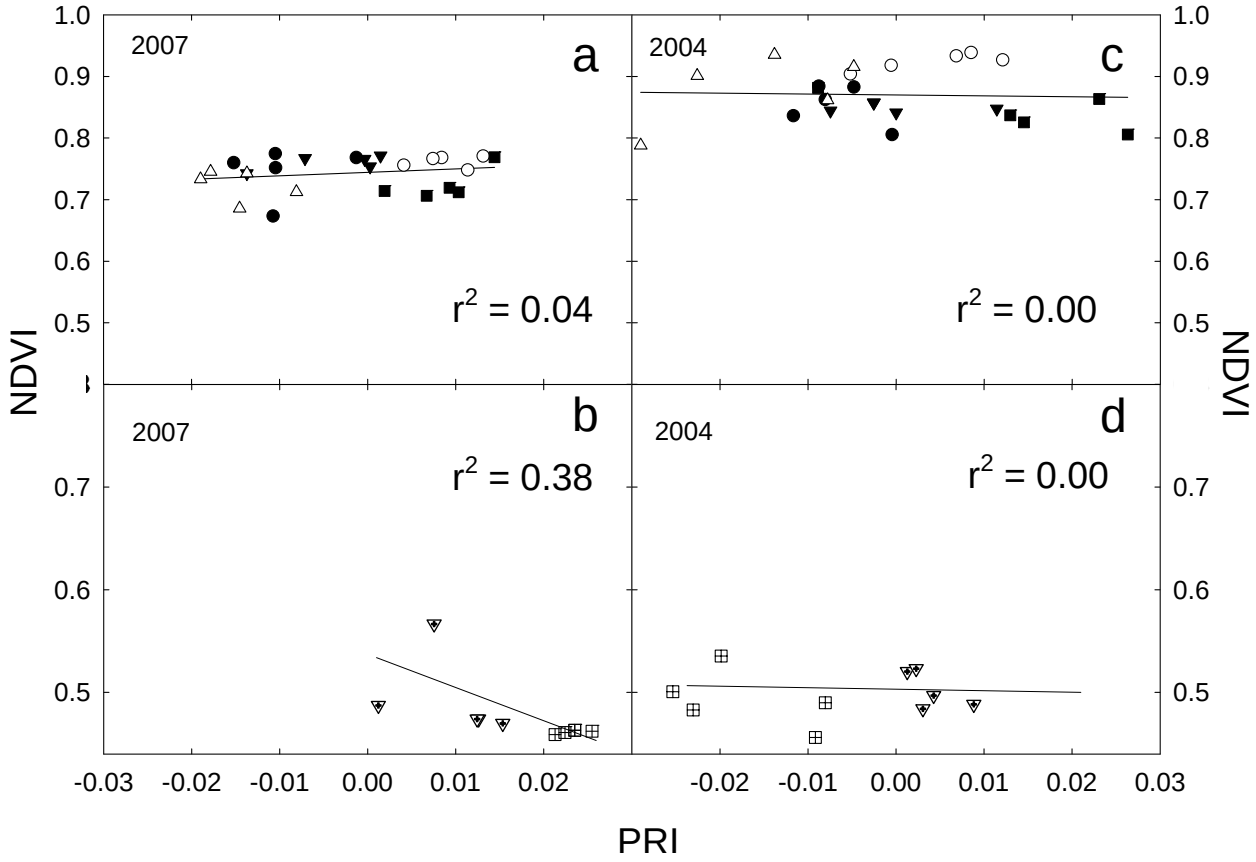
2007
a
r^2 = 0.04
2004
c
r^2 = 0.00
2007
b
r^2 = 0.38
2004
d
r^2 = 0.00
NDVI
NDVI
PRI
1.0
0.9
0.8
0.7
0.6
0.5
-0.03
-0.02
-0.01
0.00
0.01
0.02
0.03

Figure 5.8

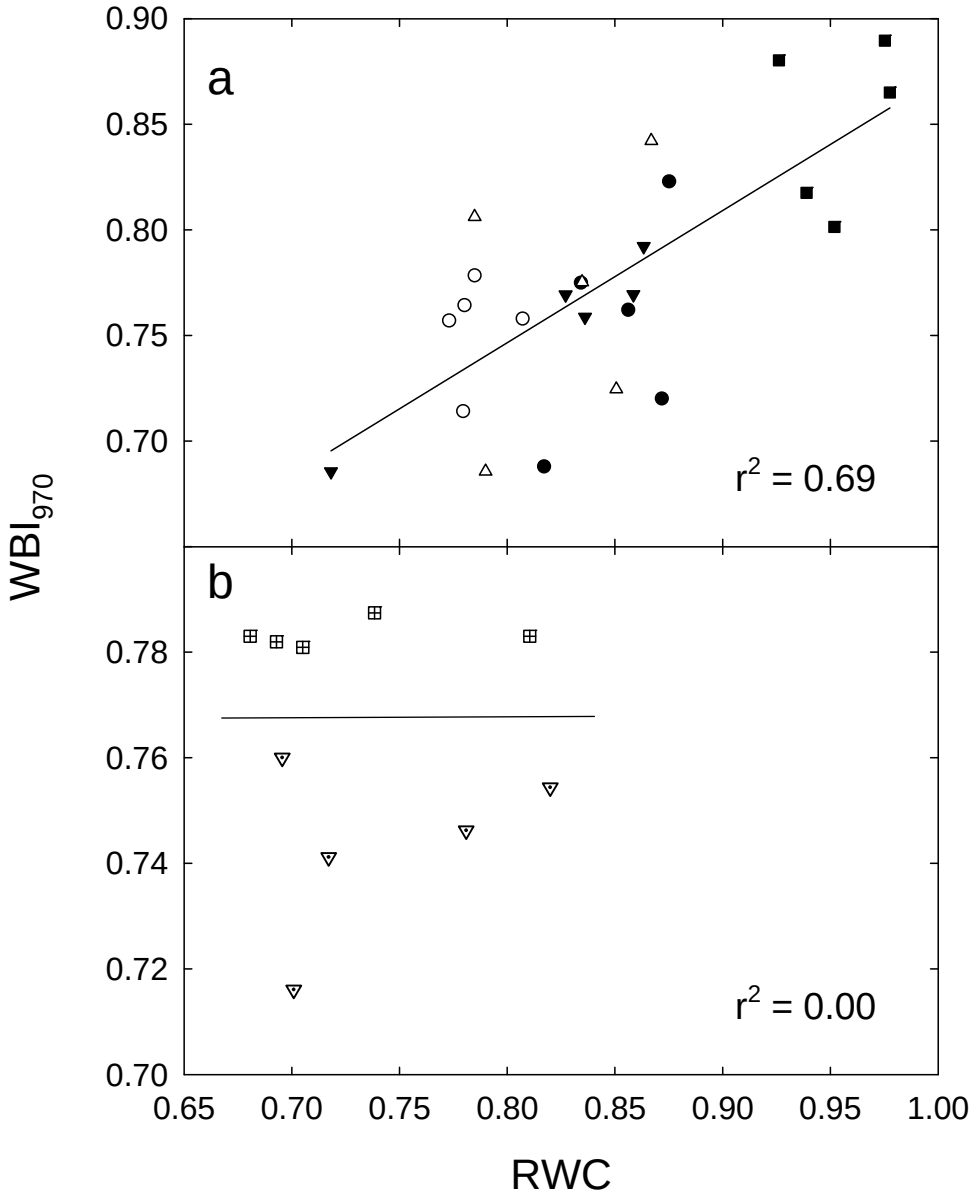
a
b
r^2 = 0.69
r^2 = 0.00
WBI_{970}
RWC
0.90
0.85
0.80
0.75
0.70
0.78
0.76
0.74
0.72
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00

Figure 5.9

VITA

Julie Christine (Zinnert) Naumann was born on April 25, 1977, in Berlin, Germany, and is an American citizen. She graduated from Broad Run High School, Ashburn, Virginia in 1995. She received her Bachelor of Science in Biology from Virginia Commonwealth University, Richmond, Virginia in 2000. She received a Master of Science in Coastal Plant Ecology from Virginia Commonwealth University, Richmond, Virginia in 2004 and was awarded the 2005 Outstanding Graduate Student of the Year Award. Julie received her PhD in Integrative Life Sciences with a focus in Plant Physiological Ecology from Virginia Commonwealth University, Richmond, Virginia in 2008.