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The Effects of Salinity Pulses of Varying Duration and Intensity on Three Freshwater Submerged Aquatic Vegetation Species

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The Effects of Salinity Pulses of Varying Duration and Intensity on Three Freshwater Submerged Aquatic Vegetation Species

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Master of Science

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by
Rebecca Wright
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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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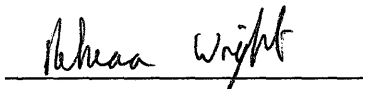

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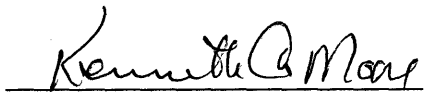

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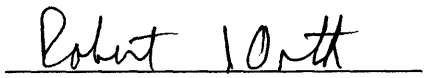

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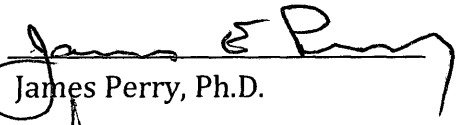

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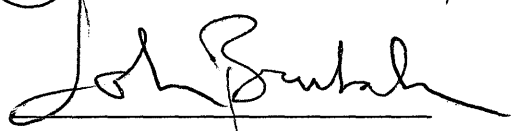

John Brubaker, Ph.D.

TABLE OF CONTENTS

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LIST OF TABLES

Table Page

LIST OF FIGURES

ABSTRACT

Salinity determines the ranges of many submerged aquatic plant species. Storm surges, water control structures, and changes in rainfall can cause salinity pulses of various durations and intensities in estuaries. These changing salinity levels can have significant effects on the growth and persistence of plants, especially in oligohaline and freshwater regions. I tested the recovery from salinity pulses of various intensities and durations on *Hydrilla verticillata*. I measured the baseline salinity tolerance of *Heteranthera dubia* for two-week pulses. I measured the effects of repeated pulses on *H. verticillata*, *H. dubia*, and *Vallisneria americana.* Finally, I tested the differences between instantaneous and gradual salinity pulses on these three species. I used various plant morphology and metabolism variables to determine stress, including growth, branch/blade number and maximum length, above and belowground biomass, maximum quantum yield and maximum electron transport rate, chlorophyll a, b, and a/b ratio, and light and dark oxygen flux. The species showed different levels of salinity tolerance. *H. dubia* was stressed at 5 PSU and survived until 10 PSU, *H. verticillata* was stressed in 3 PSU and died in 10+ PSU, and *V. americana* did not experience stress in 0-10 PSU. Both duration and intensity of pulses affected submerged aquatic vegetation (SAV), but intensity was the driving factor. Recovery from pulses was slow and plants still showed reduced growth from one-day pulses after 28 days of recovery. Repeated pulses resulted in linear decreased growth rather than the stepwise pattern expected, indicating that little recovery occurred between pulses. Gradual additions of salinity did not appreciably increase the tolerance of these species to stressful levels of salinity compared to instantaneous increases. Overall, the growth related variables (length, branching, and biomass) were much stronger indicators of stress than variables related to photosynthesis. This study will aid in the interpretation of monitoring data and enable more informed site selection for SAV restoration and *H. verticillata* removal both by defining the levels of salinity stressful to these species and identifying the role salinity pulses, even over short durations, can play in limiting the survival of both native and invasive SAV.

The Effects of Salinity Pulses of Varying Duration and Intensity on Three Freshwater Submerged Aquatic Vegetation Species

INTRODUCTION

Submerged aquatic vegetation (SAV) is a vital component of many freshwater, estuarine, and coastal systems. These aquatic macrophyte beds can provide nursery and refuge habitats, supply primary production, and improve water quality (Fonseca and Cahalan 1992, Larkum et al. 2006, Lazzari and Stone 2006, Orth et al. 2010). SAV has declined rapidly in the past several decades due in part to human impacts and environmental change (Orth et al. 2006). In the Chesapeake Bay, although there has been a resurgence of low salinity species in some areas, an increased understanding of possible stressors is still necessary for their management (Orth and Moore 1984, Carter and Rybicki 1990, Short and Neckles 1999, Moore et al. 2000, Orth et al. 2006, Orth et al. 2010).

Salinity is a major stressor of freshwater and oligohaline SAV; it often determines their distribution in estuaries (eg. Twilley and Barko 1990, Doering et al. 2001, Lirman and Cropper 2003, Frazer et al. 2006, Lacoul and Freedman 2006, Shields et al. 2012). Salinity tolerances of SAV species have usually been determined using different levels of fixed salinity concentrations; however, this does not reflect the frequent and often drastic salinity fluctuations in estuaries that may have significant effects. Pulses of high salinity have many causes including physical processes, such as climate, as well as anthropogenic alterations in river flow. Storm surge is a common cause of rapid short-term salinity pulses. During coastal storms oceanic water can be driven into an estuary by winds, waves, and low pressure systems, causing unusually high water levels and increased salinity (Li et al. 2006). These storm surges may raise salinity rapidly; in extreme cases salinity can increase by over

10 PSU within a few hours (Maryland Department of Natural Resources– Resource Assessment Service 2003, Frazer et al. 2006). There are several examples of storm surge driven salinity increases in Florida. Frazer et al. (2006) reported storm driven salinity increases up to 20 PSU in the typically tidal freshwater portion of Kings Bay. Lauer (2010) reported salinity to change by more than 12 PSU over a few hours in St. Johns River, Florida. Decreases in freshwater inflow due to seasonal changes in rainfall or drought can result in salinity increases. However this typically occurs at more gradual rates (over weeks or months) compared to storm surges (hours or days) as evidenced by continuous monitoring at many fixed sites in the Chesapeake Bay (VECOS, http://www2.vims.edu/vecos/; MDDNR Eyes on the Bay, http://mddnr.chesapeakebay.net/eyesonthebay/index.cfm).

One of the most significant causes of rapid and pronounced salinity change is the alteration of freshwater discharge for agricultural irrigation and municipal uses (Estevez 2000, Doering et al. 2001). Water control, such as the diversion or use of freshwater inflows , have the potential to decrease river discharge, thus allowing higher salinity water to move further up estuary, thereby increasing salinity (Estevez 2000, Doering et al. 2001). Water control structures are often opened and closed periodically, resulting in pulses of fresh and saline water over SAV beds; this is particularly common in Florida, where estuaries are small and ocean water can progress up the estuaries rapidly (Doering et al. 2001). Since SAV improve water quality and habitat, and are often seen as indicators of ecosystem health and restoration success, the impact of salinity on these species should be taken into considerations when determining water release. Since SAV are rooted and cannot move to areas of more suitable salinity, they are good indicators of the effects of water flow alterations. Despite this, water release regulations are sometimes based only on

the requirements of one or a few (usually commercially important) species and not the entire ecosystem (Estevez 2000). In many systems other objectives, such as the control of erosion and flooding, or human water needs are used to determine the release or use of freshwater water inflows (Estevez 2000).

Environmental stressors can have additive effects in combination with salinity stress. If plants are under stress due to one environmental factor, adding another stressor can cause more severe effects. Light and salinity are often a focus for experiments investigating additive effects on SAV (Twilley and Barko 1990, French 2001, French and Moore 2003, Dobberfuhl 2007, Boustany et al. 2010). Light levels may be an important factor in the study of salinity intrusion, as the estuarine turbidity maximum (ETM) is usually located at the freshwater-saltwater interface. The ETM is an area of increased suspended sediments trapped at the freshwater-saltwater interface by flocculation and water movement (eg. Kranck 1981, Burchard and Baumert 1998, Manning et al. 2006, Kessarkar et al. 2009). If saline water flows further up estuary, this suspended sediment may also move up estuary, shading freshwater SAV beds at the same time as the salinity pulse passes.

The compounded effects of the increased salinity and decreased light at the freshwater-saltwater interface may have a greater affect on SAV than salinity or turbidity alone. Other SAV stressors may correspond to salinity pulses due to storm surge, including increased nutrient runoff, higher water levels, changes in temperature, and scouring from high wind and wave action (Santamaría and van Vierssen 1997, Greening et al. 2006, Lacoul and Freedman 2006, Li et al. 2006). Epiphyte growth can also vary with salinity; it often increases when salinities are higher and/or plants become stressed, possibly due to damaged, less ridged leaves increasing attachment sites, and may cause a further reduction in light reaching the SAV blades (Kendrick et al. 1988, Twilley and Barko 1990).

Mechanisms of Salinity Stress

Salinity stress affects plants in a number of ways; it lowers osmotic potential and increases the diffusion of water out of the leaves (Touchette 2007). Salinity stress is partially due to the osmotic potential affecting the movement of ions across cell membranes; this requires either increasing the concentration of ions in the cytoplasm or compartmentalizing them in the cell vacuole (Parida and Das 2005, Touchette 2007). Unlike many wetland species, SAV lack salt excreting glands and cannot prevent sodium from entering their leaves, making them osmoconformers (Twilley and Barko 1990). As osmoconformers, freshwater SAV cannot exclude salinity ions in order to be resistant to saline pulses, instead recovery from these pulses would show resilience. The sodium (Na+) in saltwater can compete with K+ for uptake into the cell, thus replacing essential potassium (K+) with toxic Na+ (Twilley and Barko 1990, Touchette 2007). In *H. verticillata* and *V. americana* an increase in salinity corresponded in a decrease in K+ in leaf tissues (Twilley and Barko 1990). ATPases can help to increase the selectivity to K+ (Niu et al. 1995, Touchette 2007). Some plants can accumulate the excess Na+ in vacuoles instead of the cytoplasm (Touchette 2007). Plants may also overcome osmotic stress by increasing the concentrations of simple and complex sugars, sugar alcohols and organic metabolites, such as proline (Touchette 2007).

In aquatic and wetland species, saltwater can lead to the formation of reactive, often cytotoxic, oxygen species (ROS) such as peroxides and oxygen radicals (Rout and Shaw 2001, Parida and Das 2005). These ROS can form rapidly after salinity addition; ROS and lipid hydroperoxides were found in assays only 24 hours after salinity was raised (Lauer et al. 2010). Antioxidant enzymes for breaking down these compounds are needed in facultative halophytes (Rout and Shaw 2001, Parida and Das 2005). Therefore, these

enzymes are likely to be involved in salinity tolerance. Some freshwater SAV species produce increased superoxide dismutase (SOD) when they are exposed to salinity stress (Rout and Shaw 2001). Since these molecules may be one cause of salinity-induced damage to the plants, and they can be detected before any visible sign of stress, they may be useful in monitoring the early stages of salinity stress.

Since changes in ROS, and the enzymes that break them down, are difficult to detect, other measures of plant stress are often used. Plant growth rate and plant mortality are easily observable and ecologically relevant measures of stress, but measuring them involves tracking beds or individual plants over time and changes only become evident when plants have sustained major losses. A loss of leaf chlorophyll or a decrease in the chlorophyll a/b ratio may indicate salinity stress (Twilley and Barko 1990, Rout and Shaw 2001), but this decrease may not be evident, even in highly stressed plants (French 2001). Photosynthesis is often used as an indicator of SAV stress and has been measured using O_2 metabolism in enclosed bottles both at ambient light to measure photosynthesis and in the dark to measure respiration (Lewis and Smith 1983, Sorrell and Dromgoole 1986, Caffrey and Kemp 1991, Lewis 2004).

Photosynthetic rates are also approximated using pulse amplitude modulated fluorometry (PAM). PAM measures relative maximum electron transport rate ($rETR_{max}$) and maximum quantum yield (MQyield), using rapid light curves on dark-adapted leaves. MQyield is the rate at which photosynthesis increases with an increase in irradiance after dark adaptation and $rETR_{max}$ is the rate of photosynthesis at optimal irradiance (Beer et al. 1998, Ralph et al. 1998, French and Moore 2003).

Study species

Heteranthera dubia, *Hydrilla verticillata*, and *Vallisneria americana* are SAV species common in rivers and lakes as well as the freshwater or oligohaline zones of estuaries in all coastal US states, as well as many inland areas (USDA and NRCS 2009). They are all common in the low salinity regions of the Chesapeake Bay, often forming mixed beds (Moore et al. 2000). Although they often grow in mixed beds, they differ in terms of morphology and physiological requirements. Therefore, increasing our understanding of their individual responses to salinity stress can provide insight to the potential overall SAV community response and susceptibility to natural and anthropogenic salinity changes.

Heteranthera dubia

Heteranthera dubia (commonly called water stargrass and grassleaf mud-plantain; sometimes referred to as *Zosterella dubia*) is native to the United States. It has a branching structure with thin alternate leaves and yellow 'star-like' flowers. Its salinity range is not well studied. It is usually found in fresh water or extremely low salinity areas (Moore et al. 2000). When exposed to salinities of five PSU or higher its aboveground biomass significantly decreased compared to that of plants grown in zero PSU (Moore and Shields 2010). One objective of this study is to define *H. dubia*'s salinity tolerance more precisely.

Hydrilla verticillata

Hydrilla verticillata (commonly called hydrilla and water thyme) is highly branched; it has whorls of small pointed leaves with toothed margins. It is invasive in the United States; probably originating from southeast Asia (Cook and Lüönd 1982, Peterson et al. 2009). Its ability to grow in minimal light, form thick canopies, and grow from small stem fragments allows it to spread rapidly throughout a freshwater system (Sutton et al. 1980, Langeland 1996). Although it is an invasive species, *H. verticillata* still provides many of the

ecosystem services of native SAV, including habitat for fish and invertebrates and primary production (Staver 1994, Rybicki and Landwehr 2007). However, its rapid growth and canopy forming morphology can shade out other plant species (Langeland 1996).

Hydrilla verticillata can also have a human impact by blocking channels and impeding boat traffic (Langeland 1996). It can also fill agricultural drainage and irrigation ditches and block pumps used to control agricultural water movement, resulting in damage to crops (Langeland 1996). Control of *H. verticillata* is often ineffective, since stem fragments can grow into new plants and attempts to remove plants manually often spread populations. Control has been attempted through introduction of grass carp (Sutton and Vandiver 2006), fungal infection (Nelson and Shearer 2009) and herbicides (MacDonald et al. 2008). However, these methods can have negative effects on freshwater ecosystems and are difficult to remove from the system once the *H. verticillata* is controlled.

Hydrilla verticillata is sensitive to salinity and previous research suggests that it may be more sensitive to salinity than some other fresh water species (Table 1). In field surveys in the Chickahominy River, Virginia, it was only found growing in salinities below 2 PSU (Shields et al. 2012). Germination is stressed at very low salinities with a 50% reduction in germination at 3 PSU (Carter et al. 1987). Previous studies show that by 5 PSU growth generally ceases and plants appear stressed, although one study (Littles 2005) seems to find an exception, plants showing no stress at 5 PSU and with some plants in 5 PSU showing more growth than the controls. All other studies found a decrease or halt of growth around 5 PSU, with some stress evident as low as 2 PSU (Table 1).

The proliferation of *H. verticillata* in enclosed or semi-enclosed systems such as aquaculture and irrigation ditches is a major issue. Current methods of *H. verticillata* control, such as the use of herbicides, may also threaten the crop species and surrounding

environment. Improving our understanding of short-term (1-2 day) elevated salinity pulses on this species may help in management as well as helping to predict its spread into new areas.

TABLE 1. Summary of previous experimental results of the effect of salinity on *H. verticillata*

Citation	SAV Species	Study type	Salinity addition	Exposure time	Factors	Measurement methods	Results
Carter et al. 1987	*H. verticillata*	Mesocosm	Instant	5-6 weeks	Salinity	Germination, plant length	No propagules germinated above 9 PSU. Less than 50% germinated in 3 PSU. Over 90% were viable in 0 PSU.
Frazer et al. 2006	*V. americana, H. verticillata, Myriophyllum spicatum*	Mesocosm	Gradual	1, 2, and 7 days	Salinity	Blade/ branch number, biomass, plant number, turions,	Growth decreased at 5 PSU, plants died at 15 and 25 PSU for all durations. Salinities below 5 PSU were not tested.
Haller et al. 1974	*V. americana, H. verticillata, M. spicatum, Najas quadalupensis*	Mesocosm	Instant	4 weeks	Salinity	Biomass	Plants died at 10 PSU and above. No growth was observed at 6.66 PSU, and no stress in 0.17 and 3.33 PSU.
Littles 2005	*V. americana, H. verticillata, M. spicatum*	Mesocosm	Not noted	1, 2, and 7 days	Salinity	Blade/branch length and number, biomass, vegetative repro, turions	No stress was evident at 5 PSU, 100% mortality at 15 and 25 PSU. Salinities below 5 PSU were not tested.
Rout and Shaw 2000	*H. Verticillata, Najas indica and Najas gramenia*	Flasks	Instant	9 hours	Salinity	ROS, antioxidants, chlorophyll a/b ratio	Superoxide dismutase and peroxidases increased with salinity in all species. Ascorbate peroxidases increased in *H. verticillata*. More salt tolerant species had a stronger enzymatic response than *H. verticillata*.
Twilley and Barko 1990	*V. americana, H. verticillata, M. spicatum, Potamogeton perfoliatus*	Mesocosm	Gradual (1-2 PSU per day)	5 weeks	Salinity and light	Biomass, density, length, epiphytes, turions	A significant decrease in biomass occurred at 2 and 4 PSU, and more marked decrease at 6 and 12 PSU. Stem density and length only decreased at 6 and 12 PSU.

Vallisneria americana

Vallisneria americana (commonly called water celery and eelgrass) has long strap-like leaves originating from a single basal rosette. V. americana is more salinity tolerant than the other freshwater SAV species in this study (Haller et al. 1974, Twilley and Barko 1990, Doering et al. 2001, French and Moore 2003, Frazer et al. 2006, Boustany et al. 2010, Moore and Shields 2010, Shields et al. 2012)(Table 2). There is considerable variability in the results of salinity stress studies for *V. americana*; which may be due to possible variations in light levels, water quality, duration of pulses, as well as the specific tolerances of the *V. americana* populations used. In general, stress begins to appear between 10 and 15 PSU and plants start to die between 15 and 18 PSU. Haller et al.(1974) seems to be the largest exception: they found growth to halt at 6.66 PSU and mortality at 13.32 PSU. It is unclear why their plants appeared more sensitive to salinity.

TABLE 2. Summary of previous experimental results of the effect of salinity on *V. americana*

Citation	SAV Species	Study type	Salinity addition	Exposure time	Factors	Measurement methods	Results
Boustany et al. 2009	*V. americana*	Mesocosm	Gradual (1 PSU per day)	2.5 and 5 months	Salinity and light	biomass, growth, epiphytes, leaf area index	Plants died back in 18 PSU, but recovered when returned to 0 PSU. Growth limited at 8 PSU.
Doering et al. 2001	*V. americana*	Mesocosm	Gradual (2-3 PSU per day)	1, 5, 11, 20, 30, 50, 70 days	Salinity	shoot and blade number, blade length	Most plants died at 18 PSU. Increased exposure duration increases salinity stress. Recovery when plants were returned to fresh water.
Doering et al. 2002	*V. americana*	Field and mesocosm	Not noted	20, 30, 43, 50, 69 days	Salinity	shoot and blade number, blade length	Growth halted between 10 and 15 PSU. Mortality was seen at 18 PSU and above.
Frazer et al. 2006	*V. americana, H. verticillata, Myriophyllum spicatum*	Mesocosm	Gradual	1, 2, and 7 days	Salinity	Blade/ branch number, biomass, plant number, turions	Plants showed stress in 15 PSU, plants died in 25 PSU.
French and Moore 2003	*V. americana*	Mesocosm	Instant	~8 months	Salinity and light	Biomass, leaf area, rETR, reproduction, and photochemical efficiency.	Biomass decreased with increasing salinities. Leaf area decreased at 10 and 15 PSU. The rETR and chlorophyll did not change.
Haller et al. 1974	*V. americana, H. verticillata, M. spicatum, Najas quadalupensis*	Mesocosm	Instant	4 weeks	Salinity	Biomass	Mortality was seen at 13.32 PSU and higher. No growth was seen in 6.66 and 10 PSU, and no stress in 0.17 and 3.33 PSU.

TABLE 2. Continued

Citation	SAV Species	Study type	Salinity addition	Exposure time	Factors	Measurement methods	Results
Kraemer et al. 1999	*V. americana*	Field	Instant	Periodic monitoring 1 week-3months	Location (light and salinity)	photosynthesis, glutamine synthesis, protein, carbohydrates and nitrogen content	Plants died at 15+ PSU, but did not show stress at 12 PSU or with periodic pulses above 15 PSU. All factors except photosynthesis declined in high salinity.
Lauer et al. 2010	*V. americana*	Mesocosm	Instant	1 or 7 days	Salinity	ROS localization, respiration and photosynthetic efficiency	ROS and lipid hydroperoxides present after 1 day at 10 + 15 PSU. Respiration doubled in 15 PSU. After a week, respiration and lipid hydroperoxides increased in 13 PSU and effective yield decreased.
Littles 2005	*V. americana, H. verticillata, M. spicatum*	Mesocosm	Not noted	1, 2, and 7 days	Salinity	Branch / blade length and number, biomass, vegetative reproduction	No change in growth in 5 PSU, decrease in biomass at 15 PSU (with mortality after 7 days), and complete mortality at 25 PSU.
Moore and Shields 2010	*V. americana, H. dubia, Stuckenia pectinata*	Mesocosm	Gradual (0.5-1 PSU per day)	2 or 7 days	Salinity, Sediment type, competet-ion	Above and belowground biomass, length and density	Length decreased in 10 PSU for plants grown in sand, other salinities and sediment types showed no growth decrease.

Rate of Increase

Gradual salinity increases may affect plants less severely than rapid salinity increases since gradual pulses are thought to give the plants time to adapt and reduce shock (Twilley and Barko 1990, Doering et al. 2001). These gradual pulses have been used in many mesocosm experiments (Twilley and Barko 1990, Doering et al. 2001, Frazer et al. 2006). The unusually high levels of stress seen by Haller et al. (1974) may have been due to their use of instantaneous salinity pulses and not letting the plants acclimate. However, this has not been substantiated. For many species of seagrass adapted to oceanic salinities, gradual pulses of hypersalinity were less stressful than instantaneous addition, often by a notable amount (Koch et al. 2007b). However, no studies have explicitly compared gradual with instantaneous changes for oligohaline SAV. Studies on the tolerance of *V. americana* using gradual rates of salinity addition do not always find higher tolerance levels than studies using instantaneous salinity addition. However, changes in exposure durations, light levels, water quality, and individual population differences make comparison between studies impossible (Table 2). It may be that species with a high level of salinity tolerance mechanisms already in place may benefit from gradual exposure to stress. However, it is not evident if reducing the rate of salinity increase significantly reduces stress in plants that do not already have strong salinity tolerance mechanisms such as those found in freshwater and low salinity regions of estuaries.

Measurement Techniques

There are many methods of measuring salinity stress in aquatic macrophytes, each with possible drawbacks. Measurements of growth rates may be impractical in field studies. Identifying and re-measuring the same plant after a growth period is impossible in many field settings, particularly with fast growing plants such as *H. verticillata*, and it does not account for changes due to leaf turnover, herbivory, or sub-lethal effects on plant health, so a variety of approaches may be

necessary. The precision and accuracy of photosynthetic capacity tests such as Pulse Amplitude Modulated fluorometry, leaf chlorophyll content, and oxygen metabolism, as well as measures of growth using variables such as maximum leaf lengths, branching, and biomass, and how they compare are not well quantified for SAV.

Objectives

The goal of this study was to investigate and quantify the effects of short-term salinity increases on three SAV species found in low salinity and freshwater regions the Chesapeake Bay. Specific objectives include determining: 1) The salinities at which each of the three study species begin to experience stress, particularly *H. dubia*, where little is known about its salinity tolerance; 2) The effects of pulse intensity and duration on *H. verticillata*; 3) The effects of repeated salinity pulses on different species of SAV; 4) The effects of gradual vs. rapid salinity increase,; 5) The relative capacities for various methods to detect stress.

Understanding salinity tolerance will help quantify the potential effects of natural and anthropogenic salinity changes on ecosystem composition and SAV persistence. As global warming increases sea level and the severity of storms, the potential of the duration and intensity of salinity stress to change species distributions will increase. Understanding the effects of these pulses may help to set water control structure openings while preserving the natural ecology of an area.

METHODS

This study is comprised of five experiments: 1) quantifying the salinity stress associated by pulses of varying durations and salinities in *H. verticillata*; 2) quantifying the salinity tolerance of *H. dubia* at constant salinity levels over a two-week period; 3) quantifying the response of all three test-species to pulsed salinity for seven-day pulses with seven-day recovery periods; 4) quantifying the response of all three test-species to pulsed salinity for two-day pulses with five-day recovery period; 5) quantifying the effects of gradual vs. instantaneous salinity increases on all three species in intermediately stressful salinities. Mesocosm design and measurement techniques were constant across all experimental setups. Variables measured, salinities, and pulse times varied between experiments (Table 3). While Experiments 1 and 2 used only a single pulse in each treatment, Experiments 3, 4, and 5 are more complicated and so the patterns of salinity addition are presented in graphical form in Figure 1.

In experiment 1, *H. verticillata* was exposed to pulse salinities of either 3, 5, 10, or 15 PSU instantaneously, left for a duration of either 1, 7, 15, or 28 days and returned to 0 PSU for the remainder of the 28-day experiment. In experiment 2, *H. dubia* plants were exposed to pulse salinities of 0, 3, 5, or 10 PSU for two weeks; measurements were recorded weekly. In experiment 3, *H. dubia, H. verticillata and V. americana* were raised to 0, 5, or 10 PSU instantaneously for seven days then returned to 0 PSU for the same period, this was repeated to give two seven-day pulses with seven-day recovery periods in between (Figure 1). In experiment 4, plants of *H. dubia, H. verticillata, and V. americana* were raised to 0, 3, 5, or 10 PSU for two, two-day salinity pulses with

five-day fresh water recovery periods in between (Figure 1). Experiment 5 consisted of 4 treatments, a freshwater control (A), a constant salinity control (B), plants held in freshwater for the first week and then raised instantaneously to the designated salinity (C), and plants where the salinity was raised gradually over the first week and held stable for the second (D)(Figure 1). *Heteranthera dubia w*as raised to 5 PSU, *H. verticillata* was raised to 3 PSU, and *V. americana* was raised to 10 PSU; these were not fatal salinities but salinity levels that were likely to demonstrate stressed conditions of reduced growth that might be ameliorated by gradual salinity increases.

TABLE 3. Summary of methods.

Exp.	Plant species	Salinities (PSU)	Pulse duration(s)	Additional variables *
1	*H. verticillata*	0, 3, 5, 10, 15	1, 7, 15, and 28 days	Total lengths
2	*H. dubia*	0, 3, 5, 10	14 days	chlorophyll, oxygen metabolism
3	*H. dubia, H. verticillata, V. americana*	0, 3, 5, 10	7 days salt, 7 days recovery x2	None
4	*H. dubia, H. verticillata, V. americana*	0, 3, 5, 10	2 days salt, 5 days recovery x2	chlorophyll, oxygen metabolism
5	*H. dubia, H. verticillata, V. americana*	3 for Hv, 5 for Hd, 10 for Va	14 days, 7 days, and gradually increased then 7 days	chlorophyll, oxygen metabolism

*Max leaf length, branch/leaf number, MQyield, rETRmax, biomass, and mortality were measured in all treatments, unless noted.

Mesocosm Design

The SAV plants used in all experiments in this study were planted in 1.9-liter plastic pots filled with sediment collected near a natural SAV bed in the freshwater region of the Chickahominy River, Virginia. Each pot was 15cm tall and 17cm in diameter. Three plants were planted per pot and each pot was placed in a white, 19-liter plastic mesocosm container filled with dechlorinated tap water. The large containers were 35cm tall with a top diameter of 29cm and a basal diameter of 25.5cm. The only exception to this was Experiment 1 (*H. verticillata* concentration and duration). In that experiment, only two plants were used per mesocosm and the sediment placed directly into the 19-liter containers to 5 cm deep, instead of into a separate pot. This methodology was altered to allow for changes in salinity without excess turbidity. In Experiments 2, 3, 4, and 5, the water surface was 21 cm above the sediment in the pot.

The mesocosm containers were placed randomly in a large nursery tank of water to equalize temperature across treatments (Figure 2). Water in each container was bubbled with air and filtered with aquarium carbon filters (clear-free filters, Penn-Plax, New York). Each week water quality (temperature, pH, and dissolved oxygen (DO)) was measured using a YSI 6820V2 sonde (Yellow Spring Instruments, Ohio) to assure that these variables remained consistent across treatments. Temperature changes between the main tank and a nearby experimental container were measured using Hobo continuous temperature loggers (Onset, MA). Salinity was measured twice weekly and was kept within 0.25 PSU of the treatment values (levels were usually within 0.1). The containers were covered with clear Plexiglas to block rain and evaporation and the entire setup was covered with neutral density shade cloth to reduce irradiance to between 25 and 30 percent of ambient. Plants were kept at zero PSU for five to seven days prior to each experiment to reduce the effects

of planting stress, and then adjusted to experimental values. Entire plants for *V. americana* including intact leaves and roots, 6-10 cm apical segments of *H. dubia,* and 4-6 cm apical segments of *H. verticillata* were used for transplants. Sediment organic content was calculated using ash-free dry weight and sediment partial size was calculated according to Plumb (1981).

Stress Measurement Methods

Stress was measured using both non-destructive and destructive techniques. Nondestructive techniques were used at the start of each experiment and periodically during the studies. These included measurements of maximum blade/branch length, number of blades/branches, maximum electron transport rate, and maximum quantum yield. Destructive methods were used at the end of each experiment and included above and belowground biomass, and organic content. In Experiment 5, shoot or leaf chlorophyll a and b content were also measured. In Experiments 2 and 4, oxygen metabolism and chlorophyll a and b content were measured. Growth was determined by measuring the branch or blade number and by the length of the longest branch or blade, minus the initial values. In Experiment 1, the length of every branch on each plant was also measured and these were summed to give total branch lengths. The above and belowground weight of each plant was also determined, using the total dry weight of all of the plant material in each mesocosm container. Plant organic content was calculated using ash-free dry weights. The number of flowers was noted at each measurement time.

Photosynthetic efficiency and capacity were measured using PAM (pulse-amplitude modulated) fluorometry using a Walz diving-PAM (Diving PAM-2000, Heinz Walz, Germany). PAM fluorometry measures chlorophyll a florescence, which can be used to determine the electron-transport rate of photosystem II (Beer et al. 1998, WALZ 1998,

Durako and Kunzelman 2002, French and Moore 2003). Rapid light curves, ten-second light pulses of nine levels of increasing irradiance, were used to measure $rETR_{max}$, photosynthetic capacity, and MQyield, photosynthetic efficiency (Figure 3). Leaves were dark adapted for ten minutes with leaf clips in order to assure that reaction centers are open and the effect of ambient light levels were minimized (Beer et al. 1998, Ralph et al. 1998, WALZ 1998, French and Moore 2003). The $rETR_{max}$ was calculated by averaging the three highest consecutive rETR values from a light curve, which in most cases correspond to the plateau (French 2001). The central portion (around 10 cm from the sediment) of the second youngest healthy leaf on *V. americana* plants and the apical group of leaves of the longest branch of *H. dubia* and *H. verticillata* plants was used for PAM readings; one reading was taken per mesocosm container. Data in which the F_0 value is below 70 was discarded, since these samples had too little base fluorescence to give accurate results (K.A. Moore, personal conversation).

Photosynthesis and respiration rates were measured using oxygen metabolism. Measuring dissolved oxygen (DO) concentrations exchanged from photosynthetic tissue in water is commonly accepted as a measure of SAV photosynthesis and respiration (Lewis and Smith 1983, Caffrey and Kemp 1991, Lewis 2004, Torquemada et al. 2005). In this study, leaves were taken from one plant in each pot. A four-centimeter segment, 10 cm from the base of second order blade was used for *V. americana*. For *H. verticillata,* the top four centimeters were used, and for *H. dubia,* the apical whorl (3-4 leaves) of the longest stem was used. The leaves were cut at least 30 minutes before they were placed in the BOD (biological oxygen demand) bottles to allow air to drain from their cut lacunae (Lewis 2004); if air spaces are not cut, air trapped in airspaces may lead to erroneous data (Sorrell and Dromgoole 1986). For the last 15 minutes of the 30-minute incubations, the leaves

were dark-adapted. Then the leaves were placed in 300 ml BOD bottles in a water bath kept at the average temperature of the tanks outside (Lewis and Smith 1983). For photosynthesis measurements, BOD bottles were filled with de-chlorinated tap water bubbled with nitrogen to lower initial DO. Oxygen was measured using a DO probe initially and after two hours. For the photosynthesis measurements, the bottles were covered with shade cloth to approximately 700μmol/m^2/sec. For respiration measurements, the same basic procedures were followed; however, the water was not bubbled with nitrogen. Blackened BOD bottles were used to reduce the ambient light to zero, and the exposure time was increased to ten hours. Dry weights of samples of leaf material were used to correct photosynthesis measurements for unit weight.

Chlorophyll was measured on frozen leaf segments collected using the methods listed above for the photosynthesis and respiration tests. An extracting solution of 45% acetone, 45% dimethyl sulfoxide, 10% DI water, and 0.1% DEA buffer was used (Shoaf and Lium 1976); this was shown in preliminary tests to be far more effective than acetone alone and removed almost all of the chlorophyll with no need for cutting or grinding the samples (Wright unpublished). Samples were left in the extracting solution for 24 hours and then read on a UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Chlorophyll a, b, and total chlorophyll were calculated following equations for samples treated with DMSO (Arnon 1949). Although this method was developed for use with acetone, results were comparable to those obtained from equations developed by Wellburn for pure DMSO extraction (Wellburn 1994).

Levels of Stress

In order to standardize discussions of stress across species and experiments, four levels of stressfulness were used:

- **No stress**: plants show no significant difference in growth, morphology, and biomass from those grown in 0 PSU. Plants can probably grow at this salinity level in the field.
- **Moderate stress**: Plants show positive net growth while in the salinity treatment, but significantly less growth than in the 0 PSU control treatments. Plants may survive in these salinities, at least for short-term pulses, but they may be more susceptible to other stressors and their competitive abilities may be reduced.
- **Severe stress**: Plants show no net growth (or a net loss of tissue) while they are exposed to the salinity pulse. Plants would probably not survive permanently in this salinity.
- **Fatal**: Plants die within the experimental timeframe.

Statistical Analyses

Normality was determined by histogram appearance (McDonald and Delaware 2009). Lognormal data were transformed with a natural log correction. After transformation, Levene's test was used to determine homogeneity of variance (there were no cases where this test was violated). ANOVA and Tukey's post hoc tests were used to compare the effect of salinity level on growth after each pulse and recovery period. For post-hoc comparisons an alpha of $p=0.05$ was chosen to show significance and $p<0.05$ and >0.1 was used to identify non-significant trends. If plants died during an experiment such that there were less than three replicates in a treatment, that treatment was excluded from the statistical analysis. In cases where only two treatments remained, t-tests were used in place of ANOVAs. In some cases, especially when sample sizes were low, data were not perfectly normal, in these cases, it was noted, but ANOVAs were still used. All statistics were run in R (R Development Core Team 2012). An interpolation of summed lengths for Experiment 1 was run in SigmaPlot (Systat Software, San Jose, CA).

RESULTS

Growth, Morphology, and Biomass

Experiment 1: The effect of duration and intensity of salinity pulses on *H. verticillata*

Environmental conditions

Organic content of the sediment was 6.43% with a grain size distribution of 26.6% silt, 19.3% clay, 54.1% sand, and no gravel. Weekly measures of water quality showed no temporal trends. The mean temperature was 29.83°C, with a range of 5.16°C and a standard error of 0.201. The pH mean was 8.54, the range was 2.91, and the standard error was 0.100. Mean water column dissolved oxygen was 8.12; values ranged by 4.18 mg/L and had a standard error of 0.114.

Results

Plants exposed to the higher salinities showed high levels of mortality. At 15 PSU, the plants died at all levels of pulse duration, only one plant remained after one day of exposure (Figure 4). At 10 PSU, all exposures greater than 24 hours were fatal. The majority of plants in lower salinities did not experience mortality.

The total branch lengths at the end of the experiment minus the initial branch lengths show the effects of both salinity and duration (Figure 5). The effects of both salinity and duration can be seen compared to the horizontal line representing the control plants in 0 PSU. Plants appeared to respond linearly as exposure duration increased. In general, higher salinities resulted in slower growth, although the growth of plants exposed to 3 PSU for one day was higher than the growth of those in 0 PSU (Figure 5).

Salinities of 3 and 5 PSU were selected to examine the relative effects of salinity and duration since they were determined to cause intermediate stress in *H. verticillata*. Since most of the plants in salinities of 10 and 15 died, those salinities were classified as fatal for durations over 24 hours and were not included in the AIC analysis. Analysis indicated initial summed lengths were not significantly different between treatments, so final summed lengths (rather than initial minus final) were used. The final length data for 3 and 5 PSU treatments were log transformed to achieve normality and homogeneity of variance. An ANOVA of this data was significant for duration ($p<0.0001$), salinity ($p<0.0001$), and the interaction term ($p=0.0265$). An AIC analysis was used to calculate the effects of the single factor, additive, and interactive models. The interactive model was found to be the best representation of the data, the salinity alone and duration alone models had negligible weights (Table 4).

Table 4. Results of the AIC analysis of *H. verticillata* growth.

Model	Log likelihood	AICc	DeltaAIC	Weight
y~ duration*salinity	2.93	7.46	0.00	0.796
y~ duration+salinity	-0.047	10.20	2.74	0.202
y~ duration	-8.29	21.16	13.70	0.001
y~ salinity	-9.52	23.61	16.15	0.000

Based on the AIC analysis, longer durations of exposure were found to be more detrimental to plant growth (Figure 6). Plant growth was found to drop dramatically with salinity in all of the tested durations, but the drop is more intense in longer exposure durations. The effect of salinity pulse duration on plant growth was most apparent at intermediate salinities (Figure 7).

The peak at 3 PSU seen in the summed length data was not evident in biomass measurement (Figure 8). Other than this, the general biomass trends mirrored the changes in length. Looking at only 3 and 5 PSU again, the duration ($p<0.0001$) and the salinity effects ($p =<0.0001$) were significant; however, the interaction effect was absent. A general linear additive model also showed significant duration and salinity effects (intercept=2.8252, duration effect= -0.0572, salinity effect=-1.4816). The belowground biomass data was variable but a significant effect of salinity was seen ($p=0.0164$) (Figure 8).

Branch number followed the trends for the other growth variables indicating that this will serve well as a proxy for growth (Figure 9). Duration ($p=0.0011$) and salinity ($p<0.0001$) had a significant effect on plant branch number when 3 and 5 PSU were compared, as with biomass, an interaction was absent. The average maximum lengths of plants in each salinity treatment were lower than the lengths of the 0 PSU controls, even for the plants exposed to 3 PSU for one day, where stress was not otherwise evident (Figure 10). When 3 and 5 PSU were compared duration had a weak effect ($p=0.0280$) on maximum branch length, while salinity did not have a significant affect.

During exposure to saline pulses, plants in 3 PSU grew only slightly; they began to recover once the salinity returned to 0 PSU, placing them in the moderate stress category (Figure 11). Plants in 5 PSU suffered severe stress with a complete lack of growth while exposed to saline water and, in the longer durations, there was a net decrease in growth (Figure 12). Most of the plants exposed to 10 and 15 PSU pulses died.

Experiment 2: The salinity tolerance of *H. dubia*

Environmental conditions

The temperature, pH, and dissolved oxygen did not vary greatly among experimental containers (Table 5). Temperatures warmed over the course of the experiment (Figure 13), but the standard error between containers was less than 0.02 °C (Table 5). Regular checks of temperature in the tank and adjacent containers showed only slight differences, which averaged 0.30 degrees higher in the container with a standard error of 0.0076, this indicates that any greenhouse effect caused by the Plexiglas rainguards was minor (Figure 14). The most drastic peaks were likely due to rain events cooling the tank water more rapidly than the mesocosm containers.

Table 5. Mean water quality during Experiment 2.
Standard error values reported in parenthesis.

Day	Temperature (°C)	pH	DO%
1	19.06 (0.01)	8.12 (0.02)	101.0 (0.05)
3	20.56 (0.00)	8.20 (0.01)	100.0 (0.08)
9	22.30 (0.01)	8.17 (0.01)	101.2 (0.06)
14	26.67 (0.02)	8.54 (0.01)	104.0 (0.14)

Salinity was found to have a significant effect on the growth of *H. dubia*. The maximum length (ANOVA, $p<0.0001$, Figure 15) and branch number (ANOVA, $p<0.0001$, Figure 16) of *H. dubia* decreased with salinity. The plants in 3 PSU grew and branched comparably to those in fresh water. The plants in 5 PSU, still grew, although less than that of the control plants. Plants in 10 PSU lost length over the experiment, dropping from 8-10 cm to approximately 5 cm tall. These plants did not stop growing completely, as they gained on average 1-2 branches over the experiment.

The aboveground biomass of *H. dubia* also decreased with salinity (ANOVA, $p= 0.0027$, Figure 17). The aboveground biomass of plants grown in 10 PSU was less than that

of plants in 0 and 3 PSU, while other treatment combinations showed no significance. The aboveground percent organic content was lower for plants in 10 PSU than in 0, 3, and 5 PSU ($p<0.0001$, Figure 18). Belowground biomass and percent organic content did not change significantly, although a trend towards decreasing biomass with salinity was present (Figure 17).

Maximum branch length over time was approximately linear, with some leveling off in the 0 and 3 PSU treatments when branches reached the water's surface (~21 cm). Linear regressions were used to compare approximate growth rates (Figure 19). Plants in 0 and 3 PSU grew at comparable rates of 1.2 and 0.95 cm's/day (R^2=0.9624 and 0.9615). Plants in 5 PSU grew at less than half that rate, 0.48 cm/day (R^2=0.9154). Plants in 10 PSU decreased lengths over the study period at a rate of 0.34 cm/day (R^2=0.9941).

Based on these values, plants in 3 PSU were not subject to stress as defined here, plants in 5 PSU were subject to moderate stress (since they continued to grow), and plants in 10 PSU were subject to severe stress.

Experiment 3: The response of *H. dubia*, *H. verticillata*, and *V. americana* to repeated seven day salinity pulses

Environmental conditions

The temperature, pH, and dissolved oxygen did not fluctuate greatly among mesocosm containers (Table 6).

Table 6. Mean water quality during Experiment 3. Standard error values reported in parentheses.

	Temperature (°C)	**pH**	**Dissolved Oxygen (%)**
Initial	26.12 (0.02)	8.16 (0.01)	101.11 (0.26)
Pulse 1	26.60 (0.03)	8.27 (0.05)	101.00 (0.19)
Recovery 1	25.47 (0.04)	8.49 (0.01)	103.44 (0.31)
Pulse 2	24.07 (0.04)	8.53 (0.06)	105.13 (0.41)

Heteranthera dubia

Initial maximum length values did not differ, so the final lengths were compared directly. There were no significant differences ($p=0.1145$) among salinity treatments, but there was a trend towards decreasing maximum lengths in the more saline treatments (Figure 20). Branch number decreased with salinity ($p<0.0001$, Figure 21). Plants pulsed with 10 PSU branched less than those in 5 or 0 PSU did. Plants in the 10 PSU pulses all showed a net decrease in length and either a constant or decreased branch number, putting them into the severe stress category, 5 PSU plants showed little or no stress.

Aboveground biomass decreased significantly with salinity ($p=0.0001$, Figure 22). Plants exposed to 5 and 10 PSU pulses had less final biomass than those in 0 PSU, and plants in 10 PSU pulses grew less than plants in 5 PSU. Belowground biomass followed a similar pattern ($p<0.0001$ Figure 23). Plants in 5 and 10 PSU pulse treatments had less final biomass than those in 0 PSU and plants in the 10 PSU pulses grew less than plants in 5 PSU.

Organic content decreased with salinity in both above and belowground plant material ($p=0.0001$, above; $p<0.0001$, below, Figure 24 and 25). Above and belowground organic content was higher in 0 PSU than in the 10 PSU pulse treatment. In both treatments, organic content of plants exposed to 10 PSU was bordering on being significantly lower than plants in the 5 PSU pulses.

Based on these results, the 5 PSU plants suffered either minor or no stress and the 10 PSU plants suffered severe stress.

Hydrilla verticillata

All of the plants exposed to pulses of 10 PSU died. This treatment was labeled as fatal, so only 0 and 5 PSU were compared. Maximum length ($p=0.0070$, Figure 20) and branch number ($p=0.0002$, Figure 21) were both greater in 0 PSU than in 5 PSU. The same was true for both aboveground biomass ($p= 0.0034$, Figure 23) and belowground biomass ($p=0.0070$, Figure 24). Plant organic content did not vary among treatments.

Vallisneria americana

Vallisneria americana showed no change in maximum lengths ($p= 0.6500$) or branching ($p=0.4703$) among the treatments. Biomass aboveground showed a non-significant trend towards being greater in 0 PSU than in 10 PSU pulses ($p=0.0640$, Figure 23). Belowground biomass was slightly higher in 5 than in 10 PSU ($p=0.0497$, Figure 24). Organic content showed no trends. Given the minimal effects of the treatments, all salinities were classified as having no stress.

Experiment 4: The response of *H. dubia*, *H. verticillata*, and *V. americana* to repeated two day salinity pulses

Environmental conditions

Table 7. Mean water quality during Experiment 4. Values measured during each of the freshwater pulses. Standard error values reported in parentheses.

	Salinity	pH	Dissolved Oxygen (%)
Initial	0.14 (0.00)	8.36 (0.01)	101.18 (0.08)
Recovery 1	0.20 (0.01)	8.35 (0.02)	102.36 (0.16)
Recovery 2	0.18 (0.01)	8.55 (0.03)	102.29 (0.30)

Note: Since salinities were adjusted as measurements were taken, temperatures increased drastically over the measurement period (8am-11am). Because of this, the temperature average and error did not truly capture the field values. Temperature from a HOBO logger is reported in its place (Figure 26).

Heteranthera dubia

No plants died over the course of the experiment. The change in maximum lengths was greater in 0, 3, and 5 PSU than in 10 PSU pulses ($p=0.0002$, Figure 27). Branch number followed a lognormal pattern, and so the natural log of branch number was used in analysis. As with length measurements, the final branch number decreased as salinity increased ($p= 0.0046$, Figure 28). Plants in 10 PSU had fewer branches than those in 0 PSU. There was a non-significant trend towards plants in 3 and 5 PSU having fewer branches than those in 0 PSU pulses. Biomass and the natural log of organic content were measured for both the above and belowground sections of plants, but there were no significant differences for any of the four variables.

As with the *H. dubia* tolerance experiment (Experiment 2), salinities of 0 and pulses of 3 PSU did not result in stress. The 5 PSU pulse treatment, which was moderately stressful in the tolerance experiment, showed no stress, but it is not clear if this is due to the decreased exposure time (4 days instead of 2 weeks) or a beneficial effect of the fresh water pulses. The 10 PSU treatment was highly stressful, but not fatal, in both experiments.

Hydrilla verticillata

In this experiment three of the four plants exposed to 10 PSU pulses died. Due to this fact, 10 PSU was considered fatal to the plants and therefore was not included in the comparative statistics. Branching and organic content were corrected using a natural log transformation. The initial maximum lengths of plants in 5 PSU pulses were higher than those of plants in 0 and 3 PSU ($p= 0.0081$). Due to this, change the difference in maximum length over the experiment was used instead of total final length. The change in maximum length did decrease with increasing salinity ($p=0.0013$, Figure 27). Length increase was greater for plants in 0 PSU than for plants in either 3 or 5 PSU pulses. Final branch number also decreased with salinity showing a significant decrease between 0 and 5 PSU ($p= 0.0243$, Figure 28). Final biomass and organic content showed no change with salinity both above and belowground. The 3 PSU pulse treatment showed moderate or no stress, the plants in 5 PSU pulses experienced severe stress, and the plants in 10 PSU pulses died.

Vallisneria americana

Many of the *V. americana* plants died during the first week after planting, possibly due to transplant stress. These plants were replaced with plants of the appropriate size. No additional plants died during the experiment. After the initial acclimation period, maximum lengths and branch numbers were not different between treatments so final maximum lengths were used for statistical analyses of the effects of salinity on plant performance of this species. These final maximum lengths showed a non-significant trend towards decreasing in higher salinities ($p=0.0749$, Figure 27). Unlike the other species, the branch numbers did not need to be log transformed (possibly, because adult plants were used instead of unbranched cuttings). As with maximum length, there was a non-significant trend towards a lower number of branches in higher salinities ($p=0.0695$, Figure 28). Biomass

was not significantly different among the treatments for the above (p=0.2591) or belowground (p= 0.6546) material.

Organic content of the aboveground material did significantly decrease with salinity (p=0.0234). Plants pulsed with 5 and 3 PSU had organic contents similar to those in 0 PSU. Plants exposed to pulses of 10 PSU had a lower aboveground organic content than those in 3 PSU and showed a non-significant trend towards lower organic contents than plants in 0 and 5 PSU. The organic content of the belowground plant material was not significant (p= 0.7131). *Vallisneria americana* did not show stress in 3 or 5 PSU pulses. Plants in 10 PSU did not show a decrease in growth although the decrease in organic content may indicate moderate stress.

Experiment 5: The effects of gradual vs. rapid salinity increase.

Heteranthera dubia

The initial maximum plant lengths did not differ among treatments ($p=0.1905$). After one week the plants exposed to 5 PSU salinity (treatment B) showed a trend towards shorter plants than those exposed to freshwater (treatments A and C) ($p=0.0529$). After two weeks this trend was no longer apparent ($p=0.1116$; Figure 29).

There were no differences in the initial branch number ($p=0.3259$). After one week the data indicated a trend towards fewer branches in the 5 PSU treatment, ($p=0.0999$). There were also no significant differences in treatment effects after the second week ($p=0.1524$; Figure 30).

Hydrilla verticillata

There were no significant differences in initial maximum lengths in the treatments ($p=0.8031$). After one week, plants in 3 PSU (B) grew significantly less than those in freshwater (treatments A and C), the two freshwater treatments were the same, and plants exposed gradually to salinity (treatment D) grew at an intermediate level different from the plants in both 0 and 3 PSU ($p<0.0001$). After two weeks, plants that were permanently in 3 PSU (treatment B) showed a trend towards shorter maximum lengths than those permanently in fresh water (treatment A), but the instant and gradual pulse treatments (C and D) were not different from either fresh, 3 PSU, or each other ($p=0.0629$; Figure 29).

The initial branch numbers did not differ ($p= 0.3758$) among treatments. After one week, the plants exposed to 3 PSU salinity (treatment B) had fewer branches than those in freshwater (A and C) and those where the salinity was raised gradually (treatment D). After two weeks, the plants kept at 3 PSU (treatment B) still had fewer branches than those

maintained in fresh water (treatment A), and plants whose salinity was raised gradually (treatment D) were more stressed than those in fresh water (treatment A) (Figure 30).

Vallisneria americana

There were no apparent differences in maximum lengths initially ($p=0.5322$), after one week ($p=0.9229$) or two weeks ($p=0.9094$) exposure to 10 PSU salinity. Branch numbers also did not differ initially ($p=0.7561$) after one week ($p=0.5760$) or two weeks ($p=0.2435$).

Photosynthetic Stress Responses

Chlorophyll

In Experiment 2 (*H. dubia* only), both chlorophyll a and b per unit dry weight tended to have a slight peak at 3 PSU and decrease at 10 PSU (Figure 31). For chlorophyll a, leaf tips in 0, 3, and 5 PSU had significantly more chlorophyll than those in 10 PSU while 0, 3, and 5 were not statistically different from each other ($p=0.0001$). Chlorophyll b followed the same pattern as chlorophyll a, it was higher in 3 and 5 PSU than in 10 PSU, and there was a trend towards higher chlorophyll in 0 than in 10 PSU ($p= 0.0050$). The chlorophyll a to b ratio did not change significantly with salinity, but there was a non-significant trend towards a lower ratio in higher salinities ($p=0.0611$; Figure 32). Chlorophyll a and the a/b ratio were log transformed to achieve normality. Chlorophyll a and b showed a peak at 3 PSU and a decrease in 10 PSU.

In Experiment 3 (weeklong pulses) only *H. dubia* showed significant differences among the treatments. Chlorophyll per unit dry weight tended to peak with 5 PSU pulses and decrease in 10 PSU pulses (Figure 33). Plants exposed to 5 PSU pulses had significantly more chlorophyll a than those in the 10 PSU pulses, while plants in 0 PSU showed a non-significant trend towards having more chlorophyll a than those in 10 ($p=0.0219$). Chlorophyll b followed the same pattern as chlorophyll a, it was higher in 0 and 5 PSU than in 10 PSU ($p= 0.0034$). The chlorophyll a/b ratio increased with increasing salinity ($p<0.0001$), increasing from around 3 to an average of 17 (Figure 34). Chlorophyll a and the a/b ratio was log transformed to achieve normality. The chlorophyll a, b and a/b ratio of *V. americana* and *H. verticillata* showed no significant relationships with salinity, although the chlorophyll a/b ratio did show a non-significant increase in 5 PSU for *H. verticillata*. Chlorophyll content of plants exposed to 10 PSU was not measured for *H. verticillata* due to fatality at that salinity (Table 8). *Heteranthera dubia* showed a minor increase in

chlorophyll a and b in 5 PSU and a decrease in 10; the other species showed no change in chlorophyll.

Table 8. Non-significant p-values for measures of chlorophyll in Experiment 3.

Species	A	B	a/b ratio
Hv	0.6154	0.7354	0.09403
Va	0.1638	0.2133	0.1309

In Experiment 4 (two day pulses), none of the comparisons were significant nor were there any non-significant trends for any species. P-values are shown in Table 9. For *H. verticillata* only 0, 3, and 5 PSU were compared, due to mortality in 10 PSU. Chlorophyll a and a/b ratios were log transformed for *H. verticillata*.

Table 9. Non-significant p-values for measures of chlorophyll in Experiment 4.

Species	a	B	a/b ratio
Hd	0.1271	0.3011	0.5145
Hv	0.6197	0.499	0.803
Va	0.7142	0.7512	0.5876

Pulse Amplitude Modulated Fluorometry

The F value (a variable that indicates the signal strength of the fluorescence) was below threshold in many plants. The sample size was too low to compare statistically in almost every dataset. No apparent trends exist in the data. MQyield (Figure 35) and $rETR_{max}$ (Figure 36) are shown for the final (day 28) timepoint in the *H.* verticillata duration and intensity experiment (Experiment 1). PAM measurements were taken every week (sometimes more often) in every experiment; however, none of the data showed clear trends with salinity treatments.

Oxygen Metabolism

Oxygen metabolism was measured in the two-day pulse experiment (Experiment 4) as the change in oxygen per hour per leaf-tip dry weight in the light and dark. Oxygen change in the light was consistently positive; in the dark treatments, it was consistently negative. The increase in the light was around an order of magnitude greater than the decrease in the dark. For *H. dubia, o*xygen metabolism in the light treatment was higher in 10 PSU than in 3 PSU and showed a trend towards being greater than 0 PSU (p=0.0300) (Figure 37). For *H. verticillata* (p= 0.1334) and *V. americana* (p=0.2418), oxygen flux in the light was not significant. For both of those species, 10 PSU was not included due to plant death in *H. verticillata* and a missing data point in *V. americana.* Respiration was not significant in any of the species (p=0.3014, 0.5376, and 0.4156).

DISCUSSION

Freshwater submerged aquatic vegetation species studied here were found to be very sensitive to both the duration and intensity of salinity pulses. Even short pulses and low concentrations of salinity had noticeable effects on *H. dubia*, *H. verticillata*, and *V. americana*. The tolerance differed greatly between species from the most sensitive, *H. verticillata*, to the somewhat salt-tolerant *V. americana.* Recovery, both after single pulses and between repeated pulses, was slow, suggesting that even brief pulses of salinity may an important determinant of SAV bed community composition.

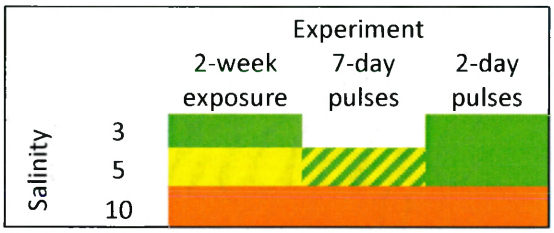
Salinity Tolerance Levels

The freshwater SAV used in this study were shown to have markedly different tolerances to both the duration and intensity of salinity stress. *Heteranthera dubia* began to show stress when exposed to between 5 and 10 PSU. *Hydrilla verticillata,* the most sensitive, experienced stress in 3 PSU. *Vallisneria americana* performed well in all of the experimental salinities.

Heteranthera dubia experienced no stress at 3 PSU and severe stress at 10 PSU across all of the experiments. Table 10 summarizes the stress of *H. dubia* to the varying durations of salinity exposure studied here. Salinities of 5 PSU sometimes caused no stress and sometimes moderate; stress was lower when the duration of exposure was brief. Mortality was not seen, but it is likely not far above 10 PSU. The salinity tolerance of *H. dubia* has not been well studied. Moore and Shields (2010) found stress at 10 PSU and no stress at 5 PSU, which supports my findings. Bergstrom et al. (2006) reported the optimal

growth range to be 0-4 PSU, and a survival range of 0-8. This supports findings reported in this study, although I found *H. dubia* is capable of surviving short pulses of 10 PSU.

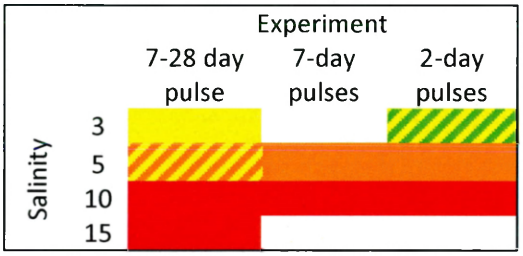
Table 10. Stress levels based on growth variables for *H. dubia*. Green = no stress, yellow = minor stress, orange= severe stress, and red = mortality. Slashed boxes indicate a stress level between the two colors.



Hydrilla verticillata is the least salinity tolerant of the species used in this study. It began to show stress around 3 PSU, was moderately to severely stressed at 5, and died in 10+ PSU (Table 11). These results confirm previous findings for fatal salinities, usually reported at 10 or 15 PSU. **Although the levels of stress seen in this study confirm others'** findings (Carter et al. 1987, Twilley and Barko 1990), some previous studies found no stress at 3 PSU (Haller et al. 1974) and some found no stress as high as 5 PSU (Littles 2005). A field study (Shields et al. 2012) shows that *H. verticillata* may be even more sensitive when other stressors and competition are present. In the Chickahominy River, VA, *H. verticillata* was restricted to upstream sites when drought years caused a salinity of 4 PSU in downstream sites. It is possible that the methods used in this study allowed for the detection stress at lower salinities or that the initial population of *H .verticillata* was more susceptible to salinity stress, since small cuttings without ample energy reserves were planted. Bergstrom et al. (2006) report the optimal growth range to be 0-5 PSU and a survival range of 0-9 PSU. This is more saline than the ranges I found. In my study, the plants were classified as **"stressed" at 3 PSU, by 5** they were no longer growing, and they could not survive 10 PSU.

Based on my work, an optimal growth range of 0-3 PSU and a survival range of 0-5 PSU may be more accurate; although different populations and growth conditions may be responsible for the discrepancy.

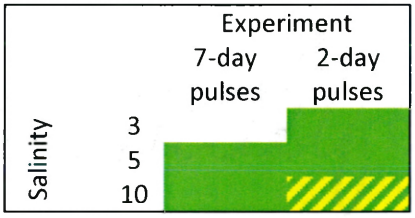
Table 11. Stress levels based on growth variables for *H. verticillata,* for experiment 1, durations over 1 day were included. Green = no stress, yellow = minor stress, orange = severe stress, and red = mortality. Slashed boxes indicate a stress level between the two colors.



Vallisneria americana is the most tolerant of the three species; it showed minor or no stress at all salinities up to 10 PSU as summarized in Table 12. Some studies have suggested that this species is stressed at 5-10 PSU and dies around 15 (Haller et al. 1974, French and Moore 2003, Boustany et al. 2010). However, other studies support the results observed here, that stress is not evident until over 10 PSU (Twilley and Barko 1990, Kraemer et al. 1999, Doering et al. 2002, Frazer et al. 2006, Moore and Shields 2010). Bergstrom et al. (2006) report the optimal growth range to be 0-5 PSU and a survival range of 0-12. This is much lower than the values found here, since stress was still not visible here at 10 PSU. The lack of stress seen may be due to the planting methods. Adult plants of *V. americana* were used in the current experiment. These plants had blades reaching the water's surface, improving light capture, and reducing the need for further blade elongation. The adult plants may also have had stored carbon reserves in their roots and leaves, which may have enabled them to survive on reserved carbon for the short pulses of high salinities.

The leaves of plants in the 10 PSU treatments appeared paler and less rigid than plants in fresh water indicating that salinity may cause noticeable stress not far above 10 PSU.

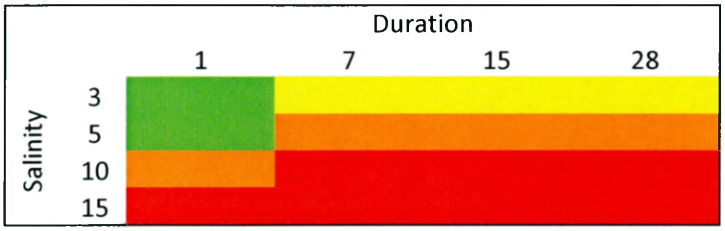
Table 12. Stress levels based on growth variables for *V. americana*. Green = no stress, yellow = minor stress, orange = severe stress, and red = mortality. Slashed boxes indicate a stress level between the two colors.



Duration and Recovery

There was an effect of pulse duration on salinity stress in *H. verticillata*, which has proved difficult to determine statistically in previous studies (Frazer et al. 2006). This effect was most noticeable when comparing pulses one week or longer with one-day pulses; however, even after 28 days of recovery, stress from one-day pulses was noticeable as summarized in Table13. The effect of duration was clearest in intermediately stressful salinities (3 and 5 PSU) (Figure 7 and Table 13). These results suggest that salinity stress can continue to affect this species for weeks after the salinity reduced to 0 PSU. Despite this, the effect of salinity appears stronger than the effect of duration.

Table 13. Growth as a factor of duration and salinity for *H. verticillata*. Green = no stress, yellow = minor stress, orange = severe stress, and red = mortality. Slashed boxes indicate a stress level between the two colors.



Also in experiment 1, a slight peak in branch number for *H. verticillata* plants exposed to 3 PSU for one day was seen in this experiment, but was not seen in the length or total biomass. This morphological change was observed by Twilly and Barko (1990). They also saw a sub-fatal increase in branch number (referred to as stem density) for *H. verticillata*, although this was observed at 6PSU, rather than the 3 PSU seen in this experiment. From personal observation, this appears to be a possible survival mechanism, where stressed *H. verticillata* will grow many short fragile branch tips, which then break off. Theoretically, these may drift to an area with more favorable conditions and then grow into new plants.

Repeated Pulses

Contrary to expectations, returning plants to fresh water after short salinity pulses did not result in a rapid recovery. Although plants left in fresh water eventually begin growing again (Experiment 1), the growth of plants exposed to repeated salinity pulses appears to be linear, rather than stepwise. The loss was not due to the inclusion of dead plant tissue in the measurements since tissue that appeared to be decaying (no visible green pigments and complete lack of rigidity) was removed before growth variables were measured. Since variables were measured after each salinity and freshwater pulse, it is possible that growth or loss were not linear and that recovery occurred at times in between measurements. Overall, however, the data did not appear follow a pattern of loss during the salinity pulses and regrowth during the freshwater recovery periods.

It is likely that stress resulting from salinity was not removed when the salinity level was returned to 0 PSU. Physiological damage caused by salinity stress may continue to stress the plants after the salinity in the water was reduced. Since free radicals and

peroxides are produced during salinity stress, some enzymes used to combat them can be impaired (Rout and Shaw 2001). The effects of salinity on enzymatic function may be one mechanism by which this occurs; however, it is unclear how long this continues after salinity stress is removed. Plants may also utilize their stores of energy during the pulse, such that they do not have the same resources available for continuing growth compared to plants not exposed to even short pulses of salinity stress. For *H. dubia*, the decline in chlorophyll a and b may have limited the plants' ability to photosynthesize, and thus decreased the oxygen produced. There were also noticeable color changes in all three species. *Vallisneria americana* turned yellow or tan in 10 PSU. *Hydrilla verticillata* became brown in stressful salinities and *H. dubia* leaf tips turned black. Although the mechanism behind this change is unclear, the change in color may prevent light from reaching the photosystem as effectively. Stressed plants also lost structural rigidity, and the leaves of all species began to feel more "gel-like" although this was not quantified, it may indicate damage to the cell membranes and vascular system due to osmotic stress.

The Effects of Gradual vs. Rapid Salinity Increase

Many studies mention the rate at which plants are exposed to stress as a possible cofactor affecting salinity tolerance (Twilley and Barko 1990, Doering et al. 2001), and the general assumption has been that gradual exposure lessens stress; however, few data are available to support these statements. My results do not support this view. When *H. verticillata* and *H. dubia* were either gradually exposed to stressful salinity or left in fresh water for a week and instantaneously raised to the same salinity there were no significant differences in length or branching. Although growth rates were low in this study (Experiment 5) making it difficult to identify significance between the growth rates of plants exposed to different pulse patterns; based on the length and branching (Figures 29

and 30), it appears that the average growth for plants exposed gradually is usually lower than plants exposed instantly. This may be because the plants were exposed to a lower level of salinity for longer periods in gradual exposure compared to rapid exposure.

The hypothesis that gradual pulses lead to lower stress was based in part on the supposition that it may allow the enzymes involved in salinity tolerance to accumulate. Rout and Shaw (2001) found that antioxidative enzymes may be able to reduce oxidative stress and that some of those, such as superoxide dismutase, do increase in reaction to salinity exposure. However, they observed these increases only 9 hours after an instantaneous pulse, indicating that gradual increase over days or weeks may not be necessary. They also found that most of the enzymes occurred in *Najas gramenia*, which has a large range of salinity tolerance. Thus, it is possible that gradual increases help plants that already have some capacity to withstand saline pulses, but do not greatly affect species that do not manufacture a wide range of antioxidative enzymes. This may be why Koch (2007a) found that species of seagrass growing in oceanic salinities were less affected by hypersalinity stress when exposed to pulses gradually.

Variable Comparisons

Overall, direct measurement of growth, branching, or biomass seem to be a more effective measure of salinity stress than any of the photosynthetic variables (MQyield, $rETR_{max}$, chlorophyll, and oxygen flux).

Photosynthetic efficiency and capacity were surprisingly even across salinity stress levels. In the examples shown (Figures 35 and 36) only one plant exposed to 15 PSU was still alive and it was only a few centimeters tall, however, both its MQyield and $rETR_{max}$ were almost identical to the plants in no or low salinity. The plants in the 28-day treatment were still in saltwater at this point, but showed no reduction in the efficiency or capacity of

photosystem II from plants in fresh water. The lack of a decline observed here may be due to the low florescence readings; the base fluorescence (Fo) was often close to the operating threshold signal levels, so it may not have been able to pick up small changes accurately. The most likely explanation is that photosystem II is not noticeably impacted by salinity stress in these plants. Although data exists for using PAM on light stress, it has rarely been used to measure salinity stress. French (2001) used PAM to measure the interactive effects of light and salinity on *V. americana*. Although she found a significant effect of salinity on MQyield, it explained very little of the variance, there was an interaction term with light levels, and stress was not evident until the plants had started to die back, about a month after the growth variables indicated stress. As with this study, she found no effect of salinity on $rETR_{max}$. It appears that changes in MQyield and $rETR_{max}$ are minor, even in salinities fatal to the plants.

Changes in chlorophyll per leaf dry weight with salinity concentrations were only significant for *H. dubia* in Experiments 2 and 3. The chlorophyll content of *V. americana* and *H. verticillata* were not significantly different in Experiment 3 and none of the species showed a change in Experiment 4. In Experiment 2, *H. dubia* chlorophyll a and b had an intermediate peak at 5 PSU and decreased at 10 PSU; in Experiment 3, the results followed a similar pattern, but the peak occurred at 3 PSU (which was not measured in Experiment 3). This peak may be due to a combination of a change in the chlorophyll content per unit leaf area and a possible decrease in the structural material and subsequent drop in the dry weight of the leaf section. No previous studies have looked at *H. dubia* chlorophyll in relation to salinity.

The lack of a response of chlorophyll to salinity in *V. americana* is concurrent with the lack of stress measured in other variables. The absence of a significant change in *H.*

verticillata was surprising, but other studies have also found no relationship between chlorophyll quantity and salinity in stressed plants (Twilley and Barko 1990, French 2001, Rout and Shaw 2001).

The chlorophyll a/b ratio of *H.* dubia showed a non-significant decreasing trend with increasing salinity in Experiment 2, but increased significantly with salinity in Experiment 3. Rout and Shaw (2001) found that the ratio of chlorophyll a to b of *H. verticillata* decreased in high salinities. There does not seem to be any published data to support a ratio increase, but the jump from a normal ratio of around 2-3 to an average ratio of 11-20 occurred in all three replicates, so it is unlikely that it was a measurement error. The change in chlorophyll a/b ratio may be due to the decline in both chlorophyll a and b and loss of plant tissue.

Oxygen metabolism was only measured for the two-day pulse experiment (Experiment 4). Since the chlorophyll quantity, photosystem efficiency, and photosystem capacity were not affected, for *H. verticillata* or *V. americana* neither was oxygen metabolism. Kraemer (1999) found no significant difference in photosynthesis using similar methods, even in fatal salinities and as plants were dying. However, in this study the oxygen metabolism for *H. dubia* increased. These results are inconsistent, as stress typically reduces photosynthetic rate. The increase was not evident until a correction for dry weight was applied, so this may be due to a decrease in dry weight per unit leaf area. More research is needed to confirm these results.

Interspecies Competition

Freshwater SAV typically grows in mixed communities of many species (Moore et al. 2000) with potentially different habitat requirements for optimum growth. Changing environmental conditions may therefore alter the composition of this community. Species

range is primarily determined by salinity (Twilley and Barko 1990, Moore et al. 2000, Doering et al. 2001, Frazer et al. 2006, Orth et al. 2010, Shields et al. 2012). Changes in salinity can restrict species with low salinity tolerance, such as *H. verticillata* to upstream lower salinity sites. However pulses of high salinity can also cause die-backs of easily stressed native species, leaving bare ground that can be rapidly colonized by *H. verticillata* after the elevated salinity levels retreat (Shields et al. 2012). Dobberfuhl et al. (2012) examined the effects of short pulses on the SAV of the St. Johns River, Florida, and found that short (one week) pulses would not have a drastic effect on SAV distribution. However, they only used data for *V. americana,* which has a much higher salinity tolerance than many species. It may be that while the distribution of SAV may remain constant after short salinity pulses, the composition of those beds may change as the less tolerant species become restricted to lower salinity regions of the estuary. Currently, there are no studies evaluating the responses of natural mixed SAV beds to short pulses of various strength and duration.

Applications and Impacts

This work emphasizes the need for continuous monitoring of salinity levels in locations where they are variable. A pulse of 10 PSU for 24 hours may be unnoticeable in data averaged over time or collected periodically, but it may be enough to cause a dieback in species such as *H. verticillata*. Salinity may be an important driver of storm surge related mortality, which is often attributed to nutrient or toxic inputs to a system, or low light levels caused by excess runoff. Short-term water quality monitoring (on the order of minutes or hours) may be necessary to understanding salinity stress, especially since plants may show clear stress 28 days after a one-day salinity pulse. This research also shows that photosynthetic measurements (chlorophyll content, PAM measurements of photosystem

condition, and direct measurements of photosynthesis, and respiration) are not effective at determining plant stress due to salinity; and only by measuring cover, length, biomass, and other physical growth properties periodically can stress be accurately detected.

The results of this study will improve future restoration and invasive species control by highlighting the need to determine site viability based on salinity variability rather than average salinity conditions alone. In an environment where salinity is variable, it may be necessary to use more salinity tolerant species for restoration efforts than average salinity values would suggest. Likewise, if salinity intrusion is likely during occasional dry periods or years in areas where *H. verticillata*, has become dominant during wet years, *H. verticillata* control may be less important.

Understanding how salinity pulses affect SAV may enable anthropogenic water control to have less drastic impacts on estuarine ecosystems. From these studies, long pulses of low salinity seem to affect these species less than short pulses of high salinity. This suggests that releasing water gradually and constantly through water control structures will have less of a negative affect than rapid pulses of very high and very low freshwater flows.

Climate change may alter salinity levels and therefore may have an effect on SAV abundance and persistence in some areas. Najjar (2010) predicts that climate change will likely increase the frequency of droughts and the intensity of storms and may alter patterns of precipitation. This will possibly result in an increase in the variability of salinity. This combined with sea level rise may result in salinity changes and pulses of salinity intruding further upstream than they have done in the past.

Conclusions

In conclusion, short-term salinity pulses can be stressful to freshwater SAV. Recovery from these pulses may be slow and the stress resulting from short pulses of salinity is visible long after the pulse has receded. Due to this slow recovery, pulsing salinity does not greatly reduce the salinity stress to the plants, even when the recovery periods are longer than the salinity pulses. The maximum level of salinity seems to be more important than the pattern of salinity change. It is also clear that freshwater SAV such as those studied here have very different levels of salinity tolerance. The invasive species *H. verticillata* has the lowest tolerance of the species investigated in this study. This indicates that salinity pulses may restrict its range to areas further upstream than other species in the freshwater mixed SAV community, while the more tolerant *V. americana* may survive into the oligohaline region of an estuary.

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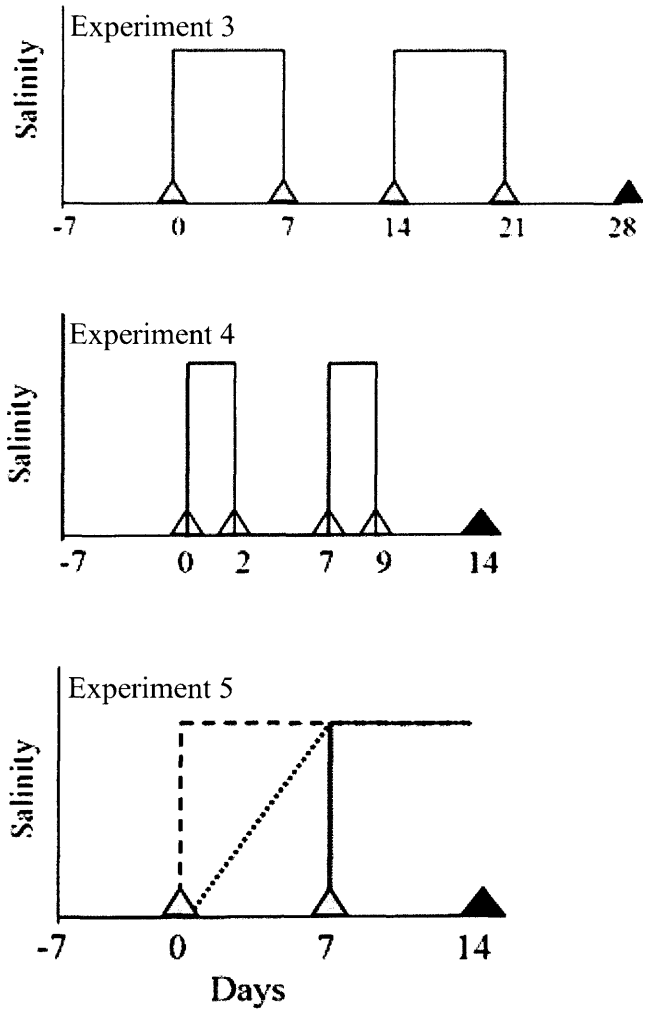

Figure 1. Salinity pulse graphical representations. Salinity levels vary between treatments. Black triangles indicate destructive sampling events, measuring all variables listed. Gray triangles indicate length, branching, and PAM. White indicate length and branching only. For all experiments, a control of plants held at 0 PSU was used. For experiment 5, the gradual increase treatment was raised once per day, for 7 days by $1/7^{th}$ of the final salinity.

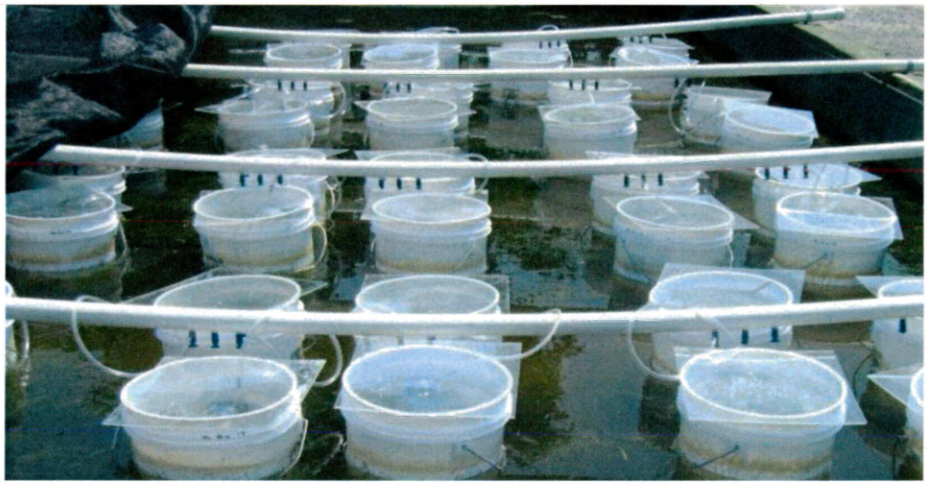

Figure 2. Experimental setup: plants in mesocosm containers and nursery tank covered with Plexiglas rain guards.

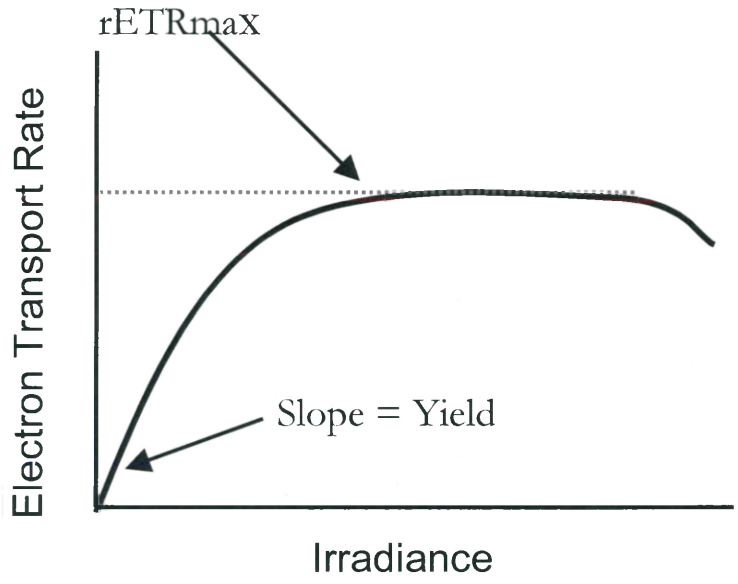

Figure 3. Definition of MQ yield and rETR max.

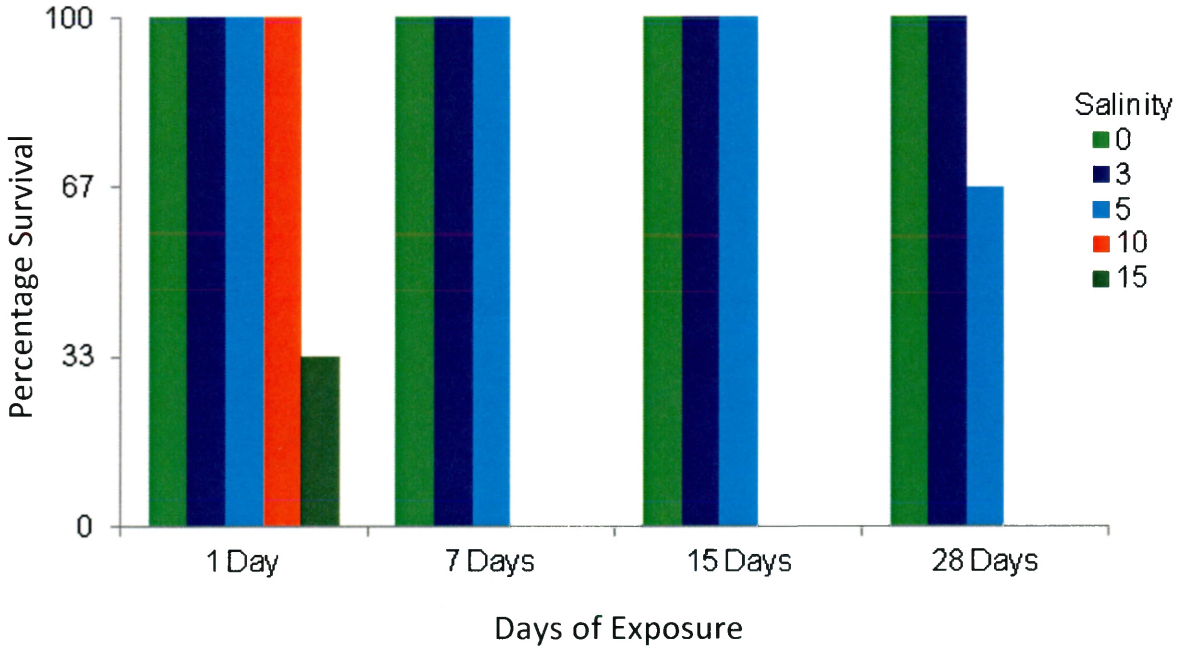

Figure 4. Percentage of replicates containing surviving *H. verticillata* after 28 days.

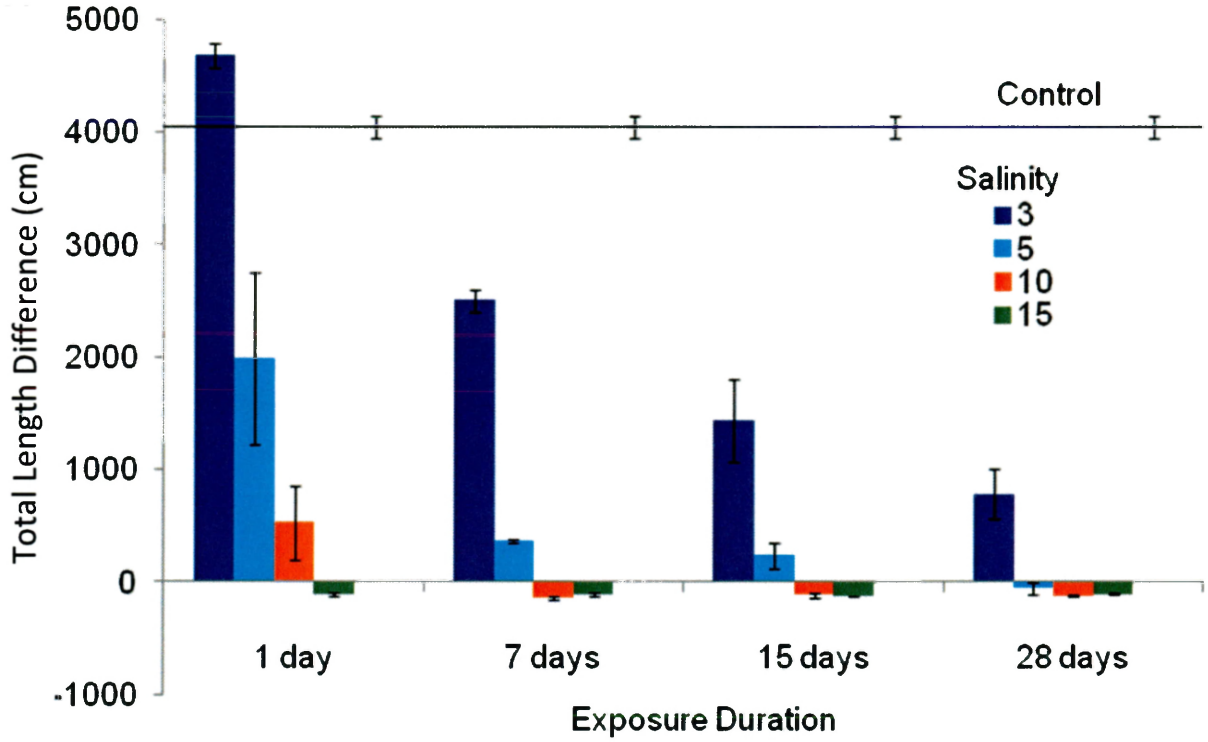

Figure 5. Final summed length of all branches of *H. verticillata* after 28 days minus the initial summed lengths. Plants were exposed to various salinities for pulses of various durations and returned to 0 PSU for the remainder of the 28 day period. Error bars represent SEM +/-1. The horizontal line shows growth of plants in 0 PSU.

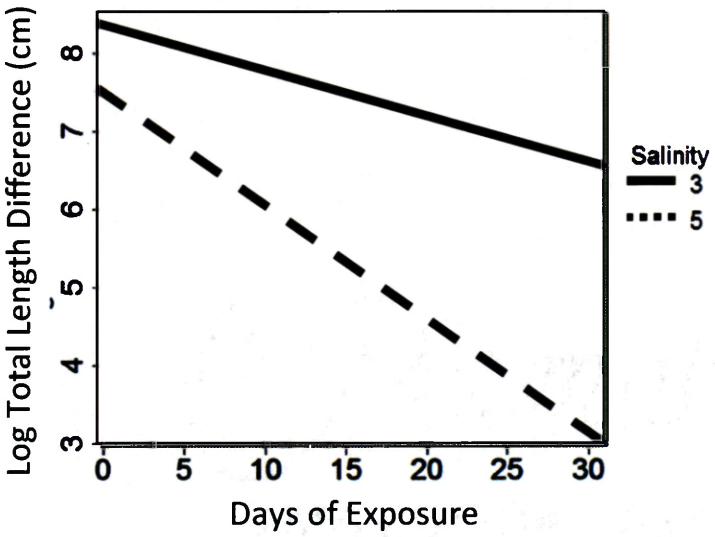

Figure 6. Plotted interactive general linear model results growth of *H. verticillata*.

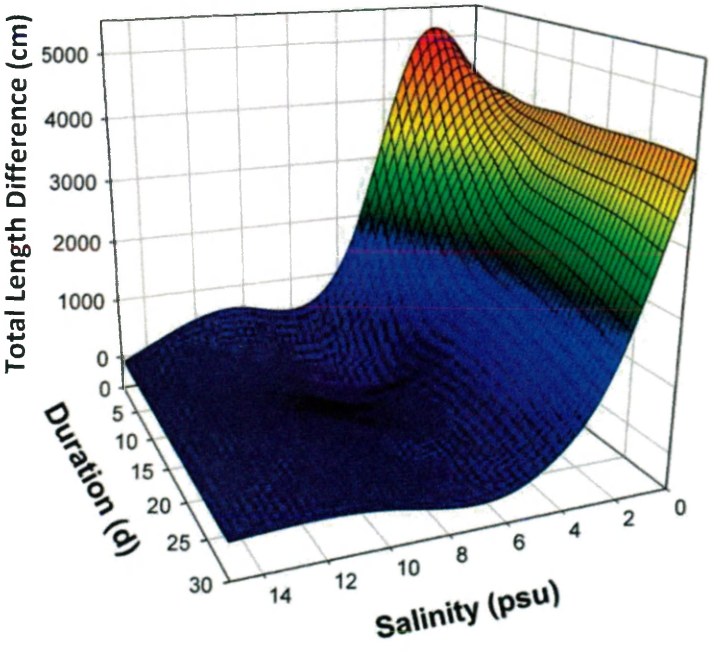

Figure 7. Interpolation of *H. verticillata* growth across salinity intensities and durations.

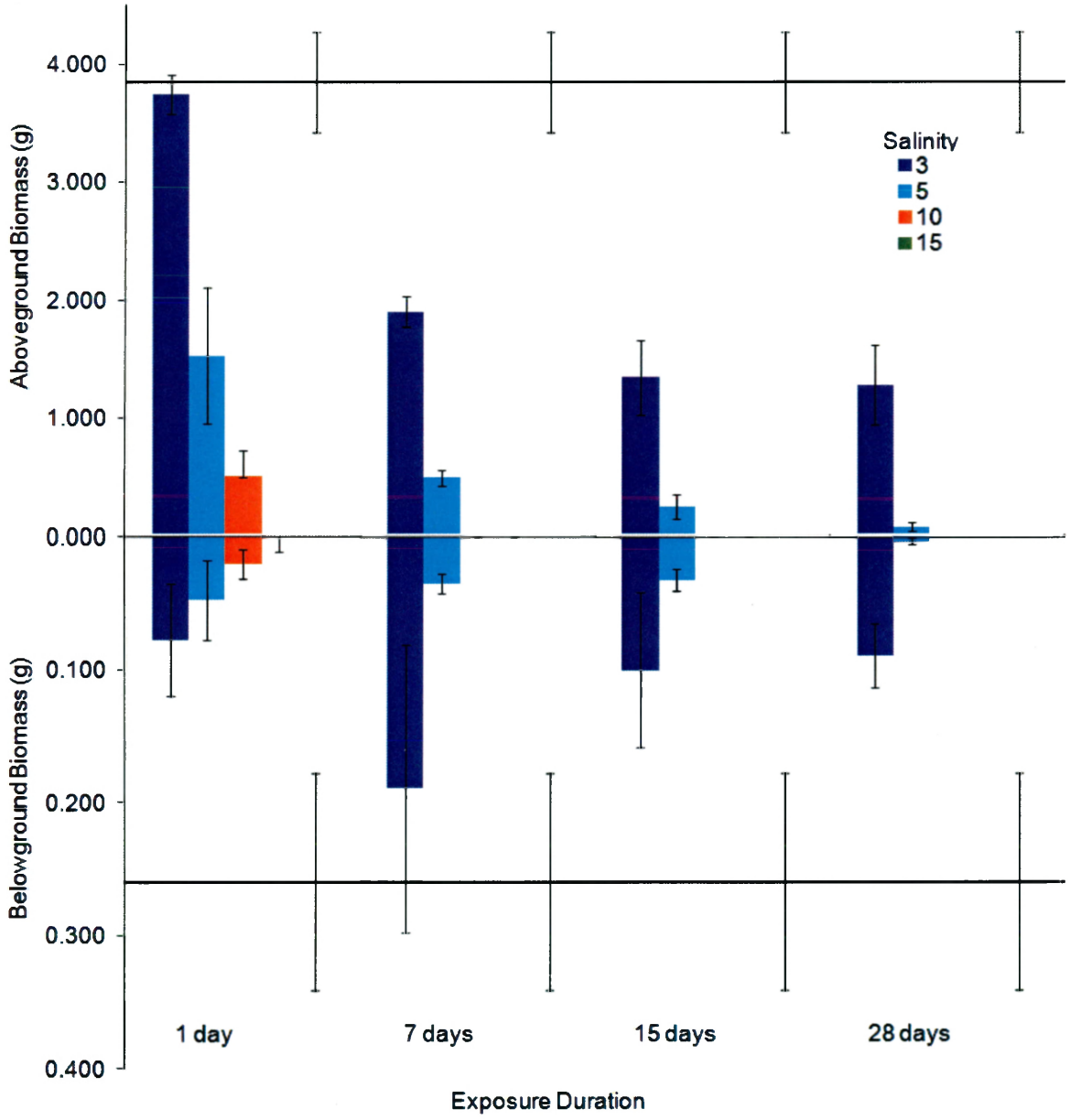

Figure 8. Above and belowground biomass of *H. verticillata* after 28 days. Plants were exposed to various salinities for pulses of various durations and returned to 0 for the remainder of the 28 day period. Error bars represent SEM +/-1. The horizontal lines shows growth of plants in 0 PSU.

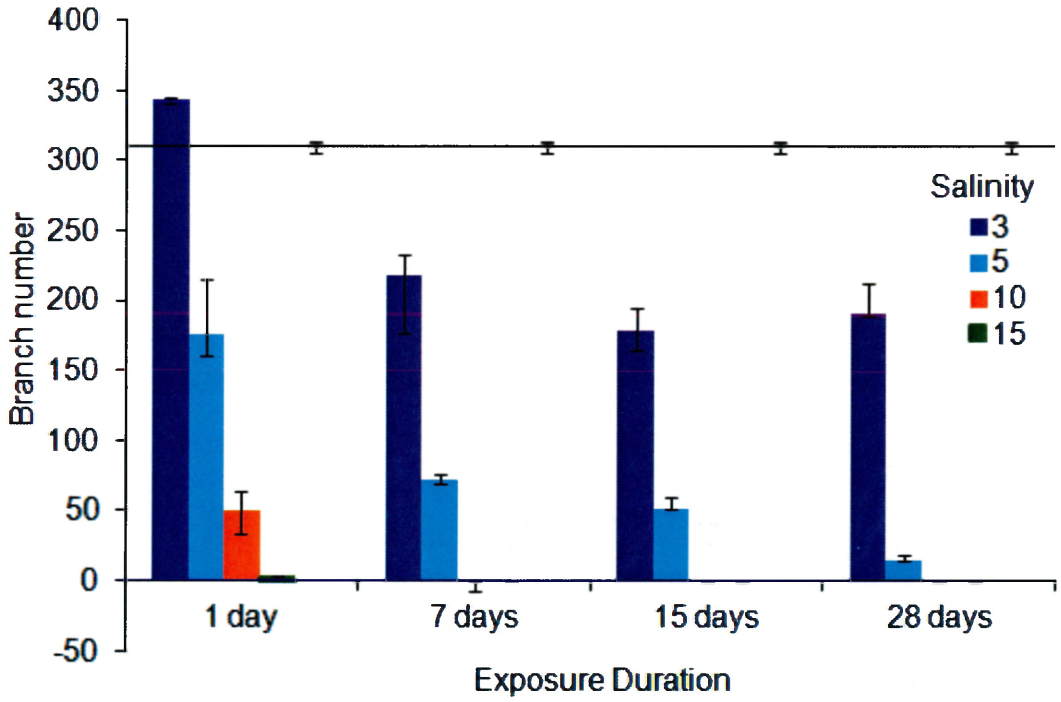

Figure 9. Branch number for *H. verticillata* after 28 days. Plants were exposed to various salinities for pulses of various durations and returned to 0 PSU for the remainder of the 28 day period. Error bars represent SEM +/-1. The horizontal line shows growth of plants in 0 PSU.

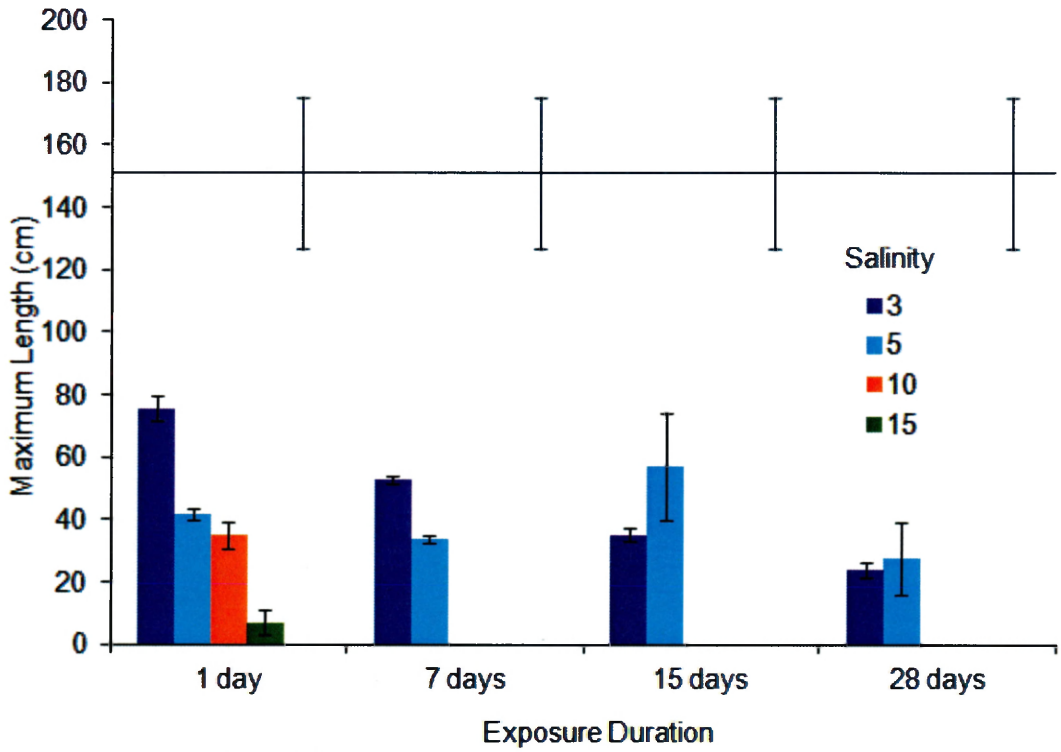

Figure 10. Maximum branch length for *H. verticillata* after 28 days. Plants were exposed to various salinities for pulses of various durations and returned to 0 PSU for the remainder of the 28 day period. Error bars represent SEM +/-1. The horizontal line shows growth of plants in 0 PSU.

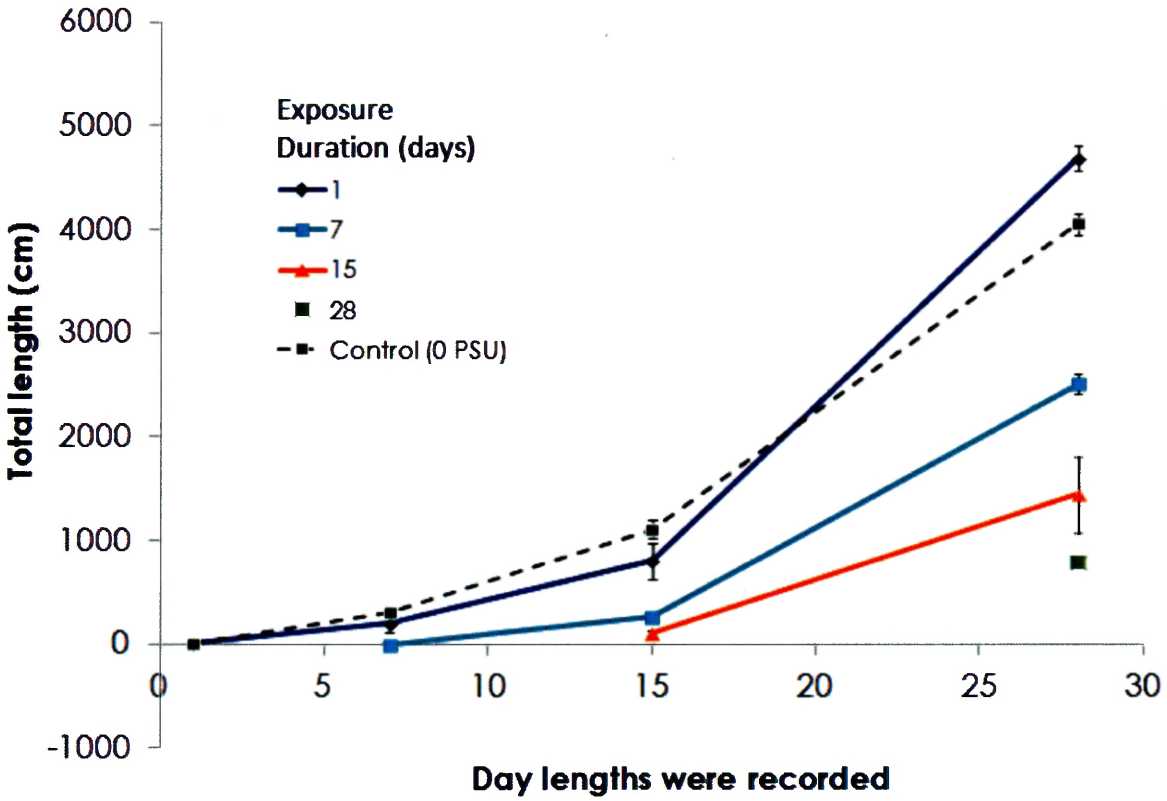

Figure 11. Length change over time for *H. verticillata* exposed to 3 PSU. Error bars represent SEM +/-1. The dotted line shows growth of plants in 0 PSU.

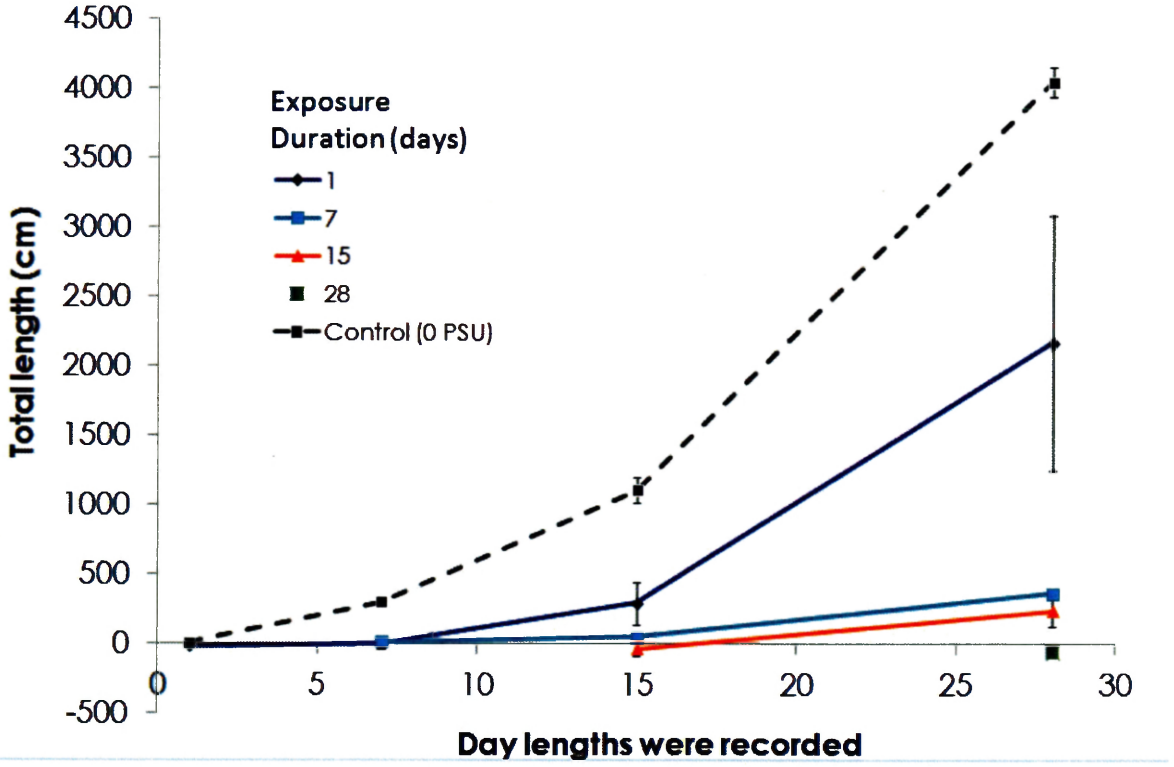

Figure 12. Length change over time for *H. verticillata* exposed to 5 PSU. Error bars represent SEM +/-1. The dashed line shows growth of plants in 0 PSU.

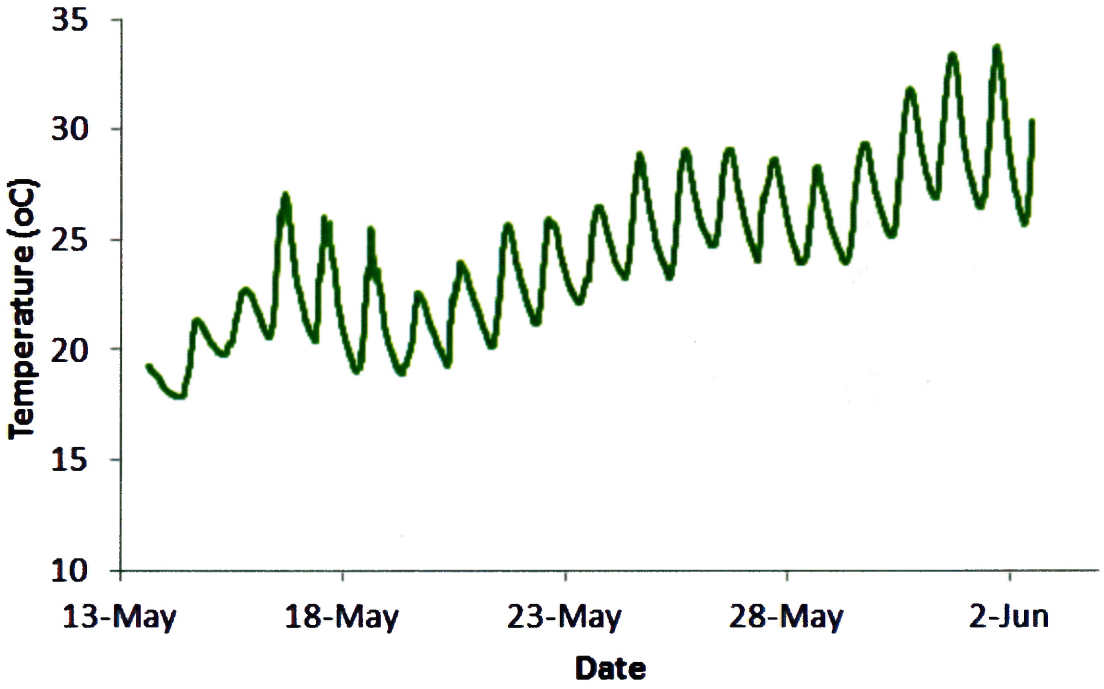

Figure 13. Temperature in a mesocosm container over the course of the experiment.

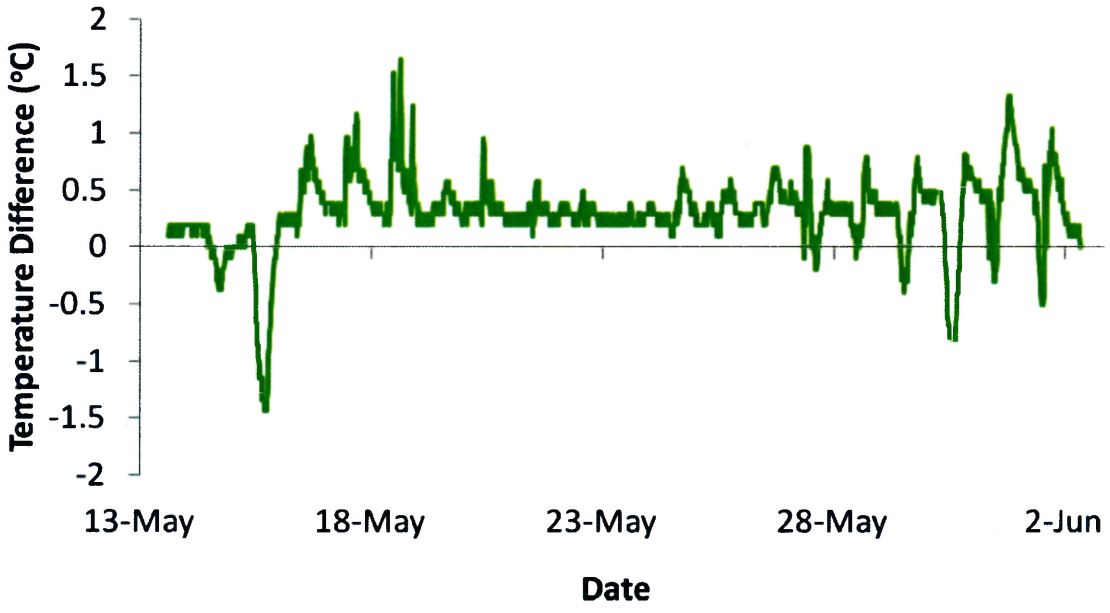

Figure 14. Difference between temperature inside a mesocosm container and the nursery tank.

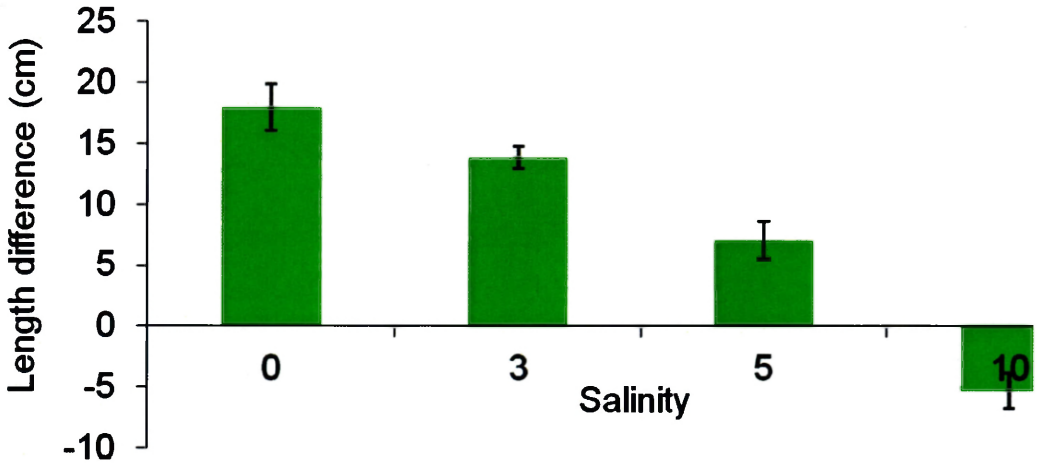

Figure 15. Change in lengths (final – initial) of *H. dubia* in each of the various salinities after 16 days. Error bars represent SEM +/- 1.

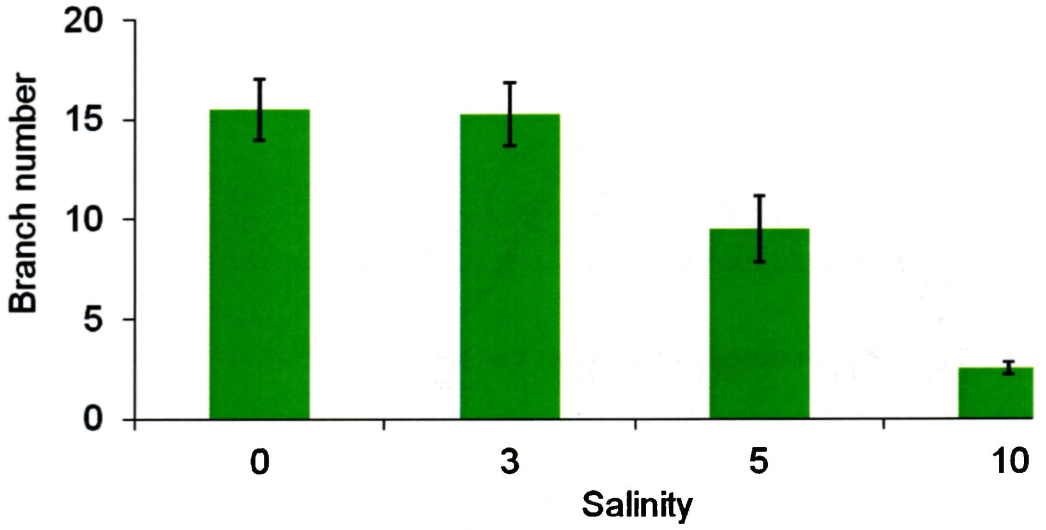

Figure 16. Number of branches of *H. dubia* in various salinities after 16 days. All plants started with one branch. Error bars represent SEM +/- 1.

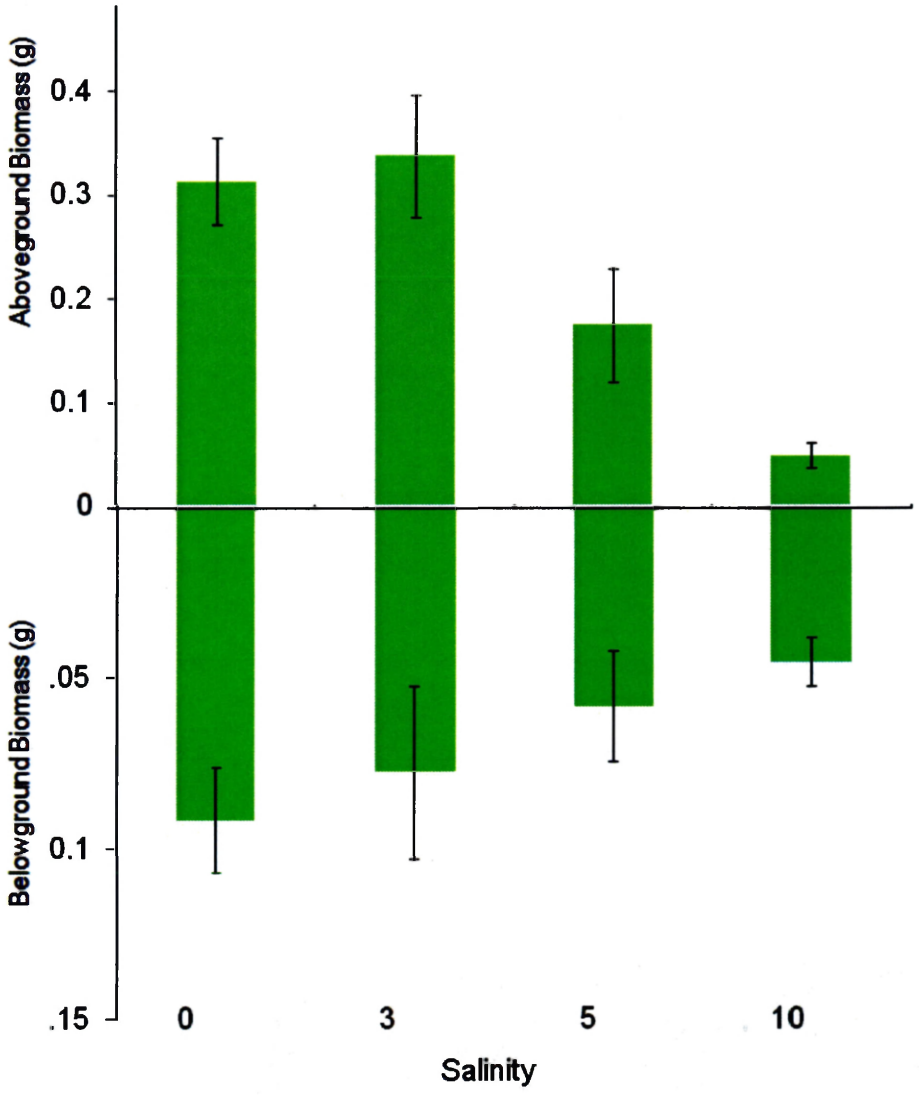

Figure 17. Above and belowground biomass of *H. dubia* in various salinities after 16 days. Error bars represent SEM +/- 1.

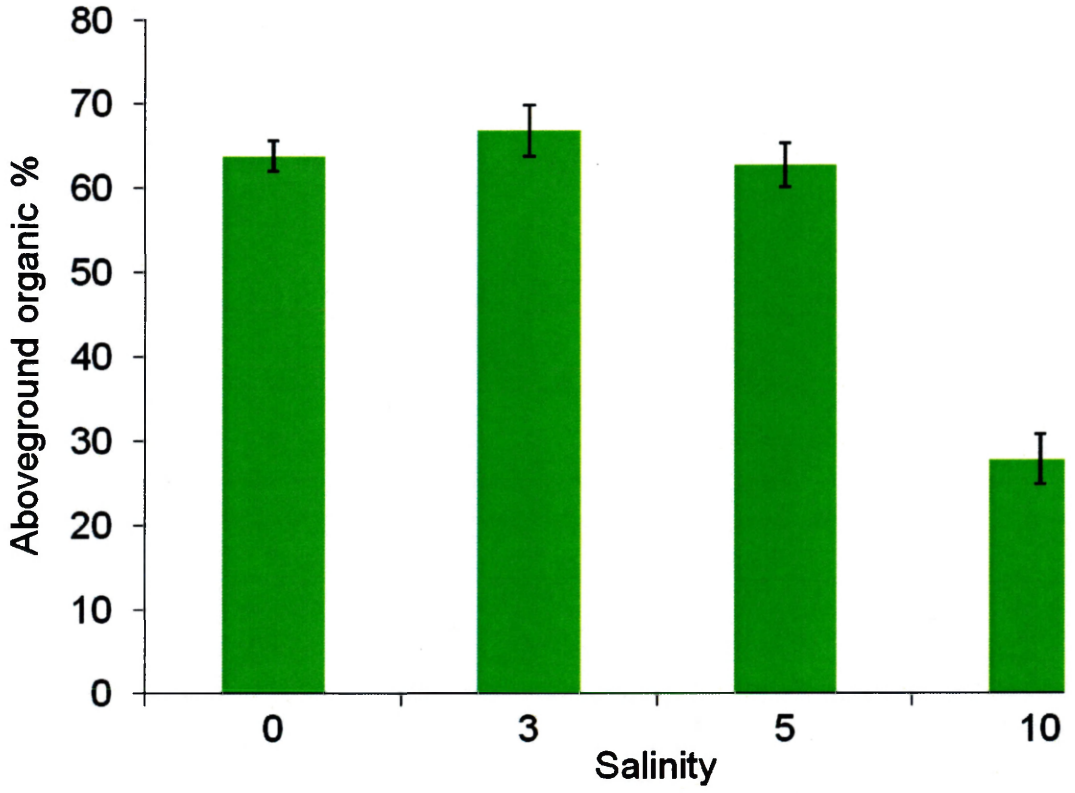

Figure 18. Aboveground organic content of *H. dubia* in each experimental salinity after 16 days. Error bars represent SEM +/- 1.

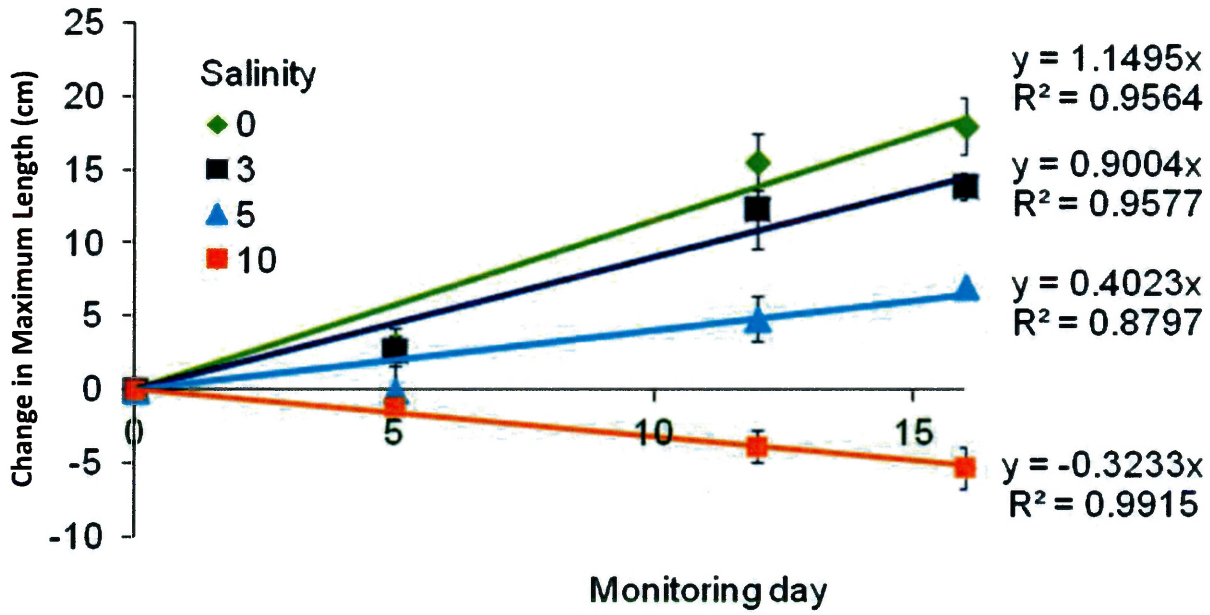

Figure 19. Average maximum branch length of *H. dubia* in each experimental salinity over time. Error bars represent SEM +/- 1.

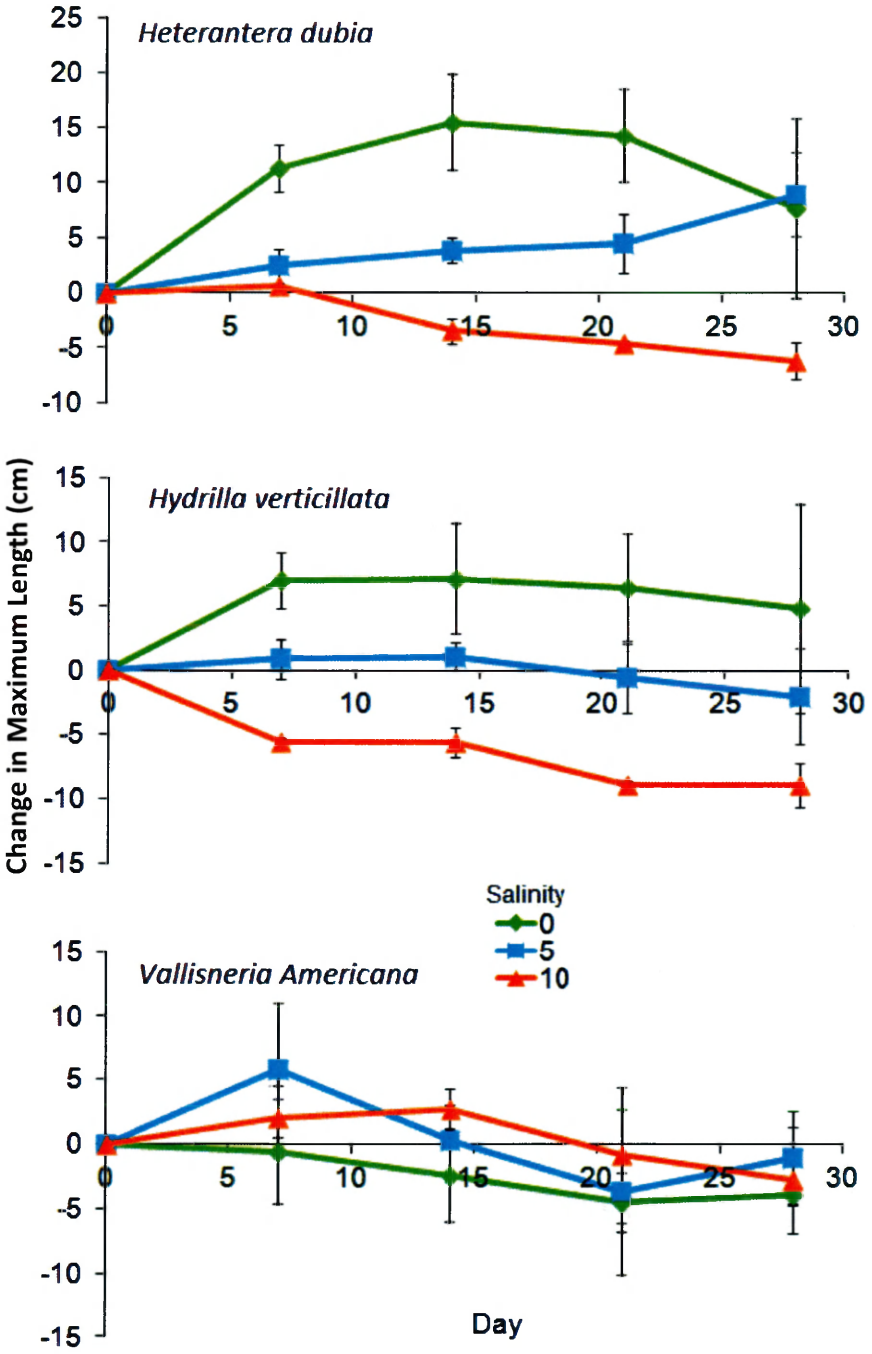

Figure 20. Maximum lengths of each species in various salinities. Plants were exposed to designated salinities on days 0 to 7 and days 14 to 21 and returned to 0 PSU at other times. Error bars represent SEM +/- 1.

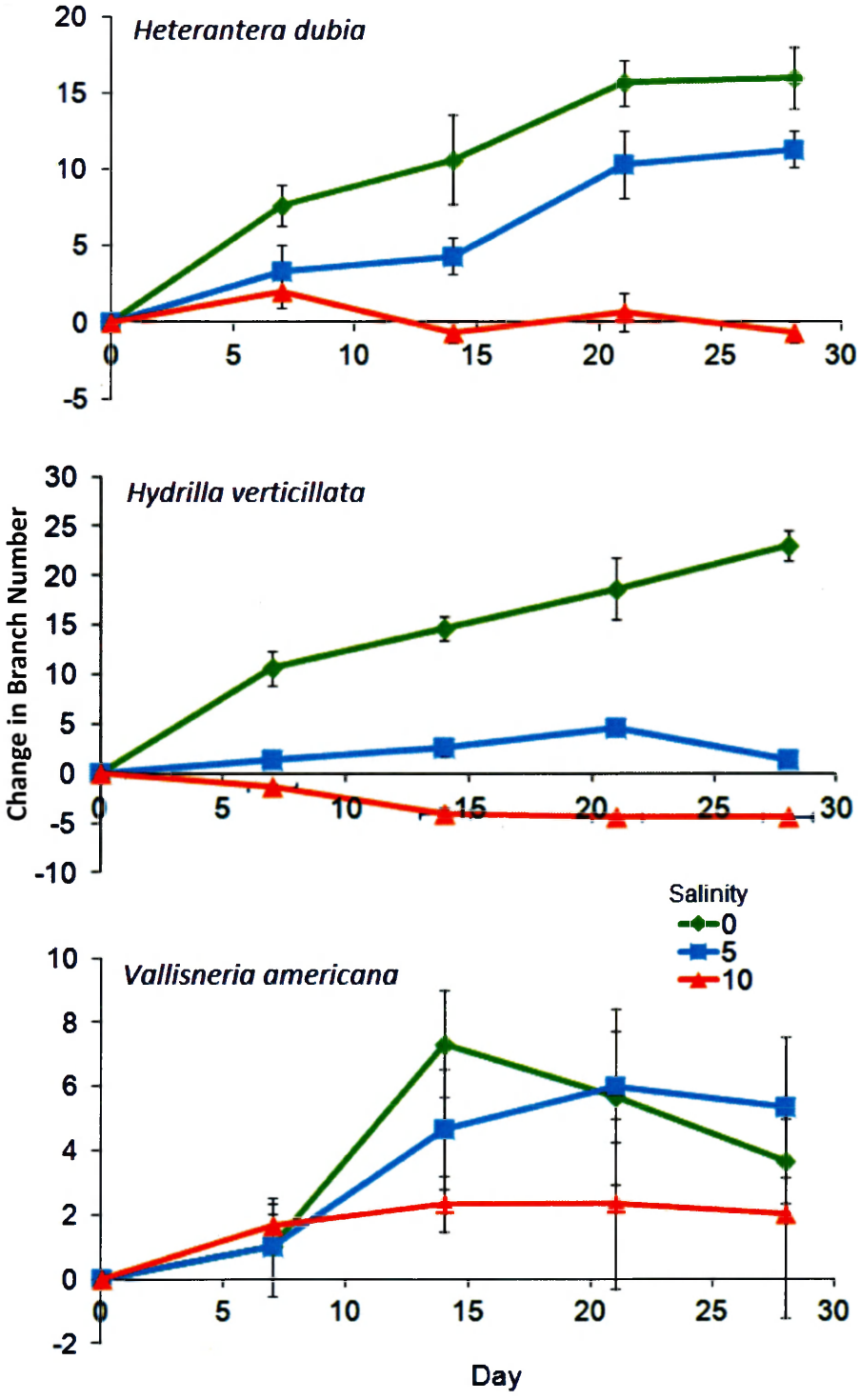

Figure 21. Branch number of each species in various salinities. Plants were exposed to designated salinities on days 0 to 7 and days 14 to 21 and returned to 0 PSU at other times. Error bars represent SEM +/- 1.

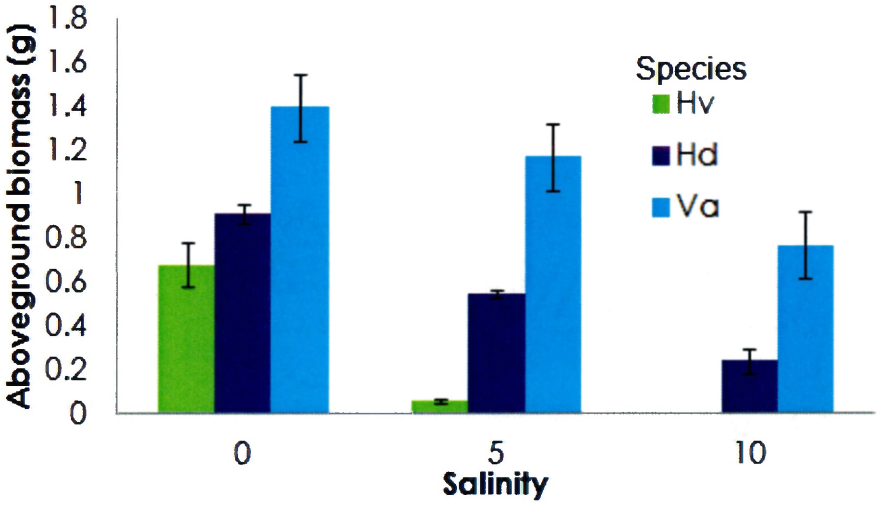

Figure 22. Aboveground biomass for each species after two seven-day salinity pulses. Error bars represent SEM +/- 1.

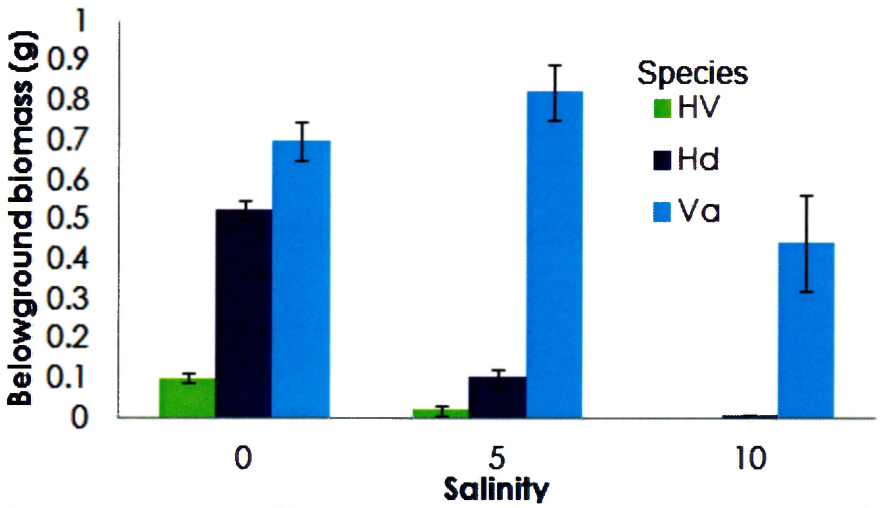

Figure 23. Belowground biomass for each species after two seven-day salinity pulses. Error bars represent SEM +/- 1

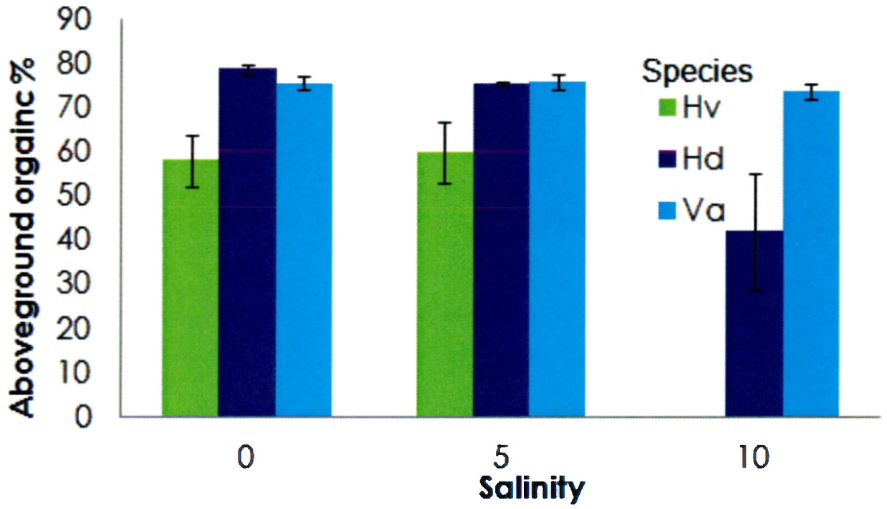

Figure 24. Aboveground organic content for each species after two seven-day salinity pulses. Error bars represent SEM +/- 1.

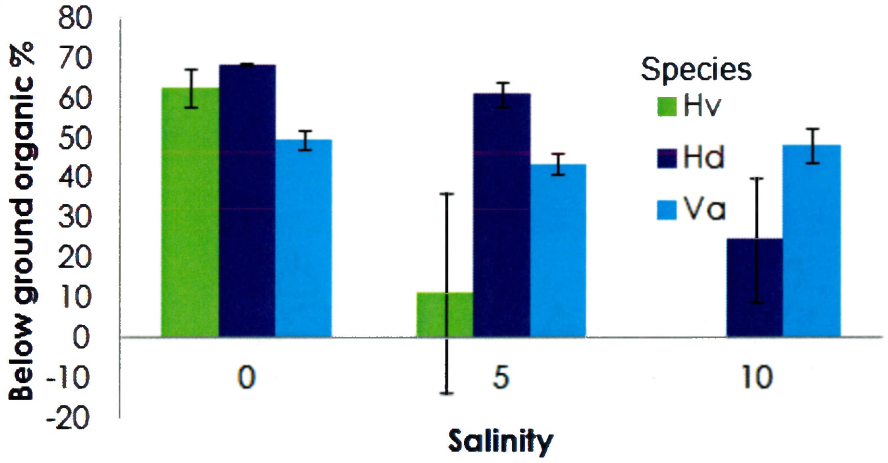

Figure 25. Belowground organic content for each species after two seven-day salinity pulses. Error bars represent SEM +/- 1.

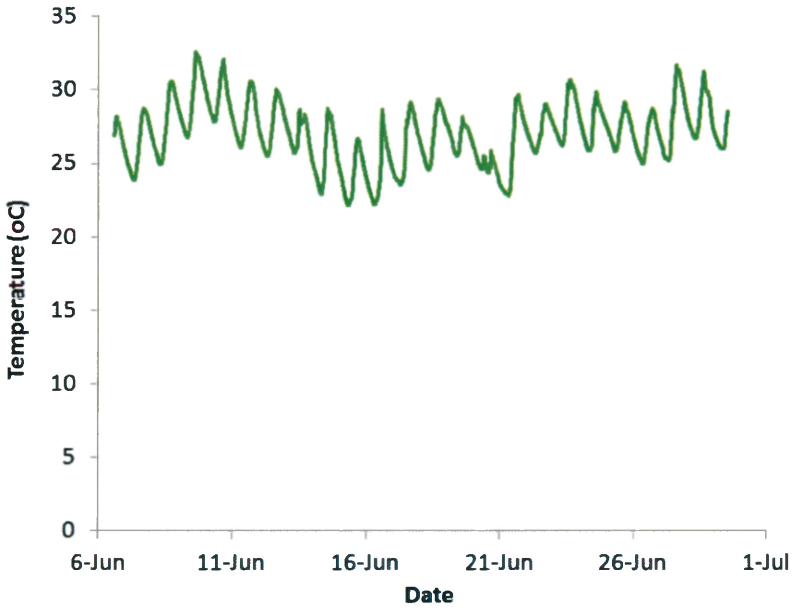

Figure 26. Temperature change over the experiment.

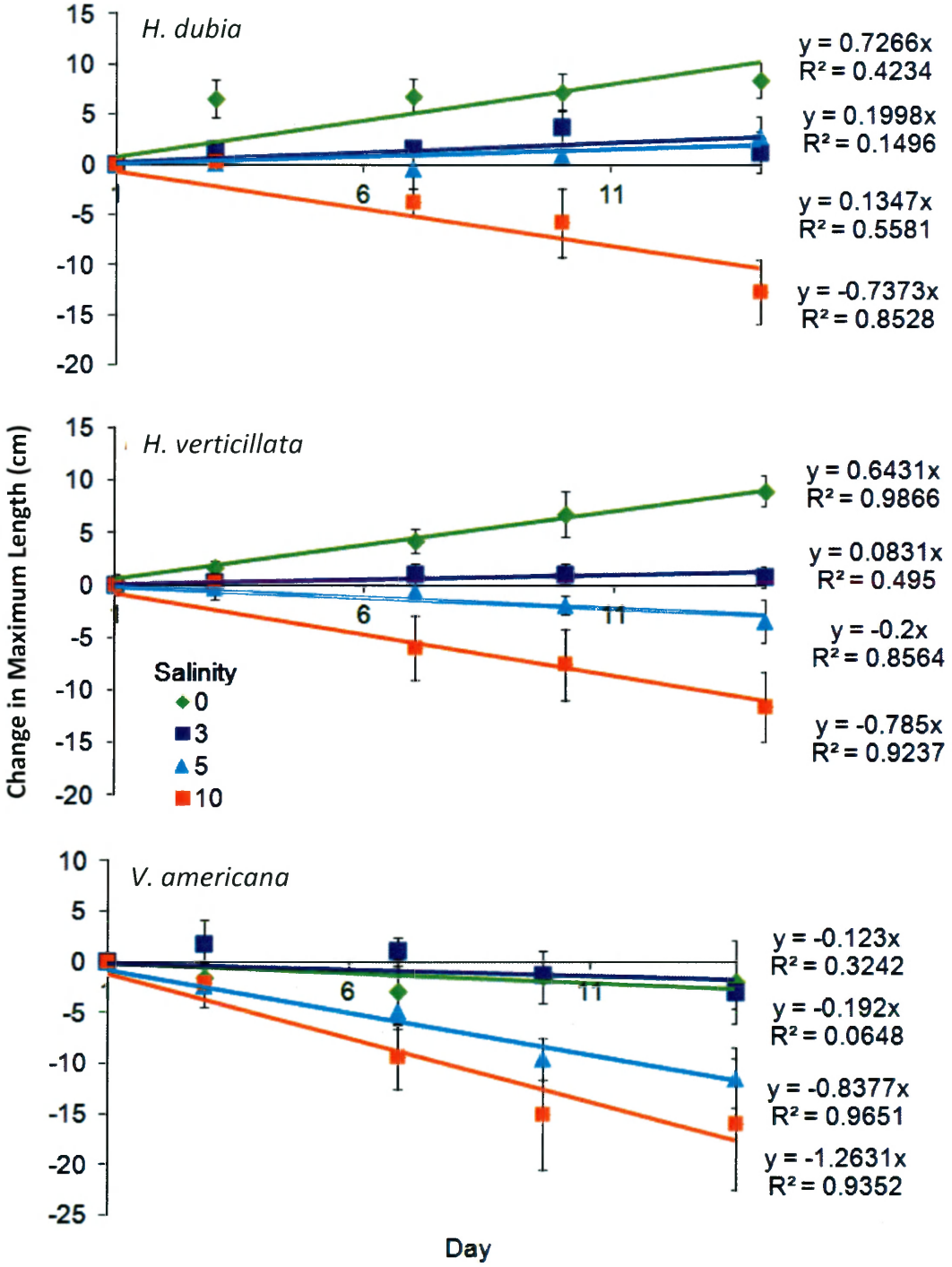

Figure 27. Change in maximum lengths for each species in various salinities over the course of the experiment. Plants were exposed to designated salinities on days 0 to 2 and days 7 to 9 and returned to 0 PSU at other times. Error bars represent SEM +/- 1.

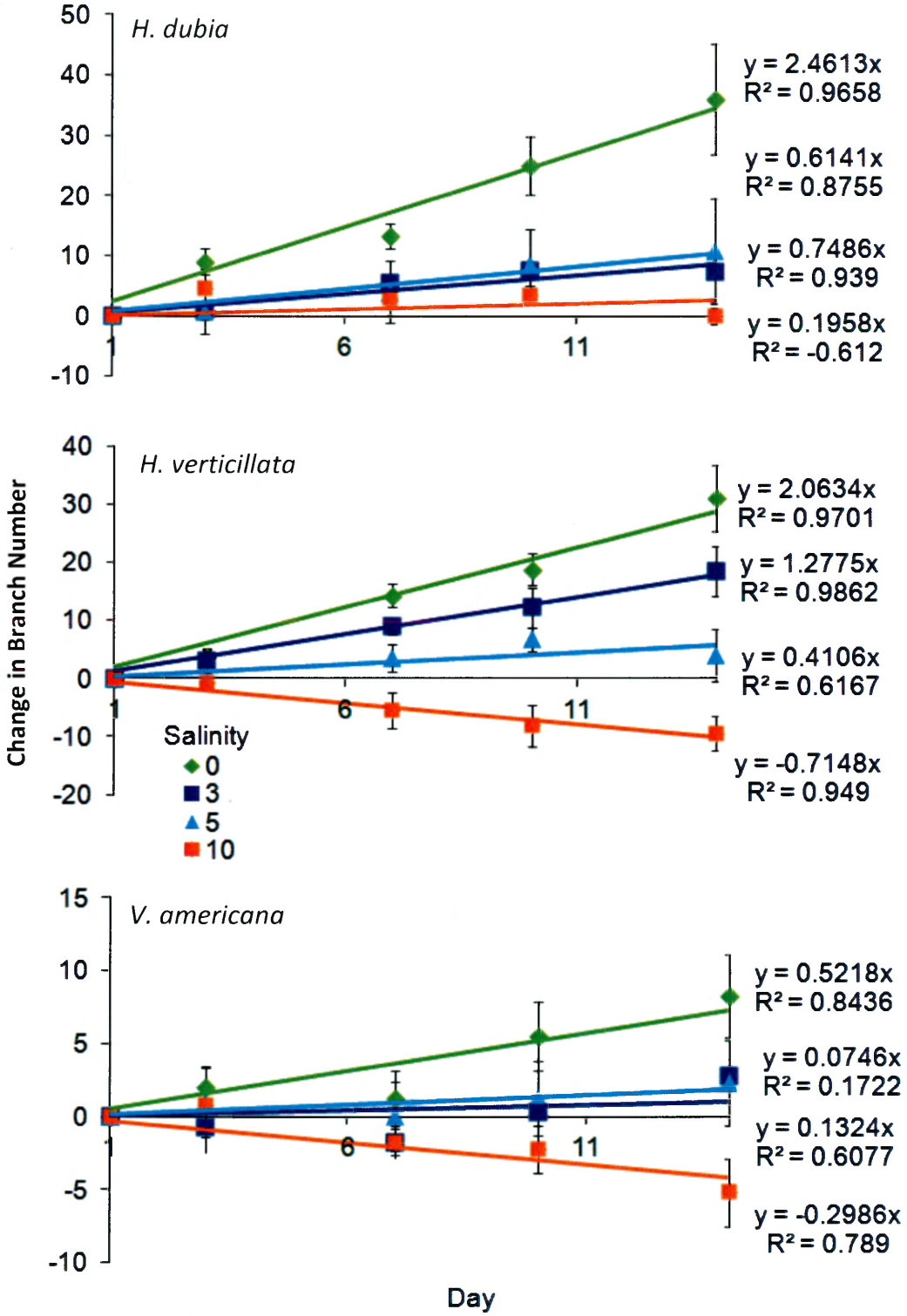

Figure 28. Change in branch number for each species in various salinities over the course of the experiment. Plants were exposed to designated salinities on days 0 to 2 and days 7 to 9 and returned to 0 PSU at other times. Error bars represent SEM +/- 1.

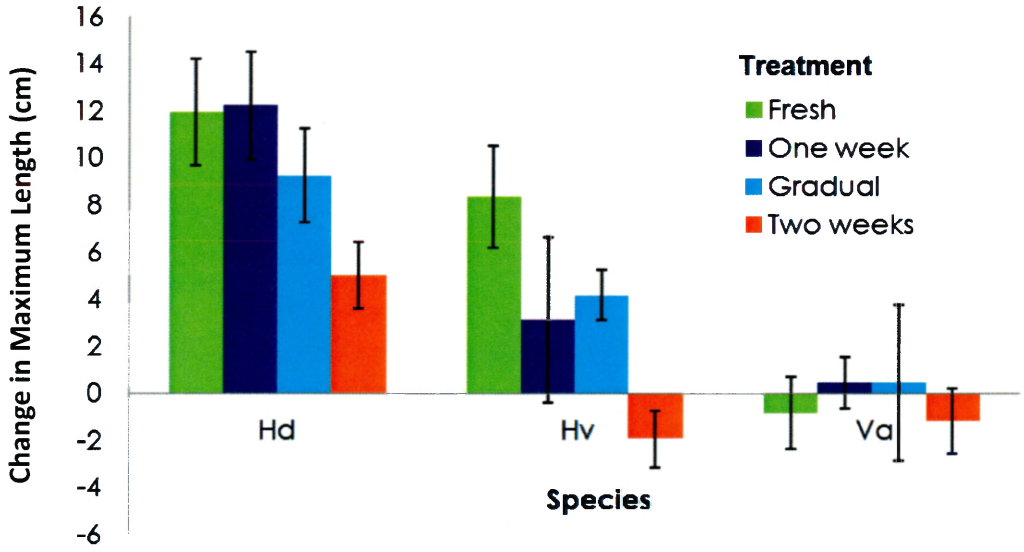

Figure 29. Change in maximum branch or blade length for each species with various salinity adjustment patterens. Error bars represent SEM +/- 1.

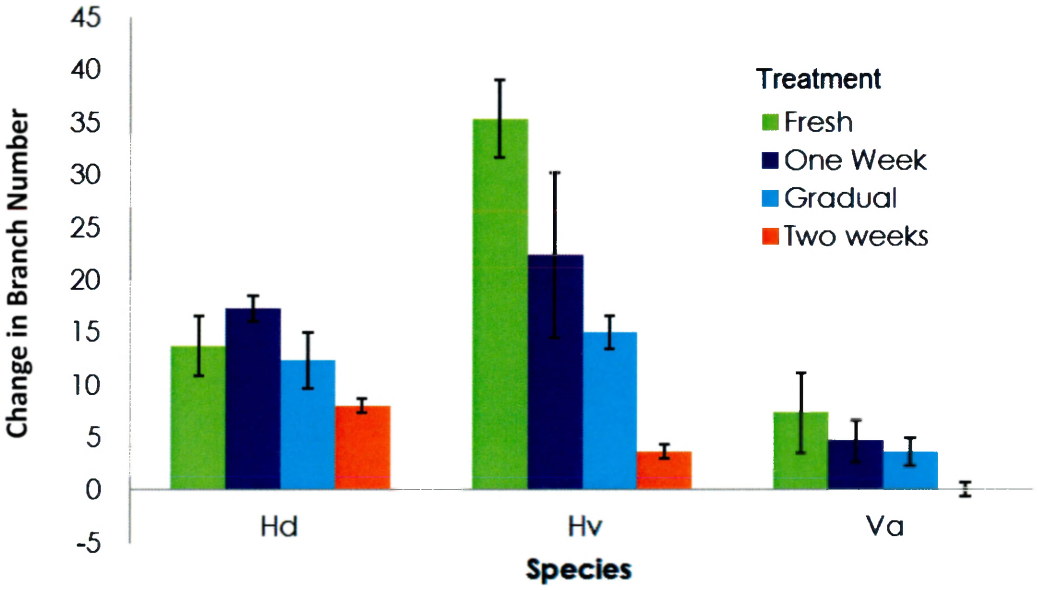

Figure 30. Change in branch number for each species with various patterns of salinity adjustment. Error bars represent SEM +/- 1.

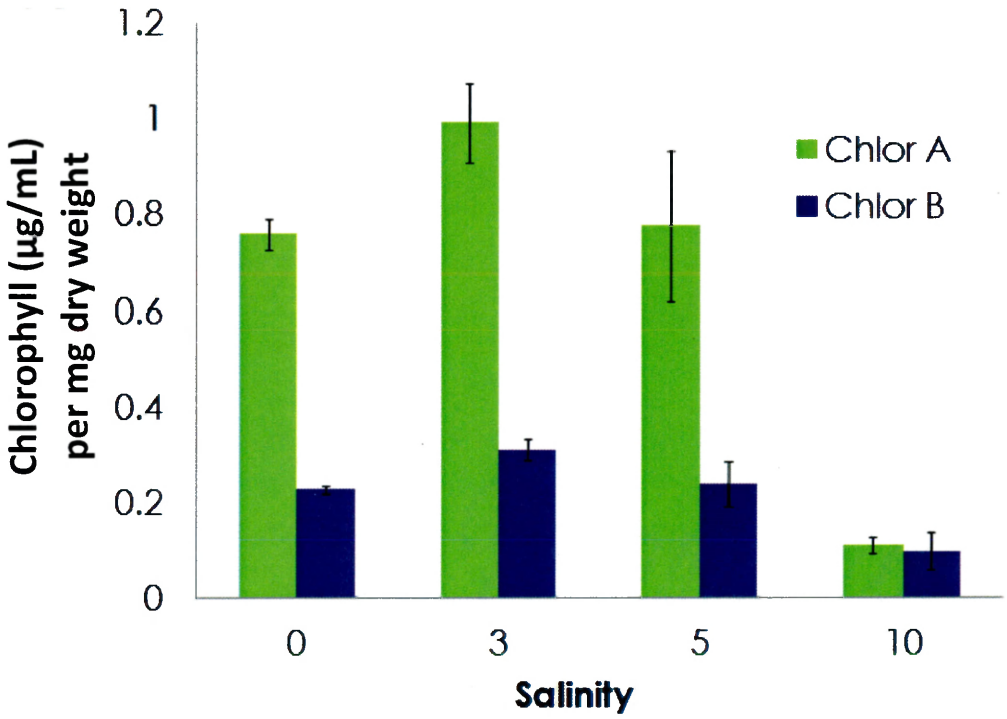

Figure 31. Chlorophyll a and b content of *H. dubia* exposed to various salinities. Error bars represent SEM +/-1.

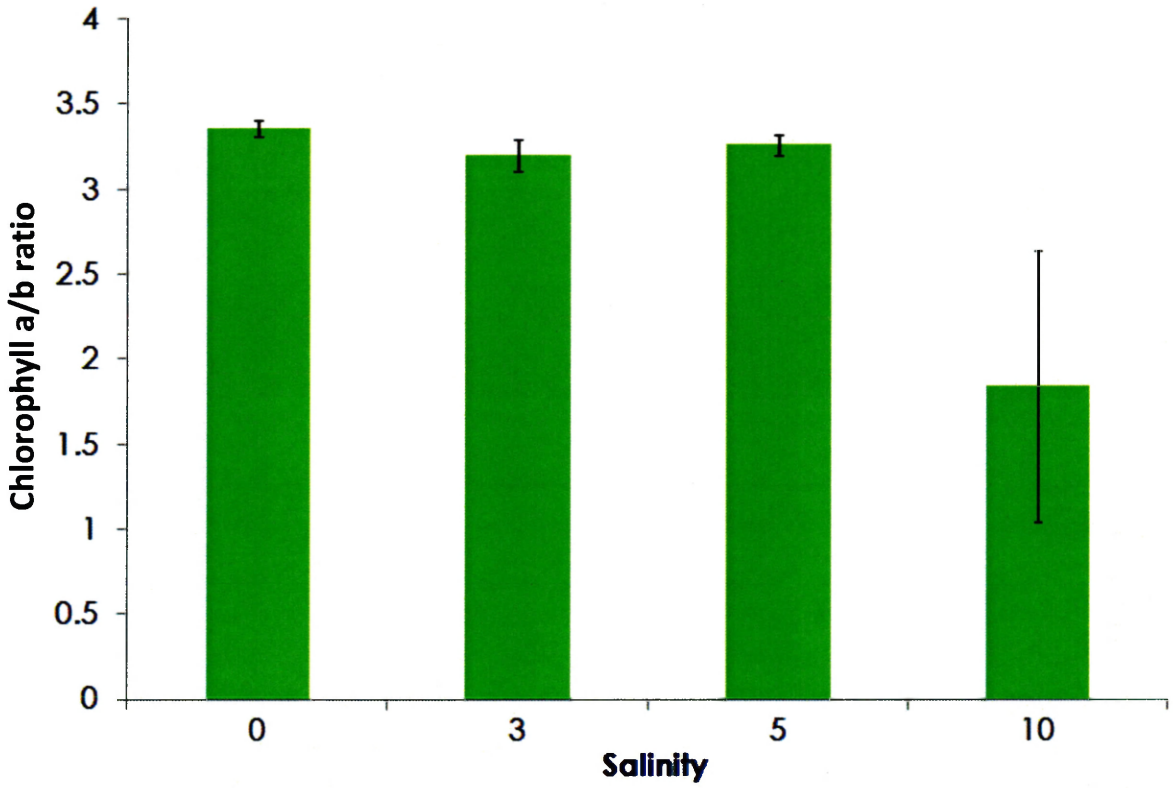

Figure 32. Chlorophyll a/b ratio of *H. dubia* exposed various salinities. Error bars represent SEM +/-1.

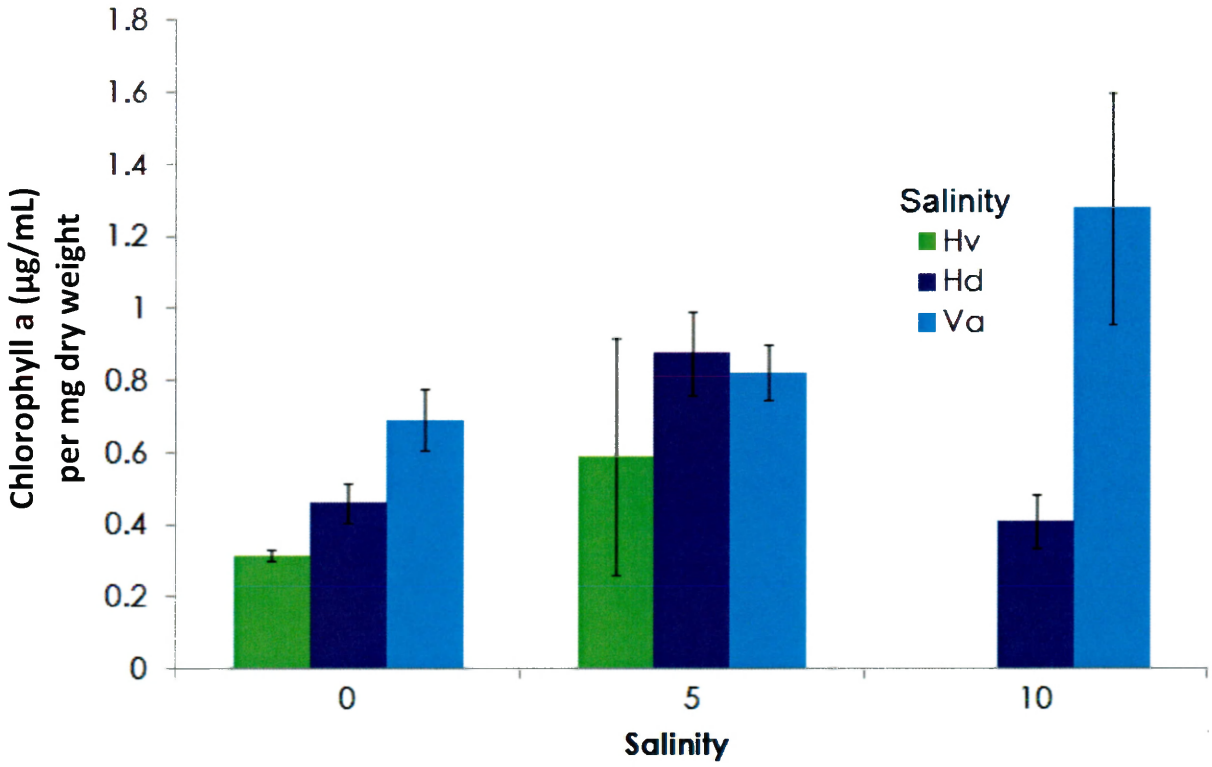

Figure 33. Chlorophyll a content of plants exposed to various salinities. Error bars represent SEM +/-1.

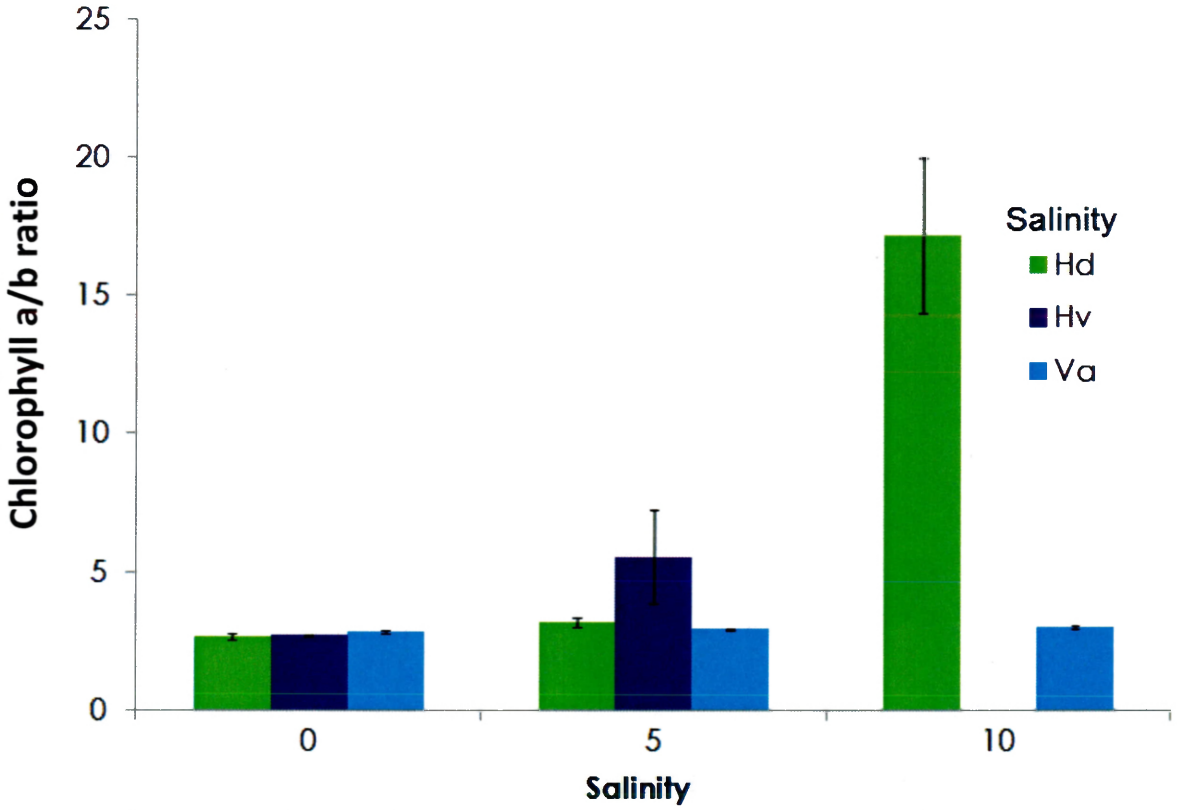

Figure 34. Chlorophyll a/b ratio of plants exposed to various salinities. Error bars represent SEM +/-1.

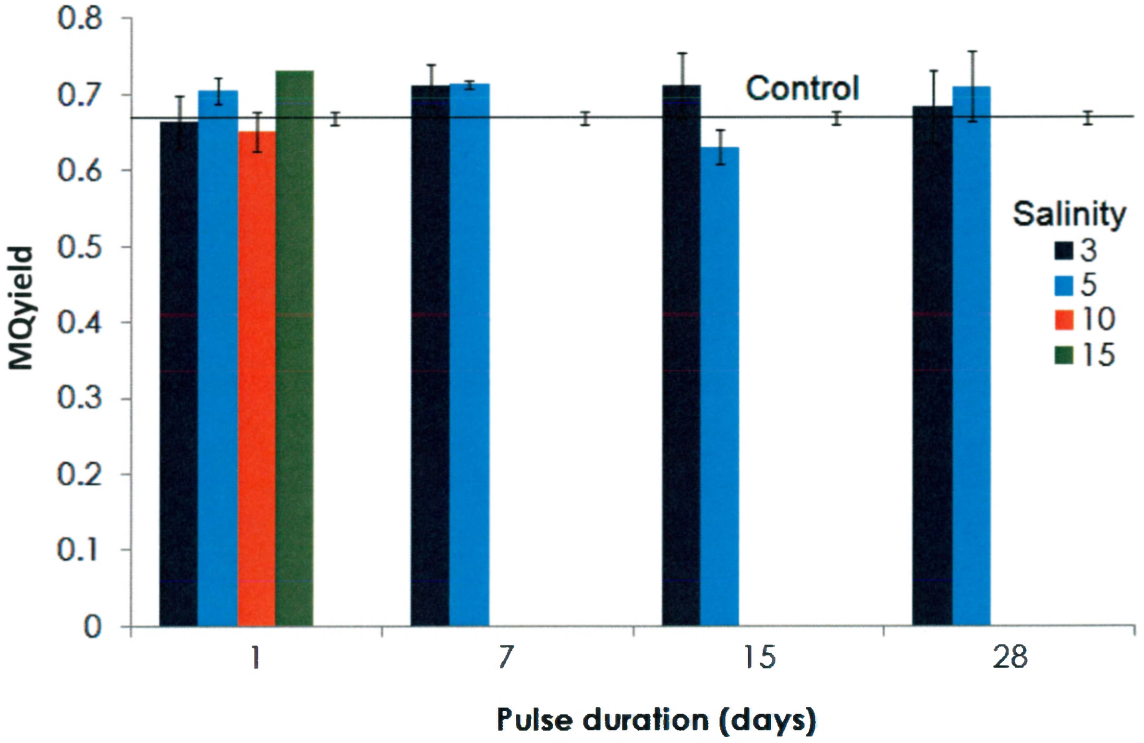

Figure 35. Maximum quantum yield after 28 days of plants exposed to various durations and intensities of salinity. Error bars represent SEM +/-1. The horizontal line shows growth of plants in 0 PSU.

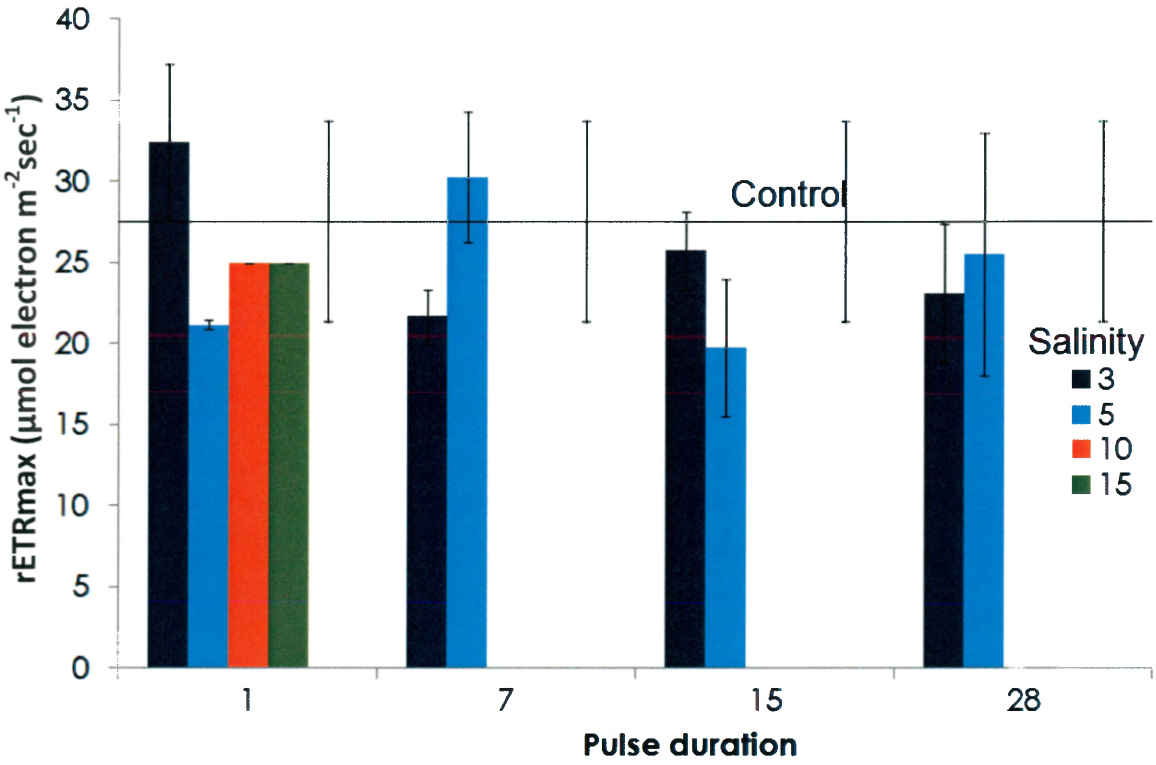

Figure 36. Maximum electron transport rate after 28 days of plants exposed to various durations and intensities of salinity. Error bars represent SEM +/-1. The horizontal line shows growth of plants in 0 PSU.

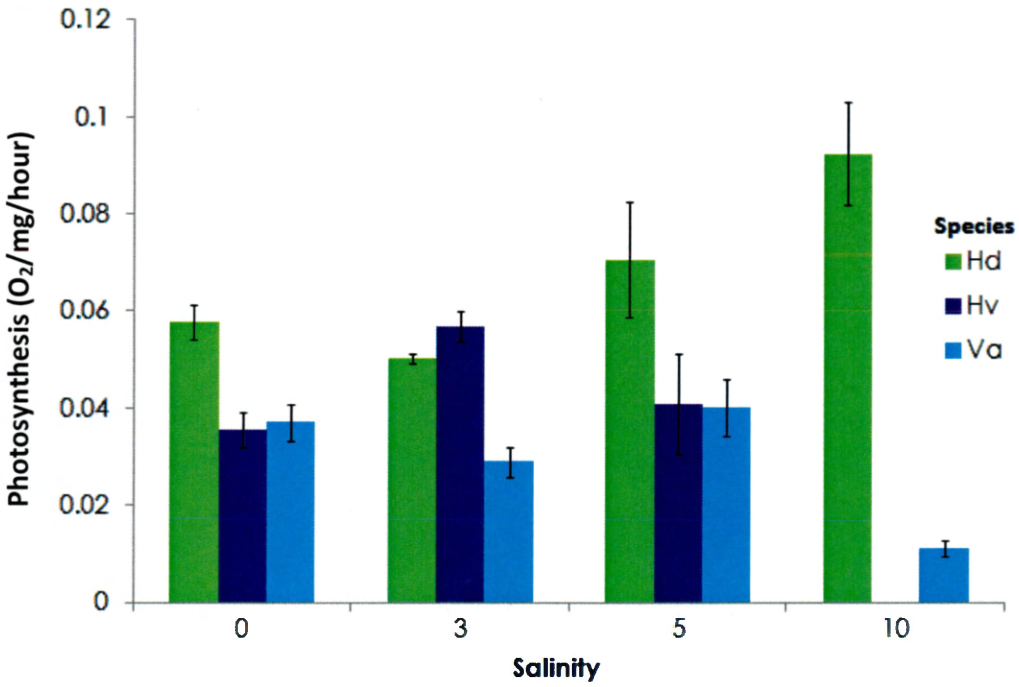

Figure 37. Oxygen metabolism in the light for plants exposed to two 2-day pulses of salinity. Error bars represent SEM +/-1.