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ORIGINAL PAPER

The effect of pulsed versus gradual salinity reduction on the physiology and survival of *Halophila johnsonii* Eiseman

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Abstract Plants of *Halophila johnsonii* Eiseman were exposed, in mesocosms, to either pulsed hyposalinity treatments of 30, 15, 10, and 8 or gradual salinity reductions of two every 2 days. When salinity was pulsed, survivorship (>80 %) and maximum quantum yields (>0.7) were high in the 30 and 15 salinity treatments, but both declined in the 10 and 8 salinity treatments. Leaf osmolality declined with respect to salinity treatment, but the difference between leaf and media osmolality remained relatively constant (675 ± 177 mmol kg^{-1}). In contrast, when salinity was gradually reduced, survivorship remained high from salinities of 30 to 4, and maximum quantum yields remained high from salinities of 30 to 6. Leaf osmolality declined linearly with respect to media osmolality and, similar to the pulsed treatments, the difference between leaf and media osmolality remained relatively constant from salinities of 30 to 2 (638 ± 161 mmol kg^{-1}). Trolox equivalent antioxidant capacity declined over time in both pulsed and gradual salinity reduction. The results indicate that *H. johnsonii* is more tolerant of hyposalinity than has previously been reported and that gradually reducing salinity extended its low-salinity tolerance threshold by approximately a salinity of 10.

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Introduction

Halophila johnsonii is one of the smallest seagrass species with a canopy height of only 2–5 cm and considerably less biomass than other seagrass genera (Kenworthy 1997; Dean and Durako 2007; Virnstein et al. 2009). It also has limited reproductive capabilities, as only female flowers have been observed (Eiseman and McMillan 1980; Kenworthy 1997). *H. johnsonii* has a restricted geographical distribution, occupying around 200 km of coastal estuaries and lagoons from Sebastian Inlet (27°51′N, 80°27′W) in the northern Indian River Lagoon (IRL) to North Biscayne Bay (25°45′N, 80°07′W), Florida (Eiseman and McMillan 1980; Virnstein and Hall 2009). Within its range, *H. johnsonii* has a patchy and disjunct occurrence, often appearing and disappearing from specific locations from one season to the next in so-called pulsating patches (Kenworthy 1997; Dean and Durako 2007; Virnstein et al. 2009). Because of its small carbon store, limited reproductive capabilities, and restricted geographical distribution, *Halophila johnsonii* is particularly susceptible to environmental disturbances and is the only marine plant listed as threatened under the US Endangered Species Act (63 FR 49035, Kenworthy 1997).

Halophila johnsonii is sensitive to changes in water quality such as turbidity, chromophoric dissolved organic matter (CDOM), temperature, and salinity (Kahn and Durako 2008). However, recent studies indicate that prolonged hyposalinity may be the most serious threat to *H. johnsonii* (Torquemada et al. 2005; Kahn and Durako 2008). Optimal growth and survival of *H. johnsonii* have been observed at a salinity of 30, decreasing under both hyper- and hyposalinities (Torquemada et al. 2005). Decreased survivorship and photosynthetic rates were observed when *H. johnsonii* was immediately exposed to

salinities of 10 and 0 (Torquemada et al. 2005), and 100 % mortality of *H. johnsonii* has been observed after 10 days in a salinity of 10 (Kahn and Durako 2008). However, *H. johnsonii* can survive for several weeks at salinities of 20 and 15 (Gavin 2010).

Because of the closed nature and restricted circulation of lagoons and estuaries, both natural and anthropogenic disturbances can drastically alter their salinities, placing submerged plants under osmotic stress. Severe storm events like hurricanes create pulsed fresh water inputs, which have severely impacted seagrass beds, including *Halophila* species (Ralph 1998; Steward et al. 2006). It is predicted that with climate change, there will be more frequent and severe rainfall events in tropical regions that will lead to hypo-osmotic conditions (Steward et al. 2006). In 2004, four major hurricanes (Charley, Frances, Ivan, and Jeanne) directly impacted the east coast of Florida and generated direct rainfall and runoff conditions that decreased water quality over much of the IRL (Steward et al. 2006). The large input of freshwater decreased salinities to $\leq$15, which led to significant declines in seagrass densities and changes in species composition (Steward et al. 2006).

Freshwater inputs from tropical storms are a natural process. However, humans have greatly modified the watersheds in Florida, primarily for flood control, through a series of canals, levees, and water control structures, diverting a larger volume of freshwater into the habitats, where *H. johnsonii* resides (IRL CCMP 1996). Freshwater inputs often occur in pulses associated with flood-control canal releases, rapidly decreasing the salinity in localized to lagoonal-scale regions. The decrease in seagrass densities following the 2004 hurricanes highlights the negative effects of pulsed and prolonged hyposalinity on seagrass survival (Steward et al. 2006). Because *H. johnsonii* is negatively affected by hyposalinity, it is important to determine the impact of freshwater releases via watershed management on its physiology and survival. This will allow the implementation of adaptive water management practices to ensure this species' continued existence.

Previous experiments have placed *H. johnsonii* directly in the treatment salinity, in what is termed a "pulsed" event (Torquemada et al. 2005; Kahn and Durako 2008; Gavin 2010). However, when gradually exposed to a change in salinity, seagrasses are able to osmoregulate (Jagels 1983) and they tolerate a salinity of 10 higher when incrementally exposed to hypersaline conditions versus exposure to a rapid pulsed event (Koch et al. 2007). In this study, *H. johnsonii* was subjected to hyposaline conditions under both pulsed and gradual salinity reductions to determine (1) its minimum salinity tolerance and (2) whether its low-salinity tolerance threshold could be further extended if salinity was gradually reduced rather than pulsed. It was hypothesized that by gradually decreasing salinity, *H. johnsonii* would have sufficient time to osmoregulate and therefore tolerate lower levels of salinity than if exposed to a pulsed salinity decrease.

Materials and methods

Collection site

Halophila johnsonii transplants were collected on May 18, 2010 from Munyon Island, Lake Worth, Florida (26°49′N, 80°02′W), using a 9 × 9 cm sod plugger. This sampling location is located near the middle of the geographic range of this species. Transplants consisted of rhizome segments with >4 leaf pairs and associated sediments, which were placed in 9 × 9 × 8 cm plastic pots. Transplants were transported in seawater-filled coolers to the University of North Carolina Wilmington, Center for Marine Science (UNCW/CMS). Upon arrival (within 24 h of collection), the plant pots were transferred to 40-L aquaria located within fiberglass vaults supplied with flow-through seawater, which was kept at near-ambient temperatures (23–29 °C). The seawater-supplied vaults acted as water baths for temperature regulation. There were four fiberglass vaults and each contained four aquaria (16 aquaria total). The aquaria were the experimental units and each pot, which contained 1 to several rhizome segments, was treated as an individual. Plants were allowed to acclimate to a control salinity of 30 for 10 days.

Experimental design: pulsed salinity reduction

In the first 3 vaults, after the 10-day acclimation, three aquaria were held at a salinity of 30, while the salinity of the other nine aquaria was immediately decreased to salinities of 15, 10, or 8. Replicate aquaria (treatment $n = 3$) were staggered in position to offset any differences associated with location within the fiberglass vaults. Controls were comprised of three staggered aquaria held at a salinity of 30 in the first 3 vaults, plus the gradual salinity control in the fourth vault ($n = 4$, see below). Physiological measurements of maximum quantum yield (pre-dawn), leaf osmolality, and total antioxidant activity, along with salinity and plant survivorship, were obtained daily for the first week, once every 2 days during the second week, and once per week for the next 2 weeks. Within treatments, each plant pot was assigned a number (1–15); plant pots were randomly sampled using a random numbers table in Excel©. Salinity was checked daily with a conductivity meter (YSI Model 80) and adjusted as needed to maintain treatment level (±0.2). Instant Ocean© salts and deionized (DI) water were used for salinity adjustments. Total

survivorship was calculated as the sum of the three replicate aquaria for each treatment.

Experimental design: gradual salinity reduction

After acclimation, the salinity of three 40-L aquaria in the fourth fiberglass vault was decreased by two every 2 days. The fourth aquarium was held at a salinity of 30 as the fourth control ($n = 4$). This gradual acclimation regime was chosen because it mimics natural field conditions and allowed for the achievement of target salinity levels in a reasonable amount of time. Previous studies have used similar adjustment rates to test the salinity tolerance of seagrasses (Kahn and Durako 2005; Koch et al. 2007). Maximum quantum yield, osmolality, and total antioxidant capacity, along with salinity and plant survivorship, were measured 24 h after salinity adjustment. Random sampling was accomplished in the same way as the "pulsed" treatments. Salinity was adjusted by adding Instant Ocean© salts or DI water.

Maximum quantum yield: F_v/F_m

Measurements of maximum quantum yield were taken using a pulse amplitude modulated (PAM) fluorometer (Mini-PAM, Walz GmbH). Maximum quantum yield is a non-destructive and non-invasive method to measure the amount of light absorbed by a plant that is directed for photosynthesis, specifically the efficiency of photosystem II, and it is a common measure of stress in seagrasses (Ralph 1998, 1999; Durako et al. 2003; Biber 2008). The leaf is exposed to a pulse of light during which two fluorescence measurements are made: one before (minimum fluorescence, F_o) and one during the burst of light (maximum fluorescence, F_m). Maximum quantum yields are calculated as F_v/F_m, where $F_v = F_m - F_o$. All measurements were taken on the second intact leaf pair back from the rhizome apical meristem. Measurements were taken at pre-dawn on dark-acclimated leaves to represent the maximum potential of photosystem II as all reaction centers are open and primary electron acceptors able to be oxidized (Murphy et al. 2003). A leaf clip was used to ensure equal distance and geometry between the fiber optic tip and leaf tissue. The fluorescence signal was obtained from approximately the middle of the adaxial side of the leaf. Maximum quantum yields nearing zero were indicative of mortality. Additionally, mortality was indicated by the absence of leaves in a plant pot.

Leaf osmolality

Leaf osmolality was measured using a Wescor VAPRO Vapor Pressure Osmometer 5520© following the protocols described by Murphy et al. (2003), Kahn and Durako (2006), and Koch et al. (2007). The osmometer measures the total concentration of dissolved particles using a vapor-point depression. When measuring solid tissue samples, a time delay had to be used to allow for vapor pressure and temperature to equilibrate. To determine the appropriate time delay for *H. johnsonii* leaf tissue, initial time delay readings were taken every 2 min for 30 min. After 10 min, there was no significant change in readings ($F_{1,32} = 0.86$, $p = 0.58$, data not shown).

To measure leaf osmolality, one mature leaf from each replicate aquarium was cut under water and submerged in a 15-mL centrifuge tube. A 10-mm section of leaf tissue was cut from the leaf, blotted with a Kimwipe to remove media liquid, and placed on the osmometer sample holder. Readings were taken after 10 min. Osmolality was also measured on 10 μL samples of the treatment media to allow for comparison of leaf tissue to treatment salinity.

Total antioxidant activity

Antioxidant activity of leaf cells was measured using the trolox equivalent antioxidant capacity (TEAC) assay (Sigma-Aldrich). The assay uses different concentrations of Trolox (1–10 μM), a vitamin E analog, as a quantitative reference for antioxidant activity (Re et al. 1999). The percent inhibition of absorbance at 734 nm is calculated as a function of decolorization by the $ABTS^{\cdot+}$ radical:

$$\% \text{ inhibition} = (A_{734\text{initial}} - A_{734\text{final}})/A_{734\text{initial}}$$

Halophila johnsonii leaves were extracted in methanol for 24 h in the dark at 7.8 °C. Absorbance of 7 mM ABTS (990 μL) at 734 nm was measured. 10 μL of the leaf methanol extract was added to the ABTS solution, and absorbance was measured 4 min after initial mixing. Decolorization in leaf extracts was compared to a Trolox standard curve (0–10 μM) to determine the level of antioxidant activity (μM Trolox g^{-1} Fresh weight) in *H. johnsonii* leaf tissue.

Statistical analyses

All values are reported as means ± standard deviation. Normality of data was tested using the Shapiro–Wilk goodness-of-fit test. When normality failed or variances were not equal, several transformations were attempted, if transformation failed, nonparametric tests (Kruskal–Wallis ANOVA on ranks) were performed to determine the effects of salinity on physiological measurements. A Friedman repeated measures ANOVA on ranks was used to compare variation in maximum yields among treatments in the pulsed salinity experiment. Two-way fixed factor ANOVAs (factor one salinity and factor two time) were

used to determine the strength of each factor and their combined interaction on leaf osmolality and antioxidant activity in the pulsed salinity treatments. Antioxidant activity data from the pulsed salinity experiment were square-root transformed. Linear regressions and one-way fixed factor ANOVAs were used to determine whether there were significant changes ($p < 0.05$) in each physiological measurement (maximum quantum yield, leaf osmolality, antioxidant activity) over time in the gradual salinity reduction. Student–Newman–Keuls (SNK) pairwise comparison tests were performed when significant treatment effects were detected in ANOVA. Statistical analyses were performed using JMP7® and SigmaPlot 11® software.

Results

Pulsed salinity reduction

In the pulsed salinity treatments, there was high total survivorship (>80 %) of *Halophila johnsonii* in the control (30) and salinity of 15 treatments over the duration of the mesocosm experiment. However, there was a relatively linear decline in survivorship in the 10 and 8 salinities over time. Total survivorship was consistently lowest in the pulsed salinity of 8 treatment (Fig. 1).

Salinity had a significant effect on maximum quantum yield (F_v/F_m) values of *H. johnsonii* ($X^2 = 21.512$, $df = 3$, $p < 0.001$). SNK pairwise comparisons indicated there was no difference in maximum quantum yields between the control (30) and salinity 15 treatments (Fig. 2). However, the maximum quantum yields of individuals in the pulsed salinity treatments of 10 and 8 were significantly lower than the control and salinity 15 treatments (Fig. 2).

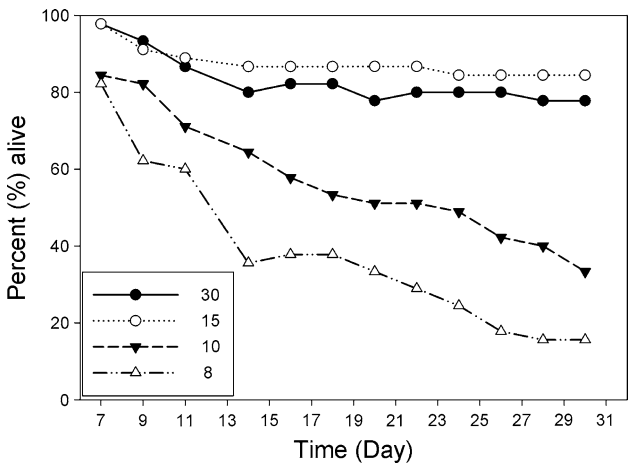

Fig. 1 Total survivorship (percent (%) alive) of *Halophila johnsonii* in each of the four pulsed salinity treatments (30, 15, 10, 8). Measurements of survivorship began on day 7 of the mesocosm experiment and represent the sum of the three replicate aquaria for each treatment

Salinity had a significant effect on leaf tissue osmolality of *H. johnsonii* in the pulsed salinity treatments, but time did not (salinity: $F_{3,104} = 96.24$, $p < 0.001$; time: $F_{12,104} = 0.69$, $p = 0.76$) nor was there a salinity × time effect ($F_{36,104} = 0.96$, $p = 0.54$). There was a direct, linear relationship between leaf and media osmolality (Fig. 3) as the slope of the line regressing leaf tissue against media osmolality does not differ significantly from one ($t_{177} = -0.113$, $p = 0.91$). The difference in osmolality between leaf tissue and media did not vary significantly among the four pulsed salinity treatments ($F_{3,104} = 0.51$, $p = 0.68$) or over time ($F_{12,104} = 0.71$, $p = 0.74$). *Halophila johnsonii* plants in the four pulsed salinity treatments maintained an internal leaf tissue osmolality that was 675 ± 177 mmol kg^{-1} greater than their media.

Antioxidant activity of *Halophila johnsonii* did not differ between pulsed salinity treatments; however, it did decline significantly over time (salinity: $F_{3,66} = 1.63$, $p = 0.19$; time: $F_{7,66} = 16.76$, $p < 0.001$). There was no salinity*time effect ($F_{21,66} = 1.31$, $p = 0.20$). Pooled treatment antioxidant activity of *H. johnsonii* declined significantly by day seven ($F_{7,90} = 14.95$, $p < 0.001$; Fig. 4).

Gradual salinity reduction

When salinity was gradually reduced, there was 100 % survivorship of *Halophila johnsonii* from salinities 30 to 22, and 80 % from salinities 20 to 4 (Fig. 5). Survivorship decreased to 60 % when a salinity of 2 was reached and finally to 0 % at salinity of zero (Fig. 5). In the gradual salinity reduction treatment, maximum quantum yield values of *H. johnsonii* remained high through a salinity of 6 (day 23), after which they began to decline significantly ($H = 37.71$, $df = 15$, $p < 0.01$; Fig. 6). SNK pairwise comparisons indicated that yields at salinities of 4 and 2 were significantly lower than salinity of 6, and salinity of 0 yields was significantly lower than all other salinities.

As salinity was gradually reduced, leaf tissue osmolality also significantly decreased ($F_{15,32} = 22.22$, $p < 0.001$; Fig. 7). There was a direct, linear relationship between leaf versus media osmolality as the slope of the line regressing leaf tissue against media osmolality does not differ significantly from one ($t_{46} = 1.12$, $p = 0.27$; Fig. 7). From salinities 30 to 2, the difference in osmolality between leaf tissue and media did not differ significantly ($F_{1,44} = 1.55$, $p = 0.15$). From salinities 30 to 2, *H. johnsonii* maintained an internal osmolality in their leaf tissue that was 638 ± 161 mmol kg^{-1} greater than their aqueous medium. It was not until freshwater conditions were reached that the difference in osmolality between leaf and media neared zero. Furthermore, the difference in osmolality between

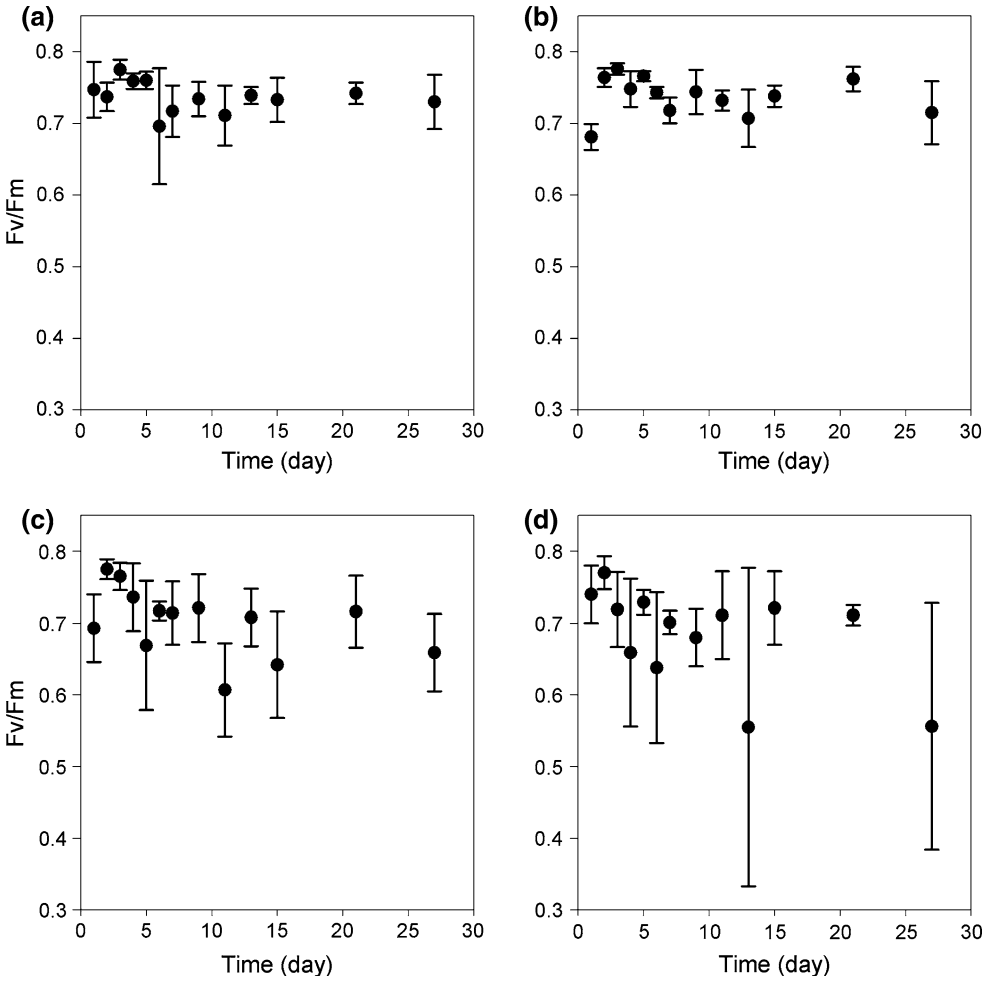

Fig. 2 Maximum quantum yields (*Fv/Fm*) of *Halophila johnsonii* in the four pulsed salinity treatments: **a** 30, **b** 15, **c** 10, **d** 8. The mean ± standard deviation are represented ($n = 3$)

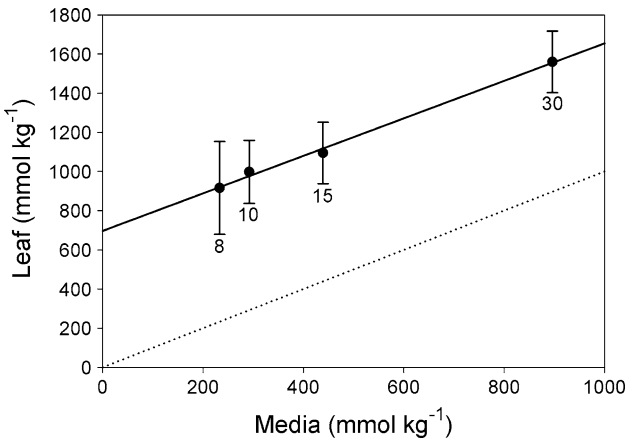

Fig. 3 Osmolality of *Halophila johnsonii* leaf tissue compared to media for each of the four pulsed salinity treatments (30, 15, 10, 8). The slope of the regression line does not differ significantly from one (*dotted line*). The mean ± standard deviation are represented (control $n = 62$, treatment $n = 39$)

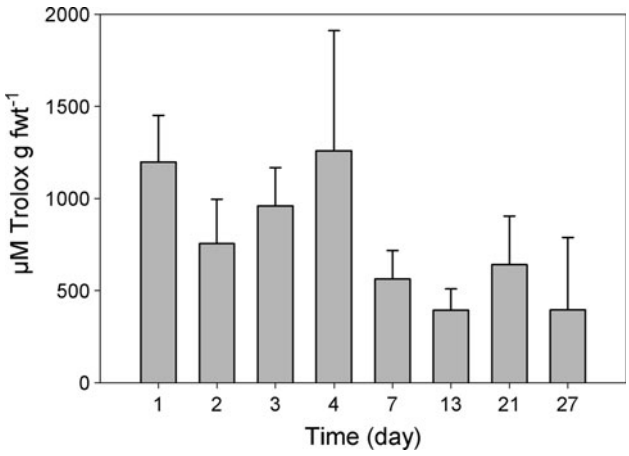

Fig. 4 Total antioxidant activity (µM Trolox g fwt^{-1}) of *Halophila johnsonii* over time using pooled data from the four pulsed salinity treatments. Data were pooled because salinity did not have a significant treatment effect on leaf antioxidant activity. The mean ± standard deviation are represented ($n = 13$)

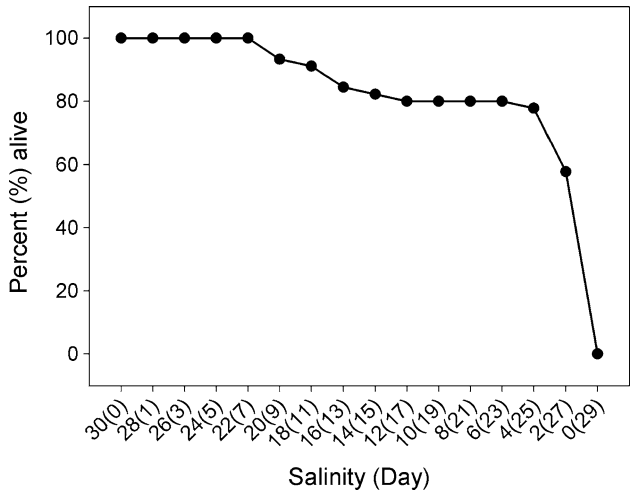

Fig. 5 Total survivorship (percent % alive) of *Halophila johnsonii* in response to gradual salinity reduction

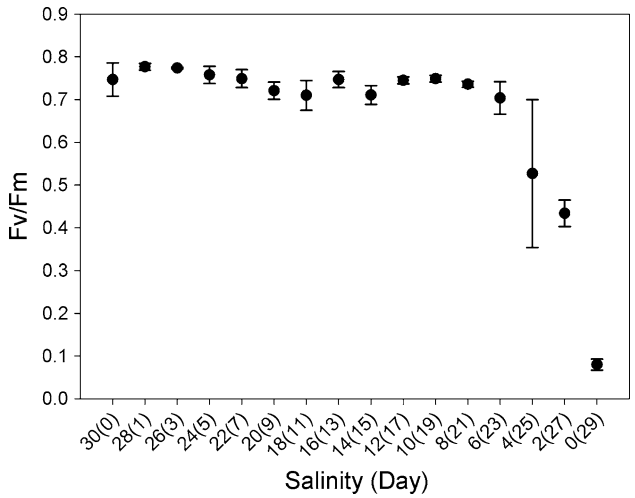

Fig. 6 Maximum quantum yields (*Fv/Fm*) of *Halophila johnsonii* in response to gradual salinity reduction over the 29-day experimental period. The mean ± standard deviation are represented ($n = 3$)

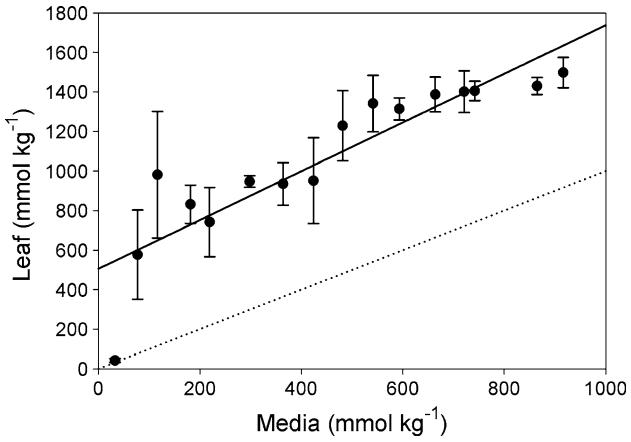

Fig. 7 Osmolality of *Halophila johnsonii* leaf tissue compared to media osmolality in response to gradual salinity reduction. The slope of the line does not differ significantly from one (*dotted line*). The mean ± standard deviation are represented ($n = 3$)

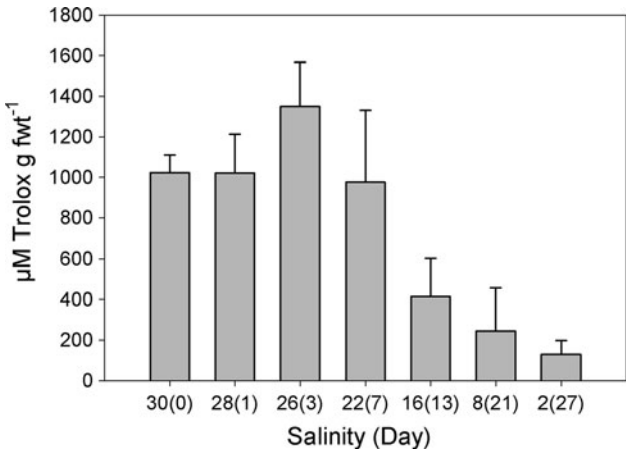

Fig. 8 Antioxidant activity (µM Trolox g fwt^{-1}) of *Halophila johnsonii* plants in response to gradual salinity reduction. The mean ± standard deviation are represented ($n = 3$)

leaf tissue and media did not vary significantly between plants in the pulsed and gradual salinity reductions ($t_{222} = 1.23$, $p = 0.22$).

As salinity was gradually reduced over time, there was a significant decline in antioxidant capacity ($F_{6,14} = 11.90$, $p < 0.001$). SNK pairwise comparisons indicated that antioxidant capacity was significantly reduced by salinity of 16 (day 13) after which it continued to decline but not significantly (Fig. 8).

Discussion

The results of this study indicate that *Halophila johnsonii* is tolerant of hyposalinity and can be considered a euryhaline species. Total survivorship of plants in the control and salinity 15 treatment remained high (~80 %), even after 30 days in the mesocosm experiment (Fig. 1). Although survivorship declined over time, 40 % of plants in the salinity 10 treatment was still alive by day 30 (Fig. 1), a survival value much higher than has previously been reported (Torquemada et al. 2005; Kahn and Durako 2008). Kahn and Durako (2008) reported 0 % survival of *H. johnsonii* plants after 10 days at a salinity of 10. The different responses of *H. johnsonii* to hyposalinity may be due to plant origin and mesocosm design. Individuals in this study were collected from Munyon Island, Florida, where the salinity averages around 32, but can vary from 37 to 15, more typical of an estuarine environment (SFWMD.gov). *Halophila johnsonii* plants from Kahn and Durako (2008) were obtained from Jupiter Inlet, Florida, where salinity usually only varies from 30 to 34, more characteristic of a marine environment (CIRP 2011).

A similar survivorship trend was observed with marine versus estuarine *Halophila ovalis* plants, where marine plants were intolerant to low salinities but estuarine plants thrived (Benjamin et al. 1999). The different salinity tolerances of *H. johnsonii* observed in this mesocosm study, and Kahn and Durako (2008) suggest that these two populations represent different ecophenes.

Another explanation for the high survivorship in the salinity 10 and 8 treatments may be due to mesocosm design. Previous studies on *H. johnsonii* have placed plants directly in treatment salinities within 24 h of collection and subsequently observed high mortality in hyper- and hyposalinity treatments (Torquemada et al. 2005; Kahn and Durako 2008; Gavin 2010). Plants in this study were allowed to acclimate for 10 days to control salinity (30) conditions before the salinity reduction treatments were initiated. This acclimation period allowed time for recovery from transplant shock and for new growth under mesocosm conditions.

Maximum quantum yield values for healthy, non-stressed seagrasses range between 0.7 and 0.8 (Ralph 1999; Durako et al. 2003). Plants in the control and salinity 15 treatment had average yields within this range (Fig. 2). However, yields of plants in the pulsed salinity 10 and 8 treatments were significantly lower, more variable over time and often fell below 0.7 (Fig. 2), indicating that photosynthetic efficiencies were compromised and that plants were stressed at these two lowest salinities. Previous studies have also measured decreases in photosynthetic parameters of *H. johnsonii* when exposed to hyposaline conditions (Torquemada et al. 2005; Kahn and Durako 2008).

There was a direct, linear relationship between leaf osmolality and treatment salinity in the four pulsed salinity treatments (Fig. 3). However, the difference in osmolality between leaf and media did not vary among salinity treatments or over time; *H. johnsonii* remained hyperosmotic to its environment by 675 ± 177 mmol kg^{-1}. The seagrasses, *Thalassia testudinum, Halodule wrightii*, and *Ruppia maritima*, also maintain an internal osmolality that is hyperosmotic to the media and show a linear trend between leaf osmolality and media salinity (Murphy et al. 2003; Kahn and Durako 2006; Koch et al. 2007).

As halophytes, seagrasses undergo both physical and mechanical changes to deal with salt stress; these include managing turgor pressure, synthesizing compatible solutes in the cytosol, and accumulating ions inside of vacuoles such that the plant is hyperosmotic to its environment and water will flux in (Touchette 2007). Under hypo-osmotic stress, plants will release ions from their vacuoles and degrade or metabolize compatible solutes (Bisson and Kirst 1995). In this mesocosm experiment, cytoplasmic non-vacuolar osmolality was measured because osmolality readings were taken on fresh *H. johnsonii* leaves (Murphy et al. 2003; Koch et al. 2007). This suggests that the concentration of compatible solutes in *H. johnsonii* leaf cells decreased under increased hyposalinity. This is consistent with data for *Ruppia maritima* in which the concentration of proline, a primary compatible solute, also decreased under hyposaline conditions (Murphy et al. 2003).

Hyposalinity stress is thought to act in the same way as high temperature stress by destabilizing membranes (Los and Murata 2004). In addition to assisting in maintaining an equal osmotic potential across a cell, compatible solutes also function to stabilize membranes (Bisson and Kirst 1995). The decreased concentration of solutes with increased hyposalinity observed in this study may explain the decrease in maximum quantum yields observed in both the pulsed and the gradual salinity reductions as stability of thylakoid membranes was reduced (Fig. 2 and Fig. 6).

Compatible solute synthesis and ion sequestering are slow processes (hours to days) and function in longer-term salinity adjustments (Murphy et al. 2003; Touchette 2007). To initially respond to salinity changes, seagrasses control water flux by varying the hydraulic conductivity of the plasma membrane and the volumetric elastic modulus properties of the cell wall (Tyerman 1982; Touchette 2007). When exposed to a hyposaline solution, there is an immediate influx of water to cells. The elastic properties of the cell wall will determine how much water fluxes in. Cells with flexible cell walls have a low volumetric elastic modulus and will swell when exposed to a high osmotic flux; cells with rigid cell walls have a high volumetric elastic modulus and prevent water from entering (Touchette 2007). *Halophila ovalis*, a euryhaline seagrass species able to tolerate salinities from 10 to 45, has a high volumetric elastic modulus (Tyerman 1982), but because of a rudimentary cuticle can adjust to hypo-osmotic conditions within 24 h (Ralph 1998). A rigid cell wall may explain why many seagrasses can tolerate hyposalinity better than hypersalinity (Ralph 1998). The leaves of *H. johnsonii* are only two cells thick at the margins, which would allow for rapid exchange between cells and the environment, and subsequent adjustment to salinity change. Further studies measuring the cell wall elasticity or turgor pressure of *H. johnsonii* would provide insight into the mechanisms used to achieve short-term osmotic equilibrium during pulsed salinity changes.

Beginning at around day 13 in the pulsed salinity reduction treatments, we observed that leaves in the lowest pulsed salinity treatments (10 and 8) were often wrinkled along the margins. Rapid leaf senescence and wrinkled leaf margins have been observed in *Halophila ovalis* under hypo-osmotic conditions (Ralph 1998; Benjamin et al. 1999). When a constant stress, such as fresh water influx from hyposalinity, is applied to a cell wall, deformation

occurs (Jagels 1973; Tyerman 1982). Because *Halophila johnsonii* is only two cells thick at the margins, any cell deformation would be observed phenotypically. Wrinkling of *H. johnsonii* leaves at the margins observed in the low salinity aquaria may be the phenotypic result of cell deformation under the constant stress of low salinity.

Total antioxidant capacity in *Halophila johnsonii* did not differ with respect to salinity but it did differ with respect to time. Under stress, free radicals and reactive oxygen species (ROS: O_2^-, OH, H_2O_2) accumulate and damage plant cells (Yang et al. 2006; Touchette 2007). To counteract these harmful products, plants produce antioxidants (xanthophylls, peroxidases, and catalases) whose concentrations increase in order to prevent damage from oxidative stress (Noctor and Foyer 1998, Touchette 2007). Therefore, it was expected that antioxidant capacity would increase under hypo-osmotic stress; however, a decrease in total antioxidant capacity was observed in all treatments, including the control (Fig. 4). One explanation for this measurable decrease may be that under hypo-osmotic stress, antioxidants are consumed at a high rate, which may be faster than they are regenerated (Lu et al. 2006). The TEAC assay depends on the antioxidant's ability to reduce the ABTS* radical (Re et al. 1999), which may be lowered if the antioxidants are being rapidly oxidized by ROS. Future studies examining the concentrations of specific antioxidants and enzymes could elucidate more detailed antioxidant responses. Another explanation for the observed decrease in total antioxidant capacity may be a mesocosm treatment effect on the plants, as antioxidant capacity of plants in the control treatments decreased over the experimental period as well.

Under gradual salinity reduction, there was a tiered stress manifestation response in *H. johnsonii*. Total antioxidant capacity declined first, which became significant by salinity of 16 (Fig. 8); maximum quantum yields ranged between 0.7 and 0.8 from salinities 30 to 6, suggesting that plants did not compromise their photosynthetic efficiencies until a salinity of 4 when yields fell below 0.7 (Fig. 6). Survivorship of plants remained high (80-100 %) over an even broader range of salinities, from 30 to 4, and did not reach 0 % until fresh water conditions (Fig. 5). Lastly, the difference in osmolality between leaf and media did not vary over salinities from 30 to 2 (Fig. 7), suggesting that plants were osmoregulating to maintain a relatively constant level of cell turgor until exposed to freshwater conditions. Since cell turgor is necessary for maintaining cell and plant form, and essential for normal cell functions (Bisson and Kirst 1995; Taiz and Zeiger 2006), it seems logical that this would be the last process to fail under hyposalinity stress.

Halophila johnsonii exposed to a gradual salinity reduction was able to extend its minimum salinity tolerance by almost 10. Gradually reducing the salinity, compared to pulsed salinity reduction, extended the thresholds of all four of the parameters measured (Table 1). When salinity was pulsed, the lowest salinity *H. johnsonii* could tolerate without showing signs of stress was 15. However, when salinity was gradually reduced, plants were able to acclimate and did not begin to show signs of physiological stress or decreased survivorship until salinity decreased below 6 (Table 1). The results of this mesocosm study also indicate that there is a primacy given to osmoregulation and the maintenance of cell turgor, as *H. johnsonii* remained at a relatively constant hyperosmotic condition to its environment, while other physiological processes, like photosynthesis and antioxidant activity, declined. This increased tolerance pattern to hyposalinity is similar to the increased tolerance to hypersalinity exhibited by *T. testudinum*, *H. wrightii*, and *R. maritima* when exposed to gradually increasing salinities versus pulsed salinity increase (Koch et al. 2007). Under hypo-osmotic stress, plants must transport inorganic ions out of their vacuoles and cells and degrade or metabolize organic solutes in order to maintain a relatively constant cell turgor (Bisson and Kirst 1995). However, since these processes are slow (hours to days), gradually reducing the salinity by two every 2 days most likely allowed enough time for these processes to occur and to counteract the influx of fresh water. With climate change, it is anticipated that there will be more storm events that will increase the volume of freshwater naturally entering the estuaries and lagoons of south Florida (Steward et al. 2006). Coupled with human alteration in watersheds, which diverts additional freshwater into these coastal systems, the resulting salinity changes may have significant implications for the species living within these systems. Understanding how reduced salinities and their rates of change affect submerged marine angiosperms, like *H. johnsonii*, will allow for the implementation of adaptive management practices to ensure their survival. The results of this study suggest that *H. johnsonii* is more tolerant of hyposalinity than has previously been

Table 1 The lowest salinity that plants were able to tolerate without showing signs of stress in response to pulsed versus gradual salinity reduction

Measurement	Pulsed	Gradual
F_v/F_m	15	6
Leaf osmolality	8	2
Antioxidant activity	Day 7	Day 13 (salinity 16)
Survivorship	15	4
Overall	15	6

Time is represented for antioxidant activity in the pulsed reduction since salinity did not have a significant effect

reported and that gradual salinity reduction further extends its tolerance threshold. Future investigations should focus on *H. johnsonii's* resilience to salinity changes and its ability to recover from low salinities at both the physical and the cellular levels.

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References

Benjamin KJ, Walker DI, McComb AJ, Kuo J (1999) Structural response of marine and estuarine plants of *Halophila ovalis* (R. Br.) Hook. f. to long-term hyposalinity. Aquat Bot 64:1–17

Biber PD (2008) Determining the salinity-tolerance of Giant Salvinia using chlorophyll fluorescence. Gulf Caribb Res 21:1–6

Bisson MA, Kirst GO (1995) Osmotic acclimation and turgor pressure regulation in algae. Naturwissenschaften 82:461–471

CIRP (2011) Salinity calculations in the Jupiter Inlet—Loxahatchee River System, FL. http://cirp.usace.army.mil. 14 Jan 2011. Accessed 1 April, 2011

Dean RJ, Durako MJ (2007) Carbon sharing through physiological integration in the threatened seagrass *Halophila Johnsonii*. Bull Mar Sci 81:21–35

Durako MJ, Kunzelman JI, Kenworthy WJ, Hammerstrom KK (2003) Depth-related variability in the photobiology of two populations of *Halophila johnsonii* and *Halophila decipiens*. Mar Biol 142:1219–1228

Eiseman NJ, McMillan C (1980) A new species of seagrass, *Halophila johnsonii*, from the atlantic coast of Florida. Aquat Bot 9:15–20

Gavin NM (2010) Localization of flavonoid compounds in *Halophila johnsonii* Eiseman in response to light and salinity variation suggests antioxidant function. Master thesis, University of North Carolina Wilmington

Indian River Lagoon Comprehensive Conservation and Management Plan (IRL CCMP) (1996) Prepared by: the Indian River Lagoon National Estuary Program sponsored by the Saint Johns Water Management District, South Florida Water Management District, and U.S. Environmental Protection Agency. p 378

Jagels R (1973) Studies of a marine grass *Thalassia testudinum* I. ultrastructure of the osmoregulatory leaf cells. Am J Bot 60:1003–1009

Jagels R (1983) Further evidence for osmoregulation in epidermal leaf cells of seagrasses. Am J Bot 70:327–333

Kahn AE, Durako MJ (2005) The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. Bull Mar Sci 77:453–458

Kahn AE, Durako MJ (2006) *Thalassia testudinum* seedling responses to changes in salinity and nitrogen levels. J Exp Mar Biol Ecol 335:1–12

Kahn AE, Durako MJ (2008) Photophysiological responses of *Halophila johnsonii* to experimental hyposaline and hyper-CDOM conditions. J Exp Mar Biol Ecol 367:230–235

Kenworthy WJ (1997) An updated biological status review and summary of the proceedings of a workshop to review the biological status of the seagrass, *Halophila johnsonii* Eiseman. Office of protected resources NMFS, NOAA. 15 Oct. 1997

Koch MS, Schopmeyer SA, Holmer M, Madden CJ, Kyhn-Hansen C (2007) *Thalassia testudinum* response to the interactive stressors hypersalinity, sulfide and hypoxia. Aquat Bot 87:104–110

Los DA, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta Biomembr 1662:142–157

Lu IF, Sung MS, Lee TM (2006) Salinity stress and hydrogen peroxide regulation of antioxidant defense system in *Ulva fasciata*. Mar Biol 150:1–15

Murphy LR, Kinsey ST, Durako MJ (2003) Physiological effects of short-term salinity changes on *Ruppia maritima*. Aquat Bot 75:293–309

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol 49:249–279

Ralph PJ (1998) Photosynthetic responses of *Halophila ovalis* (R. Br.) Hook. *f.* to osmotic stress. J Exp Mar Biol Ecol 227:203–220

Ralph PJ (1999) Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. *f.* to combined environmental stress. Aquat Bot 65:83–96

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

South Florida Water Management District (SFWMD). http://www.sfwmd.gov. Accessed 1 April 2011

Steward JS, Virnstein RW, Lasi MA, Morris LJ, Miller JD, Hall LM, Tweedale WA (2006) The Impacts of the 2004 Hurricanes on Hydrology, Water Quality, and Seagrass in the Central Indian River Lagoon, Florida. Estuar Coast 29:954–965

Taiz L, Zeiger E (2006) Plant physiology, 4th edn. Sinauer Associates, Inc., Sunderland

Torquemada YF, Durako MJ, Lizaso JLS (2005) Effects of salinity and possible interactions with temperature and pH on growth and photosynthesis of *Halophila johnsonii* Eiseman. Mar Biol 148:251–260

Touchette BW (2007) Seagrass-salinity interactions: physiological mechanisms used by submersed marine angiosperms for a life at sea. J Exp Mar Biol Ecol 350:194–215

Tyerman SD (1982) Water relations of the seagrasses stationary volumetric elastic modulus and osmotic pressure of the leaf cells of *Halophila ovalis*, *Zostera capricorni* and *Posidonia australis*. Plant Physiol 69:957–965

Virnstein RW, Hall LM (2009) Northern range extension of the seagrasses *Halophila johnsonii* and *Halophila decipiens* along the east coast of Florida, USA. Aquat Bot 90:89–92

Virnstein RW, Hayek L-AC, Morris LJ (2009) Pulsating patches: a model for the spatial and temporal dynamics of the threatened seagrass *Halophila johnsonii*. Mar Ecol Prog Ser 385:97–109

Yang Y, Jiang DA, Xu HX, Yan CQ, Hao SR (2006) Cyclic electron flow around photosystem 1 is required for adaptation to salt stress in wild soybean species *Glycine cyrtoloba* ACC547. Biol Plant 50:586–590