



Population-based variation in resilience to hyposalinity stress in *Halophila johnsonii*

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ABSTRACT.—Plants of the threatened seagrass, *Halophila johnsonii* Eiseman, from a riverine and marine population were exposed to a series of salinity treatments within mesocosms. Survival and maximum photochemical efficiencies of PSII (F_v/F_m) were measured in response to varied frequency, duration, and amplitude of hyposalinity exposures. Both populations exhibited high survival after two cycles of short, pulsed hyposalinity treatments (100% and 89%, respectively). However, two cycles of long pulses of low salinity resulted in 100% mortality for marine *H. johnsonii* and >50% mortality for riverine plants. After two cycles of gradual salinity reduction to a salinity of 5, survival for marine and riverine plants was also low (22% and 33%, respectively). F_v/F_m values of riverine *H. johnsonii* were relatively high (0.65–0.70) after a single short pulse or gradual reduction to salinity of 10. F_v/F_m values of marine plants were lower in these two treatments and exhibited greater declines following a single, long pulse to a salinity of 10 or after a single gradual reduction to a salinity of 5. F_v/F_m values showed riverine plants were also more resistant to repeated pulses of moderate low salinity than marine plants. Our results indicate wide tolerances of *H. johnsonii* to short pulses of hyposalinity, but suggest that repeated or prolonged hyposalinity stress at near-tolerance salinity levels can have additive effects on photosynthetic health and survival. Differences in resilience of marine and riverine *H. johnsonii* populations are consistent with previous suggestions that estuarine ecophenes are more tolerant to hyposalinity than marine populations.

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Halophila johnsonii Eiseman is a small-bodied seagrass with an extremely limited geographic range. Its distribution is restricted to about 200 km of coastal lagoons and estuaries of southeastern Florida from Sebastian Inlet (27°51'N, 80°27'W) in northern Indian River Lagoon to northern Biscayne Bay (25°45'N, 80°07'W), which includes man-made navigation channels (Intracoastal Waterway) that connect these larger water bodies and stormwater drainage canals managed by state water management districts (Kenworthy 1997). *Halophila johnsonii* is the least abundant seagrass within its range and is considered an opportunistic species, often occurring intertidally or at the upper and lower margins of seagrass beds where other seagrass

species are restricted (Virnstein et al. 2009). As one of the smallest seagrass species and with no known sexual reproduction (Eiseman and McMillan 1980), *H. johnsonii* has no significant genetic variation across its range and relies solely on vegetative reproduction and growth (Kenworthy 1997). It also has short shoot life spans, rapid shoot turnover, high rates of branching, and exhibits a guerilla clonal growth strategy (i.e., it rapidly colonizes disturbance or gap areas, but does not hold its ground; Kenworthy 1997, Dean and Durako 2007, Virnstein et al. 2009). These attributes make it particularly susceptible to localized extinction due to environmental disturbances or prolonged adverse environmental conditions and as a result, *H. johnsonii* is the only marine plant listed as threatened under the US Endangered Species Act (Federal Register 1998).

The waters where *H. johnsonii* occurs are generally shallow (<2–3 m) and microtidal with semi-diurnal tides that propagate through several inlets (Kenworthy 1997). Tidal forcing is strongest near inlets and diminishes rapidly with distance from the inlets so that large interior areas have restricted circulation and relatively long water residence times. Because of the closed nature and restricted circulation of these lagoons and estuaries, both natural and anthropogenic disturbances can greatly alter their salinities, placing submerged plants under osmotic stress. Severe rainstorm events and hurricanes create pulsed freshwater inputs, which have severely impacted seagrass beds, including *Halophila* species (Ralph 1998, Steward et al. 2006). It has been hypothesized that with climate change, tropical regions will have more frequent and severe rainfall events that will lead to more common hypohaline environmental conditions (Steward et al. 2006).

In 2004, four major hurricanes (Charley, Frances, Ivan, and Jeanne) directly impacted the east coast of Florida and generated runoff conditions that decreased water quality over much of the Indian River Lagoon (Steward et al. 2006). The large inputs of fresh water associated with these storms decreased salinities to <15, which led to significant declines in seagrass densities and changes in species composition (Steward et al. 2006). Extensive stormwater drainage canal systems, combined with overland and ground water discharges, also periodically input large volumes of fresh water into the coastal systems where *H. johnsonii* occurs (Steward and Green 2007). These periodic freshwater inputs expose *H. johnsonii* to large fluctuations in salinity and occasional sustained periods of salinity <15. Salinity is the only environmental factor that has been directly linked to mortality of *H. johnsonii* (Kahn and Durako 2008). Thus, reduced salinity has been identified as the most serious threat to the survival of this species (Torquemada et al. 2005, Kahn and Durako 2008, Griffin and Durako 2012).

Previous experimental studies have shown that *H. johnsonii* has a wide tolerance for salinity, with optimum rates of photosynthesis between 30 and 40 (Dawes et al. 1989, Torquemada et al. 2005). However, photosynthetic rates declined significantly after 1 hr at pulsed low salinities (<20) and after 15 d high rates of mortality (>90%) occurred at a salinity of 10 while moderate mortality (50%) occurred at salinity of 20 (Torquemada et al. 2005). In a subsequent mesocosm experiment, pulsed salinities <10 caused 100% mortality of *H. johnsonii* after 10 d, but all plants survived 1 mo at 20 (Kahn and Durako 2008). Consequently, pulsed hyposalinity tolerance threshold was estimated to be between 10–20. Griffin and Durako (2012) concluded *H. johnsonii* is tolerant of pulsed hyposalinity down to 15 before measurable stress arose. However, gradual reductions in salinity (2 d⁻¹) allow time for acclimation and

osmoregulation, extending the hyposalinity tolerance threshold of this species near 5 (Griffin and Durako 2012).

The ability of *H. johnsonii* to recover from near-tolerance limit exposures to hyposalinity is currently unknown. This information would be useful to water management to minimize negative impacts of freshwater releases on this species. Thus, the objective of the present study was to evaluate resilience of *H. johnsonii* to repeated hyposalinity stress by monitoring physiological responses during and after near tolerance-limit exposures to hyposaline conditions. Experimental treatments varied with respect to frequency, duration, and amplitude of hyposalinity exposure. Exposures to hyposalinity were followed by a return to ambient salinity (25) and recovery. Previous studies have reported riverine and marine populations of *H. johnsonii* exhibit distinctive habitat-specific responses to environmental variation indicative of physiological acclimation (Kahn and Durako 2009, Kahn et al. 2013). To examine population-based variations in hyposalinity resilience, plants of *H. johnsonii* from a riverine and marine site were exposed to the experimental conditions.

METHODS

PLANT MATERIALS.—*Halophila johnsonii* plants were collected 25 May, 2012, from the Loxahatchee River Estuary near Pennock Point (26.948046°N, 80.111365°W) and at a site adjacent to Jupiter Inlet (26.9443°N, 80.0790°W), approximately 3.5 km east of Pennock Point. The Pennock Point plants were considered our “riverine” population (mean salinity = 18.3, annual range = 3.0–37.0), while plants from Jupiter Inlet are considered to be “marine” (mean salinity = 32.8, annual range = 12.6–37.0) (Howard et al. 2011). Fifty transplants were obtained from each site. Each transplant, which consisted of a rhizome segment with a minimum of two leaf pairs of *H. johnsonii* and associated sediments, was placed into a 9 × 9 × 8 cm plastic pot. The transplants were placed into coolers filled with water from their respective collection sites and transported to the University of North Carolina Wilmington, Center for Marine Science (CMS) greenhouse the following day. After arrival at CMS, marine plants were placed in coolers filled with high-grade, filtered seawater of salinity 32, while plants collected from the riverine site were placed in coolers filled with Instant Ocean® synthetic seawater of salinity 25. Distilled H₂O was used to lower salinity 2 d⁻¹ in coolers that contained marine *H. johnsonii* until the salinity equaled 25.

EXPERIMENTAL DESIGN.—After plants from both populations reached salinity 25, they were moved outside into 15 40-L aquaria. Three pots from each population were placed in each aquaria (3 pots population⁻¹ aquarium⁻¹ = 90 total pots). Each aquarium was filled with Instant Ocean® synthetic seawater of salinity 25 and continuously bubbled with ambient air to reduce stratification and maintain dissolved oxygen and pH levels. Aquaria were randomly distributed among five fiberglass seawater-supplied vaults (three aquaria vault⁻¹) to offset any differences associated with location within the fiberglass vaults. Vaults acted as water baths and maintained a mean temperature in the aquaria of 27.4 (SD 1.36) °C during the experimental period. One liter von Stosch's (von Stosch 1964) nutrient media was added to each aquaria and replenished every 7 d to provide plants with approximately 1.21 mg L⁻¹ nitrate and 0.12 mg L⁻¹ phosphate wk⁻¹. Plants were allowed to acclimate at the control salinity of 25 for five additional days. Salinity was checked daily with a conductivity meter (YSI model

80; accuracy ± 0.5 ; Yellow Springs, OH) and adjusted as needed with distilled water and Instant Ocean® salts. Photosynthetically active radiation (PAR) was measured at canopy level using an underwater spherical quantum PAR sensor (LiCor LI-193S) attached to a LiCor LI-1400 data logger (Lincoln, NE). Mean (SD) daily PAR over the duration of the experiment was calculated from 5 min running averages logged every 15 min from dawn until nightfall [665 (SD 220) $\mu\text{mol m}^{-2} \text{s}^{-1}$].

Hyposalinity treatments were initiated after the 5-d acclimation period in the vaults. The experimental design incorporated pulsed and gradual (2 d^{-1}) reductions/increases in salinity from control levels to mimic variability that would occur during and following runoff from rain events or large rapid inputs from stormwater canal discharges. The five treatments consisted of a control with constant salinity (25), short-pulsed reduction and recovery (1 wk at 10, 1 wk at 25), long-pulsed reduction (2 wks at 10, 1 wk at 25), moderate-gradual salinity change (25 to 10 to 25), and low-gradual salinity change (25 to 5 to 25). Each treatment was randomly assigned to three aquaria and each aquarium contained three pots from each population. The aquaria were the experimental units ($n = 3$) and each pot, which contained a rhizome segment with a minimum of two leaf pairs, was treated as an individual subsample.

SURVIVAL AND PHOTOBIOLOGY.—Survival and maximum photochemical efficiency of PSII measurements were recorded pre-dawn every 2 d for the 42-d experiment. Three plants from each population were measured in each aquaria (2 populations $\times$ 3 subsamples $\times$ 15 aquaria = 90 measurements sampling $^{-1}$). Maximum photochemical efficiency (F_v/F_m , where $F_v = F_m - F_o$ and F_o is the minimum chlorophyll fluorescence at non-actinic irradiance levels and F_m is the maximum fluorescence after the fluorometer's saturating pulse) was obtained using Mini-PAM fluorometer (Walz, Germany) measurements of dark-acclimated plants. For all measurements, a leaf clip was used to set the 2 mm diameter fiber optic tip (Mini-PAM/F1) of the instrument at a standard geometry (30° from perpendicular) and distance (5 mm) to the upper surface of one leaf from the second leaf pair back from the rhizome apical meristem. Measuring light intensity and gain were set to seven and six, respectively. When the number of replicates for a treatment declined below three, zeros were recorded and averaged with remaining F_v/F_m values for that treatment. F_v/F_m values of *H. johnsonii* measured at times of low salinity were compared against control F_v/F_m values to assess sensitivity of photosystems to hyposalinity stress. In addition, F_v/F_m values of experimental treatments were assessed over recovery periods to determine whether F_v/F_m values recovered or remained significantly lower than controls. Recovery periods were defined as days when salinity returned to 25 for pulsed treatments or as days when salinity was >15 for gradual salinity treatments (Griffin and Durako 2012). Mortality was specified as the absence of leaves or F_v/F_m measurements near zero for two consecutive measurement periods (4 d). Survivorship was calculated as the sum of the three replicate aquaria for each treatment.

STATISTICAL ANALYSIS.—Kaplan-Meier survival curve analysis was used to determine whether significant changes in percent survival occurred over time. When significant treatment effects were detected, a log-rank with Holm-Sidak multiple comparisons test was performed to isolate where those differences occurred. Maximum photochemical efficiencies of PSII (F_v/F_m) are reported as means (SE). Normality of data was tested using the Shapiro-Wilk test and homogeneity of variance was tested using the Levene median test. When normality failed or variances

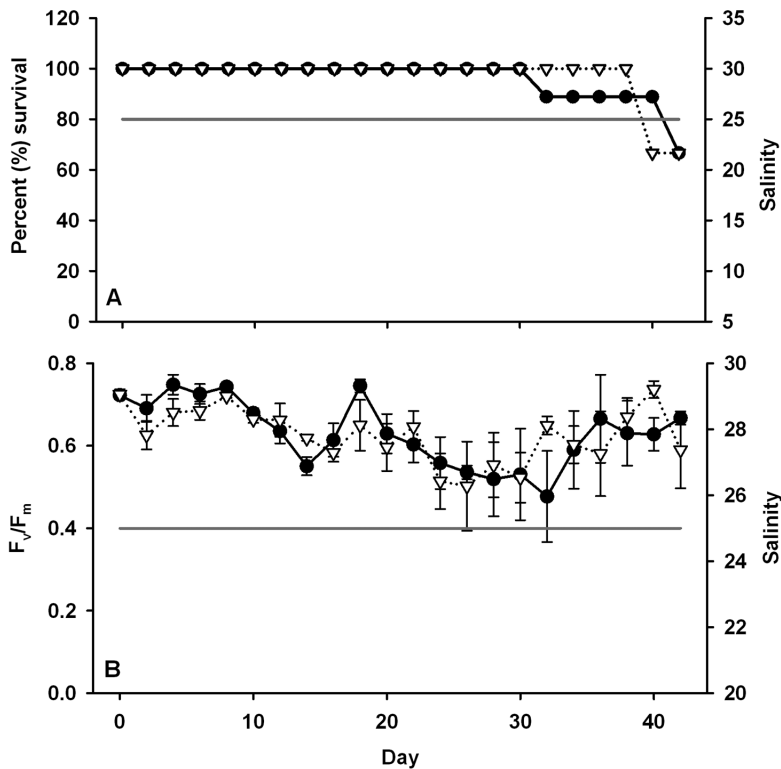


Figure 1. (A) Survival (%) and (B) mean (SE) maximum photochemical efficiency of PSII (F_v/F_m) of riverine (filled circles) and marine (open triangles) *Halophila johnsonii* in control treatments over time (day). Salinity = solid gray line, units on right-hand y-axis. There were no significant differences between the two populations.

were not equal, transformations were attempted. If transformation failed, nonparametric tests were used to compare variation in F_v/F_m values between populations (marine vs riverine) among hyposalinity treatments. For parametric data, two-way repeated measures ANOVAs, where main effects were hyposalinity treatment and population (marine vs riverine), were used to determine the strength of each factor and their combined interaction on F_v/F_m . Friedman repeated-measures ANOVA on ranks was used to compare non-parametric data. When a statistically-significant difference was identified, an all-pairwise multiple comparisons test was used to isolate where those differences occurred. The Holm-Sidak method for multiple comparison procedures was used to test parametric data; Dunn's multiple comparisons test was used for nonparametric data. Significance was determined at $P < 0.05$ for all tests and statistical analyses were performed using SAS 9.1® and SigmaPlot 11® software.

RESULTS

Control treatments of marine and riverine *H. johnsonii* had 100% survival up to day 32 and an overall survival rate of 67% (Fig. 1A). Similarly, marine and riverine *H. johnsonii* had high percent survival through two cycles of short-pulsed hyposalinity

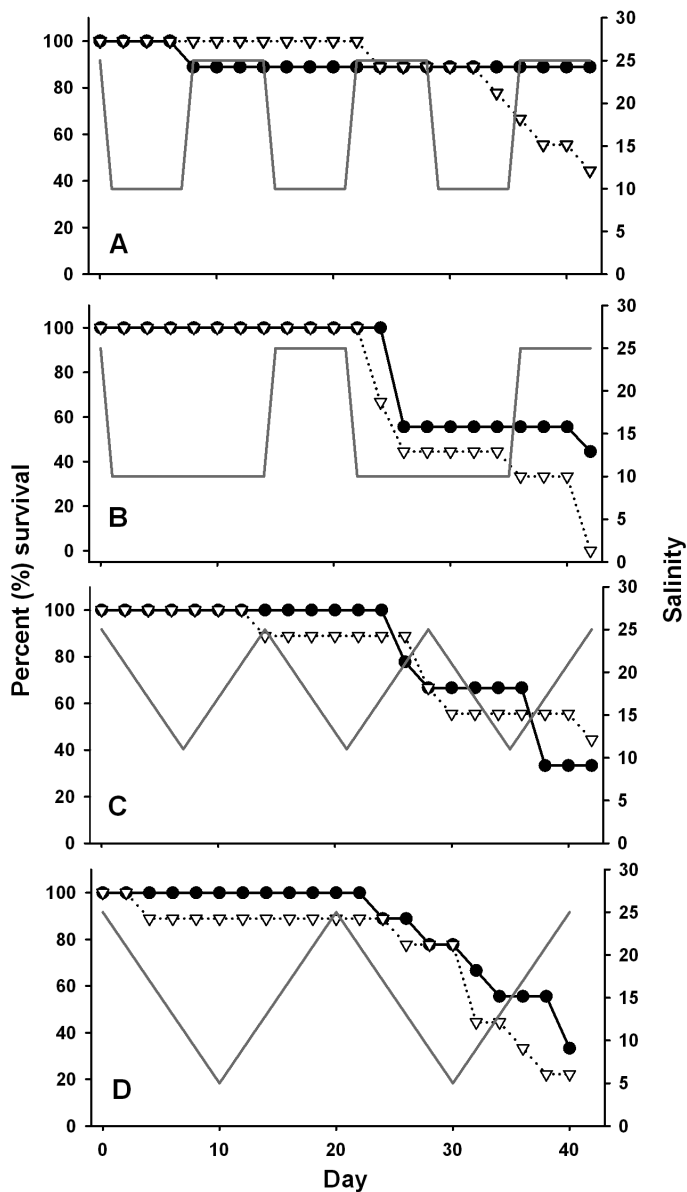


Figure 2. Survival (%) of riverine (filled circles) and marine (open triangles) *Halophila johnsonii* over time (day) in short pulsed (A), long pulsed (B), moderate gradual (C), and low gradual (D) hyposalinity treatments. Salinity = solid gray line, units on right-hand y-axis. There were no significant differences between populations within salinity treatments.

treatments (100% and 89%, respectively) and survival of riverine plants remained unchanged through a third pulse, while marine plant survival declined. In contrast, there was a marked decrease in percent survival before the end of two hyposalinity treatment cycles for marine and riverine *H. johnsonii* in all other hyposalinity treatments (Fig. 2B–D). For example, two long pulses of low salinity resulted in 100% mortality for marine *H. johnsonii* and >50% mortality for riverine *H. johnsonii* (Fig.

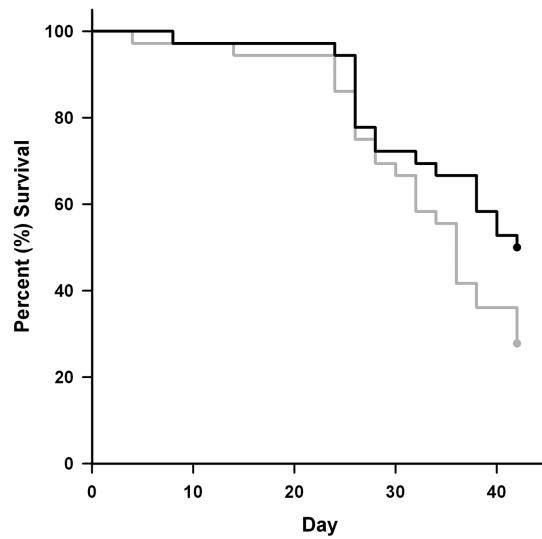


Figure 3. Total survival (%) of riverine (black line) and marine (gray line) *Halophila johnsonii* over time (day). There was no significant difference between populations ($P = 0.06$).

2B). In addition, after two cycles of low-gradual hyposalinity, survival for marine and riverine plants was also low (22% and 33% respectively, Fig. 2D) and although such low survival rates likely represent a biologically significant difference compared to controls, this result was not statistically significant due to the mortality of several control plants, which reduced our statistical power. Within hyposalinity treatments, there were no significant differences in percent survival between marine and riverine populations (log-rank test: $df = 1$, $P > 0.05$). Similarly, overall percent survival of riverine (50%) and marine (28%) populations did not significantly differ (log-rank test: $df = 1$, $P = 0.06$, Fig. 3).

Highest overall survival within riverine plants (and among all treatments) was in the short-pulsed hyposalinity treatments (89%, Fig. 2A). Likewise, highest overall mean F_v/F_m values for riverine *H. johnsonii* occurred in the short-pulsed hyposalinity treatment [0.58 (SE 0.01), Fig. 4A]. Highest overall survival within marine *H. johnsonii* was 44%, which occurred in both the short-pulsed and moderate-gradual hyposalinity treatments (Fig. 2A,C). Marine plants in the moderate-gradual hyposalinity treatment also had the highest overall mean F_v/F_m value for marine *H. johnsonii* among hyposalinity treatments [0.60 (SE 0.01), Fig. 4C]. Lowest survival and overall F_v/F_m within marine *H. johnsonii* occurred in the long-pulsed hyposalinity treatment [0% and 0.44 (SE 0.03), respectively; Fig. 4B]. This lack of survival was significantly lower than control treatments or of riverine *H. johnsonii* exposed to short-pulsed hyposalinity (log-rank test: statistic = 28.4, $df = 9$, $P < 0.001$; Holm-Sidak multiple comparisons test: $P < 0.05$). Riverine *H. johnsonii* had lowest survival in moderate and low-gradual hyposalinity treatments (33%). Although mean F_v/F_m values for riverine *H. johnsonii* in the moderate-gradual treatment [0.45 (SE 0.03)] were lower than those for the low-gradual hyposalinity treatment [0.49 (SE 0.03)] over the second half of the experiment (days 36–42, Fig. 4C,D), this difference was not significant.

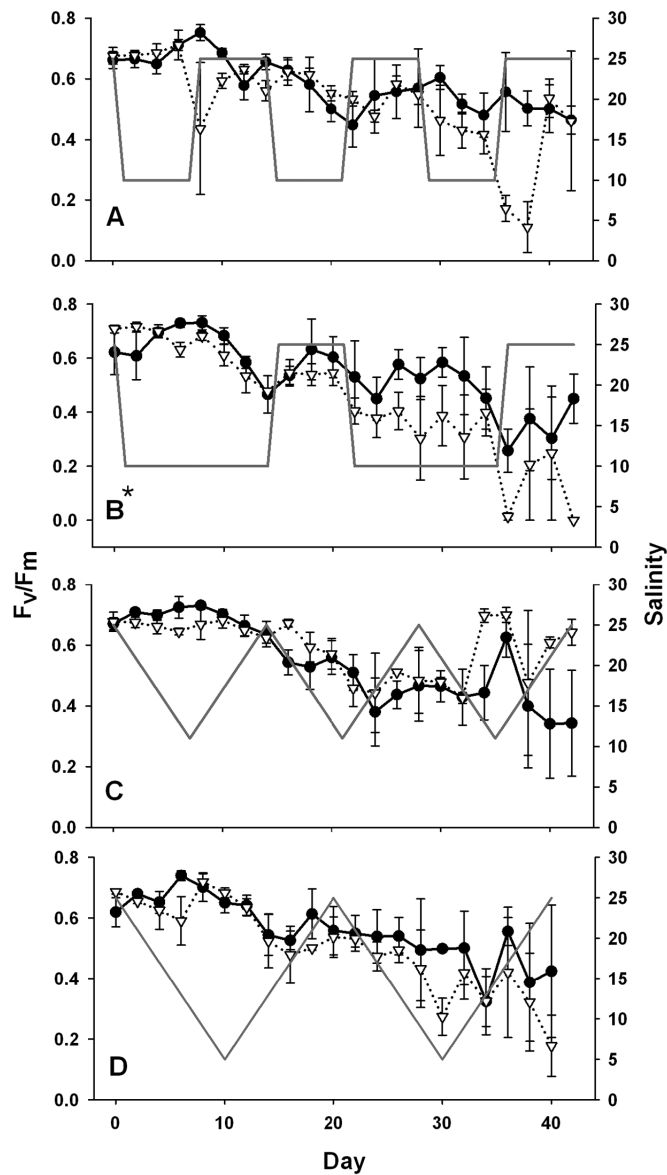


Figure 4. Mean (SE) maximum photochemical efficiency of PSII (F_v/F_m) of marine (open triangles) and riverine (filled circles) *Halophila johnsonii* over time (day) in short pulsed (A), long pulsed (B), moderate gradual (C), and low gradual (D) hyposalinity treatments (salinity = solid gray line, units on right-hand y-axis). Significant differences between populations within salinity treatments are indicated with an asterisk *.

Population source (marine vs riverine) and hyposalinity treatment had significant effects on mean F_v/F_m values (RM ANOVA: $F_{1,650} = 5.42$, $P = 0.02$; $F_{4,650} = 10.35$, $P < 0.001$, respectively). In contrast, overall mean F_v/F_m values did not vary significantly between marine and riverine *H. johnsonii* in the control treatment (Friedman RM ANOVA on ranks: $\chi^2 < 0.002$, $df = 1$, $P > 0.99$, Fig. 1B). Thus, control groups were

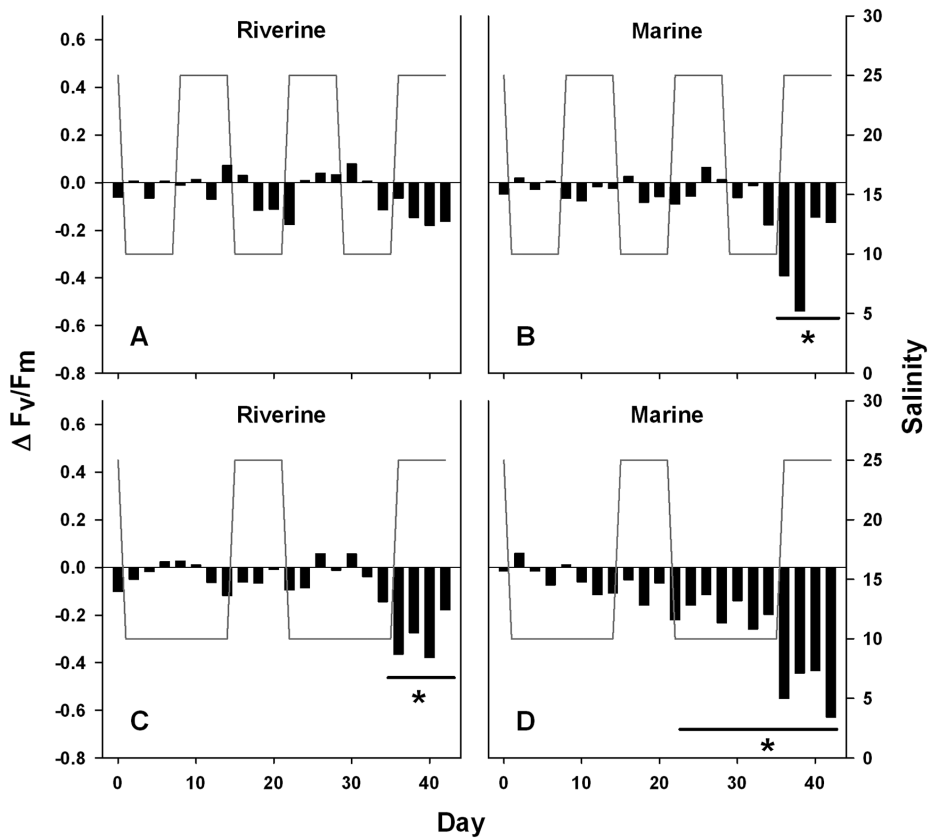


Figure 5. Differences in mean maximum photochemical efficiencies of PSII ($\Delta F_v/F_m$; black bars) between controls and riverine or marine *Halophila johnsonii* over time (day) in response to short (A, B) or long (C, D) pulsed salinity treatments (salinity = solid gray line, units on right-hand y-axis). Significant differences between control and hyposalinity treatments are indicated with an asterisk *. The black horizontal line represents the time period differences were significant.

pooled and the pooled mean value was used for the multiple comparisons vs control group tests. Mean F_v/F_m values of riverine *H. johnsonii* in each of the four hyposalinity treatments were significantly lower than controls (Dunn's multiple comparisons test: $P < 0.05$). In contrast, within marine *H. johnsonii*, overall mean F_v/F_m values were significantly lower than control values in only the long-pulsed and low-gradual hyposalinity treatments (Dunn's multiple comparisons test: $P < 0.05$). In addition, post-hoc analyses indicated that overall mean F_v/F_m values of marine *H. johnsonii* in long-pulsed marine hyposalinity treatments were significantly lower than riverine in every hyposalinity treatment (Dunn's multiple comparisons test: $P < 0.05$; Fig. 4B). For marine *H. johnsonii*, overall mean F_v/F_m values of low-gradual treatments were significantly lower than short-pulsed riverine and moderate-gradual marine values (Dunn's multiple comparisons test: $P < 0.05$).

In short-pulsed hyposalinity treatments, F_v/F_m values did not vary significantly from controls over days 0–36 for both riverine and marine *H. johnsonii*. However, over the final recovery period (day 36–42), F_v/F_m values of marine *H. johnsonii* were significantly lower than controls, while riverine values were not (RM ANOVA: $F_{1,12} = 13.7$, $P = 0.003$; Fig. 5A,B). Over the second reduction in salinity (days 22–34) of

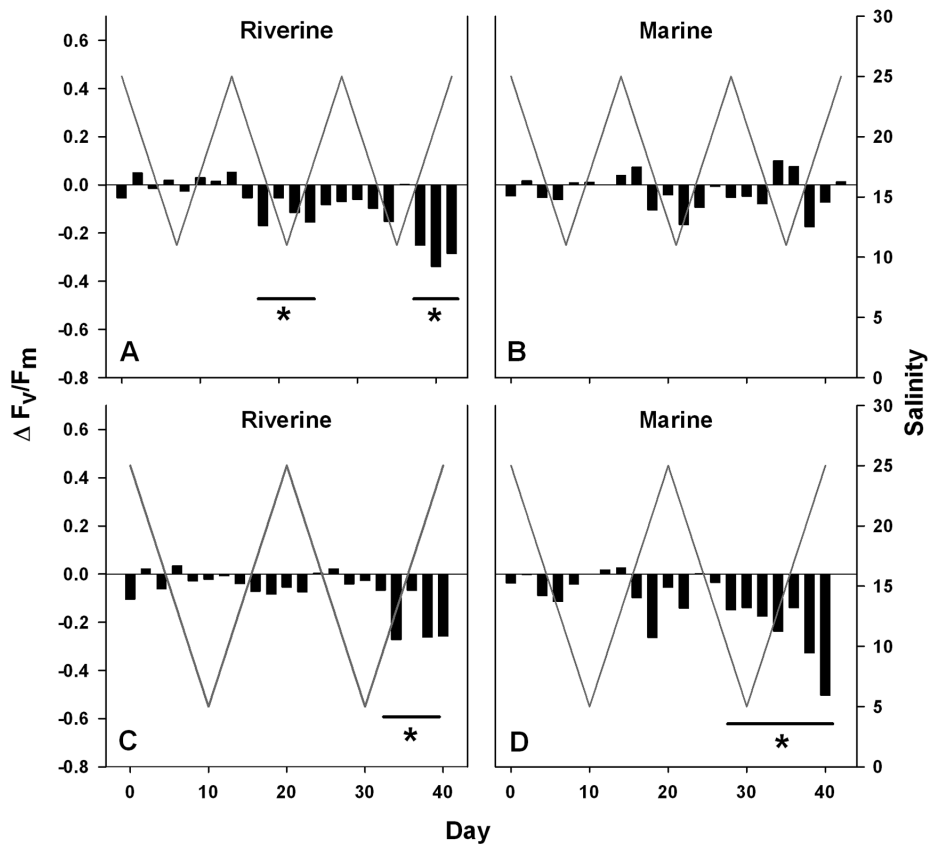


Figure 6. Differences in mean maximum photochemical efficiencies of PSII ($\Delta F_v/F_m$; black bars) between controls and riverine or marine *Halophila johnsonii* over time (day) in response to moderate (A, B) or low (C, D) gradual salinity treatments (salinity = solid gray line, units on right-hand y-axis). Significant differences between control and hyposalinity treatments are indicated with an asterisk *. The black horizontal line represents the time period differences were significant.

the long-pulsed hyposalinity treatments, F_v/F_m values of marine *H. johnsonii* were significantly lower than controls and remained significantly lower over the final recovery period (days 22–34, $F_{1,21} = 21.1$, $P < 0.001$; days 36–42, $F_{1,12} = 45.4$, $P < 0.001$; Fig. 5D). In contrast, F_v/F_m of riverine *H. johnsonii* in long pulsed treatments did not significantly decline compared to controls until the final recovery period (days 36–42, $F_{1,12} = 12.6$, $P < 0.005$; Fig. 5C). During the second cycle of the moderate-gradual hyposalinity treatment (days 14–28), F_v/F_m values of riverine *H. johnsonii* were significantly lower than controls ($F_{1,24} = 8.31$, $P = 0.01$) and remained significantly lower over the final recovery period (days 38–42, $F_{1,9} = 6.95$, $P = 0.03$; Fig. 6A). In contrast, F_v/F_m of marine *H. johnsonii* under moderate-gradual hyposalinity treatment did not significantly vary from controls over the course of the experiment (Fig. 6B). For low gradual hyposalinity treatments of riverine *H. johnsonii*, F_v/F_m was not significantly lower than controls until the final recovery period (RM ANOVA on ranks, $\chi^2 = 5.4$, $df = 1$, $P = 0.04$; Fig. 6C). F_v/F_m values of marine *H. johnsonii* in the low gradual hyposalinity treatment were significantly lower than controls from the second low-gradual hyposalinity cycle (days 28–32, $F_{1,9} = 14.85$, $P = 0.005$) through the final recovery period (days 34–42, $F_{1,15} = 12.2$, $P = 0.004$; Fig. 6D).

DISCUSSION

Our results indicate there is population-based variation in the resiliency of *H. johnsonii* to hyposalinity stress. Percent survival of marine plants declined in response to every hyposalinity treatment, being significantly lower than controls in the long-pulsed treatment. In contrast, percent survival of riverine plants exposed to short pulsed hyposalinity was higher than control values and survival rates did not significantly decline in any other treatment. In addition, chlorophyll fluorescence indicated the riverine population was more resilient to pulsed hyposalinity than the marine population while the marine population appeared more resilient to moderate, but gradual increases and decreases in salinity. These mesocosm experiment results are consistent with previous suggestions of Benjamin et al. (1999) and Griffin and Durako (2012) that tolerance to hyposalinity can vary between estuarine and marine individuals of the same species as ecophenes emerge. Wide tolerances to environmental perturbations and high morphological plasticity are common in the genus *Halophila* (Waycott et al. 2004). Benjamin et al. (1999) concluded ecotypes were responsible for differences in salinity tolerance between marine and estuarine *Halophila ovalis* (R. Brown) Hooker plants, where marine plants were intolerant to low salinities but estuarine plants thrived. Recently, Kahn et al. (2013) invoked a highly phenoplastic physiology to explain significant differences in photophysiology (acclimation to both light intensity and light quality in F_v/F_m and pigments) between inlet and riverine populations of *H. johnsonii* from Biscayne Bay in North Miami Beach, Florida. Because there is no significant genetic variation among populations of *H. johnsonii* (Kenworthy 1997), observed differences in resilience to pulsed and gradual hyposalinity between marine and riverine populations of *H. johnsonii* likely reflect emergence of locally specialized ecophenes through high physiological plasticity.

Maximum photochemical efficiency of PSII (F_v/F_m) values for healthy, non-stressed seagrasses range between 0.7 and 0.8 (Ralph 1999, Durako et al. 2003). F_v/F_m values of plants in control treatments from both populations were within this range at the beginning of the experiment [0.72 (SE 0.01), Fig. 1B], but varied over time and fell below 0.7 by the end of the experiment [0.63 (SE 0.05)]. Mortality was similar between marine and riverine populations of *H. johnsonii* at control salinities with 100% survival until day 32 and overall percent survival of 67% (Fig. 1A). These data suggest mesocosm conditions were slightly stressful, which may have contributed to the F_v/F_m values of *H. johnsonii* exposed to hyposalinity treatments not exceeding 0.7 during recovery periods.

Although survival did not differ between marine *H. johnsonii* in short-pulsed and moderate-gradual hyposalinity treatments (44%), F_v/F_m values were lower during the final recovery period (days 36–42) in the short-pulsed treatment compared to moderate-gradual treatment [0.32 (SE 0.07) and 0.58 (SE 0.07), respectively], which suggests survivors of the moderate-gradual treatment exhibited better physiological health (quality), if not better survival (quantity). In contrast, within riverine *H. johnsonii*, highest overall survival and F_v/F_m occurred in short-pulsed hyposalinity treatments [89% and 0.58 (SE 0.0), respectively; Figs. 2A, 4A], which suggests gradual reductions in salinity did not increase resilience of riverine *H. johnsonii* to hyposalinity. This result contrasts with Griffin and Durako (2012), where a gradual decrease in salinity allowed *H. johnsonii* to tolerate lower levels of salinity than if exposed to a pulsed

salinity decrease. Similar survival rates and F_v/F_m values between moderate and low-gradual hyposalinity treatments were unexpected. Rather, we expected that *H. johnsonii* would exhibit higher survival and photosynthetic performance with moderate, gradual changes in salinity. Salinity in the gradual hyposalinity treatments not only gradually decreased, it also gradually increased so plants in the gradual treatments returned to higher salinities more slowly than those in the short-pulsed treatments. As a result, short-pulsed treatment plants spent more time during recovery periods at the control salinity than plants in moderate gradual treatments. In addition, riverine *H. johnsonii* had equally low survival at moderate and low-gradual hyposalinity treatments (33%; Fig. 2C, D) and there was no significant difference in F_v/F_m values between these treatments.

Hyposalinity is associated with reduced chloroplast numbers (Iyer and Barnabas 1993) and disruption of thylakoid membranes (Kirst 1989), which impairs photosynthetic activity. Observed declines in F_v/F_m for *H. johnsonii* exposed to hyposalinity treatments may be attributed to a decline in cellular ion content, including necessary ions involved as photosynthetic cofactors (Simon et al. 1999). Initial adjustment to hyposaline conditions also results in loss or degradation of energy-rich compatible solutes that help maintain osmotic potential and stabilize membranes (Bisson and Kirst 1995). Furthermore, growth in hyposaline conditions requires increased energy demands due to elevated metabolic costs associated with maintaining internal ionic balance (Sibly and Calow 1989). *Halophila johnsonii* is considered a small-bodied, rapid-growth clonal plant with a reduced carbon storage capacity compared to larger seagrasses such as *Thalassia* sp., *Posidonea* sp., and *Zostera* sp. At reduced salinities (<30), photosynthetic rates of *H. johnsonii* decrease and dark respiratory rates increase, implying that responses to environmental stressors occur quickly in this species (Torquemada et al. 2005). Thus, it was not surprising that increased mortality closely followed declines in F_v/F_m , as cellular death would likely occur quickly once energy stores were depleted and internal ionic balances could no longer be maintained. In addition, due to metabolic costs of maintaining internal ionic balance and the limited carbon stores of *H. johnsonii*, total amount of time below the salinity-tolerance threshold of 15 may be more important than the actual rate of salinity reduction. This hypothesis may explain why survival and overall mean F_v/F_m were highest within riverine *H. johnsonii* exposed to short-pulsed hyposalinity treatments as it allowed for more days exposed to control salinities compared to the other treatments. Future studies should attempt to measure respiration rates, in addition to characterizing photosynthetic responses, to better evaluate the influence hyposalinity treatments have on the energy demands and carbon allocation patterns of *H. johnsonii* from contrasting salinity environments.

The present study highlights wide tolerances of *H. johnsonii* to short (1 wk) pulses of hyposalinity. Furthermore, it shows that repeated or prolonged hyposalinity stress at near-tolerance salinity levels can have an additive effect on *H. johnsonii* photosynthetic health and survival. Differing photosynthetic responses of marine and riverine populations to hyposalinity treatments are consistent with the presence of estuarine and marine ecophenes of *H. johnsonii*. Our observed differences in hyposalinity resilience of *H. johnsonii* plants from distinct salinity habitats emphasizes the need for experimental studies to account for the previous environmental history of sampled organisms and to examine responses over a range of conditions to determine the extent and resilience of their physiological plasticity. Hopefully, our results

provide an ecologically-relevant assessment of hyposalinity tolerance for *H. johnsonii* populations that can be used to inform water management practices throughout the geographic range of *H. johnsonii*. Knowledge of the hyposalinity resilience of *H. johnsonii* should allow management of water releases to minimize the threat of mortality to this species.

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