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Photophysiological responses of *Halophila johnsonii* to experimental hyposaline and hyper-CDOM conditions

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ABSTRACT

The endemic seagrass *Halophila johnsonii* grows intertidally to 3 m deep, in both marine and riverine influenced habitats of eastern Florida. Salinity and chromophoric dissolved organic matter (CDOM) levels widely fluctuate across this broad habitat range, changing tidally and with variable influx of freshwater from watershed runoff, river discharge and stochastic storm events. CDOM exponentially absorbs light in the UV to blue wavelengths, affecting optical water quality. *H. johnsonii* produces 15 flavonoid compounds that maximally absorb in the UV range. These flavonoids are thought to function as UV-protectants (UVP) in high-light and UV-intense environments. This mesocosm study examined the photosynthetic capacity, quantum efficiency and pigment content of *H. johnsonii* under experimental treatments of three salinities (10, 20 and 30) with and without CDOM. Main treatment effects and possible interactive effects at both short- (1 day to 1 week) and longer-term (1 month) time scales were examined. There were no significant CDOM or CDOM x salinity effects over any of the experimental treatment durations. There was 100% mortality of plants at salinity 10 after 10 days regardless of water color. UVP content of leaves was not affected by CDOM in this study, but there was significant variation in UVP in response to salinity. Our results do not support the primary role of UVP in this species as a sunscreen, but indicate that different salinity environments contribute to changes in the levels of these flavonoids. The UVP response to salinity stress response was not mitigated by a decrease in UV-radiation (increased CDOM) as *H. johnsonii* continued to put energy into the production of the carbon-rich flavonoids regardless of potential UV-stress. The experimental results indicate that prolonged hypo-salinity conditions are an important environmental factor to manage in the limited geographic range of *H. johnsonii*.

1. Introduction

Halophila johnsonii Eiseman is a seagrass that is endemic to the coastal lagoons of the East coast of Florida from Sebastian Inlet to Northern Biscayne Bay, FL (27°51′N; 80°27′W to 25°45′; 80°07′W), including much of the Indian River Lagoon (Kenworthy, 1992; Virnstein and Morris, 2007). Water quality in this region is significantly influenced by river, canal and creek discharges from the watershed, many of which are regulated by state water-management agencies. At times of high discharge, especially during the rainy season or post-storm events, salinity decreases and the presence of chromophoric dissolved organic matter (CDOM), derived from terrestrial run-off, increases (Zanardi-Lamardo et al., 2004; Steward et al., 2006). Multiple hurricanes/tropical storms impacted this region in 2004 reducing water transparency (increased CDOM and turbidity) and salinity fell from 30 to less than 15 in some regions of the Indian River Lagoon; these uncharacteristic hyposaline and high-color conditions persisted in some areas through the following year. (Steward et al., 2006).

Growth, distribution and physiology of seagrasses are significantly influenced by salinity, based upon their ability to osmoregulate to variations in environmental salinities (reviewed by Touchette, 2007). Lab studies of *H. johnsonii* indicate that hyposalinity negatively affects growth and photosynthetic activity and increases mortality (Torquemada et al., 2005). *Halophila johnsonii* and its co-occurring con-generic *H. decipiens*, which has similar morphological structure and growth habit, did not tolerate extreme hyposaline conditions (a salinity of 5) over the course of a three day acclimation period in lab culture (Dawes et al., 1989). The abundance of *H. johnsonii* at permanent monitoring sites decreases during years of increased precipitation, also suggesting that influx of freshwater and reduced salinity has a negative impact on this species *in situ* (Virnstein and Morris, 2007). Although the main effects of salinity on seagrasses are generally well known, to our knowledge, the possible interactive effects of hyposaline and hyper-CDOM conditions, which would co-occur at times of increased river discharge or runoff, have not been examined.

In the presence of CDOM, light attenuation increases exponentially with decreased wavelength, significantly decreasing the amount of high-energy UV and blue-light penetrating the water column (Kirk, 1994). It is unknown whether increased CDOM, which should decrease UV-stress, affects hyposalinity tolerance of *H. johnsonii*. CDOM is known

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to provide phytoplankton protection from UV-B radiation thereby increasing growth and decreasing photoinhibition (Nielsen and Ekelund, 1993). Although direct effects of CDOM on seagrasses have not been reported, depth distribution is influenced by species' sensitivity to UV radiation (Dawson and Dennison, 1996; Durako et al., 2003). A change in UV-B radiation entering a system may cause a shift in plant species within a system, based on species-specific spectral tolerances, thereby affecting ecosystem characteristics (Caldwell et al., 1998).

Optical water quality (e.g., UV light) is an especially relevant environmental factor to consider regarding the growth and survival of *H. johnsonii* as this seagrass produces a number of carbon-rich flavone glycosides and acetylglycosides that maximally absorb light in the UV range (330-380 nm, Krzysiak, 2006; Meng et al., 2008). This UV-absorbance peak dominates the pigment-absorption spectra of *H. johnsonii* and it is not present in the co-occurring con-generic *H. decipiens* (Durako et al., 2003). Transplant studies involving *H. johnsonii* and *H. decipiens* observed that the UV-absorbance peak in *H. johnsonii* was 30% lower in subtidal than intertidal plants supporting the suggestion that the flavonoids function as UV-protective pigments (UVP) or sunscreens (Durako et al., 2003). However, a later study examining varying UV and PAR exposure on the photobiology of *H. johnsonii* reported that UVP levels did not differ between treatments with PAR or PAR plus UVB or UVA, but responded to overall PAR levels (Kunzelman et al., 2005). These authors suggested that the flavonoids may have additional physiological roles other than solely UV protection.

Here we used controlled mesocosm experiments to investigate the physiological responses of *H. johnsonii* to hyposaline and hyper-CDOM conditions over short (1 day, 1 week) and longer (1 month) periods of time. We hypothesized that the presence of CDOM in the media may increase the tolerance of *H. johnsonii* to salinity stress by decreasing UV irradiance therefore decreasing the need to allocate energy to the production of flavonoids. This hypothesis assumed that flavonoids function primarily as sunscreens in this plant. Survival, pigment levels and photosynthetic responses under the varying salinity and CDOM conditions were assessed. Our goal was to determine how *H. johnsonii*, with its very limited geographic but broad habitat distribution, might react to changes in salinity and optical light quality resulting from tides, changes in freshwater discharge, stochastic rain events or hurricanes and whether the response patterns might provide insights into its unique distribution pattern.

2. Materials and methods

2.1. Plant material

Because of the limited size of our mesocosm system (sixteen 38 l aquaria in four fiberglass troughs), three 1-month duration experiments were run to increase replication. *Halophila johnsonii* rhizome segments of three to five leaf pairs were collected at a shallow site near Jupiter Inlet, Florida (Lat: 26° 56.692′ N, Lon: 80° 04.756′ W) on May 23rd, June 30th, and August 4th, 2006 and planted in clean play sand in 9×9×9 cm plastic pots The pots were then placed in coolers containing seawater from the collection site and transported to the University of North Carolina Wilmington Center for Marine Science in Wilmington, North Carolina.

2.2. Mesocosm experimental design

Upon arrival at the Center for Marine Science, within 24 hours post collection, treatment media were added to sixteen 38 l experimental-treatment aquaria. Treatments were assigned randomly among four fiberglass mesocosms, which were used as water baths for temperature regulation. The outdoor mesocosms were oriented in an east-west direction, to minimize shading from the trough walls. Treatment media consisted of either de-ionized (DI) water for the non-CDOM treatments or filtered Black River water for the CDOM (colored dissolved organic matter, i.e., black water) treatments, to which synthetic sea salts were added. The Black River, a tributary of the Cape Fear River in North Carolina, has CDOM levels close to the maximum reported in the literature ($a_{CDOM}(350)=29.9\ m^{-1}$, Kowalczuk et al., 2003) and extremely low levels of nutrients (Mallin et al., 2001). CDOM treatment water was collected from the Black River 3-5 days prior to plant collection and filtered through 0.7 μm GF/F filters to remove particulates, such as detritus or plankton (e.g. chlorophyll), which may have influenced light attenuation. Instant Ocean© salts were added to each base media to achieve treatment salinities of 10, 20 or 30. There were three aquaria assigned to each treatment salinity and CDOM treatment (n=3). The CDOM treatments are designated with a B. In addition, there was a single salinity 30 black-water treatment, which served as a black-water control. Nutrients were added to all treatment media in the formulation of Von Stosch's nutrient enrichment media to eliminate nutrient limitation effects.

Twelve potted *Halophila johnsonii* plants were haphazardly selected and placed in each tank at dusk. The following morning was designated day 1. A LICOR 2π sensor was placed adjacent to, and at the level of, the tanks to record incident irradiance levels for days 1, 7 and 28 from 6:00 to 20:00 (one minute intervals, 10 second average). To determine light field conditions during physiological measurements, photosynthetically active radiation (PAR) levels were averaged over the hours from 1000-1300 h, for each day 1, 7 and 28 in treatment. A SATLANTIC© spectral radiometer was used to measure percent absorption of light at 412 nm in the non-CDOM and CDOM treatments at each salinity to determine differences in the short-wavelength light field at the level of the plant canopy relative to just below the water surface. Salinity was monitored every two days and adjusted when necessary, using treatment water (DI or filtered Black River water) or Instant Ocean© salts. Any debris that landed on the surface of the water was removed to maintain an unobstructed light field for each tank.

2.3. Photosynthetic measurements

After 1, 7 and 28 days of treatment, four plants from each replicate treatment aquarium were randomly chosen for measurement. A pulse amplitude modulated (PAM) fluorometer (Mini-PAM or Diving-PAM, WALZ GmbH) was used to generate rapid light curves (RLC). The RLC was initiated by attaching a dark leaf clip (DIVING-LC) to the second leaf pair back from the rhizome apical meristem. The leaf clip held the PAM fiber optic 5 mm from the surface of the leaf blades. Eight consecutive light levels of 92, 186, 341, 531, 752, 1143, 1573 and 2406 μmol photons $m^{-2}\ s^{-1}$ were applied at 10 s intervals. An effective yield measurement (Φ_{PSII}) was taken using a saturation pulse of 0.8 s, before the actinic light was applied (~quasi-darkness, Ralph and Gademann, 2005), and at the end of each 10 s irradiance step, resulting in nine Φ_{PSII} measurements. All measurements were conducted between 1000-1300 h. Relative electron transport rate (rETR) was estimated using the following equation:

$$rETR = \phi_{PSII} \times PAR \times 0.5$$

where PAR is the actinic photosynthetically active radiation generated by the internal halogen lamp of the PAM and 0.5 assume the photons absorbed by the leaf are equally partitioned between PSII and PSI (Genty et al., 1989).

To determine $rETR_{max}$, α (initial slope) and β (down-regulation), values for rETR and the stepwise PAR values of the rapid light curves were applied to a double exponential decay function (Platt et al., 1980).

$$rETR = P_s\left(1-e^{-(\alpha PAR/P_s)}\right)e^{(\beta PAR/P_s)}$$

The P_s value is a scaling factor which was in turn used to calculate $rETR_{max}$ (Platt et al., 1980).

$$rETR_{max} = P_s[\alpha/(\alpha+\beta)][\beta/(\alpha+\beta)]^{(\beta/\alpha)}$$

In cases where no down-regulation occurred ($\beta=0$), $P_{max}=P_s$ calculated via the initial equation. All calculations were performed using SAS 9.1©.

2.4. Pigment and morphometric measurements

Following the RLC measurement, the leaf pair was removed from the plant and blade length and width (mid-blade) were measured to determine leaf area which was calculated using the equation for the area of an ellipse [(length/2)(width/2)π]. Each leaf pair was then crushed with a mortar and pestle in 6 ml of 95% acetone on ice to extract pigments. Ground leaf tissue was extracted in a refrigerator overnight in the dark and the absorbance of the supernatant measured in a 1 cm quartz cuvette the following morning. Absorbance from 300-800 nm was measured using an Ocean Optics© spectrometer and a Mini-D2T© halogen/deuterium light source. The resulting absorbance spectra were then used to calculate chlorophyll *a* and *b* concentrations by applying the following dichromatic equations:

$$[\text{chl}a] = 11.93(A_{664}) - 1.93(A_{647})$$

$$[\text{chl}b] = 20.36(A_{647}) - 5.50(A_{664})$$

Relative UV pigment content was estimated by calculating the average absorbance between 341 to 345 nm (A_{341}-A_{345}). UVP content for only months 1 and 3 were calculated; month 2 data were discarded due to an instrument malfunction; Months 1 and 3 represent early summer and late summer, respectively.

2.5. Statistical analyses

One-way ANOVA tests were run on the physiological and biological variables to determine if variation among subsamples within treatment replicates was significant; as no significant differences were detected in any of the physiological parameters, all pseudo-replicates within each replicate were pooled. The three 1-month duration experiments were then compared to determine if variation across time was significant; where no significant differences occurred between months, values from those months were pooled for those parameters. For the photosynthetic measurements, physiological parameters within each treatment were not significantly different from each other between experimental months 2 (June-July) and 3 (July-August). The first experimental month (May-June) values were significantly different from both later months. Therefore, months 2 and 3 were pooled and were designated "late summer" as they were run from late June through August and month 1 results were designated "early summer" as they represented plants collected in the end of May. Two-way ANOVAs were used to examine the significance of the main treatment effects: salinity and color, and treatment interactions (salinity×color) with a 95% probability level at each time event (1 day, 1 week and 1 month at treatment) individually for early and late summer. Values reported, unless otherwise noted, are calculated from the type III sums of squares. To compare variation at each treatment among time events, one-way ANOVA was applied. In cases where tests for normality failed, Kruskal Wallis one-way ANOVA on ranks was applied. This was also used to compare the individual treatments at each time event between late and early summer, for which type II sums of squares are reported to account for the unequal sample sizes between the late and early summer groups. In cases where differences were significant ($p<0.05$), Dunn's all-pairwise multiple comparisons tests were used to determine wherein those differences occurred. All statistical analyses were performed in SAS 9.1© or SigmaStat© 3.5.

3. Results

3.1. Mortality and morphometrics

No plants in the salinity 10 treatments survived past day 14 in any of the three 1-month runs. Mortality was low in the salinity 20 and 30 treatment plants, ranging from 4-8% of the total plants in each salinity (20 and 30) treatment over the 28-day experimental period, and there were no significant differences in mortality between these treatments over the three 1-month experimental periods.

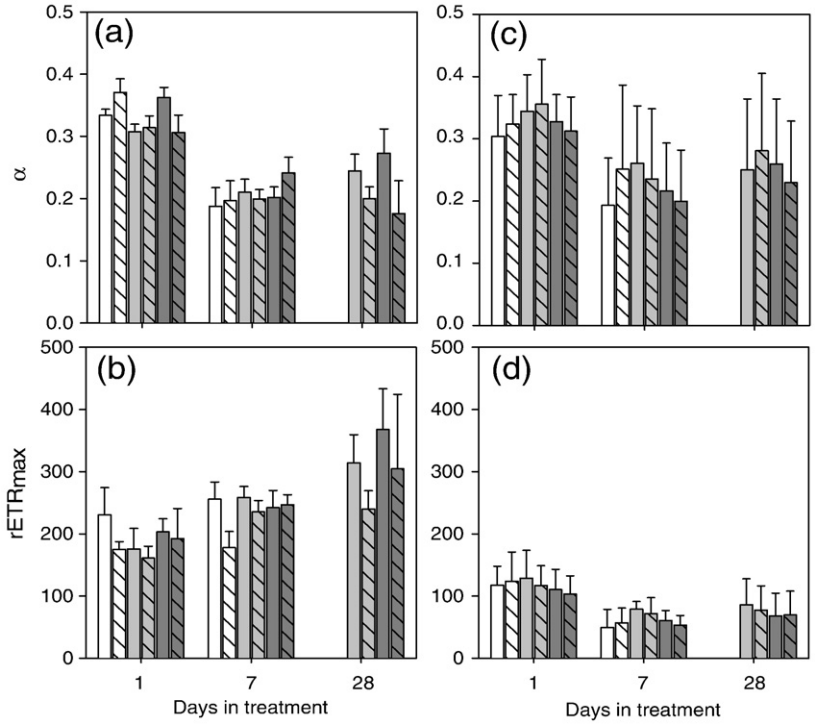

Fig. 1. *Halophila johnsonii* average values (+sterr) for α (top) and $rETR_{max}$ (bottom) for early (a, b) and late (c, d) summer over the experimental period. Treatments are salinity 10 (unfilled), 20 (light grey) and 30 (dark grey) and non-color (solid) and colored (B) (hatched).

Morphometric measurements of the sampled leaves were not significantly different among treatments at day 1, day 7 or at the end of the experimental period (28 days), nor did they vary significantly among the three months. The average blade length and width (±stdev) were 12.9 mm (±0.93) and 2.15 mm (±0.21) respectively and the average leaf area was 21.8 mm^2 (±2.7).

3.2. Photosynthetic responses

3.2.1. Early summer

Early summer plants exhibited greater values of $rETR_{max}$ over the course of the experiment than did late summer plants (compare Fig. 1b and d). In both CDOM and non-CDOM salinity 20 and 30 treatments, the greatest values of $rETR_{max}$ among the three sampling periods were observed on day 28; significantly so in treatments 20 and 30 compared to day 1 (Fig. 1b, $F=8.38$, $p=0.007$ and $F=5.59$, $p<0.001$ respectively). The values of $rETR_{max}$ on day 28 for the 20B and 30B treatments were slightly lower than the non-CDOM treatments. Salinity treatments 20 and 30 had significantly higher values of α on day 1 than days 7 or 28 (Fig. 1a, $F=13.73$, $P<0.001$ and $F=21.51$, $p<0.001$, respectively). Although on day 28 α values were greater in the non-CDOM than CDOM treatments in both salinities, the differences were not significant. In salinity 10, both CDOM and non-CDOM treatments exhibited a significant decrease in α from day 1 to day 7 (Fig. 1a, $F=47.32$, $p<0.001$). Also, in both treatments 10 and 10B, $rETR_{max}$ values were slightly, but not significantly greater on day 7 than day 1 (Fig. 1b). For plants in salinity 20 and 30 treatments that survived the 28-day experimental period, there were no significant treatment effects detected for any of the photosynthetic parameters within each time period (day 1, 7, 28).

3.2.2. Late summer

Both $rETR_{max}$ and α exhibited significant decreases from day 1 to day 7 for all salinity treatments (Fig. 1c and d, $rETR_{max}$: $F=38.77$, $F=23.53$, $F=29.38$, all $p<0.001$; α: $F=19.80$, $F=13.34$, $F=17.70$, all $p<0.001$ for salinity treatments 10, 20 and 30, respectively). Within each time period (day 1, 7 and 28), $rETR_{max}$ values were greater, though not significantly, at salinity 20 than 30 and 10 on day 7 and remained slightly greater by day 28 (Fig. 1d). No significant CDOM effects were observed among treatments for any of the parameters on any of the days nor were there any significant CDOM × salinity interactions.

3.3. Pigment analyses

3.3.1. Early summer

There was no significant CDOM effect for any pigments on day 1 (Fig. 2a, b and c). UVP levels on day 1 were higher, though not

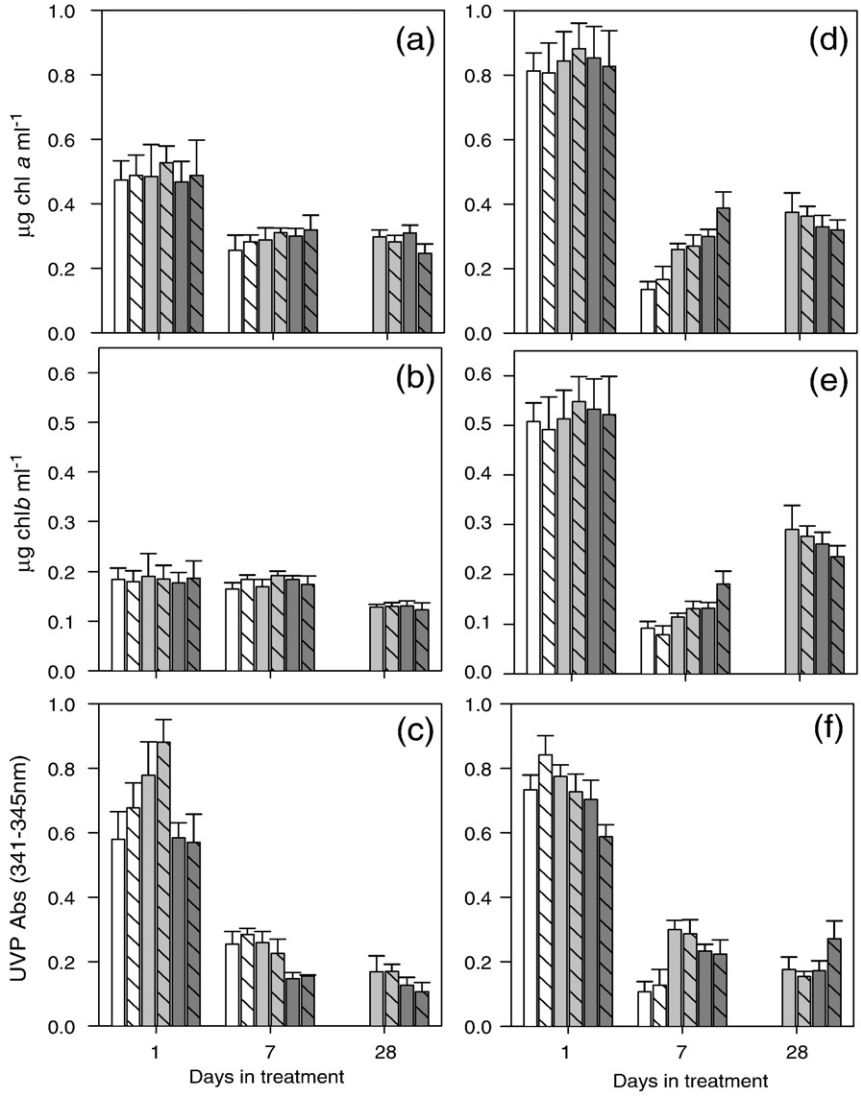

Fig. 2. *Halophila johnsonii* average values (+sterr) for chlorophylls *a* (top) and *b* (middle) and UVP absorbance (bottom) for early (a, b and c) and late (d, e and f) summer over the experimental period. Treatments are salinity 10 (unfilled), 20 (light grey) and 30 (dark grey) and non-color (solid) and colored (B) (hatched).

significantly, in plants in salinity 20, regardless of color treatment (Fig. 2c). Day 7 UVP values were significantly greater in both salinity 10 and 20 treatment plants than salinity 30 ($F=4.65$, $p=0.0156$). There was again, no CDOM effect observed. By day 28, plants in salinity 30 showed lower levels of UVP than salinity 20 plants, though it was not significant. There were also no significant CDOM or CDOM×salinity interaction effects. Day 1 values for chlorophyll *a* in all salinities (10, 20 and 30) were significantly greater than days 7 or 28 (Fig. 2a, $F=18.03$, $p<0.001$; $F=10.42$, $p<0.001$; and $F=9.11$, $p<0.001$ respectively). Values for UVP were also significantly greater on day 1 than either day 7 or 28 for all treatments (Fig. 2c, $F=33.51$, $F=63.59$, $F=16.25$, all $p<0.001$ for salinity 10, 20 and 30, respectively).There were no significant differences in chlorophyll *b* observed among treatments on any measured day over the duration of treatments (Fig. 2b). Although there was a general decline in chl *b* from day 1 to day 28, the change was not significant.

3.3.2. Late summer

On day 1, in late summer, there were no significant differences in pigment characteristics among any treatments (Fig. 2d, e and f). From day 1 to day 7, there were decreases in chlorophyll *a* and *b* and UVP levels observed in all treatments. On day 7, plants from treatment salinity 10 had significantly lower concentrations of chlorophylls *a* and *b* than those from the other two salinity treatments (Fig. 2d and e, $F=12.92$, $p<0.001$, and $F=7.61$, $p=0.0015$, respectively). These plants also had significantly lower UVP levels than plants at salinity 20 or 30 (Fig. 2f, $F=9.22$, $p<0.001$). No significant differences were observed among the two remaining treatments by day 28 and there were no significant differences observed between CDOM and non-CDOM treatments during the entire month-long experimental periods. Day 1 concentrations of both chlorophyll *a* and *b* were significantly greater in late summer than early summer in all treatments (Fig. 2d and e compared to 2a and b, $F=48.22$ and $F=15.77$, respectively $p<0.001$ for both), but there was no significant difference in UVP levels between the two periods (compare Fig. 2c and f).

3.4. Irradiance

Average mid-day values for irradiance (PAR) in early and late summer are shown in Fig. 3. For sampling days 7 and 28, early summer values of PAR were significantly greater than late summer ($p<0.05$). In early summer, days 7 and 28 PAR values were significantly greater ($p<0.05$) than day 1. There was no significant difference between day 7 and 28 values in the early summer. In the late summer, average PAR on day 28 values was significantly lower ($p<0.05$) than at days 1 or 7, which were not significantly different from each other. There were no significant differences in percent absorption of surface irradiance at 412 nm

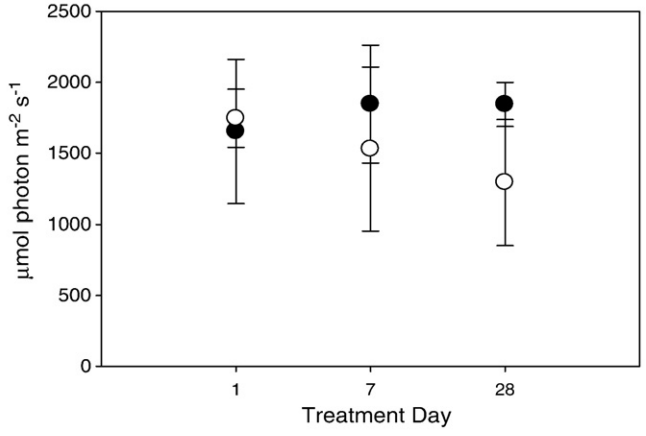

Fig. 3. Average (±stdev) PAR levels for early (filled circles) and late (open circles) summer on each day of treatment (n=180).

the CDOM treatment among salinity treatments, although the values increased with decreased salinity (46%±2.7, 47%±6.8, 49%±5.3 reduction relative to subsurface irradiance for salinities 30, 20 and 10 respectively). There were also no significant changes in percent absorption of surface irradiance over the experimental period in the CDOM treatments. There was a significant difference between light absorption of 412 nm in the CDOM treatments vs. non-CDOM (average 10%±1.5 reduction relative to subsurface irradiance) treatment ($p<0.001$).

4. Discussion

The results of this mesocosm study support previous conclusions that *Halophila johnsonii* is not tolerant of prolonged exposure to hyposalinity conditions (Dawes et al., 1989; Torquemada et al., 2005). Furthermore, a significant decrease in short wavelength irradiance from increased CDOM did not significantly affect photophysiological responses or reduce mortality rates of *H. johnsonii* in either of our hyposaline treatments, indicating that decreased UV-stress does not affect hyposalinity tolerance. All plants died in the salinity 10 treatment within 10 days, regardless of the presence or absence of CDOM. Thus, within the optical environments created in this study, decreased short-wavelength irradiance did not significantly affect survival, physiological responses or morphology in this seagrass.

Halophila johnsonii photophysiology did respond to differences in PAR levels between our early and late summer experiments and appeared to acclimate to longer-term (monthly) differences in PAR. Durako et al. (2003) and Kunzelman et al. (2005) suggested that *H. johnsonii* is high-light adapted and exhibits rapid responses in both alpha and $rETR_{max}$ to changes in the light field. Because the PAM measurements were made during the same time of day within each replicated one-month experiment, our results show that $rETR_{max}$ reflects long-term light history as well. The significantly higher PAR values observed in the early summer corresponded with the overall higher values of $rETR_{max}$. *Halophila stipulacea* also shows an increase in $rETR_{max}$ with increased irradiances while *H. ovalis* exhibits a high tolerance to high irradiance, exhibiting little mid-day depression in ETR at high afternoon irradiances (Beer et al., 1998; Beer and Björk, 2000). Campbell et al. (2007) observed an increase in $rETR_{max}$ with an increase in photosynthetic flux density for seagrasses in intertidal habitats. *Halophila johnsonii* also occurs in intertidal habitats and responded in a similar manner photosynthetically to the increased light field of the early summer.

The decrease of pigments, mainly chlorophyll *a* and UVP between day 1 and 7 in both early and late summer, is most likely a result of transplanting stress. Reduction of chlorophyll concentration commonly occurs as a general plant response to stress (Carter and Knapp, 2001). Seagrasses also generally decrease chlorophyll concentration with increased irradiance (Abal et al., 1994; Czerny and Dunton, 1995; Longstaff and Dennison, 1999) and *H. ovalis* decreases chloroplast numbers under increased UV conditions (Dawson and Dennison, 1996). In our study, seasonal higher-light light acclimation may have been the cause of lower initial levels of chlorophylls *a* and *b* in early, compared to late, summer.

Responses in the UV pigment levels were not consistent between early and late summer. In early summer there was an increase in UVP with decreased salinity after 7 days in treatment, however in late summer, the plants at salinity 10 showed lower levels of UVP than the other two salinity treatments. This indicates that salinity may affect flavonoid production, but as it was not consistent among salinity treatments, other factors may also affect production. It was clear that differences in UVP levels were not due to decreased levels of UV irradiance as there were no detectable CDOM effects in any of the experiments. In contrast, both *Halophila ovalis* and *Thalassia testudinum* increase UV-blocking pigment levels under increased UV radiation (Dawson and Dennison, 1996; Detrés et al., 2001). It appears

that under the optical environment in our study, despite the large differences in light attenuation at 412 nm between the colored and non-colored treatments, UVP levels in *H. johnsonii* did not respond to the change in UV light field. This supports the conclusion of Kunzelman et al. (2005) that in *Halophila johnsonii* the primary physiological role of the flavonoids may not be UV protection.

Halophila johnsonii produces a suite of flavonoids that exhibit very high absorption relative to chlorophyll indicating that they probably serve an important physiological function, one of which may be as UV-protective pigments (Durako et al., 2003; Krzysiak, 2006). However, flavonoids are known to have other ecological and physiological functions (Rozema et al., 2002), including acting as anti-oxidants and growth regulators and flavonoid production may increase in response to general plant stress (Yamasaki et al., 1997; Harborne and Williams, 2000). Our study does not support their primary role as a UV-photoprotector, but does suggest that changes in environmental conditions (e.g., salinity) contribute to variations in the production of these flavonoid compounds.

Salinity stress in our experiments was not mitigated by a decrease in UV-radiation (increased CDOM) as *H. johnsonii* continued to invest energy in production of the flavonoids regardless of the level of UV exposure. Our results indicate that prolonged hyposalinity, either due to regulated freshwater discharge or hurricanes/stochastic storm events, may be the most detrimental impact for this species and is an important environmental aspect to manage to maintain suitable habitat for *H. johnsonii*. It is also clear that the flavonoid compounds produced by *H. johnsonii* serve another (or multiple other) physiological functions in this seagrass. To better understand the large metabolic investment in the production of these carbon-rich compounds, further investigations into production-pathways, allocation and localization of the flavonoids are needed.

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