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RESEARCH ARTICLE

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Irradiance-induced changes in the photobiology of *Halophila johnsonii*

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Abstract The endangered seagrass *Halophila johnsonii* Eiseman, exhibits high-light adapted photophysiology consistent with its distribution in intertidal and shallow subtidal (0–3 m) coastal-lagoon habitats along 200 km of southeastern Florida. To examine the short-term responses of this seagrass to three controlled-irradiance treatments (PAR + UVA + UVB [full spectrum], PAR + UVA, and PAR only), greenhouse-acclimated plants were transferred to outdoor mesocosms during July–August 2002. Chlorophyll fluorescence, UV fluorescence, and samples for pigment extraction were collected in the greenhouse, prior to moving the plants outside and on days 1, 2, 3, 4, 6, 10, and 21 of the 24-day experiment. Typical of sun-adapted plants, effective quantum yields measured by pulse-amplitude modulated (PAM) fluorometry were relatively low in all treatments, ranging from 0.46 ± 0.09 (PAR only) to 0.58 ± 0.08 (PAR + UVA + UVB). In the PAR only treatments, there were strong effects on days 1 and 4, presumably because the irradiance in the greenhouse not only lacked all $\lambda < 400$ nm, but also had low irradiance maxima (~700 μmol photons m^{-2} s^{-1}, compared with ~1,500 μmol photons m^{-2} s^{-1} outside at midday). There were few treatment differences between PAR only and PAR + UVA treatments indicating little effect of UVA radiation on this species. Differences in effective quantum yields and relative electron transport rates between the PAR only and PAR + UVA + UVB treatments on day 4 indicated rapid acclimation to UVB radiation. Tissues of *H. johnsonii* contained compounds that absorbed strongly in the UV, with a λ_{max} at ~345 nm (depending on the extraction solvent). Absorption peak maxima and minima changed over the course of the experiment but there were no significant light-treatment differences in any pigment parameters. Percent UV shield values, measured using a newly developed UVA PAM fluorometer, were highest the day after plants were transferred from the greenhouse to the outdoor mesocosms and declined significantly to pre-treatment levels in all treatments by day 21. Percent UV shield exhibited a significant positive relationship with UV-absorbing pigment (UVP) absorbance, however, the absence of treatment effects suggests that the wavelengths inducing pigment synthesis must lie between 400 and 700 nm (PAR). The results indicate that *H. johnsonii* rapidly acclimates to high UVB and PAR which may largely explain its distribution in intertidal and shallow subtidal areas.

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Introduction

Halophila johnsonii is one of the rarest seagrasses in the world. It is patchily distributed in the intertidal and shallow subtidal (0–3 m) in coastal lagoons of southeastern Florida from Sebastian Inlet (27°51′N, 80°27′W), 200 km south to Virginia Key in southern Biscayne Bay (25°45′N, 80°07′W) (Eiseman and McMillan 1980). *H. johnsonii* is the least abundant of the seven Florida seagrass species within its restricted range of distribution (Virnstein et al. 1997), and it is listed as a threatened species under the Endangered Species Act (Federal Register 1998), the only marine plant with this designation.

Because populations of *H. johnsonii* establish in more extreme shallow environments (Dawes et al. 1989; Virnstein et al. 1997), they may be exposed to high irradiance levels, strong currents, high sediment movement, and extensive human activity. Intertidal populations of *H. johnsonii* tolerate fluctuations between low-tide exposure and high-tide submergence. Low-tide conditions include high irradiance and UV levels and possible desiccation. During high tide, plants may be exposed to either high, or drastically lower, irradiance levels. This variability is due to several different factors, but particularly tidal height (~1 m in southeast Florida) and the optical properties of the water (Koch and Beer 1996). Comparatively, subtidal populations are exposed to less daily fluctuation and lower irradiance levels overall. *H. johnsonii* does not exhibit high-light induced photosynthetic downregulation as does *H. decipiens* (Dawes et al. 1989; Durako et al. 2003). The ability to grow in such different sets of irradiance conditions between the intertidal and shallow subtidal environments lead us to hypothesize that *H. johnsonii* maintains some mechanism of photosynthetic phenoplasticity, particularly under high irradiance and UV conditions.

In a previous study (Durako et al. 2003), we investigated the photosynthetic efficiency of *H. johnsonii* and *H. decipiens* populations distributed at different depths. A reciprocal transplant experiment between intertidal and subtidal populations was also performed to assess plasticity in response to changes in the ambient light environment. Effective and maximum quantum yields, derived from chlorophyll fluorescence of photosystem II, were measured using a submersible Pulse Amplitude Modulated fluorometer (Diving-PAM, Walz, Germany). The Diving-PAM data indicated that *H. johnsonii* adapted to short-term changes in the ambient light environment and tolerated high irradiances. In response to the 4-day acclimation period of the reciprocal transplant experiment, maximum photosynthetic quantum yields (F_v/F_m) increased for intertidal plants transplanted into the subtidal while maximum relative electron tranport rates increased for subtidal-to-intertidal transplants. Acetone extracts of *H. johnsonii* leaves absorbed maximally at 345 nm; UV absorption was absent from extracts from the co-occurring congener, *H. decipiens*. The UV absorption maximum increased significantly when *H. johnsonii* was transplanted from the subtidal to the intertidal and decreased from intertidal-to-subtidal transplants. Durako et al. (2003) suggested that photosynthetic tolerance to high irradiances and the presence of UV-absorbing pigments may allow *H. johnsonii* to exploit the shallowest waters without competition from *H. decipiens*.

Literature available on the UV-responses in different marine plants is abundant and highly varied. There are generally two strategies for coping with UV radiation, protecting UV-sensitive tissues from the damage before it happens, and recovery from the damage after exposure (Vincent and Roy 1993). There are several different physiological mechanisms for acclimation to increased PAR and UV irradiance, the most common being the accumulation of UV protecting compounds. How well individual species acclimate reflects conditions to which they are evolutionarily adapted and the mechanisms available for each to employ upon short-term changes in their light environment.

The effect of UV and PAR has been examined for several seagrass species (Trocine at al. 1981; Larkum and Wood 1993; Dawson and Dennison 1996) and it has been suggested that sensitivity to UV radiation and PAR may influence seagrass depth distribution (Dawson and Dennison 1996). The present study focused on developing a better understanding of the physiological ecology of UV and PAR on *H. johnsonii*, a species known to have UV-absorbing pigments. We investigated whether UV irradiance affects the photophysiological and photochemical responses of *H. johnsonii*. Specifically, we examined whether there is: (1) a difference in the photosynthetic efficiency of plants exposed to PAR + UVA + UVB, PAR + UV and PAR only, (2) a difference in the pigment absorption spectra of plants exposed to PAR + UVA + UVB, PAR + UV and PAR only, and (3) whether there is a relationship between the level of UV radiation and UV absorption/protection in the leaves of *H. johnsonii*.

Materials and methods

Sample collection

H. johnsonii Eiseman was collected on 30 June 2002 from Haulover Park in northern Biscayne Bay (25°55′N, 80°07′W) from approximately 1–2 m depth. A 10×10 cm^2 sod plugger was used to extract at least one rhizome segment of *H. johnsonii* with at least four leaf pairs and one apical meristem from a monospecific bed. The segment, with rhizome and sediments intact, was placed in a 10×10 cm^2 peat pot. Peat pots were placed in coolers containing seawater for transport back to the Center for Marine Science greenhouse.

Once in the greenhouse, peat pots were cultivated in fiberglass troughs (115 cm L × 60 cm W × 25 cm D) with batch seawater maintained at 30°C and 29 psu, with ~10 cm water depth above the seagrass leaves . The greenhouse glass eliminated solar UV radiation ($\lambda < 400$ nm) exposing plants to PAR-only conditions for 2 weeks prior to the irradiation experiment. Maximum midday irradiance on a cloudless day averaged 700 μmol photons m^{-2} s^{-1} in the greenhouse during this period.

Experimental design

A total of 54 replicate peat pots were evenly distributed in three fiberglass troughs (115 cm L × 60 cm W × 25 cm D), arranged in east–west orientation on an outdoor platform adjacent to the Center for Marine Science greenhouse. The troughs were equipped with a

flow-through filtered seawater system that maintained a near-constant 30°C and 29 psu environment. The outdoor experiment utilized the incident solar spectrum with different filter panels constructed for the three irradiance treatments. Polycarbonate sheeting eliminated $\lambda < 400$ nm (UVA, UVB, UVC), exposing plants to PAR only. Mylar film eliminated $\lambda < 320$ nm (UVB, UVC), exposing plants to PAR + UVA. Cellulose acetate film was used to eliminate $\lambda < 280$ nm (UVC), which are also largely removed by atmospheric ozone, exposing plants to the full solar spectrum of PAR + UVA + UVB. None of the filter panel treatments significantly reduced the total intensity of the transmitted wavelengths across PAR.

Each treatment was replicated in each of the three troughs in a random block design to minimize the effects of spatial placement within the troughs. All 54 replicates were grown under one of the three irradiance treatments for 24 days from 18 July through 10 August 2002. Total spectral irradiance between 250 and 700 nm was measured in the treatment troughs using a fiber optic spectroradiometer connected to a cosine-corrected irradiance probe (Ocean Optics S2000, Dunedin, Florida, USA). PAR intensity was logged every 15 min by a Li-Cor Pyranometer Quantum Sensor (LI190SB; LiCor Instruments, Lincoln, Nebraska, USA) located at the NC NERR weather station (34°09′N, 77°51′W). Maximum midday irradiance on cloudless days averaged ~1,500 µmol photons m^{-2} s^{-1} on the outside platform.

Chlorophyll fluorescence measurements, UV fluorescence measurements, and samples for pigment extraction were collected in the greenhouse prior to the plants being moved outside (day 0), and on days 1, 2, 3, 4, 6, 10, and 21 of the experiment. From each peat pot, one leaf pair was randomly selected for fluorescence measurements. Following these measurements, three leaf pairs were randomly selected from each of the three treatments for pigment analysis. These measurements were all made between 13:00 and 16:00 h when irradiance levels were least variable.

Chlorophyll fluorescence measurements

Fluorescence was measured with a portable pulse amplitude modulated (mini-PAM) fluorometer (Walz, Germany). Short-term photosynthetic responses to increasing irradiance levels were measured using rapid light curves (RLCs). In order to minimize the effects of epiphyte cover and age-related differences in photosystem development, samples were standardized by using leaf pairs from the second node back from a primary apical bud. The tip of the instrument's fiber optic was placed ca. 2 mm from, and perpendicular to, the adaxial leaf surface using dark leaf clips (DLC). These clips were used to standardize the geometry of the leaf surface illuminated and to exclude ambient light during the RLC. Leaves were not dark-acclimated in order to assess their photophysiological state under the ambient light environment. The halogen lamp in the PAM simulates photosynthetic photon flux density (PPFD) and the internal RLC program produces nine discrete irradiance steps at 5-s intervals: 0, 135, 201, 279, 419, 578, 890, 1310 and 1905 µmol photons m^{-2} s^{-1} (values were validated using a calibrated cosine-corrected quantum sensor). Effective photosynthetic quantum yield (Y; ([Fm- F]/Fm)) was determined prior to the first illumination step, in the absence of actinic illumination, and following each consecutive illumination period. Relative electron transport rate (RETR, Beer et al. 2001) was estimated using the following equation:

$$\mathrm{RETR} = ([\mathrm{Fm} - \mathrm{F}]/\mathrm{Fm}) * \mathrm{PPFD} * 0.5 * 0.84$$

where Fm = light-acclimated maximal fluorescence, F = fluorescence yield for a given light state, PPFD = intensity of PAR at the corresponding RLC irradiance step, 0.5 assumes half of the photons are absorbed by photosystem II, and 0.84 = averaged absorption factor (AF) of terrestrial leaves, the instrument's default setting. Actual reported values of AF from seagrasses range from 0.44 to 0.72 (Beer et al. 1998; Durako and Kunzelman 2002; Runcie and Durako 2004). In the present study the instrument's default value was used because all samples were collected from a single, continuous population at uniform depth. RETR in µmol electrons m^{-2} s^{-1} were plotted against PPFD µmol quanta m^{-2} s^{-1} . Linear regression lines were fit separately to each of three regions, alpha (α; the first 4 irradiance steps), Maximum ETR (P_{max}; the y-intercept of the middle three-to-five irradiance steps), and beta (β; the last four irradiance steps).

UV fluorescence measurements

A recently developed UVA PAM fluorometer (Gademann Instruments, Germany) was used to investigate epidermal transmittance of UV radiation on the basis of chlorophyll fluorescence. Based on the theory that UV screening efficiency suppresses UV excited chlorophyll fluorescence, this instrument compares the fluorescence amplitude of a leaf sample held by a dark clip from UVA excitation (375 nm) with that of blue-green excitation (470 nm). Development of this technique was based on epidermal peels from various terrestrial plant leaves (Bilger et al. 1997; 2001). Since seagrass chloroplasts are located in the epidermal tissue, it has not yet been established whether this technique is appropriate for seagrasses.

Immediately following PAM RLCs, UVA PAM measurements were made on the adjacent leaf of the same leaf pair sampled. The tip of the instrument's fiber optic was placed ~2 mm from, and perpendicular to, the adaxial leaf surface using the DLC. The UVA PAM utilizes light-emitting diodes for quasi-simultaneous excitation of chlorophyll fluorescence by λ 375 nm and 470 nm. The measuring light does not induce significant reduction of the primary PSII acceptor Q_a. Hence, the

measured fluorescence is close to the dark-level fluorescence yield. Fluorescence is measured by the photodiode detector at $\lambda > 650$ nm. Percent UV shield is a relative measure of photosystem protection from UV radiation and is calculated from the following equation:

$$\text{UV-Shield}(\%) = 100(1 - \text{F[UV]}/\text{F[BL]})$$

where F[UV] = dark-level fluorescence yield with 375 nm excitation, and F[BL] = dark-level fluorescence yield with 470 nm excitation.

Pigment extraction and analysis

For pigment analysis, leaf pairs from the second node back from an apical were collected, manually cleaned of any epiphytes and kept dark prior to extraction. Each sample was weighed to determine wet weight and then ground in 4 ml of 100% methanol using a chilled mortar and pestle in the dark. Leaf petioles, rhizome segments and roots were also extracted separately to determine pigment distribution patterns. Methanol extracts were poured into graduated centrifuge tubes wrapped in aluminum foil and stored at 4°C overnight to allow the suspended material to settle. Absorbance spectra (250–750 nm) of the supernatants were measured in a 1-cm quartz cuvette illuminated by a halogen/deuterium light source using a fiber optic spectrometer (Ocean Optics S2000, Dunedin Florida, USA). Absorbance spectra were corrected for scattering by subtracting the 750 nm absorbance and analyzed by integrating the area under the curve using a user-defined area transform procedure in Sigma Stat (Jandel Scientific, San Rafael, California, USA). Absorbance areas were normalized by wet weight. Wellburn's (1994) extinction coefficient equations were used to calculate chlorophylls *a* and *b*, and total carotenoids.

Data analysis

Fluorescence and pigment data were statistically analyzed with SigmaStat 2.0 (Jandel Scientific, San Rafael, California). A 95% probability level ($p < 0.05$) was chosen to determine statistical significance. Normality was tested using the Kolmogorov-Smirnov test with Lilliefors' Correction. Homogeneity of variance was tested using the Levene Median test. One-way ANOVAs were used to analyze the effect of the independent variables, irradiance treatment and time, on the dependent variables, PAM-fluorescence, RLC regression parameters, and pigment data. Non-parametric analyses (ANOVA on ranks) were used when the normality or homogeneity of variance tests failed. Significant factors were tested pairwise by Tukey's multiple comparison procedure or Dunn's multiple comparison procedure on parametric and nonparametric data, respectively.

Results

Rapid-light curves

Halophila johnsonii, acclimated for 14 days in a greenhouse where all light < 400 nm was eliminated and PAR was relatively low (700 µmol photons m^{-2} s^{-1} in the greenhouse vs 1,500 µmol photons m^{-2} s^{-1} in the mesocosms), exhibited strong photosynthetic responses in the first 4 days after being placed in outside mesocosms with increased PAR, PAR + UVA and PAR + UVA + UVB (Figs. 1, 2). The rapid light curve measurements of *H. johnsonii* exposed to the three irradiance treatments revealed little difference between PAR only and PAR + UVA, indicating little effect of UVA radiation. On day 0 and after 21 d of exposure to PAR only, *H. johnsonii*'s mean RLC RETRs were higher at all irradiance levels than on days 1 and 4 (Fig. 1a). Under exposure to PAR + UVA, mean RLC RETRs at all irradiance levels were similar during the 21-day experiment (Fig. 1b). However, treatment effects could be seen between the PAR only and PAR + UVA + UVB treatments, especially in the first 4 days. Under exposure to PAR + UVA + UVB, mean RLC RETRs were generally lowest on day 1, highest on day 4, and intermediate on days 0 and 21 (Fig. 1c). All replicates had similar RLC RETRs at each irradiance level on day 0 (Fig. 2a). Mean RLC RETRs of *H. johnsonii* exposed to PAR + UVA were higher at intermediate irradiances on day 1, compared to those from the PAR only and the PAR + UVA + UVB treatments (Fig. 2b). On day 4, mean RLC RETRs increased with increased UV (Fig. 2c). The PAR + UVA + UVB treatment had the highest RETRs while the PAR only treatment had the lowest RETRs at all irradiances. By day 21, this pattern reversed, mean RLC RETRs of *H. johnsonii* exposed to PAR only were highest and those in the PAR + UVA + UVB treatments were lowest (Fig. 2d).

Regression analyses of RLCs from *H. johnsonii* provided information on induction, saturation, and inhibition. The slopes of the alpha (α) regression lines, the maximum RETR (P_{max}), and the slopes of the beta (β) regression lines are listed (Table 1). In the PAR + UVA + UVB treatment, α increased early in the acclimation process and by day 21 was similar to the PAR-only treatment. In the PAR only and PAR + UVA + UVB treatments, α was significantly higher on day 21 than on either day 1, or day 4 ($p < 0.001$; $p = 0.039$). In the plants grown under PAR + UVA, α did not significantly change. Only on day 4 was there a significant difference among the three treatments ($p = 0.003$), with the PAR + UVA + UVB treatment exhibiting a significantly higher α than the PAR only and PAR + UVA treatments. There were no significant temporal differences between maximum RETR within each of treatments. On day 4 there was a significant difference among the three treatments ($p = 0.045$), again with the PAR + UVA + UVB treatment having the

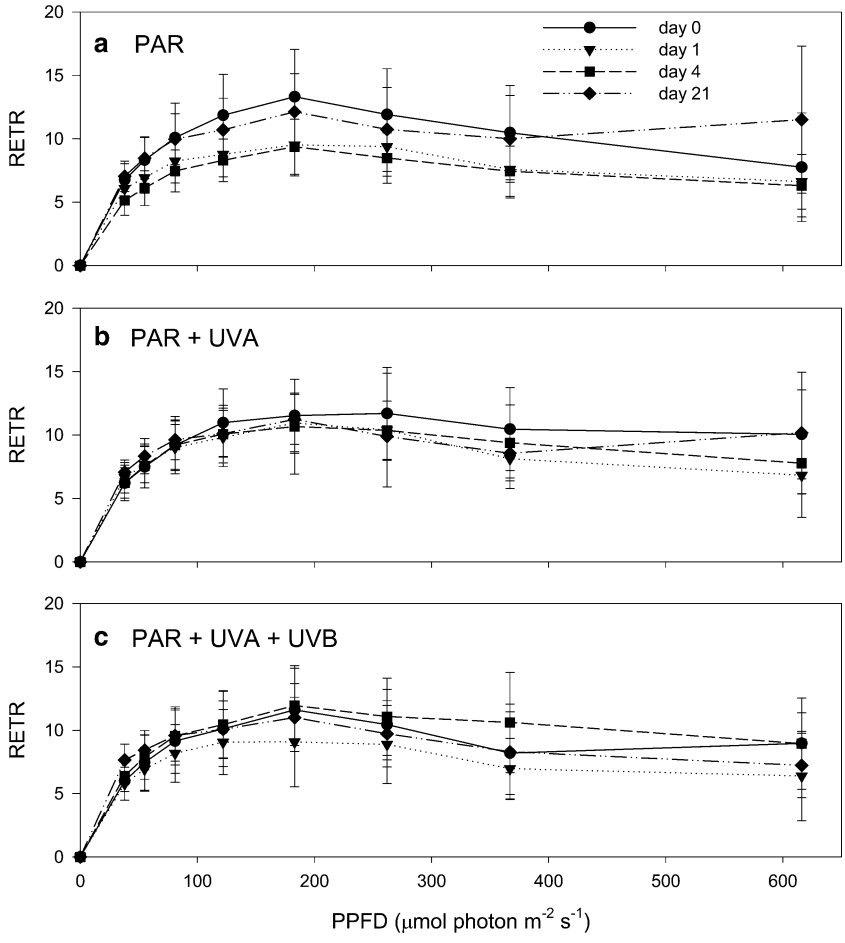

Fig. 1 *Halophila johnsonii*. Rapid light curves for plants grown under **a** PAR, **b** PAR + UVA, and **c** PAR + UVA + UVB on day 0, 1, 4, and 21. *Symbols* = mean, *error bars* = SD, $n = 18$

highest RETR values; the PAR only and PAR + UVA treatments were not significantly different from each other. There was little high-light induced downregulation evident in the RLCs. Beta values were low and did not change significantly during the 21 d of the experiment, or vary among treatments.

The first yield measurement of the RLC represents the effective photosynthetic quantum yield (Y). There was no significant change in Y exposed to PAR-only or PAR + UVA during the 21-day experiment. Y increased over the course of the experiment in the PAR + UVA + UVB treatment and it was significantly higher on day 21 than on day 0 and day 1 ($p = 0.002$). The PAR only treatment had a significantly lower Y than the PAR + UVA + UVB treatment on day 4 ($p = 0.024$); at day 4 and 21, Y increased with increased UV.

UV fluorescence

The UV shield values in *H. johnsonii* increased between day 0 and 1, followed by a decrease between day 1 and 21, in all three treatments (Table 1). In the plants grown under PAR only and PAR + UVA, percent UV shield was significantly higher on day 1 than on day 0, 10, and 21 ($p = 0.004$; $p < 0.001$). Percent UV shield was significantly higher on day 1 than on 0, 4, 10, and 21-day exposure to the PAR + UVA + UVB treatment ($p < 0.001$). There were no significant differences among the treatments on any day of the experiment.

UV-absorbing pigment analysis

Extractions of *Halophila johnsonii* tissues contained a compound, or compounds, that absorb strongly in the UV range (Table 2). This compound, or mixture of compounds, was located not only in the leaves, but also in the petioles, rhizomes, roots, as well as the apical buds (data not shown). Pigment extractions of apical meristems absorbed only in the UV range (i.e., no chlorophyll was present). Absorption spectra for leaf tissue extracted in 100% methanol had λ_{max} at 343 nm, slightly shifted from extracts in 90% acetone which had λ_{max} at 345–350 nm. Even in the same solvent, UV-absorbing peaks from different plants were not identical. Some spectra had a well-defined shoulder while others did not.

Because peak maximums and minimums shifted within a range of several nm during the experiment, integrated area under the spectral curves was used to compare UV peak 1 to UV peak 2 responses (Fig. 3).

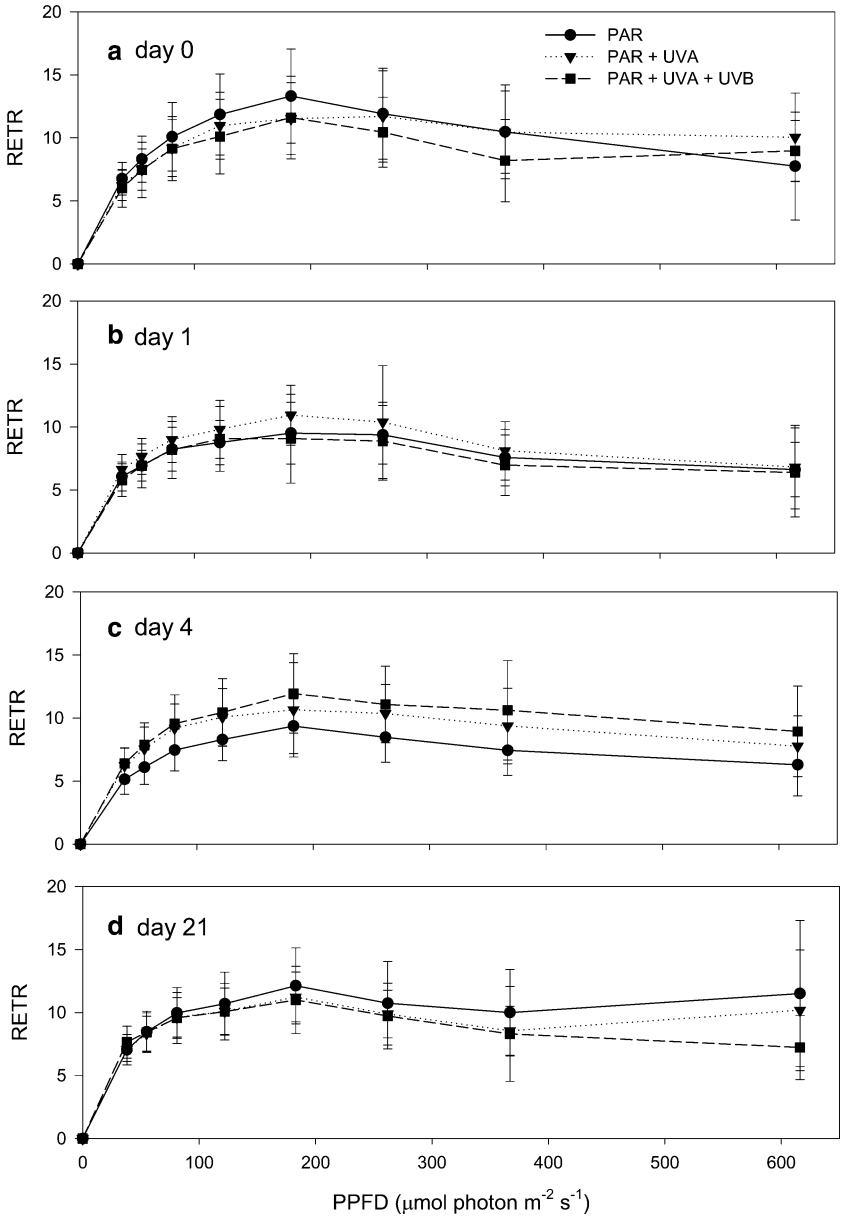

Fig. 2 *Halophila johnsonii.* Rapid light curves for plants grown under PAR, PAR + UVA, and PAR + UVA + UVB on **a** day 0, **b** day 1, **c** day 4, and **d** day 21. *Symbols* = mean, *error bars* = SD, **n** = 18

Patterns of UV absorption changed during the 21-day experiment for the three treatments (Table 2). While there were few significant differences among the different days of measurement, some patterns emerged. Both total spectral area and total UV absorbance increased from day 0 to day 1, followed by a general decrease over the 21-day experimental period. The ratio of UV peak 1 to UV peak 2 also increased from day 0 to day 1, followed by a decrease in day 2 values in the PAR and PAR + UVA treatments. The ratios fluctuated slightly during the second and third week of the experiment and equalled day 2 values by the day 21 measurement. There were no significant differences in any UV pigment parameters among the three treatments on any day of the experiment.

Comparing the total area of UV absorbance to the percent UV shield values, as measured by the UVA PAM (Fig. 4), showed that these two variables were positively correlated ($r^2 = 0.61$) and both characteristics exhibited a general decline from day 1 to day 21.

Chlorophyll and carotenoid analysis

Both chlorophyll and carotenoid concentrations followed the same general pattern as the UV absorbance (Table 2). Chlorophyll *a*, chlorophyll *b*, and total carotenoids increased from day 0 to day 1, followed by a general decrease to day 21 measurements. There were no dramatic changes in the chl *a*/*b* ratio between days 0, 1, or 2. Values increased in all three treatments on day 3, and then returned to day 0 levels on days 4, 6, and 10. Mean chl *a*/*b* ratio increased dramatically on day 21 in the PAR only treatment. There were no significant

Table 1 *Halophila johnsonii*. Summary of fluorescence parameters examined in outdoor irradiance experiment. One-way ANOVA results comparing three irradiance treatments on each day and also the different days of the experiment within each treatment

Irradiance treatment							
Fluorescence parameters		PAR		PAR + UVA		PAR + UVA + UVB	
	day						F
α	0	0.16 ± 0.03		0.14 ± 0.02		0.14 ± 0.04	0.48
	1	0.13 ± 0.02		0.15 ± 0.03		0.13 ± 0.03	1.56
	4	0.12 ± 0.03		0.14 ± 0.03		0.15 ± 0.03	6.35**
	21	0.16 ± 0.03		0.16 ± 0.03		0.16 ± 0.03	0.09
	F		8.42***		< 0.001		2.97*
RETR max	0	13.32 ± 3.73		12.42 ± 3.27		11.70 ± 3.09	0.37
	1	10.81 ± 2.72		12.56 ± 2.93		10.16 ± 4.90	1.99
	4	10.36 ± 2.68		11.95 ± 3.39		13.02 ± 3.36	3.29*
	21	11.43 ± 3.61		10.33 ± 3.45		12.01 ± 3.31	1.00
	F		1.64		1.62		1.71
β	0	0.01 ± 0.01		0.00 ± 0.01		0.01 ± 0.00	2.48
	1	0.01 ± 0.00		0.01 ± 0.00		0.01 ± 0.01	0.42
	4	0.01 ± 0.00		0.01 ± 0.00		0.01 ± 0.00	0.80
	21	0.01 ± 0.01		0.01 ± 0.01		0.01 ± 0.00	0.05
	F		0.00		2.13		1.05
Yield	0	0.53 ± 0.09		0.49 ± 0.07		0.46 ± 0.11	1.08
	1	0.52 ± 0.09		0.55 ± 0.07		0.50 ± 0.06	2.14
	4	0.46 ± 0.09		0.51 ± 0.10		0.54 ± 0.07	4.00*
	21	0.53 ± 0.10		0.57 ± 0.07		0.58 ± 0.08	1.75
	F		2.15		2.66		5.67**
% UV Shield	0	44.73 ± 8.24		50.12 ± 8.74		45.31 ± 12.16	1.62
	1	59.15 ± 9.55		61.76 ± 10.48		63.40 ± 10.46	0.79
	4	49.78 ± 12.24		51.55 ± 7.93		49.14 ± 11.94	0.24
	10	48.74 ± 10.28		47.32 ± 13.85		47.99 ± 15.49	0.05
	21	48.92 ± 13.78		44.92 ± 13.37		45.41 ± 9.65	1.99
	F		4.21**		6.07***		6.97***

Mean ± SD, F-values; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $df = 2$, $n = 18$

differences in any of the chlorophyll or carotenoid parameters among the three treatments on any day of the experiment.

Discussion

The present study supports the conclusions of Durako et al. (2003), based on in situ studies, that *H. johnsonii* is an extremely high-light adapted and UV-tolerant plant. Changes in fluorometric and pigment characteristics of *H. johnsonii* were rapid when plants were transferred from the greenhouse to the outside mesocosms. The initial one-day acclimation was followed by a period during which a new acclimation-state was reached for the duration of the experiment. During the 21-day exposure to the three different irradiance treatments, alpha increased, but P_{max}, beta, and apparent photosynthetic quantum yield exhibited little change. On day 4 there were statistically significant differences in the fluorescence variables among the three treatments; alpha, P_{max}, and apparent quantum yield were all highest in the PAR + UVA + UVB treatment. This suggests some positive UV-B effect on the photophysiology of *H. johnsonii*, as has been recently observed in sorghum (Johnson and Day 2002), but by day 21 this effect was no longer detected. UV-absorbing pigment and carotenoid levels also rapidly increased from day 0 to day 1, following the transfer of the plants from the lower irradiance and UV-free environment of the greenhouse to outside, and then declined by day 21 to below pretreatment levels. Change in UV-absorbing pigment levels is the most common plant response to elevated UV-B radiation (Searles et al. 2001).

Percent UV shield data indicated that photosystem protection of the leaves of *H. johnsonii* from UV radiation was highest on day 1. This again follows the transition from the low total irradiance and relatively UV-free conditions in the greenhouse to outside and is consistent with the pigment responses. Increase in total irradiance between the greenhouse and the outdoor troughs, rather than change in UV exposure, most likely explains the significant increase in UV shield, since it was observed in all three treatments between day 0 and day 1. After day 1, the UV shield values significantly decreased during the remaining experimental period, suggesting a change from photoprotection to photoacclimation. The same trend was observed in all three treatments, and no significant differences in percent UV shield were observed among the treatments. The positive relationship between percent UV shield values and total UVP absorbances, indicates that the UVA PAM

Table 2 *Halophila johnsonii* . Summary of pigment parameters examined in outdoor irradiance experiment. One-way ANOVA results comparing three irradiance treatments and also the different days of the experiment within each treatment

Irradiance treatment							
Pigment parameters		PAR		PAR + UVA		PAR + UVA + UVB	
	day						F
Total UV absorbance mg FW^{-1}	0	10.56 ± 2.68		10.89 ± 1.47		9.04 ± 5.08	
	1	15.96 ± 4.25		14.84 ± 11.85		13.24 ± 4.25	
	2	9.47 ± 1.37		11.09 ± 5.10		7.63 ± 2.33	
	4	8.67 ± 1.15		8.08 ± 2.0		10.57 ± 3.71	
	21	5.30 ± 0.06		5.21 ± 1.53		7.74 ± 2.09	
	F		6.87***		1.23		1.19	0.01
Area UV peak 1/ UV peak 2	0	0.39 ± 0.01		0.39 ± 0.01		0.40 ± 0.02	
	1	0.45 ± 0.04		0.43 ± 0.01		0.41 ± 0.03	
	2	0.42 ± 0.03		0.41 ± 0.03		0.42 ± 0.01	
	4	0.42 ± 0.03		0.45 ± 0.04		0.44 ± 0.04	
	21	0.45 ± 0.04		0.40 ± 0.02		0.42 ± 0.03	
	F		0.93		1.97		1.26	0.54
µg Chlorophyll a mg FW^{-1}	0	0.94 ± 0.17		0.78 ± 0.25		0.73 ± 0.10	
	1	1.41 ± 0.69		0.97 ± 0.22		0.86 ± 0.08	
	2	0.75 ± 0.03		0.58 ± 0.06		0.74 ± 0.30	
	4	0.58 ± 0.08		0.45 ± 0.10		0.72 ± 0.24	
	21	0.38 ± ± 0.11		0.53 ± 0.22		0.82 ± 0.16	
	F		4.41**		4.6*		1.40	0.53
µg Chlorophyll b mg FW^{-1}	0	0.27 ± 0.07		0.22 ± 0.06		0.21 ± 0.03	
	1	0.43 ± 0.22		0.30 ± 0.05		0.25 ± 0.02	
	2	0.24 ± 0.02		0.17 ± 0.02		0.23 ± 0.10	
	4	0.17 ± 0.02		0.14 ± 0.03		0.21 ± 0.08	
	21	0.09 ± 0.06		0.15 ± 0.06		0.22 ± 0.03	
	F		4.84**		5.192**		1.51	0.41
Chlorophyll a / b	0	3.49 ± 0.20		3.46 ± 0.20		3.47 ± 0.22	
	1	3.30 ± 0.10		3.21 ± 0.26		3.45 ± 0.06	
	2	3.22 ± 0.19		3.46 ± 0.58		3.35 ± 0.28	
	3	4.0 ± 0.59		4.97 ± 0.77		4.90 ± 0.39	
	4	3.49 ± 0.17		3.19 ± 0.13		3.41 ± 0.24	
	21	5.73 ± 3.28		3.62 ± 0.13		3.69 ± 0.25	
	F		1.33		4.25**		9.19***	0.16
Total carotenoids mg FW^{-1}	0	0.53 ± 0.12		0.44 ± 0.13		0.40 ± 0.05	
	1	0.78 ± 0.40		0.59 ± 0.11		0.47 ± 0.06	
	2	0.45 ± 0.03		0.38 ± 0.02		0.47 ± 0.18	
	4	0.38 ± 0.03		0.32 ± 0.03		0.44 ± 0.14	
	21	0.25 ± 0.06		0.30 ± 0.09		0.43 ± 0.08	
	F		3.42*		4.89**		0.73	1.25

Mean ± SD, F-values; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $df = 2, n = 18$

fluorometer is an appropriate technique for rapid assessment of the relative photosystem protection from UV radiation in seagrasses.

Pigment analysis revealed that the most dramatic changes in all photochemical compounds occurred during the first 24–48 hours of the experiment. During this period the total spectral absorbance and the UV absorbance increased. Chlorophylls *a*, *b*, and total carotenoids all increased in concentration. Both chlorophylls and carotenoids are relatively photostable except in extremely photosensitive plants (Tevini and Teramura 1998). Yakovleva and Titlyanov (2001) refer to the earliest stages of PAR or UV treatments as the induction phase of acclimation. In *Chondrus crispus* this phase was marked by a significant increase in chlorophyll *a* and carotenoids (Yakovleva and Titlyanov 2001). Prolonged exposure to excess PAR and UV without acclimation can lead to progressive pigment destruction (Yakovleva and Titlyanov 2001). The five species of seagrasses studied by Dawson and Dennison (1996), including *Halophila ovalis*, and two other marine macrophytes studied by Detrés et al. (2001), *Rhizophora mangle* and *Thalassia testudinum*, all demonstrated significant reductions in total chlorophyll and carotenoids following 6-month exposure to UV irradiance. The initial accumulation of these compounds in *H. johnsonii* rather than degradation supports the idea that this species has photochemical mechanisms associated with its high tolerance for UV irradiance and increased PAR.

Following an initial 1-day response period, the UV-absorbing pigments in *H. johnsonii* appeared to stabilize at longer-term acclimation concentrations. The acclimated concentrations were either similar to, or in most cases slightly lower than, day 0 concentrations. The ratio of UV peak 1 to UV peak 2 tended to be higher in the acclimated state due to an increase in UV peak 1

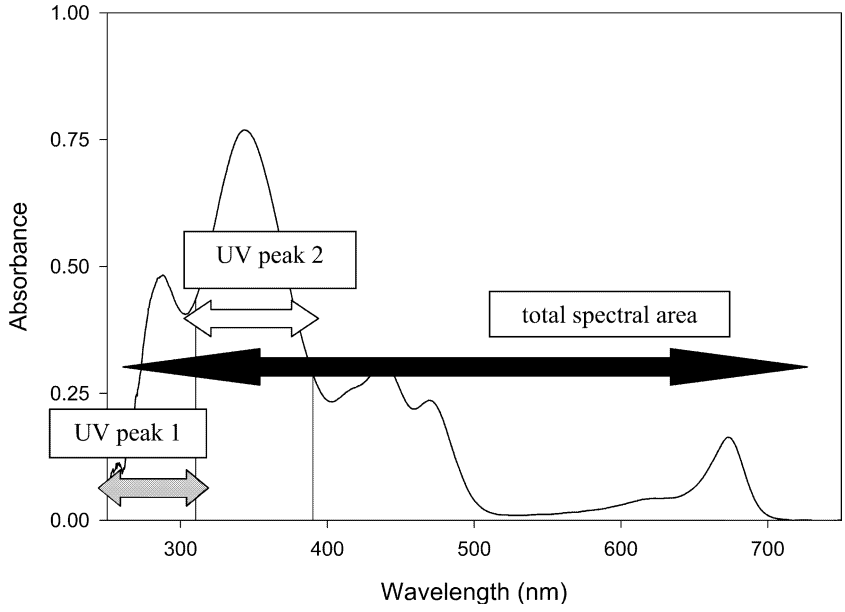

Fig. 3 *Halophila johnsonii*. Absorption spectrum of leaf pair extracted in 100% methanol showing wavelength ranges for analysis. UV peak 1 = integrated area under the curve 250–310 nm; UV peak 2 = integrated area under the curve 310–400 nm

absorbance following the initial response period. The change in the UV peak 1 to UV peak 2 ratio may be related to wavelength-dependent induction of one or more compounds. Nevertheless, there were no significant differences in pigment absorbances or concentrations among the three irradiance treatments of *H. johnsonii*, suggesting that the wavelengths that induce synthesis must be between 400 and 700 nm.

Because they are found in above- and below-ground tissues, the UV-absorbing compounds present in *H. johnsonii* appear to be constitutive. If this is the case, these compounds may have a primary physiological role other than UV-protection. The UV-absorbing compounds may confer UV protection to the plant as a result of their molecular structure, and as a secondary physiological role. If the compounds' primary physiological role is non-photochemical, this would explain why significant UV absorbance exists in apical-buds prior to chlorophyll synthesis. It would also explain why the compounds were not specifically induced by UV exposure or degraded in the absence of UV radiation. The patterns we observed are quite different than those reported for the seagrass *Thalassia testudinum* (Detrés et al. 2001). In this species, plants exposed to UV demonstrated a significant decrease in total chlorophyll and carotenoid concentrations relative to plants shielded from UV.

Halophila johnsonii has a particularly shallow depth distribution (0 – 3 m, Kenworthy 1993; Virnstein et al. 1997; Durako et al. 2003), within an extremely restricted geographic range. It persists intertidally where other seagrasses can not, but appears to be competitively excluded from many deeper subtidal environments by larger seagrasses, such as *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*, and possibly by *H. decipiens* and larger macroalgae. *Halophila johnsonii* is clearly adapted to the high PAR and UV irradiance conditions in the intertidal and shallow subtidal environments in southeastern Florida where it exists. Using fluorometric and pigment characteristics to detect UV-induced changes in *H. johnsonii* indicated a broad tolerance to varying irradiance conditions; exposure to PAR and UV irradiance, compared to PAR alone, was not significantly damaging. Coincidentally, intertidal and shallow subtidal environments are particularly susceptible to many anthropogenic disturbances. The construction of seawalls throughout the coastal lagoons of southeastern Florida has virtually eliminated much of the suitable intertidal habitat for *H. johnsonii*. In adjacent shallow subtidal areas, high turbidity, which drastically reduces light intensity, may be the primary factor limiting *H. johnsonii*. Thus, intertidal and shallow

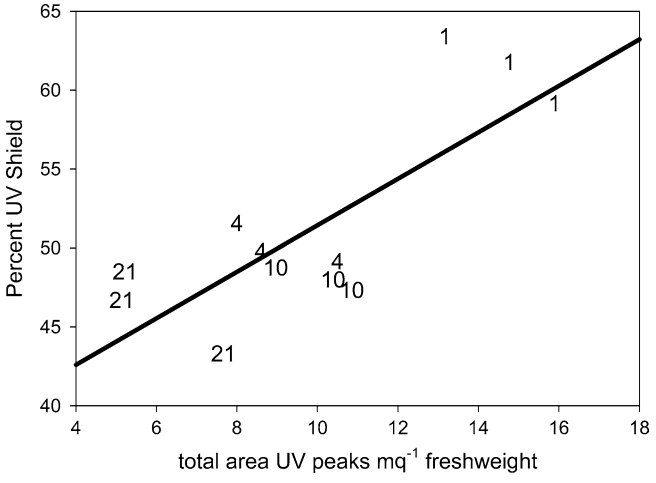

Fig. 4 *Halophila johnsonii*. Relationship between percent UV shield as measured by UVA PAM and total area UV peaks derived from analysis of pigment spectra. *Symbols* = day of measurement. r^2 = 0.61

subtidal habitats appear to be a refuge for this threatened species, and conservation of these habitats may be vital to its survival.

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