INTRODUCTION

Halophila johnsonii Eiseman is currently considered one of the rarest seagrass (submerged marine angiosperm) in the world due to its extremely restricted geographical distribution. *Halophila johnsonii* is patchily distributed in the 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) (Figure 1a). Within this range, populations are discontinuous and temporally variable (Virnstein et al. 1997). Thus, *Halophila johnsonii* is the least abundant of the seven Florida seagrass species within its range of distribution.

Populations of *Halophila johnsonii* establish in more extreme environments (Dawes et al. 1989, Virnstein et al. 1997) where there are strong currents, high sediment movement, and human activity exerting strong selective pressures. Human activities affecting this species may include, but are not limited to dredging, marine construction, and frequent boat groundings. Though *Halophila* species are small in total biomass (Philips and Menez 1988), they are able to withstand extreme ecological conditions, where some other larger bodied and more robust seagrass species can not (Williams 1988). These plants are important to coastal lagoon communities for sediment stabilization, as habitat for microfauna, and as food for grazers (Kenworthy et al. 1989). *Halophila* species have high rates of growth and decomposition, leading to a high rate of turnover that is important for nutrient cycling and primary production in the ecosystem (Kenworthy et al. 1989, Kenworthy 1993, Bolen 1997).

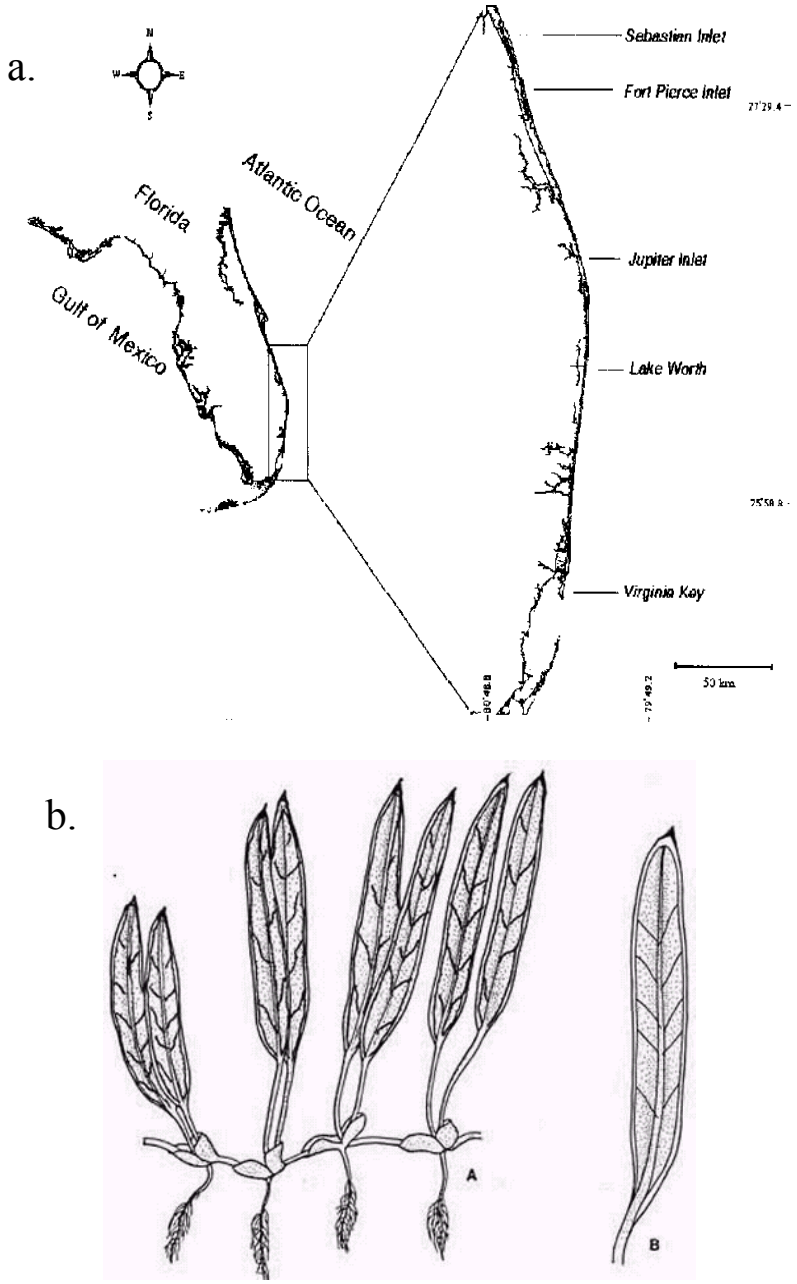


Figure 1. a. Map of *Halophila johnsonii*'s southeastern Florida distribution. b. Illustration of a *Halophila johnsonii* rhizome with leaf pairs and apical. Illustration courtesy of: Phillips and Menez 1988.

The United States National Marine Fisheries Service has listed *Halophila johnsonii* a threatened species under the Endangered Species Act (Federal Register, 1998). It is currently the only marine plant with this designation. Several critical habitat areas in southeastern Florida have also been designated for its protection. The conservation of this seagrass is vital for maintaining genetic, biological, and ecosystem diversity. Currently, large gaps exist in our understanding of *Halophila johnsonii*, primarily because this species was only recently described as a distinct *Halophila* species (Eiseman and McMillan 1980). An increasing number of scientific studies on this plant, particularly in the area of population genetics, reproductive biology, and physiological ecology will help to narrow this gap. This study was focused on the physiological ecology of depth distribution patterns observed among populations of *Halophila johnsonii*. An improved understanding of factors controlling the plant's distribution may ultimately contribute to conservation efforts.

Another species from the same genus, *Halophila decipiens*, is the only tropical seagrass species with a truly global distribution (den Hartog 1970). *Halophila johnsonii* and *Halophila decipiens* are quite similar morphologically but may be distinguished by several key differences (Eiseman and McMillan 1980). The leaves of *Halophila johnsonii* are linear with entire (smooth) margins, lack leaf hairs, and leaf cross veins diverge at 45° angles. The leaves of *Halophila decipiens* are oblong-elliptical in shape with serrulate margins, unicellular hairs on the surfaces, and cross veins that diverge at a 60° angle. *Halophila decipiens* is monoecious, produces flowers prolifically and sets seed to produce a reliable seed bank (Hammerstrom and Kenworthy 2003). Alternatively, *Halophila johnsonii* is only

known to produce pistillate flowers. Male flowers, viable fruits, and seeds have never been observed indicating an absence or rarity of sexual reproduction (Eiseman and McMillan 1980, Jewett-Smith et al. 1997). Consequently, populations are likely maintained by asexual lateral branching, or possibly by apomixis (Eiseman and McMillan 1980, Jewett-Smith et al. 1997).

These two species coexist in the coastal lagoons of southeastern Florida between 1 and 3 meters depth but with some obvious differences in zonation. *Halophila johnsonii* occurs from the intertidal zone to depths up to 3 meters (Kenworthy 1993, Virnstein et al. 1997). *Halophila decipiens* can exist from 1.5 meters to approximately 50 meters depth offshore (Kenworthy 2000). Between 1.5 and 3 meters, these two species may exist in monospecific beds or co-exist within a single bed, but only *Halophila johnsonii* occurs intertidally (Kenworthy 1993).

An aspect of *Halophila johnsonii*'s distribution that differs between the intertidal and subtidal populations is exposure to different light environments. Ultraviolet (UV) radiation (wavelengths (WL) <400 nm) and photosynthetically active radiation (PAR; 400-700 nm) influence aquatic primary production in a variety of ways (Vincent and Roy 1993). The effect of UV and PAR on other seagrass species has also been examined (Trocine at al. 1981, Larkum and Wood 1993, Dawson and Dennison 1996). While adequate levels of PAR are vital for electron excitation of photosystem II reaction centers, UV radiation and excess PAR can have a variety of deleterious effects. UV has a shorter WL, thus higher frequency energy compared to PAR. UV-B radiation (280-315 nm) is significantly more damaging than UV-A radiation (315-400 nm). UV-sensitive plants respond to exposure with

reductions in photosynthetic capacity, biomass, and chlorophyll (Tevini and Teramura 1989).

Both PAR and UV radiation are attenuated by water, but dissolved organics and suspended solids more rapidly reduce UV penetration (Booth and Morrow 1997). The waters of southeastern Florida lagoons are highly colored due to the levels of dissolved organics that can change on a short time scale. Intertidal populations of *Halophila johnsonii* must tolerate fluctuations between low-tide exposure and high-tide submergence. Low-tide conditions include high irradiance and susceptibility to desiccation. While plants are submerged during high tide they may be exposed to either high or drastically lower irradiance. This variability is due to several different factors, but particularly tidal height and water turbidity. Irradiance may further fluctuate quite rapidly due to surface ripples. Comparatively, subtidal populations are exposed to less daily fluctuation and lower irradiance levels overall. Dawes et al. (1989) demonstrated that *Halophila johnsonii* did not exhibit photoinhibition at high light intensities as did *Halophila decipiens*. The ability to grow in two such different sets of photosynthetic conditions between the intertidal and shallow subtidal environments lead us to hypothesize that *Halophila johnsonii* maintains some mechanism of photosynthetic phenoplasticity, particularly under high irradiances.

Durako et al. (in press) investigated the photosynthetic efficiency of *Halophila johnsonii* and *Halophila decipiens* populations distributed at different depths at Jupiter Island in the Indian River Lagoon and in northern Biscayne Bay. A reciprocal transplant was also performed to assess their plasticity in response to changes in the ambient light environment. Photosynthetic efficiency, derived from

chlorophyll fluorescence of photosystem II, was measured using a diving-Pulse Amplitude Modulated (PAM) fluorometer (Walz, Germany). PAM fluorescence data can be used to detect stress in response to specific variables, including UV and excess PAR (Walker et al. 1988, Larkum and Wood 1993). Furthermore, it is non-invasive and an extremely efficient way to measure seagrass photosynthetic efficiency *in situ* (Ralph et al. 1998).

Where *Halophila johnsonii* and *Halophila decipiens* co-occurred subtidally, *Halophila johnsonii* maintained a higher photosynthetic efficiency than *Halophila decipiens*. The photosynthetic efficiency of intertidal *Halophila johnsonii* varied between the two sites. *Halophila johnsonii* transplanted from shallow to deep had a higher yield than the shallow control. *Halophila johnsonii* transplanted from deep to shallow also had a higher yield than the deep control. These data indicated that *Halophila johnsonii* may be efficient at adapting to short-term changes in the ambient light environment and is capable of tolerating relatively high irradiances.

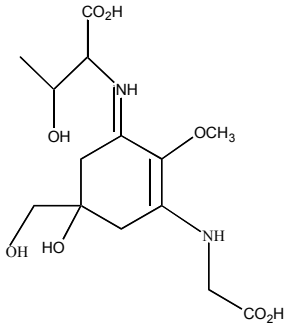
Tissue extractions were analyzed to examine differences in absorption spectra. Acetone extracts of *Halophila johnsonii* absorbed maximally at 340 nm. It appeared that the UV absorbing compound(s) was absent from extracts of *Halophila decipiens* due to the lack of significant UV-absorption. In response to the 4-day acclimation period of the reciprocal transplant experiment, the UV absorption maximum increased significantly when *Halophila johnsonii* was transplanted from deep to shallow.

Several types of compounds absorb UV wavelengths in photosynthetic organisms and are hypothesized to act as photostable compounds that protect against

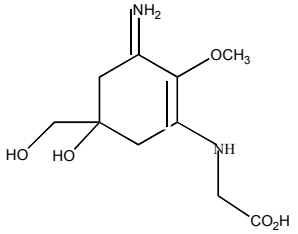
the damaging effects of excess PAR and UV radiation. Mycosporine-like amino acids (MAAs) are common UV filtering compounds synthesized by all the major algal classes. Flavonoids are typically thought to act as sunscreens in higher plants. Many chemical compounds with conjugated π-electron systems are capable of UV-screening (Cockell and Knowland 1999). Smaller molecules absorb shorter wavelengths and the more substituents added, the more intense the absorption (Cockell and Knowland 1999).

In all of the major algal divisions, MAAs are induced and accumulated in response to high UV radiation (Dunlap 1986, Sinha et al. 2001). The nineteen different MAAs have been isolated from marine organisms are all synthesized from shikimate derivatives (Cockell and Knowland 1999). The UV-absorbance property lies in the basic cyclohexanone or cyclohexenimine structure (Cockell and Knowland 1999). The absorbance of the common cyclohexanone core moiety absorbs at 255 nm but the addition of substituents shifts the absorption anywhere from 290 to 360 nm (Vincent and Roy 1993)(Figure 2). There is also evidence that some MAAs may function as biological antioxidants in response to photoxidative damage (Dunlap and Yamamoto 1995).

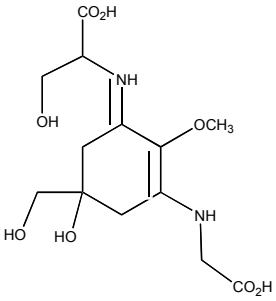
Flavonoids are also partially derived from shikimate. These compounds are nearly ubiquitous in higher plants and may be important for photoprotection in marine angiosperms. The synthesis of particular flavonoids appears to be the result of the UV-induced enzyme phenylalanine ammonia lyase (Vincent and Roy 1993). Thus, a very strong relationship emerges where plants exposed to higher UV doses



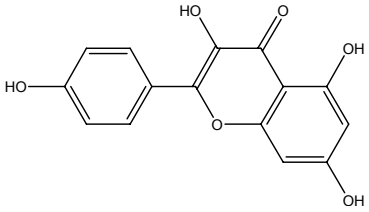
Porphyra-334 – $C_{14}O_8N_2H_{23}$
molecular weight = 347



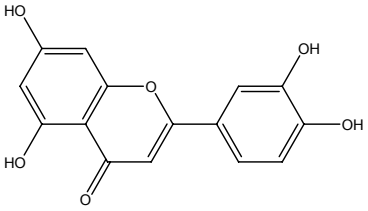
Palythine – $C_{10}O_5N_2H_{17}$
molecular weight = 245



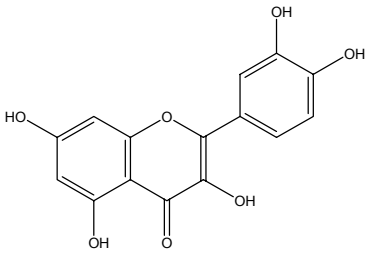
Shinorine – $C_{13}O_8N_2H_{20}$
molecular weight = 332



Kaempferol – $C_{15}H_{10}O_6$
molecular weight = 286.2



Luteolin - $C_{15}H_{10}O_6$
molecular weight = 286.2



Quercetin – $C_{15}H_{10}O_8$
molecular weight = 338.2

Figure 2. The structural formulas and molecular weights of 3 mycosporine-like amino acids: porphyra-344, palythine, and shinorine; and 3 flavonoids: kaempferol, luteolin, and quercetin

accumulate higher concentrations of flavonoids (Tevini and Teramura 1989). Compounds such as kaempferol, quercitin, luteolin, saponarin, anthocyanin and others absorb in the 280-315 nm region (Harborn and Williams 2000)(Figure 2). Abal et al. (1994) found that flavonoids in marine angiosperms tend to have absorbtion peaks between 330 and 345 nm. The diffusion of relatively rare flavonoid sulphates in hydrophytes appears to be an adaptation to the aquatic environment (Flamini et al. 2001). Yagi et al. (1994) suggested that flavonoid sulfates may act as biological antioxidants.

McMillan et al. (1983) reviewed the presence of sulfated flavonoids in several species of *Halophila*. An interesting discrepancy exists in the literature that may be resolved through the chemical analysis of *Halophila johnsonii* extracts. In the survey of sulfated flavonoids in the *H. ovalis* complex by McMillan et al. (1983), *Halophila johnsonii* was not included. However, a more recent paper by Jewett-Smith et al. (1997) referenced this earlier paper when stating that *Halophila johnsonii* did not contain sulfated flavonoids. It is of particular interest to resolve this inconsistency.

Seagrasses are believed to have evolved from terrestrial plants. Since MAAs are only known to be synthesized by algae, the precursors to higher plants, and flavonoids by higher terrestrial plants, determining the identity of the unknown UV-absorbing compound(s) synthesized by *Halophila johnsonii* could provide insight on the evolution of this UV-coping strategy. Identification of the compound(s) may also help elucidate a physiological significance.

Seagrass species sensitivity to UV radiation and PAR have been found to influence depth distribution elsewhere (Dawson and Dennison 1996). The primary objective of this study was to investigate how UV irradiance affects the

photophysiological and photochemical responses of *H. johnsonii*, which may ultimately influence the ecological distribution of this rare and threatened species. Therefore, I propose three null hypotheses:

Ho1. The photosynthetic efficiency of plants exposed to different irradiance treatments do not differ.

Ho2. The pigment spectra of plants exposed to different irradiance treatments do not differ.

Ho3. There is no relationship between the intensity of UV absorption and the photosynthetic efficiency in the leaves of *Halophila johnsonii*.

METHODS

Sample Collection

All *Halophila johnsonii* replicates were collected on June 30, 2002 from Haulover Park in northern Biscayne Bay (25º 55' N, 80º 07' W) from approximately 1-2 meters depth. A 10 x 10 cm^2 sod plugger was used to extract at least 1 rhizome segment of *Halophila johnsonii* with at least 4 leaf pairs and 1 apical meristem (see Figure 1b) from a monospecific bed. The segment, with rhizome and sediments intact, was placed in a 10 x 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, replicate peat pots were cultivated in fiberglass troughs with batch seawater maintained at 30ºC and 29 psu. The greenhouse glass eliminates solar UV radiation (WL <400nm) exposing experimental replicates 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 Arrangement

A total of 54 replicate peat pots were evenly distributed in 3 fiberglass troughs, 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 constant 30ºC and 29 psu environment. The outdoor experiment utilized the incident solar spectrum with different filter panels constructed for the 3 irradiance treatments (Figure 3). Polycarbonate sheeting eliminates WL shorter

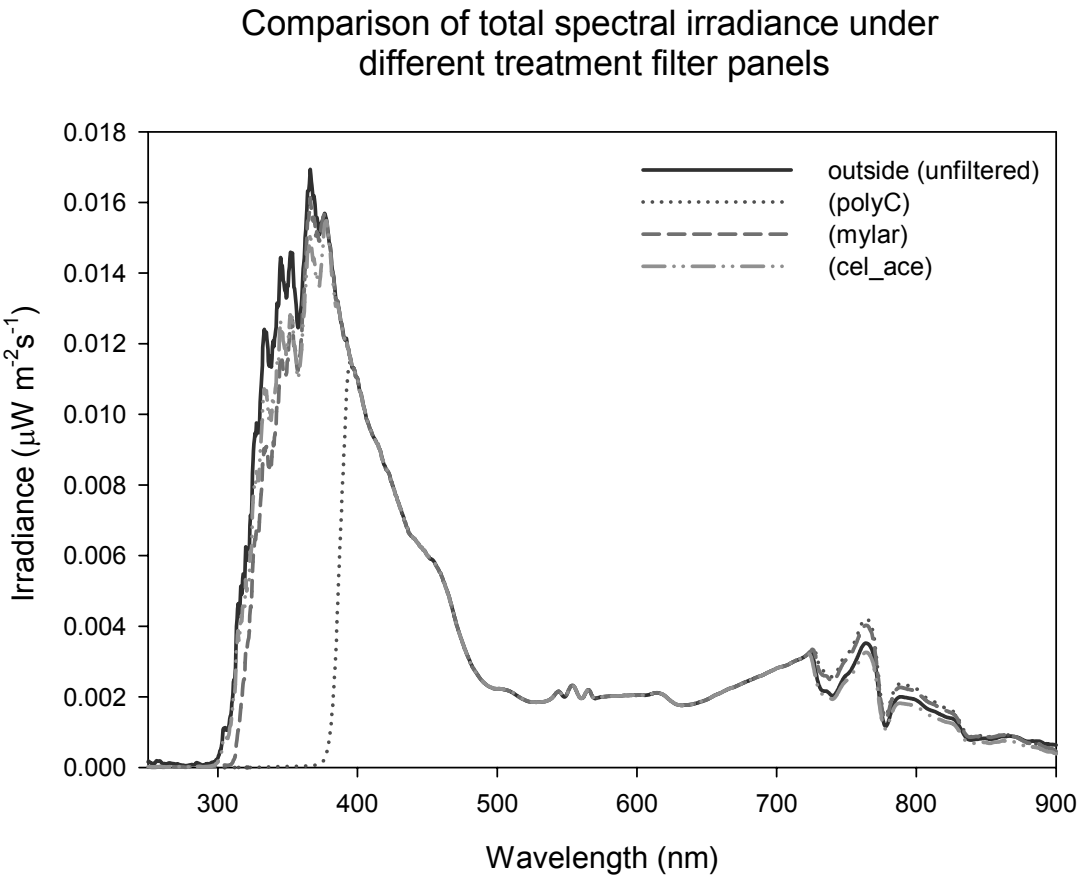


Figure 3. Comparison of total spectral irradiance under different treatment filter panels.

than 400 nm (UVA, UVB, UVC), exposing plants to PAR only. Mylar film eliminates WL shorter than 320 nm (UVB, UVC), exposing plants to PAR + UVA. Cellulose acetate film was used to eliminate WL shorter than 280 nm (UVC), which are also largely removed by atmospheric ozone, exposing plants to the full solar spectrum of PAR + UVA + UVB. Figure 3 also demonstrates that none of the filter panel treatments significantly reduced the total intensity of the transmitted wavelenths.

Each treatment was replicated in each of the 3 troughs in a random block design to minimize the effects of spatial placement within the troughs. All 54 replicates were grown under 1 of the 3 irradiance treatments for 24 consecutive days from July 18 through August 10, 2002. Total spectral irradiance between 250 and 700 nm was measured using a fiber optic spectroradiometer (Ocean Optics S2000, Dunedin, Florida, USA). PAR intensity was logged every 15 minutes by a LiCor Pyranometer Quantum Sensor (LI190SB; LiCor Instruments, Lincoln NE, USA) located at the NC NERR weather station (34º 09' N, 77º 51' W). Maximum midday irradiance on a cloudless day averaged 1500 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, and on days 1, 2, 3, 4, 6, 10, and 21 of the outdoor experiment. From each peat pot, 1 leaf pair was randomly selected for fluorescence measurements. Following these measurements, 3 leaf pairs were randomly selected from each of the 3 treatments for pigment analysis. These measurements were all made between 1 and 4 pm when irradiance levels were least variable. After day 24, 3 leaf pairs from each treatment were collected for a DNA damage assay. The remaining tissue was harvested for

chemical analysis of the photoactive compounds by High Performance Liquid Chromatography (HPLC).

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 by Rapid Light Curves (RLCs). In order to minimize 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 second 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). Apparent 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) was estimated using the following equation:

$$RETR = [(Fm'- F')/Fm'] * PPFD * 0.5 * 0.84$$

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 et al. 2002). 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} was plotted against PPFD μmol quanta m^{-2} s^{-1}. Linear regression lines were fit separately to each of 3 regions, alpha (α; the first 4 irradiance steps), Maximum ETR (P_{max}; the y-intercept of the middle 3-5 irradiance steps), and beta (β; the last 4 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 from UVA excitation (375 nm) with that of blue-green excitation (470 nm) (Gademann 2001). Development of this technique was based on epidermal peels from various terrestrial plant leaves (Bilger et al. 1997, Bilger et al. 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 ca. 2 mm from, and perpendicular to, the adaxial leaf surface using the DLC. The UVA PAM utilized light-emitting diodes for quasi-simultaneous excitation of chlorophyll fluorescence by 375 nm and 470 nm WLs. Fluorescence was measured by the photodiode detector at WL above 650 nm. Percent UV shield is a relative measure of photosystem protection from UV radiation and is calculated from the following equation:

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

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 fresh weight and then ground in 4 ml of 100% methanol using a chilled mortar and pestle in the dark. Methanol extracts were poured into graduated centrifuge tubes wrapped in aluminum foil and stored at 4˚C overnight in order for 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 FL, 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, CA, USA). Absorbance areas were normalized by freshweight. Wellburn's (1994) extinction coefficient equations were used to calculate chlorophylls *a* and *b*, and total carotenoids.

High Performance Liquid Chromatography

Samples collected for pigment extraction were pooled following spectral analysis for chemical analysis by HPLC-UV. Approximately 2 L of 100% methanol solution was filtered through a 0.7μm filter (Whatman GF/F, Kent UK) under vacuum pressure. This solution was concentrated under vacuum pressure on a rotary evaporator at 40ºC. The residue was then redissolved in 20% aqueous methanol. Samples were put on to a 10 g, 60 ml solid phase extraction (SPE) C_{18} column (Supelco, Bellefonte PA, USA). The column was eluted with 60 ml aliquots of 20%, 40%, 60%, 80%, and 100% aqueous methanol and each fraction collected separately. These fractions were again evaporated to dryness.

Approximately 0.5 g each of the 20% and 40% fraction residue were redissolved in 5% aqueous methanol and 0.02% (v:v) acetic acid. These solutions were filtered through 0.22μm pore sized filters (Millipore Millex-GS, Billerica MA, USA). Separation and analysis of the fractions was achieved using an HPLC system (Waters; Automated Gradient Controller 680, Pump 515, Dual λ Absorbance Detector 2487, Milford MA, USA). 40 μl aliquots were injected into a Zorbax C_{18} column protected with a C_8 guard column. The initial mobile phase was 5% methanol and 0.02% acetic acid (v:v) at a flow rate of 1.5 ml min^{-1}. The gradient program generated a mobile phase of 80% methanol and 0.02% acetic acid after 20 minutes. The compounds eluted from the column were detected at 214 and 340 nm.

Three MAA standards were obtained, including porphyra-334 (from *Porphyra yezoensis*), shinorine (from *Gloiopeltis furcata*), and palythine (from *Chondrus yendoi*). 1-2 μg of each standard was weighed, the three were combined and dissolved in 500 μl of

5% methanol and 0.02% acetic acid (v:v). This solution was further diluted 50 times and 40 µl were injected onto the HPLC column to calibrate retention times.

Diurnal Sampling

In situ fluorescence measurements were made under three different environmental conditions; the CMS greenhouse replicates, the CMS outdoor platform replicates, and the Biscayne Bay population at Haulover Park. The mini-PAM was used to perform RLCs on 10 randomly-selected *Halophila johnsonii* replicates growing in the greenhouse and outdoors on 15 and 17 July, 2002, respectively. Measurements were taken every 2 hours from sunrise (06:00 hrs) to sunset (20:00 hrs). A diving-PAM (Walz, Germany) was used to perform RLCs on randomly selected *Halophila johnsonii* replicates growing in northern Biscayne Bay at Haulover Park (25º 55' N, 80º 07' W) on 19 October, 2002. Two populations were selected and 8 randomly selected replicates were sampled from both a shallow, intertidal region and a deeper, subtidal region (~1.0 m mean low water). Measurements were taken every 2 hours from sunrise (08:00 hrs) to sunset (18:00 hrs).

A LiCor Pyranometer Quantum Sensor (LI190SB; LiCor Instruments, Lincoln NE, USA) logged PAR intensity every 15 minutes on 15 and 17 July 2002 for comparison with the diurnal measurements made in the greenhouse and on the outdoor platform. In situ PAR was measured by the light-sensor on the diving-PAM for comparison of the diurnal measurements made in the intertidal and subtidal populations.

Data Analysis

Fluorescence and pigment data were statistically analyzed with SigmaStat 2.0 (Jandel Scientific, Sanrafael, CA). A 95% probability level ($p<0.5$) 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, the irradiance treatment and time, on the dependent variables, fluorescence and pigment data. ANOVA with repeated measures was used to compare RLCs. 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

In situ chlorophyll fluorescence measurements of *Halophila johnsonii* exposed to different irradiance treatments revealed similar patterns of response to different biologically relevant wavelengths of solar irradiance (Table 1). Figure 4 represents the changes in the averaged RLCs on day 1, 4, and 21 under exposure to PAR and different PAR + UV treatments. After 21 days of exposure to PAR only, *Halophila johnsonii*'s RLC RETRs were significantly higher than on days 1 and 4 (Figure 4a, $p=0.037$). Under exposure to PAR + UVA, there were no significant changes among the averaged RLC RETRs during the 21 day experiment (Figure 4b). Under exposure to PAR + UVA + UVB, averaged RLC RETRs were significantly lower on day 1 than on days 4 and 21 (Figure 4c, $p<0.001$). Figure 5 represents the changes in RLCs under the 3 different irradiance treatments on days 1, 4, and 21. These data are the same as above except reorganized to show the differences among the treatments at different stages of the experiment. On day 1, the averaged RLC RETRs of *Halophila johnsonii* exposed to PAR + UVA were significantly higher then those from the PAR only and full spectrum treatments (Figure 5a, $p<0.05$). On day 4, the averaged RLC RETRs were significantly different among the 3 treatments (Figure 5b, $p<0.001$). The full spectrum was highest while the PAR only treatment was the lowest. On day 21, the averaged RLC RETRs of *Halophila johnsonii* exposed to PAR only were significantly higher than from the PAR + UVA and PAR + UVA + UVB treatments (Figure 5c, $p=0.046$).

A sample RLC from *Halophila johnsonii* replicate is shown in Figure 6, as well as the method of analysis that yielded information on induction, saturation, and

Fluorescence Parameters		PAR		PAR + UVA		PAR + UVA + UVB		
	day							F^P
alpha	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^P		**11.73*****		**1.23**		**4.56**	
Max.	1	10.81±2.72		12.56±2.93		10.16±4.90		**1.99**
RETR	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^P		**0.53**		**2.05**		**2.27**	
beta	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^P		**0.48**		**0.47**		**0.45**	
Yield	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±0.67		**4.00***
	21	0.53±0.10		0.57±0.07		0.58±0.08		**1.75**
	F^P		**3.31***		**2.06**		**6.19****	
Percent	1	59.15±9.55		61.76±10.48		63.40±10.46		**0.79**
UV	4	49.78±12.24		51.55±7.93		49.14±11.94		**0.24**
Shield	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^P		**3.36***		**7.19*****		**7.94*****	

Table 1. Summary of fluorescence parameters examined in the outdoor irradiance experiment. One-way ANOVA results comparing the 3 irradiance treatments on each day and also the different days of the experiment within each treatment. Mean ± sd, F-values; $P<0.05$ = *; $P<0.01$ = **; $P<0.001$ = ***; df = 2, n = 18.

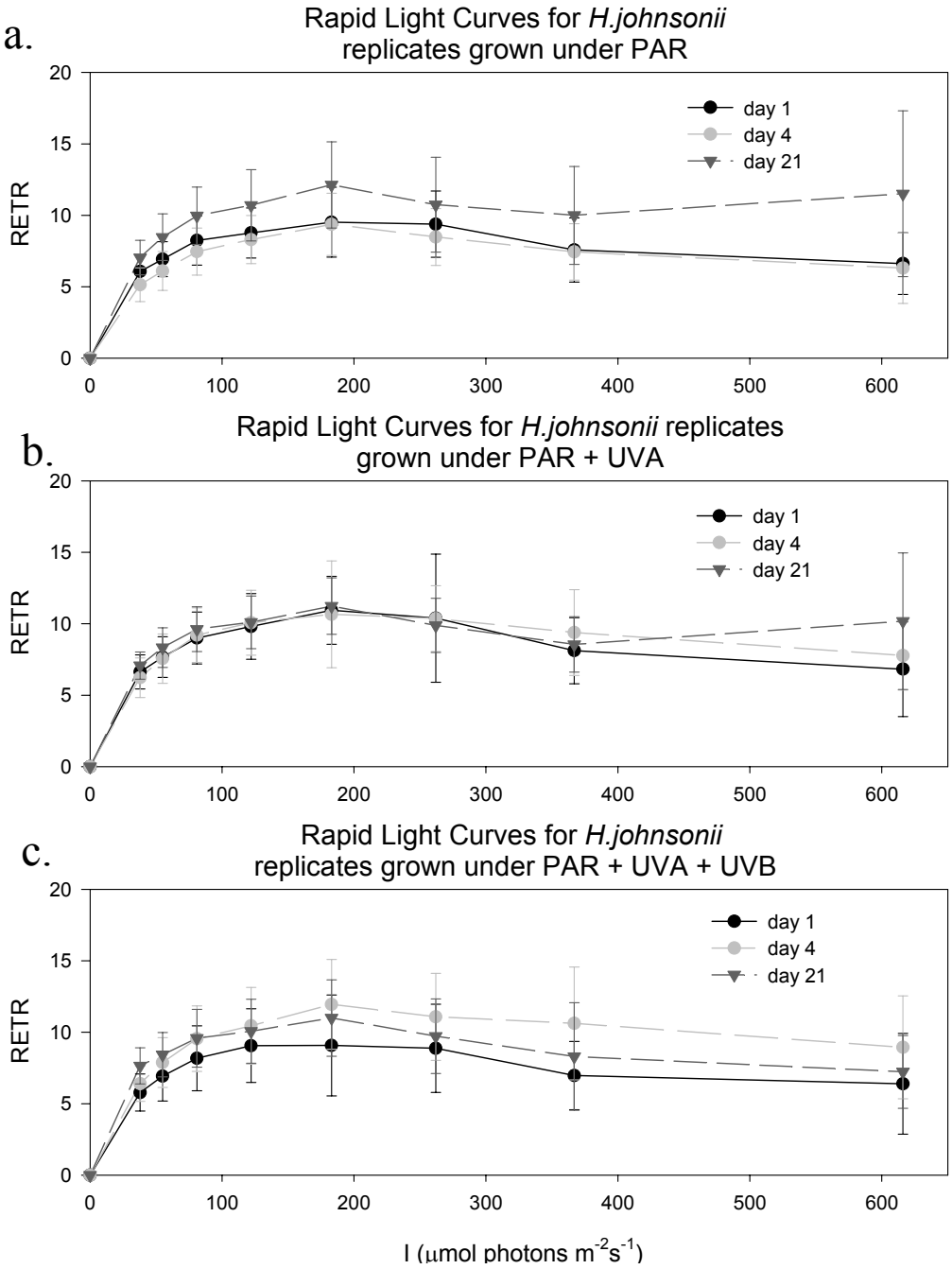


Figure 4. Rapid Light Curves for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Symbols = mean, error bars = sd, n = 18.

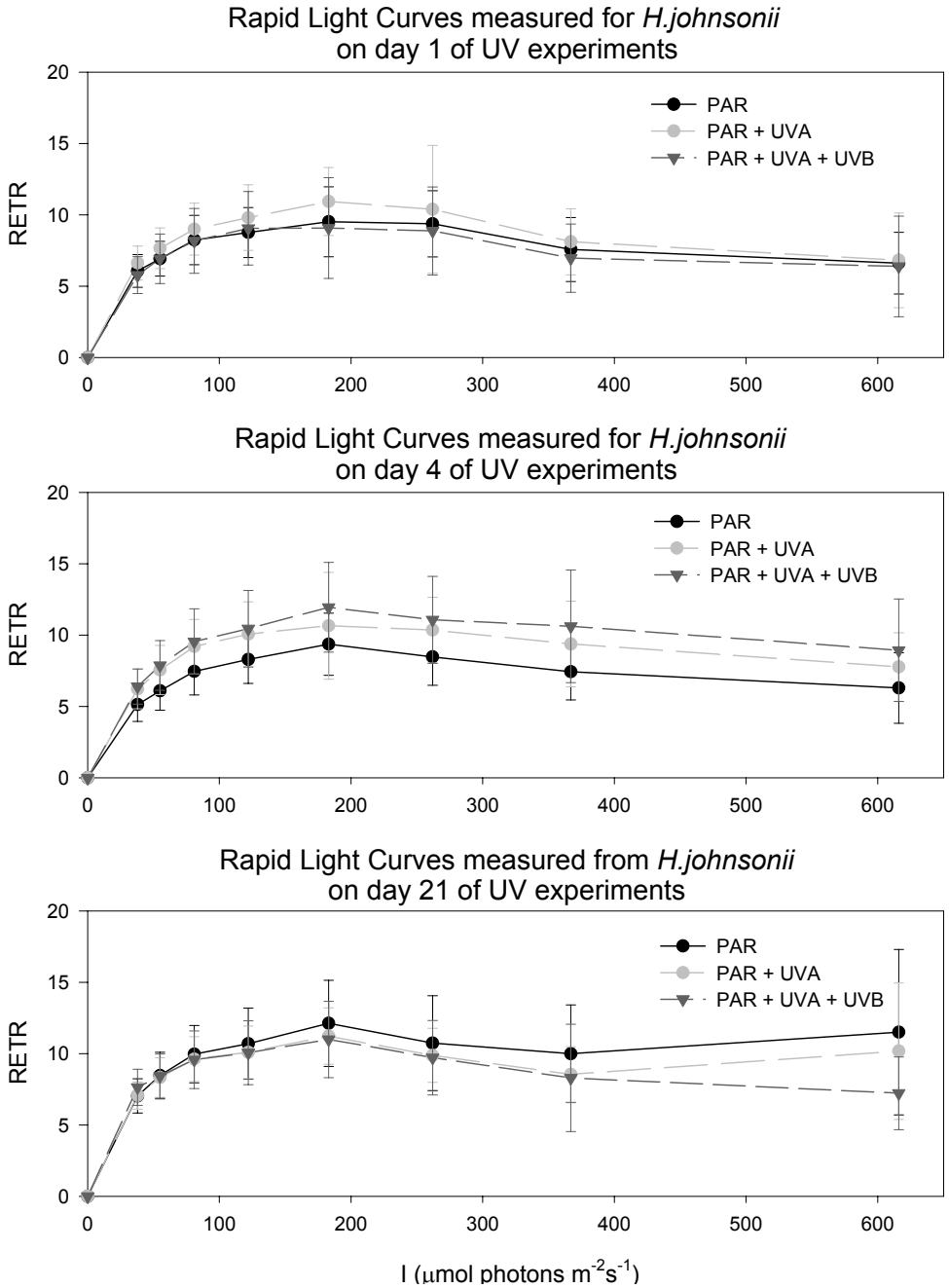


Figure 5. Rapid Light Curves for *Halophila johnsonii* replicates grown under PAR, PAR + UVA, and PAR + UVA + UVB on a. day 1, b. day 4, and c. day 21. Symbols = mean, error bars = sd, n = 18.

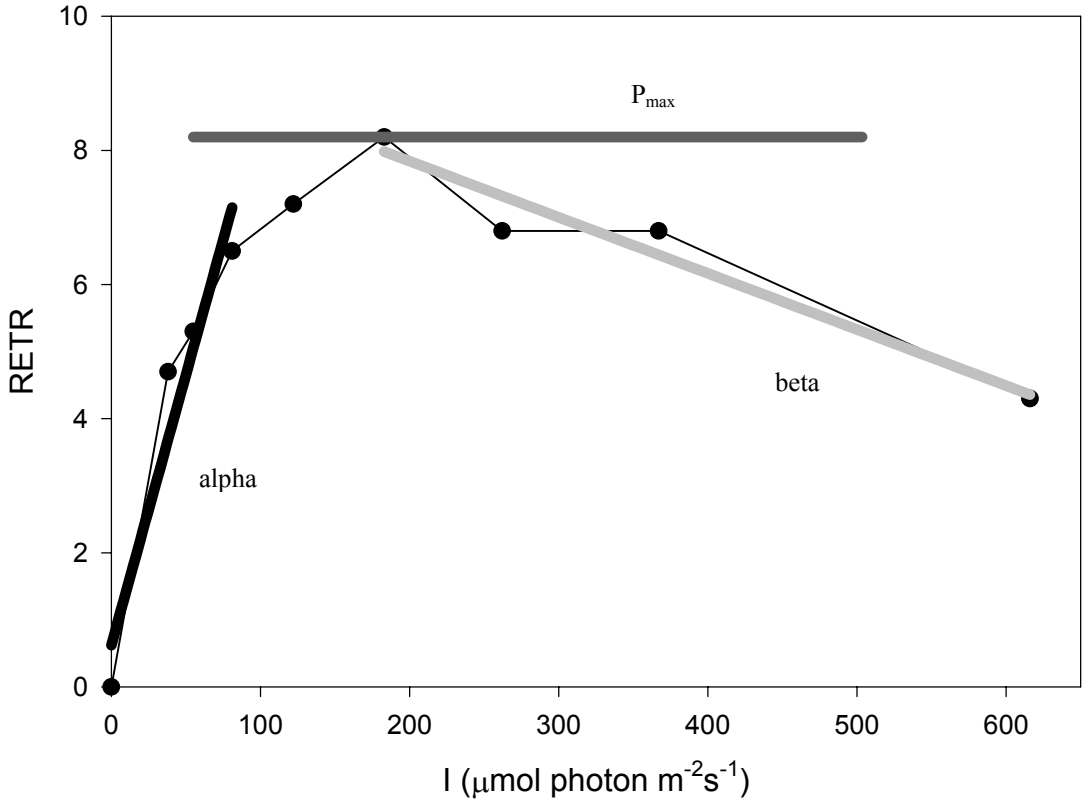


Figure 6. Sample Rapid Light Curve measured from *Halophila johnsonii* by Pulse Amplitude Modulated (PAM) fluorometry and analysis of alpha, P_{max}, and beta regression lines.

inhibition. The slopes of the alpha (α) regression lines are shown in Figure 7, the y-maximums of the P_{max} regression lines are shown in Figure 8, and the slopes of the beta (β) regression lines are shown in Figure 9. In the PAR only and full spectrum treatments, alpha was significantly higher on day 21 than on either day 1, or day 4 (Figure 7a, $p<0.001$; 7c, $p=0.015$). In the plants grown under PAR + UVA, alpha did not significantly change (Figure 7b). Only on day 4 was there a significant difference among the three treatments (Figure 7, $p=0.003$). There were no significant differences in P_{max} within each of the treatments during the course of the experiment and only on day 4 was there a significant difference among the three treatments (Figure 8, $p=0.045$). There were no significant differences in beta among the three treatments, and the values did not change significantly during the 21 days of the experiment (Figure 9).

The first yield measurement of the RLC represents the apparent photosynthetic quantum yield (Y). In the PAR only treatment, Y was significantly lower on day 4 than on days 1 and 21 (Figure 10a, $p=0.045$). There was no change in Y exposed to PAR + UVA during the 21 day experiment (Figure 10b). In the full spectrum treatment, Y was significantly higher on day 21 than on day 1 (Figure 10c, $p=0.004$). The only significant difference among the treatments was between the PAR only and full spectrum treatments on day 4 (Figure 10, $p=0.024$).

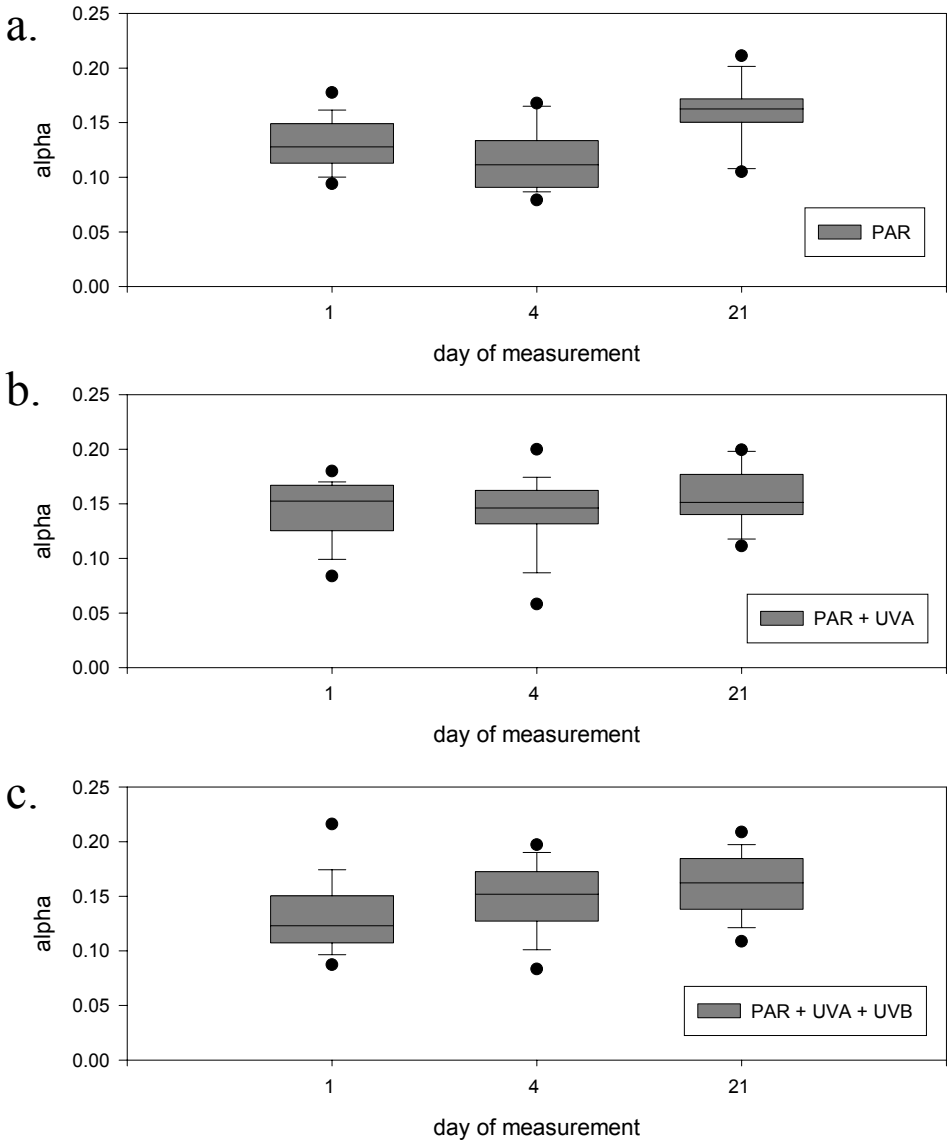


Figure 7. Comparison of alpha for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Median = solid horizontal line, box = 25th and 75th percentiles, and error bars = 10th and 90th percentiles, n = 18.

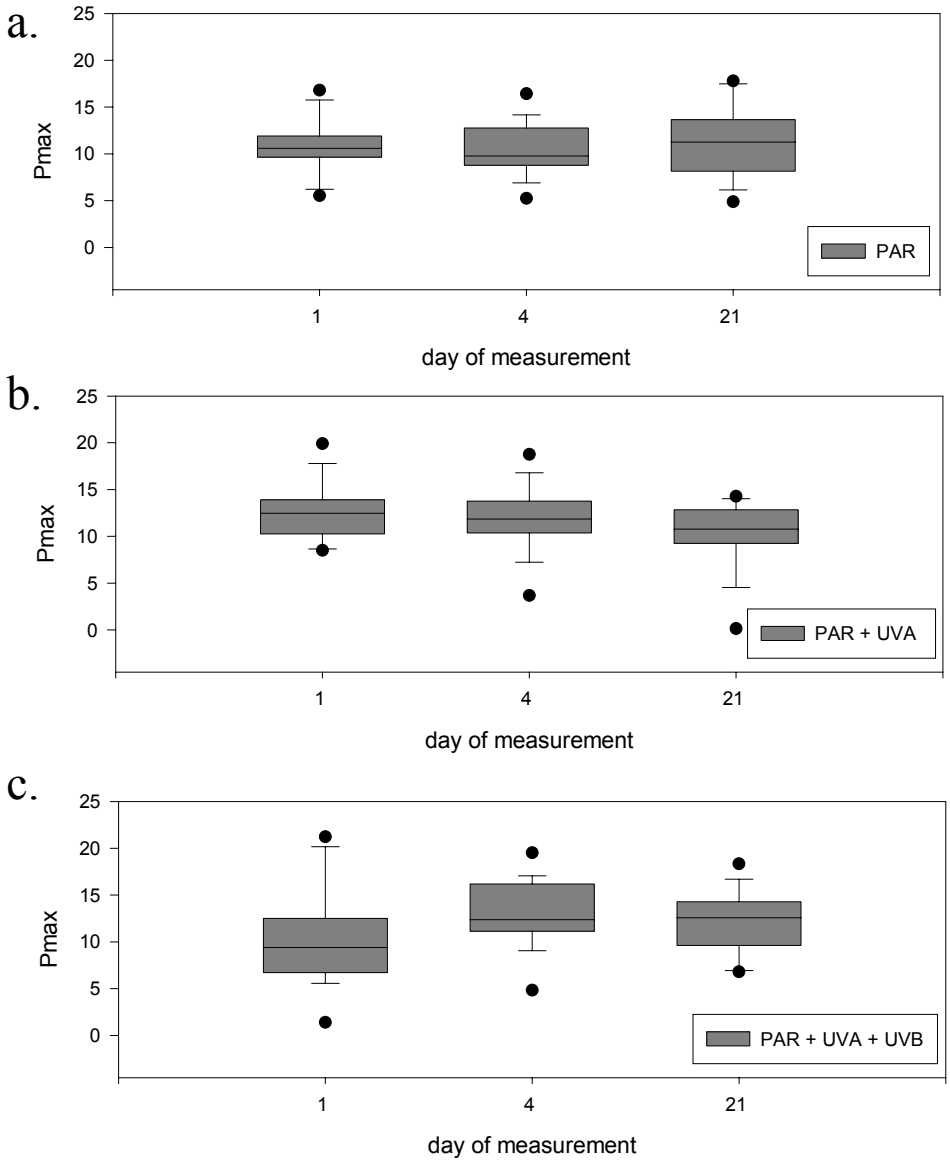


Figure 8. Comparison of P_{max} for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Median = solid horizontal line, box = 25th and 75th percentiles, and error bars = 10th and 90th percentiles, n = 18.

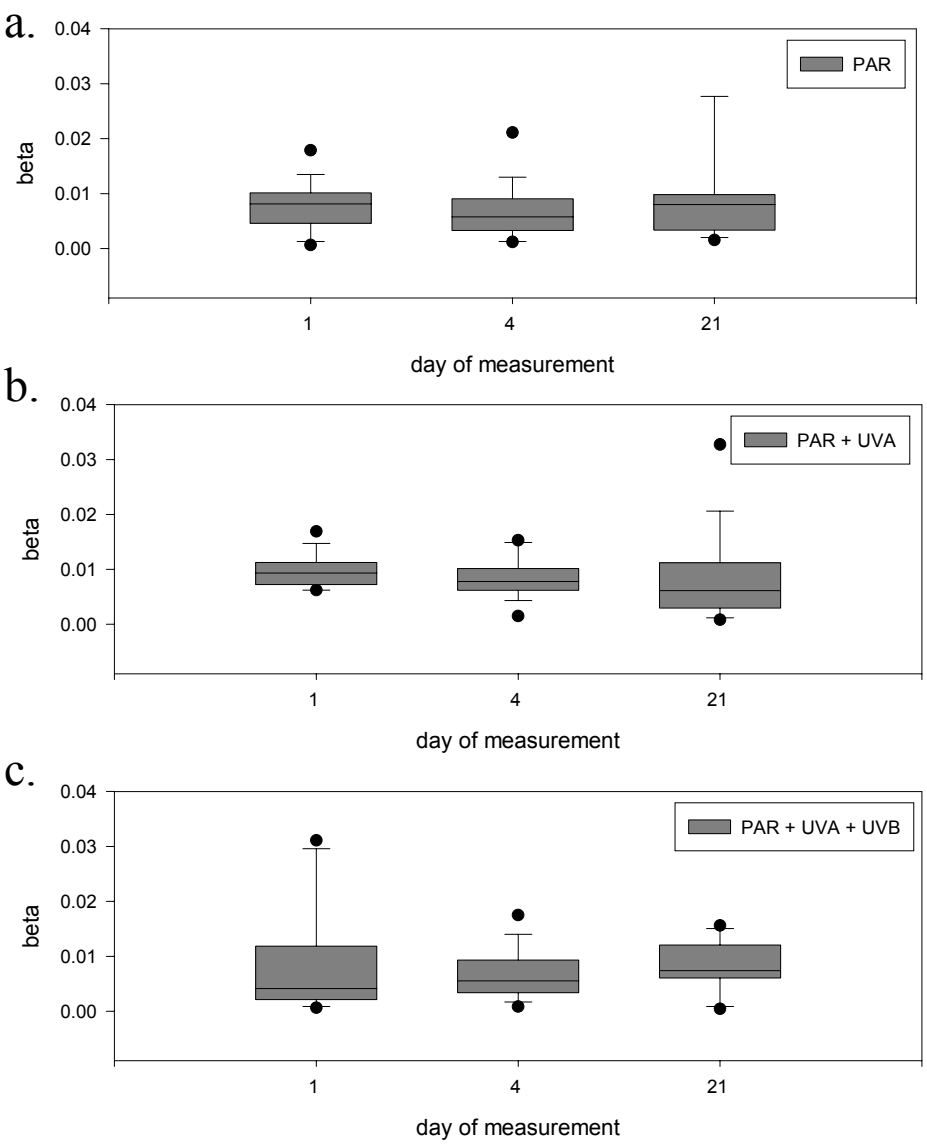


Figure 9. Comparison of beta for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Median = solid horizontal line, box = 25th and 75th percentiles, and error bars = 10th and 90th percentiles, n = 18.

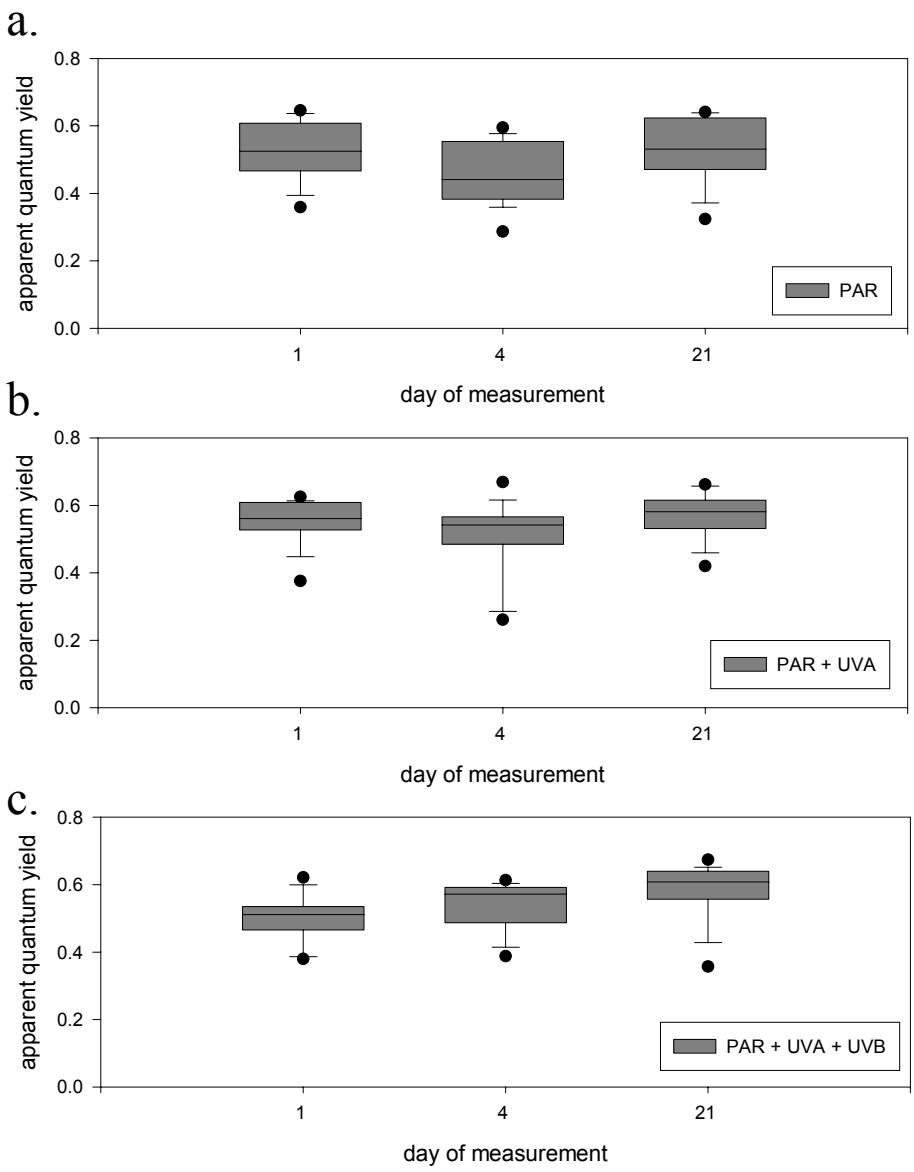


Figure 10. Comparison of apparent photosynthetic quantum yield for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Median = solid horizontal line, box = 25th and 75th percentiles, and error bars = 10th and 90th percentiles, n = 18.

UV Fluorescence

The UVA PAM data indicated that percent UV shield values in *Halophila johnsonii* changed during the UV irradiance experiment, however these changes followed the same general pattern in all three treatments (Table 1). In the plants grown under PAR only, percent UV shield was significantly higher on day 1 than on day 11 (Figure 11a, $p=0.024$). Percent UV shield was significantly higher on day 1 than after 4, 10, and 21 days exposure to both the PAR + UVA and full spectrum treatments (Figure 11b, $p<0.001$; Figure 11c, $p<0.001$). There were no significant differences among the treatments on any day of the experiment (Figure 11).

UV-Absorbing Pigment Analysis

Extractions of *Halophila johnsonii* tissue contain compounds that absorb strongly in the UV range (Figure 12). We have established that this compound, or mixture of compounds, is located not only in the leaves, but also the petioles, rhizomes, roots, as well as the apical buds. Pigment extractions from the tissue of apical meristems absorb in the UV range only prior to chlorophyll synthesis. Absorption spectra for leaf extracts in 90% acetone have λ_{max} at 350 nm, in 100% methanol λ_{max} shifts to 343 nm, and in 100% DI H_2O the λ_{max} is 290 nm. Even in the same solvent, UV-absorbing peaks do not all appears to be identical in shape. Some spectra show a well-defined shoulder while others do not.

Figure 13 shows a typical absorbance spectrum for a *Halophila johnsonii* leaf pair extracted in 100% methanol and how UV absorbance was analyzed from the scatter-corrected absorbance spectra. Because peak maximums and minimums shifted within a

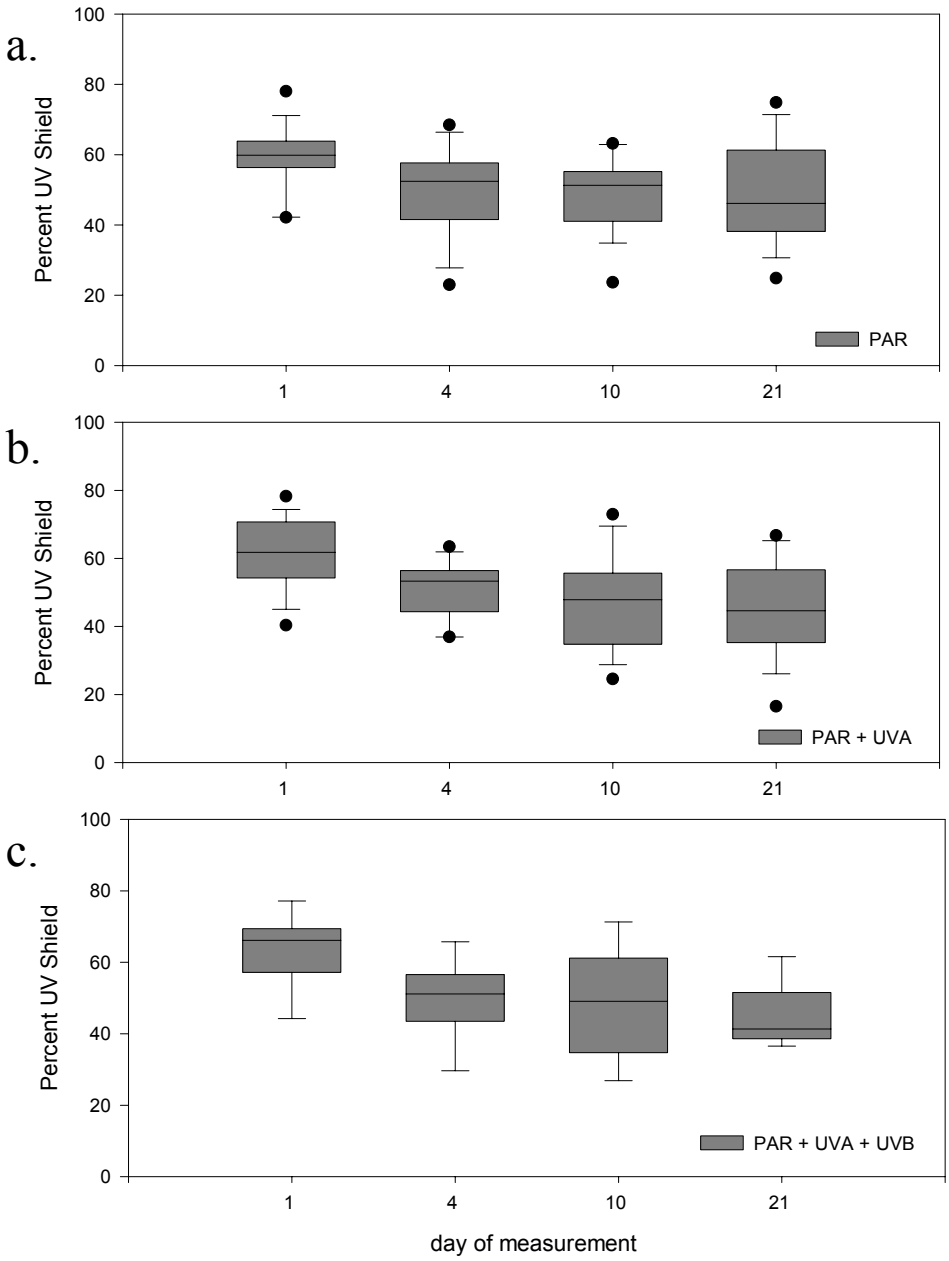


Figure 11. Comparison of percent UV shield for *Halophila johnsonii* replicates grown under a. PAR, b. PAR + UVA, and c. PAR + UVA + UVB on day 1, 4, and 21. Median = solid horizontal line, box = 25th and 75th percentiles, and error bars = 10th and 90th percentiles, n = 18.

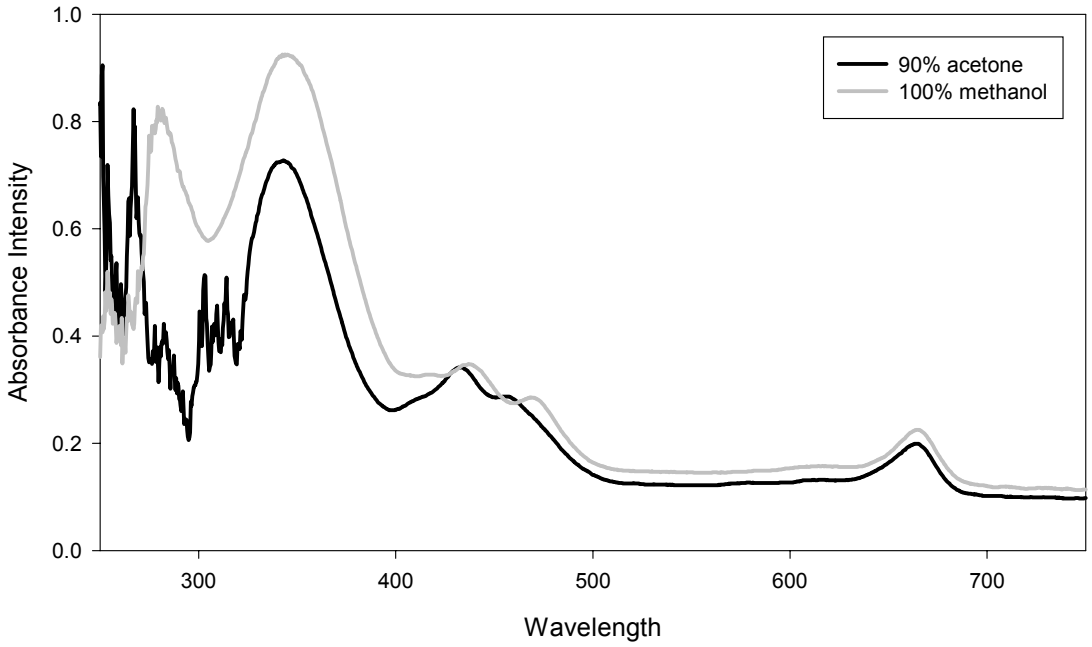


Figure 12. Comparison of *Halophila johnsonii* absorption spectra in 100% methanol and 90% acetone

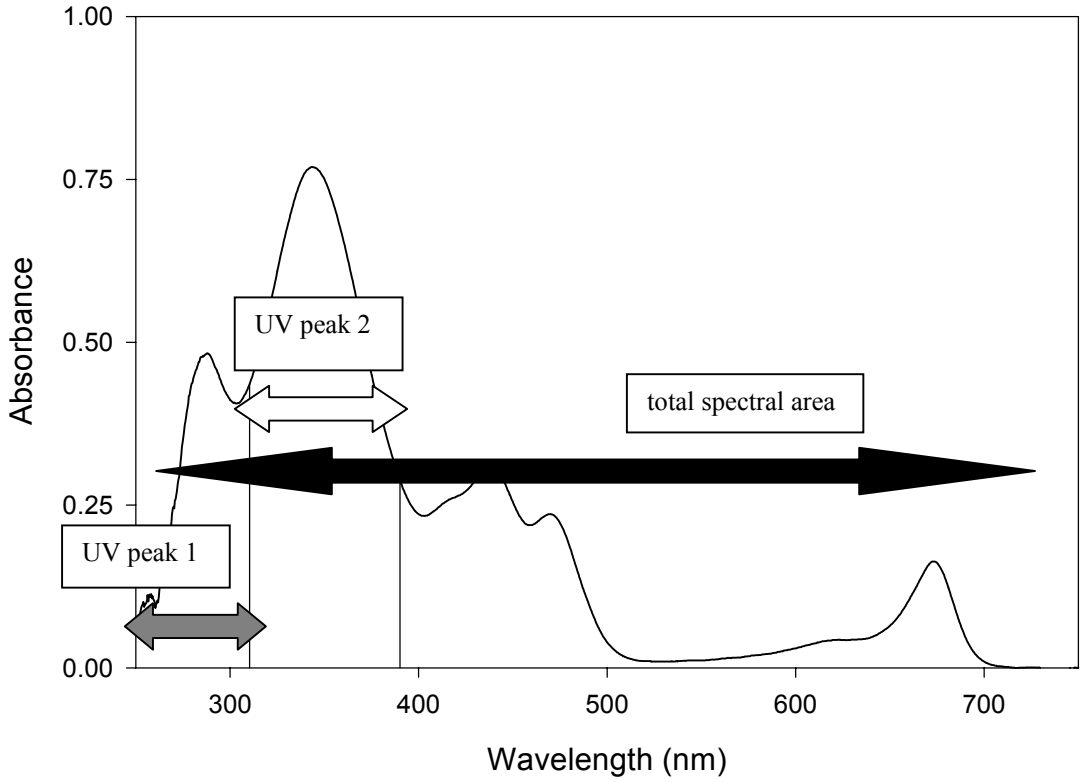


Figure 13. Absorption spectrum of *Halophila johnsonii* leaf pair extracted in 100% methanol showing wavelength ranges for analysis. UV peak 1 = the integrated area under the curve 250-310 nm, UV peak 2 = the integrated area under the curve 310-400 nm.

range of several nm during the experiment, integrating the area under the curves was the most appropriate method of analysis. Patterns of UV absorption change during the 21-day experiment for the 3 treatments are shown in Figure 14. While there are few significant differences among the different days of measurement, some interesting patterns emerge from these data (Table 2). Both total spectral area (Figure 14a) and total area UV peaks (Figure 14b) increased from day 0 to day 1, followed by a decrease in day 2 values, then remained relatively unchanged through the final measurements. The ratio of total area UV peaks to total spectral area was slightly higher on day 10 than on any previous day of measurement, though this value had decreased to the original value by day 21 (Figure 14c). The ratio of UV peak 1 to UV peak 2 increased from day 0 to day 1, followed by a decrease in day 2 values. This value slowly increased during the second and third week of the experiment back up to day 2 values by the final day 21 measurement (Figure 14d). There were no significant differences in any UV pigment parameters among the three treatments on any day of the experiment (Figure 14).

The total area UV peaks was also compared to the percent UV shield values as measured by the UVA PAM (Figure 15). The results of the regression analysis show a positive correlation between these two values ($r^2 = 0.61$).

Chlorophyll and Carotenoid Analysis

Both chlorophyll and carotenoid concentrations followed the same general pattern as the UV absorbance (Table 2). Chlorophyll *a* (Figure 16a), chlorophyll *b* (Figure 16b), and total carotenoids (Figure 16d) increased from day 0 to day 1, followed by a decrease on day 2 to values lower than the day 0 measurements. These concentrations remained constant or decreased slightly during second and third weeks to the final day 21

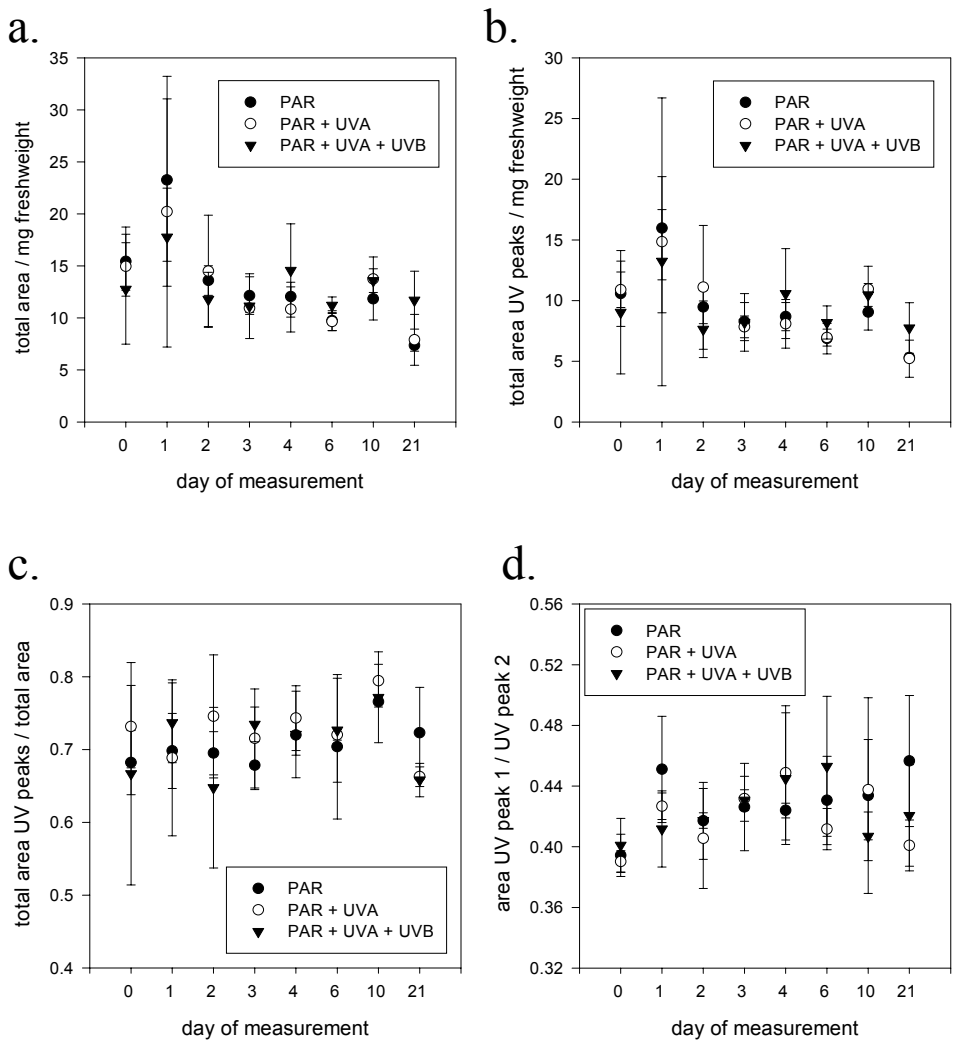


Figure 14. Integrated area under the curves for *Halophila johnsonii* replicates grown under PAR, PAR + UVA, and PAR + UVA + UVB on day 0, 1, 2, 3, 4, 6, 10, and 21. Symbols = mean, error bars = sd, n = 3.

Pigment Parameters		PAR		PAR + UVA		PAR + UVA + UVB		
	day							F^P
Total UV	0	10.56±2.68		10.89±1.47		9.04±5.08		
Absorbance	1	15.96±4.25		14.84±11.85		13.24±4.25		
mg FW^{-1}	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^P		**6.87*** **		**1.23**		**1.19**	**0.01**
Area UV peak	0	0.39±0.01		0.39±0.01		0.40±0.02		
1/ UV peak 2	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^P		**0.93**		**1.97**		**1.26**	**0.54**
µg chlorophyll	0	0.94±0.17		0.78±0.25		0.73±0.10		
a mg FW^{-1}	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^P		**4.41****		**4.6***		**1.40**	**0.53**
µg chlorophyll	0	0.27±0.07		0.22±0.06		0.21±0.03		
b mg FW^{-1}	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^P		**4.84****		**5.192****		**1.51**	**0.41**
chlorophyll a /	0	3.49±0.20		3.46±0.20		3.47±0.22		
b	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		
total	F^P		**1.33**		**4.25****		**9.19*****	**0.16**
carotenoids	0	0.53±0.12		0.44±0.13		0.40±0.05		
mg FW^{-1}	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^P		**3.42***		**4.89****		**0.73**	**1.25**

Table 2. Summary of pigment parameters examined in the outdoor irradiance experiment. One-way ANOVA results comparing the 3 irradiance treatments and also the different days of the experiment within each treatment. Mean ± sd, F-values; $P<0.05$ = *; $P<0.01$ = **; $P<0.001$ = ***; df = 2, n = 3.

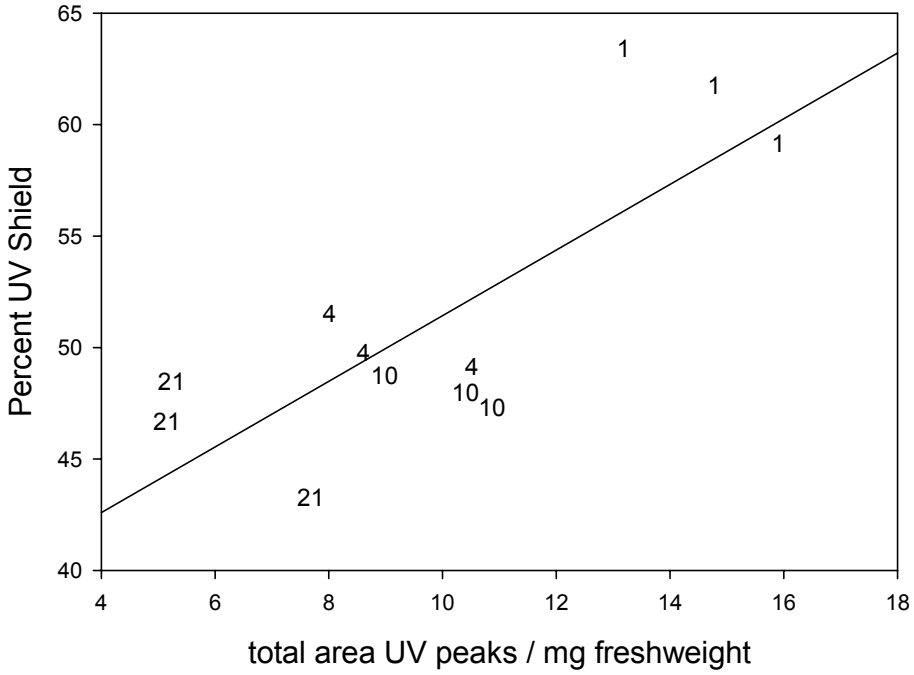


Figure 15. The relationship between percent UV shield as measured by the UVA PAM and total area UV peaks derived from analysis of pigment spectra. Symbols = day of measurement. $r^2 = 0.61$

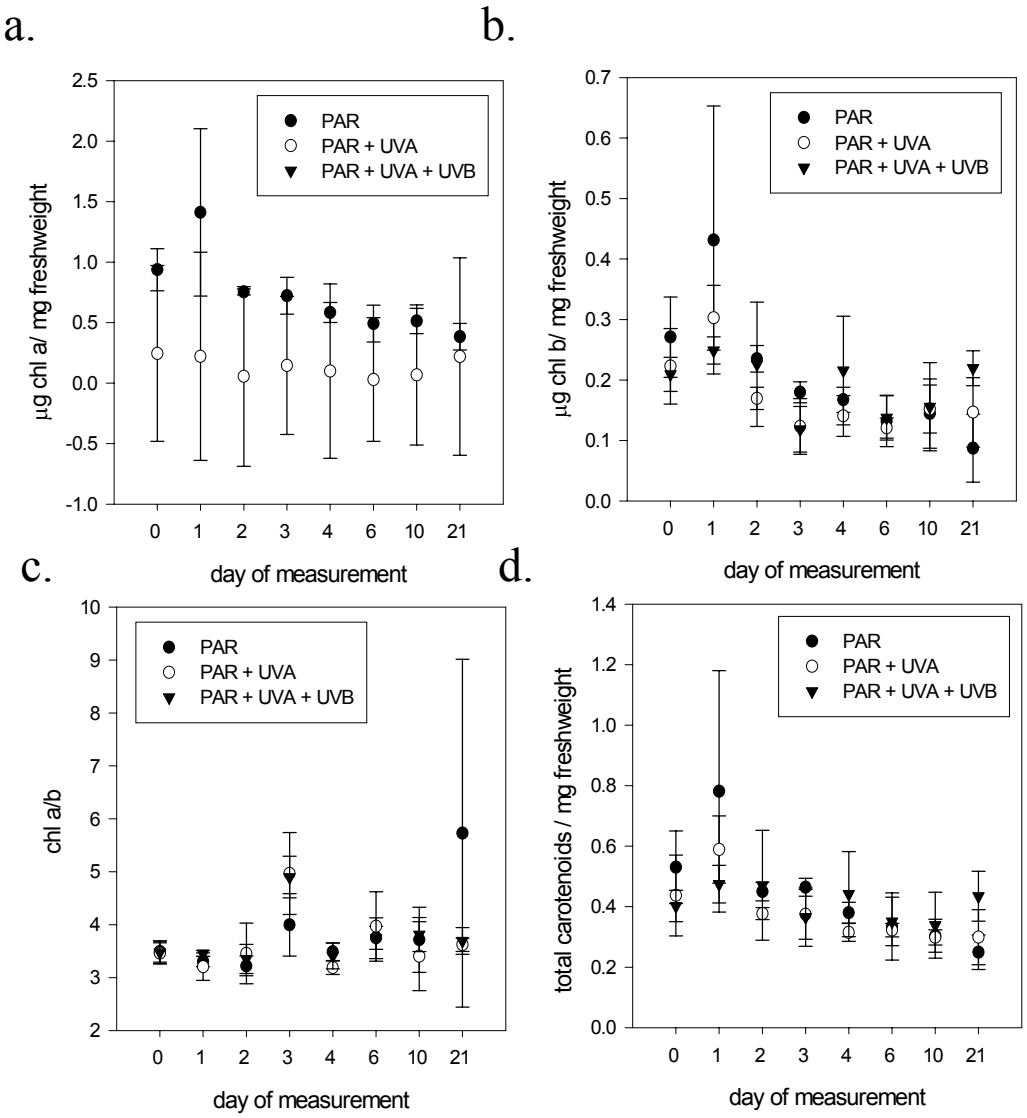


Figure 16. Pigment concentrations for *Halophila johnsonii* replicates grown under PAR, PAR + UVA, and PAR + UVA + UVB on day 0, 1, 2, 3, 4, 6, 10, and 21. Symbols = mean, error bars = sd, n = 3.

measurements. There were no dramatic changes in the chlorophyll *a*/*b* ratio (Figure 16c) between days 0, 1, or 2. Values increased in all three treatments on day 3, and then returned to day 0 levels on day 4, 6, and 10. The mean chlorophyll *a*/*b* ratio increased dramatically on day 21 in the PAR only treatment. There were no significant differences in any of the chlorophyll or carotenoid parameters among the three treatments on any day of the experiment (Figure 16).

High Performance Liquid Chromatography

The identity of the unknown UV-absorbing compound(s) from *Halophila johnsonii* samples could not be resolved from the HPLC chromatogram with the available standards (Figure 17). Detection at 340 nm revealed at least ten significant peaks (absorbance > 5) and ten lesser peaks (absorbance < 5) of unknown compounds absorbing in the UV range. All of these peaks had longer retention times than palythine (5), shinorine (23.3) and porphyra-334 (28.3) standards.

Diurnal Patterns

The different populations of *Halophila johnsonii* sampled were exposed to different light conditions and demonstrated significantly different patterns of diurnal variation (Figures 18 and 19). The population sampled in the greenhouse was exposed to a maximum irradiance of 738 μmol photons m^{-2} s^{-1} at midday (Figure 18a). The population sampled on the outdoor platform was exposed to a maximum irradiance of 1475 μmol photons m^{-2} s^{-1} at midday and significantly more irradiance throughout the day (Figure 18b). Both populations sampled in Biscayne Bay were partly shaded in the

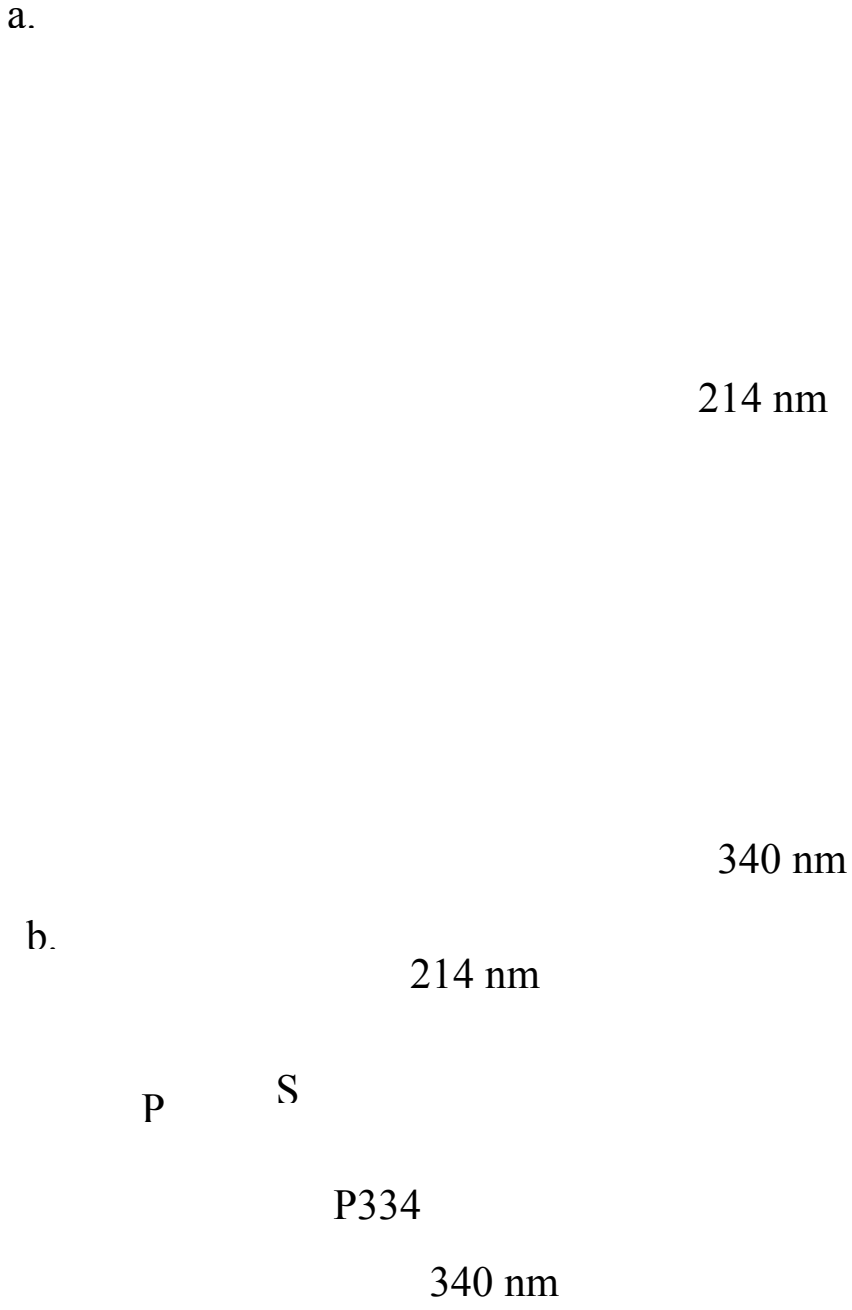


Figure 17. Comparison of HPLC chromatogram measured from a. extracts of *Halophila johnsonii* and b. prepared standards of palythine (P), shinorine (S), and porphyra-334 (P334). Absorbance at 214 and 340 nm was detected for both samples.

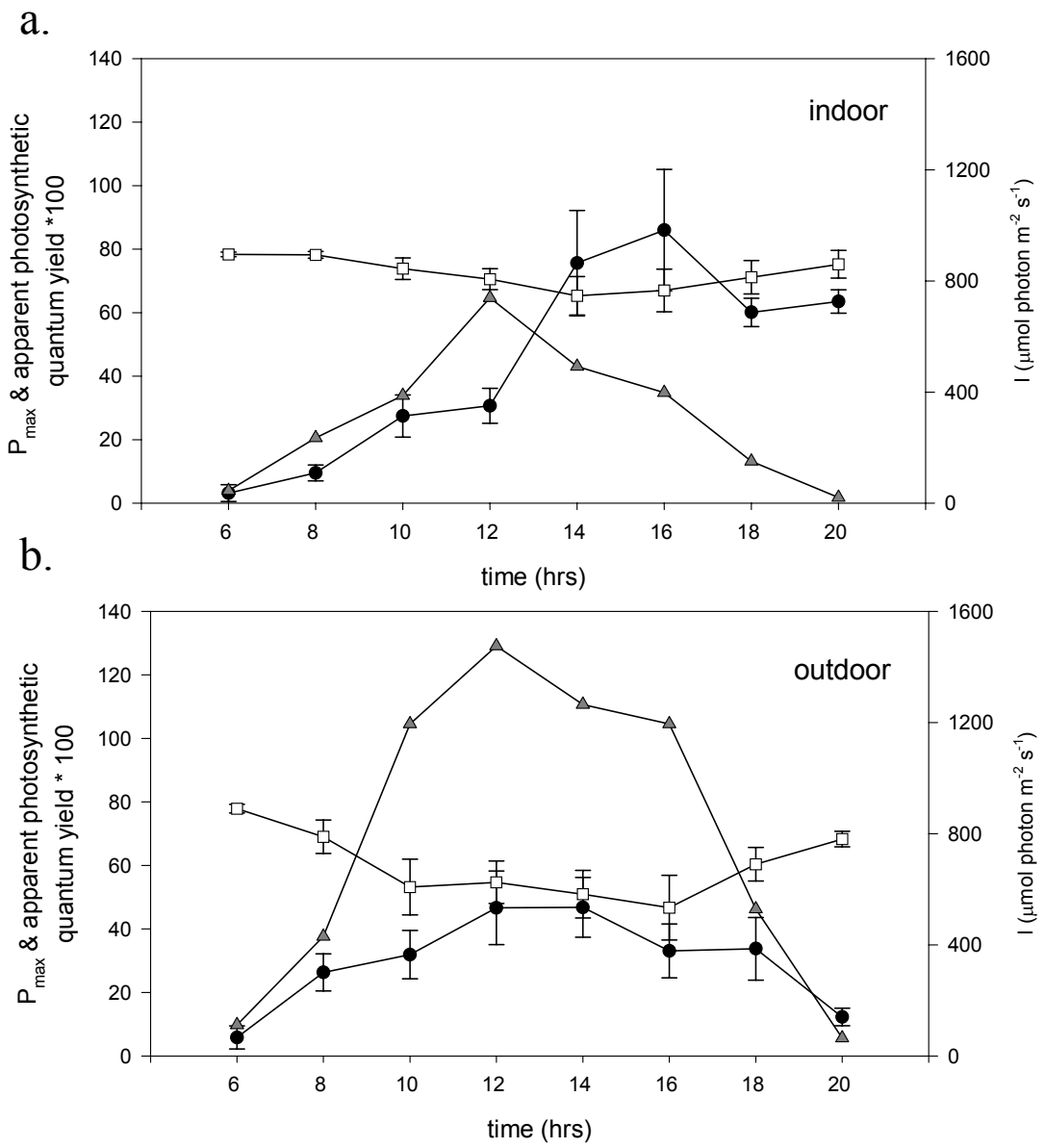


Figure 18. Diurnal changes in *Halophila johnsonii* growing a. in the greenhouse and b. on the outdoor platform. Changes in P_{max} and apparent photosynthetic quantum yield sampled at 06:00 to 20:00 hrs. Symbols = mean, error bars = sd, n = 10; ● P_{max}; □ apparent photosynthetic quantum yield; ▲irradiance.

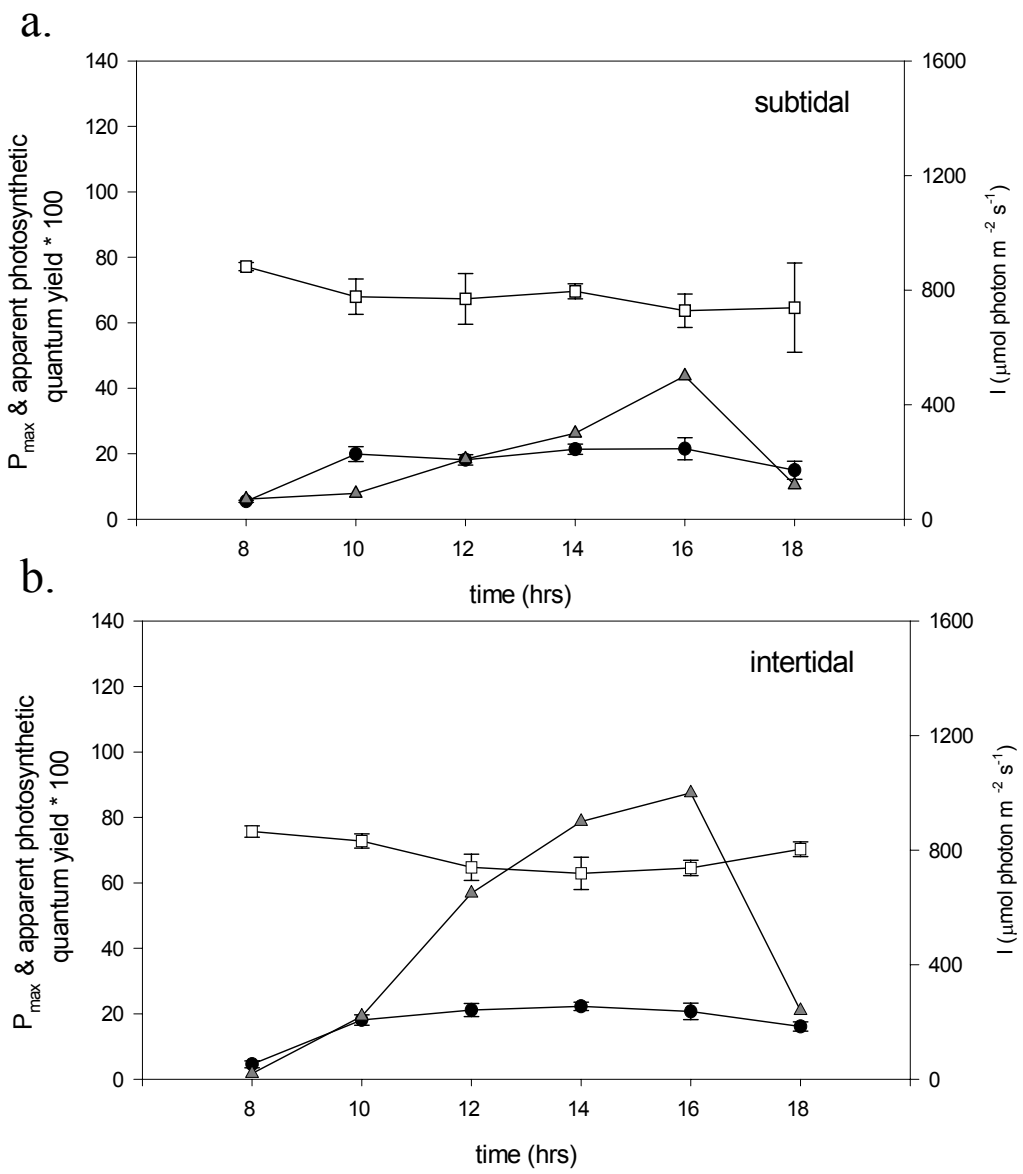


Figure 19. Diurnal changes in *Halophila johnsonii* growing a. subtidal and b. intertidal at Haulover Park in northern Biscayne Bay. Changes in P_{max} and apparent photosynthetic quantum yield sampled at 06:00 to 20:00 hrs. Symbols = mean, error bars = sd, n = 10; ● P_{max}; □ apparent photosynthetic quantum yield; ▲irradiance.

early morning, with increasing irradiance to a maximum at 16:00 hrs, followed by a rapid decrease to sunset. The maximum irradiance measured in the subtidal was 500 μmol photons m^{-2} s^{-1} (Figure 19a), and 1000 μmol photons m^{-2} s^{-1} in the intertidal (Figure 19b).

In the greenhouse (Figure 18a), apparent photosynthetic quantum yield (Y) slowly decreased from 6:00 and 8:00 hrs values to a minimum value at 14:00 hrs, which slowly increased and recovered to the early morning values by 20:00 hrs. Except for the dawn measurements, Y was significantly higher in the greenhouse replicates compared to the outdoor replicates. On the outdoor platform (Figure 18b), Y steadily decreased from the maximum values measured at dawn to a mid-day trough between 10:00 and 16:00 hrs, and then slowly increased, but did not recover by the 20:00 hrs dusk measurement. In the greenhouse, P_{max} slowly increased between dawn and mid-day but underwent more than a 2-fold increase between 12:00 and 14:00 hrs. This increase in P_{max} continued through 16:00 hrs but then steadily decreased to dusk. Throughout the day, P_{max} measured in greenhouse replicates was significantly different from the outdoor replicates, except at dawn and 10:00 hrs. In the outdoor replicates, P_{max} steadily increased from dawn to a mid-day plateau between 12:00 and 14:00 hrs, and then declined to dusk.

In the subtidal population (Figure 19a), there was a trend of decreasing Y throughout the day which was particularly significant between 08:00 and 16:00 hrs. These values were only significantly higher than the intertidal population at 10:00 and lower at 14:00 hrs. In the intertidal population (Figure 19b), Y steadily decreased from the sunrise maximum to a mid-day trough between 12:00 and 16:00 hrs, and then slowly increased, but did not recover by the 18:00 hrs dusk measurement. P_{max} was only different between the intertidal and subtidal at 08:00 and 12:00 hrs. Both populations

demonstrated the same P_{max} patterns of increase between 08:00 and 10:00 hrs to a mid-day plateau, which then decreased between 16:00 hrs and dusk.

DISCUSSION

The results of this study support the conclusions of Durako et al. (in press) that *Halophila johnsonii* is an extremely UV-tolerant plant. Monitoring changes in the fluorometric and pigment characteristics of *Halophila johnsonii* revealed a dynamic initial response period followed by a period during which an acclimation-state was reached for the duration of the experimental period. Exposure to the different irradiance conditions manipulated in this study, PAR + UVA + UVB, PAR + UVA, versus PAR alone did not induce significantly different responses in *Halophila johnsonii*.

During the 21-day exposure to the three different irradiance treatments, alpha either increased or did not change, P_{max}, beta, and apparent photosynthetic quantum yield did not change. Only on day 4 was there ever a statistically significant difference observed in any of the fluorescence variables among the three treatments.

Do to the recent development of the UVAPAM, it is unclear whether the measurements derived from this technique are appropriate for seagrasses. The percent UV shield data collected during this experiment indicated that photosystem protection from UV radiation was highest on day 1, following the period of UV-free conditions in the greenhouse, and slowly decreased during the experimental period. Nevertheless, after the initial decrease in percent UV shield, these values leveled off and remained fairly constant through day 21. Again, the same trend was observed in all three treatments, and no significant differences in percent UV shield were observed among the three treatments. The percent UV shield values measured for each of the replicates appears to have a positive relationship with the total area UV peaks (Figure 15). This relationship

indicates that the UVA PAM fluorometer is an accurate 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 1989). Yakovleva and Titlyanov (2001) refer to the earliest stages of PAR or UV treatment 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 long-term exposure to UV irradiance. The initial accumulation of these compounds in *Halophila johnsonii* rather than degradation supports the idea that this species has a high tolerance for UV irradiance and increased PAR.

Following an initial response period in *Halophila johnsonii*, the photochemical compounds appeared to stabilize at long-term acclimation concentrations. The acclimated concentrations were either the same or slightly lower in most cases, than day 0 concentrations. The ratio of UV peak 1 to UV peak 2 was higher in the acclimated

state due to an increase in UV peak 1 absorbance following the initial response period. Based on the HPLC-UV chemical analysis it appears that these two absorption peaks are the result of several different UV-absorbing compounds. The change in the UV peak 1 to UV peak 2 ratio may be related to wavelength-dependant induction of one or more of these compounds. Synthesis of the MAA shinorine is most significantly induced by the shorter wavelengths of UV-B (Sinha et al. 2002). Alternatively, synthesis of palythine, palythene, and palythinol are more affected by high PAR than by UV irradiance (Karsten et al. 1998). Nevertheless, there were no differences in pigment absorbance or concentrations among the three irradiance treatments of *Halophila johnsonii*. Therefore, the wavelengths that induced synthesis must have been between 400 and 700 nm.

The elution of several different compounds that absorbed at 340 nm in the tissue extracts of *Halophila johnsonii* all had longer retention times than the three MAA standards available. It is also evident from the strong absorbance at 214 nm that these are highly aromatic compounds, more so than the MAA standards. This evidence suggests that the unknown UV-absorbing compounds are more likely to be flavonoids. Further analysis of the UV-absorbing isolates by LC-MS and NMR will be necessary to identify these compounds with certainty.

The UV-absorbing compounds present in the tissues of *Halophila johnsonii* appear to be constitutive. If this is the case, these compound(s) 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 compound's primary physiological role is non-photochemical, this would explain why significant UV absorbance exists in apical leaves prior to

chlorophyll synthesis. This would also explain why the compound was not specifically induced by UV exposure or degraded in the absence of UV radiation.

No difference in DNA damage was detected among the different treatments (data not shown). Virtually no DNA damage could be detected in any of the *Halophila johnsonii* samples compared to a more UV sensitive plant, soybean (personal communication, Dr. Ann Stapleton). Such low DNA damage following exposure to UV irradiance could only result from efficient UV protection or exceptionally rapid DNA damage repair mechanisms. These results agree with the fluorescence and pigment data. Exposure to UV irradiance was not significantly damaging compared to exposure to PAR alone.

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. Therefore, how we detect physiological responses is also largely a function of the parameters we measure.

Detrés et al. (2001) examined photosynthetic and photoprotective pigments as well as leaf optical properties in two marine macrophytes, *Rhizophora mangle* and *Thalassia testudinum*. In these species, plants exposed to UV demonstrated a decrease in

leaf reflectance, while plants exluded from UV had a significant increase in total chlorophyll and carotenoid concentrations. Other studies have used variables such as growth to quantify the UV response. The intertidal species *Ulva rigida* (Altamirano et al. 2000) and *Ulva expansa* (Grobe and Murphy 1998) experimentally exposed to UV irradiance both demonstrated reduced growth following 7 days of exposure to UV irradiance. After 20 days however, the growth rates of UV-exposed replicates had recovered and were no longer significantly different from samples exposed only to PAR. Pigment and fluorometric analysis was used to measure the response of *Chondrus crispus* to elevated PAR and UV irradiance (Yakovleva and Titlyanov 2001). This subtidal red alga demonstrated physiological stress at the onset of the different irradiance treatments but recovered through the induction of photoprotective mechanisms. Another red alga, *Gelidium pulchellum*, was less tolerant of UV exposure, exhibiting a greater degree of photoinhibition and slower recovery, particularly when exposed to a higher background level PAR (Gómez et al. 2001). Using fluorometric and pigment characteristics to detect UV-induced changes in *Halophila johnsonii* indicated a broad tolerance to varying irradiance conditions.

Halophila johnsonii has a particularly shallow depth distribution, within an extremely restricted geographic range. It persists intertidally where other seagrasses can not, but appears to be competitively excluded from deeper subtidal environments by seagrasses, such as *Halodule wrightii*, *Syringodium filiforme*, possibly by *Halophila decipiens* and larger-bodied macroalgae. *Halophila johnsonii* is clearly adapted to the high PAR and UV irradiance to which it is exposed under natural conditions in the intertidal and shallow subtidal environments in southeastern Florida. Coincidentally,

these are the environments that are particularly susceptible to anthropogenic disturbances. Construction of residential docks and commercial marinas, dredging and the deposition of dredge spoil, boat groundings, and direct contact from human recreation may all be detrimental to *Halophila johnsonii*. The construction of seawalls throughout the coastal lagoons of southeastern Florida has virtually eliminated much of the suitable intertidal and subtidal habitat for *Halophila johnsonii*. In the adjacent shallow subtidal areas, high turbidity, which drastically reduces light intensity, may be the factor limiting *Halophila johnsonii*. Regardless, the intertidal and shallow subtidal appears to be a refuge for this threatened species, and conservation of these habitats may be vital to its survival.