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Effects of ultraviolet and photosynthetically active radiation on five seagrass species

Abstract Five seagrass species [*Halophila ovalis* (R.Br) Hook. f., *Halodule uninervis* (Forsk.) Aschers., *Zostera capricorni* Aschers., *Cymodocea serrulata* (R.Br) Aschers. (ed.) and *Syringodium isoetifolium* (Aschers.) Dandy] from Moreton Bay, Australia, were grown under increased (+25%) and ambient levels of ultraviolet (UV) radiation and photosynthetically active radiation (PAR), and various morphological and physiological responses were examined. Leaf fluorescence ratio (variable:maximum fluorescence) in conjunction with xanthophyll pigment content (violaxanthin, antheraxanthin and zeaxanthin) were used as a measure of photosynthetic efficiency. In addition, absorbance in the UV spectrum, chlorophyll content and chloroplast density were used as indicators of photosynthetic capacity. The seagrass species examined had varying degrees of sensitivity to UV radiation. *Halophila ovalis* and *Halodule uninervis* were the most sensitive species, exhibiting the largest decrease in photosynthetic efficiency and chloroplast density and the smallest increase in UV-blocking pigments in response to UV radiation. The more UV-tolerant species, *Z. capricorni*, *C. serrulata* and *S. isoetifolium*, were only significantly affected by increased levels of UV radiation, showing a gradual decline in photosynthetic efficiency and chloroplast density and the largest increases in UV-blocking pigment. UV sensitivity corresponded with leaf morphology, with thicker leaves (as in *Z. capricorni*, *C. serrulata* and *S. isoetifolium*) providing greater morphological protection for UV-sensitive organelles. Not all species were significantly affected by increasing PAR, with decreases in fluorescence ratio and increases in zeaxanthin content observed only in *C. serrulata* and *S. isoetifolium*. Sensitivity to PAR corresponded with morphological plasticity; species exhibiting a wide range of growth forms (e.g. *Halophila ovalis*, *Halodule uninervis* and *Z. capricorni*) were the least sensitive to increases in PAR. Seagrass depth-distributions in Moreton Bay appear to be influenced by species sensitivity to UV radiation and PAR, with other factors such as epiphytes, shading and nutrients also affecting species' tolerance. All species were affected to some degree by UV radiation, thus future changes in UV intensity may have repercussions on the distribution of seagrasses.

Introduction

With continued depletion of the stratospheric ozone layer by anthropogenic agents (Teramura 1983), an increase in ultraviolet radiation (UV) penetrating the water column is inevitable. The morphological and physiological effects of this increase in harmful radiation on plant communities will be largely dependent on species-specific adaptive responses to the higher levels of UV transmission.

The majority of research involving the effects of UV radiation on plants has been centred on terrestrial flora. Generally, it has been found that plants sensitive to UV-B radiation (280 to 320 nm) exhibit reductions in photosynthetic capacity, biomass, chlorophyll content and reproductive ability (Lindoo and Caldwell 1978; Worrest et al. 1978; Tevini et al. 1981; Flint and Caldwell 1984; Tevini and Teramura (1989). Morphological responses to UV radiation include epidermal deformation and blistering, lesions, reduced leaf area, increased leaf thickness and photomorphogenesis (radiation-induced change in growth form) (Cline and Salisbury 1966; Robberecht and Caldwell 1978; Tevini et al. 1981; Jackson 1987; Barnes et al. 1990).

The primary adaptive mechanisms employed by plants to cope with increased levels of UV radiation are

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based on the production of UV-absorbing compounds such as UV-blocking pigments and anthocyanins (Tevini et al. 1981; Lovelock et al. 1992). The accumulation of these compounds occurs predominantly in the epidermis, providing a UV screen for the underlying tissue. In a survey of 25 species, Robberecht and Caldwell (1978) observed that the epidermis attenuated 95 to 99% of ultraviolet radiation. Flavonoids and related pigments were reported to account for a large percentage of the attenuation.

There is limited information concerning the effects of UV radiation on marine angiosperms. Trocine et al. (1981) studied UV tolerance and photorepair capabilities of three seagrass species and noted that *Halodule wrightii* possessed a high photosynthetic tolerance for UV-B, whereas *Syringodium filiforme* and *Halophila engalmanni* were more sensitive, showing little photosynthetic tolerance. Evidence for a photorepair mechanism was only noted in *Halodule wrightii*; this mechanism decreased the degree of inhibition induced by UV-B. Trocine et al. concluded that all three species relied on epiphytic shading to reduce the degree of UV exposure. Additionally, it was speculated that the presence of flavonoids may reduce the degree of UV-induced inhibition.

Chlorophyll fluorescence has proved to be a useful tool in the detection of various stress responses in plants, including UV and high-light-induced photoinhibition (Krause and Weis 1984; Walker 1988; Larkum and Wood 1993). Under optimum environmental conditions for photosynthesis, the amount of absorbed light energy dissipated via fluorescence is relatively small (Lichtenhaler 1988). The amount of fluorescence rapidly increases as the photosynthetic efficiency of the plant decreases (Krause and Weis 1991). The ratio of variable fluorescence to maximal fluorescence ($F_v : F_m$) is typically used as an indicator of photoinhibition

(Walker 1988), with a decline in $F_v : F_m$ providing a good indicator of photoinhibitory damage (Baker and Horton 1988; Lichtenthaler 1988).

An important adaptive feature evolved by plants is the ability to dissipate excess accumulated light energy via the xanthophyll cycle (Hager 1980). It has been observed in terrestrial and marine plants that, with decreasing photosynthetic efficiency (e.g. due to high light intensities), the percentage of zeaxanthin in the total xanthophyll pool increases (Demmig et al. 1987; Demmig-Adams 1990; Demmig-Adams and Adams 1992; Lovelock and Clough 1992). Plants sensitive to high light or UV radiation may exhibit a greater de-epoxidation conversion to zeaxanthin (due to a greater accumulation of excess excitation energy) in comparison to less sensitive species.

Plant species differ considerably in their ability to tolerate UV radiation (Cline and Salisbury 1966; Tevini and Teramura 1989). Consequently, with an increase in UV radiation penetrating the water column, seagrass species most sensitive to UV may become restricted in their upper depth distribution. The present study was designed to examine the effects of increased and ambient levels of UV radiation and high PAR intensities on five seagrass species and to correlate the observed data with current depth distribution.

Materials and methods

Collection

Intact sediment cores of five seagrass species; *Halophila ovalis* (R.Br) Hook. f., *Halodule uninervis* (Forsk.) Aschers., *Zostera capricorni* Aschers., *Cymodocea serratata* (R.Br) Aschers. and *Syringodium isoetifolium* (Aschers.) Dandy, were collected from two sites at Stradbroke Island, Moreton Bay, Australia (Fig. 1): Dunwich Harbour (153°24′E; 27°28′S) and Amity Point (153°26′E; 27°24′S). All

Fig. 1 Study area. A Moreton Bay, Queensland, Australia; B generalised seagrass depth distribution in eastern Moreton Bay; C generalised schematic diagram of a seagrass transect in eastern Moreton Bay, showing various morphology types and depth distribution of each species
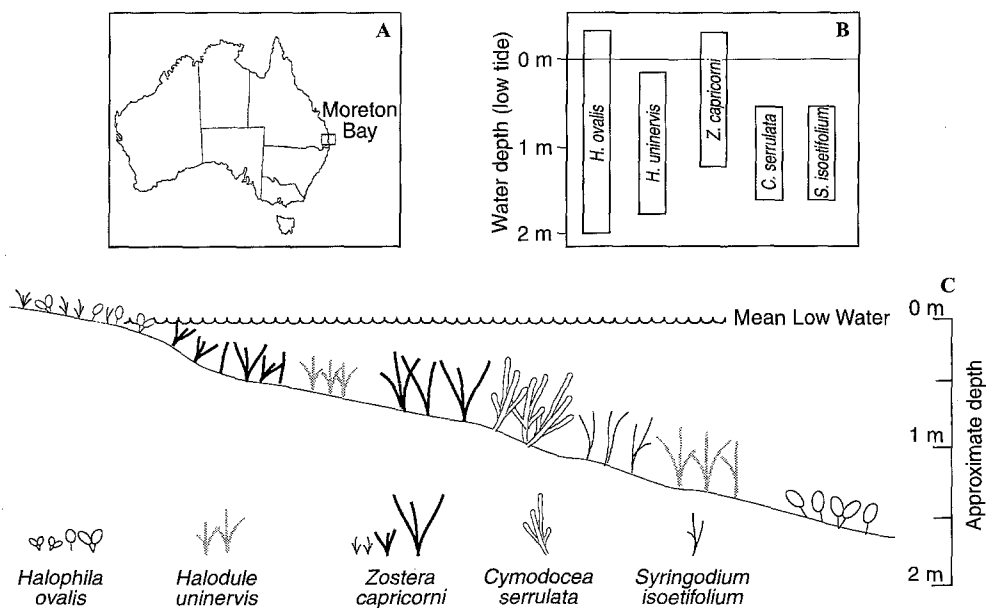
species were collected from their lower depth distribution for standardisation, and transferred to plastic pots lined with plastic bags to prevent sediment and water loss during transportation. The seagrasses were then transported to either the increased-UV experiment aquaria at the University of Queensland, Brisbane, or the ambient-UV experiment tanks at CSIRO, Cleveland.

Increased-UV experiment

The five seagrass species were cultured for 1 wk in glass aquaria at the University of Queensland under increased UV radiation and 300 μmol quanta m^{-2}s^{-1} PAR. Aquaria used filtered seawater, which was aerated and maintained at a constant temperature (23 °C) and (salinity (35‰ S)). Increased UV levels were achieved by Phillips UV-B lights (TL, 40 W/12) which simulated a 25% increase (1566 $\pm$ 80 μW s^{-1}) in current UV-B radiation levels (Wood unpublished data). Control tanks were screened from UV using transparent polycarbonate sheeting (Suntuf®) which absorbs all radiation <400 nm. UV radiation was measured spectrophotometrically according to Wood (1987) using the chemical actinometer p-nitroanisole (pNA), which has an absorbance maximum at 317 nm Dullin and Mill 1982).

Ambient-UV experiment

Fibreglass tanks were set up at CSIRO, Cleveland, Queensland with a flow-through seawater system. This system used filtered seawater at a flow rate of $\simeq$ 100 litres h^{-1} maintained at an average temperature and salinity of 23 °C and 35‰, respectively. The five seagrass species were cultured for 1 mo in two light treatments – 100 or 50% of ambient light (controlled using 50% shade-cloth) with ambient levels of UV radiation. Control tanks consisted of 100 and 50% light treatments with UV radiation screened off by transparent polycarbonate sheeting.

Fluorescence measurements

Leaves of each species were dark-adapted for a period of 30 min. In vivo fluorescence measurements were carried out using a "plant efficiency analyser" (Hansa-Tech, England). Each leaf was irradiated for 5 s, and fluorescence was measured. The fluorescence parameters examined were the ratio of variable to maximum fluorescence (F_v:F_m), initial fluorescence (F_v) and maximum fluorescence (F_m) (Bolhar-Nordenkampf et al. 1989).

Chlorophyll and UV-blocking pigment analysis

Leaf samples were ground using a mortar and pestle in 80% acetone. Acetone extracts were wrapped in aluminium foil and stored at 4 °C to allow suspended material to settle. Chlorophyll analysis and UV-blocking pigment analysis were conducted spectrophotometrically, measuring absorbance from 800 to 280 nm. Chlorophylls a and b were calculated using Arnon's (1949) equations, and UV-blocking pigment was calculated from a standardised wave scan.

Xanthophyll determinations

Leaf samples were collected and stored at -70 °C until analysis. Samples were weighed to determine fresh weight and then ground in 2 ml of 85% acetone before centrifugation at 12 000 $\times g$ for 3 min. The supernatant was decanted, and the pellet was resuspended in

85% acetone and recentrifuged, and the supernatants were combined. Xanthophyll content (violaxanthin, antheraxanthin and zeaxanthin) was determined with a dual pump Beckman System Gold HPLC and a Spherisorb ODS-1 non-endcapped, 6% carbon column (5 μm particle size, 250 mm $\times$ 4.6 mm i.d.) following a modified version of the method described by Gilmore and Yamamoto (1991). The modifications consisted of a 3 min linear gradient from 100% Solvent A to a 70%:30% Solvent A:B solution followed by a 6 min linear gradient to 100% B. Zeaxanthin content is expressed as a percentage of the total xanthophyll pool.

Light microscopy

Leaf samples were fixed in formal–seawater (4% formalin in seawater), and stored until sectioned. Epidermal shavings were sliced from fixed leaf portions (using standardised techniques) and mounted in seawater. A light microscope was used to determine chloroplast numbers per cell.

Nutrient analysis

Whole samples of *Halophila ovalis* were collected from both the intertidal and the subtidal zone at Dunwich Harbour, Stradbroke Island. The samples were cleaned and sorted into above- and below-ground biomass. The tissue was then oven-dried at 60 °C and ground to a fine powder using a Vibratory ball mill (Retsch Model MM-2) Haan, Germany. Known quantities of tissue were placed in Kjeldahl digestion tubes with 5 ml of concentrated sulphuric acid containing 2.5% salicylic acid. A Kjeldahl catalyst tablet and three 2 mm-diam glass beads were added to the digestion tubes, and the solution was mixed. After a 15 min period, 0.5 g of sodium thiosulphate was added to the mixture and again mixed. The digestion tubes were stored for a minimum of 2 h before placing in the digester block (AIM 500, Chemlab, England), where the samples were heated to 395 °C over 5 h. Samples were left to cool before diluting with distilled water. Analysis of nitrogen and phosphorus was conducted using a Chemlab Mark 1 autoanalyser. Nitrogen and phosphorus content was determined colorimetrically (Greenberg et al. 1992).

Statistics

The data were analysed on the computer software package Quattro Pro for Windows (Borland) using one-way and two-way ANOVAS.

Results

Fluorescence

The F_v:F_m ratio of all five species decreased signficantly ($p < 0.05$) under increased levels of UV radiation (Fig. 2). The greatest decrease in F_v:F_m was observed in *Halodule uninervis*, decreasing to 0.47 after 7 d (Fig. 2B). *Halophila ovalis* was similarly affected, with the F_v:F_m ratio decreasing to 0.54 after 7 d (Fig. 2A). Responses to UV radiation in *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium* were less pronounced. Initially, in *Z. capricorni*, the F_v:F_m ratio declined rapidly before increasing to a final value of 0.71 (Fig. 2C). *C. serrulata* and *S. isoetifolium* were
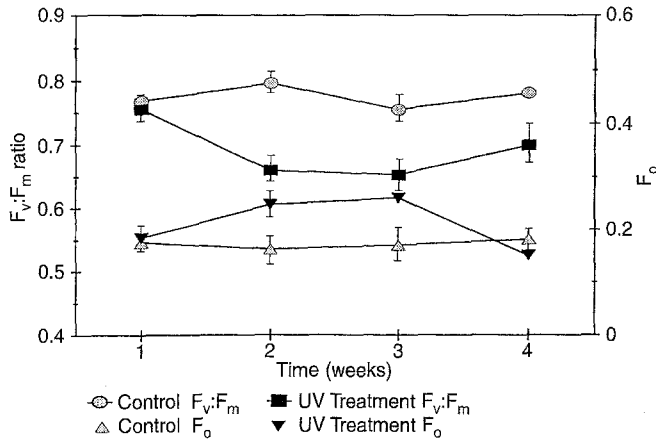
A *Halophila ovalis*

B *Halodule uninervis*

C *Zostera capricorni*

D *Cymodocea serrulata*

E *Syringodium isoetifolium*
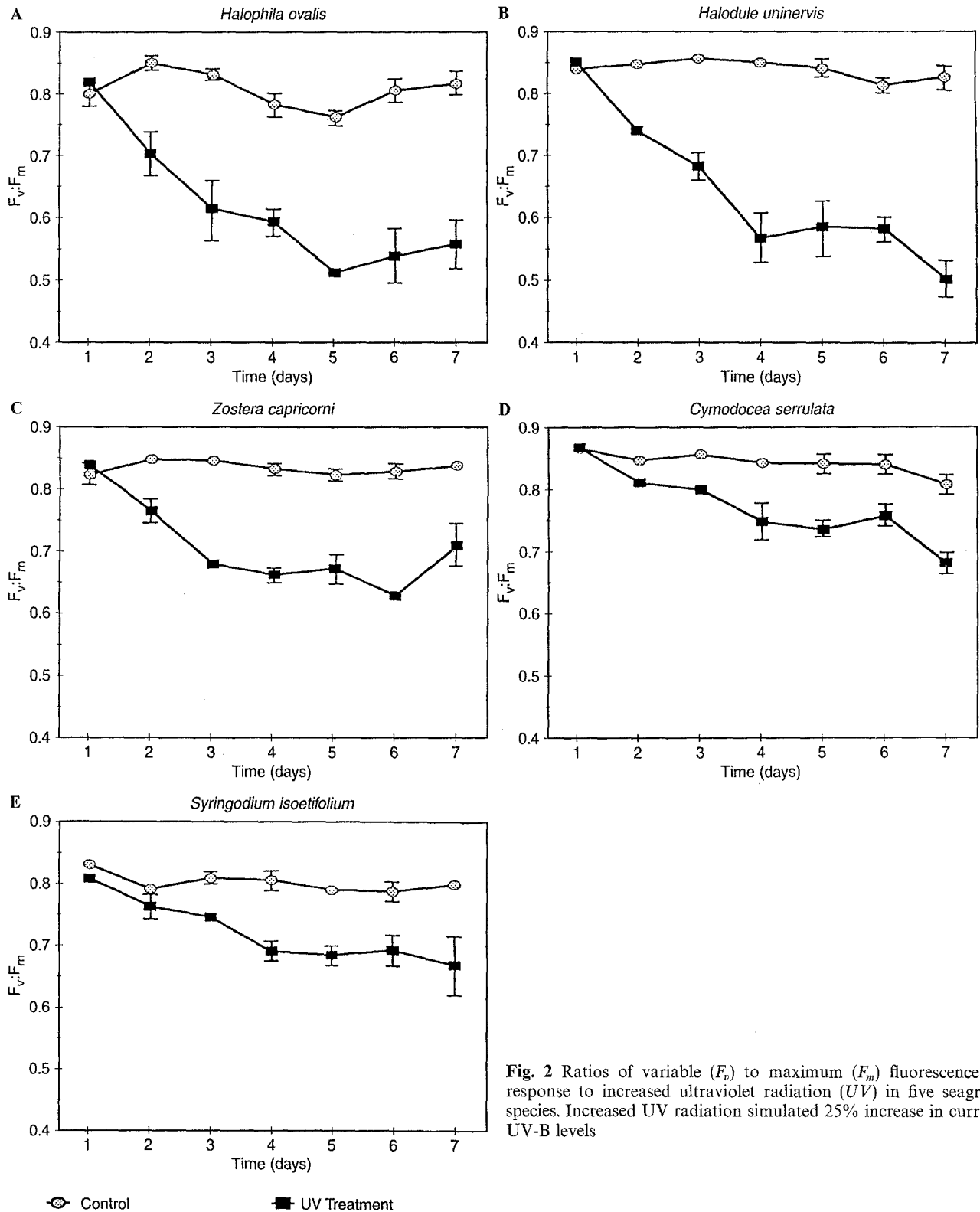
Fig. 2 Ratios of variable (F_v) to maximum (F_m) fluorescence in response to increased ultraviolet radiation (UV) in five seagrass species. Increased UV radiation simulated 25% increase in current UV-B levels

-○- Control ■ UV Treatment

the least-affected species, with gradual declines in the $F_v:F_m$ ratio of 0.68 to 0.67, respectively (Fig. 2D, E).

The greatest response to ambient UV radiation was again observed in *Halophila ovalis* and *Halodule uninervis*, with $F_v:F_m$ ratios decreasing from 0.79 and 0.82 to 0.63 and 0.64, respectively. The $F_v:F_m$ ratio in *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium* did not decrease significantly $(p > 0.05)$ in response to ambient levels of UV radiation (see Table 2).

Additionally, initial fluorescence (F_0) was monitored and was found to increase significantly ($p < 0.05$) under ambient levels of UV radiation in all species (e.g. *Zostera capricorni*; Fig. 3). The fluorescence maximum (F_m) did not vary significantly ($p > 0.05$) in the species examined.

Fig. 3 *Zostera capricorni*. Fluorescence results from ambient-UV radiation experiment. Ratios of variable (F_v) to maximum fluorescence (F_m) are plotted with initial (F_0) fluorescence over a 4 wk period

A comparison of 100 and 50% PAR treatments revealed a reversal of the previous species trend. With respect to UV radiation, the species most affected progressed from *Halophila ovalis* to *Syringodium isoetifolium*, while under high light *S. isoetifolium* was most affected, progressing to *H. ovalis*, the least affected. Under high PAR, the $F_v:F_m$ ratio decreased significantly ($p < 0.05$) only, (in *Cymodocea serrulata* (-11%), and *S. isoetifolium* (-12%; Table 3).

Pigments

Increased levels of UV radiation induced significant ($p < 0.05$) decreases in chlorophylls (mg chlorophyll $a+b\ g^{-1}$ fresh wt; Table 1) in all five species. The species most affected were *Halophila ovalis* and *Halodule uninervis*, decreasing by 72 and 53%, respectively. Smaller decreases in chlorophyll were observed in *Zostera capricorni* (-45%), *Cymodocea serrulata* (-30%) and *Syringodium isoetifolium* (-13%).

The percentage zeaxanthin in the total xanthophyll pool of the increased UV treatment plants did not vary

Table 1 Summary of parameters examined in increased-UV experiment simulating 25% increase in current levels of UV radiation. Five seagrass species were cultured in aquaria at University of Queensland, Brisbane, for 7 d under the increased UV intensity [$F_v:F_m$ ratio of variable to maximal fluorescence; $Z:VAZ$ percentage of zeaxanthin in total xanthophyll pool (violaxanthin, antheraxanthin, zeaxanthin); *, significantly different at $p < 0.05$; nd no data]

Parameter	Halophila ovalis	Halodule uninervis	Zostera capricorni	Cymodocea serrulata	Syringodium isoetifolium
Fluorescence $(F_v:F_m)$					
Control	0.84	0.86	0.84	0.81	0.80
UV treatment	0.54	0.47	0.71	0.68	0.67
% change	-35.3	-45.4	-15.5	-16.1	-16.3
F value	39.4*	64.9*	8.5*	18.1*	16.1*
Chlorophyll content (mg chl $a + b\ g^{-1}$ fresh wt)					
Control	6.6	6.8	6.2	4.2	1.9
UV treatment	1.9	3.2	3.4	2.9	1.6
% change	-71.0	-52.8	-44.9	-30.1	-14.0
F value	28.4*	53.5*	27.1*	31.9*	39.7*
% Zeaxanthin $(Z:VAZ)$					
Control	15.4	13.4	11.1	18.4	66.5
UV treatment	32.9	22.9	11.3	16.7	68.6
% change	53.2	41.5	1.8	-9.2	3.1
F value	361.4*	307.3*	0.06	0.72	0.49
UV-blocking pigment (relative absorbance)					
Control	0.5	0.4	0.5	0.4	0.4
UV treatment	0.7	0.5	1.8	0.7	0.8
% change	25.7	21.3	72.1	32.3	56.0
F value	22.9*	45.6*	11.7*	29.1*	17.8*
Epidermal chloroplasts (no. chloroplasts cell^{-1})					
Control	115.5	181.8	28.3	19.5	24.2
UV treatment	30.0	69.5	16.4	13.5	21.2
% change	-74.0	-61.8	-42.1	-30.8	-12.4
F value	10.7*	17.0*	65.2*	14.3*	7.2*
Mesophyll chloroplasts (no. chloroplasts cell^{-1})					
Control	nd	54	12	29	13
UV treatment	nd	20	8	23	23
% change	nd	-62.7	-32.8	-20.5	43.5
F value	nd	79.3*	38.9*	10.5*	24.4*

significantly ($p > 0.05$) in *Zostera capricorni*, (0%) *Cymodocea serrulata* (− 2%) or *Syringodium isoeti-folium* (− 2%). However, significant ($p < 0.05$) increases in the percentage of zeaxanthin occurred in *Halophila ovalis* and *Halodule uninervis*, increasing by 18 and 9%, respectively (Table 1).

UV-blocking pigment content increased significantly ($p < 0.05$) in all five species under increased levels of UV radiation. The smallest increases in UV-blocking pigment occurred in *Halodule uninervis* (22%) and *Halophila ovalis* (27%), while *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium* showed greater accumulation of UV-blocking pigments with increases of 72, 33 and 56%, respectively (Table 1).

In the ambient-UV experiment chlorophylls decreased significantly ($p < 0.05$) in *Halophila ovalis* (− 36%) and *Halodule uninervis* (− 22%). However, no significant ($p > 0.05$) differences were observed in *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium* (Table 2). As in the increased UV experiment, significant ($p < 0.05$) increases in the percentage of zeaxanthin in the total xanthophyll pool were observed only in *H. ovalis* (23%) and *H. uninervis* (22%), *Z. capricorni*, *C. serrulata* and *S. isoetifolium* did not increase zeaxanthin content significantly ($p > 0.05$) in response to ambient levels of UV radiation.

All species increased UV-blocking pigment content significantly ($p < 0.05$) in response to ambient levels of UV radiation. The greatest accumulation was observed in *Zostera capricorni* (+42%), *Cymodocea serrulata* (+32%) and *Syringodium isoetifolium* (+40%). *Halo-phila ovalis* and *Halodule uninervis* increased UV-blocking pigment content by 21 and 18%, respectively.

Although chlorophylls decreased in all species under high PAR, these differences were statistically insignificant at $p < 0.05$ (Table 3). Under increased levels of PAR, all species showed increases in the percentage of zeaxanthin in the total xanthophyll pool. However, significant differences ($p < 0.05$) were only observed in the high PAR-affected species *Cymodocea serrulata* (+61%) and *Syringodium isoetifolium* (+53%; Table 3). UV-blocking pigment content did not increase significantly ($p < 0.05$) in any of the seagrasses examined in response to high PAR (Table 3).

Chloroplast numbers

Chloroplast numbers per epidermal cell decreased significantly ($p < 0.05$) in all five species in response to increased levels of UV radiation. *Halophila ovalis* and *Halodule uninervis* were the most affected, both showing decreases in epidermal chloroplast numbers per cell of 74% (Table 1). Epidermal chloroplast numbers were less variable in *Zostera capricorni*, *Cymodocea serrulata* and *Syringodium isoetifolium*, with decreasing epidermal chloroplast numbers of 42, 31, and 12%, respectively. Mesophyll chloroplast numbers were similarly affected − *H. uninervis* showed the greatest response (63% decrease), ranging down to *Z. capricorni* (33% decrease) and *C. serrulata* (21% decrease). In contrast, *S. isoetifolium* increased its numbers of chloroplasts per mesophyll cell by 43%. The leaf morphology of

Table 2 Summary of parameters examined in 100% ambient-UV experiment. Five seagrass species were cultured for 1 mo under ambient UV and photosynthetically active radiation in flow-through aquaria. Further details as in legend to Table 1

Parameter	*Halophila ovalis*	*Halodule uninervis*	*Zostera capricorni*	*Cymodocea serrulata*	*Syringodium isoetifolium*
Fluorescence ($F_v:F_m$)					
Control	0.79	0.82	0.78	0.76	0.76
UV treatment	0.63	0.64	0.70	0.74	0.74
% change	− 19.9	− 21.9	− 10.4	− 2.4	− 2.1
F value	9.0*	11.5*	2.4	3.8	2.4
Chlorophyll content (mg chl $a+b$ g^{-1} fresh wt)					
Control	6.4	5.4	4.2	3.6	2.2
UV treatment	4.1	4.2	4.0	3.3	2.1
% change	− 35.9	− 21.9	− 4.9	− 7.0	− 3.2
F value	24.5*	19.8*	4.1	0.3	0.2
% Zeaxanthin ($Z:VAZ$)					
Control	49.9	48.7	47.3	71.7	80.8
UV treatment	65.1	62.3	48.2	72.9	79.7
% change	23.4	21.8	1.9	0.7	1.4
F value	17.8*	11.3*	1.4	0.7	0.3
UV-blocking pigment (relative absorbance)					
Control	2.0	1.7	1.6	1.5	1.7
UV treatment	2.6	2.0	2.7	2.2	2.8
% change	21.3	18.0	41.9	31.8	39.5
F value	28.6*	7.03*	64.3*	12.4*	9.06*

Table 3 Summary of parameters investigated in comparison of 100 and 50% of photosynthetically active radiation (*PAR*). Five seagrass species were cultured for 1 mo in two light treatments – 100 or 50% of ambient PAR (controlled using 50% shade-cloth) in flow-through aquaria. Further details as in legend to Table 1

Parameter	Halophila ovalis	Halodule uninervis	Zostera capricorni	Cymodocea serrulata	Syringodium isoetifolium
Fluorescence (F_v:F_m)					
100% PAR	0.79	0.82	0.78	0.76	0.76
50% PAR	0.80	0.84	0.81	0.86	0.87
% change	−1.3	−2.2	−3.0	−11.0	−12.1
F value	0.7	0.3	2.4	21.8*	32.4*
Chlorophyll content (mg chl $a+b$ g^{-1} fresh wt)					
100%	6.4	5.4	4.2	3.6	2.2
50%	6.5	5.8	4.4	3.6	2.2
% change	−1.7	−7.1	−2.5	−2.2	−1.8
F value	0.4	2.2	1.5	1.4	0.1
% Zeaxanthin (Z:VAZ)					
100%	49.9	48.7	47.3	71.7	80.8
50%	44.0	39.2	40.3	27.8	37.7
% change	11.8	19.5	14.8	61.2	53.3
F value	3.0	2.0	0.3	15.2*	22.8*
UV-blocking pigment (relative absorbance)					
100%	2.0	1.7	1.6	1.5	1.7
50%	1.8	1.4	1.4	1.1	1.6
% change	10.0	17.6	12.5	26.7	5.9
F value	0.3	1.8	2.74	3.4	0.1

Halophila ovalis made mesophyll chloroplast counts impractical, since there was only a small region of mesophyll tissue around the mid vein.

In the ambient-UV radiation experiment, no significant ($p > 0.05$) differences in epidermal or mesophyll chloroplast numbers per cell were observed in any of the five species examined.

Nutrient analysis

A comparison of the nutrient status between intertidal and subtidal *Halophila ovalis* indicated that intertidal *H. ovalis* had significantly ($p < 0.05$) lower tissue nitrogen ($0.91 \pm 0.1\%$ N) than *H. ovalis* occurring subtidally ($1.57 \pm 0.2\%$ N). No significant ($p > 0.05$) differences were observed in phosphorus content between the intertidal ($0.16 \pm 0.1\%$ P) and subtidal ($0.27 \pm 0.1\%$ P) *H. ovalis*.

Discussion

All seagrass species examined in this experiment displayed various degrees of sensitivity to UV radiation and high PAR intensity. Sensitivity was defined as a response to an external condition, with the most sensitive species exhibiting the most extreme response based on the data presented, whereas tolerance was interpreted as the ability of the species to endure an external condition.

In the increased-UV experiment, all species were significantly affected by UV radiation. *Halophila ovalis* and *Halodule uninervis* were the most sensitive species, with little tolerance to increased UV levels. *Halophila ovalis* and *Halodule uninervis* showed the largest reductions in the F_v:F_m ratio, chlorophyll content and chloroplast numbers per cell, and the smallest increases in UV-blocking pigment content. *Halophila ovalis* and *Halodule uninervis* were the only species to significantly increase percent zeaxanthin in response to UV radiation. *Zostera capricorni* was also sensitive to UV radiation; however, this species had the ability to rapidly acclimatise. Although an initial decrease in the F_v:F_m ratio was observed after the onset of UV treatment, *Z. capricorni* showed signs of recovery, with a stabilising of the fluorescence ratio. *Z. capricorni* exhibited reductions in chlorophylls and chloroplast numbers per cell, and increases in UV-blocking pigment content. The most tolerant species were *Cymodocea serrulata* and *Syringodium isoetifolium*, which exhibited the smallest reductions in the F_v:F_m ratio, chlorophylls and chloroplast numbers per cell, and large increases in UV-blocking pigment. The same trends were observed in the ambient-UV experiment, where *Halophila ovalis* and *Halodule uninervis* were the most sensitive to the effects of UV radiation and *Z. capricorni*, *C. serrulata* and *S. isoetifolium* were again the more tolerant species.

High PAR intensity resulted in a reversal of the UV-sensitivity trend, with *Cymodocea serrulata* and *Syringodium isoetifolium* the more sensitive species, and

Zostera capricorni, Halodule uninervis and *Halophila ovalis* the more tolerant species. Only the PAR-sensitive species, *C. serrulata* and *S. isoetifolium*, showed significant responses to PAR, namely decreases in the $F_v:F_m$ ratio and large increases in percent zeaxanthin.

Chlorophyll fluorescence reflects the primary processes of photosynthesis, such as light absorption, energy transfer and the photochemical reactions in Photosystem II (PS II) (Krause and Weis 1988). A reduction in photosynthetic efficiency of the chloroplast will result in a decrease in the ratio of variable to maximal fluorescence ($F_v:F_m$) (Krause and Weis 1991). However, it is important to distinguish between increases in F_v and decreases in F_m, as either will result in a decreased $F_v:F_m$ ratio. It has been suggested that an increase in F_0 is characteristic of destruction of the PS II reaction centres, whereas a decline in F_m may indicate an increase in non-photochemical quenching (Bjorkman 1986; Baker and Horton 1988). Monitoring of F_m and F_0 of the various species in the ambient-UV experiment revealed that the decrease in the $F_v:F_m$ ratio was due to an increase in F_0, indicating that UV radiation had a detrimental effect on the PS II reaction centres. PS II reaction-centre damage corresponded to decreased chloroplast numbers and chlorophylls. Higher $F_v:F_m$ values were observed in the more tolerant species, which had a greater percentage of chloroplasts and higher chlorophyll content. Various other researchers have also concluded that the primary site of UV-induced inhibition is the reaction centre of PS II (Norudeen and Kulandaivelu 1982; Iwanzik et al. 1983; Kulandaivelu and Norudeen 1983).

The degree of sensitivity to UV radiation exhibited by each species corresponds with their leaf morphology. The thicker leaves of *Zostera capricorni, Cymodocea serrulata,* and *Syringodium isoetifolium* provided a greater amount of morphological protection from UV radiation than the thin leaves of *Halophila ovalis* and *Halodule uninervis*. Increased leaf thickness may serve as a possible UV-protective mechanism, by placing a greater proportion of UV-sensitive organelles into deeper, more protected layers (Wellmann 1975; Robberecht and Caldwell 1983; Mirecki and Teramura 1984; Murali and Teramura 1985). Trocine et al. (1981) examined the effects of UV-B radiation on three seagrass species and suggested that the different degrees of sensitivity exhibited by each species might be due to the different sizes and widths of their epidermal cells. The thicker epidermal cells of *Syringodium filiforme* were reported to provide greater morphological protection from UV radiation than the thin cells of *Halophila englemanni* (Trocine et al. 1981).

The results of the present study indicate that the responses of seagrass to high light intensity are comparable to those exhibited by terrestrial plants. Generally, with increasing light intensity, changes in various morphological and physiological parameters associated with photosynthesis have been observed. Photosyn-

thetic rate (Bjorkman and Holmgren 1963; Singh et al. 1974), zeaxanthin content (Demmig et al. 1987; Lovelock and Clough 1992) and the chlorophyll $a:b$ ratio (Lewandowska et al. 1976; Ball and Critchely 1982) increase with increasing light intensity. However several parameters decrease under high light, including chlorophyll content (Lewandowska et al. 1976; Ball and Critchely 1982), leaf area (Logan and Krotkov 1969) and the $F_v:F_m$ ratio (Bjorkman et al. 1988; Gaus et al. 1996).

The degree of morphological plasticity exhibited by a species may influence its tolerance to high PAR intensity. Morphological variation within species has been observed by many seagrass researchers, both in the field and under experimental conditions, with many adaptations reportedly associated with photosynthetic capacity and light availability (Phillips 1960; Young and Kirkman 1975; McMillan 1981; Dennison and Alberte 1982; West 1990; Abal et al. 1994; Gaus et al. 1996). The sensitivity to high PAR of *Cymodocea serrulata* and *Syringodium isoetifolium* may be attributable to the lack of morphological plasticity occurring in these species, whereas the high-PAR-tolerant species *Halophila ovalis, Halodule uninervis* and *Zostera capricorni* all have a large degree of morphological variation (Dawson personal observation).

Several researchers have examined the effect of light intensity on the lower depth distribution of seagrass (Dennison and Alberte 1982; Dennison 1987; West 1990; Duarte 1991; Lee Long et al. 1993). However, the contribution of UV radiation and high light to upper depth distribution in seagrasses has so far been neglected. Species tolerance to UV radiation and high PAR intensity may contribute to current seagrass depth-distribution. Within Moreton Bay, *Cymodocea serrulata* and *Syringodium isoetifolium* are confined to a small sub-tidal depth range (Young and Kirkman 1975; Kirkman 1978); however, the present research indicates that it is not UV radiation that limits the upper depth-distribution of these species, but high PAR intensity. The leaf morphology and UV-blocking pigment production of these species provide a large degree of morphological protection from UV radiation. However, the large zeaxanthin production does not provide adequate protection against high PAR intensity, as indicated by the reduced fluorescence ratios. Therefore, the present study indicates that it is the lack of morphological and physiological acclimation to high PAR intensity in these species that limits their upper depth-distribution.

Zostera capricorni occurs both intertidally and subtidally, and is the most ubiquitous species in Moreton Bay (Young and Kirkman 1975; Kirkman 1978). This species appears to acclimatise very rapidly to adverse environmental conditions such as increased UV radiation and PAR levels. The leaf morphology provides adequate initial protection against UV radiation until other physiological protective mechanisms, such as UV-blocking pigment production and chloroplast

rearrangement, are initiated. The ability of this species to tolerate both UV radiation and high PAR intensity may be a factor contributing to its widespread distribution in Moreton Bay.

Halodule uninervis occurs in a wide depth distribution in Moreton Bay; however, it is confined to subtidal zones (Young and Kirkman 1975; Kirkman 1978). Its thin leaf morphology and zeaxanthin production provides little protection against the effects of UV radiation. However, the morphological plasticity of *H. uninervis* serves to increase its high PAR tolerance. Therefore, it is hypothesised that UV radiation, not PAR intensity, primarily restricts the upper depth-limit of this species.

Halophila ovalis occurs both in the intertidal and subtidal regions of Moreton Bay (Young and Kirkman 1975; Kirkman 1978). This species has a high degree of tolerance to high PAR intensity, associated with its morphological variation. However, *H. ovalis* is extremely sensitive to UV radiation, with leaf morphology and zeaxanthin production providing inadequate UV protection. This species may tolerate the intertidal region due to its ability to withstand high PAR intensities; however, its high degree of sensitivity to UV radiation should affect its distribution in this region.

The comparison of the nutrient status of intertidal and subtidal *Halophila ovalis* in the present study revealed that the intertidal specimens were more nutrient-deficient than their subtidal counterparts. Plants deficient in major nutrients such as nitrogen and phosphorus increased production of flavonoids (Harborne and Mabry 1982). Murali and Teramura (1985) studied the effects of phosphorus nutrition on plants irradiated with UV-B, and concluded that plants deficient in phosphorus were less sensitive to UV-B irradiation. The accumulation of flavonoids in response to nutrient deficiency increased the plants' tolerance to UV-B radiation. Therefore, intertidal *H. ovalis* may accumulate UV-blocking pigments in response to their nutrient-deficient status, thereby increasing UV-tolerance and hence increasing their survival in the intertidal zone. Other mechanisms utilised by *H. ovalis* may include the avoidance of UV radiation through chloroplast clumping and shading by epiphytes and other seagrass species (Drew 1979; Trocine et al. 1981; Gaus et al. 1996). Trocine et al. hypothesised that *H. englemanni* relied primarily on shading by coexisting seagrass species and epiphytes to reduce the degree of UV exposure. Additionally, it was noted in the present study that sediment displacement during ebbing tides often covered intertidal *H. ovalis* with a fine muddy film. This would presumably also act to reduce the effects of UV radiation and high PAR.

UV radiation and high PAR intensities affect seagrass upper depth-distribution; however, numerous other factors may also be influential. These include desiccation, temperature, pressure, salinity, seed-dispersal, germination, grazing and tidal and wave action.

In summary, although current UV levels only influence the depth-distribution of UV-sensitive species, increased levels have been shown to affect all the seagrass species examined. Therefore, continued increases in UV radiation may have repercussions on the upper depth-distribution of seagrasses and on seagrass-species composition. Further research into the effects of UV radiation on marine plants could be directed towards investigating changes to species composition and distribution, and the implications of this for the conservation of associated fauna.

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