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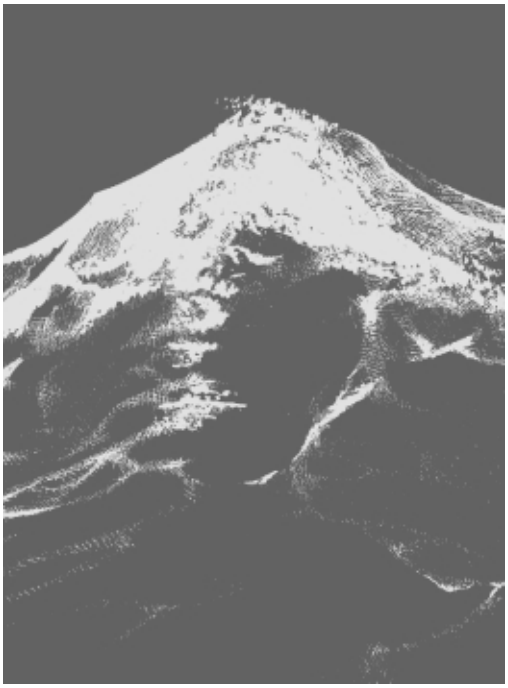

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Sensitivity of freshwater macrophytes to UV radiation: relationship to depth zonation in an oligotrophic New Zealand lake

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Abstract. The ultraviolet radiation (UVR) responses of photosynthesis by two freshwater vascular plants, *Potamogeton cheesemanii* and *Isoetes alpinus*, and the characean algae *Chara fibrosa* and *C. corallina* in Lake Coleridge, New Zealand, were investigated. Experiments comprised 4–5 h of exposure to different UV wavelengths followed by 17 h of recovery in low light. Photosynthetic competence was assessed by pulse-amplitude-modulated fluorometry. The four species showed different sensitivities to UVR, which were consistent with their upper depth limits. The shallowest-growing species, *P. cheesemanii*, was uninhibited by UVR, whereas after 5 h of exposure to UVR, inhibition of 15%, 38% and 48% was measured for *I. alpinus*, *C. fibrosa* and *C. corallina* collected from 4 m, 6.2 m and 16.5 m, respectively. Not all plants recovered fully from UVR inhibition. Plants from upper and lower depths of their growth range did not generally differ in inhibition sustained or ability to recover photosynthesis. The species with greatest tolerance of UVR also contained the highest concentrations of UVR-absorbing pigments. Freshwater macrophytes have differing abilities to tolerate UVR exposure through repair and/or protection strategies and these may be related to their vertical zonation.

Additional keywords: variable fluorescence, photoinhibition, UVR-absorbing compounds

Introduction

Research examining the biological effects of ultraviolet radiation (UVR) on aquatic environments, from molecular to ecosystem levels, has been extensive in the past 10–15 years (for reviews, see Vincent and Roy 1993; Häder *et al.* 1995; Franklin and Forster 1997; Vincent and Neale 2000). Our understanding of some of the actions and consequences of UVR for various aquatic organisms and communities has grown considerably from when stratospheric ozone thinning and associated higher levels of UVR penetration to Earth were first reported.

A significant finding of many of these studies, several of which use the simple technique of comparing performance in the absence of selected bands of UVR with that in the full ambient spectrum, is that current levels of UVR are sufficiently high to cause short- or long-term damage to organisms (e.g. Cabrera *et al.* 1997; Hazzard *et al.* 1997). It has also been shown that organisms can have markedly different tolerances of UVR (e.g. Bothwell *et al.* 1993; Dring *et al.* 1996; Vinebrooke and Leavitt 1999). These observations have given rise to considerations of what role UVR may currently play in structuring aquatic communities.

Littoral communities in lakes and oceans are often characterized by their macrophyte floras. Zonation of marine macroalgae in upper and lower subtidal regions may be related to tolerance of inhibiting irradiance in general (Hanelt *et al.* 1997; Hanelt 1998) and UVR in particular (Bischof *et al.* 1998; Bischof *et al.* 2000*b*). Differential sensitivity to UVR among marine macroalgae, as well as other organisms such as coral zooxanthellae, has been suggested to be due in part to the composition and concentration of the UVR-absorbing mycosporine-like amino acids (MAAs) within their tissues (Shick *et al.* 1995; Bischof *et al.* 2000*b*). Freshwater macrophytes also display zonation patterns along a depth gradient within the littoral zones of lakes (Wetzel 1983). As in marine systems, lower depth limits appear to be related to PAR limitation and to the abilities of different species to adjust to low irradiance (Schwarz *et al.* 1996; Sorrell *et al.* 2001). Little attention, however, has been paid to potential physiological constraints on upper depth limits. The ability to acclimate to high PAR and UVR fluxes, and thus avoid photoinhibition and photodamage, is amongst those potential constraints worthy of examination.

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Photoinhibition in response to excess photosynthetically available radiation (PAR) can be either chronic or dynamic (*sensu* Osmond 1994). Chronic photoinhibition describes reduced photosynthetic activity due to the inactivation of the D1 protein, which is integral to photosystem II (PS II; Long *et al.* 1994). Dynamic photoinhibition involves the redirection of excess photons to thermal dissipation in order to avoid photosystem damage (Powles 1984) thereby decreasing photosynthesis. For example, the xanthophyll pigment cycle decreases the quantum yield of PS II by increasing heat dissipation (Demmig-Adams and Adams 1992). The mechanisms of UVR inhibition of photosynthesis are comparable to those for high PAR with the addition of causing decreased activity of Rubisco, the primary carbon fixation enzyme (Strid *et al.* 1994; Bischof *et al.* 2000*a*). Many plants and algae use strategies to minimize photoinhibition. For example, degraded D1 proteins can be excised and replaced to reinstate PS II function (Aro *et al.* 1993; Sass *et al.* 1997). Also, cellular compounds that absorb strongly in the UVR spectrum such as MAAs in algae (Dunlap and Shick 1998; Franklin *et al.* 1999) and flavonoids in higher plants (Bornman and Teramura 1993) can reduce the potential damage incurred from UVR wavelengths (Roy 2000).

In this study, we investigated the UVR and PAR responses of maximal quantum yield for photosynthesis by four macrophyte species, including two characean algae, collected from their upper and lower depths of growth in Lake Coleridge, New Zealand. New Zealand lakes span a wide range of water clarities, but Lake Coleridge has extremely low dissolved organic carbon (DOC) concentrations such that 1% UVR (320 nm) penetrates to depths >5 m (Rae *et al.* 2001). In this study, our first objective was to determine whether upper depth limits of growth among the species are ordered by UVR tolerance and, if so, whether UVR-absorbing compounds could play a role in UVR tolerance. Secondly, we sought to identify whether differences exist within a species growing at the upper and lower depths of its growth zone.

Methods

Study site

Lake Coleridge is a large (surface area 32.9 km^2, maximum depth 200 m), oligotrophic lake of glacial origins in the central interior of the South Island of New Zealand. Being in the rainshadow on the east of the Southern Alps, and in a catchment predominantly composed of native tussock grassland and alpine herb vegetation, its DOC concentration is low at 0.4 g m^{-3} (Rae *et al.* 2001). Consequently, UVR penetrates to depths of several metres in the lake.

The littoral zone of Lake Coleridge comprises ~20% of the surface area (James *et al.* 1998), with aquatic macrophyte vegetation growing to >30 m depth in most of the lake (Schwarz and Hawes 1997). Macrophytes recorded in the lake include the vascular plants *Myriophyllum triphyllum*, *Potamogeton cheesemanii*, *Isoetes alpinus*, *Elodea canadensis* and *Ranunculus trichophyllus*, and several species of the characean algae *Chara* and *Nitella* (Schwarz and Hawes 1997). Water clarity is lowest and maximum depths of macrophytes are shallowest at the northern end of the lake where two turbid tributaries enter (Biggs and Davies-Colley 1990).

Water-column measurements and plant collection took place at the northern end of Lake Coleridge in February 2000, and experiments were conducted at NIWA's Christchurch laboratory.

Lake profiling and plant collection and culture

Water-column profiles of cosine-corrected downwelling irradiance and temperature were obtained by use of a PUV–500 radiometer (Biospherical Instruments Inc.). The radiometer measures UVR at 305, 320, 340 and 380 nm (half maximum bandwidth of 8–10 nm) and PAR (400–700 nm). Measurements were logged at 1-s intervals during up- and down-casts of the instrument, corresponding to about 20 measurements per metre. For each waveband, % transmission to depth was calculated relative to the irradiance immediately below the water surface. Vertical attenuation coefficients (K_d) were calculated from linear regression of log-transformed irradiance *v.* depth.

Four macrophytes, *I. alpinus*, *P. cheesemanii*, *Chara fibrosa* and *C. corallina*, were collected by SCUBA divers from depths of 4 and 12 m, 4.5 and 5.2 m, 6.2 and 15 m, and 16.5 and 26 m, respectively. These depths corresponded to the shallowest and deepest growth of each species at the sampling site. Plants were kept cool and dark during transport to the laboratory on the same day of collection, and were placed in aquaria with aerated lake water. The plants were kept at 14°C and 50–60 µmol photons m^{-2} s^{-1} with a photoperiod of 16L : 8D.

Spectral absorbance analyses

Immediately upon return to the laboratory, samples of each plant species were stored at -80°C for later analysis of spectral absorbance characteristics in the UVR region. Analyses were conducted by freeze-drying samples for 12 h and then weighing, grinding and extracting the plant material. Soluble UVR-absorbing compounds (e.g. MAAs and flavonoids) were extracted in 50% methanol for 1 h at 45°C followed by 24 h at 4°C, a method similar to that of Sommaruga and Garcia-Pichel (1999). Absorbance of the extracts was measured at 1 nm resolution from 250–700 nm on a Jasco model 7850 spectrophotometer. The resulting absorbance scans were normalized to the freeze-dried weight of extracted plant material.

Experiments

Two sets of experiments were conducted with the macrophytes, each using a different light source. For all experiments, plant material was exposed at 14°C to a combination of UVR and PAR in one of three treatments. The treatments were obtained through the use of glass filters (Schott, Germany) that selectively removed portions of the irradiance spectrum, and resulted in exposure of the macrophytes to PAR alone (P), PAR+UVAR (PA) or PAR+UVAR+UVBR (PAB). The filters were placed between the samples and the different light sources, which are described below. Filter types and transmission properties are listed in Table 1. Exposure of the macrophytes to the treatment conditions took place for 4–5 h, during which hourly measurements of maximal

Table 1. Percent of irradiance from each waveband (UVBR, UVAR and PAR) transmitted through the glass filters used during the experiments

Filter	Irradiance treatment	% irradiance transmitted		
		UVBR	UVAR	PAR
GG400	P (PAR only; 400–700 nm)	0	7	86
WG320	PA (PAR + UVAR; 320–700 nm)	16	87	91
WG280	PAB (PAR + UVAR + UVBR; 280–700 nm)	81	91	91

Table 2. ***(a)*** **Average irradiance output, integrated over the range indicated, of light sources used during the experiments, and** ***(b)*** **the resulting irradiance exposure for plants beneath the glass filters**

(a) Light source outputs

Source	UVBR: 280–320 nm (W m^{-2})	UVAR: 320–400 nm (W m^{-2})	PAR: 400–700 nm (μmol photons m^{-2} s^{-1})
Fluorescent bulbs (high UVR/PAR)	0.30	5.74	36.4
Sonsi lamp (low UVR/PAR)	0.47	16.52	624.5

(b) Average irradiance exposure within treatments

Source	Irradiance treatment	UVBR 280–320 nm (W m^{-2})	UVAR 320–400 nm (W m^{-2})	PAR 400–700 nm (μmol photons m^{-2} s^{-1})
Fluorescent bulbs	P	0	0.40	31.3
	PA	0.05	4.99	33.1
	PAB	0.25	5.22	33.1
Sonsi lamp	P	0	1.16	537.1
	PA	0.07	14.37	568.3
	PAB	0.38	15.03	568.3

quantum yield (measured as the ratio of variable to maximal chlorophyll fluorescence, F_v/F_m) were taken with the use of a pulse-amplitude-modulated fluorometer (PAM 2000 or Diving PAM, Walz, Germany). The system is based on one developed by Schreiber *et al.* (1986) whereby changes in the ratio of variable to maximal fluorescence, F_v/F_m, of temporarily dark-acclimated plants is used as a measure of photoinhibition and recovery (cf. Krause and Weis 1991). $F_v = F_m - F_o$ where F_o is the initial fluorescence (i.e. fluorescence when all reaction centres of PS II are active or 'open') and F_m is the maximal fluorescence measured under strong light (i.e. fluorescence when all PS II centres are 'closed'). After application of a 5 s far-red pulse (~30 μmol photons m^{-2} s^{-1}, 730 nm, PAM2000) used to oxidize the electron transport chain, F_o was measured with red light pulses (~0.3 μmol photons m^{-2} s^{-1}, 650 nm) and F_m was determined with an 800 ms saturating white light pulse (~9200 μmol photons m^{-2} s^{-1}). The exposure period was followed by 17 h of low PAR to allow for recovery from photoinhibition; F_v/F_m was measured 2–3 times during this recovery.

Experiment 1 was conducted with a high UVR/PAR ratio to assess the overall sensitivity of the macrophytes to UVR without the potential complicating factor of photorepair in the presence of moderate intensities of PAR. The PAR flux was maintained at an average of 32 μmol photons m^{-2} s^{-1} beneath the optical filters during the UVR exposure period (equivalent to <5% incident PAR at Lake Coleridge), and at 5 μmol photons m^{-2} s^{-1} during the recovery phase. White-light fluorescence tubes (Philips, Australia) were used. UVR was obtained at a flux, prior to passing through the optical filters, of 0.30 Wm^{-2} UVBR and 5.74 Wm^{-2} UVAR, using two 40 W fluorescent UVA–340 tubes (Q-Panel, Cleveland, USA). After filter transmission losses, average UVR exposure within each of the treatments (Table 2) was similar to subsurface values for specific wavelengths in Lake Coleridge (Table 3).

Experiment 2 took into account the effects of exposure to both UVR and PAR at levels representative of those subsurface at Lake Coleridge (Table 3). This was accomplished through the use of a sunshine-simulating apparatus (Sonsi; Isitec, Germany). The instrument was developed to simulate the natural underwater light spectrum at different depths and with varying amounts of stratospheric ozone depletion (see Bracher and Wiencke (2000) for a full description). The UVR/PAR ratio was lower than for Experiment 1, such that PAR-stimulated repair processes could better operate during the initial exposure period. Also, strong PAR in addition to UVR causes photoinhibition in nature; thus the combined PAR and UVR irradiance conditions to which the Experiment 2 plants were exposed were closer to those in Lake Coleridge than were the conditions obtained from the fluorescent tubes in Experiment 1 (Tables 2 and 3). The post-exposure recovery phase took place at 20 μmol photons m^{-2} s^{-1} without change to the spectrum.

Table 3. Attenuation coefficients (K_d; m^{-1}) and subsurface (0.1 m) irradiance (E_o) measured for PAR (400–700 nm; μmol photons m^{-2} s^{-1}) and four wavelengths within the UVR range (380, 340, 320 and 305 nm; W m^{-2}) at Lake Coleridge in February 2000

	PAR	380 nm	340 nm	320 nm	305 nm
K_d	0.17	0.27	0.40	0.51	0.70
E_o	1077	5.01	4.10	0.22	0.04

Incident PAR and UVR in all experiments were measured with a cosine-corrected 2 π sensor (Quantum Li–190 SA; LiCor, Nebraska, USA) and a spectroradiometer (280–780 nm, 1.3 nm dispersion) developed for use within the Sonsi (Kruse, Germany). Experiments were conducted for each species from both upper and lower limits of its depth range, using each of the experimental protocols, except in the case of *C. corallina*, which was not measured in the Sonsi apparatus.

Data analysis

The time-series experiments measuring photosynthetic response of the plants to the three irradiance treatments were analysed statistically by 1-way repeated-measures ANOVA and 2-way ANOVA followed by post-hoc assessment with the Tukey HSD test. Differences in percent inhibition after UVR exposure, relative to pre-exposure (hour 0), were analysed among plant species and irradiance treatments, or plant species and depth by 2-way ANOVA and the Tukey HSD test. Results of 1-way ANOVAs are reported on some of the figures to indicate differences among irradiance treatments for a specific plant. All analyses were undertaken with Statistica v. 5.5 (StatSoft, Inc.).

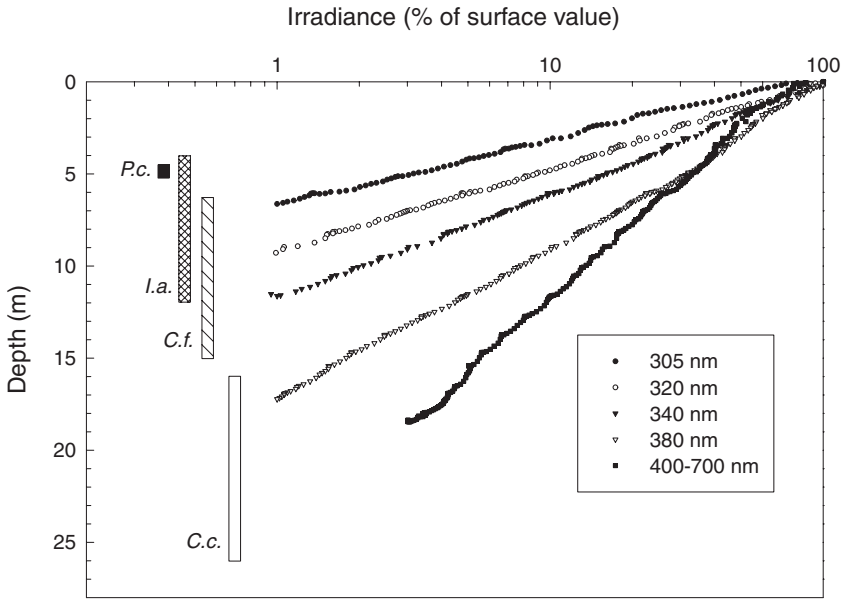

Fig. 1. Vertical profiles of ultraviolet radiation (UVR) at four wavelengths: 305, 320, 340, 380 nm (half maximum bandwidth of 8–10 nm), and photosynthetically available radiation (PAR; 400–700 nm) at the northern end of Lake Coleridge in February 2000. Vertical bars indicate the growth zone of each of four macrophytes at the sampling site: *Potamogeton cheesemanii* (*P.c.*), *Isoetes alpinus* (*I.a.*), *Chara fibrosa* (*C.f.*) and *C. corallina* (*C.c.*).

Results

Sampling site characteristics

The northern end of Lake Coleridge had an isothermal temperature profile of 14.1°C from the surface to 18 m, which was the deepest point to which we profiled at the time of sampling. Visible light penetration was deep with an attenuation coefficient (K_d) for PAR of 0.17 m^{-1}, giving a 1% PAR depth of 27 m. UVR penetration varied with waveband, with the 1% level of 320 nm light at 9 m depth (Fig. 1, Table 3).

The four plant species collected all had distinct bands of growth with overlap between some species (Fig. 1). Because of frequent fluctuations of several metres in water level in Lake Coleridge, coupled with vigorous wave action, macrophytes do not colonize the upper 4 m of the littoral zone (Schwarz and Hawes 1997).

Macrophyte sensitivity to UVR and PAR exposure

Experiment 1: *High UVR/PAR ratio*

In this series of experiments, the UVR doses applied were insufficient to cause any significant inhibition in *P. cheesemanii* over the experimental time course ($F = 0.8$, $P = 0.6$). In the other three species, there were significant responses to the UVR treatments, with repeated-measures ANOVA showing an effect on F_v/F_m of both irradiance type and duration of exposure ($F = 2.8$, 6.0 and 11.5 for *I. alpinus*, *C. fibrosa* and *C. corallina* respectively; $P < 0.01$ for all plants). These differences were ordered according to upper depth limits, such that deeper-growing species were less tolerant of UVR than shallower-growing species (Fig. 2). *C. corallina* was most affected by UVR, with F_v/F_m decreasing particularly rapidly in the PAB treatment, but with similar levels in both PA and PAB by the end of the UVR exposure period. *C. fibrosa* showed an initial rapid decline in both PA and PAB, levelling off after the first hour, to then drop between hours 3–5 with PAB most severely affected at hour 5. *I. alpinus* showed the same significant decline in both UVR treatments over time (Fig. 2). Average UVR inhibition at the end of the UVR exposure period, as a % decrease from pre-exposure (hour 0), was 15% for *I. alpinus*, 38% for *C. fibrosa* and 48% for *C. corallina* (Fig. 3*I*). Significant differences between PA and PAB were apparent only for *C. fibrosa*, with inhibition under PAB comparable to that for *C. corallina*. A ranking in terms of UVR inhibition of *P. cheesemanii* < *I. alpinus* < *C. fibrosa* ≤ *C. corallina* is similar to the ranking of upper growth depths from shallow to deep: *P. cheesemanii* = *I. alpinus* < *C. fibrosa* << *C. corallina*.

The two vascular species recovered fully from UVR exposure, but the characean species did not (Fig. 2), with final F_v/F_m values 10–15% lower than initial measurements (Fig. 3*II*).

Intraspecies comparisons showed that plants from upper and lower depths of their growth range did not differ in % inhibition of F_v/F_m after 5 h exposure, relative to

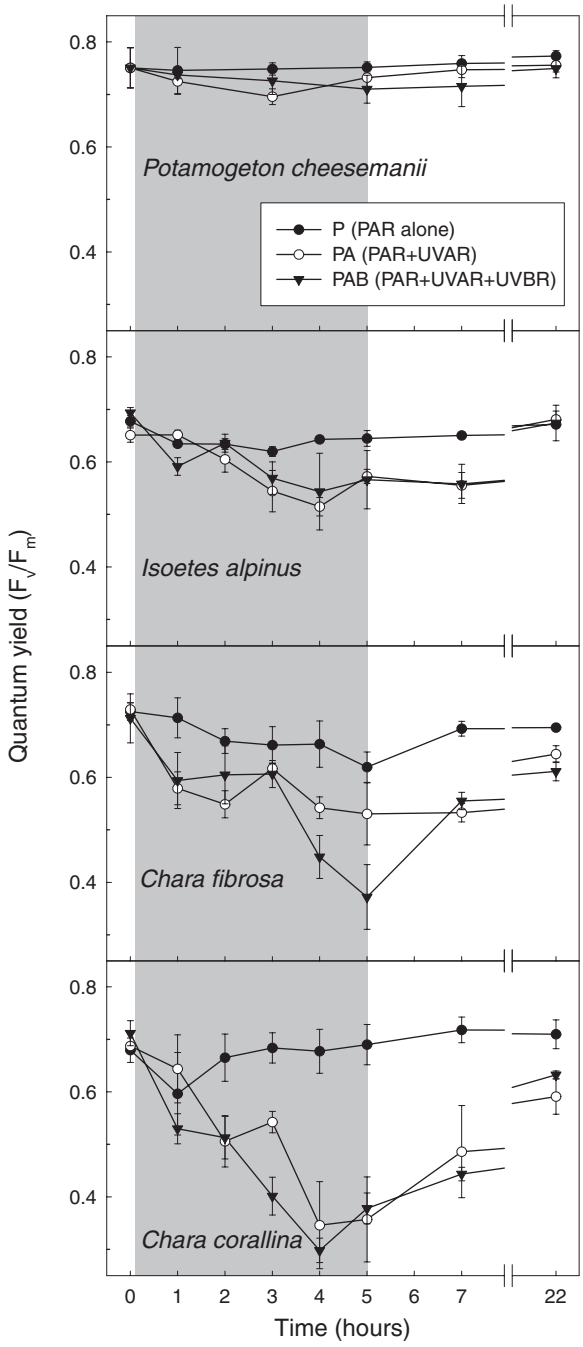

Fig. 2. *Experiment 1* (*high UVR/PAR*): Time series of maximal quantum yield (F_v/F_m; mean ± s.d.; N = 3) for four macrophyte species during a UVR exposure period (shaded area) and subsequent recovery at low PAR. The macrophytes had been collected from their shallowest depth of growth. Fluorescent bulbs gave a high UVR/PAR ratio during the exposure period.

pre-exposure (Fig. 4). The one exception was C. fibrosa from the PAB treatment only, where the shallow plant experienced 20% greater inhibition than the deep plant ($F = 7.1$, $P < 0.01$).

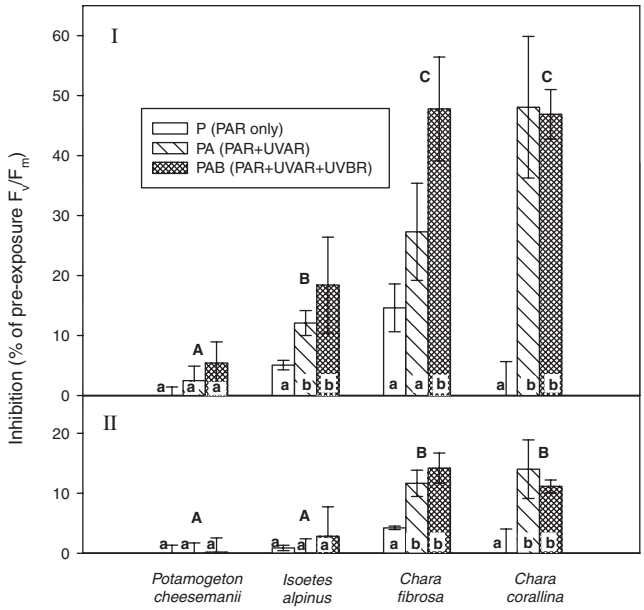

Fig. 3. *Experiment 1* (*high UVR/PAR*): Percent inhibition (mean ± s.d.; N = 3) of maximal quantum yield (F_v/F_m) at *(I)* the end of the high UVR/PAR exposure period (hour 5) and *(II)* the end of the recovery period, relative to F_v/F_m recorded pre-exposure (hour 0). The macrophytes had been collected from their shallowest depth of growth. The upper-case letter above each group of bars denotes statistically significant (P <0.01) differences among the four plant species; the lower-case letters at the base of the bars denote statistical significance (P <0.01) among the three irradiance treatments for each plant individually (1-way ANOVA).

Experiment 2: *Low UVR/PAR ratio*

When exposed to high PAR conditions in addition to UVR, all plants were inhibited by the PAR; UVR inhibition, if present, was incremental to the PAR-induced response (Fig. 5). For *P. cheesemanii*, PAR alone gave a 50% inhibition of F_v/F_m, but addition of UVR led to no further effect. For *I. alpinus* and *C. fibrosa*, PAR inhibition accounted for a 22–50% decrease in F_v/F_m, with inhibition attributed to UVR itself being an additional 2–27% (Fig. 5). Full recovery of F_v/F_m by the end of the experiment was not evident in all plants. In particular, F_v/F_m for *C. fibrosa* in the PA and PAB treatments remained at values 30–43% lower than pre-exposure (data not shown). *I. alpinus* and *C. fibrosa* plants collected from their lower depth limits were slightly less inhibited by PAR and UVR after 4 h of treatment than were the shallower plants (Fig. 5). Except in the case of the PA treatment of *I. alpinus*, these differences were small.

Spectral absorbance of plants

Spectral scans of alcoholic extracts of the four macrophytes showed varying amounts of absorbance in the UVR region, with virtually no absorbance by the two characeans and

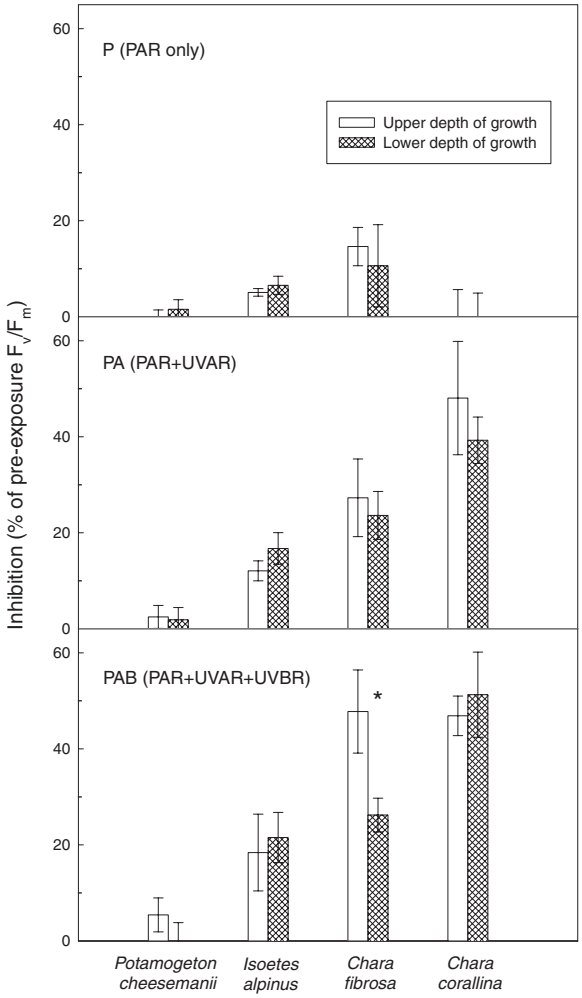

Fig. 4. *Experiment 1* (*high UVR/PAR*): Comparison of percent inhibition (mean ± s.d.; $N = 3$) of maximal quantum yield (F_v/F_m) at the end of the high UVR/PAR exposure period (hour 5) relative to F_v/F_m recorded pre-exposure (hour 0), between macrophytes obtained from their upper and lower depths of growth. *Statistically significant difference between depths ($P < 0.01$).

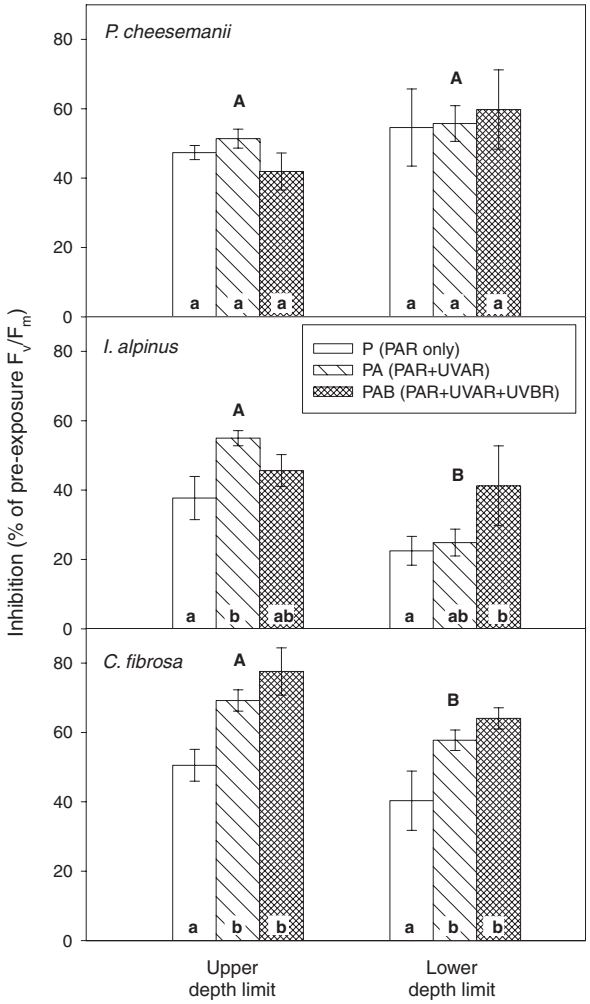

Fig. 5. *Experiment 2* (*low UVR/PAR*): Percent inhibition (mean ± s.d.; $N = 3$) of maximal quantum yield (F_v/F_m) at the end of the low UVR/PAR exposure period (hour 4). Results are shown for plants collected from both upper and lower depths of growth. Upper-case letters denote statistically significant ($P < 0.01$) differences between the two depths; lower-case letters at the base of the bars denote statistical significance ($P < 0.01$) among the three irradiance treatments for each group of three bars individually (1-way ANOVA).

definite peaks in the 310–360 nm range for *I. alpinus* and *P. cheesemanii* (Fig. 6). Of these latter plants, *I. alpinus* absorbed 35–40% less (per gram of plant material) than *P. cheesemanii*. However, the UVR-absorbance peak is a shoulder on the general increase in absorbance at low wavelengths, making the contribution to attenuation in the 310–360 nm peak slightly larger in *I. alpinus* than *P. cheesemanii*. Differences in UVR absorbance between plants of the same species from shallow and deep locations in the lake were small or not evident (Fig. 6). Wavelengths of maximal absorbance differed between *I. alpinus* and *P. cheesemanii*, with peak absorbance of the former being in the region 318–323 nm and of the latter between 332–335 nm.

Discussion

There are clearly differences in PAR and UVR sensitivity of the photosynthetic apparatus among freshwater macrophytes when tested in a laboratory setting. All plants examined in this study showed decreases in maximal quantum yield (F_v/F_m) when exposed for several hours to high PAR, and several showed additional decreases attributable to UVR. In many of these cases, a reduction in PAR and a cessation of UVR allowed F_v/F_m to recover, often to regain initial values. Dynamic photoinhibition (Long *et al.* 1994) was indicated by the reversible responses, suggesting that the shallower-growing plants were well suited to growth in high-light

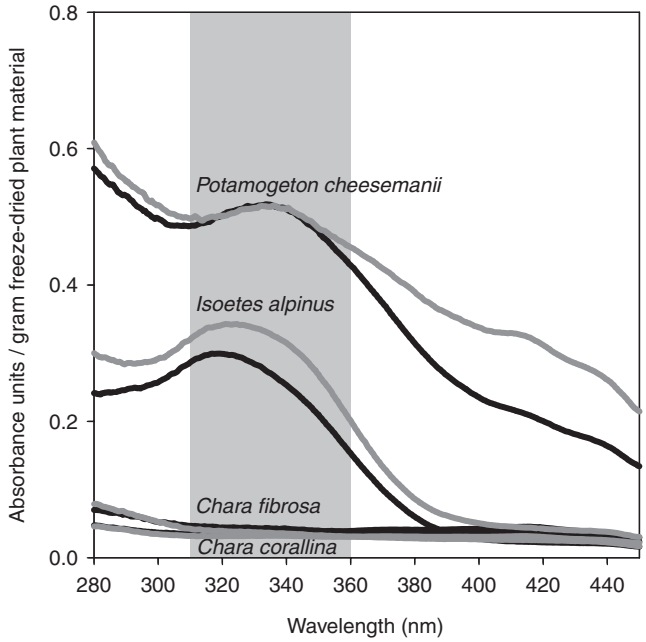

Fig. 6. Absorbance over the wavelengths 280–450 nm by four species of macrophytes, each collected from two depths. Black curves represent the lower growth limits of the plants and grey curves the upper growth limits: *P. cheesemanii* from 4.5 and 5.2 m, *I. alpinus* from 4 and 12 m, *C. fibrosa* from 6.2 and 15 m and *C. corallina* from 16 and 26 m. Vertical grey bar (310–360 nm) denotes band of typical absorbance by mycosporine-like amino acids and flavonoids.

environments with physiological mechanisms for management of excess energy. In the deeper-growing characean algae, irreversible inhibition is indicative of chronic effects in the response, although we are unable to determine the mechanism of damage involved. As with terrestrial sun and shade plants where the dominant response to exposure to high light is dynamic and chronic photoinhibition respectively (Osmond 1994), there may be variation in the dominant photoinhibitory mechanism for aquatic plants growing at different depths.

The UVR responses in our experiments were different among macrophyte species: no effect on *P. cheesemanii*, some effect on *I. alpinus* and considerable effect on both characeans. Distinct differences between PA and PAB treatments were infrequent. For the most part, both UVAR and UVBR were involved to some extent in causing a decrease in F_v/F_m in the macrophytes. UVBR inhibition was incremental to UVAR inhibition in all experiments with the characean algae, but in the case of *I. alpinus* some PAB treatments were less inhibited than PA treatments. This has been observed previously in the brown alga *Dictyola dichotoma* growing in a high-light environment (Flores-Moya *et al.* 1999). Greenberg *et al.* (1989) found that turnover rates of the D1 protein were enhanced under UVBR exposure and were greater than those measured under PAR illumination alone. Also, greater PSII turnover has been reported for a terrestrial plant that was exposed to UVR but was not exhibiting any inhibition of photosynthetic activity (Wilson and Greenberg 1993). These responses support a role for UVBR in inducing repair mechanisms. This type of positive effect mediated by UVBR was also observed in fluorescence measurements taken over a daily time course under conditions of natural irradiance on macrophytes collected from two New Zealand lakes. Shallow-growing vascular plants exposed to a PAB treatment in the Sonsi apparatus were significantly less photoinhibited, by ~10%, than plants in a PA treatment, suggesting a role for UVB in the induction of recovery processes (Hanelt *et al.* unpublished).

In treatments where plants were exposed to UVR, complete recovery from low F_v/F_m was not always attained by the end of the 17 h low-PAR period. This indicates that rates of reinstating photosynthesis and/or repairing damage vary among the species we examined. We did not measure these plants beyond the 17 h, therefore we are unable to determine if all possible recovery had taken place after 17 h (i.e. if some irreversible degradation had occurred during the UVR exposure period) or if full recovery would have been attained eventually (i.e. a slow recovery rate was operating but no permanent damage had been sustained). Rates of repair to the photosynthetic machinery and recovery of photosynthetic activity can be variable and are particularly influenced by temperature (Jensen and Knutsen 1993; Rae *et al.* 2000).

The degree of UVR inhibition to F_v/F_m appears to be related to upper depth limits of growth by the four plants in this study. *P. cheesemanii* and *I. alpinus* both have upper growth limits at ~4 m depth in Lake Coleridge, and neither was drastically inhibited by UVR: *P. cheesemanii* showed <10% inhibition (statistically not significant from pre-exposure) and *I. alpinus* showed 10–25% inhibition. That *I. alpinus* showed some inhibition while *P. cheesemanii* displayed none, yet both species have similar upper depth limits, suggests that other physiological and environmental factors also contribute to plant zonation. *C. fibrosa* from 6.2 m and *C. corallina* from 15 m were inhibited by up to 60%, with averages of 38% and 48%, respectively, when exposed to UVR for a 4–5 h period. Thus, deeper-growing species display a greater decrease of F_v/F_m in response to UVR exposure than do shallower-growing species. This type of relationship between photoinhibition and depth distribution has been reported previously for several species of marine macroalgae (Dring *et al.* 1996; Hanelt *et al.* 1997; Hanelt 1998; Bischof *et al.* 2000*b*) and zooxanthellate corals (Shick *et al.* 1995).

In addition to differential sensitivity to damage of PS II and variations in repair rates, the depth distribution and associated sensitivity to UVR may be linked to different complements

of UVR-absorbing pigments and other compounds in the macrophytes. Spectral scans revealed that *I. alpinus* and *P. cheesemanii* both absorb strongly in the 310–360 nm range; absorbance in this band has been attributed to MAAs in algae (Karentz *et al.* 1991; Dunlap and Shick 1998) and flavonoids in higher plants (Bornman and Teramura 1993). Our analyses do not indicate which UVR-absorbing compounds are present in the plants, and it is possible that they are neither MAAs nor flavonoids. The peak absorbance differed slightly in wavelength and height between *I. alpinus* and *P. cheesemanii*, therefore it is likely that type and/or concentration of UVR-absorbing compounds are different in each species. To determine this would require chromatographic separation of distinct compounds. Neither *C. fibrosa* nor *C. corallina* absorbed in the UVR region.

The distinct difference in the relative amount of absorbance by each of the plant species can also be related to depth distribution, with shallower-growing plants absorbing more strongly in the UVR wavelengths. UVR-sensitive components within the plants are protected from harmful radiation if it is absorbed by sunscreens such as MAAs or flavonoids in the epidermis. *C. fibrosa* and *C. corallina* were particularly inhibited by UVR, and also have minimal capacity to absorb UVR with sunscreens. *C. corallina* was exposed during our experiments to UVR flux that is unrealistic in terms of the natural conditions it would encounter in the lake; however, the results demonstrate the link between the presence of UVR-absorbing compounds and UVR tolerance. Decreasing concentration of MAAs has previously been related to decreasing UVR exposure associated with increasing depth for a zooxanthellate coral (Shick *et al.* 1995), plankton samples (Sommaruga and Garcia-Pichel 1999) and marine macroalgae (Bischof *et al.* 2000*b*). Despite the same lack of UVR-absorbance by the two characeans in our experiments, the upper growth limit of *C. fibrosa* is 10 m shallower than that of *C. corallina*. This suggests firstly that significant or frequent damaging UVR does not penetrate to 6 m depth where *C. fibrosa* starts to grow in Lake Coleridge, and secondly that *C. corallina* is less competitive at levels of PAR that are higher than those it experiences at 16 m depth.

The evident differences both in UVR sensitivity and UVR-absorbing ability among species with varying upper growth depths in Lake Coleridge indicate that one of two situations occurs in the macrophyte community. Either the plants growing at the shallowest region of a species' growth band have developed (or not) a tolerance to UVR and high PAR because of irradiance at that depth, or they grow at a depth for which they have the capacity to cope with the light exposure. Our examination of UVR inhibition of a single plant species between its upper and lower depths of growth shows that, for the most part, the response is related to the species and not the depth of growth. Of the four plants we examined, *I. alpinus* and *C. fibrosa* have upper limits shallow enough that they will be exposed to some UVR in the course of a sunny day, while their lower limits are at depths greater than the 1% light levels for UVBR, although they may receive some long-wavelength UVAR. We would expect to find a difference in UVR sensitivity of these species between their top and bottom limits if the shallower plants had acclimated to UVR exposure. Although differences between shallow and deep plants were observed in some UVR treatments for *C. fibrosa* in Experiment 1 and *I. alpinus* in Experiment 2, the effects were opposite to those expected with greater inhibition for shallower-growing plants. With respect to UVR-absorbing compounds, *C. fibrosa* does not appear to have any, regardless of depth of growth, and *I. alpinus* showed only a slightly higher absorbance in the shallower plant. The suggestion is that the plants are growing at depths for which they already have the capability to deal with the irradiance, rather than developing a capacity specifically for the purpose of growing at a shallower depth. Nonetheless, other physiological factors and environmental conditions will most likely be involved in determining plant zonation in the littoral zone of lakes.

Our experiments evaluated macrophyte responses to UVR and high PAR on short timescales and under conditions that are realistic to sub-surface irradiance in Lake Coleridge. They show that freshwater macrophytes differ in their photosynthetic sensitivity to UVR and in their ability to screen UVR, with both characteristics having some relationship to upper growth depths of the four plant species. Given that some macrophytes in the lake have low tolerance for UVR, any increases in the penetration depth through, for example, climate-induced decreases to DOC inputs or lake level drawdown could have consequences for competitive interactions and the littoral zonation of the plants, in addition to productivity of the littoral zone. The next step in these investigations should be to use long-term *in situ* experiments to determine whether upper depth limits would become deeper over time with greater UVR penetration, or whether plants would have the ability to acclimate to a changing underwater UVR climate.

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