Chapter 2

Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance

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ABSTRACT

The sensitivity to UV-B radiation (UVBR: 280-315 nm) was tested for littoral (*Palmaria palmata* [L.] O. Kuntze, *Chondrus crispus* Stackhouse) and sublittoral (*Phyllophora pseudoceranoides* S. G. Gmelin, *Rhodymenia pseudopalmata* [Lamouroux] Silva, *Phycodrys rubens* [L.] Batters, *Polyneura hilliae* [Greville] Kylin) red macrophytes from Brittany, France. Algal fragments were subjected to daily repeated exposures of artificial UVBR that were realistic for springtime solar UVBR at the water surface in Brittany. Growth, DNA damage, photoinhibition and UV-absorbing compounds were monitored during two weeks of PAR+UVAR+UVBR, whereas PAR+UVAR and PAR treatments were used as controls. The littoral species showed a higher UV tolerance than the sublittoral species. After two weeks, growth of *P. palmata* and *C. crispus* was not significantly affected by UVBR, and DNA damage measured as the number of cyclobutane-pyrimidine dimers per 10^6 nucleotides, was negligible. Photoinhibition, determined as the decline in optimal quantum yield, was low and decreased during the course of the experiment, coinciding with the production of UV-absorbing compounds in these species. In contrast, no UV-absorbing compounds were induced in the sublittoral species. Growth rates of *P. pseudoceranoides* and *R. pseudopalmata* were reduced by 40% compared with the PAR treatment. Additionally, constant levels of DNA damage and pronounced photoinhibition were observed after the UVBR treatments. Growth was completely halted for *P. rubens* and *P. hilliae*, whereas DNA damage accumulated in the course of the experiment. Because *P. rubens* and *P. hilliae* showed the same degree of photoinhibition as the other sublittoral species, it appears that the accumulation of DNA damage may have been responsible for the complete inhibition of growth. The results suggest an important role of DNA repair pathways in determining the UV sensitivity in red macrophytes.

INTRODUCTION

Marine macrophytes occur in coastal waters and are important components of the coastal ecosystem in terms of biomass and biodiversity. Macrophytes in the littoral zone experience large fluctuations in irradiance conditions. During low tide, littoral macrophytes are potentially exposed to full sunlight including UV-B radiation (UVBR: 280-315 nm) and UV-A radiation (UVAR: 315-400 nm). When submerged, macrophytes are subjected to reduced UVBR and UVAR due to attenuation of short wavelengths in the water column (Franklin and Forster 1997).

On the cellular level, UVBR can provoke multiple effects in marine sea grasses, macrophytes, phytoplankton and picoplankton by causing damage to nucleic acids, proteins and lipids (Karentz *et al.* 1991a, Dawson and Dennison 1996, Franklin and Forster 1997, Boelen *et al.* 2000). Short wavelength UVBR can disrupt normal base pairing of DNA by the formation of photolesions between adjacent pyrimidine bases. Cyclobutane-pyrimidine dimers (CPDs) are the most abundant photolesions induced by natural UVBR (Strid *et al.* 1994). These photoproducts inhibit transcription and replication of DNA and consequently disrupt cell metabolism and division (Buma *et al.* 1995, 2000). Efficient repair pathways have been reported for CPDs. Photolyase enzymes recognize and reverse dimerization instantaneously utilizing photons between 300 and 500 nm (Kim and Sancar 1993). Photolyase activity is an important defense against UVBR-induced DNA damage in plants, whereas complex repair pathways such as excision repair appear to have a complementary role (Quaite *et al.* 1994). Efficient light-dependent repair of CPDs was recently demonstrated in the red macrophytes *Rhodymenia pseudopalmata* and *Palmaria palmata* (Pakker *et al.* 2000a, b).

In addition, numerous studies have shown that UVBR directly influences photosynthesis by a process referred to as photoinhibition. Under photoinhibitory conditions, the efficiency of photosynthesis is reduced because of a lower optimal quantum yield of PSII (Chow *et al.* 1992). In contrast to photoinhibition induced by photosynthetically active radiation (PAR), UVBR-induced photoinhibition appears to be caused by protein damage rather than by down-regulation of PSII (Franklin and Forster 1997). Moreover, recovery from UVBR-induced photoinhibition requires additional protein synthesis and is slower compared with recovery from photoinhibition induced by PAR (Franklin and Forster 1997). Marked differences in UV sensitivity of photosynthesis have been reported between macrophyte species (Dring *et al.* 1996b). For polar macrophytes it was demonstrated that photosynthesis of deep-water species was more susceptible to UVBR compared with shallow water species (Bischof *et al.* 1998a, b). In *Chondrus crispus* and *Devaleraea ramentacea*, UV sensitivity of photosynthesis was higher in sublittoral plants than in littoral plants (Sagert *et al.* 1997, Karsten *et al.* 1998, 1999, Franklin *et al.* 1999). Differences in UVBR sensitivity in these species may be partly explained by the formation of protective UV-absorbing compounds, the mycosporine-like amino acids (MAAs) (Karsten *et al.* 1998, 1999, Franklin *et al.* 1999).

UVBR potentially inhibits the accumulation of biomass by causing a reduction in optimal quantum yield, delaying cell division, and by diverting resources to subsequent adaptation and repair processes. The balance between induction and repair of damage eventually determines the effect of UVBR on growth. Therefore, growth provides a sensitive parameter for the overall effects of UVBR on macrophytes (Dring *et al.* 1996a). Experiments with solar UVBR have indicated that current levels of UVBR can reduce the productivity of marine macrophytes (Grobe and Murphy 1994, Aguilera *et al.* 1999, Altamirano *et al.* 2000). However, studies demonstrating effects of DNA damage and photoinhibition on the productivity of macrophytes are lacking. In the present study we tested the UV sensitivity of six temperate red macrophytes to daily repeated artificial UVBR treatments. Growth, DNA damage, photoinhibition and UV-absorbing compounds were followed in the course of the experiment and were used as indicators for UVBR stress. Moreover, comparing these parameters for littoral and sublittoral species provided an opportunity to expand our understanding of the mechanisms that determine differences in UV sensitivity among species.

MATERIALS AND METHODS

Table 1. Characteristics of the red algal species used during the experiments. RGR (% per day): mean ± SD of relative growth rates during the PAR treatment calculated for 6 fragments from each species. F_v/F_m: mean ± SD of optimal quantum yield measured during the PAR treatment, calculated for 9 fragments.

Species	Habitat	RGR	F_v/F_m
Palmaria palmata	littoral	9.10 □ 0.6	0.621 □ 0.008
Chondrus crispus	littoral	5.70 □ 1.8	0.624 □ 0.013
Phyllophora pseudoceranoides	sublittoral	4.80 □ 0.8	0.578 □ 0.049
Rhodymenia pseudopalmata	sublittoral	10.9 □ 0.8	0.546 □ 0.018
Phycodrys rubens	sublittoral	9.30 □ 1.8	0.480 □ 0.060
Polyneura hilliae	sublittoral	13.5 □ 3.5	0.470 □ 0.016

Experimental set-up

The temperate red macrophytes *Palmaria palmata*, *Chondrus crispus*, *Phyllophora pseudoceranoides*, *Rhodymenia pseudopalmata*, *Phycodrys rubens*, and *Polyneura hilliae* (Table 1) were isolated from Brittany, France and grown as nonaxenic unialgal cultures. During the experiment apical fragments (1 cm) were cultivated in 1-L glass beakers containing 700 mL half strength Provasoli-enriched autoclaved seawater (Starr and Zeikus 1993), which was refreshed every 3-4 days. Aeration caused continuous motion of the fragments and ensured equal exposure during the irradiance treatments. The beakers were placed in a temperature-controlled water bath

maintained at 12 0.2ºC, the mean spring seawater temperature in Brittany (U.S. NAVY 1981). All measurements were performed in triplicate with samples obtained from three different beakers.

Irradiance conditions

PAR was provided by 2 L36W/72 Biolux fluorescent lamps (Osram, Germany) resulting in a fluence rate of 9.5 W m^{-2} (36 µmol photons m^{-2} s^{-1}). The algae were cultivated under a 16-h photoperiod. In the middle of the light period, UVBR and UVAR were provided simultaneously for 3 h per day by 2 UVA-340 lamps (Q-Panel, Cleveland, OH) and a single TL 40W/12 lamp (Philips, Eindhoven, Netherlands), providing 5.9 W m^{-2} UVAR and 0.49 W m^{-2} of UVBR. This resulted in a daily unweighted UVBR dose of 5300 J m^{-2}, and a biologically effective dose of 875 J m^{-2} and 1460 J m^{-2} when using the DNA action spectrum of Setlow (1974) and the generalized plant damage action spectrum of Caldwell (1971), both normalized to 1 at 300 nm, respectively. The UVBR dose and fluence rate are realistic for full sunlight at noon in spring in Brittany (48.4º N, 3.59º W) according to calculations with the UV model of Björn and Murphy (1985). However, accompanying UVAR and PAR levels were much lower than in natural sunlight. Covering the experimental beakers with cut-off foils created three irradiance conditions. UVR Farblos 125 µm (Digefra, München, Germany) transmitted only PAR, whereas 130 µm Folex (Dreieich, Germany) transmitted PAR+UVAR (P+A) and Ultraphan UBT 500 µm (Digefra, München, Germany) transmitted PAR+UVAR+UVBR (P+A+B) with no transmission below 294 nm. The spectral energy distribution received by the algae was measured in the beakers with a MACAM SR9910 double monochromator scanning spectroradiometer (Macam Photometrics, Livingston, U.K.) using a cosine collector (Fig. 1).

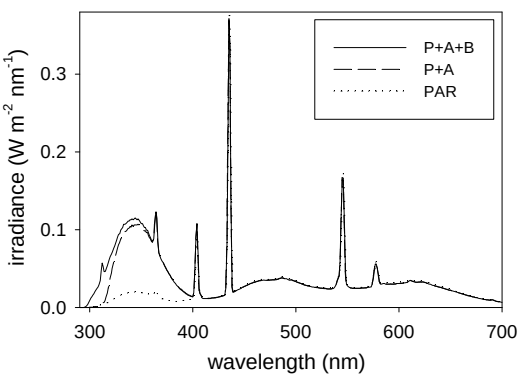


Figure 1. Spectral distribution of irradiance during UV exposure as received by the algae in the P+A+B, P+A, and PAR treatments.

Growth rates

At 2- to 3-day intervals, fresh weight of the fragments was measured using an analytical balance. The relative growth rate (RGR) was calculated after Kain (1987) and Nielsen and Sand-Jensen (1990), assuming exponential growth using the formula:

RGR (% per day) = (Ln FW_2 – Ln FW_1) / (t_2 - t_1) * 100

where FW represents the fresh weight and *t* the experimental time interval. Before UV radiation treatments, the plants were grown for 15 days under PAR. The effects of P+A and P+A+B were determined by comparing growth rates during 15 days of PAR with growth rates during the subsequent 15 days of P+A and P+A+B.

Chlorophyll fluorescence

Fluorescence measurements were executed with a PAM 2000-pulse amplitude modulated fluorometer (Walz, Effeltrich, Germany). Fragments from the experimental beakers were placed in a cooled seawater cuvette (12ºC). Measurements were performed following Bischof *et al.* (1998b). The optimal quantum yield of PSII, a measure for the efficiency of photosynthesis, was determined as the ratio of variable to maximum fluorescence (F_v/F_m) of dark-acclimated fragments. A 5-s far-red light pulse was applied before the fragments were kept in darkness for 5 min. Afterwards, minimal fluorescence F_o (0.15 µmol photons m^{-2} s^{-1}, 650 nm) and maximum fluorescence F_m (saturating white light: 0.4 s, 6000 µmol photons m^{-2} s^{-1}), were determined. To monitor induction of and recovery from photoinhibition, F_v/F_m was determined three times on day 1, 5, 9 and 15 of the experiment: before the UV treatment (overnight recovery, 20 h), directly after the UV treatment, and after 3 h of recovery under PAR. After the measurements, the fragments were replaced in the experimental beakers.

Sampling for DNA damage

Triplicate samples of algal fragments (0.015-0.03 g) were obtained directly after 1 exposure and after 15 exposures to the P+A+B treatment. Samples from the PAR and P+A treatments served as controls. The accumulation of DNA damage in *P. hilliae* was followed in a separate experiment. For this species, samples were collected directly after P+A+B exposure at 4- to 5-day intervals. All samples were frozen in liquid nitrogen and stored at –80ºC.

Extraction of algal DNA

Before extraction the samples were homogenized in liquid nitrogen. The DNA extraction procedure was modified after Doyle and Doyle (1987) using 0.9 mL CTAB extraction buffer (2% Hexadecyltrimethyl-ammonium-bromide, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.2% [w/v] Polyvinyl-polypyrrolidone, 0.01% [v/v] SDS and 0.2% [v/v]-mercaptoethanol). After extraction (30 min, room temperature) samples were washed twice with 0.6 mL chloroform-isoamyl alcohol, 24:1 (v/v) and centrifuged (10 min, 14000 rpm). DNA was precipitated with a half volume of isopropanol (1 h, 4ºC) followed by centrifugation (30 min, 14000 rpm, 4ºC). The pellet was washed with 70% cold (–20ºC) ethanol, vacuum dried and dissolved in 0.3 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The samples were treated with RNase (30 min, 37ºC) and stored at –20ºC. The DNA concentration

was quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR) and a 1420 Victor multilabel counter (Wallac Inc., Gaithersburg, MD). A dilution series with a known amount of DNA was included for calibration purposes.

Assay for the detection of CPDs

The immunoassay for CPDs was modified after Vink *et al.* (1994), Boelen *et al.* (1999) and Pakker *et al.* (2000a, b). Heat denaturated samples containing 0.1 µg DNA were transferred to a nitrocellulose membrane (Schleicher & Schuell, pore size 0.1 µm) with a Minifold I SRC96D dot blot apparatus (Schleicher & Schuell). The membrane was oven dried (2 h, 80ºC) to immobilize DNA. Nonspecific binding sites on the membrane were blocked with 5% (w/v) skimmed milk powder in PBS-T (PBS + 0.1% [v/v] Tween 20; Sigma Chemical Company St. Louis, MO) for 30 min. The membrane was incubated overnight with the H3 antibody specific for CPDs (1:3000 in PBS-T and 0.5% [w/v] skimmed milk powder) (Roza *et al.* 1988) at 4ºC. This was followed by incubation with horseradish peroxidase conjugated rabbit anti-mouse serum (1:15000 in PBS-T and 0.5% skimmed milk powder; Dako Corp. Carpinteria CA) for 2 h at room temperature. Three washing steps with PBS-T (10 min each) followed all incubations. After the addition of ECL Western blotting detection reagent (Amersham Buckinhamshire, U.K.), the membrane was sealed in a Photogene development folder (GibcoBRL, Basel, Switserland) and subsequently exposed to photosensitive ECL films (Amersham). Developed films were scanned with a UMAX PS-2400X scanner (Hsinchu, Taiwan) and gray scale values were quantified with Image QuaNT (Molecular Dynamics). A calibration series of UV-irradiated calf thymus DNA (Sigma) supplemented with unexposed DNA was included, giving 0.1 µg DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-irradiated Hela DNA with a known amount of CPDs (kindly provided by A.Vink). CPDs were quantified by comparing the gray scales of the calibration series with the gray scales of the samples within the linear range of the film.

UV-absorbing compounds

Triplicate samples were collected during the P+A+B treatment directly after the UV exposure on day 1, 5, 9 and 15. The fragments were dried on silica gel and stored in darkness at 4ºC. UV-absorbing compounds were extracted with 1 mL 25% (v/v) methanol for 2 h at 45ºC. The extracts were analyzed using a Cary 3E UV/vis double-beam spectrophotometer in quartz cells with a path length of 1 cm. Absorbance was recorded from 250 to 550 nm and was corrected for the dry weight of the fragments. No samples were collected during the P+A treatment.

Data analysis

Differences between treatments were tested for significance with a single-factor ANOVA based on groups of three replicates.

RESULTS

Growth

Growth during the PAR treatment was exponential because the RGRs of all species were constant for at least 13 days (R^2 values of Ln [fresh weight] vs. time regression > 0.99). The mean RGR values during the PAR treatment are shown in Table 1. For *P. palmata* and *C. crispus* no significant difference was observed between mean RGRs during the PAR and the P+A+B treatment (Fig. 2A). During the first and second week of the P+A+B treatment, mean RGRs of *P. pseudoceranoides*, *R. pseudopalmata*, *P. rubens* and *P. hilliae* were significantly lower compared with the PAR treatment (p=0.002 to p=1.4·10^{-6}). Mean growth rates of *P. pseudoceranoides* and *R. pseudopalmata* were approximately 25%-40% lower, whereas growth of *P. rubens* and *P. hilliae* virtually halted in the second week of the P+A+B treatment. When UVBR was excluded, mean growth rates during the first and second week were not significantly different from growth during the PAR treatment, except for *P. palmata* and *R. pseudopalmata* (Fig. 2B). The former species exhibited a 40% increase in growth rate compared with the PAR treatment (p<0.036).

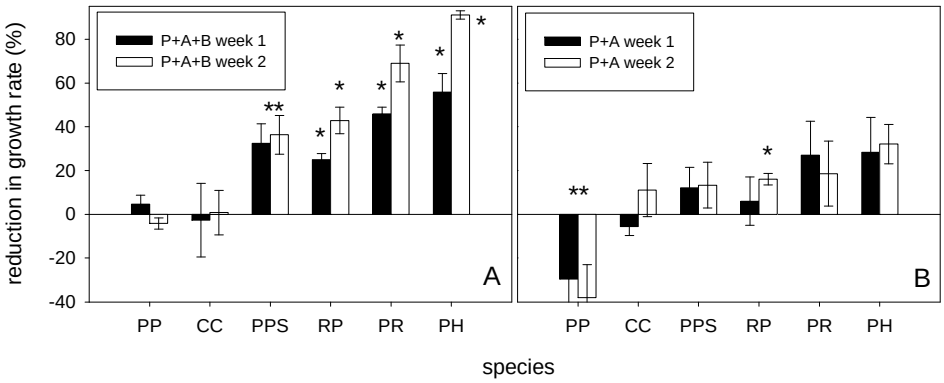


Figure 2. Reduction in growth rates during the P+A+B (A) and P+A (B) treatments compared with the PAR treatment. Mean growth rates were calculated separately for the first and second week of the P+A+B and P+A treatments. The mean and SDs are shown for three replicates. Significant differences between UV and PAR treatments are indicated with asterisks. PP, *Palmaria palmata*; CC, *Chondrus crispus*; PPS, *Phyllophora pseudoceranoides*; RP, *Rhodymenia pseudopalmata*; PR, *Phycodrys rubens*; PH, *Polyneura hilliae.*

Chlorophyll fluorescence: optimal quantum yield

Marked differences in unstressed optimal quantum yields of PSII were observed between species (Table 1). Differences in antenna size and composition may be responsible for this effect (Büchel and Wilhelm 1993). For the same species, initial F_v/F_m values of groups of replicates used in the PAR, P+A and P+A+B treatments were not significantly different before the treatments started (Fig. 3).

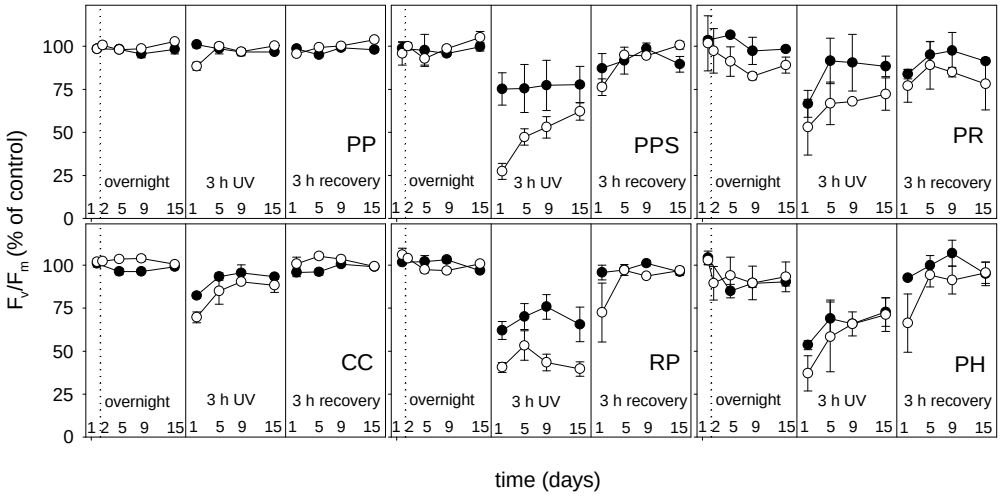


Figure 3. Changes in optimal quantum yield measured as F_v/F_m in the course of the P+A+B (○) and P+A (●) treatments, after overnight recovery (overnight), directly after 3 h of UV (3 h UV), and after 3 h of recovery under PAR (3 h recovery). Overnight recovery values on day 1 show the initial values before the UV treatment started, other overnight recovery values show recovery from treatments on the previous day. The F_v/F_m is expressed as a percentage of the corresponding mean PAR control values to facilitate comparison between the species. Means and SDs are shown for three replicates. PP, *Palmaria palmata*; CC, *Chondrus crispus*; PPS, *Phyllophora pseudoceranoides*; RP, *Rhodymenia pseudopalmata*; PR, *Phycodrys rubens*; PH, *Polyneura hilliae.*

The first P+A+B exposure resulted in a significant reduction in F_v/F_m in all species compared with the PAR exposed fragments (p=0.02 to p=1.1·10^{-6}). The F_v/F_m of *P. palmata* and *C. crispus* was reduced to 88-67% of the control values, whereas the F_v/F_m of *P. pseudoceranoides*, *R. pseudopalmata*, *P. rubens* and *P. hilliae* decreased to 55%-25% of the control values. Repeated P+A+B treatments resulted in decreased photoinhibition in *C. crispus*, *P. pseudoceranoides*, *P. rubens* and *P. hilliae*. In *P. palmata*, photoinhibition was no longer significant on day 5, 9 and 15 compared with the PAR treatment. Complete recovery from photoinhibition occurred within 3 h in *P. palmata* and *C. crispus*. In *P. pseudoceranoides*, *R. pseudopalmata* and *P. hilliae* the first P+A+B exposure was followed by recovery after 3 h of PAR to approximately 70% of the control values. In these species, recovery rates increased in the course of the experiment and F_v/F_m values after 3 h of recovery were not significantly different from the PAR treatment on day 5, 9 and 15. After overnight recovery, F_v/F_m values of P+A+B exposed fragments were not significantly different from PAR-exposed fragments in all species except *P. rubens*. In the latter species, F_v/F_m values after 3 and 20 h of recovery remained significantly lower compared with the PAR treatment (p<0.01).

Photoinhibition directly after the first P+A treatment was significantly lower than after the first P+A+B treatment in all species (p=0.002 to p=0.0007) except *P. rubens*, and remained lower than photoinhibition caused by P+A+B except in *P. hilliae* (Fig. 3). Photoinhibition during the P+A treatment became less pronounced in *C. crispus*, whereas the P+A treatment caused no significant photoinhibition in *P. palmata*. In *P. rubens* the reduction in F_v/F_m was not significant on day 5, 9 and 15 due to high variation among replicates. Recovery from photoinhibition caused by P+A was completed within 3 h in all species, and overnight recovery values were not significantly different from the F_v/F_m values of the PAR treatment. By comparing the mean decrease in F_v/F_m after the P+A and P+A+B treatments, the contribution of UVAR in the total photoinhibition could be estimated. On average, UVAR caused 2% of the total photoinhibition in *P. palmata*, 37% in *C. crispus*, 42% in *P. pseudoceranoides*, 65% in *R. pseudopalmata*, 50% in *P. rubens* and 75% in *P. hilliae*.

DNA damage

Directly after the first exposure to P+A+B, algal fragments contained 5-35 CPDs per 10^6 nucleotides (Fig. 4). CPD levels were similar for *P. palmata*, *C. crispus*, *R. pseudopalmata* and *P. pseudoceranoides* whereas samples of *P. rubens* and *P. hilliae* contained more CPDs. Differences in CPD levels between species were more pronounced after 15 days of P+A+B treatments (Fig. 4). In fragments of *P. palmata* and *C. crispus*, a significantly lower number of CPDs was found on day 15 than on day 1 ($p=8\cdot10^{-5}$, $p=7\cdot10^{-5}$, respectively). The mean number of CPDs remained constant in *P. pseudoceranoides*. A low but significant increase of CPDs was observed in *R. pseudopalmata*, whereas this was more pronounced in *P. rubens* and *P. hilliae* (p=0.02 to $p=8.8\cdot10^{-6}$). In a separate experiment, the accumulation of CPDs was followed in more detail in *P. hilliae*. For this species, the reduction in RGR coincided with the accumulation of CPDs (Fig. 5). No CPDs were detected in PAR-exposed fragments, whereas 15 days of the P+A treatment resulted in 0-5 CPDs 10^6 nucleotides^{-1} for *P. palmata*, *C. crispus*, *R. pseudopalmata* and *P. pseudoceranoides*, and 6-12 CPDs per 10^6 nucleotides for *P. rubens* and *P. hilliae* (not shown).

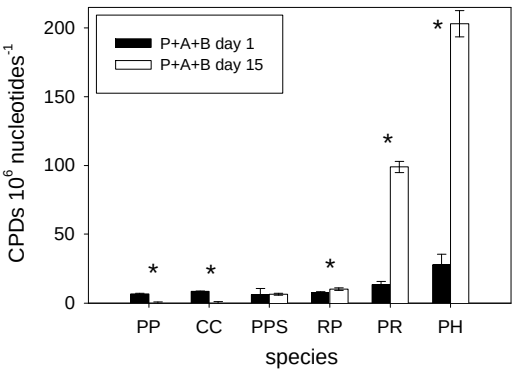
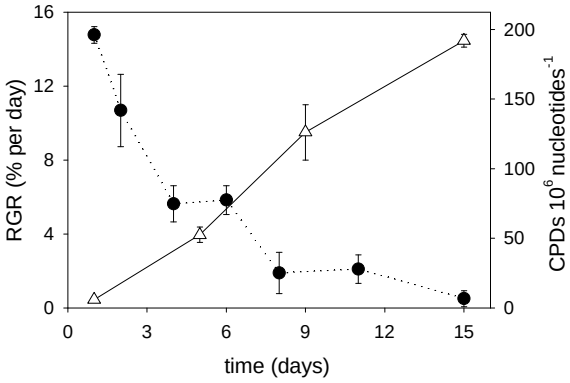


Figure 4. DNA damage determined as the number of CPDs per 10^6 nucleotides after a single P+A+B exposure (day 1) and after 15 repeated P+A+B exposures (day 15). Means and SDs are shown for three replicates. Significant differences in CPDs on day 1 and day 15 are indicated with asterisks. PP, *Palmaria palmata*; CC, *Chondrus crispus*; PPS, *Phyllophora pseudoceranoides*; RP, *Rhodymenia pseudopalmata*; PR, *Phycodrys rubens*; PH, *Polyneura hilliae*.

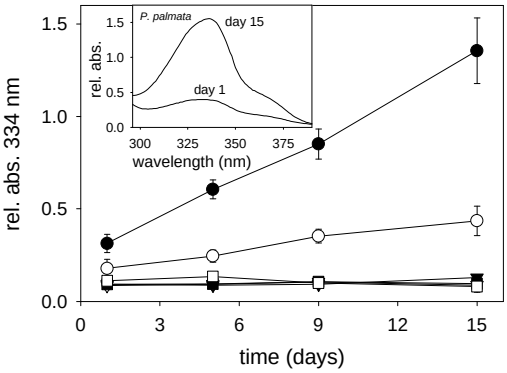
Figure 5. RGR (●) and accumulation of CPDs per 10^6 nucleotides (△) in *Polyneura hilliae* in the course of the P+A+B treatment. Means and SDs are shown for three replicates. Prior to the P+A+B treatment the fragments were growing for 15 days with a constant mean growth rate of 15% per day.



UV-absorbing compounds

Initial absorption levels in the UVAR region of extracts of *P. palmata* and *C. crispus* were significantly higher than in the other species (Fig. 6, p=0.025, p=6·10^{-5}, respectively). For both species absorption peaks were observed at 334 nm. During the course of the P+A+B experiment, absorption at 334 nm increased significantly in extracts of these species (p=0.009, p=0.0006, respectively). No absorption peaks were observed in extracts of the other species.

Figure 6. Accumulation of UV-absorbing compounds in the course of the P+A+B treatment. The relative absorption at 334 nm was used as a measure for total UV-absorbing compounds. Mean and SDs are shown for three replicates. (●) *Palmaria palmata*; (○) *Chondrus crispus*; (▽) *Phyllophora pseudoceranoides*; (▼) *Rhodymenia pseudopalmata*; (■) *Phycodrys rubens*; (□) *Polyneura hilliae*. Inset: Absorption spectra from 290 to 400 nm of extracts of *Palmaria palmata* after a single P+A+B treatment (day 1) and after 15 P+A+B treatments (day 15).



DISCUSSION

We evaluated the relative UV sensitivity of six temperate red macrophytes to UVBR levels comparable with that of full sunlight at the water surface in Brittany. These results do not allow direct extrapolation to natural irradiance conditions since the ratios of UVAR:UVBR and PAR:UVBR were lower than in natural sunlight. High levels of UVAR and PAR can increase the induction of protective and repair

mechanisms and increase UV tolerance (Takayanagi *et al.* 1994, Buchholz *et al.* 1995, Berkelaar *et al.* 1996, Hada *et al.* 1999). Furthermore, wavelengths that are most efficient for the reversal of CPDs by photolyases (360-420 nm) were underrepresented in our light sources (Pang and Hays 1991, Takeuchi *et al.* 1998, Hada *et al.* 2000). Therefore, effects of UVBR could have been overestimated in this study compared with natural irradiance conditions. However, this study does allow interspecies comparison with regard to differential susceptibilities of some of the main targets of UVBR.

Effects of P+A+B on growth were more pronounced compared with the P+A treatment. Photoinhibition and CPD induction was consistently lower during the P+A treatment, demonstrating that the UVBR component was the major cause of growth reduction in the experiments. However, solar irradiance contains higher UVAR levels compared with the experimental conditions and therefore may cause more pronounced effects in the field.

Littoral and sublittoral species differed markedly in their UV sensitivity to the daily repeated P+A+B treatments. The species with a habitat extending in the littoral zone, *P. palmata* and *C. crispus*, showed a higher tolerance to the P+A+B treatment than the sublittoral species *P. pseudoceranoides*, *R. pseudopalmata*, *P. rubens*, and *P. hilliae*. Growth rates of *C. crispus* and *P. palmata* were not significantly affected. Photoinhibition caused by UVBR and UVAR was low and recovery occurred within 3 h. Furthermore, photoinhibition became less pronounced in the course of the experiment, and this was paralleled by an accumulation of UV-absorbing compounds. The absorption maximum at 334 nm suggests that these compounds protect components of PSII against UVAR. In line with this assumption, the relative contribution of UVAR to photoinhibition was lower in these species than in the species that did not produce UV-absorbing compounds. In *C. crispus* and *P. palmata*, UV-absorbing compounds were present from the start of the experiment, indicating that these compounds are continuously synthesized in these species. Previous experiments with *Devaleraea ramentacea* (Karsten *et al.* 1999) and *C. crispus* (Franklin *et al.* 1999) also indicated increased protection against photoinhibition by MAA accumulation. In the present study, MAA accumulation during the P+A treatment was not determined. However, the induction of MAA-synthesis by PAR and UV radiation exposure was demonstrated previously for both species (Karsten *et al.* 1998, Karsten and Wiencke 1999). The low levels of CPDs in samples of *P. palmata* and *C. crispus* indicate efficient repair of photoproducts in these species. Apparently, PAR and UVAR were sufficient for repair of these lesions. The absence of CPDs after 15 days of P+A+B treatment suggests additional induction of DNA repair enzymes. Studies on terrestrial plants and macrophytes have convincingly demonstrated that repair by photolyases is the most efficient repair pathway for CPDs (Quaite *et al.* 1994, Pakker *et al.* 2000a, b). For *P. palmata*, rapid light-dependent repair of CPDs was observed after induction of these lesions with a single high dose of UVBR, whereas dark repair processed removed about 65% of the CPDs overnight (Pakker *et al.* 2000b). In addition to efficient repair, UV-absorbing compounds may

have protected DNA from UVBR by absorbing short wavelengths. However, the extracted compounds offer limited protection against CPD induction because they absorb maximally at longer wavelengths that do not efficiently induce CPDs.

In contrast to the littoral species, the sublittoral species were quite sensitive to the P+A+B treatments. Growth rates in *R. pseudopalmata* and *P. pseudoceranoides* were 40% lower during the P+A+B treatment compared with PAR. In these species, P+A+B treatments caused a more pronounced decrease in optimal quantum yield and increased CPD levels compared with the littoral species. *R. pseudopalmata* and *P. pseudoceranoides* are morphologically comparable to *C. crispus* and *P. palmata* with respect to weight:area ratios (data not shown). Therefore, morphology appears not to explain the differences in photoinhibition and DNA damage between these species. Previously, light-dependent repair of CPDs has been demonstrated for *R. pseudopalmata*, although dark repair processes appeared to be virtually absent (Pakker *et al.* 2000a).

The absence of MAAs may have caused the relatively large effects on optimal quantum yield in the sublittoral species. This was also indicated by the higher contribution of UVAR to photoinhibition, compared with the littoral species. Because the optimal quantum yields of all sublittoral species were less affected after repeated exposure to P+A+B, the induction of additional repair capacity appears also important for reducing photoinhibition. Previously, Bischof *et al.* (1999) showed that optimal quantum yield of the brown macrophyte *Alaria esculenta* acclimated to UVBR treatments by decreasing the degree of photoinhibition and increasing the recovery rate after photoinhibition. The two species found in shaded habitats in the sublittoral zone, *P. rubens* and *P. hilliae*, did not acclimate efficiently to the repeated UVBR treatments. Growth of *P. rubens* and *P. hilliae* gradually ceased in the course of the P+A+B treatment. For both species this coincided with massive accumulation of CPDs. Apparently, repair mechanisms were insufficient to remove all CPDs, either by photolyases or excision repair under these conditions, causing accumulation of CPDs in the genome. Additionally, both species are morphologically different from the other tested species as was indicated by a smaller weight:area ratio (data not shown). Therefore, the DNA of these species may be less protected, making them more vulnerable to the induction of DNA damage. The effects on photosynthetic efficiency appeared to be comparable with those observed in *R. pseudopalmata* and *P. pseudoceranoides*, suggesting that DNA damage was primarily responsible for the complete inhibition of growth of *P. hilliae* and *P. rubens*. This indicates that efficient repair of DNA damage is essential for growth under high UV conditions.

In conclusion, these experiments show that reduced growth rates during the P+A+B treatment are accompanied by accumulation of CPDs and photoinhibition. This suggests that growth reduction is the result of combined effects of damage to several cellular components, such as proteins from PSII reaction centers and DNA. The ability to repair or prevent damage eventually determines the UV tolerance of species. We demonstrated that the littoral species *P. palmata* and *C. crispus* can efficiently reduce the effects of photoinhibition and CPD formation and, as a result,

are able to maintain normal growth rates during UV exposure. The sublittoral species have a lower capacity to prevent photoinhibition and possibly a lower capacity to repair CPDs. Species such as *P. rubens* and *P. hilliae* therefore rely on protection from other macrophytes by shading and UVBR attenuation in the water column to avoid high UVBR exposure, whereas littoral species are adapted to fluctuating irradiace conditions and frequent UVBR exposure. Therefore, the structural differences in UV tolerance between the tested species appear to reflect their natural habitat in the water column. Moreover, the fixed differences in expression of UV-tolerance mechanisms such as MAA synthesis and photolyase activity may limit the distribution of sublittoral species into the littoral zone, as was suggested previously by Bischof *et al.* (1998a, b) and Maegawa *et al.* (1993).