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PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern

Michael Y. Roleda[1,4,*], Willem H. van de Poll[2], Dieter Hanelt[1,5], Christian Wiencke[3]

[1]Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Postfach 180, 27483 Helgoland, Germany

[2]Department of Marine Biology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

[3]Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

[4]Biology Department, De La Salle University, 2401 Taft Avenue, 1004 Manila, Philippines

[5]*Present address:* Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-Str. 18, 22609 Hamburg, Germany

ABSTRACT: The effects that ultraviolet radiation (UVR, 280 to 400 nm) and photosynthetically active radiation (PAR, 400 to 700 nm) had on early life stages of *Mastocarpus stellatus* and *Chondrus crispus* were studied to determine if differences in UVR tolerance could influence their recruitment success on the upper eulittoral shores of Helgoland (North Sea). Photosynthesis, germination capacity, DNA damage and carpospore repair were measured after exposures to different time lengths and intensities of PAR+UV-A+UV-B, PAR+UV-A or PAR alone, and also after recovery in low white light. Germination and photosynthesis of the low light adapted carpospores of both species were inhibited as PAR was increased. Supplemental UV-A and UV-B had a small additional effect on the F_v/F_m of *M. stellatus* but this effect was more pronounced in *C. crispus*. However, photosynthesis of both species significantly recovered after 48 h. Carpospore viability in *C. crispus* was more sensitive than in *M. stellatus* to UVR, while a higher dose was needed to achieve 50% germination inhibition in *M. stellatus*. Furthermore, UV-B-induced DNA damage, measured as cyclobutane-pyrimidine dimers (CPDs), was less in *M. stellatus* spores, which also exhibited an efficient DNA repair mechanism compared with *C. crispus*. In contrast, growth and chlorophyll *a* contents in young gametophytes of both species were not affected by repeated UV exposures. Higher total carotenoid was measured in plants exposed to UVR, indicating a photoprotection role, because photosynthesis completely acclimated to UVR after 3 d. Furthermore, DNA damage was not detected on mature fronds of both species when exposed to the full solar spectrum. Therefore, the susceptibility of carpospores to UVR could influence species recruitment to the upper eulittoral zone.

KEY WORDS: Carpospores · Gametophytes · *Mastocarpus stellatus* · *Chondrus crispus* · Germination · Cyclobutane-pyrimidine dimers · DNA repair · Pigments

INTRODUCTION

Mastocarpus stellatus Stackhouse (Guiry) and *Chondrus crispus* Stackhouse (Gigartinales) (hereafter called '*Mastocarpus*' and '*Chondrus*' respectively) are morphologically similar red algae abundantly distributed along the North Atlantic coasts and co-inhabit the rocky littoral zone (Lüning 1990). *Chondrus* has always been regarded as an abundant species within the lower eulittoral and upper sublittoral of the island of Helgoland (North Sea) (Kornmann & Sahling 1977), while *Mastocarpus* was not recorded there before 1983, when material from Iceland was accidentally introduced during a scientific campaign (Kornmann &

*Email: mroleda@awi-bremerhaven.de

Sahling 1994). Now it has successfully established and colonized all natural and man-made hard substrates, forming extensive stands that have changed the appearance of the intertidal biotopes of the island (Bartsch & Kuhlenkamp 2000).

Since the introduction of Icelandic *Mastocarpus* to Helgoland, only presumptive female gametophytes have been observed in the field and fertile plants are found all year round. *Mastocarpus* is heteromorphic and has 2 basic types of life history (Chen et al. 1974, Guiry et al. 1984). However, studies on different isolates of *Mastocarpus* showed that only apomictic or the direct-type life history is found in plants from Denmark and Iceland (Guiry & West 1983), from where the Helgoland population originated. *Chondrus* is isomorphic (Chen & McLachlan 1972) but a demographic study showed that most populations are overwhelmingly gametophytic (approximately 80%; Bhattacharya 1985, Chopin 1986, Dudgeon & Johnson 1992), although tetrasporophyte dominated populations have been also infrequently observed (Lazo et al. 1989).

Intertidal algae experience drastic environmental fluctuations on a daily basis. During low tide they can be exposed to desiccation, hyper- or hypo-osmotic shock, high or freezing temperatures and full solar radiation, depending on the season and latitude. Furthermore, stratospheric ozone depletion increases the ultraviolet-B radiation (UV-BR) component of the solar spectrum on the earth's surface (Smith et al. 1992). Recent studies have implicated the role of solar and especially ultraviolet radiation (UVR) on macroalgal zonation patterns from polar to temperate regions (Dring et al. 1996a, Hanelt et al. 1997, Bischof et al. 1998). Aside from photoinhibition of Photosystem II (PSII), UV-B damages DNA by forming cyclobutane-pyrimidine dimers (CPDs). These photoproducts inhibit transcription and replication of DNA and consequently disrupt cell metabolism and division (Buma et al. 1995, 2000), which could directly inhibit growth and survival.

Comparative ecophysiological studies on *Mastocarpus* and *Chondrus* showed that *Mastocarpus* is more tolerant of environmental stress such as temperature, desiccation and freezing (Dudgeon et al. 1989, 1990, 1995). Moreover, *Mastocarpus* was also found to be more efficient in scavenging reactive oxygen and more resistant to oxidative stress (Collén & Davison 1999), which may explain the extensive distribution of *Mastocarpus* from the upper eulittoral to the upper sublittoral zone. Recently, Bischof et al. (2000) also reported the sensitivity of *Chondrus* photosynthesis to UVR while the relative tolerance of *Mastocarpus* photosynthesis to UVR was determined to be due to its 6-fold higher total mycosporine-like amino acid (MAA) content. All previous comparative studies on the physiological response to environmental variations between these species were conducted on mature fronds (and crusts in the case of *Mastocarpus*).

Early developmental stages of macroalgae, especially 'naked' algal spores, are more susceptible to UVR than are the large developmental stages (as shown for zoospores of various brown algae by Wiencke et al. 2000). Physiological studies on the early life stages of macroalgae in response to environmental stress are lacking. Effects of UVR on photosynthesis have been studied on gametophytes and zoospores of laminariales (Dring et al. 1996b, Wiencke et al. 2000) and unicells of ulvales (Cordi et al. 2001). Other studies on the impact of UVR on the early life stages of green, red and brown macroalgae have focused either on (1) the sporulation of fertile thalli, (2) germination and photomovement of spores, (3) growth of germlings or (4) DNA synthesis in gametophytes (Huovinen et al. 2000, Swanson & Druehl 2000, Cordi et al. 2001, Makarov & Voskoboinikov 2001, Bañares et al. 2002, Flores-Moya et al. 2002, Altamirano et al. 2003a,b, Han et al. 2003). All these studies indicated high sensitivity of the early life stages of macroalgae to UV-B. In contrast to the relatively abundant information on UV-B-dependent DNA damage in higher plants and phytoplankton, information on this subject with regards to macroalgae is still limited (e.g. Pakker et al. 2000a,b, van de Poll et al. 2001, 2002a,b, Bischof et al. 2002a). Only 1 study has investigated DNA damage on macroalgal spores (Wiencke et al. 2000).

Our study focuses on UVR effects on carpospores and young gametophytes of *Mastocarpus* and *Chondrus*, in order to test if their sensitivity plays a role in the establishment of the zonation pattern on the Helgoland shoreline. This study is the first to investigate UV-B-induced DNA damage and photoinhibition on germination and growth of these early life stages. The survival of these transitional life history stages is the most critical phase leading to the successful formation of a benthic population. In the field, *Mastocarpus* is able to colonize open spaces in the upper eulittoral, while the distribution of *Chondrus* is limited to sites under canopies of *Fucus* spp. in the eulittoral, in tidepools and in the upper sublittoral. We hypothesize that differences in UVR sensitivity between *Mastocarpus* and *Chondrus* carpospores and young gametophytes affect the establishment of early post-settlement stages and growth of young juveniles in the upper eulittoral.

MATERIALS AND METHODS

Algal material. Unialgal cultures of *Mastocarpus* and *Chondrus* fronds are maintained in the Biologische Anstalt Helgoland (BAH). Carpospores from *Mastocarpus* cultures gave rise to discoid germlings with

upright fronds following the direct-type life history. The gametophytic fronds produced from the basal discs were scraped from the culture dish and maintained in aerated culture vessels. Young vegetative fronds of *Chondrus* were found to be gametophytic after acetal-resorcinol testing (Garbary & DeWreede 1988). The young fronds in culture were also observed to become fertile and to release spores. Histological cross sections of the sporulating fronds showed single-spored carposporangia. For both species, Provasoli enriched culture medium (Provasoli 1968) was used.

To obtain carpospores from both species, the culture medium of the stock gametophytes was changed and after 3 d the spore-containing medium was collected. The spores were allowed to settle for 4 h. The supernatant water was slowly sucked out by vacuum pump to obtain a concentrated stock spore suspension. The latter was then slowly and continuously agitated using a magnetic stirrer to stop the spores from settling and to derive a homogenous spore suspension. The density of the suspension was adjusted to approximately 4×10^3 spores ml^{-1} after counting the number of spores in 20 µl of suspension on a cavity slide using an inverse microscope.

Experimental light conditions. Light was provided by 3 white fluorescent lamps (Osram, L65 Watt/25S, Germany), emitting background photosynthetically active radiation (PAR) of 400 to 700 nm and 3 UV lamps (Q-Panel UVA-340, 40 Watt, Cleveland), emitting a spectrum similar to solar radiation in the range of 295 to 340 nm. Three kinds of filter foils and glass filters were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes. Experimental units were covered with the following filters: Ultraphan 280 (Digefra GmbH, Germany), Quartz glass; Folanorm 320 (Folex GmbH, Germany), Schott-WG 320 or Schott-GG 400 (Schott, Germany) corresponding to the PAR+UV-A+UV-B (PAB), PAR+UV-A (PA) and PAR (P) treatments respectively. Furthermore, 4 irradiation levels were achieved by varying the distance between the experimental units and the light source (where level 1 = highest radiation and level 4 = lowest radiation; Table 1). Irradiation was measured using a cosine sensor connected to a UV-VIS spectrometer (Marcel Kruse, Bremerhaven, Germany) below the cut-off filters. The biologically effective doses (BED) applied in each treatment were calculated using 2 action spectra for well-known biological responses: DNA damage for *Escherichia coli* (280 to 320 nm, Setlow 1974) and the generalized plant damage (280 to 312 nm) described by Caldwell (1971). Both unweighted and weighted irradiances for each treatment are compiled in Table 1.

Spore photosynthesis. From the working suspension, 5 ml were put into each 35 × 10 mm cell culture dish (5 replicates per treatment). To evaluate the effect of different radiation and exposure time treatments, carpospores of both species were exposed to the 3 radi-

Table 1. Experimental treatments applied with different irradiance levels (1 = highest, 4 = lowest) and the corresponding weighted irradiances using the biologically effective weighing function for the DNA damage of *Escherichia coli* (Setlow 1974) and general plant damage (Caldwell 1971)

Treatment		Experimental irradiance ($W m^{-2}$)			Weighted irradiance ($W m^{-2}$)	
		PAR (400–700 nm)	UVA (320–400 nm)	UVB (280–320 nm)	DNA damage (Setlow 1974)	Generalized plant damage (Caldwell 1971)
Carpospore experiment						
PAB	1	28	15.87	1.00	0.013	0.050
Ultraphan 280	2	14	10.20	0.55	0.005	0.017
(PAR+UVA+UVB)	3	14	7.48	0.35	0.002	0.007
	4	13	6.8	0.30	0.002	0.006
PA	1	27	13.77	0.04	1.41×10^{-4}	0
Folanorm 320	2	14	8.80	0.01	5.89×10^{-5}	0
(PAR+UVA)	3	13	6.10	0.01	2.41×10^{-5}	0
	4	12	6.00	0.01	1.47×10^{-5}	0
P	1	26	0.07	0	0	0
Schott-GG 400	2	14	0.04	0	0	0
(PAR alone)	3	13	0.03	0	0	0
	4	12	0.03	0	0	0
Gametophyte experiment						
Quartz (PAR+UVA+UVB)		12	6.24	0.50	0.021	0.050
WG 320 (PAR+UVA)		11	6.08	0.16	0.001	0.002
GG 400 (PAR alone)		8	0.01	0	0	0

ation conditions for 1, 4 and 8 h at 10 ± 1°C. As control, initial photosynthesis of another 5 replicates was measured without treatment. Photosynthetic efficiency, measured as variable fluorescence of PSII, was determined using a Xenon Pulse Amplitude Modulation fluorometer (XE-PAM, Heinz Walz, Germany), coupled to a PC with WinControl software (Heinz Walz GmbH, Germany). After treatments, spore suspension was poured into 5 ml quartz cuvettes and the optimum quantum yield (F_v/F_m) was measured as described by Hanelt (1998). After measurements, the spore suspension was exposed for 2 d under low white light (10 µmol photons m^{-2} s^{-1}) to recover. The control was also maintained under the same conditions.

Determination of spore mortality. For this experiment, 20 ml of working spore suspension and 20 ml of Provasoli enriched seawater were filled into 85 × 15 mm culture dishes with cover slips. The dishes were then exposed to the same combination of treatments used for photosynthesis and also to an additional treatment of 16 h exposure at 4 varying levels of radiation, (3 replicates per treatment). After the treatment, spores were allowed to germinate in low white light (10 µmol photons m^{-2} s^{-1}) for 6 d. Carpospores settled on the cover slip were scored as dead or alive by counting 300 cells per replicate using a light microscope (Olympus CH-2, Japan) equipped with 20× seawater immersion objective. Dead cells were easily distinguishable (no pigment and hollow ghost-like cells) from the live cells (pigmented and dividing cells, sometimes with germinating tubes; Fig. 1). Because dead cells were also observed in the P treatment, the percentage of dead cells for PA and PAB treatments due to the effect of PAR was calculated by using the average number of dead cells as a control value as described by Wiencke et al. (2000):

$$\text{Dead spores (\%)} = 100 \times (S_{dead} - D_C) \times L_C^{-1}$$

where S_{dead} is the number of dead spores under UV exposure, and D_C and L_C are the number of dead and living spores under white light respectively. Mortality data and BED were used to create a dose-response relationship and to calculate the BED_{50}, which is the UV dose needed to achieve 50% inhibition in germination.

Spore DNA damage and repair. In parallel with the mortality experiment, DNA damage and subsequent repair of this damage was determined after 16 h exposure to the 4 irradiation levels. From the working spore suspension, 40 ml was used for each experimental unit. For each treatment, 6 experimental units were prepared. After the irradiation treatment, 3 experimental units (as replicates) were processed immediately while the other 3 were allowed to recover for 6 d in low white light before processing. The spore samples were filtered through 44 mm 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK) and frozen at −80°C in 2 ml eppendorf tubes for further DNA extraction and analysis of CPDs.

Young gametophyte photosynthesis and growth. In a temperature controlled room, a large flow-through basin (600 × 400 × 120 mm) was placed on a shelf along with lamps as previously described. Inside the basin were cut-out PVC pipes (120 mm diameter) standing upright (70 mm height), which held the algae. The PVC pipes had a 5 mm diameter hole at the bottom for water inflow through silicon tubes and water flowed out into the basin through 4 equidistant, screen covered 10 mm diameter holes in the middle of the PVC pipes. From a reservoir, 80 l of filtered and pasteurized seawater was circulated into the basin through the PVC pipes using a submersible water pump (Eheim; Typ 1060, 38 l min^{-1}, Deizisau, Germany) which also provided water movement inside the PVC pipes. Water level in the basin was maintained at 60 mm, to simulate low tide water. During the experiment, water temperature was maintained at 10 ± 1°C. Water was changed weekly to prevent depletion of nutrients.

To determine the effects of different light treatments, glass filters corresponding to PAB, PA and P treatments

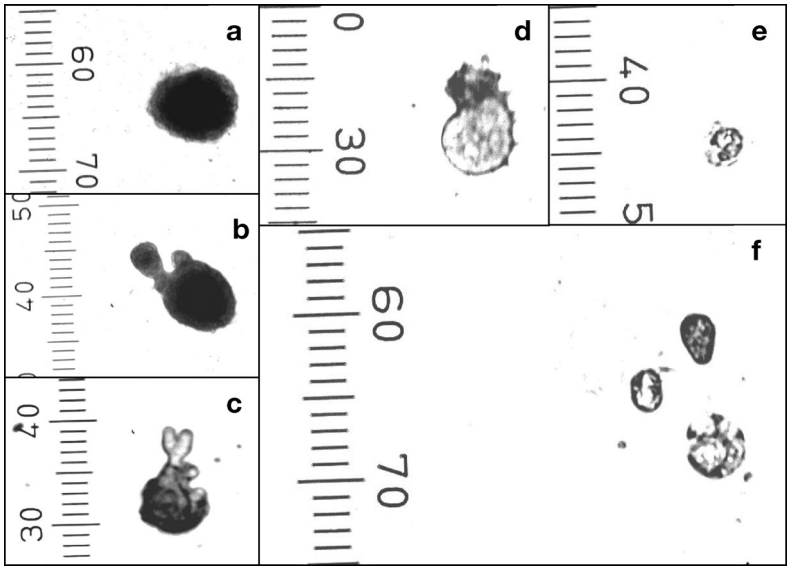

Fig. 1. Carpospores of *Mastocarpus stellatus* (a & b = germinating, d & e = dead) and *Chondrus crispus* (c = germinating, f = dead and alive). Scales are in µm

were laid on top of the standing PVC pipes. Young *Chondrus* and *Mastocarpus* thalli (average size = 20 to 27 mm^2, n = 5), previously acclimated to 46 µmol photons m^{-2} s^{-1} (≈10 W m^{-2}) white light at 10°C for 3 d, were grown for 15 d under 16:8 h light:dark (LD) cycles (05:00 to 21:00 h) with 6 h UV exposure in the middle of the light phase (09:00 to 15:00 h). Photosynthetic activity was determined by measuring the variable chlorophyll fluorescence of PSII with a Diving PAM device (Heinz Walz GmbH, Germany). Effective quantum yield ($\Delta F/F_m'$) was measured every 3 d: 1 h before UV exposure (08:00 h), after 6 h of cumulative UV exposure (15:00 h), and 2 h after UV exposure (17:00 h). Growth, in terms of weight (g) and surface area increase (mm^2), was also measured every 3 d using a weighing scale (Sartorius CP225D, Germany) and a scanner with image analysis software (Winfolia™ 5.0, Regent Instrument Inc., Canada) respectively. Growth rates were computed by plotting all data points (entire experiment period) of each replicate per treatment. They were individually fitted to an exponential equation, $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase. After the experiment, the fronds were immediately frozen in liquid nitrogen and stored at –80°C for further pigment analysis.

Outdoor exposure experiments on mature gametophytes. Vegetative gametophytic fronds of *Mastocarpus* and *Chondrus* (average size = 76 ± 22 mm^2) were collected in the Northwestern intertidal flat of Helgoland. They were cleaned of epiphytes and the meristematic tips (size = 51 to 99 mm^2) were used for the experiment. They were spread out under UV transparent (Quartz) and non-transparent (GG 400) filter glasses, incubated directly below the water surface inside a basin and exposed to the full solar radiation for 4 h (10:00 to 14:00 h) on the roof of the institute (BAH). During the experiment (18 March 2003), 11 h sunshine and 4 to 9°C air temperature was recorded in Helgoland. This is equivalent to a maximum UV radiation of 29.4 W m^{-2} UVA (= 1.2×10^6 J m^{-2}) and 1.6 W m^{-2} UVB (= 4.95×10^4 J m^{-2}) as measured by a multichannel UV-spectroradiometer (Isitec GmbH, Bremerhaven) installed on the roof of the institute. After exposure, 8 replicates per treatment were harvested and the rest of the fronds were allowed to recover overnight with screen cover under low light condition. Seawater was continuously replaced during the course of the experiment. Samples were immediately frozen in liquid nitrogen and stored at –80°C after treatment and recovery for further experiments. Three replicates were used for pigment analysis and 5 replicates for DNA extraction and CPDs analysis.

Pigment extraction and characterisation. Frozen samples were treated with 100 µl of 100% N-N-dimethylformamide and stored in darkness for approximately 16 h. Subsequent analyses for chlorophyll *a* and α- and β-carotene using HPLC were performed as described by Bischof et al. (2002b).

DNA extraction. DNA was isolated following the CTAB extraction procedure described by van de Poll et al. (2001). Spore samples were immediately treated with extraction buffer while vegetative fronds were first homogenized in liquid nitrogen. After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 µl 10 mg ml^{-1}, 30 min, 37°C; Sigma, MO, USA) and stored at –20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence spectrophotometer (Variance Scientific Instrument, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection. The immunoassay for CPDs was modified after Vink et al. (1994) and van de Poll et al. (2001). Heat denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 µm, Schleicher & Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a 2 step anti-body assay, the membrane was treated with ECL Western blotting detection reagent (Amersham, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) for different exposure times. The films were developed using X-ray film developer. Developed films were scanned using Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray-scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 µg ml^{-1} DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis. All data were tested for homogeneity of variances (Levene's test) and normality (Kolmogorov-Smirnov test). Corresponding transformations were done to heteroskedastic (unequal variances) and non-normal data. Carpospore data were subjected to multiple analysis of variance (MANOVA) to determine the main effects and interactions of species, irradiance exposure time and radiation levels on the photosynthesis, spore viability, DNA damage and repair in *Mastocarpus* and *Chondrus*. Biologically effective UV-B doses ($BED_{DNA300\ nm}$ and $BED_{plant\ damage313\ nm}$) resulting in a 50% inhibition in germination were determined

from all germination data (expressed as % of P) using linear ($y = a + bx$) and non linear ($y = a + bx + cx^2$) regressions, corresponding to the best fit curve for *Mastocarpus* and *Chondrus* respectively.

Time series measurements on the photosynthetic yield ($\Delta F/F_m'$) of the young gametophytes were subjected to repeated measures analysis of variance (RMANOVA) to determine the effects of light treatments across the sampling days. Differences in growth rates and pigment contents were tested for statistical significance using analysis of variance (ANOVA, $p = 0.05$). All test were followed by Duncan's multiple range test (DMRT, $p = 0.05$), when appropriate. Statistical analyses were done using SPSS® software.

RESULTS

Spore photosynthesis

Initial measurement of the controls showed that *Mastocarpus* spores had a higher mean (±SD) optimum quantum yield ($F_v/F_m = 0.533 \pm 0.01$) compared with *Chondrus* spores (0.386 ± 0.04). However, after 2 d in low white light (10 µmol photons m^{-2} s^{-1}), photosynthesis of the *Mastocarpus* controls had reduced to 0.453 ± 0.02 but did not vary much in *Chondrus controls* (0.394 ± 0.04). Under P treatment of around 56 µmol photons m^{-2} s^{-1} (= 12 ± 1 W m^{-2}), carpospores were photoinhibited after all exposure times (Fig. 2a,b). However, the effect in *Chondrus* was less than that in *Mastocarpus*. Additional UV-A did not further reduce the F_v/F_m of *Mastocarpus* regardless of exposure time, but significantly reduced the F_v/F_m of *Chondrus*. The combined effect of UV-A and UV-B lowered the photosynthesis of both species with increasing time, with a more pronounced effect in *Chondrus*.

After 48 h in low white light, photosynthesis of both species in all treatments was able to recover. However, the effect of long exposure times (4 and 8 h) to relatively high PAR on *Mastocarpus* and *Chondrus* carpospores of was still persistent, but better recovery was observed in *Chondrus* compared to *Mastocarpus* (Fig. 2c,d). Conversely, higher recovery was observed in carpospores of *Mastocarpus* exposed to UVR compared with those of *Chondrus*. Multiple analysis of variance (MANOVA) showed significant effect of the main factors (species, irradiance and exposure time) on carpospore photosynthesis after treatment and recovery ($p < 0.001$, Table 2). Moreover, 2-way interactions between species and irradiance as well as between species and exposure were also found to be significant in photosynthesis after treatment but only the interaction between species and irradiance was found to be significant ($p = 0.005$) after recovery. No 3-way interaction was found to be significant for carpospore photosynthesis. Duncan's multiple range test (DMRT, $p = 0.05$) also showed no homogenous subsets among the variables, such that the effect of irradiance (P, PA and PAB) and exposure time (1, 4 and 8 h) were significantly different from each other.

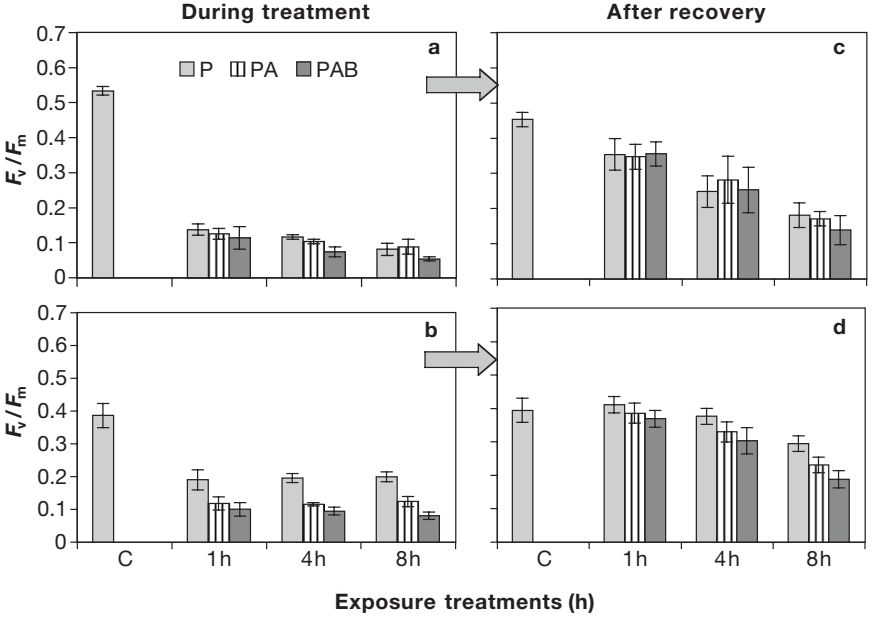

Fig. 2. (a,c) *Mastocarpus stellatus* and (b,d) *Chondrus crispus*. (a,b) Mean optimum quantum yield (F_v/F_m) of carpospores during treatment of photosynthetically active radiation, PAR = P; P+UVA = PA; PA+UVB = PAB and exposure time. PFD is 56 µmol photon m^{-2} s^{-1}. (c,d) Corresponding photosynthetic recovery after 48 h post culture in low white light (10 µmol photon m^{-2} s^{-1}). Control (= C) is without treatment and continuously maintained at 10 µmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD), n = 5

Spore mortality

Spore viability and germination capacity were also found to be low light adapted. Exposure of 1 to 8 h of high P (50 ± 5 µmol photons m^{-2} s^{-1}) was sufficient to induce significantly higher mortality in *Chondrus* (Fig. 3b) compared with *Mastocarpus* spores (Fig. 3a). After 16 h exposure to varying radiation level of P (50 to 130 µmol photons m^{-2} s^{-1}), mortality due to high PFD (photon flux density) of PAR doubled in *Mastocarpus* (Fig. 3c) and slowly increased in *Chondrus* (Fig. 3d). The spore mortality under UVR (PA, PAB) was higher in

Table 2. *Mastocarpus stellatus* and *Chondrus crispus*. Multiple analysis of variance (MANOVA) and significance values for main effects and interactions of species, irradiance, exposure time and radiation levels on the photosynthesis, spore viability, DNA damage and repair of carpospores of both algae species. *Significant; ns: not significant

Variable	Source of variation	df	*F*	p
Spore photosynthesis and viability experiment				
F_v/F_m (after treatment)	Species (A)	1	99.47	<0.001*
	Irradiance (B)	2	116.25	<0.001*
	Exposure time (C)	2	17.43	<0.001*
	A × B	2	42.53	<0.001*
	A × C	2	15.62	<0.001*
	B × C	4	1.47	0.219^{ns}
	A × B × C	4	0.71	0.585^{ns}
F_v/F_m (after recovery)	Species (A)	1	62.08	<0.001*
	Irradiance (B)	2	9.83	<0.001*
	Exposure time (C)	2	154.49	<0.001*
	A × B	2	5.81	0.005*
	A × C	2	2.61	0.081^{ns}
	B × C	4	1.57	0.191^{ns}
	A × B × C	4	0.41	0.804^{ns}
Spore mortality	Species (A)	1	692.56	<0.001*
	Irradiance (B)	2	38.67	<0.001*
	Exposure time (C)	2	45.50	<0.001*
	A × B	2	1.34	0.276^{ns}
	A × C	2	3.87	0.030*
	B × C	4	2.73	0.044*
	A × B × C	4	2.20	0.088^{ns}
Spore viability, DNA damage and repair experiment				
Spore mortality	Species (A)	1	53.39	<0.001*
	Irradiance (B)	2	101.06	<0.001*
	Radiation level (C)	3	98.75	<0.001*
	A × B	2	8.48	0.001*
	A × C	3	4.21	0.010*
	B × C	6	3.58	0.009*
	A × B × C	6	0.91	0.499^{ns}
DNA damage	Species (A)	1	10.72	0.005*
	UVB level (B)	3	45.83	<0.001*
	A × B	3	1.63	0.221^{ns}
DNA repair	Species (A)	1	51.11	<0.001*
	UVB level (B)	3	34.98	<0.001*
	A × B	3	4.81	0.014*

Chondrus compared with *Mastocarpus* across all treatments. In the first spore mortality experiment, the main factors and 2-way interactions (species × exposure time and irradiance × exposure time) were found to significantly affect spore mortality (Table 2). DMRT showed that the effect of irradiance (P vs PA vs PAB) differed significantly among treatments, while exposure times of 1 and 4 h were homogenous but significantly different from 8 h. In the second spore mortality experiment, the main effects and all 2-way interactions between species, irradiance and radiation level significantly affected carpospore mortality; no 3-way interaction was observed. Moreover, DMRT ($p = 0.05$) showed that P ≠ PA ≠ PAB and radiation level (light quantity) was also significantly different from each other.

Dose-response relationship (BED_{50}) for both *Mastocarpus* ($R^2 = 0.802$ [linear regression]; 0.776 [non linear regression]) and *Chondrus* ($R^2 = 0.780$ [linear regression]; 0.930 [non linear regression]) were highly significant ($p < 0.05$), indicating clear relationship between UV dose and inhibition of germination. A higher dose is needed to achieve a 50% inhibition of germination ($BED_{DNA\ 300\ nm}$ and $BED_{plant\ damage\ 313\ nm}$) in *Mastocarpus* (Fig. 4).

DNA damage and repair

After 16 h exposure to increasing radiation, higher DNA damage was observed in the carpospores of *Chondrus* compared with *Mastocarpus* (Fig. 5a). CPD induction in both species increased with radiation intensity. Analysis of variance (ANOVA, $p < 0.05$) showed significant differences between species and UV-B level on DNA damage (Table 3). DMRT showed that accumulation of DNA damage in UV-B level 1 = level 2 ≠ level 3 ≠ level 4. After 6 d post-culture in low white light, the amount of remaining CPDs was also significantly higher in *Chondrus* ($p < 0.001$) and in samples exposed to higher UV-BR ($p < 0.001$; Fig. 5b, Table 2). DMRT showed that DNA repair mechanism in UV-B level 1 ≠ level 2 ≠ level 3 = level 4.

The rooftop experiment simulating a low tide field condition showed no accumulation of DNA damage in exposed plants compared to non-exposed plants (data not shown).

Gametophyte photosynthesis, pigment contents and growth

Initial values of the mean effective yields ($\Delta F/F_m'$) of *Mastocarpus* and *Chondrus* gametophytes showed similar photosynthetic efficiencies with that of the carpospores, where higher $\Delta F/F_m'$ were observed in *Mastocarpus* (0.617 ± 0.03) compared with *Chondrus* (0.497 ± 0.07) (Fig. 6). Adverse reductions in the mean $\Delta F/F_m'$ of the cultured young *Mastocarpus* and *Chondrus* gametophytes were observed only during the first day of UV exposure. After 6 h of PA exposure, a comparable reduction of ~22 and 23% in the $\Delta F/F_m'$ was observed in *Mastocarpus* and *Chondrus* respectively. In the PAB treatment, a higher reduction in the $\Delta F/F_m'$ was observed in *Chondrus* (43%) than in *Mastocarpus* (30%). Two hours after the end of the first UV treatment, photosynthesis was able to recover to values comparable to pre-UV treatment. Time series measurements showed that both algae were able to acclimate to

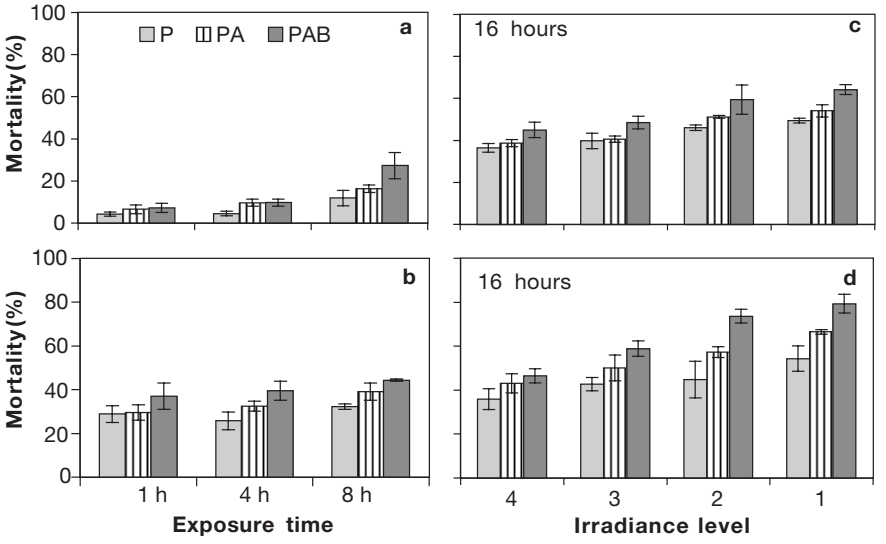

Fig. 3. (a,c) *Mastocarpus stellatus* and (b,d) *Chondrus crispus*. Spore mortality (a,b) under varying exposure time of P, PA and PAB and (c,d) under 16 h of varying irradiation levels of P, PA and PAB. Radiation level 1 = highest and 4 = lowest. PDF ranges from 50 to 130 µmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD), n = 3

UVR (Fig. 6). Statistical analysis showed that changes in the $\Delta F/F_m'$ were not significantly different between species (RMANOVA, $p > 0.05$; Table 3) across all sampling times. However, a significant effect of irradiance was observed after 6 h UVR treatment ($p < 0.001$) but complete recovery was observed after 2 h recovery in white light where $\Delta F/F_m'$ were not significantly different between treatments (Table 3).

In both the laboratory and rooftop experiments, higher chlorophyll *a* levels were measured in plants exposed to PA and PAB (Fig. 7a,b). However, the effect of irradiance was not significantly different between treatments (Table 3). Carotenoids were only determined in *Chondrus*, where different reactions to UVR were observed between young gametophytes and mature wild plants. UVR significantly reduced α-carotene

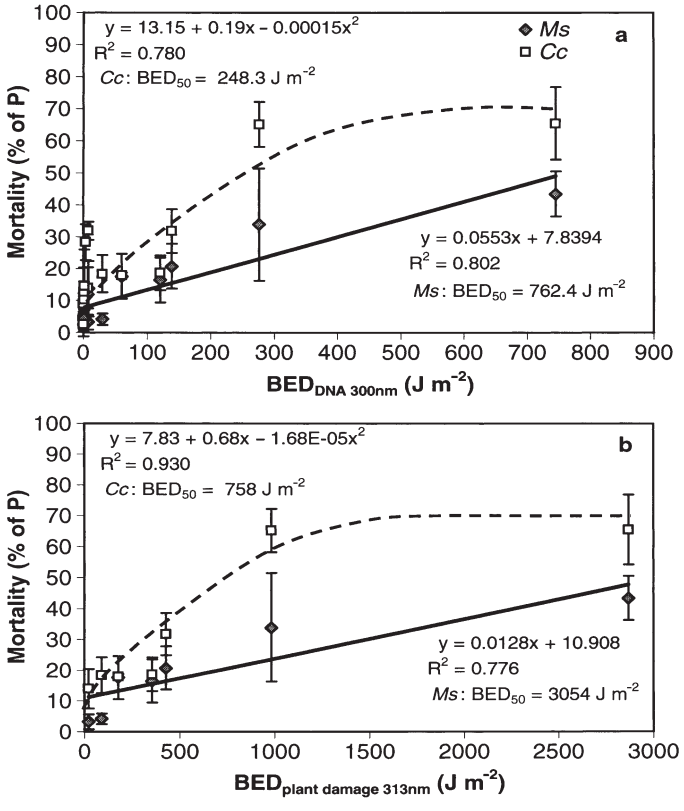

Fig. 4. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). Empirical relationship between biologically effective UVB doses and germination using action spectra for DNA damage $BED_{DNA\,300\,nm}$ (a) and generalized plant damage $BED_{plant\,damage\,313\,nm}$ (b) with corresponding BED_{50} derived from the regression equations. Vertical bars are standard deviations (SD), n = 3

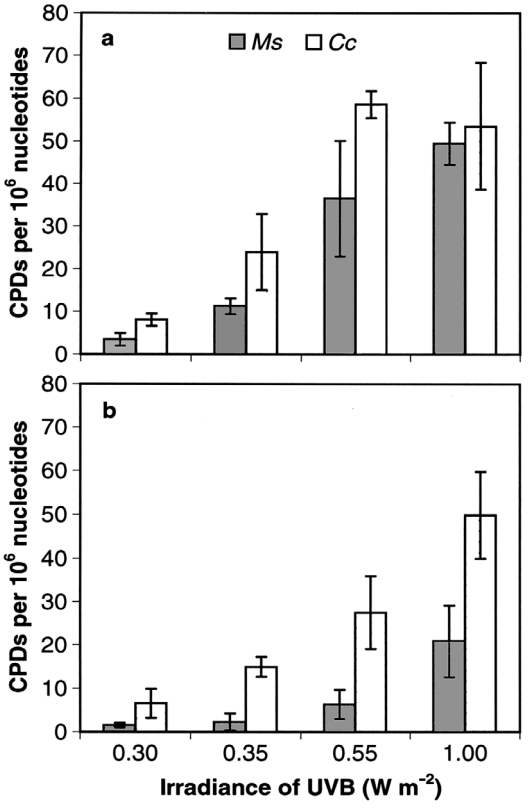

Fig. 5. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). UVB induced DNA damage (induced CPD concentrations per million nucleotides) after (a) exposure to varying levels of UVB-radiation and (b) corresponding CPD repair after 6 d recovery in low white light (10 µmol photon m^{-2} s^{-1}). Vertical bars are standard deviations (SD), n = 3

Table 3. *Mastocarpus stellatus* and *Chondrus crispus*. Repeated measures analysis of variance (RMANOVA) on the photosynthetic yields ($\Delta F/F_m'$) and analysis of variance (ANOVA) on pigments and growth of young gametophytes of both algae species. *Significant; ns: not significant

Dependent variable	Independent variable: Sampling time	Source of variation	df	*F*	p
Photosynthesis					
$\Delta F/F_m'$	08:00 (before UV)	Species (A)	1	1.72	0.201^{ns}
		Irradiance (B)	2	2.32	0.120^{ns}
		A × B	2	0.65	0.532^{ns}
	15:00 (after 6 h UV exposure)	Species (A)	1	0.83	0.372^{ns}
		Irradiance (B)	2	12.07	0.000*
		A × B	2	0.44	0.649^{ns}
	17:00 (after 2 h recovery in white light)	Species (A)	1	0.05	0.823^{ns}
		Irradiance (B)	2	2.57	0.098^{ns}
		A × B	2	0.31	0.736^{ns}
Pigments					
Chlorophyll *a*		Species (A)	1	8.03	0.015*
		Irradiance (B)	2	2.38	0.135^{ns}
		A × B	2	0.24	0.791^{ns}
C. crispus					
α-carotene		Irradiance	2	10.50	0.011*
β-carotene		Irradiance	2	7.12	0.026*
Growth					
Area		Species (A)	1	110.82	<0.001*
		Irradiance (B)	2	0.32	0.730^{ns}
		A × B	2	2.13	0.141^{ns}
Weight		Species (A)	1	95.91	<0.001*
		Irradiance (B)	2	0.28	0.758^{ns}
		A × B	2	1.95	0.164^{ns}

but enhanced β-carotene content in young gametophytes (Fig. 7c,d, Table 3). In mature thalli, α-carotene was not affected while β-carotene reacted similarly to UVR, but the effect of irradiance was insignificant. Overnight recovery of plants exposed to the full solar spectrum showed no significant change in all of the pigments examined.

Increases in surface area and wet weight as growth parameters were positively correlated in *Mastocarpus* ($r = 0.937$, $p < 0.001$) and *Chondrus* ($r = 0.980$, $p < 0.001$) (Fig. 8). Regardless of treatment, the rate of increase in surface area was faster in *Chondrus* (slope, $r = 0.077$) than in *Mastocarpus* ($r = 0.044$) (Fig. 9). Consequently, growth rate under all treatment was higher in *Chondrus* (7.0 to 8.4 % d^{-1}) than in *Mastocarpus* (4.4 to 5.2 % d^{-1}) (Fig. 9, inset a). Growth rates of UVR treated plants expressed as a percentage of PAR (Fig. 9, inset b) showed that UVR had a relatively low impact on the growth rate of both species. Under PA, growth rate was similar between *Mastocarpus* and *Chondrus*, but higher growth rate was observed in *Mastocarpus* under PAB treatment. However, statistical analysis showed insignificant effect of irradiance on the growth rate of both species (Table 3).

DISCUSSION

Carpospores of *Mastocarpus* and *Chondrus* are more susceptible to the detrimental effects of UVR than were the conspecific young gametophytes. Optimum quantum yields (F_v/F_m) of carpospores were able to recover after pulse UV exposure while effective quantum yields ($\Delta F/F_m'$) of young gametophytes were able to acclimate to chronic UV exposure. The carpospores used in our experiments were low light adapted since sporulation occurred in a low light environment and were maintained under this condition to prevent germination. In a pilot experiment, we observed a maximum germination of 95 ± 5 % under 10 µmol photons m^{-2} s^{-1} of PAR (data not shown). For *Chondrus* spores, a wide range of optimum photon flux densities (PFD) of PAR (20 to 80 µmol photons m^{-2} s^{-1} = 4.3 to 17.2 W m^{-2}) has been reported (Tasende & Fraga 1992 and references therein). However, we found that exposure of 1 to 16 h of 12 W m^{-2} PAR (50 ± 5 µmol photons m^{-2} s^{-1}) induced ~30 % mortality in *Chondrus* and photosynthesis was also reduced under these conditions in both species. Therefore, it appears that low PFD of PAR is optimal for spore germination. Our data also indicate a large impact of the PAR waveband on carpospore survival, because in the field, the UVB radiation applied in our experiments would be accompanied by a 10- to 20-fold higher PAR. Whether carpospores are capable of acclimating to higher PAR is still unknown. Physiological acclimation to high PAR can increase UVB tolerance. In higher plants, this was facilitated through photoinduction of screening compounds, increased activity of photorepair enzymes and other changes related to life under high irradiance (Warner & Caldwell 1983).

Carpospores of *Mastocarpus* were found to be more tolerant of the deleterious effect of UVR compared with *Chondrus* carpospores. Apart from the BED_{50}, differences in tolerance were also reflected in UVB induced photoinhibition and DNA damage. F_v/F_m of *Mastocarpus* spores were relatively less affected under UVR, and UV-B induced DNA damage was also lower compared with *Chondrus*. Moreover, DNA damage was also repaired faster by *Mastocarpus*. However, these differences were not observed in the growth, $\Delta F/F_m'$ and DNA damage of the gametophytic life stages of these algae. Therefore, differences in the sensitivity between *Mastocarpus* and *Chondrus* carpospores to high light and UVB radiation elucidate the

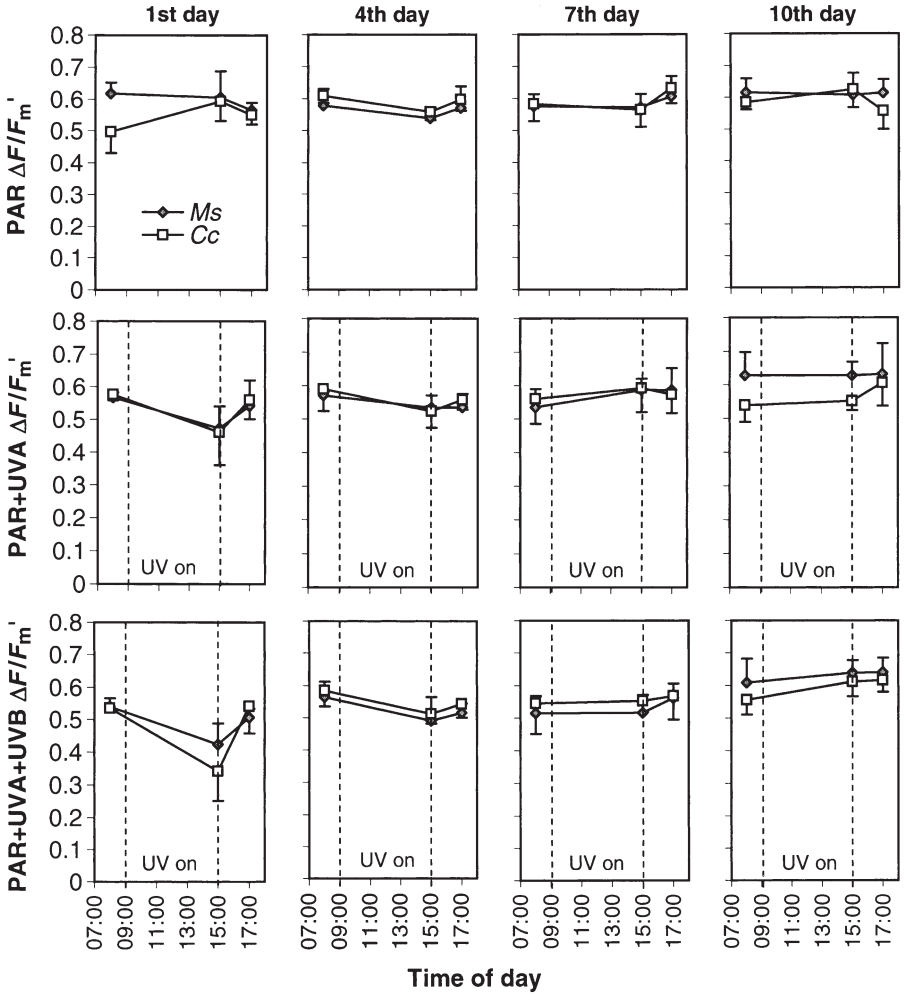

Fig. 6. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). Time series of the circadian pattern of the mean effective quantum yield ($\Delta F/F_m'$) of young gametophytes during the light phase of the 16:8 h light:dark photoperiod. Vertical bars are standard deviations (SD), n = 5

differences in their zonation. A lower sensitivity to high light and UVR would allow *Mastocarpus* to recruit at higher shore levels. Conversely, the higher sensitivity of *Chondrus* would limit recruitment at lower shore levels or under the canopy of *Fucus* spp. and *Mastocarpus*. Survival of the carpospores and recruitment of young juveniles is critical in structuring the community because once established, growth rates of young gametophytes are higher in *Chondrus* compared with *Mastocarpus*, regardless of irradiance condition. Our data concur with a previous *in situ* growth study, where 2-fold productivity was also reported in *Chondrus* (2.65 g dry wt m^{-2} d^{-1}) compared to *Mastocarpus* (1.04 g dry wt m^{-2} d^{-1}) (Dudgeon et al. 1995).

Bischof et al. (2000) reported a 6-fold higher concentration of MAAs in *Mastocarpus* when compared with *Chondrus*, and related this to the higher UV photosynthetic tolerance of *Mastocarpus*. Although MAAs were not determined in this study, we observed that photosynthesis and growth of gametophytes of both species were equally resistant to a daily repeated UV treatment. Whether carpospores of these species produce sufficient MAAs to achieve the same degree of protection to that of the gametophytes remains to be studied. The presence of UV screening compounds such as MAAs reduces the effective UVR that penetrates to UV sensitive targets in the cell. Therefore, the absence of passive protection increases the sensitivity of carpospores to UVB induced photoinhibition and DNA damage.

The higher photosynthetic rate of *Mastocarpus* compared with *Chondrus* (F_v/F_m of carpospores and $\Delta F/F_m'$ of young gametophytes) under control conditions, is attributed to *Mastocarpus*' efficient use of the available photon flux density. The initial values of the mean quantum efficiency of cultured young *Mastocarpus* and *Chondrus* gametophyte isolated from Helgoland are comparable to those reported by Bischof et al. (2000) on mature wild materials collected from the same locality. However, the complete acclimation of photosynthesis in both species after 3 d of repeated UVR exposure contradicts Bischof et al. (2000), as they reported a reduction in the mean $\Delta F/F_m'$ (with a lesser degree of photoinhibition in *Mastocarpus*) throughout the 5 d repeated exposure to PAB. This could be attributed to the higher experimental UV irradiance used in their study, which was twice the intensity we applied, or to the low PAR (25 µmol photons m^{-2} s^{-1} ≈ 5.4 W m^{-2}) they applied. Insufficient PAR and therefore unrealistically low PAR:UVB ratios, could exaggerate the UVB effects on plants (Caldwell et al. 1995, Rozema et al. 1997).

Chlorophyll *a* levels in both *Mastocarpus* and *Chondrus* were not affected by UVR but increased when supplemented with UV-A and UV-A+UV-B. Most studies reported significant damage and decrease in pigment contents under UVR (i.e. Aguilera et al. 2002, Bischof et al. 2002a,b), which can occur either (1) when protein-based pigments absorb UV energy directly and undergo photochemical degradation, (2) by photosensi-

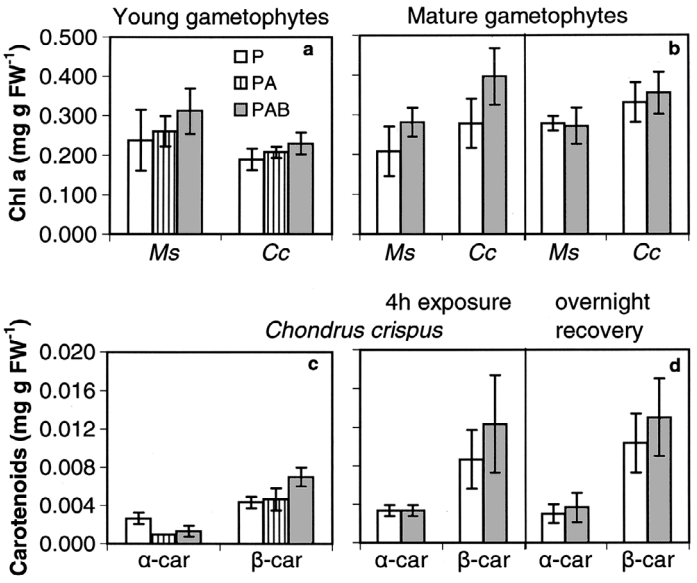

Fig. 7. *Mastocarpus stellatus* and *Chondrus crispus*. Chlorophyll *a* contents in (a) young and (b) mature gametophytes and corresponding carotenoids of *C. crispus* (c,d) exposed to P, PA, PAB. *Ms* = *M. stellatus*, *Cc* = *C. crispus*. Vertical bars are standard deviations (SD), n = 3

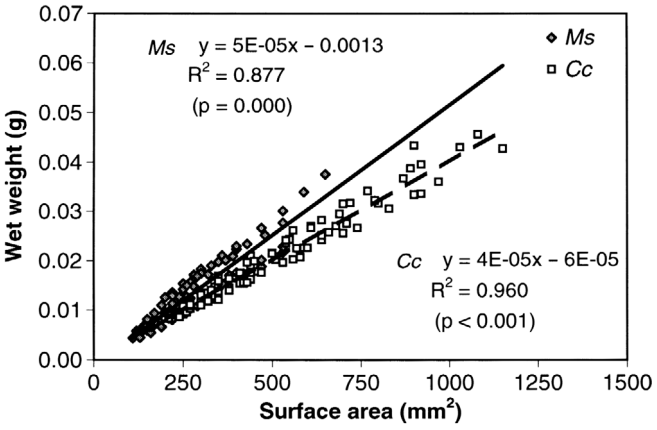

Fig. 8. *Mastocarpus stellatus* and *Chondrus crispus*. Relationship between surface area and wet weight in both algae species with corresponding regression equation. *Ms* = *M. stellatus*, *Cc* = *C. crispus*

tiser action, or (3) by oxygen radical production in addition to singlet oxygen (Vincent & Neale 2000). Although the higher chlorophyll *a* levels under PAB was statistically insignificant, the result of our study is similar to 2 other studies on *Ulva* sp., where significantly higher pigment contents were measured in the presence of UV-B (Grobe & Murphy 1998, Altamirano et al. 2000). The higher total carotenoids measured under PAB could be related to their photoprotection role (Roy 2000). Accumulation of carotenoids specifically in response to UV radiation has been previously reported in cyanobacteria and chlorophytes (Buckley & Houghton 1976, Goes et al. 1994). This mechanism could have protected the photosynthetic apparatus of *Chondrus*, which was able to acclimate its photosynthesis and growth to UVR. In *Mastocarpus*, aside from the reported higher MAA content, the thicker thalli (higher wet weight : surface area ratio) could have provided some protective mechanism against environmental stress contributing to its successful colonization in the upper eulittoral.

Exposure of mature gametophytes to natural solar radiation directly below the water surface for 4 h at noon showed no significant CPD accumulation in either *Mastocarpus* or *Chondrus*. This shows that prevention and repair mechanisms are sufficient to minimize accumulation of DNA damage. This is not surprising because both species co-exist in the eulittoral, and therefore have to be physiologically or genetically acclimated to the full spectrum of solar radiation. *Chondrus* isolates from Brittany, repeatedly exposed under comparable irradiance treatment in the laboratory for 2 wk, were also not significantly affected by UV-BR, and the number of

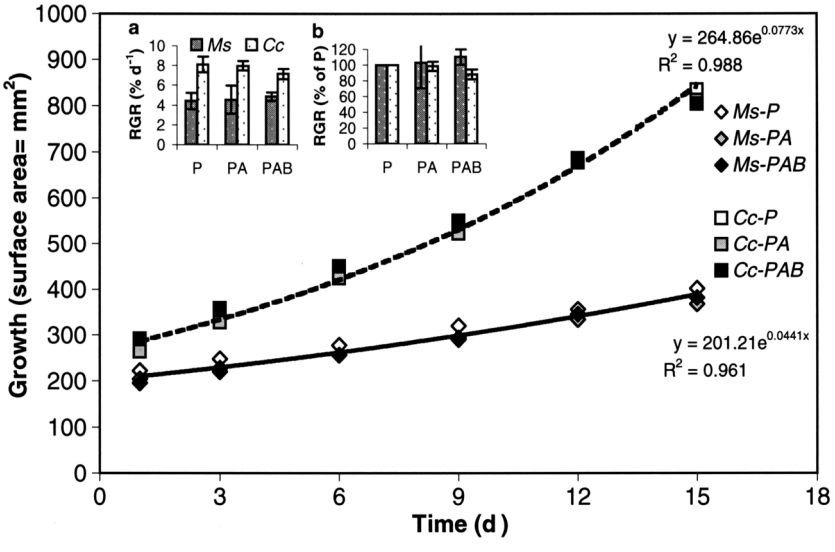

Fig. 9. *Mastocarpus stellatus* and *Chondrus crispus*. Growth, in terms of surface area increase in young gametophytes exposed to different radiation. Inlaid exponential equation is the overall growth performance of each species. Insets show relative growth rate (RGR) in % d^{-1}, per treatment (a) and growth expressed as % of PAR (b). Vertical bars are standard deviations (SD), n = 5. *Ms* = *M. stellatus*, *Cc* = *C. crispus*

CPDs per 10^6 nucleotides was found to be negligible (van de Poll et al. 2001). However, a similar outdoor experiment with several subtidal Arctic macroalgae demonstrated significant CPD accumulation after 4 h exposure to full solar radiation (van de Poll et al. 2002b).

Survival of spores is regarded as a critical life stage because once established, the vegetative plants appear not to suffer from UV-B induced DNA damage. Among kelp zoospores, the effect of UVR on spore viability was found to be correlated to the growth depth of the species (Wiencke et al. 2000) and within species, those released from adults growing in high UV environments showed higher germination and survival rates than the progeny of adults occupying lower UV environments (Swanson & Druehl 2000). In the eulittoral *Enteromorpha intestinalis*, inhibition of germination success (up to 50%) was only observed in swarmers (both spores and gametes) when exposed to elevated levels of UV-B (Cordi et al. 2001). On the other hand, in the littoral red alga *Ceramium rubrum*, 50% spore mortality was already observed after 1 h exposure to light with elevated UVR (2.2 W m^{-2} UV-B and 11 W m^{-2} UVA, Yakovleva et al. 1998). Although these experiments are not directly comparable, they suggest a high degree of variability among UV sensitivity of macroalgal spores.

There are other factors that could affect recruitment and eventual community structure. However, herbivory and wave-induced mortality were reported to have a low impact on both *Mastocarpus* and *Chondrus*. In transplant experiments to determine competitive interactions between the 2 species, coexistence was observed in the space limited intertidal flat mediated by seasonal environmental disturbances (winter storm) and physiological stress (Dudgeon et al. 1999). Aside from UV tolerance and efficient DNA repair mechanism, the apomictic reproduction (direct-type life history) of Helgolandian *Mastocarpus* may be ecologically advantageous to its successful dispersal and colonization of open spaces in the upper eulittoral. This is because the direct development female requires only 1 open space and a single establishment event; whereas a sexual female must first pass through the crustose, tetrasporophytic generation, requiring 2 open spaces and 2 establishment events (Zupan & West 1988). Moreover, spores are produced year-round, not seasonally as in sexual populations (Maggs 1988). In the field, the occurrence of foliose *Mastocarpus* on the walls and boulders around Helgoland which are always exposed during low tides (pers. obs.) could have been influenced by the hydrographic conditions (i.e. tide level and surf) during spore release (Norton 1992). Consequently, attachment and germination under favorable 'recruitment windows' (Deysher & Dean 1986, Reed et al. 1988) could have been facilitated by the presence of surface mucilage observed in both species. This blanket-like extracellular mucilaginous sheath plays a role in the ontogeny of the sporelings by influencing algal spore attachment for colonization (Fletcher & Callow 1992, Vadas et al. 1992).

In conclusion, high PAR and UV exposure could determine recruitment success. However, it remains to be determined whether there would be significant effects from exposure to UV at the magnitude and spectral composition characteristic of *in situ* environments in which PAR is already high enough to cause considerable mortality. Although mature fronds of *Mastocarpus* have been reported to be more tolerant to environmental stress (i.e. temperature, desiccation and freezing) compared with *Chondrus* (Dudgeon et al. 1989, 1990, 1995, Collén & Davison 1999), we would suggest that the foliose life stages of both species are equally resilient to UV-B stress and could persist in the rocky intertidal shore of Helgoland.

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LITERATURE CITED

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087–1095

Altamirano M, Flores-Moya A, Figueroa FL (2000) Growth, seasonality, photosynthetic pigments, and C and N content in relation to environmental factors: a field study on *Ulva olivascens* (Ulvales, Chlorophyta). Phycologia 39: 50–58.

Altamirano M, Flores-Moya A, Figueroa FL (2003a) Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophycea). Aquat Bot 75:9–20

Altamirano M, Flores-Moya A, Kuhlenkamp R, Figueroa FL (2003b) Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. Zygote 11:101–106

Bañares E, Altamirano M, Figueroa FL, Flores-Moya A (2002) Influence of UV radiation on growth of sporelings of three non-geniculate coralline red algae from southern Iberian Peninsula. Phycol Res 50:23–30

Bartsch I, Kuhlenkamp R (2000) The marine macroalgae of Helgoland (North Sea): an annotated list of records between 1845 and 1999. Helgol Mar Res 54:160–189

Bhattacharya D (1985) The demography of fronds of *Chondrus crispus* Stackhouse. J Exp Mar Biol Ecol 91:217–231

Bischof K, Hanelt D, Wiencke C (1998) UV-radiation can affect depth-zonation of Antarctic macroalgae. Mar Biol 131:597–605

Bischof K, Kräbs G, Hanelt D, Wiencke C (2000) Photosynthetic characteristics and mycosporine-like amino acids

under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? Helgol Mar Res 54:47–52

Bischof K, Peralta G, Kräbs G, van de Poll WH, Pérez-Lloréns JC, Breeman AM (2002a) Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. J Exp Bot 53:2411–2421

Bischof K, Kräbs G, Wiencke C, Hanelt D (2002b) Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 215:502–509

Buckley CE, Houghton JA (1976) A study of the effects of near UV radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. Arch Microbiol 107:93–97

Buma AGJ, van Hannen EJ, Roza L, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. J Phycol 31:314–321

Buma AGJ, van Oijen T, van de Poll WH, Veldhuis MJW, Gieskes WWC (2000) The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. J Phycol 36: 296–303

Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology. Academic Press, New York, p 131–177

Caldwell MM, Teramura AH, Tevini M, Bornman JF, Björn LO, Kulandaivelu G (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio 24:166–173

Chen LCM, McLachlan J (1972) The life history of *Chondrus crispus* in culture. Can J Bot 50:1055–1060

Chen LCM, Edelstein T, McLachlan J (1974) The life history of *Gigartina stellata* (Stackh.) Batt. (Rhodophyceae, Gigartinales) in culture. Phycologia 13:287–294

Chopin T (1986) The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans: a review. Can Tech Rep Fish Aquat Sci 1514:1–69

Collén J, Davison IR (1999) Stress tolerance and reactive oxygen metabolism in the intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. Plant Cell Environ 22: 1143–1151

Cordi B, Donkin ME, Peloquin J, Price DN, Depledge MH (2001) The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis*. Aquat Toxicol 56:1–11

Deysher LE, Dean TA (1986) In situ recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L.) C.A. Agardh: effects of physical factors. J Exp Mar Biol Ecol 103:41–63

Dring MJ, Wagner A, Boeskov J, Lüning K (1996a) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. Eur J Phycol 31:293–302

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996b) Influence of ultraviolet radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126: 183–191

Dudgeon SR, Johnson AS (1992) Thick vs thin: thallus morphology and tissue mechanics influence differential drag and dislodgment of two co-dominant seaweeds. J Exp Mar Biol Ecol 165:23–43

Dudgeon SR, Davison IR, Vadas RL (1989) Effect of freezing on photosynthesis of intertidal macroalgae: relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus* (Rhodophyta). Mar Biol 101:107–114

Dudgeon SR, Davison IR, Vadas RL (1990) Freezing tolerance in the intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus*: relative importance of acclimation and adaptation. Mar Biol 106:427–436

Dudgeon SR, Kübler JE, Vadas RL, Davison IR (1995) Physiological responses to environmental variation in intertidal red algae: does thallus morphology matter? Mar Ecol Prog Ser 117:193–206

Dudgeon SR, Steneck RS, Davison IR, Vadas RL (1999) Coexistence of similar species in a space-limited intertidal zone. Ecol Monogr 69:331–352

Fletcher RL, Callow ME (1992) The settlement, attachment and establishment of marine algal spores. Br Phycol J 27: 303–329

Flores-Moya A, Posudin YI, Fernández JA, Figueroa FL, Kawai H (2002) Photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose. J Photochem Photobiol B Biol 66:134–140

Garbary DJ, DeWreede RE (1988) Life history phases in natural populations of Gigartinaceae (Rhodophyta): quantification using resorcinol. In: Lobban DS, Chapman DJ, Kremer BP (eds) Experimental Phycology. A laboratory manual. Cambridge University Press, Cambridge, p 174–178

Goes JI, Handa N, Taguchi S, Hama T (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter *Tetraselmis* sp.: relationship to cellular pigments. Mar Ecol Prog Ser 114:259–274.

Grobe CW, Murphy TM (1998) Solar ultraviolet-B radiation effects on growth and pigment composition of the intertidal alga *Ulva expansa* (Setch.) S. & G. (Chlorophyta). J Exp Mar Biol Ecol 225:39–51

Guiry MD, West JA (1983) Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. J Phycol 19:474–494

Guiry MD, West JA, Kim DH, Masuda M (1984) Reinstatement of the genus *Mastocarpus* Kützing (Rhodophyta). Taxon 33:53–63

Han T, Han YS, Kim KY, Kim JH, Shin HW, Kain JM, Callow JA, Callow ME (2003) Influences of light and UV-B on growth and sporulation of the green alga *Ulva pertusa* Kjellman. J Exp Mar Biol Ecol 290:115–131

Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361–369

Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255–266

Huovinen PS, Oikari AOJ, Soimasuo MR, Cherr GN (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. Photochem Photobiol 72:308–313

Kornmann P, Sahling PH (1977) Meeresalgen von Helgoland. Helgol Wiss Meeresunters 29:1–29

Kornmann P, Sahling PH (1994) Meeresalgen von Helgoland: zweite Ergänzung. Helgol Meeresunters 48:365–406

Lazo ML, Greenwell M, McLachlan J (1989) Population structure of *Chondrus crispus* Stackhouse (Gigartinaceae, Rhodophyta) along the coast of Prince Edward Island, Canada: distribution of gametophytic and sporophytic fronds. J Exp Mar Biol Ecol 126:45–58

Lüning K (1990) Seaweeds: their environment, biogeography, and ecophysiology. Wiley, New York

Maggs CA (1988) Intraspecific life history variability in the Florideophycidae (Rhodophyta). Bot Mar 31:465–490

Makarov MV, Voskoboinikov GM (2001) The influence of

Ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. Bot Mar 44:89–94
Norton TA (1992) Dispersal by macroalgae. Br Phycol J 27: 293–301
Pakker H, Beekman CAC, Breeman AM (2000a) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). Eur J Phycol 35:109–114
Pakker H, Martins RST, Boelen P, Buma AGJ, Nikaido O, Breeman AM (2000b) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). J Phycol 36:334–341
Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: Watanabe A, Hattori A (eds) Cultures and collection of algae. Proceedings of U.S.-Japan Conference. Japanese Society of Plant Physiologists, Hakone, p 63–75
Reed DC, Laur DR, Ebeling AW (1988) Variation in algal dispersal and recruitment: the importance of episodic events. Ecol Monogr 58:321–335
Roy S (2000) Strategies for the minimisation of UV-induced damage. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, p 177–205
Rozema J, van de Staaij J, Björn LO, Caldwell M (1997) UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol Evol 12:22–28
Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Natl Acad Sci USA 71:3363–3366
Smith RC, Prézelin BB, Baker KS, Bidigare RR and 9 others (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952–959
Swanson AK, Druehl LD (2000) Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. Mar Biol 136:657–664
Tasende MA, Fraga MI (1992) Efecto de las condiciones de cultivo en la germinación de esporas de *Chondrus crispus* Stackh, (Gigartinales, Rhodophyta). Cah Biol Mar 33: 407–415
Vadas RL Sr, Johnson S, Norton T (1992) Recruitment and mortality of early post-settlement stages of benthic algae. Br Phycol J 27:331–351
van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat related differences in UV-B tolerance. J Phycol 37: 30–37
van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2002a) Temperature dependence of UV radiation effects in Arctic and temperate isolates of 3 red macrophytes. Eur J Phycol 37:59–68
van de Poll WH, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002b) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. Photochem Photobiol 76:493–501
Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In: de Mora S Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, p 149–176
Vink AA, Bergen-Henegouwen JB, Nikaido O, Baan RP, Roza L (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. Photochem Photobiol 24: 25–31
Warner CW, Caldwell MM (1983) Influence of photon flux density in the 400–700 nm waveband on inhibition of photosynthesis by UV-B (280–320 nm) irradiation in soybean leaves: separation of indirect and direct effects. Photochem Photobiol 38:341–346
Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa FL (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implication for depth zonation. Mar Ecol Prog Ser 197:217–229
Yakovleva IM, Dring M, Titlyanov EA (1998) Tolerance of North Sea algae to UV and visible radiation. Russ J Plant Physiol 45:45–54
Zupan JR, West JA (1988) Geographic variation in the life history of *Mastocarpus papillatus* (Rhodophyta). J Phycol 24: 223–229

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