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ORIGINAL ARTICLE

UV effects on photosynthesis and DNA in propagules of three Antarctic seaweeds (*Adenocystis utricularis*, *Monostroma hariotii* and *Porphyra endiviifolium*)

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Abstract Ozone depletion is highest during spring and summer in Antarctica, coinciding with the seasonal reproduction of most macroalgae. Propagules are the life-stage of an alga most susceptible to environmental perturbations therefore, reproductive cells of three intertidal macroalgal species *Adenocystis utricularis* (Bory) Skottsberg, *Monostroma hariotii* Gain, and *Porphyra endiviifolium* (A and E Gepp) Chamberlain were exposed to photosynthetically active radiation (PAR), PAR + UV-A and PAR + UV-A + UV-B radiation in the laboratory. During 1, 2, 4, and 8 h of exposure and after 48 h of recovery, photosynthetic efficiency, and DNA damage were determined. Saturation irradiance of freshly released propagules varied between 33 and 83 μmol photons m^{-2} s^{-1} with lowest values in *P. endiviifolium* and highest values in *M. hariotii*. Exposure to 22 μmol photons m^{-2} s^{-1} PAR significantly reduced photosynthetic efficiency in *P. endiviifolium* and *M. hariotii*, but not in *A. utricularis*. UV radiation (UVR) further decreased the photosynthetic efficiency in all species but all propagules recovered completely after 48 h. DNA damage was minimal or not existing. Repeated exposure of *A. utricularis* spores to 4 h of UVR daily did not show any acclimation of photosynthesis to UVR but fully recovered after 20 h. UVR effects on photosynthesis are shown to be species-specific. Among the tested species, *A. utricularis* propagules were the most light adapted. Propagules obviously possess good repair and protective mechanisms. Our study indicates that the applied UV dose has no long-lasting negative effects on the propagules, a precondition for the ecological success of macroalgal species in the intertidal.

Keywords Antarctica · DNA damage · Photosynthetic efficiency · *P–I* curve · Propagules · UV radiation

Abbreviations

PAR	Photosynthetically active radiation
UV-A	Ultraviolet-A
UV-B	Ultraviolet-B
UVR	UV radiation
P	PAR
PA	PAR + UV-A
PAB	PAR + UV-A + UV-B
CPDs	Cyclobutane pyrimidine dimers
F_v/F_m	Optimum quantum yield
I_k	Saturation irradiance
PFD	Photon flux density
P–I curves	Photosynthesis irradiance curves
rETR	Relative electron transport rate

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Introduction

Seaweeds are the most important primary producers in coastal waters contributing 3.2% to the global aquatic primary production (Mann 1973). In contrast to pelagic primary producers, macroalgae have complex life cycles including unicellular reproductive cells and microbenthic stages, apart from the macrobenthic thallus. Especially the early developmental stages are highly susceptible to a variety of stresses (Coelho et al. 2000). Therefore, the survival of the early phases of marine macroalgae is critical to the successful establishment of benthic populations (Vadas Sr et al. 1992).

Propagules can be exposed to high photosynthetically active radiation (PAR = 400–700 nm) and ultraviolet radiation (UVR = 280–400 nm) after release during their planktonic phase. For example kelp spores can be transported at least several kilometers in the water column and thereby be especially exposed to UVR because no protective shading by canopy algae occurs (Reed et al. 1988). The negative effects to UV exposure on cellular level include, e.g., photoinhibition and/or photodamage (Hanelt et al. 1997), protein breakdown (Lao and Glazer 1996), the production of reactive oxygen species (Rijstenbil et al. 2000) as well as damage to the DNA (van de Poll et al. 2001, 2002) and other biomolecules through the direct absorption of UVR (Vass 1997). These impacts can result in low growth rates (Roleda et al. 2006b). Moreover diversity and species richness of algal communities can be negatively affected due to UVR (K. Zacher unpublished data; Dobretsov et al. 2005).

UVR effects on macroalgae are species-specific. Different acclimation and repair mechanisms exist in species most tolerant to UV stress coming from shallow waters (Larkum and Wood 1993). Photosynthesis is a dynamic process and excessively absorbed energy, which is not utilized in photochemistry, can be converted into harmless thermal radiation until a certain point (Hanelt 1996). Maximum quantum yield of photosynthesis of, e.g., the intertidal brown alga *Alaria esculenta* can acclimate to enhanced levels of UVR within a few days (Bischof et al. 1999). Recovery after photodamage of the D1 protein of photosystem II is reflected by the new synthesis of this protein (Bischof et al. 1998). Other strategies can be avoidance of UVR or the production of screening compounds (reviewed in Franklin and Forster 1997; Bischof et al. 2006). Furthermore, DNA damage can be repaired enzymatically by light-dependent photolyases and light-independent nucleotide excision (van de Poll et al. 2002).

Although the unicellular propagules are clearly the stages most susceptible to UVR (Wiencke et al. 2006b) most UV studies have been carried out on the adult macrothalli. Some studies exist on the UV impact on spores of Arctic and temperate Laminariales and Gigartinales, showing that their sensitivity is related to their depth distribution and, hence influencing recruitment of the species in the eulittoral zone (Roleda et al. 2004b; Wiencke et al. 2006b). Antarctic intertidal algae are particularly suffering from elevated UV-B radiation (280–315 nm) during the last two decades due to stratospheric ozone depletion (>50% over this area; WMO 2003). UV-B and UV-A radiation (315–400 nm) can reach intensities of more than two and 40 W m^{-2} in spring in the studied area (King George Island, Antarctica), respectively. Furthermore, 1% of the surface irradiance of UV-B radiation can still be measured in a depth of about 15 m at clear water conditions. However, UV experiments with Antarctic macroalgae are scarce. To our knowledge these experiments are the first testing the UV sensitivity of reproductive cells from Antarctic field material.

In laboratory experiments propagules of three intertidal Antarctic macroalgal species were exposed to different light treatments to measure photosynthetic performance and DNA damage. The ability of these early developmental stages to recover from UV induced damage was also studied. The study gives valuable insights in the ecological success of Antarctic intertidal algae growing under a highly variable light regime including high UV values during spring and summer. It is hypothesized that propagules from Antarctic intertidal macroalgae can better cope with high UV levels in comparison with algae from Arctic or temperate regions.

Materials and methods

Algal material

Fertile specimen of the brown alga *Adenocystis utricularis* (Bory) Skottsberg, the green alga *Monostroma hariotii* Gain and the red alga *Porphyra endiviifolium* (A and E Gepp) Chamberlain were collected between January and March 2005 at Peñon Uno (Dallmann Laboratory, King George Island, South Shetland Islands, 62°14.80′S, 58°41.26′W). *A. utricularis* and *M. hariotii* were collected from the eulittoral were they occur together, whereas *P. endiviifolium* grows on rocks in the upper eulittoral. After collection the specimen were brought immediately to the nearby laboratory and put into filtered seawater (2°C under low light conditions) until further processing.

Spore release

Numerous individuals of each species were cleaned with tissue paper, divided randomly in five replicates and prepared for spore release in a temperature controlled room (2 ± 1.5°C). *P. endiviifolium* was put into Petri dishes with seawater for collection of monospores from the asexual thallus. Individuals of *A. utricularis* and *M. hariotii* were put in a wet chamber and left overnight under dim light. Propagules release was obtained by flooding the algae with filtered seawater in Petri dishes. Spore suspension was adjusted for *A. utricularis* spores (zoospore length around 4 μm) to ~7.1×10^4, for *M. hariotii* gametes (length around 7 μm) to ~1.57×10^4 spores ml^{-1} and for *P. endiviifolium* monospores (mean diameter 15 μm ± 2. 4 SD, $n = 32$) to ~1.12×10^4 spores ml^{-1} after counting (Sedgewick-Rafter Cell S50 spore counter, Graticules Ltd., Tonbridge, UK) to obtain the desired background fluorescence for photosynthetic measurements.

Experimental treatments

Light was provided by white fluorescent lamps (Osram GmbH, L65 Watt/25S, Munich, Germany), emitting background PAR of 400–700 nm and UV lamps (Q-Panel UV-A-340, 40 W, Cleveland, USA), emitting a spectrum qualitatively similar to solar radiation in the range of 295–340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent lamps: (1) Ultraphan transparent (Digefra GmbH, Munich, Germany), (2) Folanorm 320 (Folex GmbH, Cologne, Germany), and (3) Ultraphan URUV farblos (Digefra), corresponding to the PAR + UV-A + UV-B (PAB, 280–700 nm), PAR + UV-A (PA, 320–700 nm) and PAR (P, 400–700 nm) treatments, respectively. The available filters cut off wavelengths were slightly differing from the definition of CIE (Commission Internationale De l'Éclairage, UV-B = 280–315 nm, UV-A = 315–400nm).

Irradiance measurements

Irradiation in the laboratory was measured below the cut-off filters using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, PA, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar light). As the spectral range of the UV-A sensor extends into the UV-B region of the spectrum, UV-A radiation measurements were always made using a Schott WG320 filter (Schott, Mainz, Germany) to exclude wavelengths below 320 nm. The UV-B measurements recorded were obtained by subtracting the reading with the WG320 filter from the reading without the filter. PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1400 datalogger (LI-COR Bioscience, Lincoln, NE, USA). Irradiance under the different treatments is shown in Table 1. Furthermore, ambient UV-A and UV-B radiation in the air was measured permanently with a 32-channel single-photon counting spectroradiometer (Isitec, Bremerhaven, Germany; Hanken and Tüg 2002) at the Dallmann Laboratory.

Spore photosynthesis

Photosynthetic efficiency of reproductive cells measured as variable fluorescence of photosystem II (PSII), was determined using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of cell density (not exceeding 1 h after spore release), spore suspension was filled into 5 ml Quartz cuvettes. Optimum quantum yield (F_v/F_m) was measured after 3 min dark adaptation to determine initial photosynthetic efficiency at time zero ($n = 5$) as described by Roleda (2006a), designated as control. After that, the controls were maintained under dim white light (4 μmol photons m^{-2} s^{-1}) for 2 days before the final measurement. Photosynthesis (in terms of relative electron transport rate, rETR = PFR × $\Delta F/F_{m'}$) versus irradiance curves (*P–I* curves) were also measured in the time zero control ($n = 3$, chosen at random from the five replicates) as described by Bischof et al. (1998). The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate *P–I* curve parameters described as: $rETR = rETR_{max} \times \tanh(\alpha \times I_{PAR} \times rETR_{max}^{-1})$, where $rETR_{max}$ is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the initial slope in the light limited part of the *P–I* curve (as a measure for the electron transport efficiency) and *I* is the photon fluence rate of PAR. The saturation irradiance for

Table 1 Irradiance under the different experimental treatments

Treatments	PAR ($W m^{-2}$)	UV-A ($W m^{-2}$)	UV-B ($W m^{-2}$)
PAB (PAR + UV-A + UV-B)	4.73	4.34	0.35
PA (PAR + UV-A)	4.73	4.05	0.07
P (PAR)	4.73	0.06	0.00

Under the recovery shelf PAR irradiance was 0.86 $W m^{-2}$ (4 μmol m^{-2} s^{-1})

electron transport (I_k) was calculated as the intercept between α and the $rETR_{max}$ values. Curve fit was calculated with the Solver module of MS-Excel using the least square method comparing differences between measured and calculated data.

To evaluate the effect of different radiation and exposure time treatments, 5 ml spore suspension were filled into 35 × 10 mm cell culture dish ($n = 5$) and exposed to the three radiation conditions for 1, 2, 4, and 8 h at 2 ± 1.5°C. Spores from *A. utricularis* were exposed in another experiment for 2, 8, and 16 h (Table 2). After F_v/F_m measurements, the spore suspension was returned to their respective culture dishes and allowed to recover for 2 days under dim white light (4 μmol photons m^{-2} s^{-1}) condition. Furthermore, a time series experiment was performed exposing *A. utricularis* spores repeatedly to PAB, PA and P for 4 h daily followed by 20 h under dim white light (4 μmol photons m^{-2} s^{-1}) over a period of 5 days. Photosynthetic efficiency was measured directly after the treatment and after recovery (Table 2).

Spore DNA damage and repair

DNA damage and subsequent repair of this damage was determined after 1, 2, 4, and 8 h exposure to UV-B radiation. From the working spore suspension, 40 ml was used for each experimental unit. For each treatment, six experimental units were prepared. After the irradiation treatment, three experimental units (as replicates) were processed immediately while the other three were allowed to recover for 2 days in low white light before processing. The spore samples were filtered through 44 mm diameter 1.0–2.0 μm pore size Nuclepore® polycarbonate membrane filters (Whatman, London, UK) and frozen at −80°C in 2-ml Eppendorf tubes for further DNA extraction and analysis of cyclobutane pyrimidine dimers (CPDs).

DNA was extracted using CTAB and quantified as described by Roleda et al. (2004b). The accumulation of CPDs was determined following a two step antibody assay using anti-thymine dimer H3 (Affitech, Oslo, Norway) and rabbit anti-mouse immunoglobulins (conjugated with horseradish peroxidase, DakoCytomation, Glostrup, Denmark). Chemiluminescent detection was done using ECL Western blotting detection reagent (Amersham, Buckinghamshire, UK; Roleda et al. 2005). Developed films (using X-ray film developer) were scanned using Bio-Rad imaging densitometer (Model GS-700, Bio-Rad Laboratories, Hercules, CA, 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 (45 min exposure to 2 TL 20W/12 lamps, Philips, Eindhoven, The Netherlands) was previously calibrated against UV irradiated Hela DNA with known amounts of CPDs. CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis

A one-way ANOVA was used to test for the effects of UVR on photosynthetic efficiency and DNA damage separately for each species and each exposure time ($P < 0.05$). Prior to analysis data were tested for homogeneity of variances (Cochran´s test). Post-hoc comparisons were performed with Newman–Keuls test. Statistical analysis were done using Statistica™ 6.0 software package.

Results

UV irradiance in the field and in the laboratory

Mean daily doses of UV-A and UV-B radiation in the field (air measurements from January to February 2005)

Table 2 Different treatments for the performed experiments including measured parameters (optimum quantum yield = F_v/F_m and DNA damage = CPDs)

Species	Parameter	Treatment	Exposure	Recovery
Monostroma hariotii	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
Porphyra endiviifolium	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
Adenocystis utricularis	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
	F_v/F_m	PAB + PA + P	2, 8, 16 h	24, 48 h
	F_v/F_m	PAB + PA + P	4 h/days over 5 days	20 h/days over 5 days

Exposure and recovery time is the duration of the treatments PAB (PAR + UV-A + UV-B), PA (PAR + UV-A) and P (PAR) and recovery under dim white light, respectively. During recovery spores were exposed to a PAR of 4 μmol m^{-2} s^{-1}

are shown in Table 3 in comparison to our treatments. Exposure to artificial UV-A radiation was lower in our experiments even after 16 h of irradiance than daily doses in the field. In contrast UV-B radiation in the 8 h treatment was similar to the daily doses measured in the field (Table 3).

Photosynthesis: Irradiance curves

The *P–I* curves shown in Fig. 1a–c reveal the differences in the photosynthetic performance of spores of the three species directly after spore release. The values for α (an index of light-harvesting system efficiency) varied between 0.065 and 0.139 (Fig. 1a–c). A similar steep slope was found in *A. utricularis* and *P. endiviifolium*, whereas *M. hariotii* showed the lowest α value. Highest saturating irradiance (I_k) was measured for reproductive cells of *M. hariotii* (83 µmol photons m^{-2} s^{-1}), followed by *A. utricularis* (64 µmol photons m^{-2} s^{-1}) and *P. endiviifolium* (33 µmol photons m^{-2} s^{-1}; Fig. 1a–c). At photon fluence rates >300 µmol photons m^{-2} s^{-1}, $rETR_{max}$ slightly decreased in *A. utricularis* and *M. hariotii* (Fig. 1a, b) whereas in *P. endiviifolium* $rETR_{max}$ decreased strongly after exceeding the actinic light level of 300 µmol photons m^{-2} s^{-1}. $rETR_{max}$ was higher in *A. utricularis* ($rETR_{max}$ = 9) in comparison to *M. hariotii* ($rETR_{max}$ = 5) and *P. endiviifolium* ($rETR_{max}$ = 4).

Photosynthetic efficiency after short term exposure to UV radiation

Photosynthetic performance of the three species was affected differently by PAR, UV-A and UV-B radiation, respectively. Initial measurements of the controls showed that *P. endiviifolium* monospores had a slightly higher mean optimum quantum yield (F_v/F_m = 0.488 ± 0.04) than *A. utricularis* spores (F_v/F_m = 0.462 ± 0.11) and both had a much higher optimum quantum yield compared with *M. hariotii* gametes (F_v/F_m = 0.288 ± 0.04, Fig. 2). The changes in optimum quantum yield during treatments are shown in Fig. 2. After 1 h treatment with PAR (*P*; 22 µmol photons m^{-2} s^{-1}) the optimum quantum yield (F_v/F_m) was not affected in *A. utricularis* whereas in *M. hariotii* and *P. endiviifolium* it was already reduced to 62 and 81% (expressed as the percentage of control), respectively (Fig. 2a, c, e). Increasing exposure time did not further affect F_v/F_m in *A. utricularis* which remained still high after 8 h exposure. In *M. hariotii* highest inhibition was found after 1 h and did not significantly change with further exposure. However, in *P. endiviifolium* increasing exposure time further decreased the F_v/F_m. PAR supplemented with UV-A (PA treatment) decreased photosynthetic efficiency significantly compared to the *P* treatment in all three species during exposure. Two exceptions were the 8 h treatment of *M. hariotii* and *P. endiviifolium* where no significant UV effect compared to the 8 h exposure to higher PAR was detected. Additional UV-B (PAB treatment) generally did not reveal a further significant decrease of optimum quantum yield. Interestingly, F_v/F_m of the PAB treatment in *A. utricularis* increased again after 8 h exposure (reduction after 1 h to 37%, after 8 h to 57% of the control measurement). In all treatments photosynthetic efficiency was reduced by 55–82% due to UVR in comparison to the respective *P* treatments.

However, all species showed a complete recovery of photosynthesis after 2 days under dim white light compared to the controls (ANOVA, $P > 0.05$) and no differences between treatments were detected. In *A. utricularis* F_v/F_m increased in the controls from 0.462 ± 0.114 before treatment to 0.601 ± 0.044 (mean ± SD) after 2 days recovery and in *M. hariotii* from 0.288 ± 0.040 to 0.400 ± 0.149 (mean ± SD), respectively. However, in *P. endiviifolium* F_v/F_m decreased in the controls from 0.488 ± 0.040 at the beginning of the experiment to 0.249 ± 0.023 (mean ± SD) after 2 days (Fig. 2a–f).

A second experiment with *A. utricularis* conducted with a longer exposure time (see also light doses in Table 3) and recovery measured after 24 and 48 h followed the same pattern as described above. UVR significantly reduced optimum quantum yield of spores in comparison to the *P* treatment (Fig. 3). After 8 and 16 h additional UV-B radiation reduced F_v/F_m significantly more than UV-A alone (Newman–Keuls, $P < 0.05$ between PAB and PA). An incomplete recovery occurred after 24 h in the 16 h exposure treatments of PA and PAB (ANOVA, $F_{2,12} = 15.03$, $P = 0.0005$). However, all samples recovered in all treatments after 48 h (Fig. 3).

Table 3 UV-A and UV-B doses in the PAB (PAR + UV-A + UV-B) treatment for different exposure times and in the field (n = 50) as means ± SD of daily doses measured in January and February

	UV-A (kJ m^{-2})	UV-B (kJ m^{-2})
Laboratory		
1 h	15.62	1.26
2 h	31.25	2.52
4 h	62.50	5.04
8 h	124.99	10.08
16 h	249.98	20.17
Field daily	318.06 ± 122.39	11.20 ± 4.33

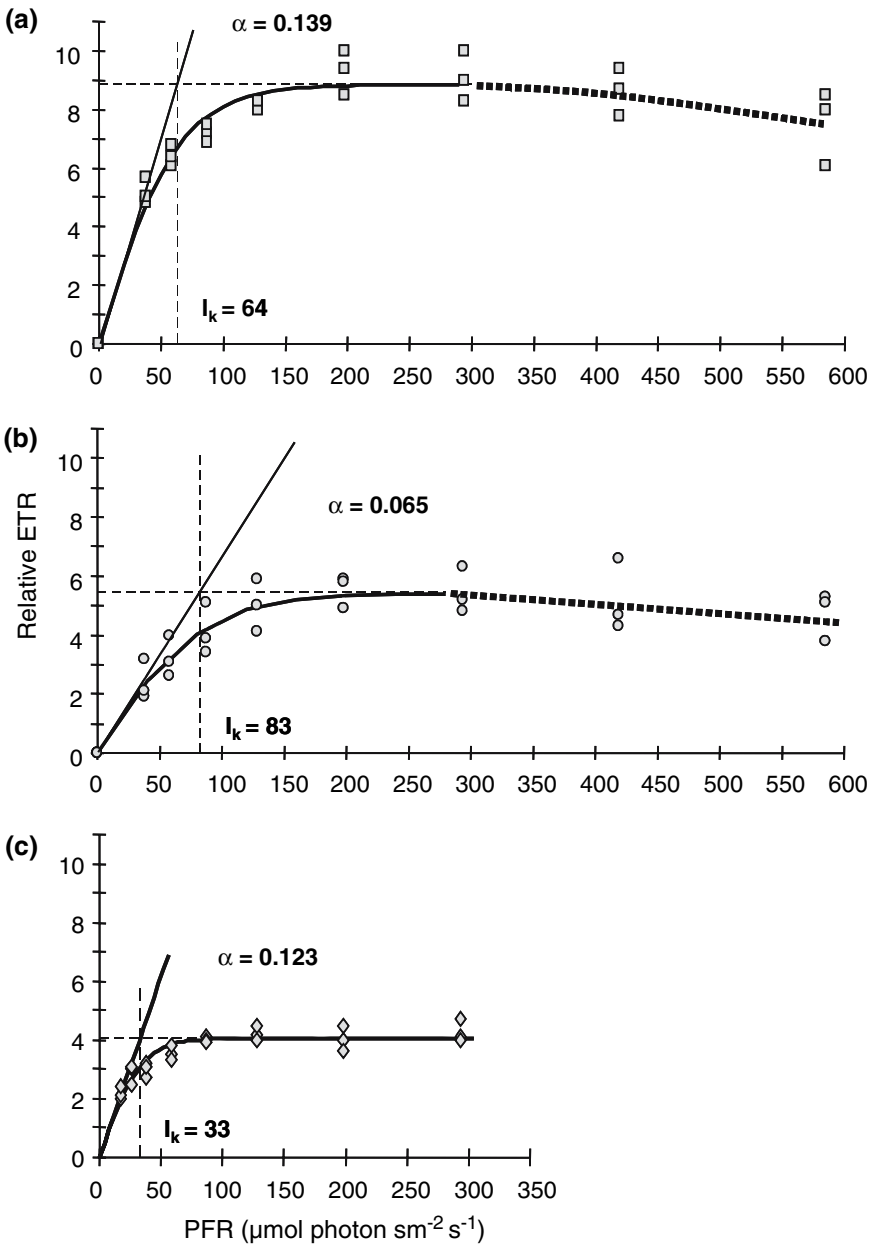

Fig. 1 Photosynthetic performance (*P–I* curves, $n = 3$) of spores of *Adenocystis utricularis* (**a**), *Monostroma hariotii* (**b**) and *Porphyra endiviifolium* (**c**) after spore release. PFR is the respective photon fluence rate of actinic white light and rETR is the relative electron transport rate

Time series of repeated UVR irradiance on *A. utricularis* spores

Repeated measurements of the same samples did not significantly affect the optimum quantum yields, as shown with the comparison of undisturbed control and disturbed control (measured at the beginning and the end of the experiment, Table 4, $P > 0.005$).

The time series measurements over a 5 days period did not show significant differences between the optimum quantum yield of the controls (maintained under 4 µmol photons m^{-2} s^{-1}) and the *P* treatments under higher PAR (22 µmol photons m^{-2} s^{-1}, $P > 0.05$). However, additional UV-A and the combination from UV-A and UV-B radiation led to a significant decrease in F_v/F_m after each of the 4 h treatments (Fig. 4). The decrease over time in the PA treatment did not significantly change (ANOVA, $F_{4, 20} = 0.91$, $P = 0.477$) during the 5 days and ranged from 48 to 56%. On the other hand, the effects of the PAB treatment changed over time (ANOVA, $F_{4, 20} = 5.36$, $P = 0.004$). After a significant increase in F_v/F_m from days 1 to 2 (Newman–Keuls, $P = 0.020$), F_v/F_m dropped significantly from days 2 to 5 (Newman–Keuls, $P = 0.003$) after 4 h exposure. Optimum quantum yield was reduced to 41% (on day 2) and to 28% (on day 5) in comparison to the control.

A complete recovery was measured after 20 h under dim white light with one exception on day 4 (PAB sig-

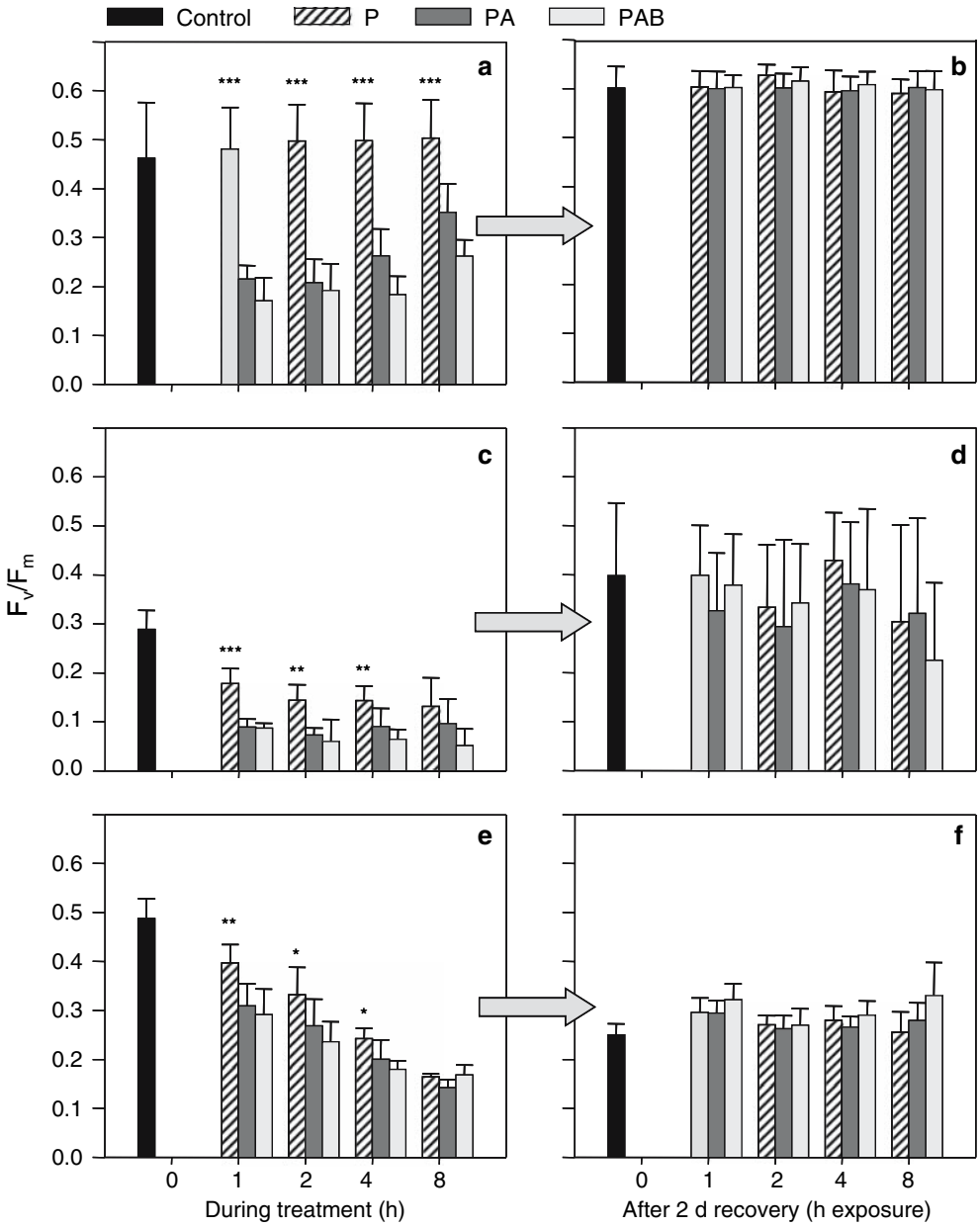

Fig. 2 Mean optimum quantum yield (F_v/F_m) ± SD ($n = 5$) of reproductive cells of *Adenocystis utricularis* (**a, b**) *Monostroma hariotii* (**c, d**) and *Porphyra endiviifolium* (**e, f**) after exposure to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) and after 2 days of recovery, respectively. Control (C) is without treatment and continuously maintained under 4 μmol photons m^{-2} s^{-1} white light. *Asterisks* indicate significant differences between the *P* and PA and/or PAB treatment. Significance levels were defined as follows: ***$P < 0.001$, **$P = 0.001$–0.01, *$P = 0.01$–0.05

nificantly different from *P*, ANOVA, $F_{2,\ 12} = 5.00$, $P = 0.026$; Fig. 4).

DNA damage and repair

No detectable DNA damage (measured as CPD concentrations per million nucleotides, CPD Mbp^{-1}) was found in *P. endiviifolium* monospores and only minimal DNA damage in propagules of *A. utricularis* (2, 4, and 8 h treatment) and *M. hariotii* (4 and 8 h treatment; Fig. 5) after exposure to PAB. CPD induction significantly increased in both species from 2 (4) to 8 h PAB exposure time (ANOVA, $F_{1,6} = 11.95$, $P = 0.008$ and $F_{1,4} = 7.85$, $P = 0.049$ for *A. utricularis* and *M. hariotii*, respectively). After 2 days recovery under dim white light all species were able to repair the DNA damage.

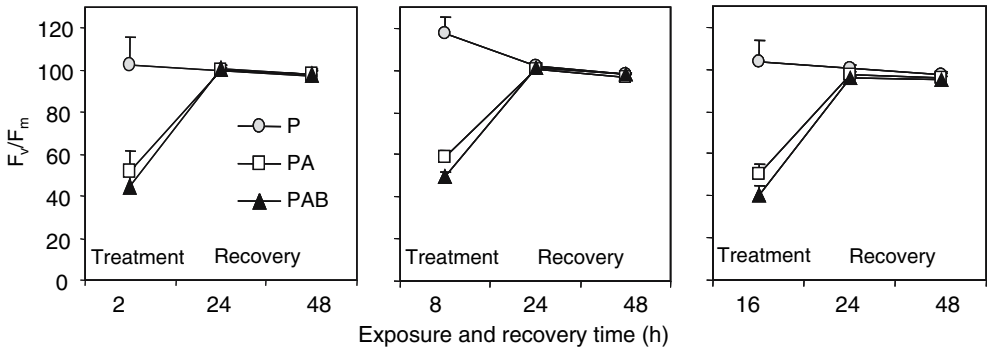

Fig. 3 Mean optimum quantum yield (F_v/F_m) ± SD ($n = 5$) expressed as percentage of the respective control of *Adenocystis utricularis* spores after exposure (treatment 2, 8, and 16 h) to PAR (*P*), PAR + UV-A (*PA*) and PAR + UV-A + UV-B (*PAB*) and after 24 and 48 h of recovery, respectively. F_v/F_m of controls were 0.4574 ± 0.0541 (*treatment*), 0.6072 ± 0.0209 (24-h recovery) and 0.6408 ± 0.0113 (48-h recovery), respectively

Table 4 Mean optimum quantum yield (F_v/F_m ± SD) of untreated zoospores of *Adenocystis utricularis* (controls) after release and at different time series intervals (see Fig. 4 for treatment effects)

Day	Hours	Disturbed control	Undisturbed control
0 initial	0	0.579 ± 0.031	0.571 ± 0.028
1	4	0.598 ± 0.020	–
	24	0.642 ± 0.014	–
2	28	0.631 ± 0.012	–
	48	0.643 ± 0.011	–
3	52	0.640 ± 0.012	–
	72	0.649 ± 0.006	–
4	76	0.652 ± 0.005	–
	96	0.658 ± 0.007	–
5	100	0.653 ± 0.007	–
	120	0.665 ± 0.007	0.669 ± 0.005

Disturbed control is without treatment, continuously maintained under 4 µmol photons m^{-2} s^{-1} white light and measured at the same time intervals as the treated samples. Undisturbed control was measured once before the start and at the end of the experiment to determine weather samples get disturbed due to the measurements. In the meantime it was continuously maintained under 4 µmol photons m^{-2} s^{-1}

Discussion

Our experiments showed that propagules from the Antarctic intertidal are well fitted to survive in their extreme habitat, although this life stage is the most susceptible to environmental stress factors. This study is among the first testing UVR effects on intertidal propagules of seaweeds and the first with Antarctic species.

In the laboratory experiments a UV-A:UV-B ratio of 12:1 is emitted by the lamps, whereas in the field the proportion of UV-A is more than two times higher. Even stronger is the difference in the ratio between PAR:UV-A:UV-B which was ~790:19:1 ($n = 112$) in the field in air (data not shown) and only 13.5:12.4:1 in the laboratory. The lower doses in the laboratory in relation to field air measurements were chosen to take the absorption by the water column into account. For example only 55% of UV-A and 60% of UV-B radiation reached the sample area in 10 cm water depth. Therefore, the 8-h treatment reflects the most natural situation in terms of daily UV doses for *A. utricularis* and *M. hariotii* whereas *P. endiviifolium* is exposed to higher doses due to its occurrence at the high tide level. However, maximal irradiances and doses in the intertidal are generally highly variable, depending, e.g., on tide level, water turbidity and weather conditions.

The I_k of *P. endiviifolium* is much lower than the I_k values of *A. utricularis* and *M. hariotii*. Low light adapted macroalgae have a saturation point ranging between 14 and 52 µmol m^{2-} s^{-1} (Hanelt et al. 2003) characterizing spores of *P. endiviifolium* as strongly shade adapted. In contrast propagules of *A. utricularis* and *M. hariotii* seem to be less strongly shade adapted.

These results are in agreement with measured I_k values for the adult thalli. Weykam et al. (1996) showed that the I_k values of adult Antarctic Rhodophyta are low compared to Chlorophyta or Phaeophyta. Results on zoospores of Arctic Laminariales generally showed a high shade adaption (I_k between 13 and 18 µmol m^{-2} s^{-1}, Roleda et al. 2006a). In contrast, I_k values of zoospores of cold temperate *Laminaria* species range between 20 and 40 µmol m^{-2} s^{-1} (Roleda et al. 2005) while kelp zoospores from the warm temperate regions have higher I_k ranging from 41 to 77 µmol m^{-2} s^{-1} (Amsler and Neushul 1991). The geographical trend with low values in the polar and higher ones in warmer regions corresponding to the increasing solar irradiance from the poles to the equator (Roleda et al. 2006a) could not be confirmed for Antarctic propagules with saturating irradiances within the range of temperate species. However, I_k values are also

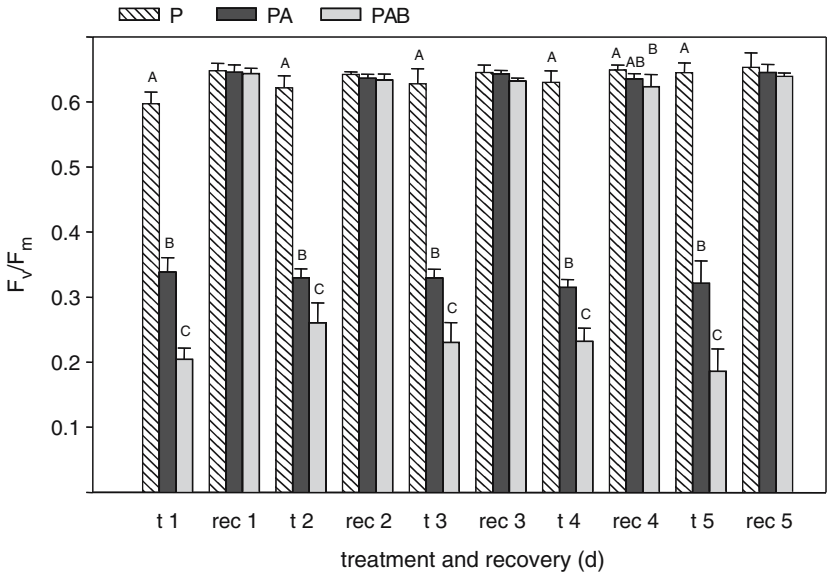

Fig. 4 Time series of repeated UV irradiation on spores of *Adenocystis utricularis*. Mean optimum quantum yield (F_v/F_m) ± 1 SD (n = 5) of spores after 4 h exposure (t 1 to t 5) to PAR (*P*), PAR +UV-A (*PA*) and PAR + UV-A + UV-B (*PAB*) and after subsequent 20 h of recovery (rec 1–5) repeated over 5 days, respectively. *Different letters* indicate significant differences between the treatments

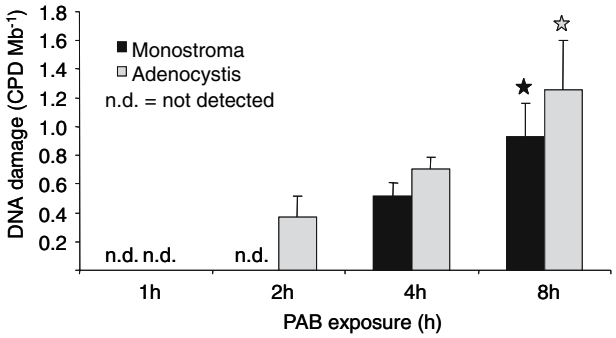

Fig. 5 UV-B induced DNA damage (mean ± SD, n = 3, induced CPD concentrations per million nucleotides) in *Adenocystis utricularis* spores and *Monostroma hariotii* gametes after exposure to different doses of PAB (PAR + UV-A + UV-B, 1, 2, 4, and 8 h). CPDs were not detected in *Porphyra endiviifolium* and in all species after 2 days recovery under dim white light (4 µmol photons m^{-2} s^{-1}) no more CPDs were detected. Significant differences among the different exposure times for each species are marked with *asterisk*

dependent on algal zonation on the shore with higher values measured for macroalgae from shallower water depth (Roleda et al. 2006a). In our study only intertidal species were tested explaining the relatively high I_k values. $rETR_{max}$ was not inhibited by actinic light <300 µmol m^{-2} s^{-1} showing good adaptations to higher PAR levels according to the occurrence of the adult thalli in the upper eulittoral. Eulittoral algae are periodically exposed to air where they experience a variety of stressful environmental conditions, e.g., very high light intensities (Davison and Pearson 1996). The potential for acclimation and recovery of the photosynthetic apparatus to high or damaging radiation conditions is therefore an important pre-requisite for the recruitment and ecological success of algae growing in the intertidal (Roleda et al. 2006a).

In general, photosynthetic efficiency (F_v/F_m) of freshly released propagules was lower compared to young or adult macrothalli as also shown in other studies (Wiencke et al. 2000; Roleda et al. 2004b). This can be attributed to the development stage of the chloroplast in different life stages. Laminariales zoospores with thin plasmalemma and one chloroplast per cell, e.g., are more sensitive to light stress (Roleda et al. 2006a). In contrast to the other two species optimum quantum yield of *Porphyra* spores decreased during the experiment in the controls maybe due to non-optimal cultivation conditions for this species.

Reaction to P, PA and PAB exposure in propagules was species-specific, indicating a higher light sensitivity of the green algae *M. hariotii* and the red algae *P. endiviifolium*. High light conditions during e.g., low tide or high water transparency might therefore influence their propagules survival more negatively than in *A. utricularis*. However, the reaction of propagules to high light stress remains to be tested.

Reduction of photosynthetic efficiency while exposed to high PAR is a protective mechanism to dissipate energy absorbed by PSII as heat via the xanthophylls cycle to avoid photodamage (dynamic photoinhibition; Osmond 1994). UVR exhibited an additional effect in the reduction of F_v/F_m in all species. The measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar but the mechanisms behind PAR and UVR induced inhibition of photosynthesis are different (Hanelt et al. 2003). UVR exhibits adverse effects on photosynthesis causing a direct molecular damage due to the absorp-

tion by biomolecules (Vass 1997). Depression of photosynthetic performance by UVR is, e.g., implicated to the damage of the oxidizing site of the reaction center of photosystem II (Franklin et al. 2003).

In contrast to eulittoral *A. utricularis*, spores of sublittoral Arctic Laminariales reacted already with a strong depression of F_v/F_m under PAR of 22 µmol photon m^{-2} s^{-1} (Roleda et al. 2006a). Furthermore Laminariales zoospores from Helgoland (Germany) were not able to recover after 8 h exposure to PAB in a comparable experiment (Roleda et al. 2005) indicating that the Antarctic intertidal *Adenocystis* is better acclimated to PAR and UVR. Results from irradiance experiments with intertidal carpospores from the red algae *Chondrus crispus* and *Mastocarpus stellatus* from the North Sea (Helgoland, Germany) showed that spores of these species react more sensitive to PAR and UVR than *P. endiviifolium* monospores (Roleda et al. 2004b). This difference is surprising as at least *M. stellatus* is able to grow in the upper eulittoral zone as well (Roleda et al. 2004b).

The monostromatic thallus of *Monostroma arcticum* from the Arctic is, in comparison to other investigated Arctic Chlorophyta and eulittoral Phaeophyta quite light sensitive, fast photoinhibited but recovering slowly, an indication of chronic photoinhibiton (Hanelt 1998). The monospores from *M. hariotii* exibit a similar behavior, as they were more light sensitive than the brown alga *A. utricularis*, but less sensitive than the red alga *P. endiviifolium*.

Short time experiments (8-h exposure) gave evidence of a possible acclimation of the photosynthetic apparatus of *A. utricularis* to UV-A and UV-B radiation as found in other studies with brown algae (Bischof et al. 1999; Roleda et al. 2004a). However, no such effect was observed after exposure to 16 h and after repeated exposure over a period of 5 days. In contrast to the earlier experiments additional UV-B significantly decreased photosynthetic efficiency further and inhibition was highest after 5 days suggesting a higher degree of damage due to repeated exposures. Apparently spores of *A. utricularis* lack acclimation abilities and are not able to diminish the inhibition caused by repeated UV exposure. After 20 h under dim white light, however, F_v/F_m recovered completely.

The non-detectable DNA damage in *P. endiviifolium* spores and minimal CPD formation in *A. utricularis* and *M. hariotii* propagules indicate effective shielding of the DNA and/or fast repair mechanism in the Antarctic intertidal propagules. The degree of damage due to UVR was observed to be related to cell size as DNA damage was observed to decrease in species (*Adenocystis*, *Monostroma*, *Porphyra*) with increasing cell size (4, 7, and 15 µm, respectively). This might be attributed to the increasing pathway for UV-B penetration through the cytoplasm (filtering effect; Swanson and Druehl 2000). In other studies, UV induced damage was related to thallus thickness (Franklin and Forster 1997; Johansson and Snoeijs 2002), e.g., thinner and relatively translucent species showed more DNA damage than thicker ones. An effective DNA repair mechanism was also observed in spores of Arctic and temperate Laminariales and Gigartinales but initial CPD formation was much higher (Roleda et al. 2004b, 2005).

DNA damage can be repaired through photolyase enzyme (light-dependent), nucleotide excision and recombination repair (light-independent; van de Poll et al. 2002). *M. arcticum* from Spitsbergen was not able to repair UV-B induced CPD formation probably due to low photolyase activity which has an important role in removing the majority of CPDs (van de Poll et al. 2002). The small amount of DNA damage in the tested Antarctic species might therefore be related to high photolyase activity. Another possibility is shielding due to UV absorbing compounds. However, whether the tested propagules are able to produce some kind of UV protective substance or have a high photolyase activity remains to be studied. Anyway, the ability of the propagules to cope with UV-B induced DNA damage seems to be crucial for the vertical zonation of the macrothalli at the coastline. If not repaired, DNA lesions can disrupt metabolism, cell division and impair growth and germination. Most macroalgae in Antarctica occur only in the subtidal (Wiencke et al. 2006a) and few are able to recruit in the intertidal partly due to their capacity to successfully repair DNA damage.

In general, exposure to the UV doses used in our laboratory experiment should not affect the survival and success of the investigated intertidal algae on short term view as all species recovered effectively from UV induced damage. However, in the field, maximal light intensities can be much higher especially when low tide coincides with noon and cloudless weather conditions. Longer exposure to ambient radiation over more than 8 h can take place and PAR would be much higher when cells are suspended within the euphotic layer of the water column. Therefore, field experiments on propagules are of great importance also taking into account parameters like germination and growth as integrative parameters of all physiological processes. Nevertheless, laboratory experiments give valuable insights in physiological mechanisms and common adaptations. Another important point is that these experiments were performed with field grown material as Swanson and Druehl (2000) hypothesized that kelp

spores might be pre-adapted to the UV conditions of their parent plants. If so, experiments with cultured material would not reflect the actual situation in the field and might overestimate UV effects because culturing usually takes place under PAR light only. Generally, the propagules studied here seem to be better adapted to UVR than temperate or Arctic ones. On the other hand, most previous studies were performed with subtidal species, mostly Laminariales which makes a direct comparison difficult. More comparative studies on related species and their reproductive cells respectively, from different geographical regions but similar zonation would improve our knowledge about the species-specific reactions and adaptations to (elevated) UVR.

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