## RESEARCH PAPER

# Impact of ultraviolet radiation on cell structure, UV-absorbing compounds, photosynthesis, DNA damage, and germination in zoospores of Arctic *Saccorhiza dermatodea*

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## Abstract

**Stratospheric ozone depletion leads to enhanced UV-B radiation. Therefore, the capacity of reproductive cells to cope with different spectral irradiance was investigated in the laboratory. Zoospores of the upper sublittoral kelp *Saccorhiza dermatodea* were exposed to varying fluence of spectral irradiance consisting of photosynthetically active radiation (PAR, 400–700 nm; =P), PAR+UV-A radiation (UV-A, 320–400 nm; =PA), and PAR+UV-A+UV-B radiation (UV-B, 280–320 nm; =PAB). Structural changes, localization of phlorotannin-containing physodes, accumulation of UV-absorbing phlorotannins, and physiological responses of zoospores were measured after exposure treatments as well as after 2–6 d recovery in dim white light (8 μmol photon $m^{-2}$ $s^{-1}$). Physodes increased in size under PAB treatment. Extrusion of phlorotannins into the medium and accumulation of physodes was induced not only under UVR treatment but also under PAR. UV-B radiation caused photodestruction indicated by a loss of pigmentation. Photosynthetic efficiency of spores was photoinhibited after 8 h exposure to 22 and 30 μmol photon $m^{-2}$ $s^{-1}$ of PAR, while supplement of UVR had a significant additional effect on photoinhibition. A relatively low recovery of photosystem II function was observed after 2 d recovery in spores exposed to $1.7 \times 10^4$ J $m^{-2}$ of UV-B, with a germination rate of only 49% of P treatment after 6 d recovery. The amount of UV-B-induced DNA damage measured as cyclobutane–pyrimidine dimers (CPDs) increased with the biologically effective UV-B dose ($BED_{DNA}$). Significant removal of CPDs indicating repair of DNA damage was observed after 2 d in low white light. The protective function of phlorotannins has restricted efficiency for a single cell. Within a plume of zoospores, however, each cell can buffer each other and protect the lower layer of spores from excessive radiation. Exudation of phlorotannins into the water can also reduce the impact of UV-B radiation on UV-sensitive spores. The results of this study showed that the impact of UVR on reproductive cells can be mitigated by protective and repair mechanisms.**

Key words: Cyclobutane–pyrimidine dimers, germination, photosynthesis, ultraviolet radiation, UV-absorbing compounds.

## Introduction

Several features in *Saccorhiza dermatodea* (Bachelot de la Pylaie) J. E. Areschoug indicate its primitiveness in kelp-like brown algae (Henry and Cole, 1982). Its zoospores possess an eyespot in the chloroplast and its gametophytes are monoecious with antheridia formed in catenate series on specialized laterals (Henry, 1987). In this species, gametogenesis and sporogenesis are strongly regulated by temperature and daylength (Henry, 1988). Vegetative growth of gametophytes and sporophytes occurs throughout summer. At the onset of shorter daylength in autumn, sporogenesis begins. Gametogenesis is restricted to temperatures

below 13 °C, but maturation of gametangia requires 6 weeks at 0 °C (Henry, 1987). Therefore, this species has a limited circumboreal and Arctic distribution (Norton, 1972; Keats and South, 1985). In Kongsfjorden on Spitsbergen, sporophytes of *S. dermatodea* occur from the low tide level down to a depth of 13.5 m (Wiencke *et al.*, 2004*a*).

The capacity to tolerate and recover from photoinhibiting irradiance of photosynthetically active radiation (PAR) among young and adult macroalgal thalli is a major factor for the determination of seaweed depth distribution (Han and Kain, 1996; Hanelt *et al.*, 1997). In addition, exposure to ultraviolet radiation (UVR) has also been reported to influence the vertical distribution pattern of seaweeds (Dring *et al.*, 2001; Roleda *et al.*, 2004, 2005*a*). In this context, enhanced UV-B radiation at the earth's surface due to the lowering of the stratospheric ozone concentration (von der Gathen *et al.*, 1995; Stähelin *et al.*, 2001) may directly affect seaweeds and their communities.

Experiments performed among different life history stages showed that early developmental stages of seaweeds such as gametes and spores are more vulnerable to environmental stresses when compared with juvenile and adult macrothalli (reviewed by Coelho *et al.*, 2000). Exposure to increased UVR induces spore mortality and photoinhibition of photosynthesis (Wiencke *et al.*, 2000, 2004*b*; Roleda *et al.*, 2004, 2005*a*). The primary targets for the UV suppression of photosynthetic activity appears to be the degradation of the D1 protein and part of the D1/D2 heterodimer; the major structural complex within photosystem II (PSII; Aro *et al.*, 1990; Melis *et al.*, 1992; Jansen *et al.*, 1993). Exposure to UV-B radiation also causes DNA damage in algal spores (Wiencke *et al.*, 2000; Roleda *et al.*, 2004, 2005*a*). Most UV-B-induced lesions involve dimerization of adjacent pyrimidine bases resulting in the formation of cyclobutane–pyrimidine dimers (CPDs). These lesions disrupt cell metabolism and division (van de Poll *et al.*, 2001).

Responses to UV damage are counterbalanced by protection strategies such as avoidance, screening, photochemical quenching, and repair. Avoidance strategies include reproductive seasonality and diel periodicity in spore release (Reed *et al.*, 1988; Amsler and Neushul, 1989) to ensure reproductive success by synchronizing propagule production and release with the onset of favourable environmental conditions (Kinlan *et al.*, 2003). Screening includes extracellular (cell wall) and intracellular formation of UV-absorbing compounds. Important UV screening compounds are mycosporine-like amino acid (MAA) in red macroalgae and scytonemin in cyanobacteria (Franklin *et al.*, 2003). UV sunscreens used as photoprotective mechanisms in planktonic organisms (1 to <10 µm size class cells) can afford considerable benefits but only at the expense of relatively high energetic investment and with restricted efficiencies (Garcia-Pichel, 1994).

In brown algae, the secondary metabolites phlorotannins were invoked to act as UV-screening pigments. In addition to UV screening, phlorotannins serves as herbivore deterrents, digestion inhibitors, and antibacterial agents (Schoenwaelder, 2002). Phlorotannins also have various primary roles in reproduction, fertilization, spore attachment, and cell wall construction (Arnold, 2003). Schoenwaelder and Clayton (1998*a*) have shown that prior to cell division in zygotes, physodes form a distinct line across the centre of cells, followed by accumulation of other cell wall constituents. Phlorotannins are also secreted into cell walls of zygotes and adult sporophytes (Schoenwaelder and Clayton, 1998*a*, *b*, 2000; Lüder and Clayton, 2004).

In kelp zoospores (3–5 µm), the protective potential of phlorotannin was regarded to enhance germination capacity (Wiencke *et al.*, 2004*b*). Kelp phlorotannin exudates from macroalgal sources have been invoked to reduce the impact of UV-B radiation by forming UV refugia for kelp zoospores within the water column (Swanson and Druehl, 2002). In *S. dermatodea*, the response to UV stress has been studied for the germination capacity of zoospores (Wiencke *et al.*, 2004*b*, 2006), and photosynthesis and growth of juvenile and adult sporophytes (Aguilera *et al.*, 1999; Karsten *et al.*, 2001; Roleda *et al.*, 2005*b*). Apart from these studies, not much is known about other physiological responses of this species to enhanced UVR. The development of a new generation of gametophytic and sporophytic life stages is dependent on the survivorship of zoospores. It is hypothesized that the negative impact of UVR on zoospores can be mitigated by different protective and repair mechanisms whereby the survival strategy is important for providing new recruits to sustain kelp communities in coastal marine environments.

## Materials and methods

### Algal material

Fertile sporophytes of *S. dermatodea* were collected between May and June 2004 by scuba-divers in Kongsfjorden at Prins Heinrichøya close to Ny Ålesund (Spitsbergen, 78°55′ N, 11°56′ E). Blades with sori were abscised from five different individuals, cleaned of epiphytes, blotted with tissue paper, and kept in darkness in a moist chamber at 0 °C overnight or for a maximum of 2 d. To induce rapid release of zoospores, sori were immersed in 5–10 ml of filtered (0.2 µm pore size) seawater at ±15 °C and exposed to natural light close to a window pane (Wiencke *et al.*, 2006). The initial zoospore density was counted by use of a Neubauer chamber (Brand, Germany). Stock suspensions were diluted with filtered seawater to give the spore densities required for each experiment.

### Light treatments

Two exposure desks were prepared where lamps were hanging 40 cm (shelf 1) and 30 cm (shelf 2) above the table to produce different irradiance conditions. PAR was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany), and UVR was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA), emitting a spectrum similar to solar radiation in the

range 295–340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes. Cell culture dishes were covered with one of the following filters: Ultraphan URUV farblos (Digefra GmbH, Germany); Folanorm (Folex GmbH, Germany), or Ultraphan transparent, corresponding to the PAR (P=22–30 µmol photon m$^{-2}$ s$^{-1}$), PAR+UV-A (PA, UV-A=5.65–7.03 W m$^{-2}$), and PAR+UV-A+UV-B (PAB, UV-B=0.47–0.58 W m$^{-2}$) treatments, respectively. UVR was measured using a cosine sensor connected to a UV-VIS Spectrometer (Marcel Kruse, Bremerhaven, Germany) below the cut-off filters. The equivalent biologically effective doses (BEDs) were calculated using action spectra for DNA damage (280–320 nm; Setlow, 1974) and general plant damage (280–312 nm; Caldwell, 1971). PAR was measured using a cosine quantum sensor attached to a Li-Cor data logger (LI-1000, Li-Cor Biosciences, Lincoln, NE, USA). Weighted and unweighted irradiances used in the experiments are presented in Table 1.

## Absorbance spectra

To determine the presence of UV-absorbing compounds, 80 ml of zoospore suspension containing $2.64 \times 10^6$ spores ml$^{-1}$ was placed into 85 mm×15 mm culture dishes covered with corresponding cut-off filters and exposed under different light treatments for 23 h on shelf 2. A portion of the zoospore suspension was kept in the dark (dark control). After treatments, samples were placed into quartz cuvettes and scanned in the 250–700 nm waveband using a Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere. Absorbance spectra of the zoospore suspension, zoospore, and the medium (filtrate) were measured from: (i) a zoospore suspension with seawater as reference; (ii) a zoospore suspension with filtrate as reference; and (iii) filtrate with seawater as reference, respectively. The filtrate was obtained by filtering the zoospores out of the suspension through a 44 mm diameter 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK) using a vacuum pump at 400–600 mbar to minimize damage to the cells.

## Chlorophyll fluorescence measurements

Photosynthetic efficiency, measured as variable fluorescence of 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 spore density ($3 \times 10^5$–$4 \times 10^5$ spores ml$^{-1}$, not exceeding 1 h after spore release), the spore suspensions from the different sporophytes were poured into 5 ml quartz cuvettes and the maximum quantum yield ($F_v/F_m$) was measured to determine initial photosynthetic efficiency at time zero ($n$=5) as described by Roleda *et al.* (2006*a*), designated as control. Controls measured at time zero were placed into corresponding culture dishes (35 mm×10 mm Corning™, Corning Inc., NY, USA). To evaluate the effect of different irradiation intensities, fresh spore suspensions (5 ml) were poured into cell culture dishes and exposed under the three radiation regimes in shelves 1 and 2 for 8 h ($n$=5, per treatment combination) at 7±1 °C. After treatments, $F_v/F_m$ was determined and the spore suspension was returned to the same culture dish and cultivated under dim white light (8 µmol photon m$^{-2}$ s$^{-1}$) for recovery. Time zero control was also maintained under the same conditions. Measurements of photosynthetic recovery were done after 48 h exposure to the dim white light condition. Settled and germinating spores were slowly resuspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes.

## DNA damage and repair

DNA damage and its subsequent repair were determined after exposure to the whole light spectrum at different exposure times on shelf 1 (1, 4, 8, and 16 h) and shelf 2 (8 h and 16 h). From the spore suspension ($3 \times 10^5$–$4 \times 10^5$ spores ml$^{-1}$), 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 d in low white light before processing. Settled and germinating spores were resuspended from the bottom of the Petri dishes by jetting pressurized seawater from a wash bottle. The spore samples were filtered through a 44 mm diameter 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK) and frozen at –80 °C. DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) and quantified as described by Roleda *et al.* (2004). 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 performed using enhanced chemiluminescence (ECL) western blotting detection reagent (Amersham, Buckinghamshire, UK) (Roleda *et al.*, 2005*a*). Developed films (using X-ray film developer) were scanned using a Bio-Rad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and grey 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 20 W/12 lamps, Philips, Eindhoven, The Netherlands) was previously calibrated against UV-irradiated HeLa DNA with known amounts of CPDs (kindly provided by A Vink). CPDs were quantified by comparing the grey scales within the linear range of the film.

## Light microscopy

Structural changes of zoospores were studied after 8 h and 16 h exposure to treatments corresponding to P, PA, and PAB, followed by 2 d and 6 d of recovery under dim white light. Freshly released zoospores of three individual sporophytes ($n$=3) were diluted with filtered seawater to a concentration of $3 \times 10^5$–$4 \times 10^5$ spores ml$^{-1}$. For each treatment combination (spectral composition and exposure time), nine experimental units were prepared. Culture dishes (53 mm in diameter and 12 mm high) containing a paper filter at the bottom and a coverslip (18×18 mm) on the paper filter were filled with 10 ml

**Table 1.** *Experimental irradiance and corresponding biologically weighted irradiances using the biologically effective weighing function for general plant damage (Caldwell, 1971) and DNA damage of* Escherichia coli *(Setlow, 1974)*

| | Experimental irradiance | | | Weighted irradiance (W m$^{-2}$) | |
| --- | --- | --- | --- | --- | --- |
| | PAR (400–700 nm) (µmol photon m$^{-2}$ s$^{-1}$) | UV-A (320–400 nm) (W m$^{-2}$) | UV-B (280–320 nm) (W m$^{-2}$) | General plant damage (Caldwell, 1971) | DNA damage (Setlow, 1974) |
| Shelf 1 | 21.8 | 5.65 | 0.47 | $3.51 \times 10^{-2}$ | $1.47 \times 10^{-2}$ |
| Shelf 2 | 29.5 | 7.03 | 0.58 | $4.48 \times 10^{-2}$ | $1.60 \times 10^{-2}$ |

of zoospore suspension, covered with corresponding cut-off filter foils and exposed to 8 h and 16 h at irradiances on shelf 2 (Table 1), at 7±1 °C. Zoospores settled on the filter and on the coverslip within minutes. After exposure, one-third of the material of each treatment was processed immediately and two-thirds were cultivated further for 2 d and 6 d under dim white light. At sampling time, the coverslip with settled spores was removed from the filter, put on a slide, covered with another coverslip (22×22 mm), and examined for structural changes using an Axioplan imaging light microscope (Zeiss, Germany). Microscopic pictures were taken with a digital camera (Canon 70, Japan).

### Germination

Culture dishes (53 mm×12 mm) were filled with filtered seawater, and 2–5 drops of the zoospore suspension containing ~$3×10^5$–$4×10^5$ spores ml$^{-1}$ from different sporophytes were added to each dish. The dishes were covered with corresponding cut-off filters and exposed to different radiation conditions for 8 h and 17.5 h on shelf 2. After the treatment, spores were allowed to germinate in low white light (8 μmol photon m$^{-2}$ s$^{-1}$) for 3 d and 6 d. Spores were scored as germinated or not germinated by counting 300 cells per replicate using a light microscope (Zeiss, Germany) equipped with a ×20 seawater immersion objective. A spore was classified as germinated when at least a germ tube was formed. Dead and living cells were not differentiated. Since non-germinated cells were also observed under P, the germination rate under PA and PAB treatments was expressed as a percentage of P.

### Statistical analysis

Data were tested for homogeneity (Levene statistics) and normality (Kolmogorov–Smirnov test) of variance. Corresponding transformations (square roots) were done to heteroskedastic and non-normal data. Photosynthetic efficiency and amount of CPDs after exposure to varying fluence of different combinations of spectral irradiance (P, PA, and PAB) and after recovery were tested using analyses of variance (ANOVAs, $P < 0.05$). The difference in germination rate (raw data) between treatments was tested using repeated measure analysis of variance (RMANOVA, $P < 0.05$). All analyses were followed by Duncan's multiple range test (DMRT, $P = 0.05$). Statistical analyses were done using the SPSS program (SPSS, Chicago, IL, USA).

## Results

### Absorption spectra

Zoospore suspension (Fig. 1A), zoospores (Fig. 1B), and filtrate (Fig. 1C) showed a strong absorbance below 350 nm. The absorbance maxima in the 250–280 nm range is ~4–5 times higher compared with the chlorophyll and xanthophyll peaks at 675 nm and 440 nm in the zoospores, respectively (Fig. 1B). There was an increase in absorbance in the UV waveband after exposure of the zoospore suspensions for 23 h to the different radiation regimes compared with the dark control (Fig. 1A). In zoospore suspensions, the highest increase was observed under PAB treatment and the lowest under P, but this was significantly higher compared with the dark control (Fig. 1A). Much of this UV-absorbing compound was found within the cell in the dark control and P treatment (Fig. 1B). In the filtrate, the lowest absorbance was determined in the dark control and after exposure to

the P condition. The absorbance was highest in the PA and PAB treatments (Fig. 1C).

### Photosynthetic efficiency

Exposure to different fluences (J m$^{-2}$) of light (P, PA, and PAB) significantly reduced optimum quantum yields ($F_v/F_m$) of the zoospores (Table 2). After 8 h exposure to a combination of different fluence rates (W m$^{-2}$) of PAR, UV-A, and UV-B, photoinhibition of photosynthesis was already reduced by 83–88% under P treatment. A further
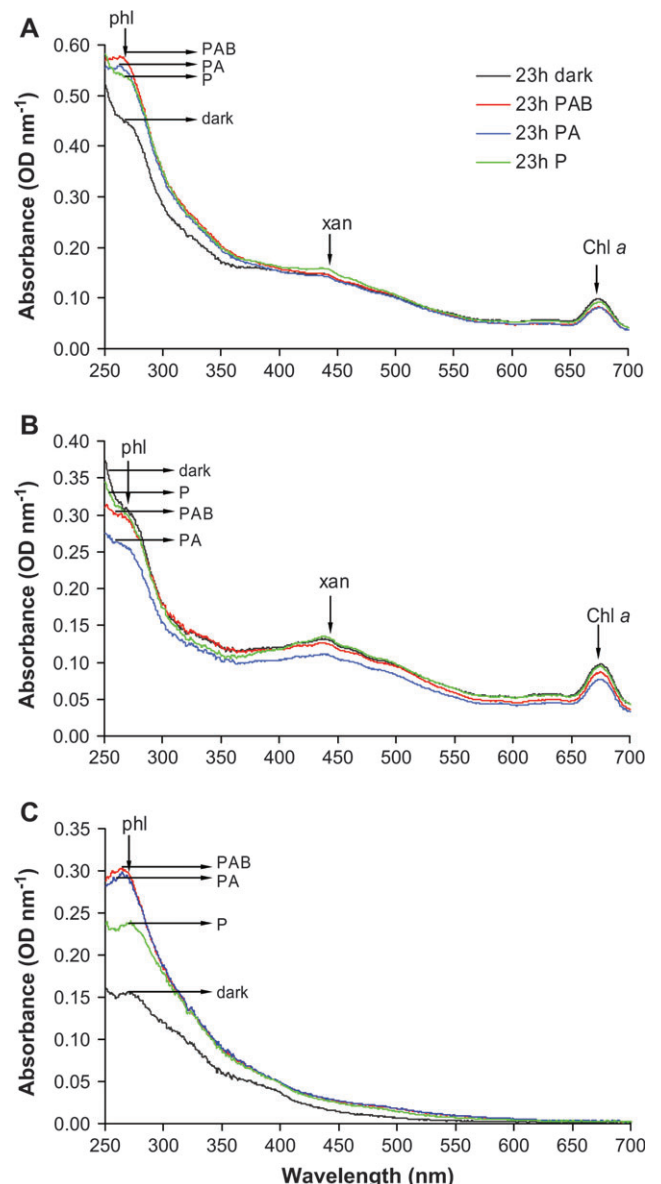


**Fig. 1.** Absorption spectra in (A) zoospore suspension, (B) zoospores, and (C) filtrate of *Saccorhiza dermatodea* exposed to 23 h of photosynthetically active radiation (PAR; P=30 μmol photon m$^{-2}$ s$^{-1}$), PAR+UV-A (PA; UV-A=7.0 W m$^{-2}$), and PAR+UV-A+UV-B (PAB; UV-B=0.6 W m$^{-2}$). Absorption maxima at 675, 440, and 265 nm are characteristics for chlorophyll a (Chl *a*), xanthophylls (xan), and phlorotannins (phl), respectively. Zoospore density is $2.64×10^6$ spores ml$^{-1}$.

decrease in $F_v/F_m$ of zoospores to 92% and 94–96% of control was observed under PA and PAB, respectively. After 2 d recovery in dim white light, the photosynthetic efficiency of germinating spores in the untreated control was not significantly different from that of the freshly released zoospores. The percentage recovery relative to the control among treated spores was highest in P at 45–60%, followed by PA at 39–52%, and lowest in PAB at 14–42%. The effect of spectral composition (P, PA, and PAB) on $F_v/F_m$ after treatment was significantly different, as shown by the ANOVA ($P < 0.001$). Similarly, spectral composition as well as fluence rates (high and low) had a significant effect on the recovery of photosynthetic efficiency (Table 3).

### DNA damage and repair

Induction of CPDs increased significantly with increasing UV-B dose (ANOVA, $P < 0.001$). After 2 d recovery in

**Table 2.** *Mean optimum quantum yield ($F_v/F_m$) of Saccorhiza dermatodea zoospores after exposure to 8 h of photosynthetically active radiation (PAR=P), PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB) at two light regimes on shelf 1 and shelf 2 and after 2 d recovery in dim white light (8 μmol photon $m^{-2}$ $s^{-1}$)*

The control was measured after spore release and recovery under the same dim light condition as that of the treated samples. Corresponding statistical analysis is shown in Table 3.

| Treatment | | | Photosynthetic efficiency ($F_v/F_m$) | | |
|---|---|---|---|---|---|
| Light | Shelf | Total fluence (J $m^{-2}$) | After release | After exposure | 2 d recovery |
| Control | | | 0.539±0.04 | | 0.579±0.03 |
| P | 1 | $1.35×10^5$ | | 0.094±0.04 | 0.345±0.09 |
| | 2 | $1.83×10^5$ | | 0.067±0.03 | 0.260±0.07 |
| PA | 1 | $2.98×10^5$ | | 0.044±0.02 | 0.304±0.04 |
| | 2 | $3.85×10^5$ | | 0.044±0.01 | 0.226±0.04 |
| PAB | 1 | $3.11×10^5$ | | 0.035±0.02 | 0.244±0.07 |
| | 2 | $4.02×10^5$ | | 0.023±0.01 | 0.079±0.02 |

**Table 3.** *Analysis of variance and significance values for the effect of irradiance (P, PA, and PAB) and intensity (high and low) on the photosynthetic yields ($F_v/F_m$) of zoospores of Saccorhiza dermatodea after exposure and recovery*

The effect of fluence (J $m^{-2}$) as an independent variable was also statistically tested.

| Dependent variable | Independent variable | df | F | P |
|---|---|---|---|---|
| $F_v/F_m$ (after treatment) | Irradiance (A) | 2 | 11.756 | <0.001* |
| | Intensity (B) | 1 | 1.718 | 0.202 ns |
| | A×B | 2 | 0.578 | 0.569 ns |
| | Fluence | 5 | 5.277 | 0.002* |
| $F_v/F_m$ (after recovery) | Irradiance (A) | 2 | 16.006 | <0.001* |
| | Intensity (B) | 1 | 26.789 | <0.001* |
| | A×B | 2 | 1.779 | 0.190 ns |
| | Fluence | 5 | 17.048 | <0.001* |

* Significant; ns, not significant.

dim white light, an efficient DNA repair mechanism was observed (Fig. 2). DNA damage was completely repaired in spores exposed to low fluence of biologically effective UV-B ($BED_{DNA}$). At an intense BED, the remaining DNA damage was significantly higher (ANOVA, $P < 0.001$). DMRT ($P=0.05$) showed three significantly different subsets in response to UV-B dose (see inset letters and numbers in Fig. 3).

### Changes in cellular structure

Freshly released zoospores of *S. dermatodea* are ~5 μm in diameter. Cells are filled with several physodes (black arrows, Fig. 3A, C, E, G), phlorotannin-containing globular vesicles appearing light to dark grey in the light microscope, yellow to greenish elongated chloroplasts (green arrows, Fig. 3D, E, H, P), and an orange eyespot (red arrows, Fig. 3A, D). The eyespot is a photoreceptor containing carotenoids, with maximal absorption at 420–490 nm. Several physode-like vesicles were present in the medium (grey arrows, Fig. 3A).

After 8 h exposure to different light treatments, the physodes are observed in all cells but more prominent under PAB (Fig. 3C). After 2 d of recovery in dim white light, germination was observed in the P and PA treatment (Fig. 3D and E, respectively), but not under PAB (Fig. 3F). Furthermore, in the PAB treatment, pigmentation of chloroplasts and eyespot was lost in many cells (Fig. 3F). After 6 d of recovery in dim white light, the germination tubes were developed in the P treatment (Fig. 3G) and often contained physodes. In the PA treatment, germination was delayed (Fig. 3H). In the PAB treatment, many cells were not germinated and appeared dead (Fig. 3I).

Drastic differences in spore structures were evident after 16 h exposure (Fig. 3J–L). The P- and PA-treated zoospores appeared healthy (Fig. 3J and K, respectively), while many PAB-treated spores had lost their pigmentation (Fig. 3L). The remaining pigmented zoospores under PAB deteriorated after 2 d or 6 d in dim white light (Fig. 3O and R, respectively). The same was observed in PA-treated zoospores after 2 d and 6 d in dim white light (Fig. 3N and Q, respectively). Dead cells often contained enlarged physodes and underwent autolysis (Fig. 3Q, R). Under P treatment, few cells germinated after 2 d and 6 d in low white light (Fig. 3M and P, respectively), while most cells appeared pale and did not germinate.

### Germination

Spore germination after 8 h and 17.5 h exposure to high PAR (=P treatment) and post-cultivation under dim white light (8 μmol photon $m^{-2}$ $s^{-1}$) for 3 d and 6 d ranges between 16% and 23%. Therefore, the germination rates under different UVR treatments were expressed as a percentage of the germination under the P treatment. When exposed to $2.0×10^5$ J $m^{-2}$ UV-A radiation (8 h exposure
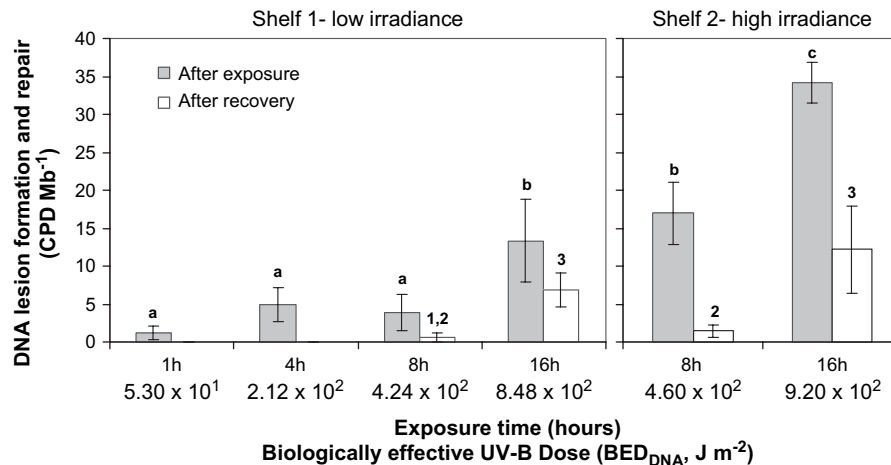
**Fig. 2.** UV-B-induced DNA damage (CPD formation per million nucleotides) in zoospores of *Saccorhiza dermatodea* after increasing time of exposure to different irradiance of PAB (PAR+UV-A+UV-B) and corresponding DNA damage repair after 2 d of recovery in dim white light (8 μmol photon $m^{-2} s^{-1}$). The biologically effective UV-B dose calculated using weighing function for DNA damage, $BED_{DNA}$ (Setlow 1974), is shown on the *x*-axis. Vertical bars are standard deviations (SD, *n*=3). ANOVA showed a significant effect of treatment (*P* <0.001). Letters (after exposure) and numbers (after recovery) on the graph show the result of DMRT (*P* <0.05); different notations refer to significant differences between mean values.

under PA treatment), there was no significant difference between germination after 3 d and 6 d of recovery under P compared with PA (Fig. 4A). In contrast, after exposure to $4.4 \times 10^5$ J $m^{-2}$ UV-A radiation (17.5 h exposure under PA treatment), germination rates decreased significantly down to 56% after 3 d of recovery (Fig. 4B). After 6 d of recovery, germination rates in PA-treated spores increased but with high variation between sporophyte source (Fig. 4B). Like UV-A radiation, UV-B radiation had a strong dose-dependent inhibitory effect on the germination rate. After exposure to low UV-B radiation (8 h exposure under PAB treatment), >50% of spores were dead and there was no or little recovery potential. After exposure to the 2-fold UV-B dose (17.5 h exposure under PAB treatment), germination rates went down to almost zero and there was no recovery at all after three more days of recovery. RMANOVA (*P* < 0.001) showed a significant difference between treatments. Corresponding DMRT (*P*=0.05) showed that P = PA>PAB in 8 h-exposed zoospores and P>PA>PAB in 16 h-exposed zoospores.

## Discussion

This study shows for the first time the different physiological responses in zoospores of *S. dermatodea* exposed to UVR-depleted light and to the full light spectrum. Cellular damage was observed in spores exposed to light supplemented with UVR. Photoinhibition and recovery of photosynthetic capacity as well as germination capacity respond according to DNA damage and repair capacity. Extrusion of phlorotannin-containing physodes into the medium and accumulation of these UV-absorbing compounds was also found to be induced not only under UVR treatment but also under PAR.

Absorption within the UV waveband of the spectrum is characteristic of the compound phlorotannin. Isolated phlorotannins from *Fucus gardneri* Silva absorb strongly in the UV region, with a peak at 265 nm (Henry and van Alstyne, 2004). Due to their absorption characteristics, phlorotannins play a role in UV defence because their synthesis is inducible by UV-B radiation (Pavia *et al.*, 1997; Pavia and Brock, 2000; Schoenwaelder, 2002). In this study, however, it was found that phlorotannin synthesis is not only inducible by UV-B radiation but also by PAR and UV-A. Furthermore, exudation of this compound into the medium was observed microscopically, and spectrophotometric measurements showed a corresponding strong absorbance in the external medium within the UV waveband. Phlorotannin exudates in coastal seawater can at low concentrations reduce the impact of UV-B exposure to UV-sensitive kelp meiospores (Swanson and Druehl, 2002).

Increasing PAR fluence from $1.35 \times 10^5$ to $1.83 \times 10^5$ J $m^{-2}$ (8 h exposure to 4.7 and 6.3 W $m^{-2}$) effectively depressed the photosynthetic capacity of zoospores of *S. dermatodea*. Reduction of photosynthetic capacity and quantum efficiency is a protective strategy in plants exposed to high fluence of PAR to be able to dissipate excess energy absorbed by PSII as heat to avoid photodamage. UVR exhibited an additional effect in the reduction of photosynthetic capacity of zoospores. 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). Depression of photosynthetic performance by UV radiation is implicated in the impairment of the oxidizing site and reaction centre of PSII (Grzymski *et al.*, 2001; Turcsányi and Vass, 2002; Franklin *et al.*, 2003). Despite the 48 h
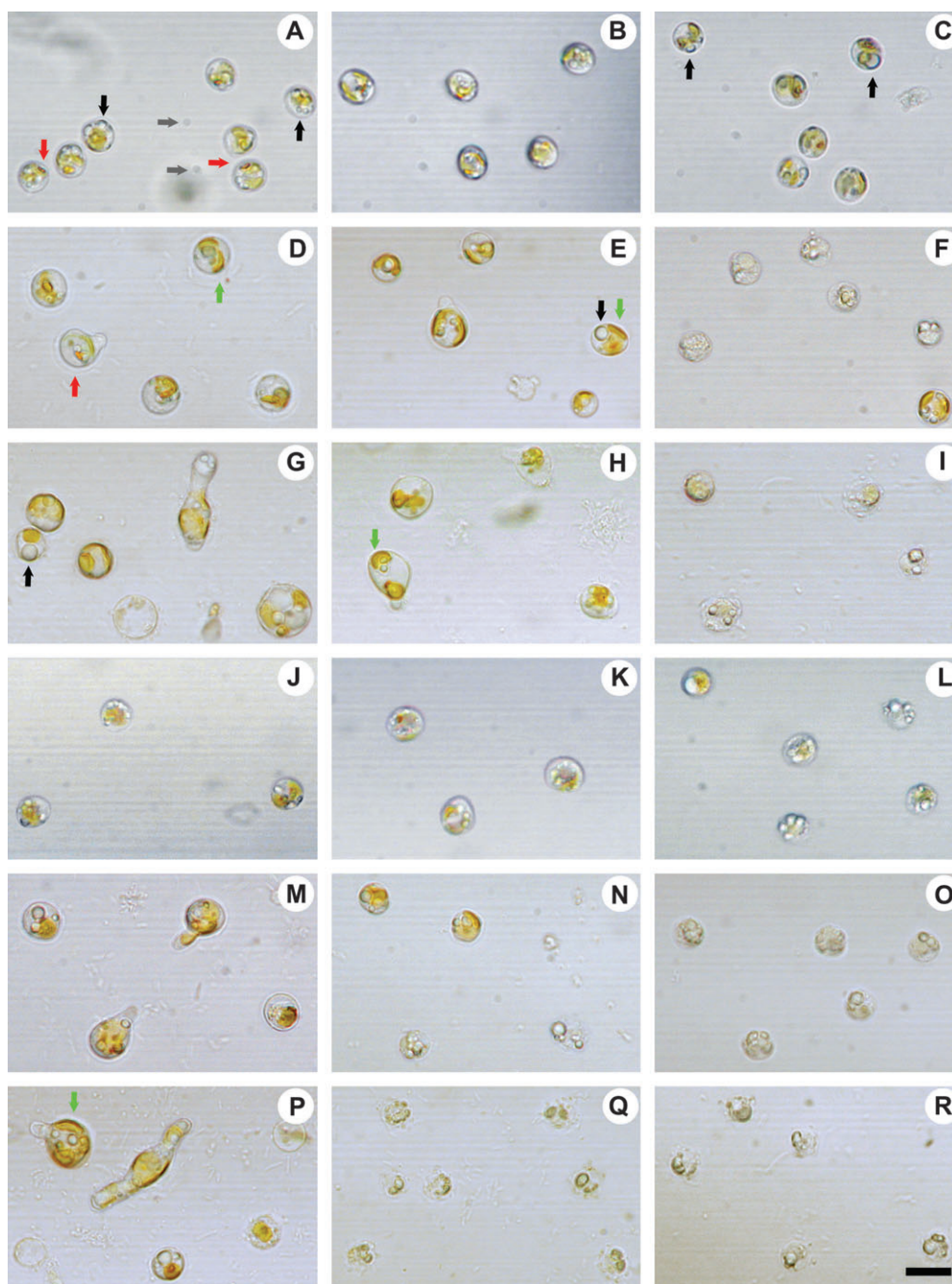
**Fig. 3.** *Saccorhiza dermatodea* spores after 8 (A–C) and 16 (J–L) h exposure to PAR (A, J), PAR+UV-A (B, K), and PAR+UV-A+UV-B (C, L) on shelf 2. Corresponding recovery, after exposure treatments, under dim white light (8 µmol photon $m^{-2}$ $s^{-1}$) for 2 d (D–F and M–O, respectively) and 6 d (G–I and P–R, respectively). Black arrows are physodes appearing light to dark grey; grey arrows are vesicles in the medium; red arrows are eyespots appearing orange; green arrows are chloroplasts appearing yellow to green. Scale bar=5 µm.

recovery period in dim white light after exposure to high fluence of PAB, minimal photosynthetic recovery was observed in spores. This is attributed to photodamage of PSII. During photo-inactivation, *de novo* synthesis of D1 protein is required to regain functional PSII; therefore, a longer recovery time is required. Under a lower fluence rate, the photosynthetic capacity of P-, PA-, and PAB-treated *S. dermatodea* spores was already fully functional

after 24 h recovery in low white light (Roleda *et al.*, 2006*a*). Since low light adaptation is the general feature of brown algal zoospores, as presented here, immediate settlement and access to substrate under a low light microenvironment will facilitate recruitment success.

The capacity of zoospores to repair UV-B-induced DNA damage was previously reported among temperate Laminariales (Roleda *et al.*, 2005*a*). In the present study,
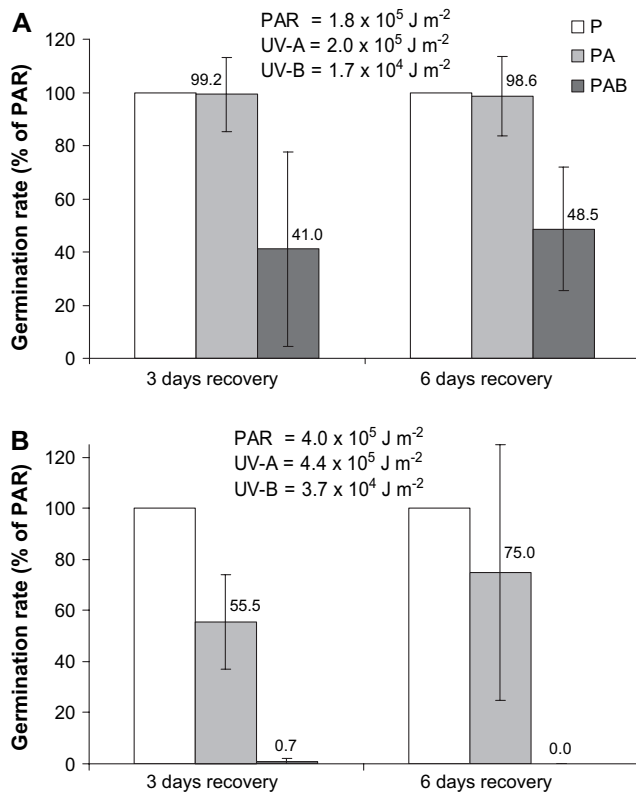
**A**



PAR = 1.8 x 10⁵ J m⁻²
UV-A = 2.0 x 10⁵ J m⁻²
UV-B = 1.7 x 10⁴ J m⁻²

99.2          98.6
41.0          48.5

3 days recovery          6 days recovery

**B**

PAR = 4.0 x 10⁵ J m⁻²
UV-A = 4.4 x 10⁵ J m⁻²
UV-B = 3.7 x 10⁴ J m⁻²

55.5          75.0
0.7           0.0

3 days recovery          6 days recovery

**Fig. 4.** Spore germination, expressed as a percentage of P treatment in *Saccorhiza dermatodea*, on days 3 and 6 of recovery in dim white light (8 µmol photon m⁻² s⁻¹) after an exposure time of (a) 8 h amd (b) 17.5 h of photosynthetically active radiation (PAR=P), PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB). Insets are equivalent doses of PAR, UV-A, and UV-B. Vertical bars are standard deviations (SD, *n*=5). ANOVA and RMANOVA (*P* <0.001) showed a significant difference between treatments. DMRT (*P* <0.05) showed germination in (A) P=PA>PAB and (B) P>PA>PAB.

zoospores of *S. dermatodea* were also able to repair DNA damage effectively. The DNA repair mechanism can be mediated by light-dependent photolyases and/ or light-independent nucleotide excision repair (Pakker *et al.*, 2000). At approximately the same UV-B dose, DNA damage sustained by the upper sublittoral Arctic *S. dermatodea* zoospores is comparable with the upper sublittoral temperate *Laminaria digitata* from Helgoland (Roleda *et al.*, 2005*a*). The sensitivity of spores to UV-B-induced DNA damage and their capacity to repair DNA lesions can influence recruitment and the upper depth distribution limit of macroalgae (Roleda *et al.*, 2004, 2005*a*). After 16 h exposure and 2 d recovery, effective DNA repair was still observed in the cell, but an increase in exposure time inhibits germination. Increasing exposure time to PAB treatment from 16 h to 17.5 h (a 90 min increase in exposure time, with a corresponding increase in UV-B dose) has adverse impact on the cell viability, where the germination rate was observed to be very minimal.

The light microscopic data revealed that in the 8 h PA-treated spores, germination is comparable with P-treated

spores. This is also reflected by the high recovery of photosynthetic efficiency. Increasing exposure to PA, however, leads to cell damage with visible photo-destruction and increased mortality. The decrease in pigmentation is also evident in the absorption spectra taken after 23 h exposure to P, PA, and PAB. The chlorophyll peak at 675 nm and the xanthophyll peak at 440 nm are both reduced in zoospores after PA and PAB treatment. The increase in phlorotannin absorption at 265 nm in the filtrate of PA- and PAB-treated suspensions demonstrates the release of physodes to the medium. Under PAB treatment, enlarged physodes are formed, especially in cells without pigmentation prior to cell death. This was previously also described for zoospores of *L. digitata* after 24 h exposure to PAB (Wiencke *et al.*, 2004*b*). The protective function of phlorotannin has been reported in other *Fucus* embryos whereby fewer physodes in *F. serratus* influenced their susceptibility to UVR compared with *F. spiralis* (Schoenwaelder *et al.*, 2003). In Arctic Laminariales zoospores, an increase in number and size of phlorotannin-containing physodes was observed after UV exposure, which probably contributed to protection against cellular damage (Wiencke *et al.*, 2004*b*).

Considering the uneven localization of physodes within the cell, phlorotannin-containing physodes do not offer optimum protection against UV radiation for each single cell. In this context, it must be noted that the UV-protective function of cellular physodes is density dependent (Roleda *et al.*, 2006*b*). Different concentrations of zoospore suspensions attenuate UVR in Arctic *S. dermatodea* and *Alaria esculenta*, but not in *Laminaria digitata* (Roleda *et al.*, 2006*b*), thereby effectively reducing UV fluence before reaching other cells and physiological targets. For example, a plume of zoospores released into the water could serve as buffers protecting the lower layer of spores from excessive radiation. At the start of the experiment, free physodes were already observed in the medium, possibly released from the phlorotannin-rich paraphyses cells of the sori of the sporophytes, giving additional UV protection to the UV-sensitive kelp meiospores. Although an active release of phlorotannins from *S. dermatodea* zoospores into the medium was not microscopically observed, there is a clear increase in the phlorotannin content after exposure to PA and PAB compared with P and dark control as measured spectrophotometrically.

Higher germination rates were previously reported after laboratory exposure to the whole light spectrum in spores of *S. dermatodea* obtained from sporophytes collected in autumn (Wiencke *et al.*, 2004*b*). The lower germination rate reported in the present study could be attributed to the reproductive biology of the species. A new generation of the normally annual sporophytes is established from gamete-releasing gametophytes in spring. Vegetative growth of sporophytes occurs throughout summer, and sporogenesis begins during short days in autumn. The exception to this pattern occurs when sorus-bearing sporophytes survive

the winter under ice (Henry, 1988). Therefore, the fertile sporophytes present in autumn previously exposed and pre-conditioned to strong solar radiation during summer are naturally more robust compared with the over-wintered senescing sporophytes acclimated to darkness or low light collected in spring. Hence, the performance of spores is also dependent on the condition of the sporophytes.

In the field, no additional UVR effect was observed on the viability of the *S. dermatodea* zoospores. The germination rate was not significantly different after exposure to the P, PA, and PAB conditions (Wiencke *et al.*, 2006). Under high PAR in the field, UVR tolerance is enhanced by increasing activity of photorepair enzymes (Warner and Caldwell, 1983), where a 10-fold higher $BED_{50}$ is needed to inhibit germination of zoospores exposed to ambient solar radiation compared with artificial laboratory irradiation (Roleda, 2006). On the other hand, the high UVR:PAR ratio applied in the laboratory exhibits an additional UV-B effect on photosynthesis and germination (Wiencke *et al.*, 2000, 2004*b*; Roleda *et al.*, 2005*a*). A higher order of magnitude in the UVR:PAR ratio has been reported to intensify the UV effect on plants (Caldwell *et al.*, 1995; Rozema *et al.*, 1997). The difference in germination rate previously reported by Wiencke *et al.* (2004*b*) is attributed to the sampling month of the fertile sporophytes, which shows that there is seasonality in physiological performance of the spores. Vital spores can acclimate to a wide range of photon flux densities or photosynthetic efficiency is able to recover after photoinhibition. Ecologically, the recovery process will also be dependent on the settlement of spores in a suitable low-light environment. Prolonged exposure to high PAR can inhibit recruitment of kelps to shallow water by killing their post-settlement stages (Graham, 1996).

In conclusion, individual cells were found to be highly sensitive to UVR. The protective function of phlorotannins also seems to be dependent on the density of the spore suspension with restricted efficiency for a single cell. A plume of zoospores can, however, act as a buffer protecting the lower layer of spores from excessive radiation. Zoospore suspensions of *S. dermatodea* recover their photosynthetic functions after light stress (Roleda *et al.*, 2006*a*) while the DNA damage repair mechanism can enhance germination capacity. These protective and repair mechanisms should be coupled with avoidance strategies such as negative phototaxis, habitat selection (settlement under the canopy), seasonal reproduction, and timing of zoospore release (avoid release at midday) to minimize UV-induced damage and facilitate successful recruitment.

## Acknowledgements

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