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SENSITIVITY OF ANTARCTIC *UROSPORA PENICILLIFORMIS* (ULOTRICHALES, CHLOROPHYTA) TO ULTRAVIOLET RADIATION IS LIFE-STAGE DEPENDENT[1]

Michael Y. Roleda

Institute for Polar Ecology, Wischhofstraße 1-3, Bldg. 12, D-24148 Kiel, Germany

Gabriela L. Campana

CONICET, Instituto Antártico Argentino, Cerrito 1248 (C1010AAZ), CA de Buenos Aires, Argentina

Christian Wiencke[2]

Section Functional Ecology, Department Seaweed Biology, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Dieter Hanelt

Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-Str. 18, D-22609 Hamburg, Germany

Maria Liliana Quartino

Instituto Antártico Argentino, Cerrito 1248 (C1010AAZ), CA de Buenos Aires, Argentina

and Angela Wulff

Department of Marine Ecology, Marine Botany, Gothenburg University, Box 461, SE 40530 Gothenburg, Sweden

The sensitivity of different life stages of the eulittoral green alga *Urospora penicilliformis* (Roth) Aresch. to ultraviolet radiation (UVR) was examined in the laboratory. Gametophytic filaments and propagules (zoospores and gametes) released from filaments were separately exposed to different fluence of radiation treatments consisting of PAR (*P* = 400–700 nm), PAR + ultraviolet A (UVA) (PA, UVA = 320–400 nm), and PAR + UVA + ultraviolet B (UVB) (PAB, UVB = 280–320 nm). Photophysiological indices (ETR_{max}, E_k, and α) derived from rapid light curves were measured in controls, while photosynthetic efficiency and amount of DNA lesions in terms of cyclobutane pyrimidine dimers (CPDs) were measured after exposure to radiation treatments and after recovery in low PAR; pigments of propagules were quantified after exposure treatment only. The photosynthetic conversion efficiency (α) and photosynthetic capacity ($rETR_{max}$) were higher in gametophytes compared with the propagules. The propagules were slightly more sensitive to UVB-induced DNA damage; however, both life stages of the eulittoral inhabiting turf alga were not severely affected by the negative impacts of UVR. Exposure to a maximum of 8 h UVR caused mild effects on the photochemical efficiency of PSII and induced minimal DNA lesions in both the gametophytes and propagules. Pigment concentrations were not significantly different between PAR-exposed and PAR + UVR–exposed propagules. Our data showed that *U. penicilliformis* from the Antarctic is rather insensitive to the applied UVR. This amphi-equatorial species possesses different protective mechanisms that can cope with high UVR in cold-temperate waters of both hemispheres and in polar regions under conditions of increasing UVR as a consequence of further reduction of stratospheric ozone.

Key index words: **carotenoids; cyclobutane pyrimidine dimers; DNA damage; gametophytes; *P–E* curves; photosynthetic efficiency; pigments; propagules; rapid light curves; *Urospora penicilliformis*; UVR; xanthophyll**

Abbreviations: **CPD, cyclobutane pyrimidine dimer; PAM, pulse amplitude modulated; *P–E* curve, photosynthesis-irradiance curve; RLC, rapid light curves; UVR, ultraviolet radiation**

U. penicilliformis has a haplodiplontic and heteromorphic life history. It is distinguished by a uniseriate filamentous gametophyte with multinucleate cells and multicellular rhizoids and a unicellular, uninucleate *Codiolum*-like sporophyte (Hanic 2005, Lindstrom and Hanic 2005). Sexual reproduction is anisogamous with biflagellated gametes produced by unisexual filaments. Zygotes develop into stalked, free-living epilithic *Codiolum*-stage producing quadriflagellated zoospores. Asexual reproduction of gametophytes producing zoospores and

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[2]Author for correspondence: e-mail Christian.Wiencke@awi.de.

parthenogenetic development of gametes into *Codiolum* phase have also been observed (Nagata 1971, Lokhorst and Trask 1981, Hanic 2005).

Green algae form a conspicuous component of the intertidal community contributing a significant fraction of the total coastal primary production, of which ~34% of the macroalgal net primary production is consumed by herbivores (Duarte and Cebrián 1996). Occurrence of *U. penicilliformis* in the intertidal is not only affected by herbivory (Harley 2006) but also depends on its seasonal development. Maximum biomass is usually attained in spring (Rico and Gappa 2006).

Over Antarctica, the naturally occurring springtime ozone levels are ~25% lower than springtime ozone levels over the Arctic (Fahey 2003). As the stratosphere of the polar latitudes became affected by anthropogenic chlorine and bromine, an average net springtime ozone loss of 60%–70% has been a recurring phenomenon intensifying ambient UVB radiation on the biosphere (Herman et al. 1996). The mechanistic effect of elevated UVR on algae has been extensively studied in the laboratory, and numerous deleterious effects at the physiological, biochemical, and ultrastructural levels have been revealed (Holzinger and Lütz 2006). The species-specific sensitivity to UVR among other abiotic factors effectively shapes the algal zonation pattern in coastal benthic communities (Bischof et al. 2006).

UVR studies on macrothalli of green macroalgae have been performed under artificial laboratory conditions (Grobe and Murphy 1997, Holzinger et al. 2006) and in outdoor tank cultures or in situ (Grobe and Murphy 1994, Altamirano et al. 2000a,b, Bischof et al. 2002a). Interactive effects of PAR and temperature (Choo et al. 2004), PAR and nutrients (Henley et al. 1991), and UVR and temperature (Rautenberger and Bischof 2006) have also been investigated in adult stages. Among propagules, the impact of UVR as an environmental stress factor on photosynthesis and germination of Ulvales was studied (Cordi et al. 2001, Han et al. 2004). Studies on *U. penicilliformis* are limited to temperature adaptation and ultrastructural details and their implications on biogeography and taxonomy, respectively (Sluiman et al. 1982, Bischoff and Wiencke 1995).

Comparison between different early life-history stages of various kelp and kelp-like species and Gigartinales from the Northern Hemisphere showed that spores are more susceptible to UVR compared with their corresponding juvenile sporophytic and gametophytic life stages (reviewed by Roleda et al. 2007a and references therein). To our knowledge, no comparative physiological study in response to UV stress has been conducted in different life-history stages of any green macroalga, and particularly not in *Urospora*.

This study investigated the impact of UVR on the photosynthesis and DNA damage and repair in filamentous gametophytes and propagules (gametes and zoospores) released from specimens of the natural population of *U. penicilliformis* in King George Island (South Shetland Islands) off the Antarctic Peninsula. The impact of UVR on the photosynthetic pigments of propagules was investigated. We hypothesize that the propagules are more susceptible to UVR compared with the filamentous gametophytes. The sensitivity of propagules, as a recruit source of the macroscopic thalli, to UVR among other abiotic and biotic factors can impact the seasonal abundance of visible gametophytes in the Southern Hemisphere under the seasonal influence of the Antarctic Ozone Hole.

MATERIALS AND METHODS

Algal material. Filaments of *U. penicilliformis* attached to rocks were collected in spring (October 2004) by hand during low tide in the upper eulittoral of Barton Peninsula, King George Island (Antarctica, 62°13.46′ S, 58°42.33′ W). Several rocks with lush filaments were selected and assigned as different replicates.

In the laboratory, each rock was processed separately. Filamentous gametophytes were carefully gleaned off the rocks and were cleaned of epiphytes. Plants were immersed in 5–10 mL filtered (0.2 μm pore size) seawater at ±5°C and exposed under white light (±16 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) to induce release of propagules (Fig. 1). Biflagellated gametes and quadriflagellated zoospores, sexually produced by unisexual filaments and asexually produced by gametophytes, respectively, were observed. Hereafter, the mixture of gametes and zoospores will be collectively referred to as propagules (Fig. 1). Due to logistical constraints, the relative proportion of gametes to zoospores was not determined. The relative proportion was assumed to be the same between replicates and experiments. Freshly released propagules were collected and maintained under low light (±10 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) conditions. The initial cell density was counted by using Sedgewick-Rafter Cell S50 spore counter (Graticules Ltd., Tonbridge, England). Mean cell size (diameter = 6 μm and length = 20 μm) of freshly released propagules was measured under light microscope (Axioplan imaging; Zeiss, Jena, Germany). Microscopic pictures were taken with a digital camera (Canon PowerShot A80; Tokyo, Japan). Stock suspensions were diluted with filtered seawater to give densities between 4×10^3 and 5×10^3 propagules $\cdot$ mL^{-1} among the five replicates.

Irradiation treatments. White fluorescent tubes (L65 Watt/25S; Osram, Munich, Germany) and UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA) were used to provide PAR (400–700 nm) and UVR (280–400 nm), respectively. To cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes, cell culture dishes were covered with glass filter (gametophyte setup) or filter foil (propagule setup). The filters used were WG 280 or Ultraphan transparent (Digefra GmbH, Munich, Germany) (cutoff below 280 nm = PAR + UVA + UVB treatment), WG 320 or Folanorm (Folex GmbH, Dreieich, Germany) (cutoff below 320 nm = PAR + UVA treatment), and GG 400 or Ultraphan URUV farblos (cutoff below 400 nm = PAR treatment). The optical properties of the filters were characterized by Bischof et al. (2002a). These filter foils cut off wavelengths slightly differing from the UVR definition of CIE (Commission Internationale De l'Éclairage, UVB = 280–315 nm, UVA = 315–400 nm), but these are commonly used by environmental researchers due to the practical availability of filter material.

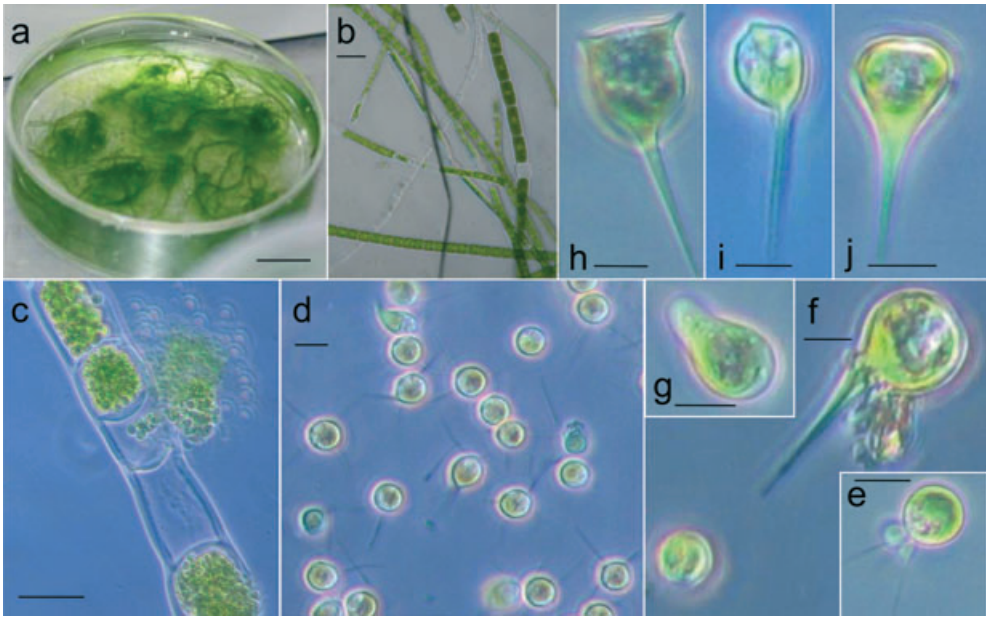

FIG. 1. *Urospora penicilliformis*. (a, b) Vegetative filaments, (c) sporulating filament, (d–f) reproductive cells (mixture of zoospores and gametes) and germling (g). Mature vegetative cell and reproductive cell structures are as described by Nagata (1971), Lokhorst and Trask (1981), Sluiman et al. (1982), and Hanic (2005). Zoospore (h) four-flagellated quadrate acuminate with anterior hooks. Gametes biflagellate; male gamete (i) ovoid to irregularly spindle-shaped and female gamete (j) ovoid-elliptical and larger than male. Mean reproductive cell (propagule) size is 6 ± 1.3 μm diameter and 20 ± 5.5 μm length (n = 196 and 89 cells, respectively). Scale bars: (a) = 1 cm, (b) = 300 μm, (c) = 100 μm, and (d–j) = 5 μm.

UVR was measured using a Solar Light PMA 2100 radiometer equipped with the UV-A Sensor PMA 2110 and the UV-B Sensor PMA 2106 (Solar Light, Philadelphia, PA, USA). Adjusted UVR below the cutoff filters was 4.34 W · m^{-2} UVA and 0.40 W · m^{-2} UVB. The available PAR measured using a cosine quantum sensor attached to a LI-COR data logger (LI-1000; LI-COR Biosciences, Lincoln, NE, USA) was 22 μmol photons · m^{-2} · s^{-1} (~4.73 W · m^{-2}).

Chl fluorescence measurements. Photosynthetic activities of filamentous gametophytes and propagule suspension were determined by measuring the variable chl fluorescence of PSII. Rapid photosynthesis (in terms of relative electron transport rate, rETR = PFR × $\Delta F/F'_m$) versus irradiance (E) curves (P–E curve) of single filament and propagule suspension were measured in triplicates using a Water PAM device (Walz, Effeltrich, Germany) described by Roleda et al. (2006a). Low and high actinic light intensities making up 12 points (17–1,458 μmol photons · m^{-2} · s^{-1}) were used. The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate P–E curve parameters described as follows:

$$\mathrm{rETR} = \mathrm{rETR}_{max} * \tanh(\alpha * E_{PAR} * \mathrm{rETR}_{max}{}^{-1}) \quad (1)$$

where rETR_{max} is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the electron transport efficiency, and E is the photon fluence rate of PAR. Curve fit was calculated with the Solver Module of MS-Excel (Microsoft Corp., Redmond, WA, USA) using the least squares method comparing differences between measured and calculated data (Roleda et al. 2006a). The saturation irradiance for electron transport (E_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote (rETR_{max}).

Approximately 10 g wet weight of filaments was spread out evenly in culture dishes (45 mm × 10 mm) with filtered seawater and covered with respective filters representing the P, PA, and PAB treatments. Effective quantum yield ($\Delta F/F'_m$, n = 9) was measured using a Diving PAM device (Walz), at initial (07:00 h); at 1, 2, 4, and 8 h after the start of UV exposure (09:00, 10:00, 12:00, 16:00 h); and at the same time interval after the end of UV exposure (17:00, 18:00, 20:00, 00:00 h).

Immediately after adjustment of propagule density, the suspension was filled into 5 mL Quartz cuvettes, and the optimum quantum yield (F_v/F_m) was measured using a Water PAM device (n = 5). After 3 min dark incubation, F_o was measured with a red measuring light pulse (~0.3 μmol photons · m^{-2} · s^{-1}, 650 nm), and F_m was determined with a 800 ms completely saturating red light pulse (~2,750 μmol photons · m^{-2} · s^{-1}). Controls measured at time zero were filled into corresponding culture dishes (35 mm × 10 mm, Corning™; Corning Inc., Corning, NY, USA). To evaluate the effect of different radiation treatments (three levels: P, PA, and PAB) and exposure times (four levels: 1, 2, 4, and 8 h), samples of fresh propagule suspension (not exceeding 1 h after release) were filled into each of the culture dishes (total experimental units = 60). Samples corresponding to the five replicates were exposed to each treatment combination of radiation and exposure time at 2 ± 1.5°C. After treatments, F_v/F_m was determined, and the suspension was returned to the same culture dish and cultivated under dim white light (4 ± 1 μmol photons · m^{-2} · s^{-1}) at the same temperature for recovery. Time zero control was also maintained at the same condition. Measurements of photosynthetic recovery were made after 24 h in dim white light condition. Germinating cells were slowly resuspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes.

DNA damage and repair. The number of DNA lesions in terms of cyclobutane pyrimidine dimers (CPDs) was measured after exposure to the whole light spectrum (PAR + UVA + UVB = PAB) and after postcultivation under PAR only. From the gametophyte-containing setup, PAB-exposed

filaments (~5 mg) were harvested after 2, 4, and 8 h exposure to PAB treatment. Repair of DNA lesion was determined in samples after 2 and 4 h postcultivation under PAR only (22 μmol photons · m^{-2} · s^{-1}). From the propagule suspension, 40 mL (4×10^5–5×10^5 propagules · mL^{-1}) was used for each experimental unit. For each treatment, six experimental units were prepared. After the irradiation treatment, three experimental units (corresponding to the three replicates) were processed immediately, while the other three (parallel of the three replicates) were allowed to recover for 24 h in low white light (4 ± 1 μmol photons · m^{-2} · s^{-1}) before processing. Germinating cells were resuspended from the bottom of the petri dishes by jetting pressurized seawater from a wash bottle. The suspensions were filtered through 44 mm diameter 1.0 μm pore-size Nuclepore® polycarbonate membrane (Whatman, Maidstone, Kent, UK). Blotted dry filaments and propagule-containing filters were individually filled into 2 mL Eppendorf tubes and frozen at −80°C until further analysis.

DNA was extracted using 2% CTAB extraction buffer and quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, Palo Alto, CA, USA) (Roleda et al. 2005). The accumulation of cyclobutane pyrimidine dimers (CPDs) was determined following a two-step antibody assay using antithymine dimer H3 (Affitech, Oslo, Norway) and rabbit antimouse immunoglobulins (conjugated with horseradish peroxidase, DakoCytomation, Glostrup, Denmark). Chemiluminescent detection was subsequently performed using ECL Western blotting detection reagent (Amersham, Buckinghamshire, UK). 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) (Roleda et al. 2005). A calibration series of UV-irradiated calf thymus DNA (Serva Electrophoresis GmbH, Heidelberg, Germany) 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 (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Pigment extraction and HPLC analyses. Preparation of reproductive cell suspensions followed the above procedure for DNA damage. After 8 h exposure to different radiation treatments, the suspensions (control and treated) were filtered through 44 mm diameter 1.0 μm pore-size Nuclepore® polycarbonate membrane (Whatman) and frozen in liquid nitrogen. The filters were put in 4.5 mL 100% MeOH, and the solution with the filter was sonicated for 45 s using a Vibra-cell sonicator equipped with a 3 mm diameter probe (cf. Wright and Jeffrey 1997). The HPLC-analysis continued according to Wright and Jeffrey (1997), using an absorbance diode-array detector (Spectra-Physics UV6000LP; Newport Corp., Spectra-Physics Finnigan, Thermo Fisher Scientific, Waltham, MA, USA). The columns used were a 150 × 3.20 mm C18 Phenomenex (Ultracarb 3 μm ODS [20]; Phenomenex, Torrance, CA, USA) and a 4 × 3.0 mm C18 guard column (SecurityGuard; Phenomenex). The HPLC system was calibrated with pigment standards from DHI, Water and Environment, Denmark. Peak identities were further confirmed by an online recording of absorbance spectra (400–700 nm) described in Wright and Jeffrey (1997). Dominating pigments detected were neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxantin, chl *b*, chl *a*, and betacarotene. Pigments are expressed as ratios to chl *a* (weight/weight).

Statistical analysis. Data were tested for homogeneity (Levene Statistics) of variance. Corresponding transformations (square roots) were made to heteroskedastic data. Response variables ($\Delta F/F'_m$, F_v/F_m, CPD, and pigments) were tested using analyses of variance (repeated measure analysis of variance, RMANOVA, and one-way ANOVA; $P < 0.05$) followed by Duncan's multiple range test (DMRT, $P < 0.05$). Statistical analyses were performed using SPSS software (Chicago, IL, USA).

RESULTS

P–E curve parameters (Fig. 2) showed significantly higher (ANOVA, $P < 0.05$) saturating irradiance (E_k) in gametophytes (252 μmol photons · m^{-2} · s^{-1}) compared with propagules (87 μmol photons · m^{-2} · s^{-1}) of *U. penicilliformis* (hereafter called *Urospora*). The light-harvesting performance and photosynthetic conversion efficiency (α) and photosynthetic capacity ($rETR_{max}$) were likewise significantly higher (ANOVA, $P < 0.05$) in gametophytes (α = 0.175, R^2 = 0.86; $rETR_{max}$ = 44) compared with

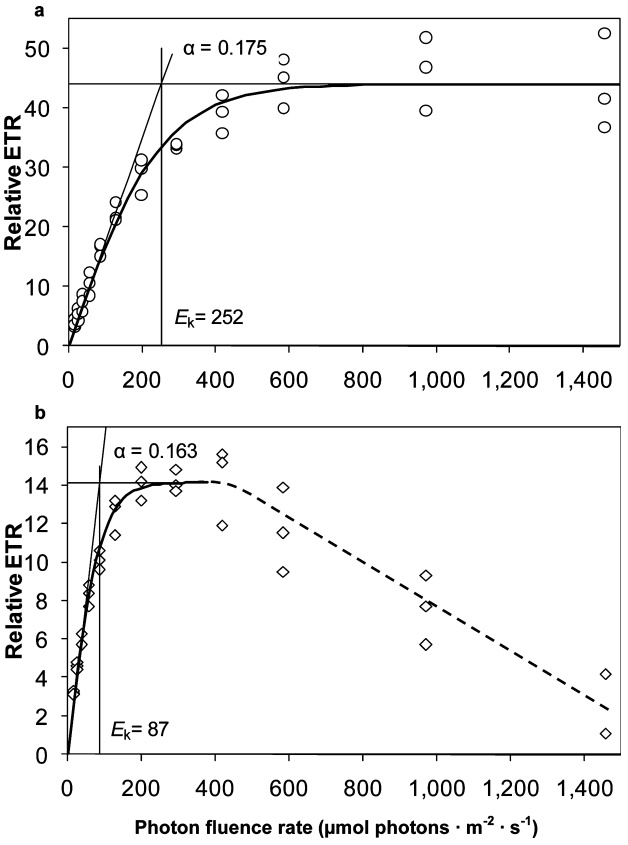

FIG. 2. Rapid photosynthesis-irradiance (*P–E*) curves of gametophytic filament (a) and propagules (b; zoospores and gametes) from *Urospora penicilliformis* (n = 3). PFR is the respective photon fluence of actinic light, and ETR is the electron transport rate. Saturating irradiance (E_k) is estimated as the point at which the initial slope (α) crosses the maximum photosynthesis ($rETR_{max}$) using the hyperbolic tangent model of Jassby and Platt (1976). Maximum effective quantum yields are 0.687 ± 0.03 and 0.501 ± 0.04 for filament and propagules, respectively.

propagules ($\alpha = 0.163$, $R^2 = 0.95$; $rETR_{max} = 14$). During *P–E* curve measurements, $rETR_{max}$ in gametophytes did not decrease until the highest actinic light level of 1,458 μmol photons · m^{-2} · s^{-1} was reached, whereas the $rETR_{max}$ of propagules decreased above 419 μmol photons · m^{-2} · s^{-1}.

Exposure to PAR did not influence the effective quantum yield ($\Delta F/F'_m$) of filamentous gametophytes. After 1 h exposure under PAR + UVR, a 12% reduction in $\Delta F/F'_m$ was observed in both the PA- and PAB-exposed gametophytes relative to P treatment (Fig. 3). After 6 h, photoinhibition of photosynthesis was 25% and 29% in PA and PAB treatments, respectively. The PA- and PAB-exposed gametophytes were observed to recover 94% of their photosynthetic function already 1 h after UV lamps were switched off. Further increase in $\Delta F/F'_m$ was observed during the course of the day and in darkness. RMANOVA ($P < 0.05$) showed a significant effect of radiation treatment on $\Delta F/F'_m$ (Table 1). Gametophytes exposed to PA and PAB were, however, not significantly different from each other (DMRT, $P = 0.05$; PA = PAB < *P*).

After exposure to different fluence of PAR (as a function of exposure time), the optimum quantum yield (F_v/F_m) of propagules decreased slightly but was not significantly different between control and P-treated propagules (Fig. 4a). Exposure to light supplemented with UVR significantly decreased the optimum quantum yields (31%–36% in PA and 33%–43% in PAB) of propagules relative to P treatment (ANOVA, $P < 0.001$; Table 1), but no significant difference were observed in F_v/F_m of UVR-treated propagules (DMRT, $P = 0.05$, PAB = PA < *P*). The increasing fluence of the different light treatments also did not exhibit significant effects on the F_v/F_m of propagules (ANOVA, $P = 0.515$).

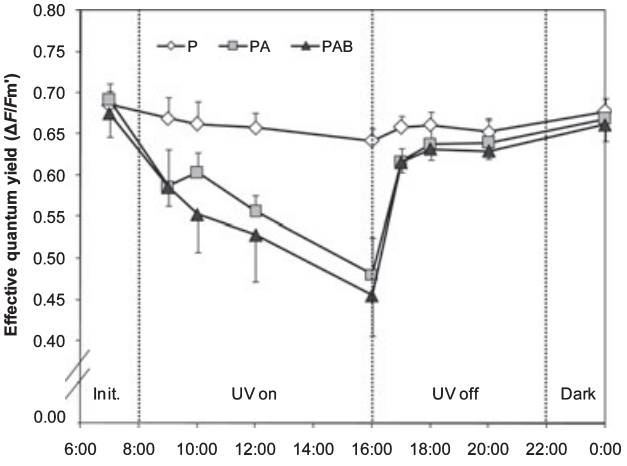

FIG. 3. Mean effective quantum yield ($\Delta F/F'_m$) in gametophytic filaments of *Urospora penicilliformis* exposed to PAR (P), PAR + UVA (PA), and PAR + UVA + UVB (PAB) during the light (with and without UVR supplement) and dark phase of the day. Photon flux density (PFD) is 22 μmol photons · m^{-2} · s^{-1}. Vertical bars are standard deviations (SD, $n = 9$). Corresponding statistical analysis is shown in Table 1.

TABLE 1. Analysis of variance (repeated measure, RMANOVA, and one-way ANOVA) and significance values for the main effects and interaction of main factors (radiation treatment and exposure time) on the photosynthesis of *Urospora penicilliformis* exposed to different radiation treatments.

Experiment/variable	Source of variation	df	*F*-value	*P*-value
Photosynthesis				
Filaments				
$\Delta F/F'_m$ (after treatment)	Radiation	2	52.729	<0.001*
$\Delta F/F'_m$ (after recovery)	Radiation	2	12.277	<0.001*
Propagules and germlings				
F_v/F_m (after treatment)	Radiation (A)	2	95.347	0.001*
	Exposure time (B)	3	0.772	0.515^{ns}
	A × B	6	0.448	0.843^{ns}
F_v/F_m (after recovery)	Radiation (A)	2	6.953	0.002*
	Exposure time (B)	3	4.893	0.005*
	A × B	6	0.715	0.639^{ns}
Propagules and germlings				
DNA damage	UVB dose	2	47.770	<0.001*
DNA damage repair	UVB dose	2	2.614	0.153^{ns}
Filaments				
DNA damage	UVB dose	2	9.782	<0.013*

*, significant; ns, not significant.

Radiation treatments consist of PAR (P), PAR + UVA (PA), and PAR + UVA + UVB (PAB).

After 24 h recovery in dim white light, optimum quantum yields of germlings of previously untreated (control) and treated propagules increased (Fig. 4b). Recovery of photosynthesis was higher in UVR-pretreated propagules compared with PAR-treated alone (ANOVA, $P = 0.002$, DMRT, $P = 0.05$, PAB = PA > *P*). Higher photosynthetic recovery was also observed in germlings preexposed to higher fluence of light treatments (ANOVA, $P = 0.005$, DMRT, $P = 0.05$, 4 h = 8 h ≥ 2 h ≥ 1 h).

DNA damage in terms of CPD formation was 12 to 15 times higher in propagules (2.2–5.4 CPD · Mb^{-1}) compared to the filamentous gametophyte (0.2–0.5 CPD · Mb^{-1}) of *Urospora* (Fig. 5). The amount of CPD significantly increased with increasing UVB dose in both life stages (ANOVA, $P < 0.05$) investigated. After 24 h under photoreactivating light, the germlings were able to repair 44%–61% of the DNA damage (Fig. 5a). The remaining DNA damage (1.2–2.1 CPD · Mb^{-1}) in propagules preexposed to different UVB doses was not significantly different after recovery. In gametophytic filaments, the CPDs accumulated after 8 h exposures were repaired by 71% already after 2 h recovery in photoreactivating light. A further 36% repair of DNA lesions was observed after 4 h (Fig. 5b).

Pigment composition and concentrations were only characterized in propagules due to accidental loss of filament samples during transport. The protocol utilized for HPLC analysis of pigment extracts allowed separation of cellular chl and six carotenoids characteristic of chlorophytes (Table 2). Traces of fucoxanthin and diadinoxanthin were also detected in the chromatogram that could be

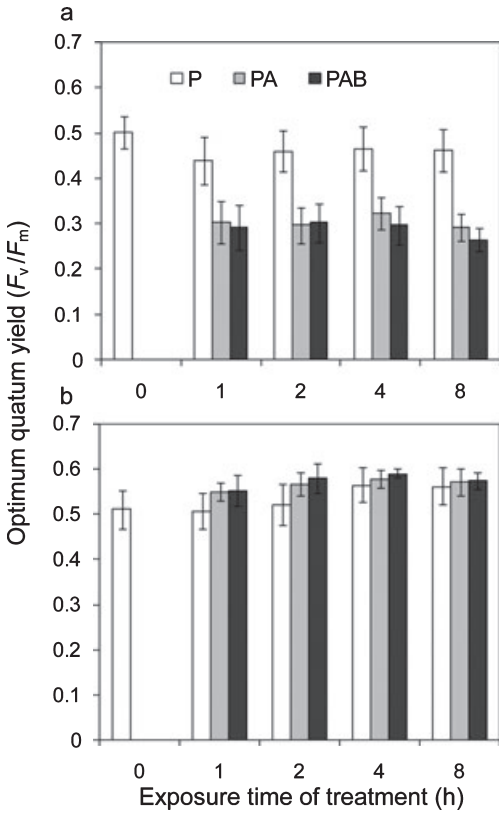

FIG. 4. Mean optimum quantum yield (F_v/F_m) in propagules of *Urospora penicilliformis* during treatment (a) to PAR (P), PAR + UVA (PA), and PAR + UVA + UVB (PAB) at different exposure times. Corresponding photosynthetic recovery of germlings (b) was measured after 24 h postculture in dim white light (4 μmol photons · m^{-2} · s^{-1}). Vertical bars are standard deviations (SD, $n = 5$). Analysis of variance (ANOVA) is presented in Table 1.

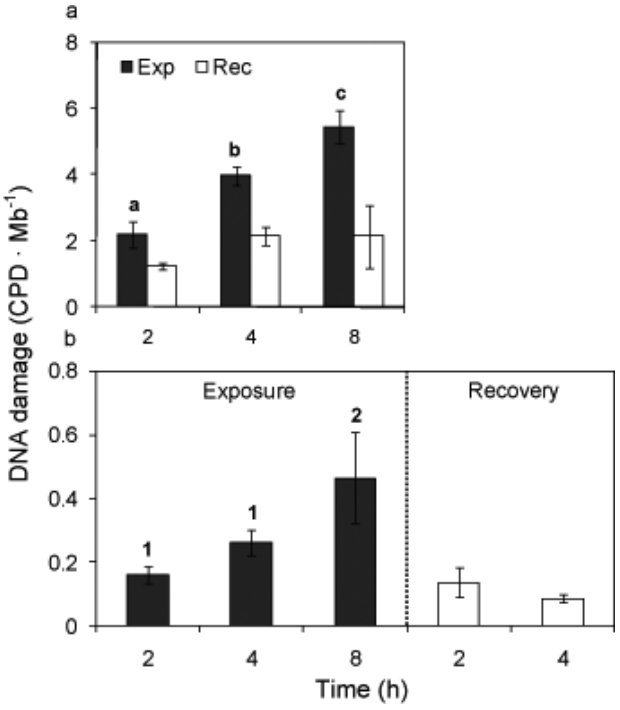

FIG. 5. UVB-induced DNA damage (cyclobutane pyrimidine dimers [CPD] per million nucleotides) in propagules (a) and gametophytic filaments (b) of *Urospora penicilliformis* after exposure to increasing time of PAR + UVA + UVB (shaded bars) and remaining DNA damage after postcultivation under PAR only (open bars). DNA damage repair in propagules was determined after 24 h recovery vis-à-vis exposure treatment; gametophytes were exposed in time series up to a maximum of 8 h and allowed to recover for 2 and 4 h. Vertical bars are standard deviations (SD, $n = 3$). Notations on graph show result of post hoc Duncan's multiple range test (DMRT, $P = 0.05$) after analysis of variance (ANOVA); different letters and numbers refer to significant difference between treatments. No significant difference in CPD concentrations after recovery was detected.

TABLE 2. Concentration of major photosynthetic and accessory pigments in propagules (zoospores and gametes) of *Urospora penicilliformis* after release and 8 h exposure to different radiation treatment consisting of PAR (P) only, PAR + UVA (PA), and PAR + UVA + UVB (PAB).

	Treatment			
Pigments (ratio to chl *a* [w/w])	Control	*P*	PA	PAB
Neoxanthin	0.12 (±0.02)	0.09 (±0.04)	0.10 (±0.05)	0.09 (±0.03)
Violaxanthin	0.08 (±0.01)	0.07 (±0.02)	0.09 (±0.04)	0.06 (±0.02)
Antheraxanthin	0.03 (±0.01)	0.04 (±0.01)	0.05 (±0.02)	0.04 (±0.01)
Lutein	0.23 (±0.03)	0.19 (±0.06)	0.23 (±0.07)	0.21 (±0.07)
Zeaxanthin	0.06 (±0.01)	0.07 (±0.02)	0.09 (±0.05)	0.06 (±0.02)
Chl *b*	1.44 (±0.16)	1.17 (±0.35)	1.34 (±0.48)	1.24 (±0.52)
Chl *a*	1.00 (±0.00)	1.00 (±0.00)	1.00 (±0.00)	1.00 (±0.00)
Betacarotene	0.14 (±0.02)	0.10 (±0.03)	0.10 (±0.02)	0.09 (±0.03)
Chl *a*/chl *b*	0.70 (±0.07)	0.90 (±0.25)	0.69 (±0.15)	0.93 (±0.45)
Carotenoids	0.66 (±0.08)	0.54 (±0.16)	0.66 (±0.25)	0.54 (±0.18)
Chl/Car	3.70 (±0.26)	4.12 (±0.54)	3.67 (±0.57)	4.21 (±0.43)

One-way analysis of variance (ANOVA) showed no significant difference between treatment and control.

attributed to minor diatom contamination. Different radiation treatment had no significant impact on reproductive cells' pigment concentrations in relation to chl *a*.

DISCUSSION

This study showed that the green macroalga *U. penicilliformis* occurring in the eulittoral was not severely affected by the negative impacts of artificial UVR. Exposure to a maximum of 8 h UVR caused mild effects on the photochemical efficiency of PSII and induced minimal DNA damage in both the filament (gametophyte) and propagules (zoospores and gametes). The major pigment composition in propagules of a green macroalga was first reported here, where concentrations were not negatively influenced by experimental radiation treatments.

The photosynthetic parameters (E_k and α) showed that photosynthesis of *Urospora* propagules is shade adapted compared with the adult plants. Low-light adaptation is observed to be the general characteristic feature of propagules of macroalgae (Amsler and Neushul 1991, Roleda et al. 2004, 2005, 2006a,b, 2007b, 2008, Wiencke et al. 2007, Zacher et al. 2007). This finding might be related to different chl antenna size and chloroplast number and integrity. Mature vegetative cells (filamentous gametophytes) of *Urospora* are multinucleate, while zoospores and gametes contain only one chloroplast, with the male gametes having a poorly developed chloroplast and a fast swimmer, and the female gametes having a well-developed chloroplast and distinct eyespot but moving slower (Lindstrom and Hanic 2005). On the other hand, zoospore chloroplast extends for a considerable length into the tail, which is devoid of organelles like mitochondria and Golgi bodies (Sluiman et al. 1982). Survival of macroalgal recruits will therefore be dependent on the diel pattern of propagule release (spores and gametes) and their immediate settlement on substrate at depths, under algal canopies, or interjacent bushy turf algae where the prevailing low-light microenvironment will be suitable for their germination.

Zoospores of *Urospora* are specialized not only in structure (unique flagellar apparatus, cf. Sluiman et al. 1982) but also in behavior. A few minutes after release, *Urospora* zoospores are observed to swim downward and start gliding on the surface of the microscope slide that may well be facilitated by the secretion of mucilage that covers the cell surface of the zoospore and the tip of the tail (Sluiman et al. 1982). Motile propagules have the opportunity to escape from high PAR and UVR, which is the case of *Urospora* propagules. Production of mucilage may also protect cells against short wavelengths. In another mucilage-producing unicellular green alga, *Micrasterias denticulata*, measurements on isolated slime produced by the algae showed strong absorption in the UVB range (Lütz et al. 1997). The thick mucilage layer, which is secreted through cell wall pores and surrounds the *Micrasterias* cell, was speculated to have a similar function and contain similar compounds as in cyanobacteria (Garcia-Pichel and Castenholz 1991).

Exposure to PAR did not affect photosynthesis of *Urospora* gametophytes and propagules. Previous studies on polar macroalgae showed a PAR dose-dependent decrease in optimum quantum yield in propagules of sublittoral species (Roleda et al. 2006a,b, 2007b, 2008, Wiencke et al. 2007) but not in the eulittoral *Adenocystis utricularis*, *Monostroma hariotii* (Zacher et al. 2007), and *Urospora* (this study). Reduction in photosynthetic efficiency was observed only when filaments and propagules were exposed to light supplemented with UVA and UVA + UVB. However, no significant additional UVB effect was observed. Photoinhibition of photosynthesis under UVR can be attributed to possible damage to the oxidizing site and reaction center of the PSII (Grzymski et al. 2001, Lesser et al. 2002) and decreased activity of the primary carbon fixation enzyme RUBISCO (Bischof et al. 2000, 2002b). A fast turnover of D1 protein may be responsible for the fast reversible photoinhibition of photosynthesis in eulittoral macroalgae such as *Urospora* observed in this study. As photoprotection, epidermal UVR screening in higher plants has been reported to provide UVB protection by UV-absorbing compounds located in the epidermis (Bilger et al. 2001, Nybakken et al. 2004). Cell walls of *Urospora* filaments are microfibrillar, two-layered, outermost electron-dense, and covered by a thin gelatinous layer, while plasmalemma of zoospores is covered with granular to fibrillar material that may well be mucilage (Sluiman et al. 1982, Lindstrom and Hanic 2005). Whether an active UV-screening substance is localized on the cell wall of *Urospora* remains unknown.

The traces of fucoxanthin and diadinoxanthin most likely originated from diatoms that are epiphytic on the filaments. Although the filaments were thoroughly cleaned in filtered seawater, a few diatom cells could have been introduced into the propagule suspension. Such contamination could not be totally avoided since we were working with field-collected materials. We are, however, confident that the diatom density was minimal and did not significantly contribute to the photosynthetic parameters measured.

It is noteworthy that the concentrations of all major pigments in propagules of *Urospora* were not significantly different between treatments and to the control. Photoinhibition of photosynthesis under UVR was, therefore, not correlated to pigment concentration, which implied a transient UVR effect on photosynthetic efficiency. Consequently, the concentration of the xanthophyll pigments, known to contribute some photoprotective function during light stress (e.g., Laurion et al. 2002), did not increase

under UVR; contrary to the xanthophyll cycle reported in other UVR-stressed macroalgae (e.g., Fredersdorf and Bischof 2007). A similar response showing dynamic recovery in photoinhibition of photosynthesis and insignificant difference in pigment concentration between PAR and PAR + UVR treatments was also observed in Arctic *U. penicilliformis* gametophytes (filaments) exposed to a higher dose of PAR and UVR (Roleda et al. 2009). This finding shows that the mechanism of UV-stress tolerance and/or resistance in eulittoral green turf algae periodically exposed to multiple stresses (i.e., high PAR and UVR, elevated temperature, osmotic stress and desiccation) is not yet clear and needs further study. For example, under desiccation, certain cyanobacteria are able to deactivate PSII activity and dissipate light energy absorbed by pigment-protein complexes to avoid photodamage (Fukuda et al. 2008). Desiccation also increases thermotolerance in intertidal marine algae exposed periodically on a daily basis during low tide (Hunt and Denny 2008, and references therein). Despite the beneficial role of desiccation reported in several physiological studies, it is thought to cause disruptive effects by most intertidal ecologists.

Among Antarctic macroalgal reproductive cells exposed to a comparable UVB dose, significantly lower DNA damage was observed in propagules of *Urospora* compared with the gametes of the sublittoral *Ascoseira mirabilis* (Roleda et al. 2007b) but comparable with tetraspores and carpospores of the subtidal *Gigartina skottsbergii* (Roleda et al. 2008). Moreover, propagules of the eulittoral *Adenocystis utricularis* and *Monostroma hariotii*, and the upper eulittoral *Porphyra endiviifolium* sustained lesser to nondetectable DNA lesions, respectively (Zacher et al. 2007). Sensitivity of different propagules to UVR leading to DNA damage is not only related to depth distribution of the adult plants but also to the size of the propagules. Among kelps, the reported prevalence of larger, more UV-tolerant meiospores originating from species or populations from sites exposed to high UV radiation suggests that kelp meiospores are preadapted to the UV conditions of the parent plant (Swanson and Druehl 2000, Roleda et al. 2005, 2006a, Wiencke et al. 2006).

Filamentous *Urospora* gametophytes sustained significantly less DNA damage compared with propagules exposed to the same UVB dose. In multicellular filaments, the relatively thick cell walls may be able to selectively filter short UV-wavelengths from reaching the UV-sensitive targets (i.e., chloroplast and nucleus) compared with the ''naked'' propagules. Aside from UV-screening by cell walls, intracellular mechanisms (e.g., UV-absorbing compounds) are also important for UV protection. UV-absorbing compounds include mycosporine-like amino acids (MAAs), physode-bound phlorotannins, and scytonemin, which partially provide UVA and UVB screening in Rhodophyta, Phaeophyceae, and cyanobacteria, respectively (Karsten et al. 1998, Schoenwaelder 2002, Franklin et al. 2003).

Possible UV-absorbing compounds in green macroalgae, if present, are not well studied. Excretion of 3,6,7-trihydroxycoumarin, a group of UV-absorbing compounds with maximum absorption at 332–348 nm, was previously described in the green alga *Dasycladus vermicularis* (class Ulvophyceae) (Gómez et al. 1998, Pérez-Rodríguez et al. 1998, 2001, 2003), which cannot sufficiently provide UVB-screening protection. Another UV-absorbing compound identified as 324 nm-MAA occurs only in green macroalgae belonging to class Trebouxiophyceae and absent in members of Ulvophyceae and Chlorophyceae (Karsten et al. 2005, 2007). The phylogenetic pattern observed in MAA synthesis suggests that another type of UV-absorbing compound may be present in *Urospora*. Absorption spectra of methanol extracts from thalli of *Ulva pertusa* (class Ulvophyceae) showed strong absorption below 300 nm (Han and Han 2005). The presumptive UV-absorbing compound, however, remains to be characterized.

Induction of UVB-absorbing flavonoids in terrestrial plants by UVR is well studied. Flavonoids are products of phenolic polymer metabolism that occur in gymnosperms and angiosperms but are lacking in most algae. Simple phenolics, however, occur in lower plants and act as UV filters (Rozema et al. 1997). A study on UV-induced changes in gene expression of marine macroalgae recently detected an up-regulation in the expression of genes encoding for chalcon synthase, a key enzyme involved in flavonoid synthesis, in the green macroalgae *Acrosiphonia* sp. (class Ulvophyceae) after exposure to enhanced UVB radiation (Kremb 2007).

This study confirmed our hypothesis that propagules are more susceptible to the negative impacts of UVR compared with adult life stages. However, green turf algae inhabiting the upper- to mid-eulittoral, like *Urospora*, are generally tolerant to UVR. Previously, the photosynthetic performance of the supralittoral green macroalgae *Prasiola crispa* under UVR was determined to be mildly affected, and the ultrastructure under UVR was not significantly altered compared with the control (Holzinger et al. 2006).

Considering the 25% lower naturally occurring springtime ozone levels over Antarctica compared with the Arctic (Fahey 2003), eulittoral Antarctic macroalgae can be exposed to a higher intensity of solar UVB radiation compared with their Northern Hemisphere counterparts. The applied UV treatment in this experiment is relatively moderate compared with the daily springtime UV dose recorded at Dallmann Laboratory/Jubany Station (King's George Island), which ranges from 300 to 600 $kJ \cdot m^{-2}$ UVA and 10 to 20 $kJ \cdot m^{-2}$ UVB (Richter et al. 2008). *Urospora* distributed mostly in

cold temperate waters of both hemispheres, and also in Arctic and Antarctic seas inhabiting hard substrates in the middle-upper intertidal zone and the splash zone, is adapted to cope with naturally high UV radiation. Even with a high UVR:PAR ratio, which is suggested to exaggerate UVR effects on some macroalgae (cf. Fredersdorf and Bischof 2007), the data presented demonstrate that *U. penicilliformis* from the Antarctic is rather insensitive to the applied artificial irradiation treatment. The photoprotective mechanisms involved, whether physical or biochemical, however, remain to be elucidated. Further study using higher irradiation intensities is necessary to describe a more realistic scenario.

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