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Susceptibility of spores of different ploidy levels from Antarctic *Gigartina skottsbergii* (Gigartinales, Rhodophyta) to ultraviolet radiation

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Haploid tetraspores and diploid carpospores from Antarctic *Gigartina skottsbergii* were exposed in the laboratory to photosynthetically active radiation (400–700 nm = P), P + ultraviolet (UV)-A radiation (320–700 nm = PA) and P + UV-A + UV-B radiation (280–700 nm = PAB). Photosynthetic performance, DNA damage and repair, spore mortality, and an initial characterization of the UV-absorbing mycosporine-like amino acids (MAAs) were studied. Rapid photosynthesis vs irradiance (*E*) curves of freshly released spores showed that both tetraspores and carpospores were low-light adapted (E_k = 44 ± 2 and 54 ± 2 µmol photons m^{-2} s^{-1}, respectively). The light-harvesting and photosynthetic conversion efficiencies were similar (α = 0.13), whereas photosynthetic capacity in terms of optimum quantum yield (F_v/F_m) and relative electron transport rate ($rETR_{max}$) were significantly higher in carpospores. Photoinhibition and recovery of photosynthesis were not significantly different between spore ploidy but were significantly affected by radiation and exposure time treatments. Accumulation of DNA damage was UV-B dose dependent and significantly higher in tetraspores than in carpospores. After 2 days postcultivation, DNA lesions were completely repaired in spores exposed to UV-B dose less than 1.2×10^4 J m^{-2}. The dynamic recovery of photosynthetic capacity as well as effective DNA repair mechanism contributed to the relatively low spore mortality (4–14%). A substantial amount of UV-screening MAAs shinorine and palythine were observed for the first time in spores of Gigartinales. This study on stress and physiological characterization of seaweed propagules is important to understand recruitment dynamics and life history phase dominance in the field.

KEY WORDS: Carpospore, Cyclobutane pyrimidine dimer, DNA damage repair, Life history, Mycosporine-like amino acids, Optimum quantum yield, P-E curve, Tetraspore

INTRODUCTION

Gigartina skottsbergii Setchell *et* Gardner is one of the main raw material sources for the carrageenan industry in Chile (Buschmann *et al.* 2001). This species occurs in the subtidal and is confined to southern South America and the Antarctic region, and the northern limit is at about 40°S (Wiencke & tom Dieck 1990; Ramirez & Santelices 1991). Biomass production may potentially be negatively affected by solar ultraviolet radiation (UVR, 280–400 nm). The loss of stratospheric ozone over Antarctica during the past two decades has aroused concern about the effects of increased solar UV-B radiation on marine ecosystems.

Exposure to solar UVR has numerous deleterious effects on different life history stages of macroalgae. It can reduce productivity, affect reproduction and development, and increase mutation rate. Consequently, it can change the performance of the species in the field and influence the community structure (Bischof *et al.* 2006). Comparison between different early life history stages of various Laminariales and Gigartinales from the Northern Hemisphere showed that spores are more susceptible to UVR than their corresponding juvenile sporophytic and gametophytic life stages (Roleda *et al.* 2007a). However, no study has focused on the UVR susceptibility of spores from different life history phases of a specific red macroalga. The relative susceptibility of the different spore ploidy levels might play an important role in the development and spatial frequency distribution of the respective adult life history phases or influence life history phase dominance within a population under the annual influence of the Antarctic ozone hole and the general thinning of the ozone layer.

This study investigated the impact of UVR on the photosynthesis, cell structure, DNA damage, and repair in tetraspores and carpospores isolated from different life history phases of a natural population of *G. skottsbergii* in King George Island (South Shetland Islands, West Antarctic). Initial characterization of mycosporine-like amino acids (MAAs) present in tetraspores of this species was shown for the first time. We hypothesized that diploid spores, which may benefit from genetic buffering conferring better cellular regulation, are more vigorous and tolerant to environmental stress than haploid spores.

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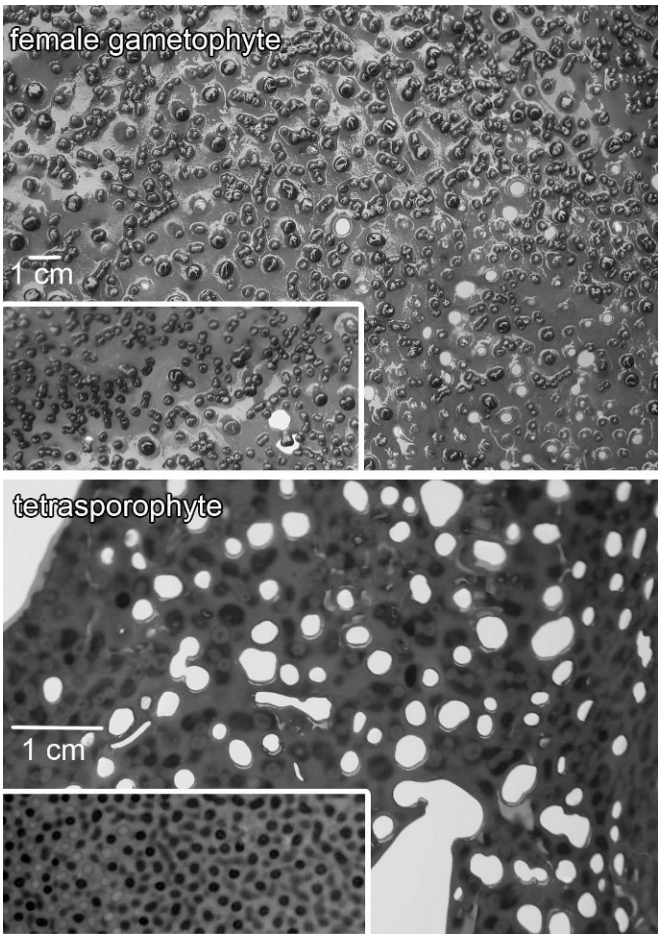


Fig. 1. *Gigartina skottsbergii* (top) haploid female gametophyte with protruding cystocarps containing diploid carposporophytes, where the spore resulting from fertilization (2n) is cloned to form the diploid carpospores and (bottom) diploid tetrasporophyte with tetrasporangial sori containing 2n cell, which undergoes meiosis to form haploid tetraspores.

MATERIAL AND METHODS

Algal material

Tetrasporic and cystocarpic *G. skottsbergii* were collected in spring (October 2004) by scuba diving at Peñon de Pesca, King George Island, Antarctica (62°14.22′S, 58°41.96′W). In the laboratory, tetrasporangial sori and cystocarps (Fig. 1) were excised from five different sporophytes and female gametophytes, respectively. The sporangia-containing tissues collected from each individual were immersed in 5–10 ml of filtered (0.2-μm pore size) seawater at ± 5°C and exposed under white light (15 ± 1 μmol photons m^{-2} s^{-1}) to induce spore release. Freshly released spores were collected and maintained under low white light (15 ± 1 μmol photons m^{-2} s^{-1}). The initial spore density was counted by use of Sedgewick-Rafter cell S50 spore counter (Graticules, Tonbridge, UK). Stock suspensions were diluted with filtered seawater to give densities between 4×10^3 and 5×10^3 spores ml^{-1} among the five replicates.

Irradiation treatments

White fluorescent tubes (Osram, L65 W/25S, Munich, Germany) and UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA) were used to provide photosynthetically active radiation (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 one of the following filters: Ultraphan transparent (Digefra, Germany); Folanorm (Folex, Germany) or Ultraphan URUV farblos corresponding to the PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) and PAR (P) treatments, respectively. 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} UV-A and 0.40 W m^{-2} UV-B, corresponding to 9.5×10^{-5} and 4.7×10^{-3} W m^{-2} weighted irradiance, respectively, using biological effective weighing function for DNA damage of *Escherichia coli* (Setlow 1974). 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}).

Light microscopy

Mean cell sizes (diameter) of freshly released tetraspores and carpospores were measured under light microscope (Zeiss Axiolab, Germany). Gross structural changes after exposure to different light treatments and 2 days post-cultivation in low white light were observed and micrographs were taken with a digital camera (Canon PowerShot A80, Japan). Spores were scored as dead (pale and unpigmented) or alive (darkly pigmented). Spore mortality was determined by counting 100 cells per replicate ($n = 3$).

Chlorophyll fluorescence measurements

Photosynthetic efficiencies of both spore types were measured as variable fluorescence of photosystem II (PSII) using a water pulse amplitude modulation fluorometer (water PAM) consisting of emitter–detector (water ED) unit and PAM control universal control unit connected to a personal computer operated with WinControl software (Heinz Walz, Effeltrich, Germany) (Roleda *et al.* 2006a). Immediately after adjustment of spore density, the suspension was filled into 5-ml quartz cuvettes to determine optimum quantum yield (F_v/F_m) inside the ED unit at time zero ($n = 5$). After 3 min of dark incubation, F_0 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 600-ms completely saturating white light pulse (~ 275 μmol photons m^{-2} s^{-1}).

Rapid photosynthesis (in terms of relative electron transport rate, rETR = photon fluence rate × $\Delta F/F_m'$) vs irradiance (E) curves (P-E curve) was measured in the control ($n = 3$, chosen at random from the five replicates). Spore suspension was exposed to increasing actinic light making up to 10 points of 17, 26, 38, 58, 87, 128, 198, 294, 419, 585 μmol photons m^{-2} s^{-1}. Each actinic light treat-

ment was 30 s before application of the saturating pulse to determine rETR. Data points were plotted and curve fits were calculated with the solver module of MS-Excel using the least-squares method comparing differences between measured and calculated data. The hyperbolic tangent model of Jassby & Platt (1976) was used to estimate P-E curve parameters, described as:

$$\mathrm{rETR} = \mathrm{rETR}_{\max} * \tanh\left(\infty * E_{\mathrm{PAR}} * \mathrm{rETR}_{\max}^{-1}\right)$$

where rETRmax 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. 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 ($\mathrm{rETR}_{\max}$).

Controls measured at time zero were poured into corresponding culture dishes (35 mm × 10 mm; Corning™, 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 spore suspension (not exceeding 1 h after release) were poured into different culture dishes (total experimental units = 60 per life history phase). Samples corresponding to the five replicates were exposed to each treatment combination of radiation and exposure time at 2°C ± 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. The controls were also maintained at the same condition. Measurements of photosynthetic recovery were made after 2 days. 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 in tetraspores and carpospores were determined after exposure to the whole light spectrum at different exposure times of 1, 2, 4, and 8 h. For each life history phase (n = 3), 40 ml of spore suspension (4×10^3–5×10^3 spores 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 2 days in low white light (4 ± 1 μmol photons m^{-2} s^{-1}) before processing. Settled and germinating spores 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, UK) and frozen at −80°C. The DNA was extracted using 2% cetyltrimethyl ammoniumbromide extraction buffer and quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR, USA) (Roleda *et al.* 2005). The accumulation of cyclobutane pyrimidine dimers (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 subsequently done using ECL™ Western blotting detection reagent (Amersham Buckinghamshire, UK). Films were developed and scanned using Biorad imaging densitometer (model GS-700, Bio-Rad Laboratories, Hercules, CA, USA), and grey scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems) (Roleda *et al.* 2005). CPDs were quantified by comparing the grey scales of the samples against the standard (UV-irradiated Hela DNA with known amounts of CPDs) within the linear range of the film.

MAA extraction and analysis

In parallel to DNA damage exposure treatments, tetraspores were also exposed to 8 h of P, PA, and PAB. Untreated control (freshly released spores) and treated spore suspensions were filtered through GF/C filters, frozen in liquid nitrogen, and stored at −80°C until further analysis. Spore MAAs were extracted in 2 ml of 25% MeOH in a water bath (45°C) for 2 h. The suspension was centrifuged, and the supernatant was analyzed using high-performance liquid chromatography (HPLC) described by Hoyer *et al.* (2001). The mobile phase with 25% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) was run in isocratic reverse phase at a flow rate of 0.7 ml min^{-1}. The MAAs were detected online with a photodiode array detector (Spectraphysics UV6000LP) at 330 nm, and absorption spectra (280–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification of MAAs was done by comparison of spectra and retention time described in thallus extracts of *G. skottsbergii* (Hoyer *et al.* 2001). Because of the lack of commercially available standards, the MAA concentrations are expressed as absorbance units (peak area) at 330 nm.

Statistical analysis

Data were tested for homogeneity of variance (Levene statistics). Corresponding transformations (square roots) were made to heteroskedastic data. Response variables (photosynthesis, DNA damage, and MAA content) were tested using analyses of variance (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

Isolated spores from different life history stages of *G. skottsbergii* showed that carpospores were larger in size (27 ± 2.0 μm, n = 50) compared with tetraspores (23 ± 1.8 μm, n = 140). Size difference is significant (ANOVA, $P < 0.001$).

The optimum quantum yield of PSII (F_v/F_m, Table 1) and electron transport rate ($\mathrm{rETR}_{\max}$, Fig. 2) of freshly released spores was significantly higher (ANOVA, $P < 0.005$) in carpospores (F_v/F_m = 0.403 ± 0.03; $\mathrm{rETR}_{\max}$ =

Table 1. Mean absolute values (± SD) of spore photosynthetic efficiency (optimum quantum yield, F_v/F_m) after treatment to photosynthetically active radiation (PAR = P); PAR + UV-A radiation (PA); PAR + UV-A + UV-B radiation (PAB).

		Optimum quantum yield (F_v/F_m)							
		After exposure				After recovery			
Ploidy	Treatment (h)	Control (T_0)	P	PA	PAB	Control (T_2)	P	PA	PAB
n		0.307 ± 0.07				0.371 ± 0.05			
	1		0.140 ± 0.04	0.087 ± 0.02	0.067 ± 0.01		0.368 ± 0.04	0.350 ± 0.06	0.344 ± 0.06
	2		0.094 ± 0.02	0.062 ± 0.01	0.047 ± 0.02		0.359 ± 0.06	0.325 ± 0.06	0.309 ± 0.06
	4		0.093 ± 0.02	0.042 ± 0.01	0.031 ± 0.01		0.325 ± 0.04	0.265 ± 0.03	0.246 ± 0.04
	8		0.076 ± 0.02	0.027 ± 0.00	0.013 ± 0.01		0.279 ± 0.04	0.231 ± 0.04	0.228 ± 0.03
2n		0.403 ± 0.03				0.434 ± 0.02			
	1		0.169 ± 0.04	0.087 ± 0.02	0.075 ± 0.01		0.429 ± 0.04	0.404 ± 0.03	0.376 ± 0.04
	2		0.109 ± 0.02	0.063 ± 0.01	0.060 ± 0.01		0.422 ± 0.05	0.375 ± 0.03	0.363 ± 0.04
	4		0.105 ± 0.03	0.055 ± 0.01	0.044 ± 0.01		0.416 ± 0.03	0.371 ± 0.02	0.352 ± 0.02
	8		0.094 ± 0.02	0.050 ± 0.01	0.042 ± 0.01		0.373 ± 0.01	0.282 ± 0.04	0.298 ± 0.02

Different spore ploidies are tetraspores (n) and carpospores (2n). Photon flux density is 22 μmol photons m^{-2} s^{-1} (~ 4.73 W m^{-2}). Photosynthetic recovery was initiated in dim white light of 4 ± 1 μmol photons m^{-2} s^{-1} after treatment. Control at time zero (T_0) was measured after spore release; whereas, control at day 2 (T_2) was measured after postcultivation at the same dim light condition with that of the treated samples. Note: All SD values below the average for uniformity.

6.87 ± 0.18) than in tetraspores (F_v/F_m = 0.307 ± 0.07; $rETR_{max}$ = 5.60 ± 0.08). The alpha (α), a measure of light-harvesting performance and photosynthetic conversion efficiency, were comparable between the different spore ploidy (α = 0.13 ± 0.01; ANOVA, P = 0.899). The saturating irradiance, E_k, was, however, significantly higher (ANOVA, $P < 0.001$) in carpospores (54 ± 2 μmol photons m^{-2} s^{-1}) than in the tetraspores (44 ± 2 μmol photons m^{-2} s^{-1}).

Exposure to 1 h of 22 μmol photons m^{-2} s^{-1} PAR (= 7.2 × 10^4 J m^{-2}) already reduced the optimum quantum yield (F_v/F_m, Table 1) by 53% and 58% relative to controls in tetraspores and carpospores, respectively. Increasing fluence of PAR (as a function of exposure time) further reduced the F_v/F_m in both tetraspores (15–21%) and carpospores (15–19%; Fig. 3). In irradiance supplemented with UV-A, additional reduction in F_v/F_m relative to P treatment was 11–18% in tetraspores and 11–21% in carpospores. PAR supplemented with the whole UVR spectrum further reduced the F_v/F_m relative to PA treatment by 4–7% in tetraspores and only 1–3% in carpospores. The decrease in photosynthesis was not significantly different between spore ploidy (Table 2; ANOVA, P = 0.259) but was significantly different between radiation treatment (ANOVA, $P < 0.001$; DMRT, P = 0.05; PAB > PA > P) and exposure time (ANOVA, $P < 0.001$; DMRT, P = 0.05; 8 h > 4 h > 2 h > 1 h).

Two days of cultivation after treatment under dim white light allowed photosynthetic recovery in all treatments. Recovery of PSII function was not significantly different between spore ploidy but was significantly different between radiation treatment and exposure time (Table 2; ANOVA, $P < 0.001$). DMRT (P = 0.05) showed that photosynthetic recovery was dependent on the pre-exposure radiation (P > PA = PAB) and exposure time (1 h = 2 h > 4 h > 8 h) treatments.

DNA lesions measured as number of CPDs (10^6 $nucleotides^{-1}$; Fig. 4) increased significantly with increasing dose of UV-B radiation (ANOVA, $P < 0.001$). Significantly higher DNA damage was observed in haploid tetraspores compared with diploid carpospores (ANOVA, $P < 0.001$). After the exclusion of UVR, an efficient DNA damage repair mechanism was observed. DNA lesions were completely repaired in spores exposed to UV-B dose less than 1.2 × 10^4 J m^{-2} (8 h of 0.4 W m^{-2} UV-B). In spores exposed to higher UV-B dose, DNA damage repair rate was higher in tetraspores (1.2 × 10^5 $nucleotides^{-1}$ h^{-1}) compared with carpospores (6.4 × 10^4 $nucleotides^{-1}$ h^{-1}) but the remaining DNA damage 2 days postcultivation under dim white light was significantly lower in carpospores (0.843 ± 0.01 CPDs 10^6 $nucleotides^{-1}$) compared with tetraspores (0.872 ± 0.02 CPDs 10^6 $nucleotides^{-1}$; t test, P = 0.039).

MAAs measured as absorbance units at 330 nm (Table 3) showed three different peaks at retention times of

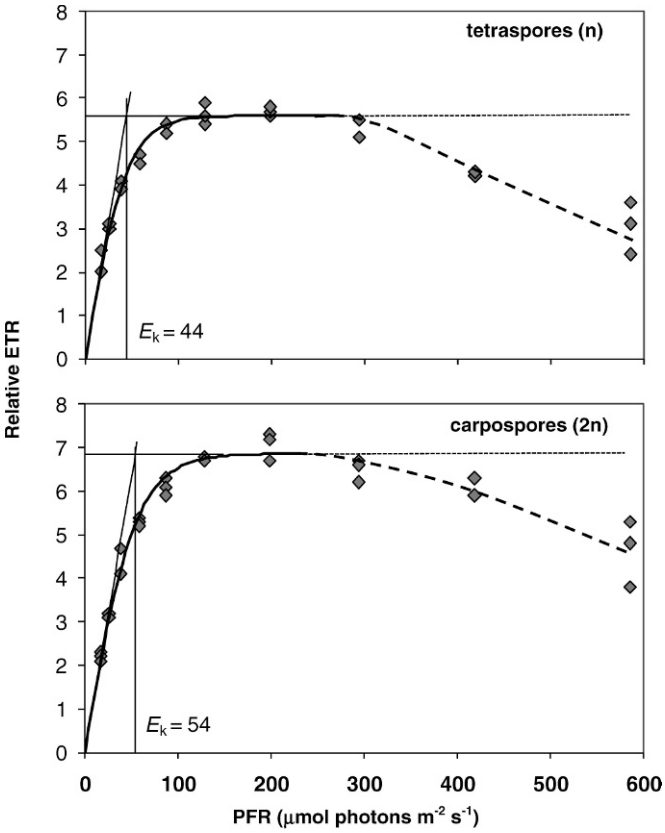


Fig. 2. Photosynthesis–irradiance curves of tetraspores (n) and carpospores (2n) from *Gigartina skottsbergii* (n = 3) immediately after release from sporangium. PFR is the respective photon fluence of actinic light, and ETR is the electron transport rate. Saturating irradiance (E_k) is estimated as the point at which the initial slope crosses maximum photosynthesis ($rETR_{max}$) using the hyperbolic tangent model of Jassby & Platt (1976). Maximum effective quantum yields are 0.307 ± 0.07 and 0.403 ± 0.03 for haploid and diploid spores (n = 5) respectively.

3.28, 3.38, and 4.43 min. In comparison with the spectra and retention times reported by Hoyer *et al.* (2001) on extracts of adult thallus of *G. skottsbergii*, the peaks at 3.28 and 3.38 were then identified as shinorine (λ_{max} = 330 nm) and palythine (λ_{max} = 319 nm). The peak at 4.43 (λ_{max} = 318 nm) is speculated to be a derivative substance. Total MAAs in tetraspores of *G. skottsbergii* were not significantly different between control and treatment (ANOVA, P = 0.084).

Viable spores were observed after 8-h exposure treatments and 2 days postcultivation in low white light (Figs 5–12). Significantly higher spore mortality was observed in tetraspores (11–14%) compared with carpospores (4–7%) (Fig. 13; ANOVA, P = 0.001). The negative impacts of different light treatments on spore mortality were, however, not significantly different between P, PA, and PAB treatments (ANOVA, P = 0.341).

DISCUSSION

This study showed an intrinsic difference in the physiological performance of diploid and haploid spores in terms of F_v/F_m, $rETR_{max}$, and E_k. Experimental exposure to different radiation treatments showed that both spore ploidies were, however, equally susceptible to PAR and UVR. Accumulation of UVB-induced DNA damage was dose dependent and higher in haploid than in diploid spores, contributing to higher cell mortality in tetraspores. The insignificant difference in spore mortality between PAR and PAR+UVR treatments was attributed to the efficient DNA damage repair mechanism that sustained cell viability after UVR treatment.

Photosynthetic parameters (F_v/F_m, ETR_{max}, and E_k) were observed to be significantly higher in carpospores than in tetraspores. This may be due to other factors such as light acclimation capacity and may not be directly due to the ploidy level. A possible relationship between photosynthetic capacity and ploidy levels remains to be demonstrated with other species and life history phases.

Photosynthetic efficiency of spores is already lower than that of adult thalli and after exposure treatments decreased to fluorescence ratios below 0.1 (Table 1), which may not be reliable anymore. Despite the methodological limitation, the measured F_v/F_m values were coherent among replicates and treatments (radiation × exposure time) so that an additional UV effect can be assumed. The reduction in F_v/F_m under PAR treatment was not solely due to the photon fluence rate (E = 22 µmol photons m^{-2} s^{-1}), which was lower than the extrapolated E_k (44–54 µmol photons m^{-2} s^{-1}), but rather due to long irradiation period, i.e. the total fluence of PAR applied (H = fluence rate * exposure time in seconds; expressed in J m^{-2}). For the low-light-adapted spores exposed to excess fluence of PAR, an increase in thermal energy dissipation regulated by carotenoids diminishes the quantum yield of PS II. Under excessive PAR fluence, degradation of D1 protein of PSII can occur (Adams & Demmig-Adams 1992).

Despite the artificial laboratory irradiance, with the absence of relatively high PAR, the UV irradiances comparable with those encountered in the field had a negative impact on the physiology and photochemistry of reproductive cells of *G. skottsbergii*. UVR can additionally depress photosynthetic performance by possible damage to the oxidizing site and reaction center of PS II (Grzymski *et al.* 2001; Turcsányi & Vass 2002). Degradation of parts of the D1/D2 heterodimer, the major structural complex within PSII, is mediated by UV-B (Richter *et al.* 1990), whereas UV-A radiation is found to be damaging for PSII by decreasing the electron flow from reaction centers to plastoquinone (Grzymski *et al.* 2001), affecting electron transport both at the water oxidizing complex and the binding site of the secondary quinone acceptor in PSII (Q_B) quinone electron acceptor (Turcsányi & Vass 2002).

After exposure to UVR, DNA damage was lower in carpospores (2n) compared with tetraspores (n). After UVR exclusion and 2 days of recovery in dim white light, DNA damage repair rate was higher in tetraspores, but the remaining DNA damage was lower in carpospores. These conform to the theoretical model stipulating that diploid cells are more resistant to DNA damage; whereas, haploid cells are efficient replicators (Long & Michod 1995). Photorepair of DNA lesions by photolyase reverse dimer-

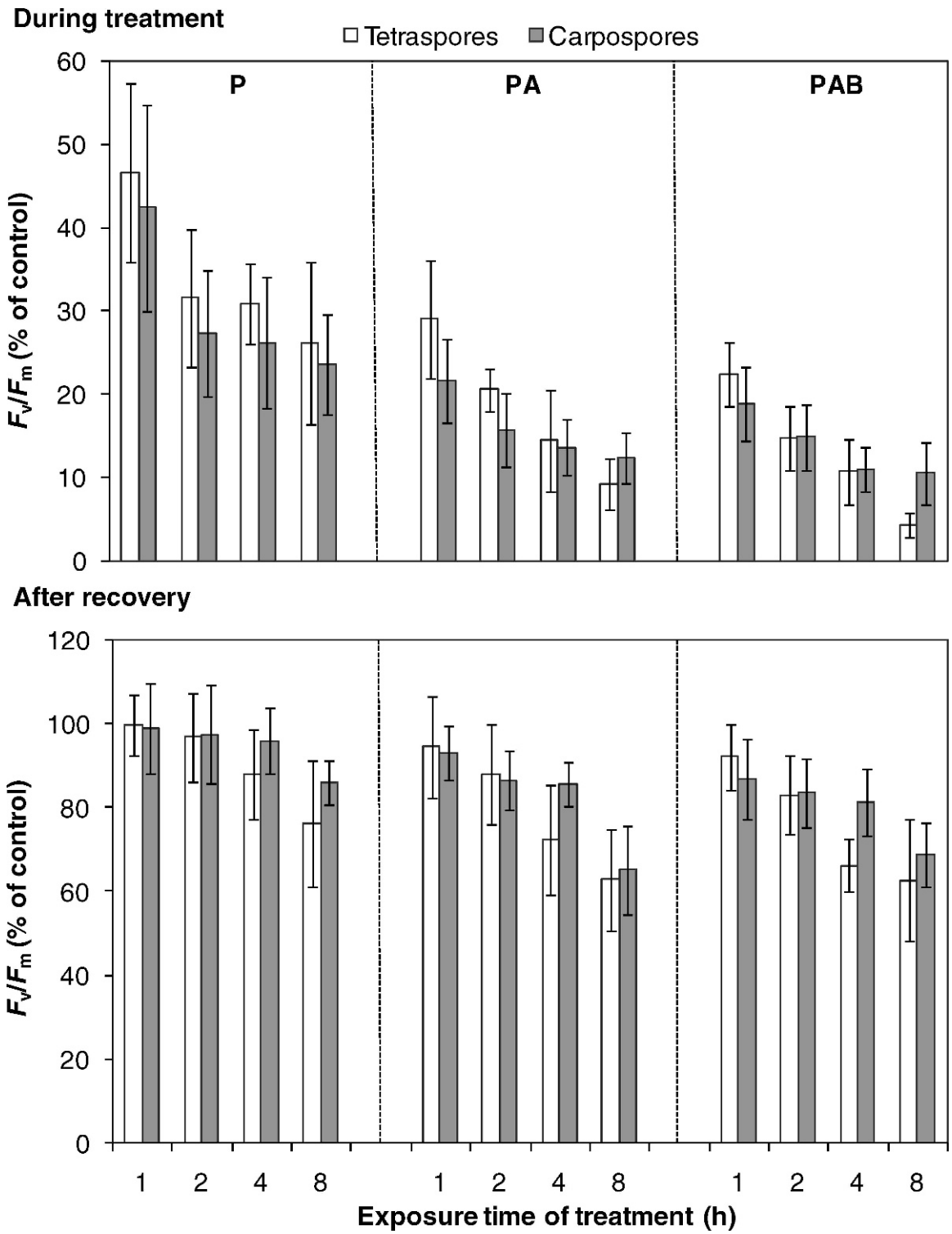


Fig. 3. Mean optimum quantum yield (F_v/F_m) of tetraspores (n) and carpospores (2n) during treatment (top) to photosynthetically active radiation (PAR = P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) at different exposure times expressed as percentage of control. Mean absolute values are shown in Table 1. Corresponding photosynthetic recovery (bottom) was measured 48 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 2.

ization of adjacent pyrimidine bases under sufficient PAR (and UV-A). Other repair mechanisms include nucleotide and base excision repair and recombination repair (van de Poll *et al.* 2002).

Because of logistic constraints, only MAA contents from tetraspores were successfully characterized. This study showed for the first time the presence of UV-absorbing compounds in an unicellular life history stage of a red

Table 2. Analysis of variance (ANOVA) and significance values for the main effects and interactions of independent variables on photosynthetic efficiency, DNA damage and repair, MAA synthesis (tetraspores), and mortality in spores of *Gigartina skottsbergii*.

Experiment/variable	Source of variation	df	*F*-value	*P*-value
Photosynthesis				
F_v/F_m (after treatment)	ploidy (A)	1	1.292	0.259[ns]
	radiation[1] (B)	2	118.443	< 0.001*
	exposure time (C)	3	45.773	< 0.001*
	A × B	2	2.313	0.104[ns]
	A × C	3	0.694	0.558[ns]
	B × C	6	0.752	0.609[ns]
	A × B × C	6	0.782	0.586[ns]
F_v/F_m (after recovery)	ploidy (A)	1	2.125	0.148[ns]
	radiation (B)	2	23.054	< 0.001*
	exposure time (C)	3	33.990	< 0.001*
	A × B	2	0.028	0.972[ns]
	A × C	3	2.431	0.069[ns]
	B × C	6	0.970	0.450[ns]
	A × B × C	6	0.330	0.920[ns]
DNA damage				
CPD induction	ploidy (A)	1	19.963	< 0.001*
	UV-B dose (B)	3	23.071	< 0.001*
	A × B	3	1.424	0.273[ns]
DNA damage repair	ploidy	1	9.109	0.039*
MAAs synthesis				
Total MAAs	radiation	3	3.195	0.084[ns]
Spore mortality	ploidy (A)	1	19.055	0.001*
	radiation (B)	2	1.177	0.341[ns]
	A × B	2	0.195	0.825[ns]

[1] Radiation treatments consist of photosynthetically active radiation (PAR = P), PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB).
* Significant; ns, not significant).

seaweed. Whether there is a significant difference in MAA contents between spore ploidy remains to be studied. In adult life history stages, MAA contents in tetrasporophytic and gametophytic thalli of *G. skottsbergii* are not significantly different (Hoyer *et al.* 2001). Further studies on the synthesis of MAAs under different radiation treatments among different red macroalgal spores and ploidy levels are also needed.

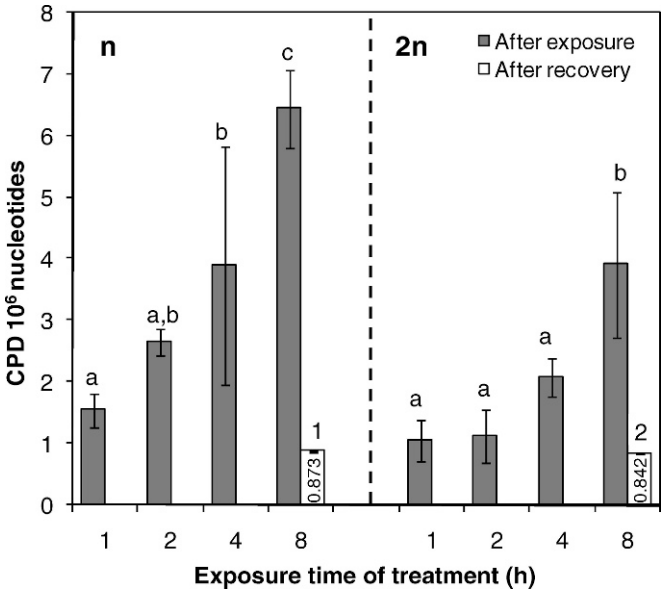


Fig. 4. UV-B-induced DNA damage (induced CPD concentrations per million nucleotides) in tetraspores (n) and carpospores (2n) after exposure to increasing time of PAR + UV-A + UV-B (shaded bars) and corresponding CPD repair (open bars) after 2 days of recovery in 4 μmol photons m^{-2} s^{-1}. Vertical bars are standard deviations (SD, $n = 3$). Letters on graph show result of *post hoc* Duncan's multiple range test (DMRT, $P = 0.05$) after ANOVA; different letters and number refer to significant difference between means.

The effective DNA damage repair mechanism effectively contributed to cell viability and low mortality. Under exposure to comparable UV-B doses, DNA damage in carpospores of *G. skottsbergii* (this study) was similar to that in carpospores of *Mastocarpus stellatus* but lower than in carpospores of *Chondrus crispus* (Roleda *et al.* 2004). DNA damage repair was, however, more efficient in both spore ploidy types of *G. skottsbergii* compared with carpospores of *M. stellatus* and *C. crispus*. This was consequently responsible for the low UVR-induced (PAB treatment) spore mortality in tetraspores (14%) and carpospores (7%) of *G. skottsbergii* compared with carpospores of *M. stellatus* (27%) and *C. crispus* (44%) (Roleda *et al.* 2004). The data on mortality have to be supported in future experiments by use of vital/lethal stains. However, as structural observations on spores and zygotes of different macroalgal species exposed under UVR showed loss of chloroplast pigmentation, ruptured cell wall, disintegrated cells, and inability to germinate and divide (Schoenwaelder *et al.* 2003; Wiencke *et al.* 2004, 2007; Roleda *et al.* 2006b), we believe that our criteria characterized spore mortality quite well.

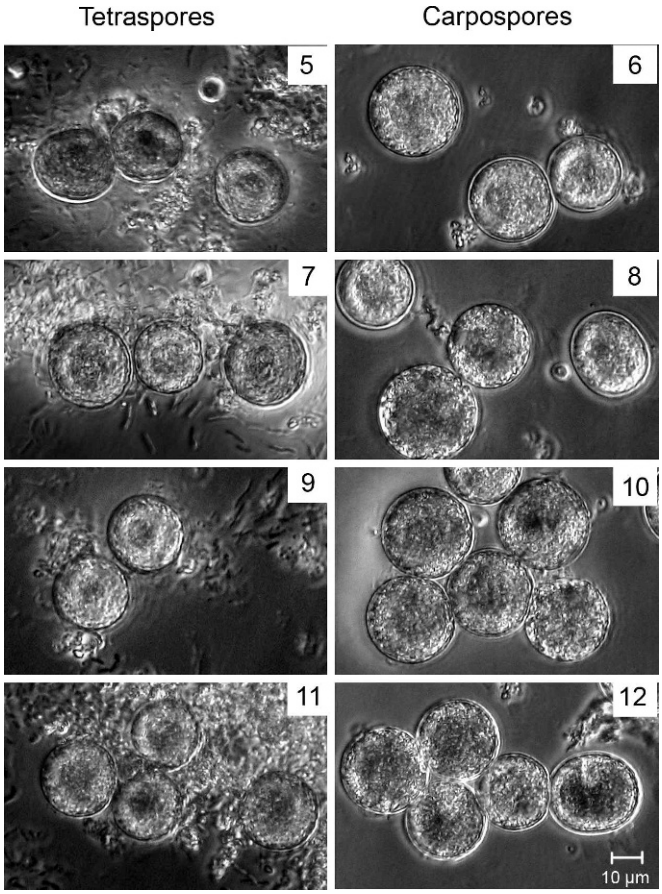
The size difference between carpospores and tetraspores of *G. skottsbergii* conform to a previous study on the spore size in the Florideophyceae, where diploid carpospores were observed to be generally larger than haploid tetraspores

Table 3. Mean mycosporine-like amino acid content of freshly released tetraspores (control) and after 8-h exposure to PAR (P), PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB) expressed as absorbance units at 330-nm peak areas. Analysis of variance showed no significant effect between control and treatments.

	Total MAA (10^5 absorbance units)		
Treatment	Shinorine	Palythine	Peak 4.43
Control	0.999 ± 0.08	11.383 ± 1.45	0.441 ± 0.04
P	0.872 ± 0.08	11.325 ± 0.44	0.531 ± 0.04
PA	0.709 ± 0.09	10.235 ± 0.67	0.491 ± 0.06
PAB	0.701 ± 0.15	13.027 ± 1.63	0.668 ± 0.10

(Ngan & Price 1979). The larger spore size among Gigartinales (> 20 μm, this study and Roleda *et al.* 2004) compared with zoospores (5 μm) of kelp and kelp-like brown macroalgae (Roleda *et al.* 2005, 2006b), and gametes of *Ascoseira mirabilis* (3 μm; Roleda *et al.* 2007b), possibly contributed to lower DNA damage due to the longer pathlength for UVR to reach the nucleus in larger cells (Garcia-Pichel 1994). Propagules of three Antarctic macroalgae, *Adenocystis utricularis*, *Monostroma hariotii*, and *Porphyra endiviifolium*, inhabiting the upper eulittoral zone, also exhibited an inverse correlation between increasing cell size and amount of DNA damage (Zacher *et al.* 2007). Aside from the functional significance of spore size, the hypothesis that diploid microscopic stages may be more resistant to the deleterious effects of UVR was also previously raised by Altamirano *et al.* (2003) when comparing the sensitivity of zygotes of *Fucus* species (> 40 μm) and zoospores of Laminariales.

Stock assessment of wild *G. skottsbergii* along a 50-m transect showed that reproductive thalli were predominantly tetrasporophytic (T) rather than gametophytic (G) (mean T : G ratio = 8 : 1; Zacher and Roleda unpublished data). In this study, we have shown that carpospores are more tolerant to UV-B-induced DNA damage and incurred lower spore mortality under different radiation treatments, which may partially explain the dominance of tetrasporophytes in the population of *G. skottsbergii* in Potter Cove. Our empirical and stock assessment data present an initial evidence for future validation of the hypothesis that spore susceptibility to UV radiation is one of the factors that may influence the phase dominance of the macrothalli population, possibly together with other environmental factors. Further exhaustive studies are, however, needed to unequivocally validate this hypothesis. Our observation, however, contradicts the reported pattern of gametophytic



Figs 5–12. Gross structural cell morphology and integrity of tetraspores (left column) and carpospores (right column) in control (5,6) and 8-h treatment of PAR (7,8), PAR+UV-A (9,10), and PAR+UV-A+UV-B (11,12). Micrographs (scale = 10 μm) were taken after exposure treatments and 2 days of recovery in low white light.

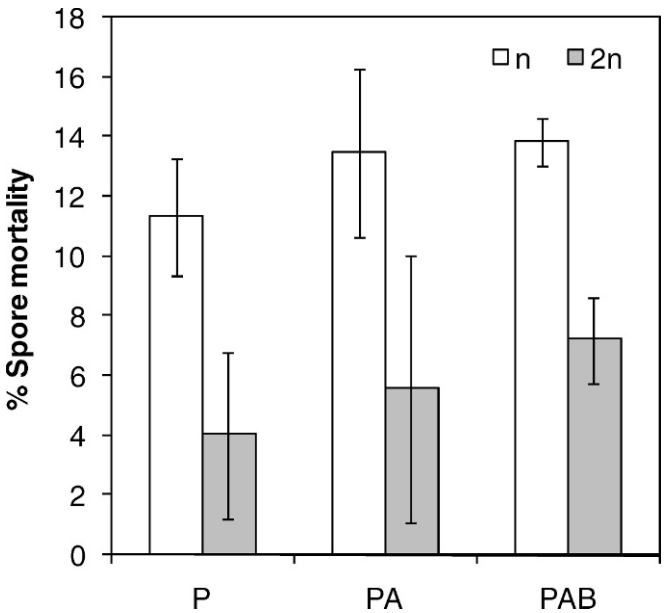


Fig. 13. Spore mortality in haploid tetraspores (n) and diploid carpospores of *Gigartina skottsbergii* after 8-h exposure under PAR (P), PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB) and 2 days recovery in low white light. Vertical bars are standard deviations (SD, $n = 5$). Analysis of variance (ANOVA) is presented in Table 2.

phase dominance in most Gigartinales species and that of *G. skottsbergii* population in South America (Piriz 1996; Avila *et al.* 1999; Faugeron *et al.* 2004). Phase dominance of specific macroalgal life stage may result from differences in spore production between generations (Scrosati & De Wreede 1999; Thornber & Gaines 2004) or fertilization rate (Fierst *et al.* 2005). In the triphasic life history of red macroalgae, the carposporophyte phase represents an adaptation to minimize the effect of both low and variable fertilization rates, where the cloning of the zygote amplifies the production ratio of carpospores relative to tetraspores (Searles 1980).

The higher tolerance of carpospores to UVR stress conforms to the genetic buffering hypothesis, which proposes that diploids benefit from better cellular regulation and are, therefore, more vigorous and tolerant to stress (Raper & Flexer 1970). Diploids are often thought to have fitness advantage over haploids because diploids, with two copies of every gene, are able to survive the effects of deleterious recessive mutations. However, masking in diploids may be disadvantageous by allowing mutations to persist over time (Otto & Marks 1996). The higher rate of DNA damage repair in haploid tetraspores may prove advantageous in the sense that deleterious mutations are purged more rapidly from haploid populations compared with diploid populations, which tend to mask deleterious alleles (Orr & Otto 1994; Hughes & Otto 1999). The existence and potential ecological advantages of alternation of generation between haploid–diploidy is thought to be the exploitation of a broader range of ecological niches, especially in environments that vary over space and time (Willson 1981). The extreme Antarctic environment characterized by low sea and air temperatures, annual extremes in light regime, wind speeds, disturbance, and isolation (Peck *et al.* 2006), as well as the naturally lower net springtime ozone levels (Fahey 2003) might have favored diploidy, driving a shift in phase dominance in the local population. The phase-specific adaptation theory that confers different mortality rates requires further studies to be conclusively demonstrated.

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