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Screening capacity of UV-absorbing compounds in spores of Arctic Laminariales

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Abstract

The functional significance of phlorotannins as ultraviolet radiation screens in brown algae is presented. Spectral analysis of zoospore suspensions of the three Arctic Laminariales *Saccorhiza dermatodea*, *Alaria esculenta* and *Laminaria digitata* showed strong absorption in the UV waveband, characteristic of phlorotannins. An induction in the synthesis of the UV-absorbing compound in zoospore suspensions of *S. dermatodea* and *A. esculenta* was observed as an increase in absorbance in the UV region after 8 h exposure to the whole light spectrum. Transmission of UVR was also negatively correlated with zoospore density in both these species but not in *L. digitata*. 'Biofilters' constructed from UV-transparent acrylic sheet, containing zoospore suspensions or solutions of phloroglucinol showed varying capacity to protect zoospore cultures from the lethal effects of ultraviolet radiation. Phloroglucinol protects the zoospores from damage by screening out the much harmful shorter UV-B spectra (280–290 nm). Cultured spores of *A. esculenta* and *L. digitata* after exposure to the whole light spectrum covered by filters containing phloroglucinol showed high rates of germination, unlike controls covered by seawater-only filters that showed 100% mortality. Biofilters containing zoospore suspensions act as buffers and showed variable UV-protection properties on the germination of its conspecifics. At highest zoospore density ($\sim 4 \times 10^6$ spores ml⁻¹), zoospores were observed to screen UV radiation maintaining viability among shielded spores in all species investigated. The protective function of zoospore film is, however, density-dependent in *L. digitata*. At lower spore density, UV-screening function in *S. dermatodea* and *A. esculenta* is attributed to their capacity to accumulate and release UV-absorbing compounds into the medium. Ultraviolet radiation transmission by zoospore suspensions of *Saccorhiza* and *Alaria* decreased during exposure to the whole light spectrum which is consistent with the earlier observation of enlarged phenolic vesicles following UVR exposure. The increase in vesicle size and the corresponding increase in UV-absorbing capacity may contribute to greater tolerance of UVR exposure in both species.

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1. Introduction

An increasing awareness of the environmental stress caused by increased levels of UVR attributable to global stratospheric ozone depletion has stimulated interest in the impact of elevated levels on marine macroalgae.

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UV-B radiation (UVBR) represents a very significant ecological impact that can potentially threaten the survival of species and thereby the health and diversity of marine coastal ecosystems. UVBR causes a range of deleterious effects in algae including damage to photosystem II, thylakoid membranes, microtubules, DNA and the formation of superoxide radicals (Franklin and Forster, 1997). The early life stages are particularly susceptible, and UVBR has been shown to inhibit germination, photosynthesis and survival of spores and zygotes of various species of brown algae (Dring et al., 1996; Huovinen et al., 2000; Swanson and Druehl, 2000; Wiencke et al., 2000, 2004; Makarov and Voskoboinikov, 2001; Flores-Moya et al., 2002; Altamirano et al., 2003; Schoenwaelder et al., 2003; Henry and Van Alstyne, 2004; Roleda et al., 2005, 2006). In plants, UVBR induces genes of the phenylpropanoid pathway that lead to the synthesis of phenolics such as flavonoids, lignin and tannins, compounds that, like phlorotannins, absorb UVBR and have broad defence-related functions (Jordan, 1996, 2002). The UV-screening function of phenolic compounds in higher plants is widely accepted; indeed Rozema and co-workers (1997, 2002) argued that this function made phenolics critical to the success of plant life on land.

Phlorotannins are polymers of phloroglucinol, unique to the Phaeophyceae. They occur in cells in vesicles known as physodes and are also deposited in cell walls (Schoenwaelder and Clayton, 1998a, 1999). Their functional significance has been the subject of some debate for well over a century (see Ragan, 1976; Ragan and Glombitza, 1986; Schoenwaelder, 2002a). However, it is only in the past few years that the probability that phlorotannins have multiple roles has been acknowledged (Arnold and Targett, 2002, 2003). Their strategic disposition in brown algal thalli, concentrated in cells of the outer epidermal layers (Tugwell and Branch, 1989; Schoenwaelder, 2002a), is consistent with a generalist role in defence against herbivores. Numerous experimental studies have examined the importance of phlorotannins as chemical defence agents, and have confirmed their effectiveness against a range of invertebrate herbivores and some species of fish (Targett and Arnold, 1998; Amsler and Fairhead, 2006).

Experimental evidence to support the UV-protective role of phlorotannins in brown algae is rather more preliminary. Swanson and Druehl (2002) showed that seawater containing phlorotannin exudates of *Macrocystis* increased survivorship of germinating *Laminaria groenlandica* spores exposed to UVBR. Schoenwaelder et al. (2003) linked higher numbers of physodes in *Fucus spiralis* embryos with a greater tolerance to elevated

levels of UV-A radiation (UVA) and UVBR, compared with more susceptible *Fucus* spp. In addition, they used phloroglucinol filters to screen out UVR and allow normal development of embryos of susceptible *Fucus serratus*. Embryos of *F. gardneri* are rather susceptible to UVR but UV-tolerance was developed and is related to the increase in phlorotannin concentrations during their maturation (Henry and Van Alstyne, 2004).

Recently it was demonstrated that zoospores of Arctic *Alaria esculenta* and *Saccorhiza dermatodea* are less sensitive to UVR or have a better capacity to recover from UVR-induced stress than zoospores of species from deeper water, *Laminaria digitata*, *L. saccharina* and *L. solidungula* (Wiencke et al., 2004). During the experiments, enlargement of physodes in *A. esculenta* and *S. dermatodea* was observed and inferred a possible protective function of these enlarged compartments against UVR. The aims of the present study were to measure the UV-absorbing properties of zoospore suspensions of species of Arctic Laminariales and to investigate their potential capacity to protect spores from the damaging effects of UVR. Accumulation and extrusion of UV-absorbing compounds were also investigated in zoospore and gametophyte stages. In addition we tested whether phenolics, in the form of the commercially available phloroglucinol has an effective screening capacity to protect the delicate zoospores from damage caused by UVR.

2. Methodology

2.1. Algal material

Fertile sporophytes of *S. dermatodea* (Pyl.) J. Ag., *A. esculenta* (L.) Grev., and *L. digitata* (Huds.) Lamour., were collected between May and June 2004 by SCUBA divers in Kongsfjorden at Prins Heinrichøya or Blomstrandhalvøya close to Ny Ålesund (Spitsbergen, 78°55'N, 11°56'E), Svalbard, Norway. Blades with sori were abscised from three different individuals per species, cleaned of epiphytes, blotted with tissue paper and kept in darkness in a moist chamber at 0 °C overnight up to a maximum of 2 days. To induce rapid release of zoospores, sori were immersed in 5–10 ml filtered (0.2 µm pore size) seawater at ±15 °C and exposed to natural light close to a window. The initial zoospore density was counted by the use of a Neubauer chamber (Brand, Germany). Stock suspensions were diluted with filtered seawater to give spore densities appropriate for each experiment. Due to the extent of the experimental work, fertile sporophytes were collected several times and sori were processed separately. A dilution series was made from a known zoospore suspension (e.g. 100%, 80% 60%, 40% 20%). Spores were also used

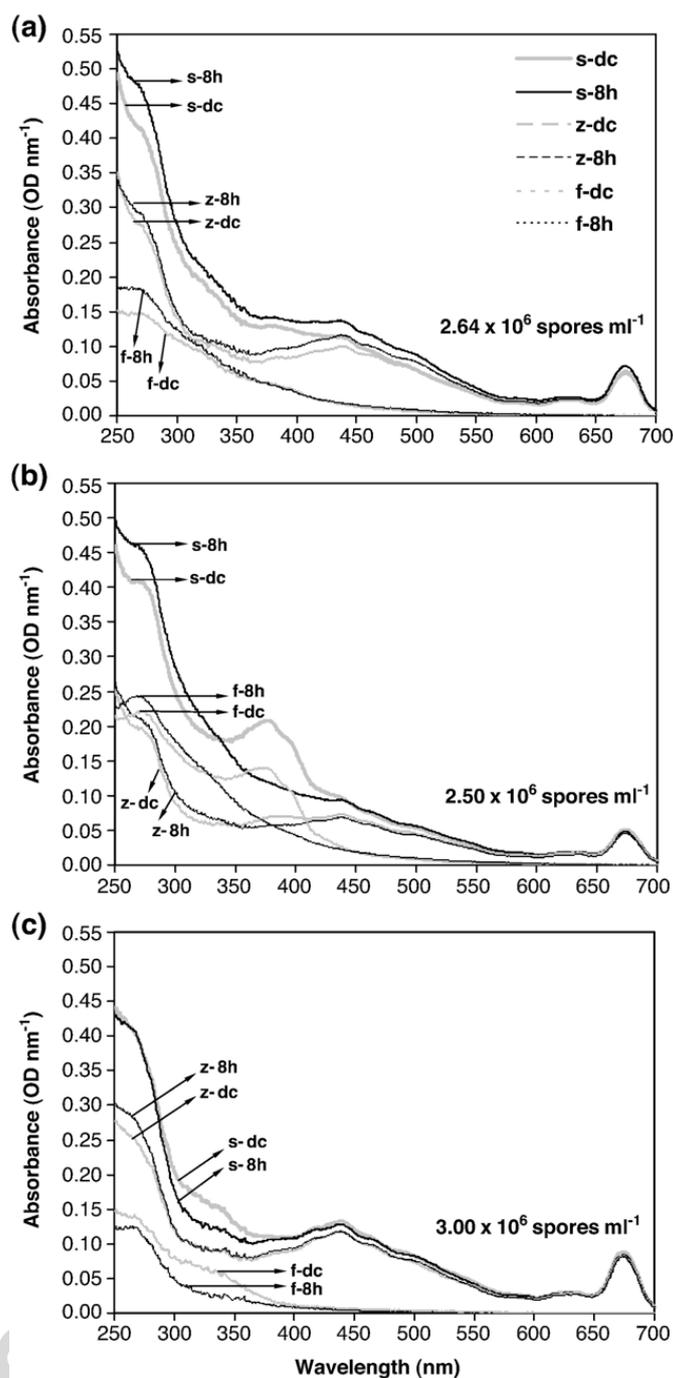


Fig. 1. Absorbance spectrum of zoospore suspension (=s), zoospores (=z) and filtrate (=f) of dark control (=dc) and 8 h exposure (=8 h) to the whole light spectrum of PAR+UV-A+UV-B in (a) *Saccorhiza dermatodea* (b) *Alaria esculenta* and (c) *Laminaria digitata*. Insets show zoospore density.

to establish gametophyte cultures in filtered seawater for each species investigated.

2.2. Light regime

Fluorescent tubes were hung 30 cm above the experimental set-up. Photosynthetically active radiation (PAR) was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany). Ultraviolet radiation

(UVR) was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA), emitting a spectrum similar to solar radiation in the range 295 to 340 nm. Ultraviolet radiation was measured using a cosine sensor connected to a UV-VIS Spectrometer (Marcel Kruse, Bremerhaven, Germany) at 7.03 W m^{-2} UV-A and 0.58 W m^{-2} UV-B. This is equivalent to a biologically weighted dose (erythema weighted, UV_{ery}) of 105 mW m^{-2} , measured with an ELUV-14 datalogger (El Naggar et al., 1995),

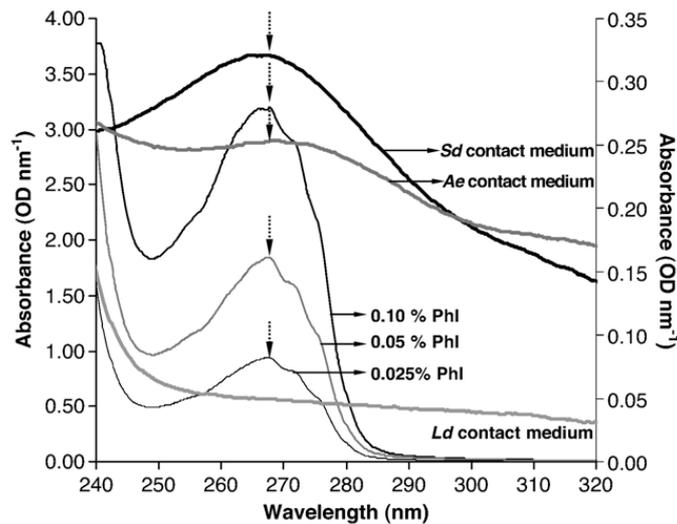


Fig. 2. Absorbance spectrum of phloroglucinol solutions and contact medium obtained from gametophyte cultures of Arctic Laminariales *Saccorhiza dermatodea* (*Sd*), *Alaria esculenta* (*Ae*) and *Laminaria digitata* (*Ld*).

calibrated to the standard CIE-87 erythral response after McKinlay and Diffey (1987). Photosynthetically active radiation (PAR) was measured using a cosine quantum sensor attached to a LI-COR datalogger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska, USA) to be $29.5 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ($\sim 6.34 \text{ W m}^{-2}$).

2.3. Absorbance spectrum

To determine the presence and induction of synthesis of UV-absorbing compounds, 80 ml zoospore suspension containing 2.5×10^6 – 3.0×10^6 spores ml^{-1} , was placed into 85 mm \times 15 mm culture dishes covered with Quartz glass filters and they were exposed to the full spectrum for 8 h. A portion of the zoospore suspension was kept in the dark (dark control). After treatments, samples were trans-

ferred into quartz cuvettes and scanned in the 250–700 nm waveband using Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere. Absorbance spectrum of the zoospore suspension, zoospore and the medium (filtrate) was measured in triplicate from: (1) zoospore suspension using seawater as reference, (2) zoospore suspension with filtrate as reference, and (3) filtrate with seawater as reference, respectively. The filtrate was obtained by filtering the zoospores out of the suspension through 44 mm diameter 1.0 μm pore size Nuclepore® polycarbonate membrane (Whatman, UK) using a vacuum pump at 400–600 millibars to minimize damage to the cells. In addition, absorption spectra of the contact medium of gametophytes grown from zoospores of different species were measured. Spectral characteristics of the screening medium used,

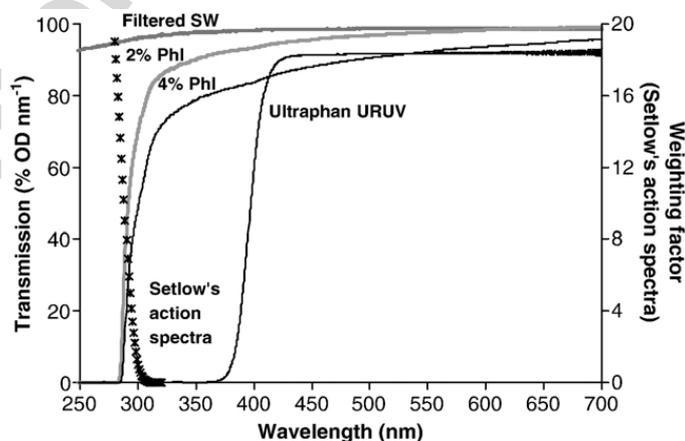


Fig. 3. Spectral transmittance of the different screening medium used for germination experiments includes: Filtered seawater (SW), 2% and 4% phloroglucinol (Phl) and Ultraphan URUV farblos filter foil. The weighting factor of the action spectra for DNA damage is plotted in the second Y-axis (Setlow, 1974).

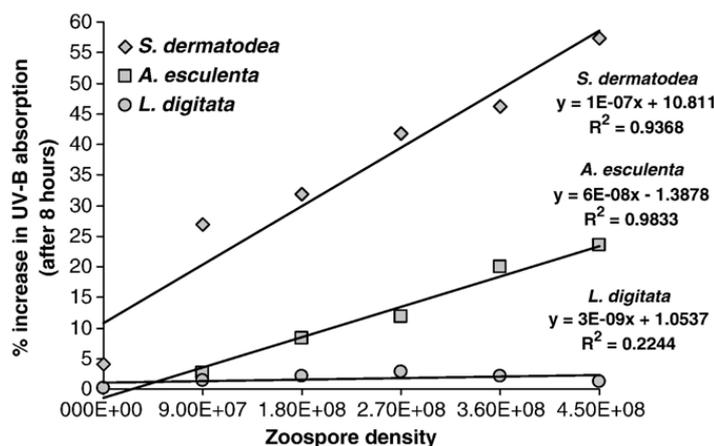


Fig. 4. Percent increase in UV-B absorption as a function of zoospore density and synthesis of UV-absorbing compounds in different species of Laminariales. Y-axis was computed from the difference in UV transmission through the Plexiglas containing zoospore suspension before and after 8 h exposure to the whole light spectrum (PAR+UV-A+UV-B) in the laboratory. UV-B was measured using Solar Light PMA2100 photoradiometer under the biofilters.

i.e. seawater, phloroglucinol (Fluka Chemie GmbH, Switzerland) and filter foil were also determined.

2.4. UV-B transmission

Biofilter holders similar to the ones used by Schoenwaelder et al. (2003) were made from UV-transparent acrylic (Acrylite OP-4, CYRO Industries, New Jersey, USA). In order to investigate the capacity of zoospores themselves to absorb UV-A and UV-B radiation, a dilution series of zoospore suspensions from *S. dermatodea*, *A. esculenta*, and *L. digitata* were placed inside the UV-transparent biofilter holders and exposed to the whole light spectrum for 8 h at 5 ± 2 °C. Transmission of UV-A and UV-B radiation was measured below the biofilters before and after the exposure treatment using a Solar Light PMA 2100 radiometer equipped with the UVA sensor PMA

2110 and the UVB Sensor PMA 2106 (Solar light, Philadelphia, USA). As the spectral range of the UVA sensor extends into the UVB region of the spectrum, UVAR measurements were always made using a Schott WG320 filter (Schott, Mainz, Germany) to exclude wavelengths below 320 nm. The UV-B measurements recorded were obtained by subtracting the reading with the WG320 filter from the reading without the filter. Percent increase in UV-B absorption was computed accordingly. In another set-up, transmittance of effective UV-B dose was measured below the biofilter containing *S. dermatodea* zoospore suspension using ELUV-14 UV-Data logger for 5 h.

2.5. Spore germination under biofilters

To investigate whether zoospore suspensions have the potential to screen out UVR and thus protect other spores

Table 1

Mean percent transmission of different spectral irradiance through the screening medium and the corresponding germination rates of *Alaria esculenta* and *Laminaria digitata* determined after 16 h exposure to the whole light spectrum and 6 days cultivation under dim white light ($10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)

Screening medium	Mean % transmission						Germination rate (%)	
	UV-B				UV-A	PAR	<i>A. esculenta</i> *	<i>L. digitata</i> ^{ns}
	280–285	285–290	290–300	300–320	320–400	400–700		
Filtered seawater	94.7±0.2%	95.1±0.1%	95.7±0.2%	96.4±0.2%	97.6±0.4%	98.6±0.1%	0%	0%
2% Phloroglucinol	0.6±1.1%	22.3±13.5%	58.8±8.2%	79.9±4.8%	90.6±2.1%	97.3±1.5%	87.6±3.4%	92.4±2.4%
4% Phloroglucinol	0.05±0.1%	8.0±7.3%	37.5±8.2%	62.5±7.2%	79.3±3.3%	91.5±3.1%	87.7±5.9%	93.4±2.5%
Ultraplan URUV filter					7.2±14.4%	90.8±3.9%	97.4±2.6%*	92.5±5.5%
Mean weighting factor (Setlow's action spectra)	16.4±2.0	10.8±2.1	3.8±2.3	0.2±0.3				

The mean weighting factor for the different UV-B ranges is shown using Setlow's action spectra for DNA damage where shorter UV-B radiation can trigger higher magnitude of biological response. * refers to significant difference and ns non-significant difference between screening medium (excluding filtered SW) on the germination rate of each species (ANOVA, $P < 0.001$).

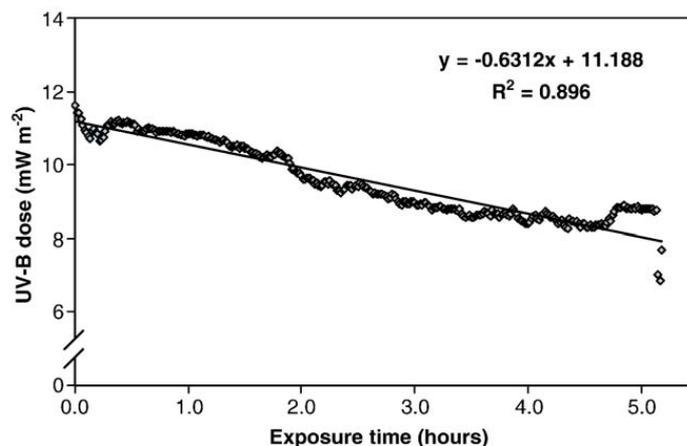


Fig. 5. Decrease in transmittance of effective UV-B dose through *Saccorhiza dermatodea* zoospore suspension after 5 h exposure to the whole light spectrum (PAR+UV-A+UV-B) in the laboratory as a function of increase in synthesis of UV-absorbing compound. Transmitted UV-B dose was measured using ELUV-14 dosimeter under the Plexiglas containing zoospore suspension.

from damage, a germination experiment was conducted under the biofilters. Culture dishes (53 mm × 12 mm) were filled with filtered seawater and two to five drops of the zoospore suspension containing approximately 3×10^5 – 4×10^5 spores ml^{-1} from different sporophytes of each species to each dish. The dishes were covered with different biofilters of their respective species and exposed to the whole light spectrum for 8 h at 5 ± 2 °C. In parallel, separate experimental units were prepared and covered with Ultraphan URUV farblos (Digefra GmbH, Germany) cutting off UV spectrum corresponding to PAR treatment. After treatment, spores were allowed to germinate in low white light (8 ± 2 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 6 days at 5 ± 2 °C. Spores were scored as germinated or not germinated by counting 300 cells per replicate using a light microscope (Zeiss Axioplan) equipped with 25× 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 PAR treatment, germination rates under different biofilters were expressed as percent of PAR.

To determine the UV-protective capacity of phloroglucinol, other biofilter holders were prepared containing 4% and 2% phloroglucinol solution as described by Schoenwaelder et al. (2003) and containing seawater. Culture dishes for the germination experiment were prepared as described above and exposed to 16 h at 5 ± 2 °C of the whole spectrum of the experimental radiation below the phloroglucinol-containing biofilters.

2.6. Statistical analysis

Germination data were tested for homogeneity of variances (Levene Statistics). Corresponding transforma-

tions (square root) were made to heteroskedastic data. The effect of biofilters on germination rates for each species was tested using analyses of variance (ANOVA, $P < 0.01$), followed by Duncan's multiple range test (DMRT, $P < 0.05$). Statistical analyses were carried out using the SPSS program (SPSS, Chicago, IL, USA).

3. Results

Spectral analysis of the zoospore suspensions, zoospores and filtrates showed strong absorption below 360 nm in all species investigated (Fig. 1). The absorbance maxima within the 250–280 nm wavebands are characteristic of phenolics in the form of phloroglucinol (Fig. 2). Comparison between the dark control and 8 h exposure to the whole light spectrum, however, showed an increase in UV-absorption only in *S. dermatodea* and *A. esculenta* (Fig. 1a and b). Higher UV-absorption was observed in the zoospore suspensions in general as well as in the zoospore and filtrate fraction in both species. In the *L. digitata* zoospore suspension, lower UV-absorption was observed within the 280–400 nm region and also at shorter wavelengths down to 250 nm, after 8 h exposure compared to the dark control. With the zoospore fraction, however, a slight increase in UV-absorption was observed from 250–280 nm (Fig. 1c).

Absorption spectra of the contact media of *Saccorhiza* and *Alaria* gametophytes increase toward the shorter UV wavelength and showed a peak at 267 nm similar to that of phloroglucinol solution (Fig. 2). Compared to filtered seawater, phloroglucinol solution allows much of the UVR to be transmitted through the screening medium (Fig. 3) but effectively cuts off the shorter wavelength in the range of 280–290 nm. Transmission of the lethally-weighted shorter wavelengths of 280–285 nm (Setlow's

action spectra) is only 0.6% and 0.05% through the 2% and 4% phloroglucinol solution respectively (Table 1). At 285–290 nm, 8 and 22% of the harmful UV-B radiation is transmitted through the phloroglucinol solutions.

A thin film of zoospores was formed inside the plexi-glass container used as a biofilter. The capacity to absorb UV-B radiation as a function of zoospore density was observed in *S. dermatodea* and *A. esculenta* but not in *L. digitata* (Fig. 4). The linear increase in UV-B absorption

was steep in *S. dermatodea* and gradual in *A. esculenta*. A flat line was observed in *L. digitata*. Moreover, transmittance of effective UV-B dose through an absolute density of zoospore suspension in *S. dermatodea* was also observed to decrease after 5 h treatment (Fig. 5).

The function of different zoospore density as a protective biofilter buffer on the germination of its conspecifics was observed (Fig. 6). Germination rate, expressed as percent of PAR control (Ultraplan URUV filter), showed

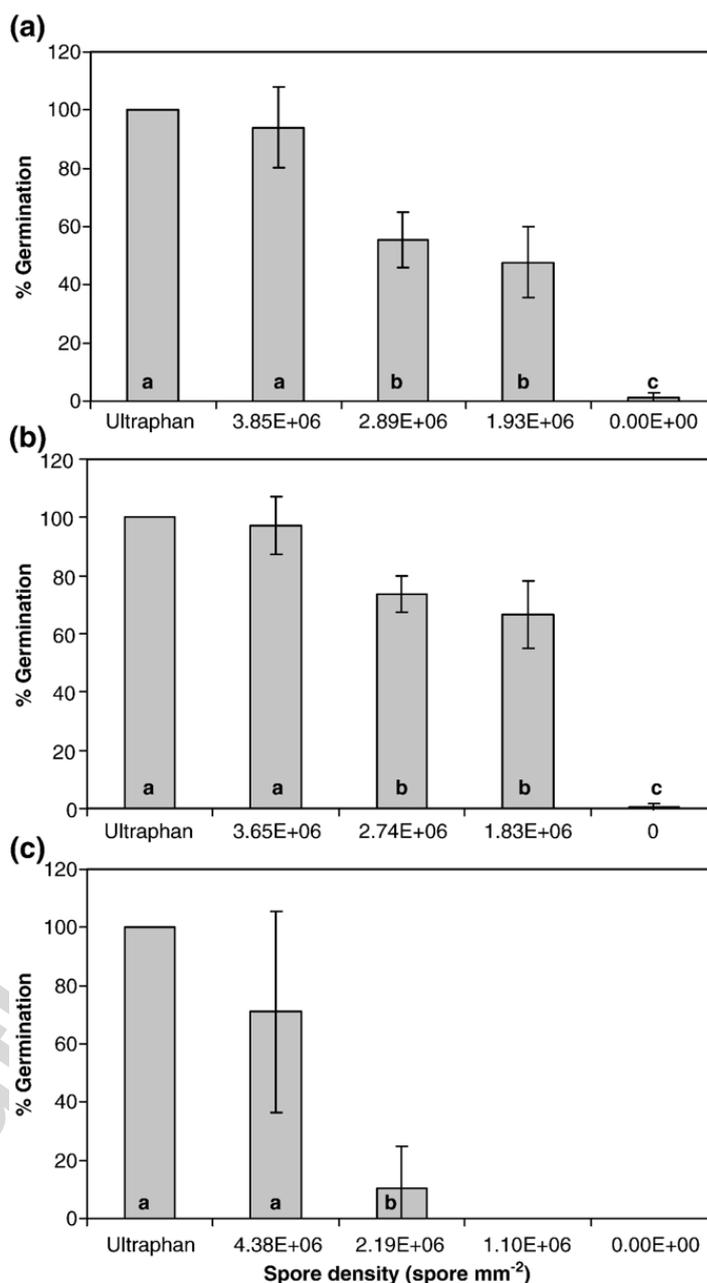


Fig. 6. Spore germination, expressed as percentage of control (PAR only=Ultraplan 395 filter) in (a) *Saccorhiza dermatodea*, (b) *Alaria esculenta* and (c) *Laminaria digitata*, 6 days after exposure to 8 h of the whole light spectrum of PAR+UV-A+UV-B under varying concentration of zoospore suspension as UV-biofilter. Post-cultivation is at low white light ($8 \mu\text{mol photon m}^{-2} \text{s}^{-1}$). Vertical bars are standard deviations (SD, $n=3$). Analysis of variance showed significant variation between treatments (ANOVA, $P<0.01$). Letters on graph show result of Duncan multiple range test (DMRT, $P<0.05$); different letters refer to significant differences between mean values.

that at highest zoospore density ($\sim 4 \times 10^6$ spores ml^{-1}), zoospores can screen UV radiation and maintain viability among shielded spores in all species investigated. At a lower zoospore density, the protective function was only effective in *S. dermatodea* and *A. esculenta*. In biofilters with zoospore density of approximately 2×10^6 – 3×10^6 spores ml^{-1} , a higher germination rate was observed in *A. esculenta* compared to *S. dermatodea* and very low in *L. digitata*. In seawater biofilter, minimal germination was still observed in *S. dermatodea* and *A. esculenta* and 100% mortality in *L. digitata*. Analysis of variance (ANOVA, $P < 0.01$) showed, significant protective effect of biofilters as UV-screen among the different species investigated. Duncan's multiple range test (DMRT, $P = 0.05$) showed different groups with significant difference in mean values (see letters inset in Fig. 6). In contrast, germination in spores covered by filters containing 2% and 4% phloroglucinol solutions showed $> 88\%$ in all species (Table 1). Germination rate of spores after exposure to the whole light spectrum under different screening medium (excluding seawater) and 6 days recovery under low white light is not significantly different in *Laminaria*. In *Alaria* significantly higher germination rate was observed under Ultraphan URUV filter (ANOVA, $P < 0.001$, DMRT, $P = 0.05$).

4. Discussion

Our study has demonstrated that the UV-screening capacity of Laminariales zoospores is species specific. *S. dermatodea* and *A. esculenta* can attenuate UVAR and UVBR, and that transmission is inversely proportional to the zoospore density. In *L. digitata*, the protective function of zoospore film is density-dependent. In *S. dermatodea* and *A. esculenta*, the UV-screening function even at lower zoospore density is attributed to their capacity to accumulate and release UV-absorbing compounds into the medium. No accumulation and sustained release of UV-absorbing compounds was observed in the spores and gametophytes of *L. digitata*. Phloroglucinol as screening medium could protect the zoospores from damage by screening out the much harmful shorter UV-B spectra (280–290 nm).

The ability to absorb UVR may well be a common property of brown algal zoospores and possibly also many other brown algal cell types, both reproductive and vegetative. No previous studies have examined the UVR transmission properties of intact, living cells of brown algae although Ragan and Craigie (1980) measured absorption by extracted high molecular weight phlorotannins and several researchers including, recent-

ly, Swanson and Druehl (2002) have measured UVR absorption by exudates. UV-absorption of zoospore suspensions from *Laminaria* species from Helgoland including *L. digitata* is based both on the absorption by the freshly released zoospores itself as well as by exudates in the medium (Roleda et al., 2005). Attenuation by living zoospores is probably attributable to the highly characteristic phlorotannin-containing phytodes in addition to absorption by nucleic acids and proteins which are present in all living cells. The presence of other chemical exudates, aside from phlorotannin, in the contact medium of gametophytes and the cellular structure of intact zoospores is responsible to the higher absorbance at 290–320 nm compared to the commercially available phenolic compound phloroglucinol.

Zoospore viability is observed to be affected by the UVBR. Phloroglucinol screens out these lethally-weighted shorter wavelengths responsible for DNA damage (Setlow, 1974). After treatment, spores successfully germinated when allowed recovering for 6 days in low white light. Germination capacity of Laminariales spores increases after 3 days when further cultivated for 6 days in low white light (Wiencke et al., 2004) and this is related to the DNA damage repair capacity in spores (Roleda et al., 2004, 2005). Photosynthetic recovery of zoospores is more dynamic. Kinetics of photosynthetic recovery after exposure to the whole light spectrum is faster in *Saccorhiza* compared to *Laminaria* spp. Photosynthetic capacity of *S. dermatodea* spores was restored to 100% relative to the control after 24 h recovery in low white light while *Laminaria* spp. spores require longer than 48 h (Roleda et al., 2006).

The insignificantly higher germination rate in *A. esculenta* compared to *S. dermatodea* covered with biofilters containing approximately 2×10^6 – 3×10^6 spores ml^{-1} is attributed to the observed clumping of *S. dermatodea* spores in the Plexiglas container making an uneven film of protective cell layers. A film of cells plus exudates containing UV-absorbing compound can provide more UV-screening function compared to exudates alone. The UV-screening function of seawater containing phlorotannin exudates of *Macrocystis* is, however, observed to be sufficient to increase the survivorship of germinating *L. groenlandica* zoospores exposed to UVBR (Swanson and Druehl, 2002).

The spore clumping behaviour observed especially in *S. dermatodea* could also serve as UV-refugia for underlying cells. Cells and tissues of brown algae that absorb UVR have the potential to protect any underlying living cells from the harmful effects of high levels of UVR. In the fucoid brown alga, *Hormosira banksii*, Schoenwaelder (2002b) observed superficial meristoderm cells damaged by excess solar radiation while the inner cells

remain healthy, apparently shielded by the phlorotannin-rich dead outer tissue.

Incident ultraviolet radiation can easily penetrate through the thin plasmalemma of zoospores damaging the nucleus and chloroplast. With only one chloroplast per cell (Henry and Cole, 1982), zoospores are more susceptible to photodamage compared to multi-cellular life history stages. Intracellular self-shading in macroalgal thalli and the nonuniformly shaped and unevenly spaced cells cause multiple scattering (Grzymski et al., 1997) which can attenuate up to 95% of the incident UVBR and yet transmit between 70–80% of the visible radiation (Robberecht and Caldwell, 1983). However, the striking and typical arrangement of physodes around the periphery of *Laminaria* spp. and *S. dermatodea* zoospores illustrated by Wiencke et al. (2004, Figs. 3a–c) suggests the possibility that physodes *in situ* may afford the centrally positioned nucleus some level of protection against UVR. In other brown algae, physodes in furoid eggs and zygotes are similarly concentrated in the outer region of cytoplasm (Schoenwaelder and Clayton, 1998a,b; Schoenwaelder and Wiencke, 2000). However, even in a different sub-cellular location, physodes would probably help to screen out harmful UVR, if the rotating movements of the zoospores are taken into consideration. At single cell level, the UV-protective function of phlorotannins could entail heavy energetic investment and with restricted efficiencies (Garcia-Pichel, 1994). At a population level, a large number of gametes are released at the same time and a cloud of spore could buffer each other from the harmful UVR. Moreover, it has also been suggested that kelp-derived phlorotannin exudates in coastal seawater may act as UVR screens at an ecosystem level (Swanson and Druehl, 2002).

Further support for a role in protection against UVR comes from experimental studies showing phlorotannins are induced in response to UVR. The phlorotannin content of *Ascophyllum nodosum* explants (Pavia et al., 1997; Pavia and Brock, 2000) and *Macrocystis integrifolia* blades (Swanson and Druehl, 2002) increased after exposure to UVR. Our measurements showing increase in UVR absorption and a drop in UVR transmission in zoospore suspension and biofilters containing *S. dermatodea* and *A. esculenta* zoospores after exposure to 8 h PAR+UV-A+UV-B, are explained by the synthesis of additional phlorotannins. The enlarged physodes observed in zoospores of *A. esculenta* and also *S. dermatodea* after exposure to the same UVR+PAR regime used in the present study (Wiencke et al., 2004) are further evidence for the induction of phlorotannin synthesis. In *L. digitata*, no increase in UVR absorption was observed in the zoospore suspension, biofilters and gametophyte contact

medium. We plan to conduct further quantitative studies to investigate this response in a wider range of kelp species.

The present study supports the general view that phlorotannins have multiple functions (Schoenwaelder, 2002a) in brown algae, and shows that, as a constitutive and inducible defence, they offer protection against UVR (an abiotic stress) as well as biotic stresses such as herbivory (Amsler, 2001; Amsler and Fairhead, 2006). In this respect, the functions of phlorotannins in brown algae are comparable with those of phenylpropanoids in higher plants, and the induced response confers an increased level of protection following exposure to stress. Various types of phenylpropanoids (a common class of plant phenolics) with varying defence-related characteristics are induced by different biotic and abiotic stresses (Dixon and Paiva, 1995). UVBR induces the synthesis of several phenylpropanoids, including a range of flavonoids, some having UVR-absorbing and also antioxidant properties (Jordan, 2002; Winkel-Shirley, 2002; Cooper-Driver and Bhattacharya, 1998). Flavonoids are synthesised predominantly in epidermal cells and provide an internal sunscreen regarded as the plant's 'first line of defence against UVBR-induced damage' (Jordan, 2002). UVBR upregulates the chalcone synthase (CHS) gene that controls a key step in phenylpropanoid metabolism (Dixon and Paiva, 1995).

In conclusion, species-specific *in vivo* phlorotannin content and induction of synthesis upon exposure to the whole light spectrum have ecological consequences. The increase in size of phlorotannin-containing physodes previously reported in *S. dermatodea* and *A. esculenta* (Wiencke et al., 2004) and the corresponding increase in UV-absorbing capacity presented in the present study can contribute to greater tolerance of UVR exposure in both species. Results of this study are consistent with our field germination experiments performed on *S. dermatodea*, *A. esculenta* and *L. digitata*. In these experiments, zoospores were exposed to ambient solar radiation at different water depth and cultivated in the laboratory at low-light conditions (Wiencke et al., 2006). In all species, germination rates of zoospores exposed to PAR alone were similar at all depths investigated. However, species-specific response to UVR exposure was observed. Zoospores of *S. dermatodea* germinated in all water depths were investigated while germination of zoospores of *A. esculenta* was strongly inhibited in 0.5 m water depth. Zoospores of *L. digitata* were most susceptible to UVR where failure of germination was observed in 0.5 and 1.0 m water depths.

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