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# Zoospores of Three Arctic Laminariales Under Different UV Radiation and Temperature Conditions: Exceptional Spectral Absorbance Properties and Lack of Phlorotannin Induction

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## ABSTRACT

**Phlorotannins have often been considered to act as UV-protective compounds in zoospores of brown algae. However, only the absorption characteristics of zoospores under UV exposure have been determined and no data are available on the actual content of phlorotannins or on temperature–UV interactions. Therefore, we determined the absorbance spectra and the phlorotannin contents in zoospore suspensions of three Arctic species (*Saccharina latissima*, *Laminaria digitata*, *Alaria esculenta*), and in the media surrounding zoospores after exposure to different radiation (400–700, 320–700, 295–700 nm) and temperature (2–18°C) conditions for 8 h. Absorption typical of phlorotannins with a maximum at 276 nm was monitored in zoospore suspensions as well as in the media surrounding zoospores, but the results depended strongly on radiation treatments and on zoospore densities. Surprisingly, the content of UV-absorbing phlorotannins subsequent to different exposures did not change in any of the three species. The observed exceptional absorption properties could, therefore, not be related to phlorotannin contents. These findings are discussed in light of a strong phlorotannin investment from sporophytes during spore release and a minor UV-protective role of phlorotannins for zoospores of Arctic kelp species.**

## INTRODUCTION

Kelps, brown algae of the order Laminariales, are the key species in many seaweed forests and as such, very important structural elements of benthic coastal ecosystems. Furthermore, kelps are seasonally abundant in the planktonic phase as each fertile sporophyte of Laminariales produces approximately $10^9$–$10^{11}$ zoospores every year, which are released into the sea (1). The zoospores, with a size of 5 $\mu$m, are the most vulnerable life stage in the life cycle of Laminariales (2,3). Consequently, laminarian zoospores might be strongly affected by global climate changes, especially in the Arctic regions. Here, the temperature will increase dramatically up to 3–6°C during the next century (4) and UV-B radiation (UVBR) will be enhanced due to an approximate 20% depletion of the stratospheric ozone layer by the year 2020 (5).

However, it is well known that UVBR becomes seasonally strongly absorbed in coastal seawaters due to the presence of organic matter, including phlorotannins from seaweeds (6,7). Phlorotannins occur only in brown algae and were thought to be UV-B protective compounds due to their absorption capacity in the UV-B region (8). As a result of their phlorotannin exudation into the surrounding seawater, Laminariales were thus considered to be able to alter their spectral environment and to shade themselves from detrimental UV radiation (UVR) (9). However, the magnitude of UV-B absorption by Laminariales' exudates is extremely dependent on their actual concentration, on temperature and on differing qualities of spectral radiation (9,10).

The phlorotannin content of seaweeds can depend on spectral light qualities: In macrothalli of *Ascophyllum nodosum* and *Fucus vesiculosus* which have been exposed to sunlight, the phlorotannin levels are higher than thalli in shaded conditions (11). A similar comparison exists between UVR-exposed blades of *Macrocystis integrifolia*, *A. nodosum* and *Saccharina latissima* (L.) Lane, Mayes, Druehl, Saunders comb. nov. and UVR-shielded blades (8,9,12). But, the patterns of phlorotannin induction were not consistent. For example, phlorotannin levels in *Desmarestia anceps* and *Desmarestia menziesii* sporophytes increased in both the UVR and the non-UVR treatment (13). In a review by Amsler and Fairhead (14), it was pointed out that differences exist in the phlorotannin content of kelps in various ontogenetic stages as well as within the same and between different species under various stress factors. As far as we are aware, studies determining the effects of temperature on phlorotannin induction still have to be carried out. There are also no available data on the phlorotannin content in the vulnerable laminarian zoospores, nor on Arctic kelp species nor with regard to interactive effects of UVR and elevated temperature.

The main goal of the present study was, therefore, to investigate the absorption properties of and phlorotannin levels in zoospore suspensions of three Arctic kelp species (*S. latissima*, *Laminaria digitata* [Huds.] Lamour., *Alaria*

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*esculenta* [L.] Grev.) under three radiation and four different temperature conditions in context to global climate changes. Three major questions are addressed: (1) To which extent do different radiation conditions and different temperatures affect the absorbance properties and the UV-B protection of Laminariales' zoospores in suspensions? (2) Do the phlorotannin levels change in zoospore suspensions in response to different radiation and/or temperature exposure? (3) How many phlorotannins are available in the medium surrounding the zoospores?

## MATERIALS AND METHODS

*Algal material*. Fertile individuals of *S. latissima*, *L. digitata* and *A. esculenta* were collected by scuba divers from a depth of 4–8 m in Kongsfjorden (Spitsbergen, 78°55′N, 11°56′E) in May and June 2006. After having been wiped clean with tissue paper, the fertile algal parts were stored in a moist, dark chamber at 2 ± 1°C for 2 days. The release of zoospores was induced by the immersion of sori in 0.2 μm filtered seawater. The obtained zoospore suspensions were then filtered through 20 μm gauze (Nytal HD 20; Hydro-Bios, Kiel, Germany).

Zoospore densities were counted with a Neubauer chamber (Brand, Wertheim, Germany) under 200× magnification in an Axioplan microscope (Zeiss, Jena, Germany). The samples were adjusted to the required zoospore density by the addition of seawater (see *Experimental conditions*). Zoospore suspensions of three to five individuals per species were subsequently allotted to separate culture dishes by dispensettes (Brand): 5 mL into 35 × 10 mm culture dishes (Corning TM, Corning, Inc., New York) for absorption measurements as well as LC/MS analysis and 40 mL into 85 × 15 mm glass culture dishes for Folin Ciocalteau assay. Only zoospore suspensions with approximately 75–100% motile zoospores were used for experiments.

*Experimental conditions*. Zoospore suspensions of *L. digitata*, *S. latissima* and *A. esculenta* ($1.0–8.0 \times 10^5$ zoospores $mL^{-1}$) were exposed in environmentally controlled chambers to temperatures of 2, 7, 12 and 18 ± 1.4°C as well as to three light treatments by exposure to (1) photosynthetically active radiation PAR (P), (2) PAR + UV-A radiation (PA) or (3) PAR + UV-A + UV-B radiation (PAB) for 8 h. Additionally, three concentrations of *A. esculenta* zoospore suspensions ($3.7 \pm 0.1 \times 10^6$, $1.9 \pm 0.1 \times 10^6$, $6.8 \pm 1.2 \times 10^5$ zoospores $mL^{-1}$) were exposed to P, PA and PAB at 7 ± 1.0°C for 8 h.

For the generation of light treatments, culture dishes were covered with filter foil off-cuts, transparent for wavelengths of 400–700 nm (P; URUV Ultraphan UV farblos, Difrega, Munich, Germany), 320–700 nm (PA; Folanorm SF-AS, Folex GmbH, Cologne, Germany) and 295–700 nm (PAB; URT 140 Ultraphan UV farblos, Difrega), respectively. Radiation was generated by fluorescent tubes (PAR: 36 W, Powertwist true light® II, Duro-Test® Lighting, Inc., Philadelphia; UVR: UV A-340 tubes, 40 W, Q- Panel, Cleveland, OH) which were, if necessary, dimmed by black net gauze (1.4 mm mesh size). Radiation was measured by a cosine corrected LI-COR 2π sensor attached to a LI-COR Li-190 radiometer (LI-COR Bioscience, Lincoln, NE) and by the cosine corrected sensors PMA 2110 (UV-A radiation, UVAR) and PMA 2106 (UVBR), the latter both connected to radiometer PMA 2100 (Solar Light, Glenside, PA). UVR measurements were carried out under a WG 320 glass filter (Schott, Mainz, Germany) to exactly cut wavelengths below and above 320 nm. Specimens were irradiated with $20 \pm 5$ μmol $m^{-2}$ $s^{-1}$ PAR, $6.73 \pm 0.81$ W $m^{-2}$ UVAR and $0.35 \pm 0.04$ W $m^{-2}$ UVBR. The biologically effective doses were computed using spectral measurements with a cosine sensor attached to UV–VIS spectroradiometer (Ramses SAM 80f6 to IPS 104; TriOS Optical Sensors, Trios, Oldenburg, Germany) and the formula for generalized plant damage (15). Generalized plant damage for zoospores amounted to 140 mW $m^{-2}$ (P), 170 mW $m^{-2}$ (PA) and 530 mW $m^{-2}$ (PAB), respectively.

In addition, the phlorotannin monomer phloroglucinol and its isomer pyrogallol (Merck, Darmstadt, Germany) were diluted in 5 mL deionized water (10 μg $L^{-1}$) and exposed for 8 h at 7 ± 1.0°C to five radiation conditions: (1) P, (2) PA, (3) PAB, (4) A (320–400 nm, UG 5 glass filter [Schott] combined with foil Folanorm SF-AS) and (5) AB (280–400 nm, UG 5 glass filter). For detailed irradiance see Table 1.

**Table 1.** Irradiation conditions in phloroglucinol and pyrogallol experiments.

| Treatments | P | PA | PAB | A | B |
|---|---|---|---|---|---|
| PAR (μmol $m^{-2}$ $s^{-1}$) | 21 | 21 | 21 | – | – |
| UVAR (W $m^{-2}$) | – | 5.7 | 5.7 | 4.4 | 4.8 |
| UVBR (W $m^{-2}$) | – | – | 0.6 | – | 0.46 |

PAR = photosynthetically active radiation; PA = PAR + UVAR; PAB = PA + UVRB; A = UVAR; AB = UVAR + UVBR.

*Absorption*. Absorption of initial, light- and temperature-exposed zoospore suspensions of *A. esculenta*, *L. digitata* and *S. latissima*, of zoospores and of their surrounding media obtained by filtering zoospore suspensions through 1.2 μm filters (GF/C filter; Whatman, Maidstone, UK) was measured in the range of 240–700 nm (quartz glass cuvettes 6030-UV; Hellma, Müllheim, Germany) with a spectrophotometer (UV 2401PC; Shimadzu, Kyoto, Japan) including an integrative sphere ($n$ = 4–5). Filtered seawater (0.2 μm) was used as a reference to obtain the absorption spectra of zoospore suspensions and of the surrounding media. Absorption of zoospores was assessed by measuring zoospore suspension *versus* surrounding medium acting as reference. Absorption properties of initial, dark- and light-treated zoospore suspensions of *A. esculenta* (triplicates of three concentrations) and phloroglucinol as well as pyrogallol solutions ($n$ = 5) were also examined. Absorption of chemicals was measured *versus* deionized water in a Hitachi spectrophotometer (U-3310; Tokyo, Japan).

*LC-MS analysis*. Mass spectral experiments were performed on an ABI-SCIEX-4000 Q Trap, triple quadrupole mass spectrometer equipped with a TurboSpray® interface coupled to an Agilent model 1100 LC. The liquid chromatograph equipment included a solvent reservoir, an in-line degasser (G1379A), a binary pump (G1311A), a refrigerated autosampler (G1329A/G1330B) and a temperature-controlled column oven (G1316A). Mass spectrometric analyses for phloroglucinol were performed in five replicate measurements. The analytical column (250 × 2 mm) was packed with 5 μm (pore size 200 Å) Aqua C18 (Phenomenex, Aschaffenburg, Germany) and maintained at 25°C. The flow rate was 0.2 mL $min^{-1}$ and gradient elution was performed with two eluants, A and B. Eluant A was 2 mM ammonium formate and 50 mM formic acid in water and B was 2 mM ammonium formate and 50 mM formic acid in acetonitrile/water (95:5 vol/vol). The gradient was as follows: 12 min column equilibration with 1% B, then 15 min linear gradient to 49.5% B, then 1 min until return to initial conditions (1% B). Five microliters of the sample was injected.

Multiple reaction monitoring experiments were carried out in the positive ion mode by selecting the following transitions for phloroglucinol and pyrogallol (precursor ion > fragment ion): $m/z$ 127 > 81 (quantifier), $m/z$ 127 > 109 and 127 > 99 (qualifiers). Dwelling times of 200 ms were used for each transition. For these studies the following source parameters were used: curtain gas: 10 psi, temperature: 200°C, ion-spray voltage: 5500 V, nebulizer gas: 11 psi and auxiliary gas: 10 psi, interface heater: on, declustering potential: 10 V. The following parameters were used for compound fragmentation: collision gas: high, entrance potential 10 V, collision energy 30 V and collision cell exit potential 12 V. Phloroglucinol was quantified by calibration with an external standard.

*Phlorotannin quantification*. Zoospores of *L. digitata*, *S. latissima* and *A. esculenta* were broken up with sterile finger tips in petri dishes before or after exposure to the different light and temperature conditions. Parallel to this, the medium surrounding the zoospores was separated from initial and exposed zoospore suspensions by filtration (1.0 μm Nucleopore Track-Etch Membrane PC MB; Whatman) at 300–400 mbar using a vacuum pump. Forty milliliters of zoospore suspensions and 40 mL of surrounding media (triplicates each) were treated with 40 mL deionized water, stored at −20°C, lyophilized (alpha 1–4 LSC; Christ, Osterode am Harz, Germany) and dissolved in 4 mL deionized water. For analysis 1 mL aliquots of lyophilized samples were mixed with 1 mL Folin Ciocalteau reagent (1 *N*) and after 3 min 2 mL of 20% sodium carbonate was added.

Mixtures were maintained in darkness for 45 min and centrifuged at 1600 ***g*** for 8 min. To quantify phlorotannin contents, absorption of Folin Ciocalteau reagents was measured in supernatants at 730 nm using Shimadzu spectrophotometer (cuvettes 1.5 mL PS; Plastibrand, Wertheim, Germany). During each assay known concentrations of phloroglucinol solutions were used as interim standards.

*Statistics*. After the homogeneity of variances was tested with Cochran's test ($P < 0.01$), effects of independent factors (temperature, UVR, individual, suspension and surrounding medium) were estimated by one or two factorial Models I ANOVA ($P < 0.05$). The data were log, sin or square root transformed should it be necessary to meet the assumptions for ANOVA. *Post hoc* multiple mean comparisons were accomplished by using the Tukey HSD test ($P < 0.05$). An unpaired *t*-test was applied to detect significant differences between the phlorotannin amounts $L^{-1}$ of three species. All statistical analyses were performed with Statistica software Version 7 (StatSoft, Inc.) in accordance with Sokal and Rohlf (16).

## RESULTS

### Absorption properties

The peak at 675 nm typical of chlorophyll *a* was characteristically very low in zoospore suspensions of *S. latissima* (OD 0.00–0.04 $nm^{-1}$), *L. digitata* and *A. esculenta* (OD 0.01–0.02 $nm^{-1}$, compare also Roleda *et al.* [17]). However, zoospore suspensions and surrounding media of three species

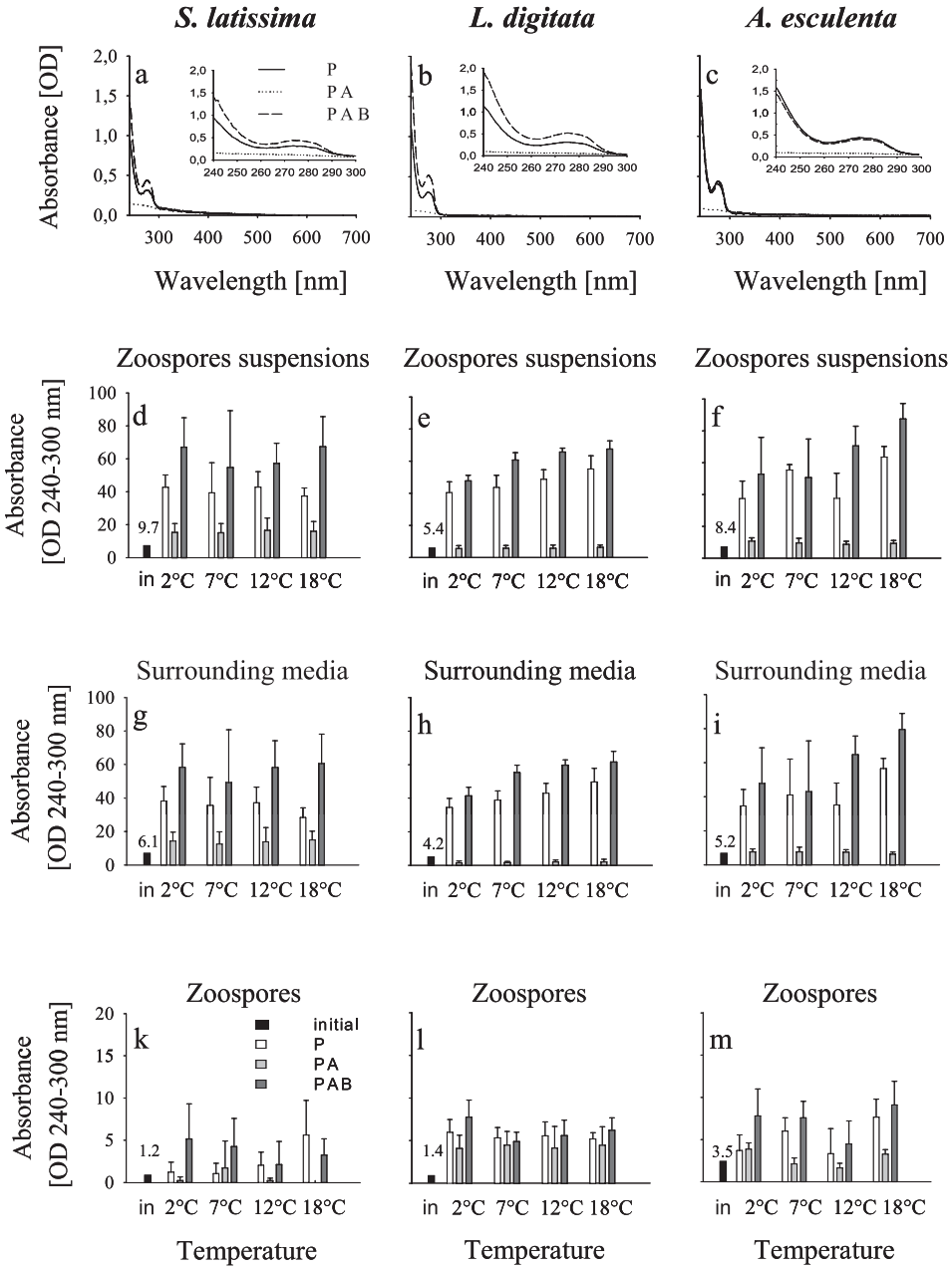


**Figure 1.** Spectral absorbance from light-exposed zoospore suspensions (7°C) of *Saccharina latissima* ($2.4 \pm 1.1 \times 10^5$ zoospores $mL^{-1}$), *Laminaria digitata* ($4.9 \pm 1.1 \times 10^5$ zoospores $mL^{-1}$) and *Alaria esculenta* ($6.8 \pm 0.9 \times 10^5$ zoospores $mL^{-1}$) is given in (a–c), whereas insets enlarge the data range of OD 240–300 nm. The integral of the absorbance (OD 240–300 nm) from initial, light- and temperature- exposed zoospore suspensions, surrounding media of zoospores and zoospores ($n$ = 4–5) of three species is presented in (d–m). in = initial; P = 400–700 nm; PA = 320–700 nm; PAB = 295–700 nm.

showed a very strong UV-C and a weaker UV-B absorption with a peak at 276 nm in P and PAB treatments at all temperatures, albeit not in freshly released or PA-treated specimens (Fig. 1a–c, DPA—depressed phlorotannin typical absorption). The P and PAB-induced absorption changes (IAC) measured in zoospore suspensions and surrounding media in the range of 240–300 nm were higher under PAB in comparison with P exposure in *L. digitata* at 7–18°C ($P \leq 0.001$) and in *A. esculenta* at 12°C ($P \leq 0.03$) (Fig. 1d–i). However, the P and PAB-induced absorption was similar in other temperature treatments ($P \geq 0.76$), and in *S. latissima* under all temperature conditions ($P \geq 0.10$).

The absorption of P and PAB-treated suspensions and surrounding media of *L. digitata* and *A. esculenta* increased significantly at elevated temperatures ($P < 0.05$) with a linear slope of 6.46 (P, $R^2 = 0.87$) or 15.02 (PAB, $R^2 = 0.80$) in *L. digitata* and 5.98 (P, $R^2 = 0.57$) or 11.69 (PAB, $R^2 = 0.82$) in *A. esculenta*. A temperature-dependent absorption increase was, however, not the case in suspensions and surrounding media of *S. latissima* ($P \geq 0.84$, Table 2). The inherent absorption of zoospores (240–300 nm) was only significantly different between PAB and initial or PA treatments in *A. esculenta* at 7 and 18°C ($P < 0.01$). Nevertheless, a trend to an IAC/DPA pattern was also apparent in zoospores of other temperature treatments and two other species (Fig. 1k–m, Table 2).

Furthermore, the DPA depended on the density of zoospores as examined in *A. esculenta* suspensions, by way of an example (Fig. 2a–c). At the lowest density of $6.8 \times 10^5$ zoospores $mL^{-1}$, the IAC/DPA pattern was very distinct. But at a concentration of $1.8 \times 10^6$ zoospores $mL^{-1}$, the absorption of the PA-treated suspensions approached the absorption obtained in the P and PAB treatments (Fig. 2a–c). Moreover, in suspensions with the highest spore density of $3.9 \times 10^6$ zoospores $mL^{-1}$, there was always an IAC (Fig. 2a).

The UV-B range of 300–320 nm, mainly occurring in natural seawaters, was only absorbed to maximal 16% per wavelength (mean 3–8%) by low dense zoospore suspensions of three species. This was independent of temperature and/or radiation treatments ($P \geq 0.09$). With increasing zoospore densities in suspensions of *A. esculenta*, the absorption capacity for the wavelengths 300–320 nm increased but did not differ within radiation treatments ($P \geq 0.08$). Lowest dense zoospore suspensions of *A. esculenta* absorbed a maximum of 7%, medium dense suspensions a maximum of 15% and highly dense zoospore suspensions a maximum of 30% per wavelength in the range of 300–320 nm.

In an effort to understand the chemical basis of the IAC/DPA, we tested the absorbance properties of the monomer of phlorotannins phloroglucinol and its isomer pyrogallol. The phloroglucinol and pyrogallol showed the same IAC/DPA patterns as biological samples. Absorption (240–300 nm) of phloroglucinol and pyrogallol solutions was induced after P exposure and proved highest after PAB exposure (Table 3). Absorption differed among P and PAB-exposed phloroglucinol ($P < 0.001$) and pyrogallol solutions ($P \leq 0.016$, Table 3) and absorption of both treatments was also significantly different from all other treatments of phloroglucinol or pyrogallol solutions ($P < 0.001$). In comparison with initial specimens (Table 3), dark, PA, A and AB-treated phloroglucinol and pyrogallol solutions slightly increased their absorption (240–300 nm). The absorption (240–300 nm) of dark PA, A and AB-treated phloroglucinol and pyrogallol solutions did not differ from each other ($P \geq 0.35$), but was significantly different from the absorption of fresh phloroglucinol ($P \leq 0.001$) and pyrogallol solutions ($P \leq 0.023$), except in the latter case which did not differ with dark treatment of pyrogallol ($P = 0.07$).

### Analysis of molecular weights

Treated phloroglucinol solutions revealed probable changes at the molecular level. However, in initial or light-treated specimens, no compounds with other molecular weights other than 126.1 DA, representing either phloroglucinol or its collision-induced ion products (Fig. 3a), were detected by LC-MS. In contrast to the absorption measurements, the quantities of phloroglucinol amounts showed no significant differences among initial and light treatments ($P = 0.37$, Table 3, see Fig. 3b for qualifier and quantifier).

### Phlorotannin contents

The general amounts of phlorotannins were $3.2 \pm 1.2$ μg phlorotannins $L^{-1}$ in *S. latissima*, $3.2 \pm 0.9$ μg $L^{-1}$ in *L. digitata*

**Table 2.** Two-way analysis of variances (ANOVA) of absorption in zoospore suspensions, the surrounding media and the zoospores of *Saccharina latissima* ($n = 4$), *Laminaria digitata* ($n = 5$) and *Alaria esculenta* ($n = 5$) after 8 h exposure to four different temperature and three radiation conditions.

| | | | Zoospore suspensions | | Surrounding media | | Zoospores | |
|---|---|---|---|---|---|---|---|---|
| Species | Source of variation | d.f. | *F* | *P* | *F* | *P* | *F* | *P* |
| *Saccharina latissima* | Temperature (A) | 3 | 0.27 | 0.84 | 0.25 | 0.86 | 0.71 | 0.55 |
| | Irradiation (B) | 2 | 36.49 | > 0.01* | 36.25 | > 0.01* | 6.48 | > 0.01* |
| | A × B | 6 | 0.31 | 0.93 | 0.33 | 0.92 | 1.90 | 0.11 |
| *Laminaria digitata* | A | 3 | 58.80 | > 0.01* | 95.51 | > 0.01* | 1.02 | 0.39 |
| | B | 2 | 464.26 | > 0.01* | 633.23 | > 0.01* | 5.81 | > 0.01* |
| | A × B | 6 | 37.38 | > 0.01* | 57.58 | > 0.01* | 0.89 | 0.51 |
| *Alaria esculenta* | A | 3 | 3.74 | 0.02* | 2.93 | 0.04* | 7.21 | > 0.01* |
| | B | 2 | 221.24 | > 0.01* | 295.42 | > 0.01* | 18.11 | > 0.01* |
| | A × B | 6 | 2.70 | 0.03* | 2.84 | 0.02* | 1.33 | 0.27 |

*Significant differences of dependent variables.

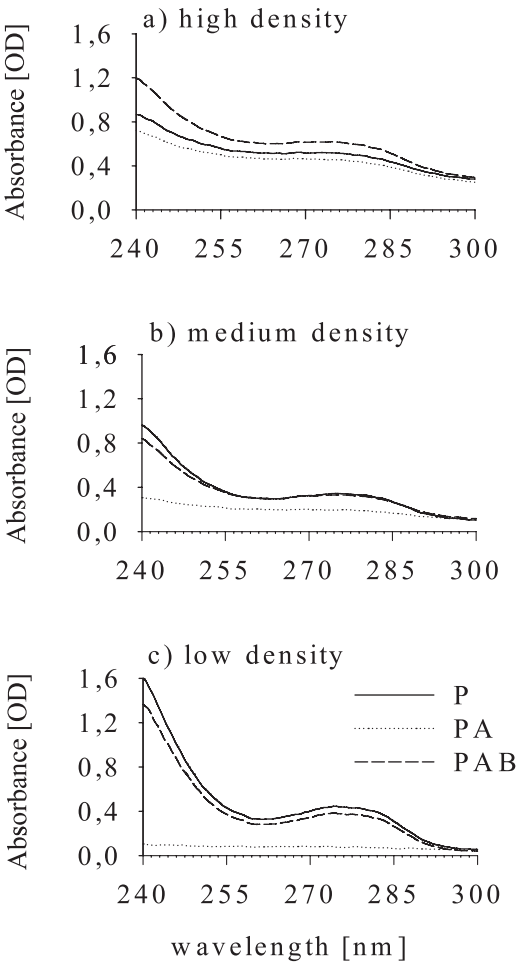


**Figure 2.** Absorbance spectra of irradiated *Alaria esculenta* zoospore suspensions (mean, $n = 3$) with (a) high density: $3.7 \pm 0.1 \times 10^6$ zoospores $mL^{-1}$, (b) medium density: $1.9 \pm 0.1 \times 10^6$ zoospores $mL^{-1}$ and (c) low density: $6.8 \pm 1.2 \times 10^5$ zoospores $mL^{-1}$. P = 400–700 nm; PA = 320–700 nm; PAB = 295–700 nm.

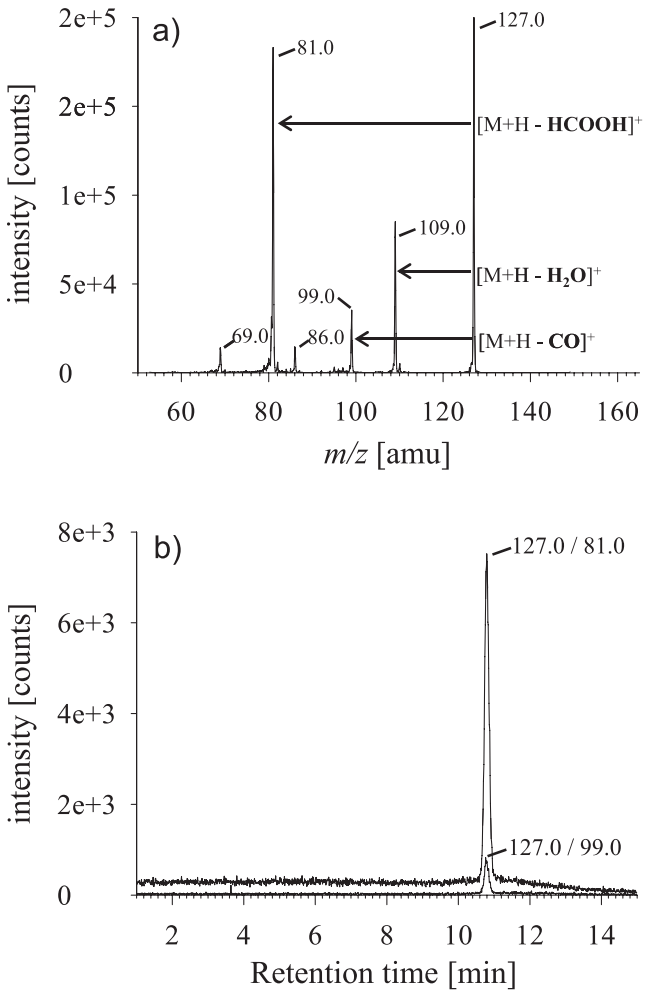


**Figure 3.** (a) Collision-induced dissociation mass spectrum of 10 $\mu g\ L^{-1}$ phloroglucinol and (b) retention times of transitions of phloroglucinol pseudomolecular ion fragments determined by multiple reaction monitoring (quantifier: transition $m/z$ 127 > 81; qualifier $m/z$ 127 > 99).

**Table 3.** Absorbance (OD 240–300 nm) and peak area $m/z$ = 126.1 of phloroglucinol (10 $\mu g\ L^{-1}$) and absorbance (OD 240–300 nm) of its isomer pyrogallol (10 $\mu g\ L^{-1}$) of initial, dark- and light-treated solutions.

| | Phloroglucinol | | Pyrogallol |
|---|---|---|---|
| | Absorption | Peak area | Absorption |
| Initial | $0.54 \pm 0.02^{a}$ | $8130 \pm 1311^{a}$ | $0.09 \pm 0.11^{a}$ |
| Dark | $0.89 \pm 0.18^{b}$ | $8736 \pm 584^{a}$ | $0.35 \pm 0.14^{ab}$ |
| P | $3.57 \pm 0.31^{c}$ | $8322 \pm 1219^{a}$ | $8.37 \pm 6.29^{c}$ |
| PA | $0.97 \pm 0.06^{b}$ | $6816 \pm 580^{a}$ | $0.66 \pm 0.16^{b}$ |
| PAB | $5.77 \pm 0.77^{d}$ | $6094 \pm 616^{a}$ | $22.64 \pm 6.46^{d}$ |
| A | $0.86 \pm 0.07^{b}$ | $6476 \pm 616^{a}$ | $0.40 \pm 0.11^{b}$ |
| AB | $0.91 \pm 0.07^{b}$ | $5888 \pm 522^{a}$ | $0.53 \pm 0.11^{b}$ |

Values are mean ± SD, $n = 5$. Lower-case letters indicate significant differences among treatments of one isomer. P = 400–700 nm; PA = 320–700 nm; PAB = 295–700 nm; A = 320–400 nm; AB = 295–400 nm.

or $3.9 \pm 1.3$ $\mu g\ L^{-1}$ in *A. esculenta* zoospore suspensions and $3.1 \pm 1.5$, $2.8 \pm 1.1$ or $2.3 \pm 0.8$ $\mu g\ L^{-1}$ in their surrounding media, respectively. Amounts of phlorotannins $L^{-1}$ neither differed among zoospore suspensions and surrounding media of one species ($P > 0.05$) other than that of *A. esculenta* at 12°C ($P = 0.029$), nor among suspensions of three species, nor among contact media of three species ($P \geq 0.059$), and also not in response to temperature ($P \geq 0.62$) or radiation exposures ($P \geq 0.14$). Regarding individual amounts of phlorotannins $L^{-1}$, only individual 1 of *L. digitata* was distinct from individuals 2 and 3 ($P \leq 0.049$), whilst within the other two species individuals did not differ significantly ($P \geq 0.07$).

## DISCUSSION

This study clearly shows that the phlorotannin content of zoospores and zoospore suspensions does not change after exposure to different radiation and temperature conditions as inferred from the spectrometric absorption measurements. Another important result is that the phlorotannins in the surrounding medium probably originate mainly from sporophytic exudates and not from the spores.

### Exceptional absorption properties

An absorption in the UV-C and UV-B range with a similar curve progression and a maximum at approximately 276 nm as observed at all temperatures in P and PAB treatments of *S. latissima*, *L. digitata* and *A. esculenta* (Fig. 1a–c) was described to be typical of laminarian and fucoid phlorotannins (8,18,19). Moreover, the temperature dependence of absorption (Fig. 1e,f,h,i) was comparable to the increased UVR absorption of nondialyzable phlorotannin-polymer fractions of *A. nodosum* at higher temperatures (10). However, persistent DPA after PA exposure as obtained in our experiments has not yet been reported (Fig. 1a–i, Table 3). As a consequence of the mirrored response patterns of the DPA and the gametogenesis of Laminariales after PA exposure (3), a relation between the phlorotannin absorption status and the enhancing PA effects on Laminariales can be presumed. An absence of phlorotannin typical UVR absorption was equally monitored in freshly released exudates from thalli of *F. vesiculosus* (20), in freshly released zoospore suspensions of Laminariales (Fig. 1d–i), in dark A and AB-treated phlorotannin monomers (Table 3) as well as very occasionally in P treatments of *A. esculenta* zoospores (data not shown) and of pyrogallol solutions (Table 3).

The phenomenon of irradiation-specific IAC/DPA is certainly based on a chemical process as the IAC/DPA phenomenon similarly occurred in phloroglucinol and pyrogallol solutions (Table 3). The chemical mechanism behind this absorption phenomenon was not entirely ascertained in this study, but a number of probable chemical reactions could be ruled out. A polymerization of phloroglucinol monomers could be rejected due to the observed peaks which are only typical of phloroglucinol and its collision-induced products (Fig. 3), albeit an increase in UV absorption by substitution of monomers is well known (21). Although several authors have suggested that an enriched UV absorption is due to a photodegradation or oxidation of phloroglucinol (22–24), the IAC/DPA patterns were not reflected by the stable mass of radiation-treated phloroglucinol molecules (Table 3). An isomerization of phloroglucinol to pyrogallol under P and PAB exposure could also be precluded as the UVR absorption of phloroglucinol and pyrogallol did not react antagonistically to the different radiation conditions (Table 3). Another explanation for the irradiation-specific IAC/DPA of phloroglucinol monomers, without changing their molecular weight, could be in accordance with the considerations of Swanson and Druehl (9). They suggested that absorbance changes in phlorotannins are due to photoinduced electronic transitions within aromatic molecules. However, should this be the case, it ought to be taken into account that these assumed electronic transitions in phloroglucinol molecules are only stable for a few microseconds (25), other than the absorbance patterns (IAC and DPA) observed in this study which retained their stability for several hours. Certainly, further investigations regarding exceptional absorbance properties of phloroglucinol and laminarian exudates are required.

### The UV-protective role of exuded phlorotannins

Another interesting observation of our study was the dependence of the IAC/DPA phenomenon on zoospore concentration. The absence of a DPA at higher densities of *A. esculenta* zoospores (Fig. 2) correlated with results of Roleda *et al.* (17). They revealed only minor differences among the strong UVR absorption of P, PA and PAB-treated dense zoospore suspensions of *S. latissima*, *A. esculenta* and *Saccorhiza dermatodea* from Spitsbergen. Moreover, dense zoospore suspensions of the same species provided a higher UV-B protection for underlying zoospores, whereas less dense suspensions showed no or strongly reduced UV-B protective effects (17). Aside from the UV-B protection by dense zoospore suspensions, UV experiments using more natural lower densities exhibited highly detrimental UV-B effects on the germination, the ultrastructure, the photosynthesis and the DNA of Laminariales' zoospores, especially at elevated temperatures (3,26–28).

Consequently, the activity of the repair mechanisms seems to be more crucial for the survival of zoospores in shallow waters than UV-B protection by phlorotannins. This is in fact the case with respect to the recovery of photosynthesis (29). Certainly under artificial UVR conditions, the recovery of photosynthesis is more active in species inhabiting shallow waters (*e.g. A. esculenta*) than in species from greater depths (*e.g. S. latissima*) (30,31). In view of the manifold reports on UV-B damage to Arctic zoospores (reviewed in 29, 32) and as a result of the very low UV-B absorption of phlorotannins in less dense zoospore suspensions which we observed, it is of importance to reconsider the hypothesis of Swanson and Druehl (9). The latter suggest that kelp populations may be able to promote UVR refugia for their zoospores and other kelp residents by exuding phlorotannins. Nevertheless, the different susceptibility to UVR during zoospore germination of *A. esculenta* and *L. digitata* collected in field UVR experiments at different dates (31) could probably be the result of the changing phlorotannin content in the seawater. Prospective research should therefore be aimed at investigating the seasonally varying UV protective role of phlorotannins for zoospores of Arctic Laminariales.

### Lack of phlorotannin induction

After the exposure of kelp zoospores to UVR, the following microscopical observations were often made: The physodes containing phlorotannin became enlarged due to the aggregation of several small ones, an exocytosis of physodes into the surrounding medium took place and the number of physodes increased (33–35). On the other hand, the detection of quantitative differences in the phlorotannin content of macroalgae was regarded only possible under conditions of high formation rates, which also depended on the general metabolic turnover of cells (34,35). Therefore, the discrepancy between the fact that physodes in *A. esculenta* zoospores seem to be enlarged and extruded after 8 h of exposure to PA and PAB (36) and the fact that the phlorotannin levels stay constant is most likely due to a too low phlorotannin production within this time. In support of this hypothesis, phlorotannin accumulations in exudates from *M. integrifolia* sporophytes were not detected after 4 h but only after 8 h of PA exposure (37) and also in the case of *A. nodosum* sporophytes, not after 4 weeks but after 7 weeks of PAB exposure (9). The necessity of a phlorotannin induction time longer than the duration of the experiment was likewise considered as a reasonable explanation for any differences

among phlorotannin contents of P, PA and PAB-treated juvenile thalli and embryos of *Fucus gardneri* after a 3 week exposure (19). Aside from too short experimental time for the detection of phlorotannin induction, perhaps the sensitivity of the Folin Ciocalteau assay is too low. The Folin Ciocalteau method did, however, prove to be most appropriate for small sample volumes and small phlorotannin concentrations ([8], R. Koivikko, personal communication).

### Maternal phlorotannin investment

Phlorotannins detected in the medium surrounding the zoospores may not only be released by the zoospores, but may at least partially originate from the light-, temperature- and desiccation-stressed sori during spore release. Corresponding to this hypothesis, the highest phlorotannin exudation rates caused by stress, shock or injury were detected in thalli of *A. nodosum* and *Ecklonia radiata* during the first few minutes of immersion (38,39). The assumption that maternal phlorotannin release occurs is supported by the high and constant phlorotannin amounts in the media surrounding zoospores revealed in this study. It is also supported by the fact that free physodes were already observed in freshly released zoospore suspensions of *L. digitata* and *S. dermatodea*, thought to have originated in part from phlorotannin-rich paraphyses located in sorus tissues of sporophytes ([40], U. Lüder, unpublished). On the basis of these observations we hypothesize that sporophytes (sori) contribute strongly to phlorotannin release and that there is not a significant level of *de novo* synthesis of phlorotannins by zoospores within 8 h, at least not under laboratory conditions.

## CONCLUSION

Unfortunately, there are no field data available in the literature regarding naturally exuded phlorotannin amounts during spore release. However, phlorotannin concentrations measured in the medium surrounding zoospores (2.8–3.1 $\mu g\ L^{-1}$) as well as those of phloroglucinol solutions (10 $\mu g\ L^{-1}$) investigated in this study coincide with predicted phlorotannin exudation rates of 1.4–11.2 $\mu g\ L^{-1}\ h^{-1}$ for kelp beds of *E. radiata* (41) and *M. integrifolia* (9). Therefore, the exceptional absorption patterns and the low UV-B absorption by phlorotannins, as revealed in this study, are very likely to occur regularly in the field. Zoospores of Laminariales might therefore only be UV-B protected by a very large phlorotannin exudation, for example 0.57–0.63 mg phlorotannin $L^{-1}$, as observed in the contact seawater of a large kelp bed of *M. integrifolia* (42).

Drastic losses of European kelp populations have been reported in the last century and more kelp reductions are expected in the future (43). Zoospores released from more sparse kelp populations will either completely lack any UV-B protection due to too low extruded phlorotannin concentrations into the seawater or kelps will have to increase their excretion in order to maintain a phlorotannin-provided UV-B protection for their zoospores. But phlorotannin exudation may not increase in times of global warming, as the amount of phlorotannins did not change in response to temperature in our study and exudation rates of *L. digitata* and *Laminaria agardhii* sporophytes were constant or even decreased slightly at elevated temperatures (44). However, in addition to UV-B protection, maternal phlorotannin investment during spore release could also offer other advantages for zoospores, for instance with respect to the algicidal, bactericidal and antioxidative functions of phlorotannins (45–47).

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