Universität Bremen GLOMAR AWI Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft

Phlorotannins as UV-protective substances in early developmental stages of brown algae

DISSERTATION

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vorgelegt von

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Erkennen heißt nicht zerlegen, auch nicht erklären.

Es heißt Zugang zur Schau finden.

Aber um zu schauen, muss man erst teilnehmen.

Das ist eine harte Lehre.

Antoine de Saint-Exupéry

Contents

List of Abbreviations

%	Percent
~	Approximately
<	Smaller than
>	Greater than
°	Degree
°C	Degree Celsius
µ	Micro
µg	Microgram
µm	Micrometer
µmol	Micromole
Acetyl-CoA	Acetyl-Co-enzyme A
Apr	April
Aug	August
C	Carbon
CIE	Commission Internationale de l'Éclairage
CPD	Cyclobutane pyrimidine dimer
d	Days
Da	Dalton
Dec	December
DNA	Desoxyribonucleic acid
DOC	Dissolved organic matter
DU	Dobson unit
E	East
e.g.	Exampli gratia (for example)
EM	Electron microscopy
FA	Fatty acid
FC	Folin-Ciocalteu
FD	Folin-Denis
Feb	February
GC	Gas chromatography
h	Hours
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
IR	Infrared
Jan	January
Jul	July
Jun	June
kDa	Kilo Dalton
kg	Kilogram

km	Kilometer
L	Lipid globules
LM	Light microscopy
m	Meter
MAA	Mycosporine-like amino acid
Mar	March
mol	Mole
mRNA	Messenger Ribonucleic acid
MUFA	Monosaturated fatty acids
N	North
n	Number of replicates
NBT	Nitro-blue tetrazolium
nm	Nanometer
Nov	November
Oct	October
OH	Hydroxyl
p	Probability
PA	Photosynthetically active radiation (PAR) + UV-A (320-700 nm)
PAB	Photosynthetically active radiation (PAR)+UV-A +UV-B (280-700 nm)
PAR	Photosynthetically active radiation (400-700 nm)
PS II	Photosystem II
PSU	Practical salinity unit
Publ	Publication
PUFA	poly unsaturated fatty acids
ROS	Reactive oxygen species
s	Second
S	South
SAFA	Saturated fatty acids
SD	Standard deviation
Sep	September
UV-A	Ultraviolet radiation A (320-400 nm)
UV-B	Ultraviolet radiation B (280-320 nm)
UV-C	Ultraviolet radiation C (220-280 nm)
UVR	Ultraviolet radiation (220-400 nm)
W	Watt [SI Unit]
WSC	West Spitsbergen Current
λ	Wavelength [nm]

Summary

Macroalgae are distributed globally from the tropical to the Polar regions and grow preferably on hard bottom substrates. Their perennial representatives form submarine forests in rocky coastal habitats of temperate and Arctic regions. Communities of macro- and microalgae present a dominant group of coastal benthic ecosystems and are known to be important primary producers, sediment stabilizers and provide shelter and nutrition to reams of aquatic orga-nisms. Macroalgae exhibit a complex developmental cycle involving microscopic life stages. Due to their small size, the single-celled zoospores and few-celled juvenile gametophytes are fully exposed to environmental factors such as UV radiation (UV) and water temperatures. Consequently, they display the most sensitive stage of the entire macroalgal life-cycle.

Without solar radiation, life on earth would not be able to exist in its present form. Radiation reaching the surface of the earth is dependent of the solar angle and atmospheric conditions as cloud cover and aerosols. Solar radiation can be divided into infrared radiation, photosynthetically active radiation (PAR; 400-700 nm) and ultraviolet radiation (UV-A; 320-400 nm) and (UV-B; 280-320 nm). Most of the high energetic short wavelength radiation consisting of UV-B and partly UV-C is usually absorbed by the stratospheric ozone layer. However, in the last 50 years, the ozone content in the atmosphere has significantly diminished due to growing emissions of synthetic chlorofluorocarbon molecules. At the same time, global mean temperature increased due to the so-called green house effect. These global environmental changes may affect ecosystems to a hitherto unknown extent. In terms of sensitivity and vulnerability towards environmental variability, Polar ecosystems react rather fast and in a distinct way and consequently function as an early indicator for commencing changes.

Due to the penetration into the water column, solar UVR and PAR can cause multiple negative radiation impacts on aquatic organisms, especially on algal communities. Effects at the intracellular level may be linked to the community level. Together with the species' sensitivity and adaptation to prevent damages by UVR, UVR may cause changes in species composition on the community level and in species dependent depth distribution (vertical zonation patterns) if irradiances of UV-B are further increasing.

The aims of the present study therefore involve the depiction of acclimation to UVR and PAR in the protective and metabolic mechanisms of brown algal juvenile life stages of the species *Alaria esculenta, Laminaria digitata, Saccharina latissima* and *Saccorhiza dermatodea* as well as the species' adaptive potential to enhanced temperatures. Including a comparative approach of field and laboratory experiments, the present study integrates various radiation

regimes and intensities, temperature experiments and exposure times. For a broader understanding of effects, a variety of methods investigating e.g. intra- and extracellular levels of phlorotannins by the Folin-Ciocalteu method, fatty acid determination by gas chromatography, determination of reactive oxygen species and electron microscopy was applied to several juveniles of various Arctic macroalgae from Spitsbergen (Kongsfjorden). Within radiation treatments, wavelength dependent effects were distinguished by the help of cut-off foils separating applied radiation spectra in PAR only (P=PAR), PAR+UV-A (PA) and PAR+UV-A+UV-B (PAB).

The various experiments revealed that **spore germination** success varies between species and is strongly related to the respective temperatures and radiation climate. At 2 °C, decreased germination was only observed in *A. esculenta* and *S. latissima* while at 17 °C, decreased germination was present in all species except *S. latissima.* Optimum germination temperature varied with species and was 7°C for *L. digitata* and *S. dermatodea,* 12 °C for *A. esculenta* and 17 °C for *S. latissima* spores. Different light regimes in the laboratory and field experiments revealed strong variations in spore germination rate. While all spores under low PAR controls and PA exhibited similar high germination rates, irradiation with the full solar spectrum (PAB) lead to a decrease in germination compared to controls in all temperature treatments of *L. digitata* and *A. esculenta* spores and in the temperature sensitive treatments of 2 °C (*S. latissima)* and 17 °C PAB (*S. dermatodea)*. In the field experiment, high PAR compared to low PAR exhibited a strong decrease in germination rate already in the controls while high PAR + UVR treatment affected the most *S. latissima* spores compared to all other species. In contrast, experiments simulating enhanced UV-A and UV-B radiation due to ozone depletion revealed only minor effects in *S. latissima* spores while spores of *A. esculenta* remained un-affected.

Among the developmental stages of brown algal spores and juvenile gametophytes, **phloro-tannins** seem to play a major role in external protection against UVR due to their ability to absorb in the UV wavelength range and their antioxidant activity upon cell damage. This study was able to reveal that phlorotannin contents vary between species, habitat and de-velopmental stage as well as according to seasonal and environmental parameters Phlorotannin contents within zoospores and their surrounding medium are correlated with seasonal maturity of the investigated parental algae and with their different adaptations to their habitats. Intra- and extracellular phlorotannin levels of spores were also strongly depending on collection depths of the parental algae and thus distinctly related to the vertical zonation pattern of macroalgae at Kongsfjorden based on their different depth-depending sen-

sitivities to UVR. Furthermore, intra- and extra-cellular phlorotannin contents were species specific and an increase could only be observed on mid-term scales after approximately 10 days (induction).

Production of **phenolic compounds** unquestionably is costly to the algae leading to increased metabolic or nutrient demands, and thus, decreasing growth rates. It is suggested that storage compounds in spores might fuel phlorotannin synthesis by degrading fatty acids via β-oxidation to Acetyl-Coenzyme A. Fatty acid content and composition was shown to vary with species and developmental stages as well as under low/high PAR and UVR exposures. Under low PAR, monounsaturated and polyunsaturated **fatty acids** were oxidized upon UV-A and UV-B exposure and total fatty acid content decreased dramatically compared to their low light controls. Under high PAR, total fatty acids decreased about 50-75% in all treatments illustrating the underestimated effect of high PAR on early developmental stages.

Reactive oxygen species formation (ROS) displays a central element of UVR-toxicology. Summarizing investigated observations of germination success, plastoglobuli formation within the chloroplast, lipid peroxidation, ROS formation, and phlorotannin levels lead to the conclusion that phlorotannins might be able to "buffer" ROS formation to some extent by their antioxidant activity as earlier suggested for mycosporine-like amino acids upon high PAR and UVR exposure.

Consequently, sensitivity to visible and UVR of various macroalgal species is determined by their radiation tolerance and the protective potential of their parental tissue. As still only few data are available on interactive effects of PAR, enhanced UVR and temperatures, predictions of future consequences for algal recruitment and survival as well as on the community level remain difficult. While enhanced UVR in combination with low PAR in the laboratory was not affecting algal propagules, field experiments under high PAR showed detrimental effects leading to a decrease of spore germination.

Most studies conducted in the past have focused on UVR effects while the present study reveals that high PAR was always underestimated and might play a more pronounced role than usually expected. Ozone depletion might be of greater importance for smaller organisms such as spores and bacteria which are physically less protected from UVR damage, especially in marine environments where concentrations of chromophoric dissolved organic matter are low and UVR transparency is high. Stratospheric ozone depletion over the Arctic in combination with rising temperatures is therefore expected to have a strong impact on the survival of macroalgal zoospores and finally the vertical zonation of Arctic and cold temperate macroalgal species around Spitsbergen.

Zusammenfassung

Makroalgen sind von den tropischen bis zu den Polarregionen weltweit verbreitet und siedeln sich bevorzugt auf Hartbodensubstraten an. Ihre mehrjährigen Vertreter bilden Unterwasserwälder in felsigen Küstenhabitaten der temperaten und arktischen Regionen. Mikro- und Makroalgengemeinschaften stellen eine dominante Gruppe der benthischen Küstenökosys-teme dar. Neben ihrer Aufgabe als wichtige Primärproduzenten und Sedimentstabilisierer bieten sie Schutz und Nahrung für unzählige im Wasser lebende Organismen. Makroalgen zeichnen sich durch einen komplexen Entwicklungszyklus mit Mikrostadien aus. Durch ihre geringe Größe sind die einzelligen Zoosporen und die mehrzelligen juvenilen Gametophyten Umweltfaktoren, wie z.B. UV-Strahlung und Wassertemperatur, vollkommen ausgesetzt und stellen das empfindlichste Stadium des gesamten Entwicklungszyklus der Makroalgen dar.

Ohne Sonnenstrahlung würde es das Leben auf der Erde in seiner jetzigen Form nicht geben. Die Strahlung, die die Erdoberfläche erreicht, ist abhängig vom Winkel der Sonne und von atmosphärischen Gegebenheiten, wie Wolkenbedeckung und Aerosolvorkommen. Die Strahlung der Sonne wird eingeteilt in Infrarotstrahlung, photosynthetisch aktive Strahlung (PAR; 400-700 nm) und ultraviolette Strahlung (UV-A; 320-400 nm) und (UV-B; 280-320 nm). Energiereiche kurzwellige Strahlung besteht aus UV-B und teilweise aus UV-C Strahlung und wird im Allgemeinen durch die stratospherische Ozonschicht absorbiert. Jedoch hat der Ozongehalt der Atmosphäre aufgrund des Austosses von synthetischen Fluorchlorkohlenwasserstoffmolekülen in den letzten 50 Jahren signifikant abgenommen. Im gleichen Zeitraum erhöhte sich die globale Mitteltemperatur durch den sogenannten Treibhauseffekt. Diese globalen Umweltveränderungen könnten Ökosysteme in einem bisher unbekannten Ausmass beinträchtigen. Polare Ökosysteme zeichnen sich durch eine besondere Empfindlich- und Verletzbarkeit gegenüber Umweltveränderungen aus, auf die sie deshalb relativ schnell und ausgeprägt reagieren. Aus diesem Grund werden polare Ökosysteme als Frühindikatoren für beginnende Veränderungen gesehen.

Solare UV- und photosynthetisch aktive Strahlung können tief in die Wassersäule eindringen und eine Vielzahl von negativen Strahlungseinwirkungen auf aquatische Organismen im Besonderen Makroalgengemeinschaften hervorrufen. Effekte auf zellulärer Ebene können mit Effekten auf der Gemeinschaftsebene verknüpft sein. Bei einer weiteren Zunahme von UV-B Strahlung, vermag sich die Artzusammensetzung innerhalb der Gemeinschaftsebene und die

artbezogene Tiefenverteilung (vertikale Zonierungsmuster) in Zusammenhang mit der Empfindlichkeit und Adaption einer Art, sich vor UV-Strahlung zu schützen, verändern.

Die Zielsetzung dieser Arbeit beschäftigt sich daher mit der Schilderung von Akklimatisationen gegenüber UV und PAR innerhalb der Schutz- und Stoffwechselmechanismen von juvenilen Braunalgen-Stadien der Arten *Alaria esculenta, Laminaria digitata, Saccharina latissima* und *Saccorhiza dermatodea* sowie mit dem spezifischen Vermögen einer Art, sich an erhöhte Wassertemperaturen anzupassen. Eine vergleichende Herangehensweise aus Freiland- und Laborexperimenten ermöglicht es in dieser Arbeit, verschiedene Strahlungsregime- und stärken, Temperaturexperimente und Expositionszeiten miteinander zu vereinen. Für ein umfassendes Verständnis der Effekte auf einige juvenile arktische Makroalgen von Spitzbergen (Kongsfjorden), wurden eine Vielfalt von Methoden angewandt, wie z.B die Bestimmung der intra- und extrazellulären Phlorotanningehalte durch die Folin-Ciocalteu-Methode, die Fettsäurebestimmung mit Hilfe von gaschromatographischen Messungen sowie die Bestimmung von reaktiven Sauerstoffspezies (ROS) und elektronenmikroskopische Untersuchungen. Wellenlängenabhängige Effekte innerhalb der Strahlungsbehandlungen konnten durch abschirmende Folien unterschieden werden, die die angewandten Strahlungsspektren in PAR (P=PAR), PAR+UV-A (PA) und PAR+UV-A+UV-B (PAB) teilten.

Die verschieden Experimente zeigen, dass der **Keimungserfolg der Sporen** zwischen den Arten verschieden ist und in Zusammenhang mit den jeweiligen Temperaturen und dem Strahlungsklima steht. Bei 2 °C wurde eine verminderte Keimung in *A. esculenta* und *S. latissima* Sporen beobachtet, während bei 17 °C die Keimung in allen Arten außer *S. latissima* abnahm. Die optimale Keimungstemperatur war in allen Arten verschieden und lag bei 7 °C für *L. digitata* und *S. dermatodea*, bei 12 °C für *A. esculenta* und bei 17 °C für *S. latissima* spores. Verschiedene Strahlungsregimes der Freiland- und Laborexperimente ließen große Abweichungen in der Keimungsrate erkennen. Während alle Sporen innerhalb der Schwachlichtbehandlungen in den Kontrollen und PA-Behandlungen ähnlich hohe Keimungsraten zeigten, war eine Verminderung der Keimung im Vergleich zu den Kontrollen bei Bestrahlung mit dem gesamten Sonnenspektrum (PAB) in allen Temperaturbehandlungen von *L. digitata* und *A. esculenta* Sporen sowie in den Temperaturbehandlungen bei 2 °C (*S. latissima*) und 17 °C (*S. dermatodea*) festzustellen. Unter Starklicht in den Freilandexperimenten nahm die Keimungsrate im Vergleich zu den Schwachlichtexperimenten schon innerhalb der Kontrollen ab, während die Starklicht+UV-Behandlungen am meisten *S. latissima* Sporen im Vergleich zu allen anderen Arten

beeinträchtigte. Im Gegensatz dazu konnten in Experimenten, die erhöhte UV-A und UV-B Strahlung aufgrund der Ozonausdünnung simulierten, nur geringe Auswirkungen auf *S. latissima* Sporen gezeigt werden, *A.esculenta* Sporen hingegen wiesen keine Beeinträchtigungen auf.

Innerhalb der Sporen und Gametophyten von Braunalgen scheinen **Phlorotannine** einerseits aufgrund ihrer Fähigkeit im UV Bereich zu absorbieren als auch durch ihre antioxidative Aktivität eine bedeutende Schutzrolle vor UV-Schäden innerhalb und außerhalb der Zelle zu haben. Diese Studie konnte zeigen, dass sich Phlorotanningehalte sowohl innerhalb der Arten, der Habitate und Entwicklungsstadien als auch hinsichtlich saisonaler und umweltbedingter Parameter unterscheiden. Phlorotanningehalte innerhalb der Zoosporen und des sie umgebenden Mediums stehen in Verbindung mit dem saisonalen Reifegrad der untersuchten adulten Algen (Mutteralgen) und ihren verschiedenen Anpassungen an ihre Habitate. Intra- und extrazelluläre Phlorotanningehalte der Sporen waren ebenfalls abhängig von der Sammeltiefe der Mutteralgen und stehen somit in Zusammenhang mit der Vertikalzonierung der Makroalgen im Kongsfjord, die auf den unterschiedlichen tiefenabhängigen Empfindlichkeiten gegenüber UV-Strahlung beruht. Desweiteren waren die intra- und extrazellulären Phlorotanningehalte artspezifisch und ein Anstieg konnte erst nach ca. 10 Tagen beobachtet werden (Induktion).

Die Produktion von **phenolischen Verbindungen** ist für die Algen unumstritten kostspielig, was mit einem erhöhten Stoffwechsel und Nährstoffbedarf und folglich abnehmenden Wachstumsraten einhergeht. Speicherstoffe in Sporen könnten zur Phlorotanninsynthese durch den Abbau von Fettsäuren via β-Oxidation zu Acetyl-Coenzym A beitragen. Die Zusammensetzung und der Gehalt der **Fettsäuren** variierten im Experiment sowohl innerhalb der Arten und Entwicklungsstadien als auch unter Schwach- und Starklichtexposition. Unter Schwachlicht und innerhalb der UV-A and UV-B Exposition wurden einfach ungesättigte und mehrfach ungesättigte Fettsäuren oxidiert und der Gesamtgehalt an Fettsäuren sank drastisch im Vergleich zu den dazugehörigen Schwachlichtkontrollen. Unter Starklichexposition sank der Gesamtfettsäuregehalt um 50-75% in allen Behandlungen und veranschaulicht somit die unter-schätzten Effekte von Starklicht auf junge Entwicklungsstadien.

Die Bildung von **reaktiven Sauerstoffspezies (ROS)** stellt ein zentrales Element der UV-Toxikologie dar. Bei der zusammenfassenden Betrachtung aller Ergebnisse aus den Keimungsraten, der Bildung von Plastoglobuli innerhalb der Chloroplasten, der Lipidperoxi-dation, der Bildung von ROS sowie den Phlorotanningehalten läßt sich schlussfolgern, dass

Phlorotannine aufgrund ihrer antioxidativen Eigenschaften die Fähigkeit besitzen könnten, ROS Bildung, so wie Mycosporin-ähnliche Substanzen unter UV und Starklichtexposition, abzupuffern.

Folglich ist die Empfindlichkeit der verschiedenen Makroalgenarten gegenüber sichtbarer und UV-Strahlung bestimmt durch ihre Strahlungstoleranz und das Schutzpotential des Muttergewebes. Vorhersagen über die Auswirkungen auf die Nachkommenschaft, den Fortbestand der Algen sowie auf die Gemeinschaftsebene in der Zukunft gestalten sich weiterhin schwierig, da bisher nur wenige Daten über die interaktiven Effekte von PAR, erhöhter UV-Strahlung und Temperaturen erhältlich sind. Während erhöhte UV-Strahlung in Kombination mit Schwachlicht in Laborversuchen keine Effekte auf die Algensporen hatte, zeigten Expositionen unter Starklicht in den Freilandexperimenten nachteilige Effekte in Form von verminderter Keimung der Sporen.

Die meisten vorherigen Studien haben sich mit den Auswirkungen von UV-Strahlung beschäftigt. Währenddessen konnte die vorliegende Studie aufdecken, dass Starklicht bisher unterschätzt wurde und eine größere Rolle spielt als bisher angenommen. Die Ozonausdünnung könnte für kleinere Organismen wie Sporen und Bakterien vor allem im marinen Millieu von größerer Bedeutung sein, da sie physikalisch gesehen weniger geschütz gegenüber UV-Schäden sind und die Konzentrationen von chromophorischem gelöstem organischem Material gering und die UV-Durchlässigkeit hoch ist. Es wird erwartet, dass die stratosphärische Ozonausdünnung über der Arktis in Verbindung mit steigenden Temperaturen eine starke Auswirkung auf den Fortbestand der Algensporen und letztendlich auf die Vertikalzonierung von arktischen und gemäßigten Makroalgen um Spitzbergen herum haben wird.

List of Publications

This thesis is based on the following papers, referred to by their Roman numbers:

Publication	
I	**Steinhoff FS**, Graeve M, Wiencke C, Wulff A and Bischof K (submitted). Lipid content and fatty acid biosynthesis in zoospores/ developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential fuel for phlorotannin production. Polar Biology
II	**Steinhoff FS**, Wiencke C, Wuttke S and Bischof K (under revision). Effects of water temperatures, UV radiation and low versus high PAR on phlorotannin content and germination in zoospores of *Saccorhiza dermatodea* (Tilopteridales, Phaeophyceae). Phycologia
III	**Steinhoff FS**, Bischof K, Wuttke S and Wiencke C (under revision). Is a changing environment affecting phlorotannin production and germination in Arctic kelp zoospores? European Journal of Phycology
IV	**Steinhoff FS**, Graeve M, Bischof K and Wiencke C (manuscript). Phlorotannin production and lipid oxidation as a potential protective function against high visible and UV radiation in gametophytes of *Alaria esculenta* (Alariales, Phaeophyceae)
V	Karsten U, Wulff A, Roleda MY, Müller R, **Steinhoff FS**, Fredersdorf J and Wiencke C (2009). Physiological responses of polar benthic algae to ultraviolet radiation. Botanica Marina 52: 639-654
VI	Müller R, Desel C, **Steinhoff FS**, Wiencke C and Bischof K (submitted). UVB and temperature-induced reactive oxygen species in gametophytes of three Arctic kelp species (Laminariales, Phaeophyceae). Plant Biology

Declaration of contribution

Publications conducted (I-IV) were initiated, conceptualized, conducted and written by Franciska S. Steinhoff. As a Co-Author of Publication V, F.S. Steinhoff was contributing by writing the chapter “cell biological and ultrastructural changes”. In Publication VI, the Co-authorship included the scientific design, experimental work, data analysis and evaluation as well as writing of the electron microscopic part.

1. Introduction

1.1. Seaweeds

While the term seaweed traditionally describes (Lobban and Harrison 1994) macroscopic multi-cellular green, red and brown algae (macroalgae), the term kelp is used for brown algae of the order Laminariales (Phaeophyceae; Bartsch et al. 2008). Their microscopic and often unicellular representatives are defined as microalgae. Macro- and micro-algal communities present a dominant group in coastal ecosystems and are known to be important primary producers (Mann and Chapman 1975). Although only covering 0.6 % of the sea bottom, they contribute with 5% to the global oceanic primary production (Smith 1981). Seaweeds are distributed globally from the Tropics to the Polar regions (Lüning 1990; Bischof et al. 2006), grow preferably on hard bottom substrates and their perennial representatives form submarine forests in rocky coastal habitats of temperate and Arctic regions (Steneck et al. 2002).

Due to their high productivity and the often-extensive vertical structure formed by their fronds, kelp forests provide food and shelter for juvenile fishes and other species (e.g. Dayton 1994; Duffy and Hay 2000; Bartsch et al. 2008). By attachment to their substrates, seaweeds are able to stabilize sediments and reduce alongshore currents and water motion considerably (Jackson and Winant 1983) leading *inter alia* to less nutrient discharge out of the system.

The life cycle of seaweeds is characterized by a strong seasonal dependence influencing their growth, reproductive and photosynthetic performance and their sensitivity to biotic and abiotic factors (summarized in Kain 1979 and Bartsch et al. 2008). Additionally, different sensitivities and adaptations to environmental factors as e.g. UVR and high PAR lead to a distinct zonation patterns on rocky shores (Lüning 1990; Welch et al. 1992; Wiencke et al. 2004; Bischof et al. 2006).

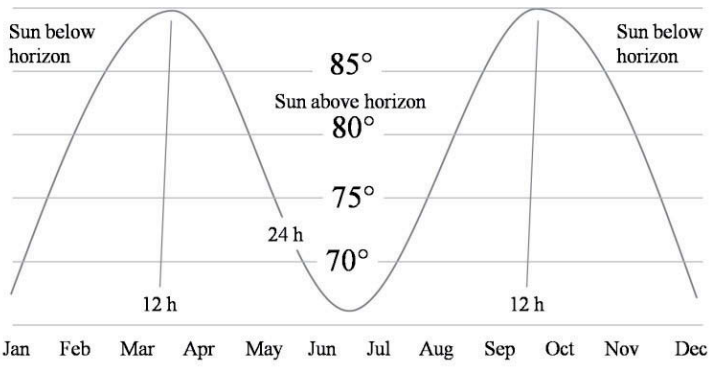


Fig 1 Daylength north of the Polar circle in relation to latitude (modified after Lüning 1990). Polar day at 80°N last from mid-April to end of August while Polar night lasts from mid-October to mid February. Between mid-February and mid-April and early-September and mid-October twilight conditions occur.

1.1.1 Seaweed communities in the Arctic

The Arctic marine flora is characterized by low abundances, scarcity of species, oversize (gigantism) but longevity of thalli (Kjellmann 1883; Lüning 1990) due to low water temperatures and a lack of light during periods of darkness. At 80°N, light for photosynthesis is only available from mid-February to mid-October (see Fig 1) with continuous day light around the summer solstice (mid-April to end of August). But sea ice and snow coverage in Polar regions allow PAR to penetrate into the water column only from early summer (June) to autumn (early September). The ice opening is also associated with higher temperatures and a higher input of melt water and sediment derived from the glaciers. As a consequence of mixing processes, the sediment load in the water column causes higher attenuation (Jerlov 1976; Hanelt et al. 2001) leading to less light availability and consequently less photosynthetic activity in polar summers.

As mentioned above, Polar regions are denoted by ice coverage and drifting ice. As a result of abrasion processes by drifting ice blocks, colonization of the littoral and upper sublittoral (3-5 m water depth) by perennial species is physically difficult (Svendsen 1959; Wiencke et al. 2004). Consequently only the Polar coastal zones from the lower littoral down to depths allowing sufficient light penetration are suitable for macroalgal vegetation. Different sensitivities and adaptations to the radiation regime and ice scour have lead to zonation patterns of the Polar macroalgal communities as described e.g. by Wiencke et al. (2004) for an Arctic fjord system.

Seaweeds are distributed up to 80°N (Lüning 1990). The southern boundary of the Arctic region "nearly follows the 0°C February isotherm (10°C August isotherm) or approximately the southern ice limit in winter and spring" (Lüning 1990) distinguishing Arctic species from their representatives of cold-temperate regions (Bolton and Lüning 1982; Lüning 1990; tom Dieck 1993). In Polar regions, seaweeds display a year-round essential carbon sink by producing high amounts of biomass with maximum wet biomass in the upper sublittoral of 4.5 kg m^{-2}, in the mid sublittoral 6.5 kg m^{-2} and in the lower sublittoral 0.9 kg m^{-2} (Wiencke et al. 2004). After degradation, seaweeds display a carbon source by providing dissolved and particular detritus (Amsler et al. 1995). But how are algae able to sustain the seasonal variations in light availability (Polar night and Polar day)? Some algae species use their storage reserves for forming a new blade during the Polar night (e.g. *Laminaria solidungula*; Chapman and Lindley 1980) enabling the algae to grow in the darkness and be prepared for photosynthesis as soon as the light is available. Lüning (1990) described

additionallya dark-tolerance of their spores/gametophytes of 6 months to assure the reproductive cycle.

1.1.2 Developmental life-cycle of kelps

Kelps are characterized by a complex heteromorphic (haplodiplontic) life cycle (Fig 2). The diploid macrothallus (sporophyte) is composed of a holdfast (rhizoid), the stipe (cauloid) and the blade (phylloid) (i.a. Kain 1979). Within the phylloid, the fertile tissue (sorus) of Laminariaceae and Saccharinaceae bearing unilocular sporangia is located while Alariaceae generate their fertile tissue in separate sporophylls (Kain 1979).

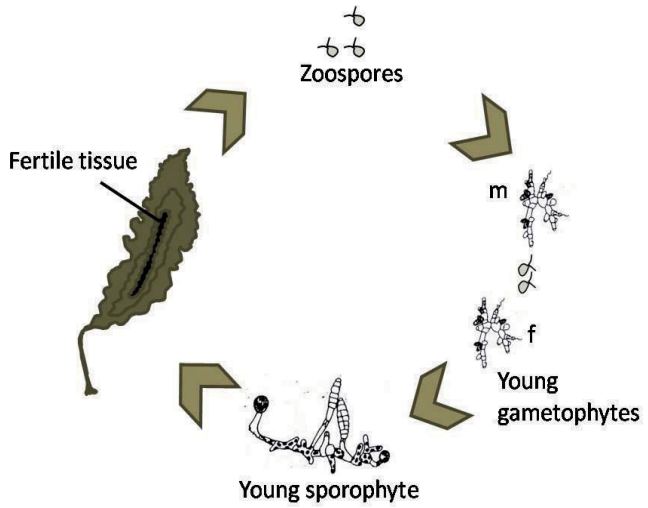


Fig 2 Developmental cycle of the brown alga *Saccharina latissima.* Microscopic zoospores are released by the fertile tissue of the parental algae into the water, settle and grow into juvenile female (f) and male (m) gametophytes. Gametophytes release gametes, become fertilized and the zygote develops into a young sporophyte. The young sporophyte is the juvenile stage of the adult algae.

Induction of fertile tissue seems to be related to the life-cycle pattern, abiotic factors as well as size and weight of the sporophyte (Bartsch et al. 2008). The sorus releases haploid male and female meiospores (Fig 2) in some species probably controlled by a circadian rhythm (reviewed by Bartsch et al. 2008) and artificially induced by osmotic and temperature changes (Kain 1979). With the help of water motion and their flagella, zoospores are able to swim up to 72 h in the water column before settlement, dependent on their swimming behavior, photosynthetic efficiency and the light environment inhabited (Reed et al. 1990, 1992, 1999). Surprisingly, brown algal zoospores show neither signs of phototaxis nor geotaxis to be geared to their substrates. Later on, during the process of settlement, adhesion vesicles are extruded from the cytoplasm of the zoospore leading to adhesion to the substrate (Henry and Cole 1982). After settlement, spores grow into microscopic haploid female and male gametophytes forming a germination tube and the gametophytic cell. In a few-cell stage, gametangia of the female gametophyte become fertilized by spermatozoids released by the male gametophyte. The attraction of the spermatozoids is coordinated by the hormone lamoxirene as reviewed by Pohnert and Boland (2001). The resulting zygote now develops into a new adult macrothallus/ sporophyte (van den Hoek et al. 1995).

1.1.3. Ultrastructure of kelp zoospores

Upon release, zoospores of the order Laminariales are ~4µm in size and characterized by a cell membrane, a nucleus with nucleolus, one or more chloroplasts, several mitochondria, endoplasmatic reticulum, Golgi vesicles, dictyosomes, several vesicle types as e.g. adhesion vesicles and two flagella for zoospore dispersal and settlement (Loiseaux 1973; Henry and Cole 1982; Steinhoff et al. 2008). The occurrence of eyespots is diversely discussed among brown algae (Henry and Cole 1982) but zoospores of Laminariales lack an eyespot (Henry and Cole 1982). Additionally, spores contain lipid globules with fatty acids known to fuel zoospore swimming and metabolism (Reed et al. 1999). Small globular vesicles within the spore cell indicate physodes (Crato 1892) containing polyphenolic phlorotannins (Fig 3). Physodes are often found in groups while lipid globules were distributed throughout the cell (Ragan 1976; Steinhoff et al. 2008).

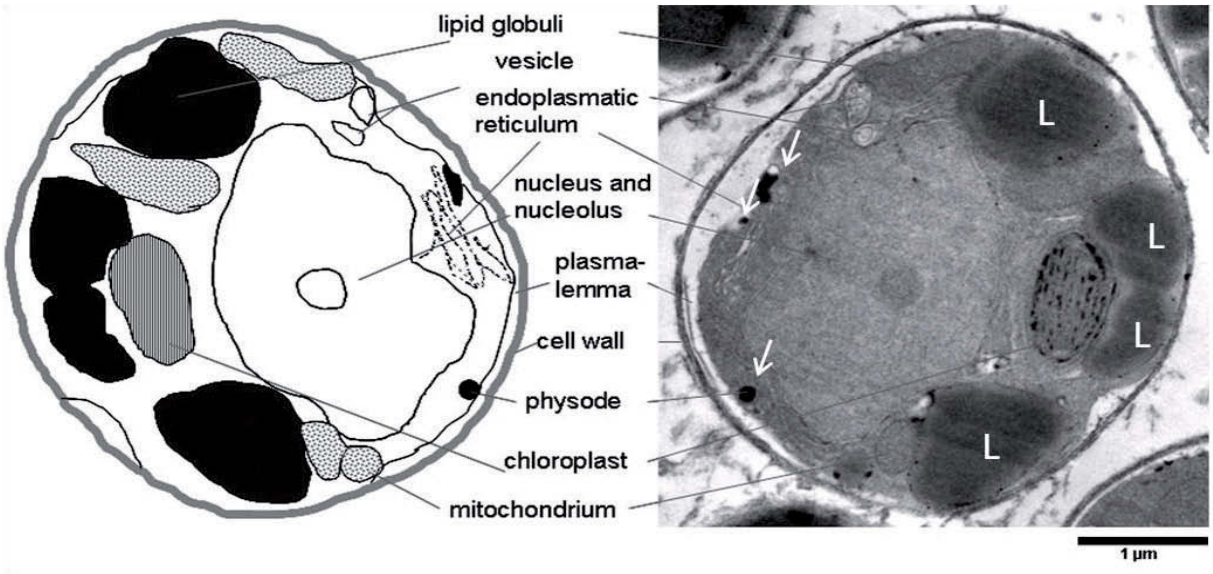


Fig 3 Electron micrograph of a *Laminaria hyperborea* zoospore 8 h after release (modified after Steinhoff et al. 2008). White arrows indicate phlorotannin containing physodes and white L lipid globules.

1.2. Stratospheric ozone layer, greenhouse effect and variability of UV radiation

Without solar radiation, life on earth would not be able to exist in its present form. Solar radiation reaching earth's surface (Fig 4) can be divided according to the Comission Internationale de l'Éclairage (CIE) in infrared radiation with wavelengths (λ) > 700 nm, visible radiation (photosynthetically active radiation PAR) λ=400-700 nm and UVR (λ=280-400 nm).

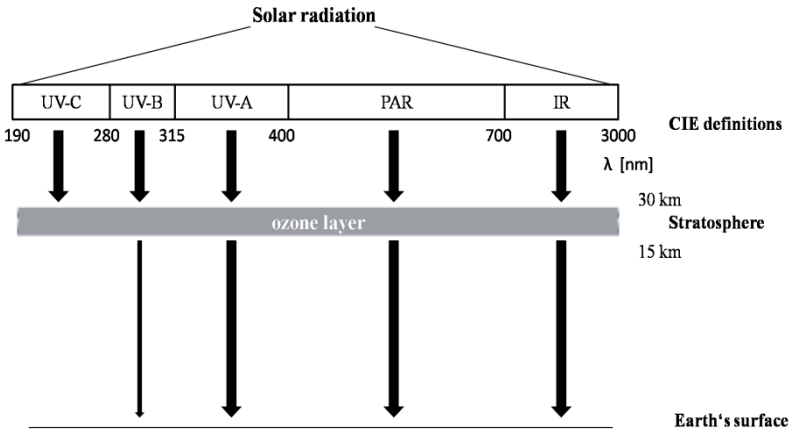


Fig 4 Solar radiation spectrum reaching the Earth's surface. By the ozone layer, short and high energetic wavelengths are filtered out of the solar spectrum. Wavelengths (λ) are given according to CIE

According to the properties of the cut-off filter foils used in UVR experiments, photo-biologists have "redefined" UV-B radiation to 280-320 nm (Franklin et al. 2003).

1.2.1. Stratospheric ozone layer, UV radiation and PAR

To prevent high energetic short wavelength from reaching earth's surface, the most energetic fraction of the solar radiation (Rowland 2006) as UV-C and parts of the UV-B wavelength range are absorbed by the stratospheric ozone layer. The ozone layer is located between 10 and 30 km within the stratosphere (Rowland 2006) and formed in turn by the action of short-wavelength solar radiation on oxygen molecules (Madronich 1993). In the last 50 years, the ozone content has significantly diminished due to growing emissions of synthetic chlorofluorocarbon molecules (Rowland 2006). As a result, a radiation shift to shorter wavelengths was detected (compare Fig 4). Due to the high energy short wavelengths contain, the decrease in ozone is almost exponentially correlated with the increase of negative impacts on organisms. At a wavelength of 300 nm, a 10% decrease in ozone is accompanied with a doubling in irradiance (Frederick et al. 1989). The Polar regions are especially affected by stratospheric ozone depletion. Entire ozone column loss of Polar zones in 2005 ranged from 130 DU in the Arctic to 157 DU in the Antarctic (Newman and Rex 2007) while the trend in total ozone column loss on the Northern hemisphere (30°-60°N) was 8.7 DU for 1979-1996 (Bodeker and Waugh 2007) . Nevertheless, ozone depletion over the Arctic is relatively less pronounced compared to the Antarctic. In contrast, the interannual variability of stratospheric ozone concentration is rather high (Bodeker and Waugh 2007). Consequently, changes in ozone are very difficult to detect and chemistry-climate models predict that the first signals of ozone layer recovery in the Arctic are likely to be overseen (Bodeker and Waugh 2007).

As mentioned above, ozone depletion allows more short-wavelength radiation to reach the biosphere with the potential to affect organisms negatively (Environmental Effects Assessment Panel 2006). While changes in ozone affect mostly UV-B radiation, cloud cover

caused by atmospheric changes, haze and absorbing aerosols and solar angles affects both UV-B and UV-A on a daily to annual basis (Madronich 1993; Herman et al. 1999). Changes in UVR in the underwater environment are apart from the atmospheric factors additionally dependent on the optical properties of the water body (Jerlov 1976; Kirk 1994; Hanelt et al. 2001; compare section 2.1).

PAR is the prerequisite for carbon assimilation and oxygen production on earth. Irradiance of PAR is, as UVR, dependent of the solar angle and atmospheric conditions as cloud cover and aerosols. In the study area Kongsfjorden, up to 1400 µmol photons m^{-2} s^{-1} (~300 W m^{-2}) have been measured on a sunny day in the atmosphere (Bischof et al. 1998a). Depending on their habitat, plants have established strategies to adapt to the availability of PAR by either avoiding light stress (high or low irradiances of PAR) or adjust to their habitats by e.g pigment accumulation (Aguilera et al. 2002). Although much weaker energetically than UVR, PAR has the ability to penetrate much deeper into the water (Wetzel 2003) enabling photosynthesis but leading also to various effects on aquatic organisms (e.g. Hoffman et al. 2003) and community structure (Zacher et al. 2007, 2009).

1.2.2. Impact of UV radiation and high PAR irradiances on algae

The impact of UVR and high PAR is not only limited to macroalgae but rather extends to several aquatic organisms in the euphotic zone. Negative radiation impacts can occur from the cellular to the community level (Wulff 1999; Wahl et al. 2004) and are related to the previously discussed high energetic properties of UV-B radiation. UV-A radiation effects play a minor role, but are known to be positively correlated with e.g. formation of gametangia during the developmental cycle (reviewed in Bartsch et al. 2008). UVR and their impacts on macroalgae and polar benthic algae are described extensively by Bischof et al. (2006), Hanelt et al. (2007) and Karsten et al. (2009), respectively.

Similar to the broad overview about UVR effects on seaweeds, there are several studies investigating high PAR effects. Studies so far have revealed that effects of intense PAR as photoinhibition (Altamirano et al. 2004), pigment destruction (Yakovleva and Titlyanov 2001), reduced settlement and a decline of germ tube density (Cie and Edwards 2008) on macroalgae are comparable to effects exhibited by the high energetic UVR (Bischof et al. 1999; Fredersdorf and Bischof 2007).

Earlier studies on photobiology have shown that on a cellular level UVR inactivates DNA and mRNA (Harm 1980; Karentz et al. 1991), affects enzymes (Döhler 1985; Lesser 1996; Bischof et al. 2000), formation of reactive oxygen species (ROS) by impairment of

photosynthesis (Bischof et al. 2002; Lesser 2005), membranes (Poppe et al. 2002, 2003; Lesser 2005) and lipids, affects nuclei (Steinhoff et al. 2008) and inhibits cell division (Calkins and Thordardottir 1980).

Different sensitivities within the life history of seaweeds reviewed by Roleda et al. (2007) seem to be related to their morphology. Unicellular or few celled stages are more easily penetrated by solar radiation while adult stages have developed a shielding capacity through their thallus structure (Franklin and Forster 1997; Swanson and Druehl 2000; Altamirano et al. 2004). Seaweeds have therefore developed protective mechanisms as for instance quenching of UVR-produced toxic intermediates (Vincent and Roy 1993), photoenzymatic repair (Karentz et al 1991), and formation of UV-protective substances such as mycosporine-like amino acids (Nakamura et al. 1982; Karsten et al 1998) and phlorotannins in brown algae (Pavia et al. 1997; Swanson and Druehl 2002; Schoenwaelder 2002).

Due to the coupling of effects from the intracellular to the community level and the resulting sensitivity and adaptation to UVR (Dring et al. 1996), species composition within the community level (Bischof et al. 2006 references therein) and local/species dependent depth distributions (vertical zonation patterns) as e.g. described by Bischof et al. (1998, 1998a), Wiencke et al. (2004) might lead to changes by increasing levels of UV-B.

1.3. Climate change and enhanced temperatures in the Arctic

Earth's atmosphere serves as a greenhouse creating a natural greenhouse effect. Due to the anthropogenic release of gases to the atmosphere (Solomon et al. 2007), e.g. carbon dioxide, methane, water vapour, nitrous oxide, hydro- and perfluoro-carbons, the natural carbon flux, dominated by natural processes as photosynthesis is not able to balance greenhouse gas emissions and absorption processes. The continuous increase of greenhouse gases in the atmosphere leads to global warming in the atmosphere related to absorption and reflection processes of solar radiation and in contrast to a decrease in temperature in the stratosphere related to an enhanced cloud formation. Polar stratospheric cloud formation (PSC) causes a negative feedback by even more extensive ozone depletion (Rowland 2006) affecting especially the Polar stratosphere.

The Polar climate is characterized by a large interannual and larger time-scale natural variability (Räisänen 2002). Furthermore, incomplete data sets, poor resolution of climate models in Polar regions and complex atmosphere-land-cryosphere-ocean-ecosystem inter-

action and their feedbacks lead to uncertainties in climate change predictions (Christensen et al. 2007). It is known that generally warmer mean temperatures will increase the probability of extreme warm days while the probability of extreme cold days decreases (Mitchell et al. 1990). With climate models as e.g. the A2(B1) scenario, a considerable and comparable (Chapman and Walsh 2007) mean warming of 5.9 °C was projected over the Arctic at the end of the 21st century. Due to melting sea ice and glacier input, temperature amplitudes of Arctic waters will be much larger than temperatures predicted over land (Christensen et al. 2007) leading very likely to major changes in ecosystem structure and function, interaction and shift of species (Solomon et al. 2007).

1.4. Protective, stress and storage compounds in zoospores

Macroalgal zoospores are equipped with different mechanisms to survive radiation stress, grazing and spatial dispersal. In the following, phlorotannins as potential UV-protective and scavenging substances of photoproducts as reactive oxygen species (ROS) as well as fatty acids as storage and precursor compounds will be introduced.

1.4.1. Phlorotannins

Among brown algae (Phaeophyceae), only one group of polyphenolics is present - the phlorotannins (Targett and Arnold 1998; Jormalainen et al. 2003). Phlorotannins are polymeric substances composed of the monomer phloroglucinol (1,3,5-trihydroxybenzene; Fig 5) with molecular weights from 126 to 650 kDa (Ragan and Glombitza 1986). Phlorotannins are located in spherical and membrane-bound vesicles, the so-called physodes (Crato 1892; Ragan 1976; Schoenwaelder and Clayton 1999) which are distributed throughout the cell and the cell walls.

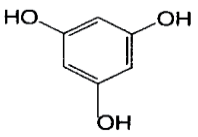


Fig 5 The phlorotannin monomer phloroglucinol (1,3,5-trihydroxybenzene).

Phlorotannins are divided in 6 subgroups: 1 Fucoles, 2. Phlorethols, 3. Fucophlorethols, 4. Fuhalols, 5. Eckols and 6. Carmalols (Ragan and Glombitza 1986) distinguished by their type of bonding. Fucols are e.g. connected by aryl-aryl bonds, Phlorethols by aryl-ether bonds (Glombitza and Pauli 2003) and Fuhalols by para- and ortho-arranged ether bridges containing one additional OH-group in every third ring (Koivikko 2008). Eckols are characterized by a substitution of at least one three-ring moiety with a dibenzodioxin element by a phenoxyl group at C-4 while Carmalols are further derivates of phlorethols containing a dibenzodioxin moiety (Ragan and Glombitza 1986).

Phlorotannins are discussed to be formed via the acetate-malonate pathway (poly-

ketide pathway) involving a polyketide synthase-type enzyme complex (Arnold and Targett 2002). Up to 20% of brown algal DW can consist of phlorotannins (Ragan and Glombitza 1986) indicating their multifunctional role and importance. In Tab. 1, biological functions and responses of phlorotannins are summarized.

Tab. 1 Study examples of biological functions and responses of phlorotannins in several brown algae (supplemented and modified after Koivikko 2008). Impact on phlorotannins is marked by the following signs: (+) increase/positive, (-) decrease/negative, (o) no response, (v) variable

Phlorotannin functions and responses	impact	Species	Reference
Antioxidation	-/v	*Ascophyllum nodosum, Sargassum muticum, Laminaria digitata, Laminaria hyperborea Dictyota cervicornis, Dictyota ciliolata,*	Connan et al. 2006, 2007
	-/v	*Dictyota crenulata, Lobophora variegate, Padina gymnospora, Sargassum pteropleuron, Sargassum ramifolium, Turbinaria tricostata*	Zubia et al. 2007
UVR protection	+	*div. brown algae*	Reviewed in Schoenwaelder 2001
UV refugia	+	*Macrocystis integrifolia, Laminaria groenlandica*	Swanson and Druehl 2002
Screening capacity	+	*Alaria esculenta, Laminaria digitata, Saccorhiza dermatodea*	Roleda et al. 2006a
	+	*Alaria esculenta, Laminaria digitata, Saccharina latissima*	Müller et al. 2009
Defense			
Inducible	+	*Ascophyllum nodosum*	Svensson et al. 2007
Water-borne cues	+	*Ascophyllum nodosum, Fucus vesiculosus*	Toth and Pavia 2000
Constitutive			
Herbivore performance	-	*Fucus vesiculosus*	Jormalainen et al. 2005
Feeding preference	-	*Fucus vesiculosus*	Kubanek et al. 2004
Other functions			
Polyspermy block	+	*Acrocarpia paniculata, Hormosira banksii*	Schoenwaelder 1996
Antifouling substances	+	*Fucus vesiculosus, Fucus evanescens*	Wikström and Pavia 2004
Antiallergic activity	+	*Ecklonia cava*	Li et al. 2008
Inhibition of cytochrome P450 activity	+	*Fucus vesiculosus*	Parys et al. 2009
Inhibition of enzymatic activity	-	*Eisenia bicyclis*	Shibata et al. 2003
Responses to			
High photosynthetically active radiation	-	*Cystoseira tamariscifolia*	Abdala-Díaz et al. 2005
Nutrient enrichment	-	*Fucus gardneri*	Van Alstyne and Pelletreau 2000
Mechanical Wounding	+	*Ecklonia radiate*	Lüder and Clayton 2004
Variations in phlorotannin contents			
genetic	v	*Fucus vesiculosus*	Koivikko et al 2008
seasonal	v	*Sargassum muticum*	Plouguerné et al. 2006
spatial	v	*Fucus vesiculosus*	Hemmi and Jormalainen 2004
Within algae	v	*Ascophyllum nodosum*	Toth et al. 2005
	v	*Ascophyllum nodosum, Sargasum muticum, Laminaria digitata, Laminaria hyperborea*	Connan et al. 2006
Developmental stage	v	*div. brown algae*	Van Alstyne et al. 2001
Depth distribution	v	*div. brown algae*	Van Alstyne et al. 1999
	v	*Alaria esculenta, Laminaria digitata, Saccharina latissima, Saccorhiza dermatodea*	

The most important property of phlorotannins for this study was their ability to absorb in the UV wavelength range (Pavia et al. 1997; Swanson and Druehl 2002; Schoenwaelder et al. 2003; Roleda et al. 2006, 2006a, 2007; Karsten et al. 2009).

1.4.2. ROS (reactive oxygen species)

The production and subsequent activity of reactive oxygen species (ROS) displays a central element of UVR toxicology (Vincent and Neale 2000). Reactive oxygen species evolved within the aerobic metabolic processes of photosynthesis and respiration and cause oxidative damage to proteins, DNA and lipids (Apel and Hirt 2004). They are produced in mitochondria, chloroplasts and peroxisomes. Under physiological steady state conditions ROS are scavenged by different antioxidative defense components (Lesser 2005) as e.g. phlorotannins.

Environmental factors as temperature and UV-B radiation may perturb the equilibrium between damage and repair as proposed by Malanga and Puntarulo (1995) leading to an increase of intra- and extra-celluar ROS levels (Elstner 1991; Lesser 2005) and to an increase in the activity of the antioxidant enzymes superoxide dismutase and catalase (Malanga and Puntarulo 1995; Bischof et al. 2003; Rautenberger and Bischof 2006). Thus the determination of ROS in different life-history stages of kelps will contribute on the cellular level to understand how changes in environmental conditions affect kelps.

1.4.3. Fatty acids in kelps

Fatty acids (FA) are essential storage compounds of macroalgae feeding manifold metabolic processes as e.g. energy source during kelp spore dispersal and settlement (Reed et al. 1992, 1999). Fatty compositions in various seaweeds were measured and summarized by Jamieson and Reid (1972) and Pohl and Zurheide (1979). Membrane lipid and therefore fatty acid composition is changed in response to the alteration of major environmental factors as temperature and light (Harwood 1984; Becker et al. 2010) leading to various impacts on organisms. UVR and temperature are known to affect major biochemical constituents as FA (Hessen et al. 1997) and differences in the UV-B susceptibility may involve differences in lipid metabolism (Kramer et al. 1991).

Thus, investigations of fatty acid composition and their consumption processes under various simulated conditions will shed light on metabolic processes and costs of different life history stages of kelps and will lead to a deeper understanding on impacts of environmental factors

1.5. Aims of the thesis

Global environmental change leads to a multitude of research efforts in relation to the ecosystem. The shift in the solar spectrum towards shorter wavelengths (stratospheric ozone depletion) and enhanced water temperatures will especially affect coastal algal communities (Bischof et al. 2006) and their most sensitive developmental stages (Roleda et al. 2007). Polar ecosystems are especially vulnerable to environmental variability and could, consequently, function as an early indicator for environmental changes. Macroalgae present a dominant and important group of Polar coastal ecosystems and their unicellular and few-celled developmental stages display the most vulnerable part of the entire life-cycle. Thus, studies on zoospores and gametophytes of brown algae were carried out on the Arctic island of Spitsbergen.

This study aims to shed light on protective and metabolic mechanisms of brown algal juvenile life stages. Especially polyphenolic substances known as phlorotannins will be investigated in terms of induction and formation processes (Publ. I, II, III, IV), metabolic costs (Publ. I, IV) and photo-protective substances (Publ. II, III, V). A comparative approach, including laboratory (Publ. I, II, III, IV, VI) and field experiments (Publ. II, III), was carried out to elucidate interactive effects of solar radiation and temperature (Publ. II, III, IV, VI) as well as the impact of low and high PAR on various species among brown algae (Publ. II, III, IV). For a broad understanding of effects, various methods as e.g. electron microscopy, fatty acid analysis, determination of phlorotannin contents and amounts of reactive oxygen species (ROS) are included.

In detail, the following questions are addressed:

- How is UVR stress reflected in cellular ultrastructure and the expression of reactive oxygen species in juvenile stages of kelps (Publ. VI)?
- Are UV-protective phlorotannins detectable in early life stages and are variations in phlorotannin contents with species measurable (Publ. II, III)?
- Is the internal composition of phlorotannins in the zoospores controlled by external factors such as time, temperature, UVR and PAR (Publ. I, II, III, IV)?
- Are storage lipids involved in phlorotannin metabolism (Publ. I, IV)?
- What is the protective potential of phlorotannins among spores of different brown algal species and what are the expected ecological consequences (Publ. II, III, V)?

2. Methodological considerations

The experiments presented were performed between June 2006 and July 2009. Over time, methods, experimental design and analysis were further refined. In the following, all methods used will be explained and discussed in general. Detailed descriptions are given in the Material and Methods section of the related publications as indicated by their roman numbers.

2.1. The Kongsfjord, an Arctic Fjord system

Located on the northwest coast of Spitsbergen, Kongsfjorden is characterized by a length of 26 km, a width ranging from 3-8 km and a maximum depth of ~400 m (Hanelt et al. 2001). The sublittoral is mostly steep and rocky with shallower soft-bottom parts caused by strong deposition of sediments from four glaciers (Hanelt et al. 2001). The water masses are influenced by Atlantic and Arctic waters (Fig 6) as well as the weak tidal current ranging up to 2 m (Ito and Kudoh 1997). Light availability is influenced by the annual cycle of Polar day /Twilight/Polar night conditions (see Fig 1). On sunny days, PAR in air can range up to 1400 µmol photons m^{-2} s^{-1} (~300 W m^{-2}), UV-A to 19 W m^{-2} and UV-B to 1.09 W m^{-2} (Bischof et al. 1998a).

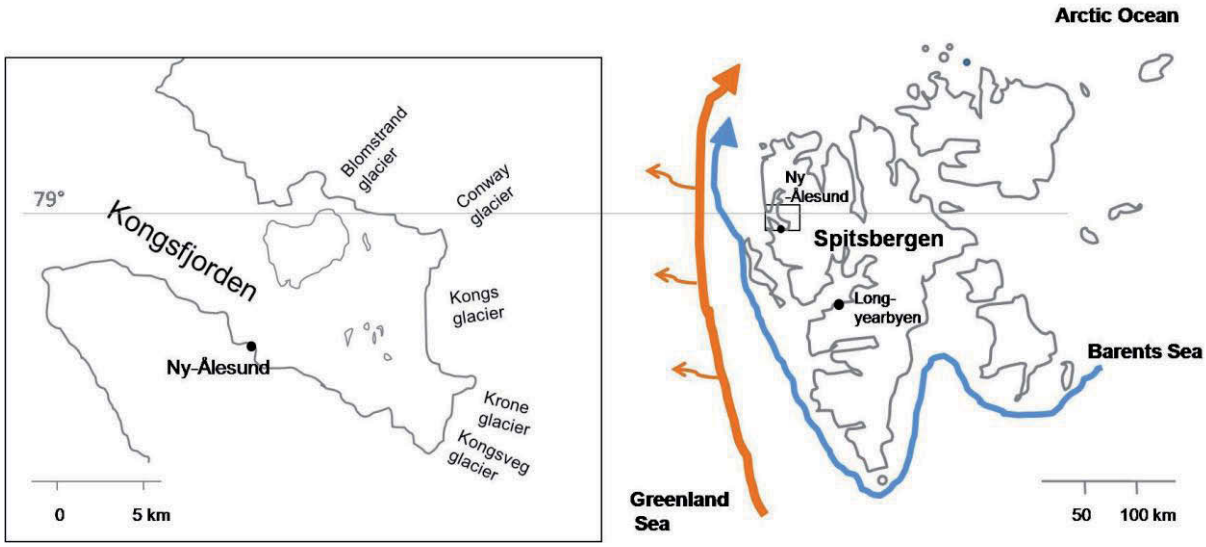


Fig 6 Study area Kongsfjorden in the North-West of Spitsbergen. Kongsfjorden is influenced by the West Spitsbergen Current (WSC, red arrow) from the West, the Arctic-type coastal water (blue arrow) and from glacier ice and their melt water from easterly directions.

2.1.1. The physical environment of Kongsfjorden

2.1.1.1. Water temperature

Due to the water exchange of the North-Atlantic Current with the West Spitsbergen Current (WSC) (Fig 6), water masses entering Kongsfjorden are relatively warm and salty compared to the east-western Svalbard Fjords. The water masses can be regarded as Transformed Atlantic Waters with salinities >34.7 PSU and temperatures >1°C (Svendsen et al. 2002). Interannual water temperature variations are rather high and dependent on warm water intrusions from the Atlantic Waters, river discharge, turbid melt water inflow and wind direction (Svendsen et al. 2002). Mean water temperatures of Kongsfjorden in the summer season (Fig 7) can range from 2.3 to 6.5 °C (Brey 2009).

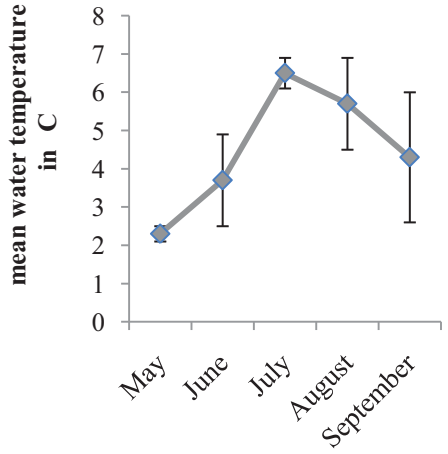


Fig 7 Mean water temperature [°C] in Kongsfjorden during the summer season (Ny-Ålesund, Spitsbergen, Norway). Data modified after Brey (2009).

2.1.1.2. Underwater radiation regime

Optical properties of the water column are depending on a variety of factors e.g. solar zenith angle (geographic latitude), stratospherical ozone depletion levels, attenuation and absorption processes due to dissolved organic matter (DOC), inorganic sediments and organic particles such as phytoplankton as well as ice-coverage and season.

The seawater of Kongsfjorden is characterized by low contents of DOC, but high sediment particle and freshwater inflow due to glacier and river input (Svendsen et al. 2002) in the summer season. Hence, changes in turbidity exhibits the main factor controlling irradiance penetration of UVR and PAR into the water column. Hanelt et al. (2001) regarded the water masses of Kongsfjorden after the coastal water classification of Jerlov (1976) as coastal water type 1 (clear waters) and type 9 (turbid water masses). Waters of the coastal water type 1 are characterized by clear water with a transmission window in the blue and highest solar energy transmittance at approx. 475 nm (Jerlov 1976). In this stage, high PAR and UVR can penetrate deeply into the water column with potentially damaging effects on marine organisms (Hanelt et al. 2001; Hop et al. 2002).

2.1.2. Study area and sample collection

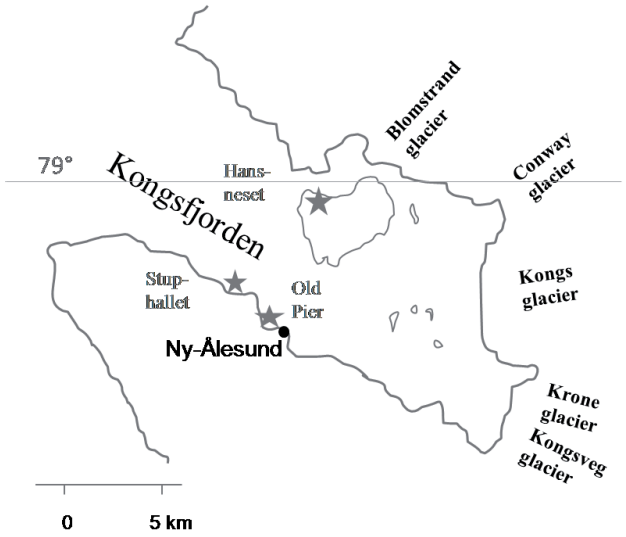


Fig 8 Algae collection sites in Kongsfjorden. Fertile algae were collected at Hansneset, Stuphallet and the Old Pier as indicated by grey stars.

Fertile algae were collected by SCUBA diving at Hansneset, the Old Pier and at Stuphallet in Kongsfjorden (see Fig 8) and were kept fully hydrated in the dark at ambient temperatures until arrival in the laboratory (for e.g. conservation of total phenolics, Kleiner 1991). An overview on species collected, sampling depth and collection site of the parental algae and the investigated developmental stage, is given in Tab 2.

Tab. 2 Overview about all species tested, their investigated developmental stage, collection site and sampling depth of the parental algae.

Species	Order Family	Developmental stage investigated	Collection site (parental algae)	Sampling depth (parental algae)	Publication
Alaria esculenta	Laminariales Alariaceae	•Zoospores •Juvenile gametophytes	Hansneset	3-4 m	III, IV
Laminaria digitata	Laminariales Laminariaceae	•Zoospores •Juvenile gametophytes	Hansneset	6 m	III, VI
Saccharina latissima	Laminariales Laminariaceae	•Zoospores •Juvenile gametophytes	Hansneset Stuphallet Old Pier	8 m 4-6m	I, III VI
Saccorhiza dermatodea	Tilopteridales Phyllariaceae	•Zoospores	Hansneset	4-5 m	II

2.2. Experimental design, set-up and investigated parameters

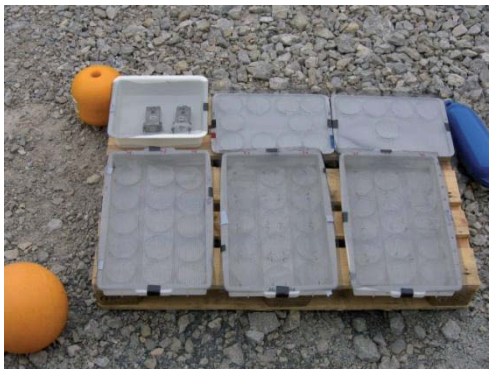
This study intended to investigate the interactive effects of UVR and temperatures on phlorotannins (Publ. I, II, III, IV), germination (Publ. II & III), fatty acid composition (Publ. I & IV) and reactive oxygen species (Publ. VI) on various brown algal species in the laboratory and in the field (summary of variables in Tab. 3).

Field experiments (Publ. II and III) were performed in the Arctic during summer 2007 simulating clear water conditions in Kongsfjorden by using experimental tanks and gauze ashore (Fig 9). Different treatments within the radiation conditions were obtained under high PAR (field) and low PAR (laboratory) conditions and by additional usage of cut-off filter foils. By cutting off different wavelength of the solar radiation spectra, wavelength effects can be distinguished: (1) PAR only (controls), (2) PAR and UV-A radiation (→PA) or (3) PAR and UV-A+UV-B (→PAB). Temperatures of 2, 7, 12 and 17 °C were adjusted in temperature controlled climate rooms.

Tab. 3 Summary of variables obtained for the different experiments.

	Publication					
	I	**II**	**III**	**IV**	**V**	**VI**
Species	*Saccharina latissima*	*Saccorhiza dermatodea*	*Alaria esculenta* *Laminaria digitata* *Saccharina latissima*	*Alaria esculenta*		*Laminaria digitata* *Saccharina latissima*
Duration of experiment	20 d	8 h	8 h	20 d		8 h/10 d
Temperature tested	7°C	2,7,12,17°C	2,7,12,17°C	7°C	Review	2,7,12,18°C
PAR low	•	•	•	•		•
PAR high		•	•	•	Polar	
UV-A		•	•	•		•
UV-B		•	•	•		•
Phlorotannins Spores+ Filtrate	•	•	•	•	benthic	
Fatty acid composition	•			•	algae	
Germination		•	•			
ROS						•
Electron (EM) / Light (LM) microscopy	EM	LM				EM /LM

a



b

Fig 9 Experimental exposure of zoospore material (a) in the laboratory and (b) in the field.

In our studies ultraviolet fluorescent lamps and PAR fluorescent lamps were used to obtain stable radiation conditions in the laboratory. As the radiant flux in fluorescent lamps is temperature dependent (Björn and Teramura 1993) leading quickly to a decrease in radiation flux with lower temperatures (<20°C). To compensate this effect, lamps were positioned lower in the 2°C compared to the 17°C treatment to match the desired irradiances in all temperature treatments.

2.2.1. Underwater simulations

In the field experiments, an underwater radiation climate of Kongsfjorden in spring was simulated. However, it is hardly possible to perfectly mimic under water condition *ex situ*. Hence, a compromise between the experimental set-up and the simulation of the most natural radiation conditions has to be made.

Simulation ashore had various advantages: a quick handling without e.g. losing sample material during transport onboard, adjustable radiation and temperature conditions without turbid water influences, some kind of weather independency in the use of boat and diving facilities and short transports to preserve sample material in the actual stage after exposure. On the other hand, simulation of the underwater radiation climate ashore is very challenging as discussed in Publ. II and III considering e.g. spectral composition, differences in attenuation, melt water and sediment input, clouds and water movements. Nevertheless, comparison of different studies and conditions can be obtained by e.g. calculating erythemally weighted doses of UVR (UV_{ery}) or doses (Publ. III, IV) to relate actual impacts, exposure times and radiation regimes. In future studies we should try to standardize radiation climate during periods of exposure as suggested e.g. by Wulff (1999).

2.3. Experimental design in relation to statistics

In ecological data sets, statistical analyses including complex data sets are usually tightrope walks trying to combine independency of variables and ecological truth (Underwood 1997; Wulff 1999).

In our study, we tried to evaluate experimental outcomes (interactive effects) with the help of the statistical computing environment of "R" (R-project organization; GNU) in combination with empirical approach (as suggested by Underwood 1997) to overcome problems and errors of e.g. predesigned work sheets and not applicable data sets. Due to the high number of parameters used in our study, we decided to first apply the parametric analysis of variance, the Shapiro-Wilk test, to test if a sample of data is normally distributed (Sokal and Rohlf 1995). Afterwards samples were tested with a multi-comparison test (Tukey-HSD) to obtain statistically significant differences within treatments. Tukey's honest significance differentiation (HSD) procedure is based on the distribution of range (Saville 1990) and is reliable for a small number of experimental treatments as used in our case. Interpretations in our study were made on the basis of the statistical probability of $p<0.05$. Nevertheless, ecologists should be aware of trends and often insignificant data ($p>0.01$; $p>0.05$) in ecological data sets which could lead to clarifiaction and understanding of complex ecological systems as suggested by Zacher (2007).

2.4. Radiation treatments and measurements

As mentioned above, the spectral quality and quantity of solar radiation determine its impacts on organisms. Thus, during radiation experiments, the radiation regime should be controlled and adjusted, especially under field conditions. While radiation conditions are very stable under laboratory conditions, field irradiances can change due to variable meteorological conditions and changing solar elevation (Josefsson 1993). Considering these differences, a radiometer (Solar light PMA 2100) recording irradiances of UV-A and UV-B almost instantaneously was used for radiation measurements in the laboratory while for spectral radiation measurements of the solar radiation in the field experiments the spectroradiometer of the NDACC (Network for detection of atmospheric composition change) was used (for details compare Publ. II and III). Spectral data are highly dependent on the resolution of the spectrometer (bandpass) determined by the width of the slits and dispersion of the grating (Josefsson 1993). Spectral measurements assign each wavelength to the actual obtained energy with the ability to describe biological effects as a function of wavelength. Weighting

functions vary in addressing their effects to different wavelengths mainly in the UV-B wavelength range. The use of cut-off foils to determine wavelength specific effects in combination with weighting function help in addition (Björn and Teramura 1993) to bridge a problem often occurring in experiments obtained under artificial UVR - unnatural ratios of PAR:UVR and UV-A:UV-B. In our study (Publ. III, IV), we used calculations of the erythemally weighted UVR (UV_{ery}) after McKenzie et al. (2004) assuming ozone concentrations of ~320 DU (*in situ* values measured over Ny-Ålesund in August 2007). Although our experiments were not designed to mimic natural conditions perfectly, UVR doses were adjusted as natural as possible. Nevertheless, laboratory experiments should be seen as a more mechanistic approach than as a duplicate of field experiments.

Underwater measurements of the environment of Kongsfjorden were obtained in July and August 2007 and 2009 with a LiCor underwater sensor (LiCor, Lincoln, NE, USA) and UVR with underwater sensors of a Solar light PMA 2100 (Solar light Co., PA, USA) and Underwater UVR Logger X-2000 (Gigahertz-Optik, Germany). For logistic reasons, *in situ* underwater measurements were unfortunately only obtained during periods of turbid melt water inflow or cloudy days (compare Fig 12 in the results section) in the experimental summer season and excluded a comparison to simulated clear water conditions in spring. Consequently, radiation conditions simulated in the field experiments were related to literature data of Bischof et al. (1998a), Hanelt et al. (2001) and Brey (2009) obtained in Kongsfjorden at 2-5 m water depth between May and June.

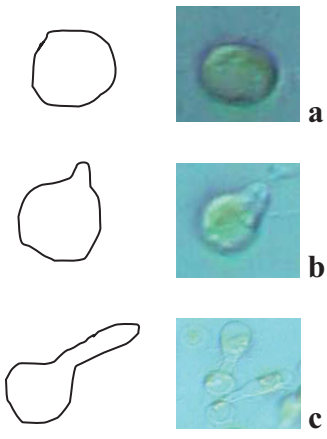


Fig 10 Determination of non-germinated (a) and germinated (b,c) spores. Spores exhibiting a bulge structure or a germ tube (b) were considered as germinated. Modified after Steinhoff (2006).

2.5. Germination as a fitness parameter

Zoospore germination success was determined from at least 300 settled spores per replicate six days after exposure. Germinated and non-germinated spores were evaluated under 200 x magnification (Plan-Apochromat, Zeiss, Germany) in a light microscope (Axio Scope, Zeiss, Germany). Spores were counted as germinated by indication of an obvious germination tube (compare Fig 10).

As discussed in Publ. V, the integrative parameter "germination" describes the ability of the spore/zygote to cope with certain impacts as e.g. UVR. The balance of damaging

effects and the repair and protective mechanisms spores/zygotes exhibit, are displayed in the germination pattern relating high germination rates with a high level of spore/ zygote fitness.

2.6. Ultrastructure of gametophytes and reactive oxygen species (ROS)

To determine UVR effects on the ultrastructure of juvenile gametophytes, *S. latissima* gametophytes were carefully removed from the Petri-dish bottom after 10 days of incubation and slowly centrifuged to separate them from the surrounding medium. Fixation for electron microscopy followed the protocol of Steinhoff et al. (2008) including, aldehyde and osmium fixation as well as embedding into Spurr's artificial resin. Electron micrographs were obtained from ultrathin cuts as described in detail in Publication VI.

Due to the fact that phlorotannins are *inter alia* discussed to have an antioxidative potential, we studied whether reactions related to reactive oxygen species as e.g. enhanced production of the plastoglobuli (stained black during osmium fixation) within the chloroplasts are already visible at the ultrastructural level. Plastoglobuli are often found in aging or damaged thylakoids (Senser et al. 1975) indicating either stress reactions or irreversible cell damage. Additional assays to determine intra- and extracellular reactive oxygen species via confocal laser scanning microscopy and spectrophotometric analysis were obtained by staining of superoxide anions with nitro-blue tetrazolium (NBT) as described in Publ. VI.

2.7. Quantitative and qualitative analysis of fatty acids

Fatty acid composition and total fatty acid content were investigated over a period of 20 days in zoospores and juvenile gametophytes of *S. latissima* and *A. esculenta*. Investigation of fatty acid composition and consumption processes (Publ. I) present a base line study in zoospores and juvenile gametophytes of *S. latissima.* The fatty acid experiment was further developed to obtain additional effects of UVR in combination with high and low irradiances of visible light (PAR) under nutrient enriched conditions during the development process of *A. esculenta* (Publ. IV). Originally, the second experiment (Publ. IV) was intended to be a continuation and further development of the first experiment. Unfortunately in summer 2009, no fertile *S. latissima* thalli were available to conduct the experiment as planned. The decision

to obtain the experiment with species of *A. esculenta* was made on the basis of similar thalli maturity and affinity of species.

Additionally, phlorotannin contents were measured both in the spores and the filtrate (surrounding medium, Publ. I and IV) to link fatty acid metabolism and phlorotannin production mechanisms (Publ. I). Further details both for fatty acid component determination and phlorotannin analysis are addressed in the material and methods sections of the respective publications (I and IV).

2.8. Phlorotannin extraction

The objective of preparing extracts is usually to transfer the chemicals which represent the physiological and living stage into solution and avoid those chemicals representing the pathological stage (Waterman and Mole 1994) within three steps: 1. collection and preservation of the sample, 2. physical degradation before extraction and 3. extraction of phytochemicals.

Extraction protocols for phenolics are highly variable in extraction time, solvent and temperatures as summarized by Waterman and Mole (1994). The yield of phenolics extracted from the samples should be optimized by adapting extraction protocols to the own needs and sample size and not simply trust methods which appear to have worked out in the past.

The extraction protocol was developed together with Dr. Riitta Koivikko by extraction samples with different solvents (Methanol, Methanol:deionized water, Ethanol, Ethanol:deionized water, Acetonitril, Aceton, Aceton: deionized water). Additionally, various degradation studies as filter break up by usage of a cell mill, grinding in liquid nitrogen with mortar and pestle as well as shaking and elution processes in the solvent were performed. Purification of extracts via solid phase extraction (SPE) was tested but seemed unnecessary due to very low amounts of polysaccharides within the spores.

The most suitable phenolic compound extraction in kelp zoospores was developed and obtained at room temperature as also recommended by Waterman and Mole (1994) by:

1. Filtering zoospores directly after exposure and transfer of filters into liquid nitrogen leading to an immediate preservation of the spore's biological /chemical state and an inhibition of destructive enzymes due to low temperatures. Frozen

filters were transported back to the laboratory and melted before extraction procedure started.

2. Filters were physically destroyed by extensive vortexing to remove all phenolics from the solid residue of the zoospores. Extraction processes from fresh and wet material are faster versus from dry material as all components do not need to be rewetted and essentially unstuck before they enter the extract (Waterman and Mole 1994).
3. As an excellent solvent of phenolics (Cork and Krockenberger 1991) in zoospores, 7:3 Acetone:deionized water (further details in publication I, II, III and IV) was chosen.

To the filtrates (surrounding medium of spores), 50% deionized water was added before freezing to obtain osmotic and physical break-up of physodes within the medium. Freeze-drying of spore extract and filtrate can be regarded as "safe" method to preserve phenolics (Waterman and Mole 1994) while the necessary re-solution of freeze-dried material before quantitative measurement might have lead to a decrease in phlorotannin contents. If samples are not processed fast enough, enzymes and substrate reaction are able to restart and the phenol group could be easily transformed into a reactive group of quinine upon exposure to air, resulting in variations in extractable phenolics (Cork and Krockenberger 1991).

2.9. Quantitative phlorotannin determination

There are various commonly used methods in ecological studies for total phenolic content and examples of their use as reviewed by Singleton et al. (1999), Schofield et al. (2001) and Amsler and Fairhead (2006): one of the most common spectrophotometrical assays is the Folin-Ciocalteu method. Spectro-photometric assays determine the concentration of a particular substance or group of substances by measuring the light at a particular wavelength (220-850 nm) which is absorbed by a liquid sample (Waterman and Mole 1994). The Folin-Ciocalteau (1927) method was developed from the Folin-Denis method (1912, 1912a) and is more sensitive to reduction by phenolics and less prone to precipitation and interference by non-phenolics compared to the Folin-Denis method. Therefore the Folin-Ciocalteu method has been recommended over the Folin-Denis method (Waterman and Mole 1994) and it is gaining acceptance among ecologists as recent studies show (van Alstyne 1999, 1999a; Connan et al. 2004; Kubanek et al. 2004; Pavia and Toth 2000; Toth and Pavia 2001; Wikström et al. 2006). The method additionally does not require species-specific standards

e.g. the DMBA assay (Amsler and Fairhead 2006) and the variation in reactivity of different phlorotannin fractions is rather small. The Folin-Ciocalteu reagent is composed of sodium tungstate, phosphomolybdic acid, hydrochloric acid, orthophosphoric acid, lithium sulphate, sodium carbonate and deionized water (Waterman and Mole 1994). The reaction is based on the oxidation of the phenolate ion under alkaline condition and the reduction of the phosphotungstic-phosphomolybtic complex to a blue coloured solution (chromophore). Nevertheless, the structure of the inorganic complex formed could not yet be resolved (as reviewed in Schofield et al. 2001).

However, the Folin-Denis method was not specifically developed to determine only hydrolysable phenolics as phlorotannins but rather total phenolics and tyrosine in proteins (Folin-Dennis 1912, 1912a) by quantifying total phenolic hydroxyl groups in the extract (Hagerman and Butler 1994). Due to the fact that we lack specific analysis for phlorotannins and that it is widely agreed that phlorotannins are the only phenolic compounds brown algae contain (Targett and Arnold 1998; Jormalainen et al. 2003), the Folin-Ciocalteu method was the most applicable and reliable analysis (Singleton et al. 1999; Mueller-Harvey 2001) for quantifying the total concentration of phenolics (phlorotannins) in our study (Publication I, II, III, and IV).

The Folin-Ciocalteau method was modified after Koivikko et al. (2005) using 2 ml Folin-Ciocalteu solution and 1 ml 20% sodium carbonate. To maximize the sensitivity of the assay, the measurements were obtained at 730 nm, the maximum absorption of the chromophore. Ascorbic acid to prevent phlorotannins from oxidation was not added as proposed by Waterman and Mole (1994) and Koivikko et al. (2005) due to analytical interferences with the preferred assay of Folin-Ciocalteu. Detailed descriptions of the method applied can be found in Publ. I, II, III and IV.

3. Summary of results

3.1. UV radiation at Ny-Ålesund and in Kongsfjorden

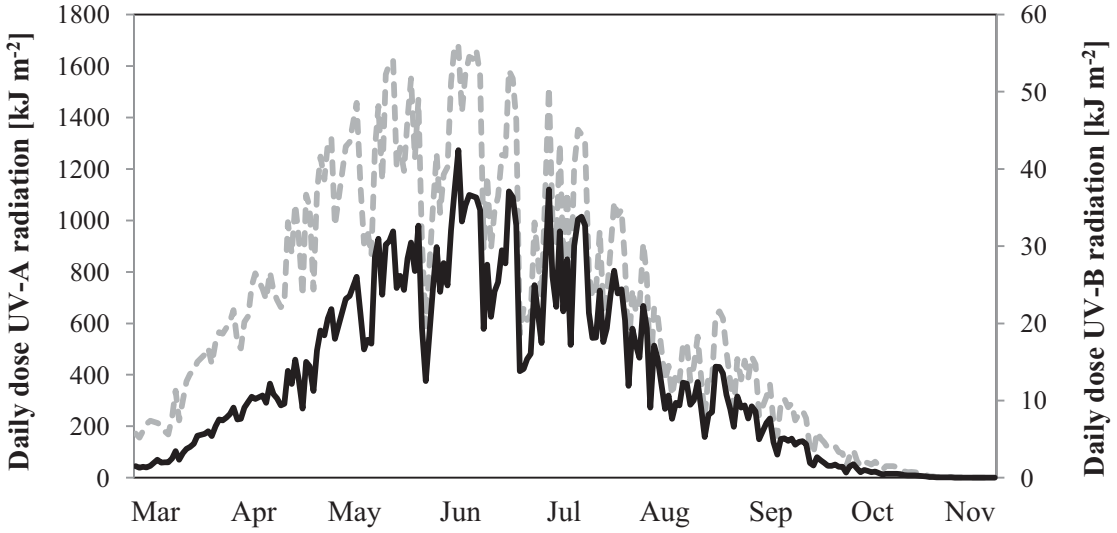


Fig. 11 UVR measurements (in air) during the Arctic summer season 2007 in Ny-Ålesund. Daily UV-A (dashed grey line) and UV-B dose in kJ m^{-2} (black solid line, secondary axis).

The radiation climate, especially in Polar regions, is highly variable on a daily and yearly base. Surface radiation measurements in the field (in air) of the year 2007 are shown in Fig. 11 for March until November. Highest UVR doses were measured in June with daily doses of up to 1678 kJ m^{-2} UV-A and 42 kJ m^{-2} UV-B and lowest values in March and October during twilight conditions. Daily variations in UVR doses are related to weather conditions such as clouds and fog (compare chapter 1.2.1 and 2.1).

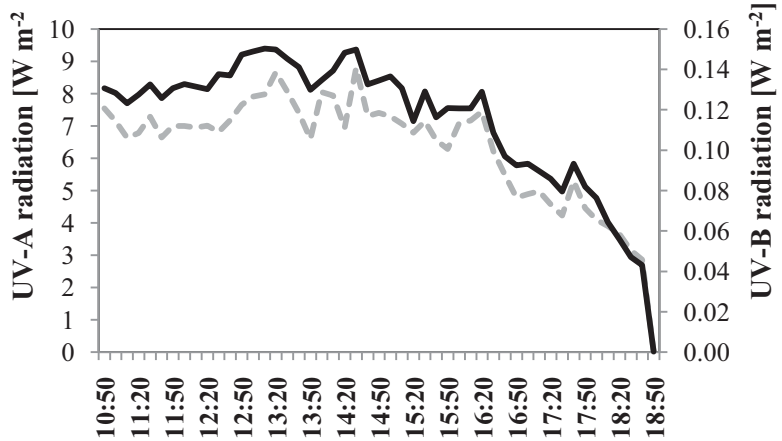


Fig. 12 Underwater UV irradiances [W m^{-2}] measured with an UVR Logger on the 10th June 2009 exemplarily for a 8 h exposure. UV-A (dashed grey line) and UV-B (black solid line) were measured in 3 m water depth of Kongsfjorden (in front of the harbor, Ny-Ålesund). Attenuation of UVR can be explained by cloudy weather conditions and visible sediment load of the water column.

To illustrate previously mentioned fluctuating underwater radiation conditions in Kongsfjorden during Arctic summer, underwater irradiances for June the 10th are shown (Fig. 12). Irradiation conditions in Kongsfjorden are strongly dependent on ice conditions, melt water inflow of glaciers, their sediment load, phytoplankton blooms, wind

directions and mixing processes within the water column. As described in chapter 2.1, in early spring (May-June) where glaciers are still frozen and melt-water-rivers have not formed yet, UVR and PAR are able to penetrate deeply into the water of Kongsfjorden. In contrast, with rising temperatures in late spring and early summer, glaciers start to melt and the input of their sediments and fresh water via melt water rivers begins and increases the attenuation of PAR and UVR in the water column (Hanelt et al. 2001; Steneck et al. 2001). In Fig. 12, underwater irradiances for an early summer day with slightly turbid waters and cloudy weather are shown where UV-A and UV-B irradiances in a water depth of 3 m can range up to 8.3 W m^{-2} and 0.15 W m^{-2}, respectively.

3.2. Effects of a changing environment on spores

3.2.1. Germination of various brown algal zoospores

Tab. 3 displays a summary of germination rates (Publ. II and III) as a function of UVR and PAR exposure as well as of various temperatures in spores of *A. esculenta, L. digitata, S. dermatodea* and *S. latissima*. Global change scenarios of Publication III are highlighted in grey.

Germination success varied between species and was strongly related to surrounding temperatures and radiation climate (Tab. 3). At 2 °C, germination was decreased only in *A. esculenta* and *S. latissima* while temperatures of 17 °C decreased germination in all species except *S. latissima*. *S. latissima* zoospores showed their maximum germination in the control treatment of 17 °C with 97% germinated spores. At 7 °C, spores of *L. digitata* and *S. dermatodea* exhibited highest germination rates in their controls. *A. esculenta* spores showed maximum germination rates at the control treatment of 12 °C.

Treatments including low PAR and UV-A showed no statistical differences in all species except for the 2°C PA treatment of *S. latissima* and the 17 °C treatment of *L. digitata.* Irradiation with the full solar spectrum (PAB) lead to a decrease in germination compared to controls in all temperature treatments of *L. digitata* and *A. esculenta* spores and in the 2 °C PAB treatment of *S. latissima* and the 17 °C PAB treatment of *S. dermatodea*.

High PAR (scenario b) compared to low PAR exhibited a strong decrease in germination rate in the controls (HLPAR) and the HLUV treatments where *S. latissima* spores seemed to be the most affected compared to all other species.

In contrast, enhanced UV-A and UV-B radiation (HUV PA and HUV PAB; scenario c) showed only slight effects in *S. latissima* spores while spores of *A. esculenta* remained unaffected.

Tab. 3 Summary of germination in 6 days old germlings of *A. esculenta, L. digitata, S. dermatodea* and *S. latissima* as an indicator of fitness:
Three different global change scenarios (8h exposure) were simulated: the interactive treatments of temperature (2 °C-17 °C) and UVR (PAR-control, PA-PAR+UV-A, PAB-PAR+UV-A+UV-B), UVR and high PAR (HL PAR, HL PA, HL PAB) and under enhanced UVR (HUV PAR, HUV PA, HUV PAB). n.d. - not determined. * indicate statistically different treatments ($p<0.05$)

	Temperature	**Light treatment**	**Germinated spores in %**			
Publication ***Species***			***III*** ***Alaria esculenta***	***III*** ***Laminaria digitata***	***II*** ***Saccorhiza dermatodea***	***III*** ***Saccharina latissima***
	2 °C	***PAR***	49.7±7.5*	70.3±3.9	93.2±0.8	66.9±7.7*
		PA	55.7±3.4*	67.5±4.4	94.0±2.1	39.3±7.3*
		PAB	48.4±1.9*	41.6±12.0*	89.7±6.1	28.6±22.9*
	7 °C	***PAR***	73.2±6.8	72.7±12.5	98.1±0.8	74.1±9.2
		PA	76.8±3.1	62.7±2.2	98.5±0.7	74.9±9.4
		PAB	65.6±2.3	36.0±5.4*	92.7±1.9	75.1±6.3
Global change scenario: enhanced water temperatures	**12 °C**	***PAR***	87.3±2.1	57.2±4.9	96.7±1.8	81.4±0.6
		PA	80.6±4.8	68.7±5.15	95.5±1.7	79.3±2.7
		PAB	64.8±3.2	49.2±8.8*	90.9±8.0	81.1±6.7
	17 °C	***PAR***	42.2±38.6	35.1±4.6*	72.1±20.2*	97.0±0.0
		PA	78.4±8.3	60.5±10.9*	85.9±17.2	91.7±3.0
		PAB	9.9±17.1*	13.9±9.7*	21.4±18.1*	86.6±1.3
Global change scenario: High PAR	**7 °C**	***HLPAR***	31.2±2.7*		82.0±0.0	9.9±7.9*
		HL PA	20.8±4.6*	n.d.	35.6±49.9*	18.5±3.4*
		HL PAB	5.2±8.9*		9.6±16.6*	0*
Global change scenario: enhanced UVR	**7 °C**	***HUV P***	79.0±5.6			88.23±4.7
		HUV PA	80.1±0.7	n.d.	n.d	62.6±16.6
		HUV PAB	58.9±2.4			72.6±9.35

3.2.2. Phlorotannin content in zoospores and their surrounding medium

3.2.2.1. Phlorotannin variability over time

Phlorotannin content within the zoospores and the surrounding medium (filtrate) was measured over a time period of 20 days in spores and juvenile gametophytes of *A. esculenta* (Publ.

IV) and *S. latissima* (Publ. I) to investigate the effect of time on phlorotannin production and exudation.

While initial phlorotannin contents within both species were similar (0.31 µg phlorotannin ml spore solution^{-1}), phlorotannin content in the surrounding medium of *A. esculenta* spores was significantly higher than the initial filtrate obtained in *S. latissima* (Tab. 4). During development under low PAR conditions, phlorotannin content increased with time in juvenile gametophytes *A. esculenta* until day 15 and within filtrates until the end of the experiment. Until day 20, phlorotannins decreased only within the zoospores of *A. esculenta*. In contrast, phlorotannins within zoospores of *S. latissima* did not vary over time but showed a significant increase until day 20 within the filtrates. Among high PAR treatments, juvenile gametophytes of *A. esculenta* showed a significant increase of phlorotannins until day 10 and a significant decrease from day 10 to 20. Filtrates obtained from the high PAR treatment showed a significant decrease over the exposure period of 20 days.

Tab. 4 Summary of phlorotannin content as a function of time (initial-20 days) and irradiances of photosynthetically active radiation (low and high PAR) during the development from zoospores (initial) to juvenile gametophytes (day 5-20) in *A. esculenta* and *S. latissima.*

Species	Phlorotannin content in µg phlorotannins ml spore solution^{-1} initial	5 d	10d	15d	20 d	Publication
LOW PAR						
A.esculenta Spores	0.31±0.04	0.42±0.13	1.09±0.21	1.42±0.25	0.93±0.15	IV
S. latissima Spores	0.18±0.00	0.15±0.02	0.17±0.01	0.18±0.01	0.17±0.01	I
A.esculenta Filtrates	5.64±0.30	5.75±0.07	8.39±0.20	11.75±2.02	12.75±1.51	IV
S. latissima Filtrates	0.23±0.01	0.33±0.02	0.39±0.02	0.48±0.09	0.47±0.02	I
HIGH PAR						
A.esculenta Spores	0.31±0.04	0.30±0.10	0.62±0.62	0.45±0.46	0.24±0.01	IV
A.esculenta Filtrates	5.64±0.30	4.79±0.28	10.24±1.02	9.3±0.9	11.9±0.00	IV

3.2.2.2. Phlorotannin variability under UVR and in various temperatures

Upon 8 h exposure (Fig. 13), phlorotannin contents within spores and filtrates of *A. esculenta, L. digitata, S. dermatodea* and *S. latissima* showed species and treatment dependent significant differences compared to their initial (release) values. It has to be noticed, that

differences neither in the spores themselves nor their filtrates within the treatments of PAR only, PAR+UV-A and PAR+UV-A+UV-B could be detected.

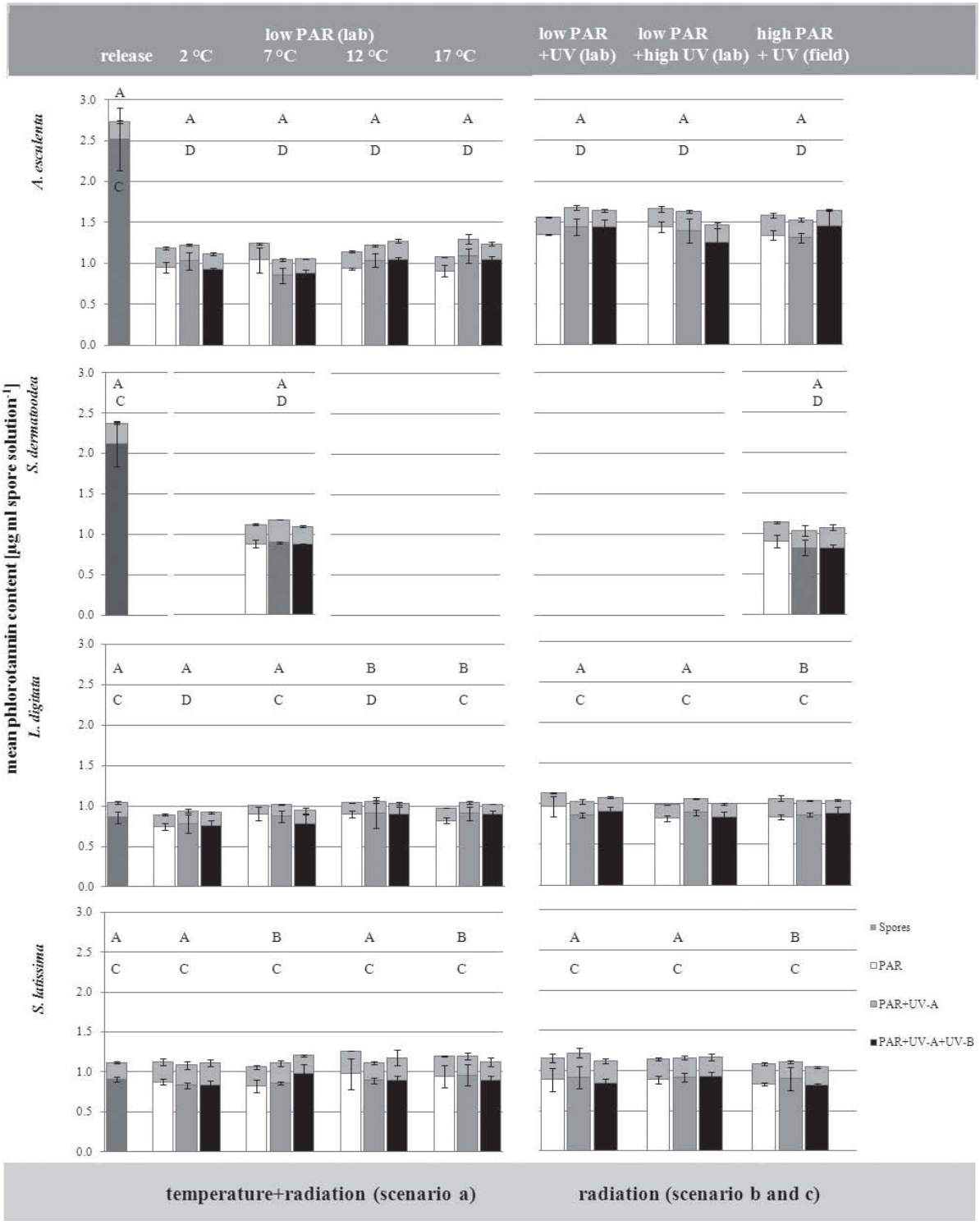


Fig. 13 Sum of phlorotannins in zoospores (upper part of the bars) and filtrates (lower part of the bars) in 4 investigated zoospore species: (1) *A. esculenta*, (2) *S. dermatodea*, (3) *L. digitata* and (4) *S. latissima* displayed as an interactive parameter of various temperatures (2-17°C) and radiation conditions (Publ. II and III). The global change scenarios a, b and c are shown according to Publication III. White bars indicate PAR (control), medium grey bars PA (PAR+UV-A) and black bars PAB (PAR+UV-A+UV-B) treatments. Capital letters indicate significant ($p<0.05$) differences during a pair-wised comparison test (Tukey-HSD).

In A. esculenta, only the phlorotannin content within the filtrate decreased about 66% compared to the initial, while phlorotannin content in zoospores remained unaffected. Zoospores of *S. dermatodea* exhibited a similar pattern with significant differences of phlorotannin contents within the filtrates 8 h after release under low and high PAR conditions and no differences in phlorotannin contents within the spores. Contrary to the species collected in the upper littoral, zoospore's phlorotannin contents of *L. digitata* and *S. latissima* showed significantly lower contents at temperatures of 12 °C and 17 °C (*L. digitata*) as well as 7 °C and 17 °C (*S. latissima*) compared to the initial. Nevertheless, only filtrates of *L. digitata* showed significantly lower phlorotannin contents compared to the initial at 2 °C and 12 °C. Among UVR treatments, Spores of *S. latissima* showed significantly lower phlorotannin contents after exposure to high PAR compared to the initial.

3.2.2.3. Phlorotannin content and vertical zonation pattern of algae

Fig. 14 displays the summary of all investigated spore species and their phlorotannin contents during release (Publ. II and III). Spores of *A. esculenta*, *S. dermatodea* and *L. digitata* contained similar amounts of phlorotannins while phlorotannin content in spores of *S. latissima* was significantly higher compared to all other investigated species.

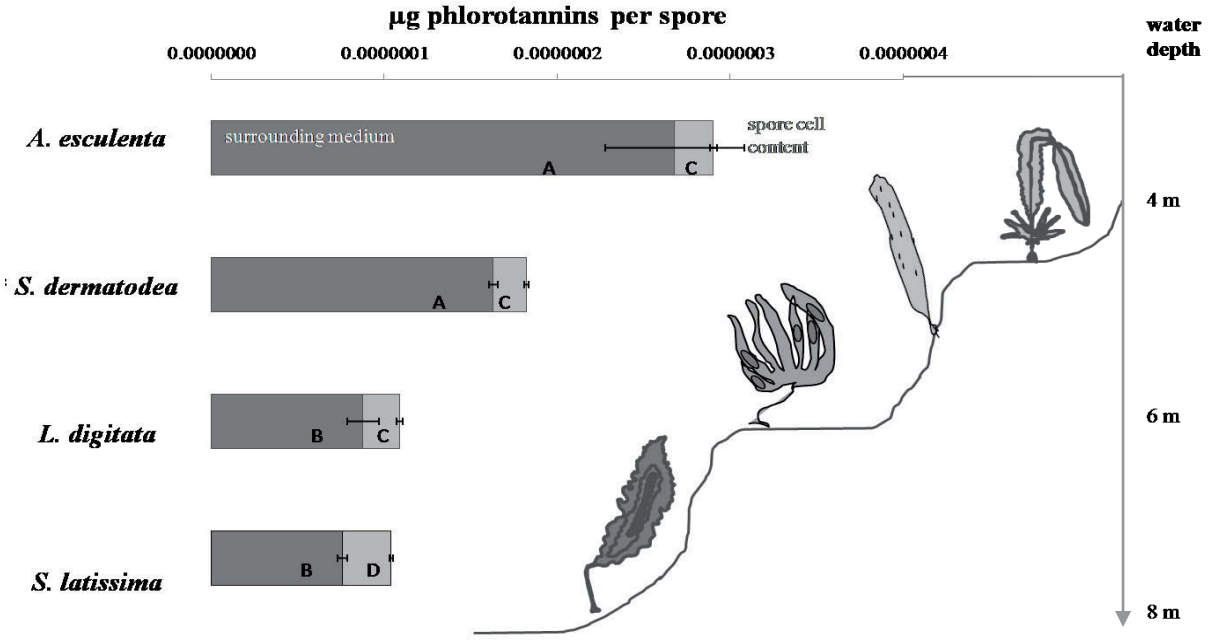


Fig. 14 Summary of phlorotannin content during release within zoospores and the surrounding medium (filtrate) in correlation with collection depth of the parental algae (Publ. II and III; Figure modified after Publ. III). The shallow water species *A. esculenta* and *S. dermatodea* released significantly more phlorotannins with their zoospores than species collected in deeper waters as *L. digitata* and *S. latissima*. In contrast, only zoospores of the alga *S. latissima*, collected at 8 m, contained significantly higher amounts of phlorotannins compared to all other species. Capital letters indicate significant differences.

Phlorotannin content in the filtrate differed significantly with species and was highest in spores released by parental algae collected in the upper sublittoral (*A. esculenta* and *S. dermatodea*) and lowest in spores released by parental algae collected in the lower sublittoral (*L. digitata* and *S. latissima*).

3.2.3. Fatty acid composition kelp zoospores and juvenile gametophytes

Different species exhibit different fatty acid compositions leading to characteristic fatty acid composition patterns. In Fig. 15, a summary of relative major fatty acid (FA) composition of *S. latissima* and *A. esculenta* spores and juvenile gametophytes (Publ. I and IV) is shown. FA composition in spores was measured directly after release (initial) while FA composition in juvenile gametophytes was obtained after an incubation period of 20 days. While the first two major FA 18:1(n-9) and 16:0 are similar in spores of both species, the second two major FA are different with 18:0 and 18:3(n-3) in spores of *S. latissima* and 20:5(n-3) and 18:2(n-6) in *A. esculenta*.

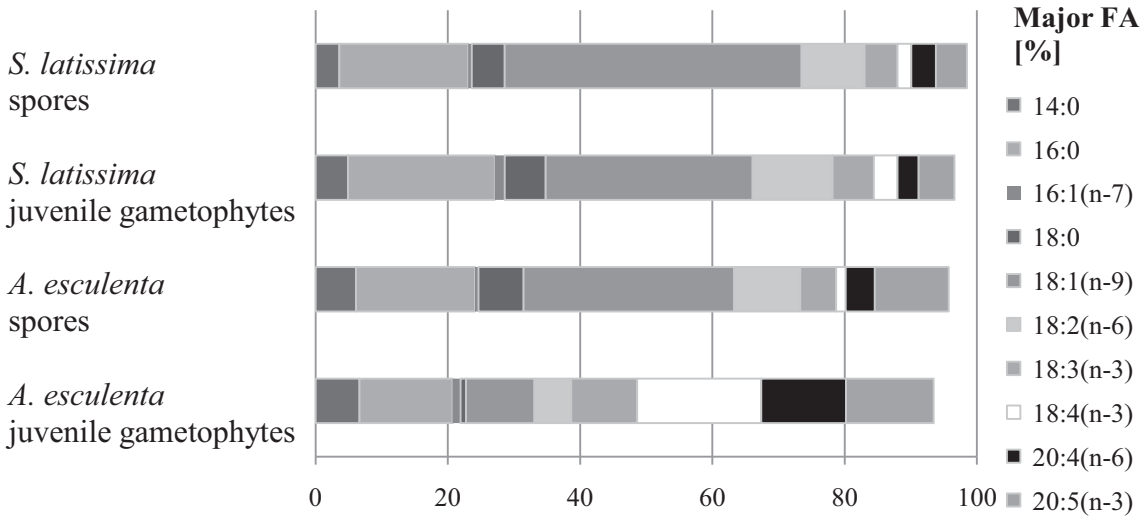


Fig. 15 Summary of major fatty acid (FA) composition in % of *S. latissima* and *A. esculenta* spores and juvenile gametophytes (Publ. I and IV) displayed as sums of FA.

Furthermore, the ratios of saturated to monounsaturated FA (SAFA:MUFA), saturated to polyunsaturated FA (SAFA:PUFA) and monounsaturated fatty acids to polyunsaturated FA (MUFA:PUFA) were determined for both spores and juvenile gametophytes of *A. esculenta* and *S. latissima* (Tab. 5). Compared to spores of *S. latissima* containing higher amounts of MUFA than SAFA and PUFA, spores of *A. esculenta* had more SAFA than MUFA and PUFA. During development from spores to juvenile gametophytes under low PAR conditions, MUFA content in both species decreased.

PUFA content in *S. latissima* spores remained relatively stable while PUFAs in *A. esculenta* increased among low PAR control treatments (Tab 5). Total FA content in *A. esculenta* spores and gametophytes was a multiple higher than in *S. latissima*.

Tab. 5 Summary of fatty acid ratios: saturated to polyunsaturated FA (SAFA:PUFA), saturated to monounsaturated FA (SAFA:MUFA) and monounsaturated fatty acids to polyunsaturated FA (MUFA:PUFA) in spores and juvenile gametophtes of *A. esculenta* and *S. latissima* (Publ. I and IV)

Publication	I	IV
	S. latissima	*A. esculenta*
Initial		
SAFA:MUFA	0.64	1.18
SAFA:PUFA	1.06	1.33
MUFA:PUFA	1.67	1.13
Day 5		
SAFA:PUFA	0.55	0.98
SAFA:MUFA	0.80	0.67
MUFA:PUFA	1.43	0.68
Day 10		
SAFA:PUFA	0.65	4.77
SAFA:MUFA	0.77	3.81
MUFA:PUFA	1.19	0.80
Day 15		
SAFA:PUFA	0.85	1.70
SAFA:MUFA	0.85	0.39
MUFA:PUFA	1.00	0.23
Day 20		
SAFA:PUFA	0.98	1.86
SAFA:MUFA	1.02	0.36
MUFA:PUFA	1.04	0.19

3.2.3.1. Effects of time, UV radiation and low vs high PAR on fatty acid content and composition

In Fig. 16, effects of UVR among low vs high PAR on total fatty acids (FA) are summarized for *A. esculenta* (Publ. IV). Among low PAR irradiation treatments (Fig. 16a), total FA content decreased from the control treatment P, to PA and the PAB treatment at each collection day (initial 0, 5, 10, 15, 20; compare trend lines). By comparing control treatments over the exposure period of 20 days, total FA content increased towards day 5 but decreased again to initial levels until the end of the experiment. In contrast, among high PAR irradiation treatments (Fig. 16b), only on day 5 and 10 a similar pattern of a decrease from the control treatment P to PA and the PAB treatment could be observed while on day 15 and 20, total FA content was lowest within the control treatments and highest in the PA or PAB treatments. Generally, total FA content under high PAR (Fig. 16b) was decreased about 50-75% after 15 and 20 days compared to treatments obtained under low PAR (Fig. 16a).

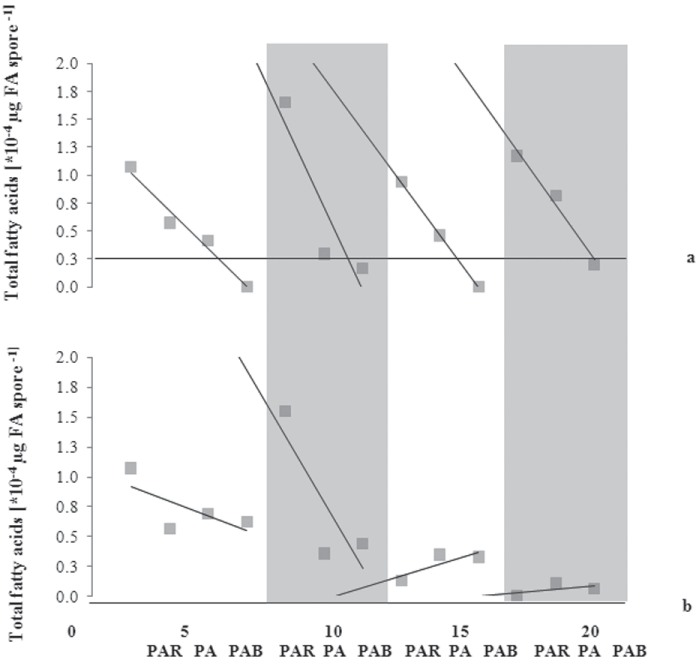


Fig. 16 Total fatty acids per *A. esculenta* spore upon UVR exposure and low PAR (a) vs high PAR (b) treatment. Black lines indicate trend lines.

Trend lines comparing total FA contents within the different UVR treatments indicate a more distinct decline-pattern of total FA between control, PA and PAB treatment among low photosynthetically active radiation than among high PAR treatments.

3.2.3.2. Coherence of fatty acids and phlorotannins

The monounsaturated FA diminishing the most during development from spores into juvenile gametophytes was 18:1(n-9) in *S. latissima* and *A. esculenta*. Even though early developmental stages of *S. latissima* contained much less total FA and consequently less 18:1(n-9) than their representatives of *A. esculenta*, a similar correlation between the fatty acid 18:1(n-9) and the phlorotannin exudation was observed in both species (Fig. 17).

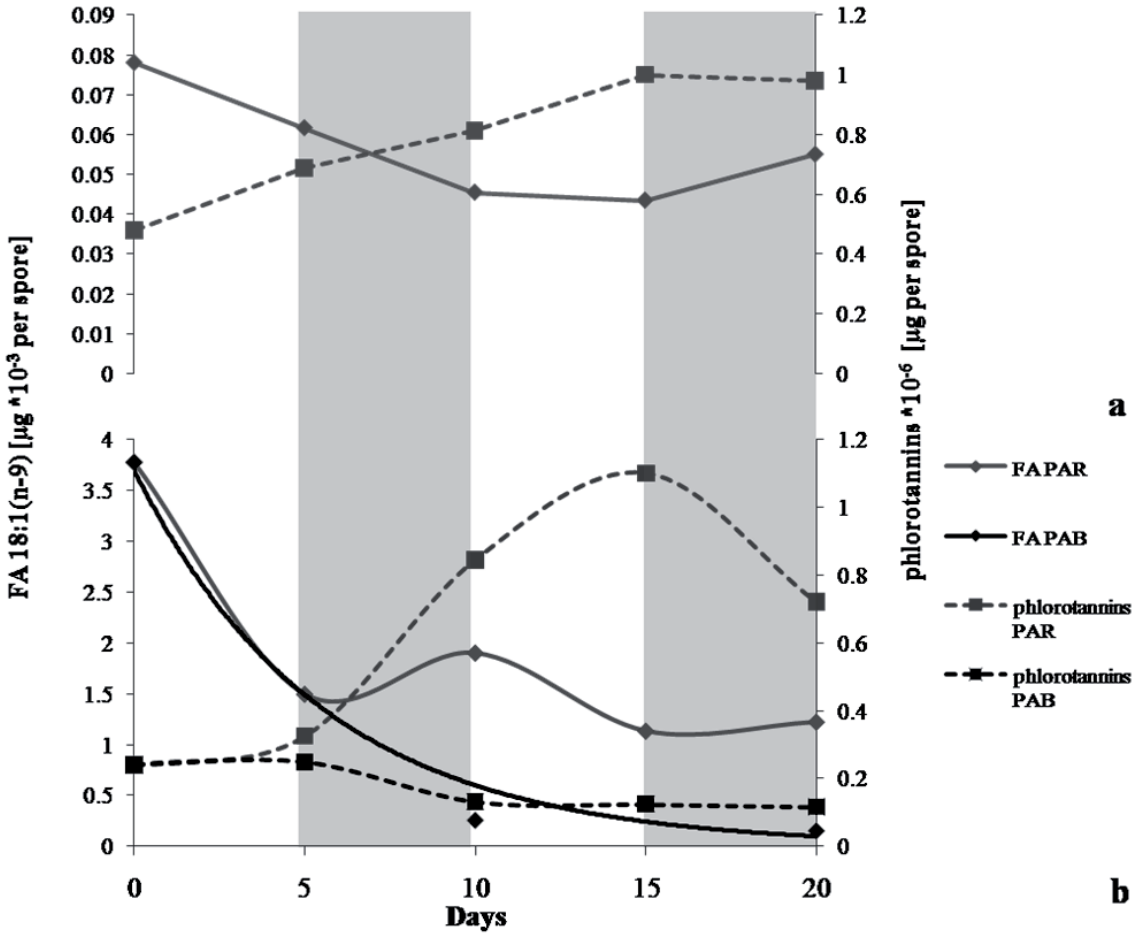


Fig. 17 Correlation between fatty acid 18:1(n-9) [µg FA*10^{-3} per spore] and exuded phlorotannins [µg phlorotannin *10^{-6} per spore] over 20 days in (a) *S. latissima* – Publ. I and (b) *A. esculenta*- Publ. IV. FA 18:1(n-9) is indicated by grey (PAR treatment) and black (PAB treatment) solid lines while phlorotannin content is indicated by grey dashed lines (PAR treatment) and black dashed lines (PAB treatment). Note secondary-axis for phlorotannin content. For the FA PAB treatment, an exponential trend line was used due uncertain data on day 5 and day 15.

Under low PAR conditions, both species showed a decrease of the FA 18:1(n-9) until day 5 and an increase in phlorotannin production until day 15. As shown in Publ. IV, the FA 18:1(n-9) and phlorotannin production/exudation in spores/juvenile gametophytes of *A. esculenta* exhibited a different pattern among exposure to UV-B radiation (Fig. 17b) compared to the previously shown pattern under low PAR only (Fig. 17a and b). Both FA 18:1(n-9) and phlorotannin content decreased under UV-B exposure with time.

3.2.4. Microscopical observations of UV radiation and temperature effects

Spores and juvenile gametophytes of *L. digitata* and *S. latissima* were examined by electron microscopy (Publ. I and IV) directly after release and after 10 days incubation while spores/juvenile gametophytes of *S. dermatodea* were investigated by light microscopy after 8h exposure to various temperatures and light treatments + 6 days recovery (Publ. II).

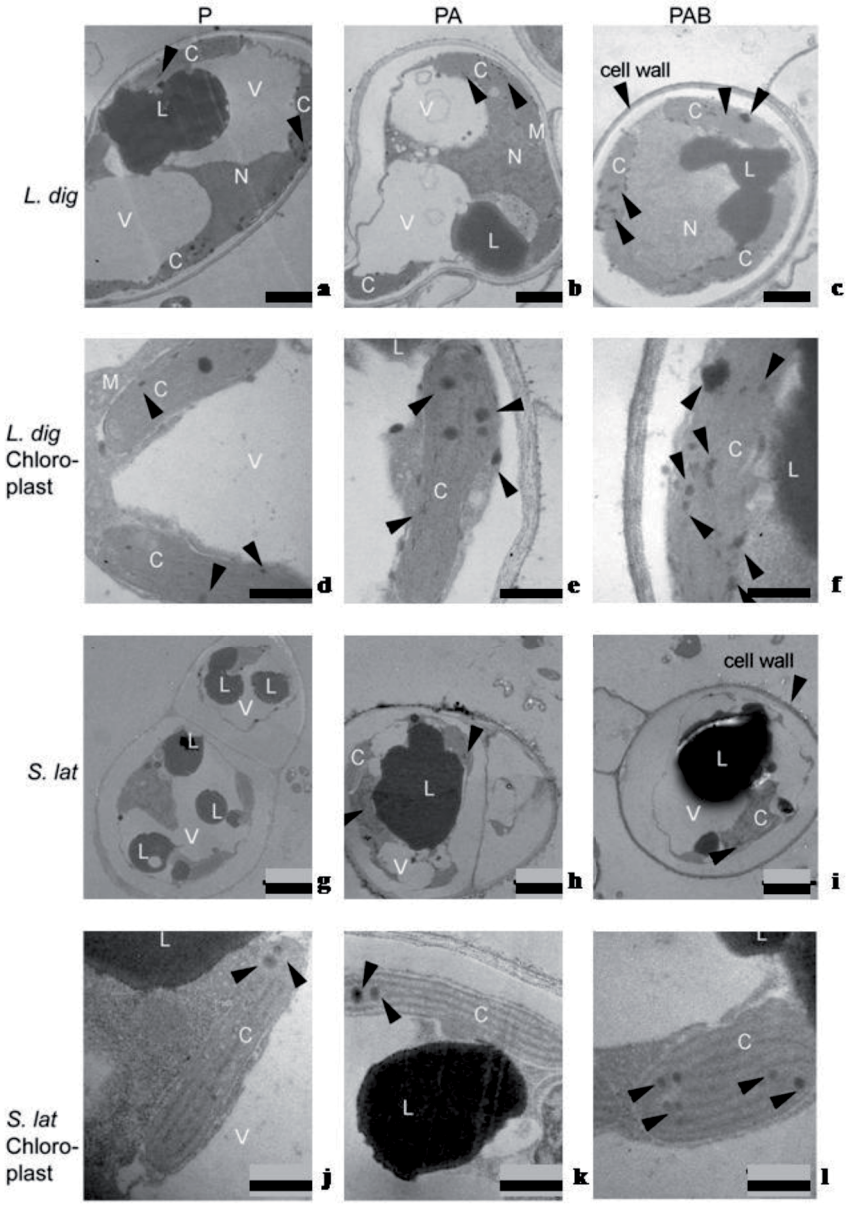


Fig. 18 Electron- micrographs of 10 days old gametophytes of *L. digitata* (a - f) and *S. latissima* (g - l) after irradiation with PAR (a, d, g, j), PA (b, e, h, k) or PAB (c, f, I, l) for 8 hours. A - c and g - i show the entire gametophytes (scale 2 µm) while d - f and j - l focus on one chloroplast exemplarily (scale 500 nm). Plastoglobuli in the chloroplasts are highlighted by black arrows. C = chloroplast, L = lipid globule, N = nucleus, V = vacuole. Figure modified after Publication VI.

After release (data not shown), *L. digitata* and *S. latissima* zoospores contain one nucleus with nucleolus, one chloroplast, several mitochondria and vacuoles as well as several lipid globules. The control treatments (P=PAR) showed after 10 days incubation under low PAR (Fig. 18) intact juvenile gametophytes of *L. digitata* (Fig. 18a, d) and *S. latissima* (Fig. 18g, j) with an established cell wall, several chloroplasts, mitochondria, nucleus, vacuoles and corroded lipid globules (compare Publ. I, Fig. 1).

In the PA and PAB treatment of both species, (Fig. 18b-c, e-f *L. digitata*; h-i, k-l *S. latissima*), an increase of plastoglobuli compared to the control treatments within the chloroplasts was observed. While thylakoid structure seemed disrupted additionally to plastoglobuli formation in *L. digitata,* juvenile gametophytes of *S. latissima* possessed intact thylakoids with more distinct and globular plastoglobuli (see arrows Fig. 18).

Investigations of *S. dermatodea* spores/juvenile gametophytes (Publ. II, Fig. 3) by light microscopy correlated with observations during germination (Tab. 3). After six days, no differences in spore development at 2, 7 and 12°C under the three light treatments (P, PA and PAB) could be observed. All spores had formed a germination tube and the germlings were equal in size (~25-30 µm). When exposed to 17 °C PAR and PA, the germlings were shorter with relatively thick germ tubes while under the 17°C PAB condition, spores showed only little signs of germination. Additionally, we observed dark globular pigmented cell structures (probably phlorotannin containing physodes) within the early gametophytes at 2°C PAR and PA, 7°C P, 12°C PAB, at 17°C PA and at 17°C PAB (Fig. 3 arrows). The observed germination process in the entire high PAR field treatment was different from all other treatments. Although spores germinated under high PAR, their germination tubes were usually thinner. Under high PAR+UV-A (PA), we observed germinated spores with thin germination tubes in addition to spores that had not germinated, appearing almost destroyed. Barely germinated or “viable” spores were found in the high PAR field PAB treatment where only remains of cell organelles were visible (compare Publ. II, Fig. 3).

4. Synoptic Discussion

4.1. Experimental radiation treatments and measurements

As mentioned above, the sensitivity of an organism to radiation is in general a function of wavelength (Madronich 1993). A match between natural solar radiation condition and artificially produced radiation spectra within laboratory experiments is therefore desirable but difficult to obtain (Holm-Hansen et al. 1993). Unfortunately, there are discrepancies in natural UVR ratios and ratios emitted by UV-fluorescent lamps possibly overestimating UVR effects under artificial UVR experimental conditions (Franklin and Forster 1997; Fredersdorf and Bischof 2007). Natural solar radiation exhibits ratios of UV-B:UV-A:PAR of 0.6:10:100 (Franklin and Forster 1997). While ratios obtained in field experiments of the present study (0.4:12:100) were close to the natural ratio, low PAR radiation in combination with high UV irradiances in the laboratory experiments with ratios of 8:114:100 (UV-B:UV-A:PAR) very likely overestimated UVR effects (Fiscus and Booker 1995; Fredersdorf and Bischof 2007) but helped to understand effects of low and high photosynthetically active radiation in combination with UVR on early developmental life-stages of brown algae.

UV-fluorescent lamps for instance often emit their radiation with peaks at 253.7 nm (Björn and Teramura 1993) close to the maximum absorption of e.g. nucleic acids, amino acids and phlorotannins. To bridge this gap, we used UV-fluorescent lamps with radiation peaks at 340 nm and additionally decided to use cut-off filter foils to distinguish wavelength effects and to evaluate UV irradiances under artificial radiation conditions as suggested by (Björn and Teramura 1993). Field experiments of Publ. II and III, simulating underwater experiments ashore, were discussed already in the Methodological consideration chapter. Consequently, additional comparison of radiation conditions for estimation of biological responses (Madronich 1993) using a weighting function to approximate the action spectrum for the radiation effect (e.g. UV_{ery}) were made as discussed in Publication II, III and IV. Nevertheless, our studies were not designed to mimic natural conditions perfectly and should be seen as a more mechanistic approach.

Reliable UVR data are also dependent on the strength and weaknesses of the instruments used for UVR measurements. For instance, the accuracy of the UV-B sensor (Solar light PMA 2100) was enhanced by using a cut-off filter (WG 320 Schott) as discussed in Publ. II.

Additionally, an intercomparison of UVR instruments was carried out in June 2009 within the framework of the ARCFAC project QAARC by Dr. Julian Gröbner (PMOWRC, Davos, Switzerland). This cross check under solar radiation conditions showed the high accuracy of the broadband radiometer Solar light within the UV-A range, but also an overestimation of UV-B up to ~30% (considering the broadband covered) compared to a spectro-meter. Nevertheless, variations of measured values can occur due to differences in calibration towards different sources, accuracies and saturation states of sensors, stray light and differences in the angle of incidence (Josefsson 1993) and should be considered in future studies (Gröbner et al. 2010).

4.2. Zoospores, a challenging developmental stage?

As most ecological experiments, investigating interactive effects is complex. Experiments with zoospores particularly face some challenges and require more compromises to the treatments and the experimental set-up as discussed in the following.

The challenge in working with zoospores starts already during release. Release of zoospores has to be induced artificially by simulating stress conditions such as dryness, darkness and temperature change. As investigated by Amsler and Neushul (1989) the majority of spores are released with this technique within the first hour. Under natural conditions, release of zoospores is dependent on season and induced hormonally by their sporophytes (reviewed by Bartsch et al. 2008). During artificially induced spore release, preconditioning of sporophytes can therefore not be considered and can lead to differences in sensitivity and fitness of the spores (compare Publ. II). Alternative collection of microscopic zoospores by nets in the water column is almost impossible due to difficulties in timing of spore release and low abundances. Additionally, distinction of different species seems to be difficult under natural mixed conditions (Graham and Mitchell 1999). Each algal individual released their zoospores in the same amount of surrounding seawater and solutions were mixed afterwards to obtain experimental spore solution. This technique allowed obtaining an average of different age classes and maturity grades among the parental algae to mirror natural conditions as much as possible. Nevertheless, due to collection, release and settling tendencies during experiments (Publ. I-IV), zoospore experiments generally suffer to some extent from artificiality almost unfeasible to overcome and should be considered during data interpretation of spore experiments.

4.3. The notional role of phlorotannins in brown macroalgae

Up to 20% of brown algal dry weight can consist of phlorotannins (Ragan and Glombitza 1986). As described previously in chapter 1, phlorotannins exhibit a multifunctional protective role against several abiotic and biotic factors due to their chemical characteristics. However, the most important properties of phlorotannins for this study were their abilities to absorb in the UVR wavelength range (Pavia et al. 1997; Swanson and Druehl 2002; Schoenwaelder et al. 2003; Roleda et al. 2006, 2006a, 2007; Karsten et al. 2009) and to possess antioxidant activity (Connan et al. 2006; Zubia et al. 2007).

Recent studies have investigated absorbance maxima of tissue phlorotannins (260-280 nm) in the UV-C wavelength range. Nonetheless, these absorbance maxima are observed within wavelengths which are much shorter than wavelengths algae are exposed to in their natural habitats (Pavia et al. 1997; Swanson and Druehl 2002). Additional shoulder peak absorbancies of phlorotannins (280-320 nm) observed by Pavia et al. (1997) and the ability of phlorotannins to function as antioxidants (Zubia et al. 2007) may contribute to the reduction of intracellular exposures of UV-B (Swanson and Druehl 2002).

Phlorotannin contents can vary with species, morphology of the algae, habitat and developmental stage as well as with seasonal and environmental parameter as reviewed in Tab. 1. These constraints have to be taken into considerations when interpreting results and dis-tinguish effects of applied experiments within the present studies:

4.3.1. Phlorotannin induction in relation to season and species?

Seasonal variation in phlorotannin contents were *inter alia* observed by Van Alstyne et al. (1999) and Van Alstyne et al. (2001) comparing *Alaria marginata, Costaria costata, Egregia menziesii* and *Hedophyllum sessile.* In May 1993, phlorotannin concentration within species was 80-600% higher than in 1998 (Van Alstyne et al. 2001) indicating either strong seasonal or inter-annual variations in phlorotannin content. Plouguerné et al. (2006) observed seasonal variations of phlorotannin contents in the temperate alga *Sargassum muticum.* Highest phenolic contents within adult specimen of *S. muticum* coincided with the reproductive period from May to September and were interpreted as chemical protection response of the receptacles to solar radiation and/or grazing. Nevertheless, seasonality is interwoven with several other parameters. Species specific genetic determination of phlorotannin expression, thallus morphologies (Stiger et al. 2004) and response to biotic and abiotic factors lead for instance to maximum phlorotannin contents in summer within the order Fucales while in the

order Laminariales phlorotannin contents are maximal during winter (Connan et al. 2004). Phlorotannin contents in our study were correlated with seasonal dependent maturity of investigated parental algae as discussed in Publ. II and III and hence confirm these observations. Nevertheless, phlorotannin content of spores and their surrounding medium during release is related to the amounts the parental tissue contains and can consequently not be regarded as completely independent (as discussed also later on).

Differences in phlorotannin content could be observed already among various brown algal families and yet down to the species level. The brown algal order Fucales for instance exceeds phlorotannin levels of Laminariales by a factor of ten (Connan et al. 2004). Several studies among adult algae confirm species specific differences in phlorotannin content and expression as reviewed by Amsler and Fairhead (2006). Publication III compared for the first time intra- and extracellular phlorotannin contents of four different brown algal spore species (Fig 13 and 14) directly after release and revealed that species specific differences in phlorotannin content of macroalgae occur already at their single-celled stages.

4.3.2. Phlorotannin expression in relation to habitat and age of the algae?

Due to the stratospheric ozone depletion over the Arctic (Bodeker and Waugh 2007) more shorter wavelength of the UVR spectrum reach the earth`s surface (mainly UV-B; 280-320 nm) leading to a high impact on Arctic Ecosystems (Hessen 2002). Underwater measurements of UVR by Bischof et al. (1998a) and Hanelt et al. (2001) revealed UV-B penetration down to a 1% depth of 13 m during clear water in spring and a decreasing UV-B transparency due to the turbid melt water input in early summer.

As we discussed above, phlorotannin levels can be highly variable within different species and due to seasonal variations. But to what extent influences the habitat phlorotannin levels? Phlorotannins are known to differ with different habitats (Hay and Fenical 1988, Hay 1996). Phlorotannin contents were higher in algae from grazer- and nutrient-rich sites (Stiger et al. 2004) or differed with collection sites (Plouguerné et al. 2006). An increase in phlorotannin content in nutrient-rich waters was additionally shown by Arnold et al. (1995) and Peckol et al. (1996). Spores developed in the fertile tissue of their parental algae seem to reflect the physiological stage and fitness, maturity and adaptations of the parental alga as discussed in Publ. II and III. Parental algae of the same species collected at different sites would therefore very likely “transfer” their adaptations to their habitat reflected *inter alia* in the phlorotannin content to their reproductive stages (“transfer”-hypothesis). Comparing intra- and extracellular phlorotannin levels of *S. latissima* spores (Publ. I and III), collected at

different sites in Kongsfjorden, revealed that intra- and extracellular phlorotannin levels were doubled and almost quadrupled at Hansneset compared to the Old Pier. This would support the "transfer"-hypothesis in the investigated species of the order Laminariales, Tilopteridales and Alariales. Another explanation might be derived from the underwater radiation regimes at the collection sites Old Pier and Hansneset. During Arctic Summer the collection site Old Pier is characterized by a high sediment load of the incoming glacier melt water. More UVR and photosynthetically active radiation would therfore be attenuated by the sediment-loaded water masses at Old Pier compared to the collection site Hansneset. As a consequence, algae at Old Pier would be low light adapted and very likely express less photo-protective substances as e.g. phlorotannins than their high light exposed representatives at Hansneset.

Additionally, different developmental stages exhibit species specific levels of phlorotannin contents. While phlorotannin contents in juveniles of *E. menziesii, Fucus gardneri, H. sessile* and *Lessoniopsis littoralis* were higher relative to concentrations of adult species, phlorotannin concentrations in *Nereocystis luetkeana* were higher in adult specimen (Van Alstyne et al. 2001). Studies of Stiger et al. (2004) observed less phenolic compounds in juveniles compared to adult stages. Within zoospores, phlorotannins are located in physodes randomly distributed throughout the cell (Publ. II and II) while phlorotannins in adult algae are located in the cell walls (Schoenwaelder and Clayton 1998) and the cytoplasma (Schoenwaelder and Clayton 1999) as well as in the outer epidermal cell layer, cortical cells and in the innermost medullary tissue (Lüder and Clayton 2004).

Due to the small cell size of spores, intracellular phlorotannin contents are rather small. In our studies, changes in intracellular phlorotannin content could not be detected during development from spores into juvenile gametophytes in *S. latissima* (Publ. I) and increased in *A. esculenta* (Publ. IV). More phlorotannins are exuded from the juvenile gamtophytes in both species to the surrounding medium than integrated into the cells themselves leading to the conclusion that either phlorotannins might play a major role in external rather than in internal cell protection or that intracellular phlorotannin contents might have been diminished immediately after formation.

4.3.3. Phlorotannin expression in relation to radiation tolerance and exposure time, Reactive oxygen species and vertical zonation?

Studies so far dealing with radiation effects on juvenile developmental stages have either used PAR only (Cie and Edwards 2008), low PAR+UVR (summarized in Roleda et al. 2007; Müller et al. 2008, 2009) or intermediate PAR+UVR (Hoffman et al. 2003; Wiencke et al. 2006). In these studies, either exposure times were too short to detect changes in phlorotannin content (Müller et al. 2009). Bischof et al. (1999) observed an acclimation difference in *A. esculenta* sporophytes upon low and high light exposure and with or without additional UVR.

Effects of increased UVR were not similar to photoinhibitory effects of high light suggesting that UVR might stimulate photoprotective mechanisms by blue light but also cause chloroplast displacement (Bischof et al. 1999). Moreover, an increase in recovery rate was more pronounced upon low PAR and UVR exposure than upon exposure to high PAR (Bischof et al. 1999) as can be supported in the present study by observations of germination pattern in several brown algal spores.

Therefore Publ. IV displayed the first direct comparison of low vs high PAR effects in combination with effects of UV-A and UV-B radiation on Arctic macroalgal juveniles over an exposure period of 20 days (Publ. IV) observing total intra- and extracellular phlorotannin levels. In comparison to other studies, our results support the theory of non-short-term induction of phlorotannins (Müller et al. 2009) and variable phlorotannin levels with species (Publ. III) and radiation conditions (Publ. I, II, III, IV).

Pavia et al. (1997) exposed *A. nodosum* sporophytes to significantly higher radiation conditions with 0.6 W m^{-2} UV-B for 2 weeks compared to our study leading to a~ 30 % increase in mean phlorotannin concentrations. Similar results were obtained by Henry and Van Alstyne (2004) in *F. gardneri* and by *S. muticum* (Plouguerné et al. 2006) suggesting high photo-protective activity of phlorotannins. Additionnally, studies of Abdala-Diaz et al. (2005) suggest that the rapid synthesis and and turnover time of phenolic compounds in the brown alga *Cystoseira tamariscifolia* might serve as photo-protective mechanisms against high irradiances. Nevertheless, an increase in intracellular phlorotannin levels in the present studies was only detectable after 5-10 days exposure within UVR and non-UVR treated juvenile gametophytes *A. esculenta* (Publ. IV) while low PAR only treatment showed no effects on *S. latissima* juveniles. Instead, phlorotannins were exuded into the surrounding medium with

significantly increasing levels over time (Publ. I and IV) and significantly lower levels among UV-B exposure (Publ. IV).

To understand interactive effects of radiation, antioxidants (phlorotannins) and lipids better, reactive oxygen species (ROS) in juveniles of *L. digitata* and *S. latissima* were studied. Investigations showed plastoglobuli both in almost intact and disintegrated chloroplasts of the investigated species (Publ. VI). A general morphogenic relationship between chloroplasts and plastoglobuli was proposed by Tivini and Steinmüller (1985) indicating that maximum plastoglobuli enrichment was strongly correlated with a minimum in thylakoid development. Holzinger et al. (2004) and Steinhoff et al. (2008) reported plastoglobuli within the chloroplasts of red and brown algae after exposure to UV-B radiation. Ultrastructural observations (Publ. VI) lead to the assumption that upon UVR exposure the photosystem (PS) II within the chloroplasts might be damaged caused by a degradation of the D1 protein (Asada 1994) and a hindered repair of damage of PS II (Nishyama et al. 2001). The xantophyll-cycle seems to be another target for UV-B radiation (Bischof et al. 2002) impairing the response to high radiation stress by thermal energy dissipation and would lead to formation of ROS within photosynthesis resulting in photooxidation of the photosynthetic apparatus (Bischof et al. 2002 references therein). Additional cellular injury by ROS caused the peroxidation of lipids (Lesser 2005) leading to less MUFA and PUFA content within the spore/gametophytes cells (Publ. I and IV). Nevertheless, it has to be considered that ROS may function as second messengers leading to apoptosis after irreversible cell damage (Johnson et al. 1996) or the expression of transcriptional factors (Lesser 2005) as a response to change in environmental conditions. Ahn et al. (2007) investigated antioxidant activities by comet assay for protecting effects of phlorotannins against H_2O_2-mediated DNA damage. Their results show that the investigated phlorotannins phloroglucinol, eckol and dieckol in *Ecklonia cava* have potential radical scavenging activities. In addition, protective effects of the phlorotannins against H_2O_2-mediated DNA damage increased with increased concentrations in the samples (Ahn et al. 2007). Meng et al. (1989) concluded that polyphenols in general undergo progressive polymerization *in vivo* producing high molecular weight, non-dialyzable polyphloroglucinols (Ragan 1976) which are readily oxidized (Crato 1892) to form low molecular weight phlorotannins (Koch et al. 1980).

Summarizing investigated observations of plastoglobuli formation within the chloroplasts (Publ. IV), lipid peroxidation (Publ. I and IV), ROS formation and phlorotannin levels lead to the conclusion that phlorotannins might be able to “buffer” ROS formation to some

extent by their antioxidant activity as mycosporine-like amino acids (Karentz et al. 1991a; Kim et al. 2001) explaining the decrease in external phlorotannin levels. However, only little is known about the effect of externally generated ROS on the growth and health of aquatic organisms (Kieber et al. 2003). Internal phlorotannin levels seemed to have remained stable but it can be assumed that internally produced phlorotannins are oxidized immediately leading to no or only minor changes in intracellular phlorotannin content.

As mentioned earlier, discrepancies in radiation effects on phlorotannins could probably be addressed to different species, developmental stages and adaptations of the investigated algae leading to difficulties in interpreting results clearly. In Publ. III, we therefore tried to exclude factors known to affect phlorotannins by collecting various algal species at the same site and within the same seasonal period. Hence, observations in parental algal collection depth and intra- and extracellular phlorotannin levels of their spores (Fig. 14) were correlated to the vertical zonation pattern of macroalgae at Kongsfjorden (Wiencke et al. 2004; Roleda et al. 2006) based on different sensitivities to UVR (Bischof et al. 2006; Zacher 2007).

Species from the upper sublittoral are exposed to higher irradiances of photosynthetically active radiation and UVR than their representatives in deeper habitats. We could observe that upper littoral species as *A. esculenta* and *S. dermatodea* released their zoospores with more phlorotannins than species of the lower sublittoral as *L. digitata* and *S. latissima.* In addition, spores of *S. latissima* exhibited less germinated spores after exposure to high PAR than spores of *A. esculenta* while differences in germination between species under enhanced UV-A+UV-B exposure were only minor. Consequently, it can be suggested that sensitivity towards visible and UVR of various macroalgal spore species is determined by the radiation tolerance of their parental tissue.

4.3.4. Phlorotannins - protective compounds for recruits?

Combining results of the present study and literature data, it can be assumed that phlorotannins are highly variable due to inter- and intraspecific variations, developmental stage, habitat and sensitivities to biotic and abiotic factors. Likewise are the multiple roles and tasks of phlorotannins within different species and their developmental stages very likely to be determined by interaction of a variety of biotic and abiotic factors (Pavia et al. 1997).

Phlorotannins among the developmental stage of brown algal spores and juvenile gametophytes seemed to play a pronounced role in external protection against UVR by absorbing in the UV wavelength range and by their antioxidant activity upon cell damage (Publ. III, IV). Phlorotannins were released by the parental algae upon spore release and exuded by juvenile gametophytes in our experiments after 5 days (Publ. I and IV). In contrast, internal phlorotannin levels upon release seemed rather too small to have any effective protection mechanisms on short term-scales (Publ. IV).

Due to the fact that we still lack information about the biosynthesis of phlorotannins, attempts to monitor and resolve phlorotannin synthesis at genetic and enzymatic levels are pressing (Amsler and Fairhead 2006; Koivikko 2008) and would shed additional light on their roles and protective functions.

4.4. Fatty acid composition, variable or reliable metabolic parameter?

Production of phenolic compounds certainly results in a cost to the algae leading to increased metabolic or nutrient demands and decreasing growth rates (Hay and Fenical 1988; Steinberg 1995; Stiger et al. 2004). However, costs are difficult to detect and to quantify (Pavia and Åberg 1996). Here, determination of total FA and FA composition came into play:

In Publication I, a possible linkage between the fatty acid 18:1(n-9) and phlorotannin formation was suggested for the first time based on the high availability of the FA 18:1(n-9) and the information that phlorotannins might be formed via the acetate-malonate pathway (poly-ketide pathway) involving a polyketide synthase-type enzyme complex (Arnold and Targett 2002). The sensitivity of FA towards environmental parameters as nutrients (Hessen et al. 1997 references therein), temperature (Floreto et al. 1993) and radiation (Dennis and Shibamoto 1990 references therein; Hessen et al. 1997) made the determination of total FA and separation into SAFA, MUFA and PUFA a reliable parameter for reconstructing interactions with secondary metabolites as phlorotannins as well as understanding radiation effects on spore metabolism, growth and development in the present study (Publ. I and IV). Never-theless, intra- and interspecific variability (Jamieson and Reid 1972; Banaimoon 1992; Fleurence et al. 1994; Graeve et al. 2002) as well as differences of FA within different developmental stages (compare Reed et al. 1999) have to be considered and evaluated in terms of morphology, habitat and adaptation to environmental factors.

4.4.1. Fatty acid composition in relation to species

Investigations about algal lipids obtained in recent years suggest a relation between chemical composition of algae and their taxonomy (Khotimchenko et al. 1990, references therein). However, studies of FA in Phaeophyta have not been systematic so far (Khotimchenko 1998). Fatty acids among algae are known to be very complex due to a greater range of fatty acids present compared to higher plants (Jamieson and Reid 1972).

Despite the fact that macroalgae are known to differ in their fatty acid composition due to latitudinal or habitat-specific adaptations (Khotimchenko and Vaskovsky 1990; Banaimoon 1992; Fleurence et al. 1994; Graeve et al. 2002) and due to growth conditions and abiotic factors as temperature and radiation (Becker et al. 2010), our results confirm that there is either a wide intraspecific range within lipid composition (see Reed et al. 1999, *Macrocystis pyrifera*) or a large difference within different developmental stages of the same species. These observations particularly apply to the fatty acids 16:0, 18:1(n-9), 18:1(n-7), 20:4(n-3) and 20:5(n-3) which considerably differed in their proportions. Often, spores and gametophytes contained much lower concentrations of the FA 20:5(n-3) than their adult representatives.

In spores of *S. latissima* and *A. esculenta*, we observed the occurrence of equal fatty acids in both species but in a different sequence pattern. While the first two major fatty acids were equal in both species, the sequence of the other FA listed was different (Publ. I and IV). *S. latissima* spores FA concentration and composition was comparable to data of *Laminaria hyperborea* spores (Graeve and Bartsch 2002, unpubl. data). Studies on lipid composition of sporophytes of *S. latissima* (Jamieson and Reid 1972) and *Laminaria solidungula* (Graeve et al. 2002) corroborate the occurrence of 18:1(n-9), 16:0 and 18:2(n-6) as the major fatty acids in the present study. However, fatty acids 16:0, 18:1(n-9), 18:1(n-7), 20:4(n-3) and 20:5(n-3) considerably differed in their proportions.

Contrary to the findings of Jamieson and Reid (1972) and Graeve et al. (2002), we did not observe a dominance of the fatty acids 18:4(n-3) and 20:5(n-3) in *S. latissima* but could observe an increase in these two FA during the development of *A. esculenta* to become dominant FA in juvenile gametophytes of *A. esculenta* (Publ. IV). As shown by Becker et al. (2010), ratios of polyunsaturated and saturated fatty acids in algae respond to environmental factors as a change in temperature. To obtain reliable data and to minimize uncertainties on

change of FA related to environmental factors, an appropriate number of experimental individuals among one species together with comparable starting conditions have to be considered.

4.4.2. Fatty acid composition in relation to sensitivity to UVR or/and high PAR

Fatty acid profiles are known to change under UVR exposure by an increase in lipid peroxidation (Malanga and Puntarulo 1995) leading to an increase in short-chained fatty acids and a decrease in poly-unsaturated fatty acids (Dennis and Shibamoto 1990, Wang and Chai 1994, Hessen et al. 1997). In contrast, visible light (PAR) influences primarily the content of saturated and polyunsaturated fatty acids (Khotimchenko and Yakovleva 2004 references therein). In Publ. IV, a comparable analysis of low vs high PAR in relation to UVR effects was carried out for the first time on early life stages of *A. esculenta*. While we could observe high FA content among all low PAR controls (Fig. 16a), there were significant trends to lower total FA contents from the low PAR controls to the related PA and PAB treatments. The effect of visible light as suggested by Khotimchenko and Yakovleva (2004) were observed in the present study (Pub. IV), where saturated fatty acids of 16:0 and 18:0 increased in all treatments while especially the polyunsaturated fatty acid 18:2(n-6) decreased. Total FA concentration decreased under high PAR exposure about 50-75% of the initial content after 15 days in all treatments. The impact of UV-B radiation leading to a decrease in fatty acids (Goes et al. 1994; Wang and Chai 1994) could only be observed under low PAR exposure. Consequently, high PAR seems to have more impact on total FA and FA composition than high energetic short wavebands of UV-B. Results suggest that interactive effects of high PAR and UV-B on *A. esculenta* juveniles might enhance FA peroxidation (Kramer et al. 1991) leading to feed back stress responses as formation of ROS (Bischof et al. 2002) and antioxidants (e.g. phlorotannin).

4.5. Short- and mid- term radiation and temperature effects on brown algal propagules

Earlier studies on photobiology have shown that on a cellular level UVR for instance inactivates DNA and mRNA (Harm 1980; Karentz et al. 1991), affects enzymes (Döhler 1985; Lesser 1996), formation of ROS by impairment of photosynthesis (Bischof et al. 2000; Lesser 2005), membrane lipids (Poppe et al. 2002, 2003; Lesser 2005) and lipids (Publ. IV), affects nuclei (Steinhoff et al. 2008) and inhibits cell division (Calkins and Thordardottir 1980). By

analyzing dimeric photoproducts as cyclobutandimers and pyrimidins (CPD) within the DNA (Harm 1980; van de Poll et al. 2001; Wiencke et al. 2000), changes of molecule conformations by absorption of solar UVR could be confirmed (Karentz et al. 1991).

DNA damage caused by solar radiation is a very complex wavelength dependent interplay between intrinsic mutagenicity and lethality (Mitchell and Karentz 1993; Garces and Davilla 1982). Impacts of solar radiation on the cellular level as e.g. inhibition of photosynthesis (Strid et al. 1990; Hanelt 1992; Bischof et al. 1998a) are closely related to effects on a community level as e.g. reduced growth (Calkins and Thordardottir 1980; Ekelund and Björn 1980; Wiencke et al. 2000), hindered release of zoospores (Makarov and Voskoboinikov 2001) and recruitment (reviewed by Franklin and Forster 1997; Aguilera et al. 1999; Wiencke et al. 2000, 2004; Roleda et al. 2006).

Macroalgal spores are able to swim up to 72 h in the water column after release (Reed et al. 1992) and are therefore exposed to abiotic and biotic factors on short- and mid-term scales. Short-term effects in the present study were related to results obtained 8 h after spore release while mid-term effects described effects investigated 5 until 20 days after spore release.

Results of the present study reveal that spores of different species are differentially susceptible to temperature, UVR and high PAR effects. Parental efforts and conditioning seems to play a major role by “transferring” UVR tolerances to their reproductive stages (Publ. II and III). By release of phenolic substances as phlorotannins so called UV-refugia (Swanson and Druehl 2002) are created to protect spores from harmful radiation and to deter grazers. Phlorotannin release seems to be coupled with the vertical zonation pattern of the parental algae (Bischof et al. 2006) by higher amounts of released phlorotannins in upper littoral species compared to species from the lower sublittoral (Publ. III). The hypothesis of a time dependent induction of phlorotannins (Pavia et al. 1997) could be supported by a lack of phlorotannin formation on a short term scale (Publ. II and III) as suggested by Müller et al. (2009) and a significant phlorotannin formation at mid-term scale within juvenile gametophytes of *S. latissima* and *A. esculenta* (Publ. I and IV).

Short-term effects of high PAR on macroalgal propagules illustrated the underestimation of PAR intensities compared to UVR (Publ. II, III, IV). In the present study, high PAR seemed to have a more detrimental impact on spore germination and total FA

content and FA composition than assumed before (Publ. II, III, IV). High PAR exhibited in combination with short waveband UV-B lethal effects (Bischof et al. 2002) and should be considered as inter-active parameter together with elevated temperatures in future studies.

In times of global warming, studies investigating interactive effects of temperature and UVR on macroalgal spores are pressing but scarce (Müller et al. 2008, 2009). The present study reveals that Arctic spores of *A. esculenta, L.digitata, S. dermatodea* and *S. latissima* are able to survive elevated water temperatures of 12°C in combination with UVR and low PAR showing only minor decrease in germination under UV-B compared to controls in *A. esculenta* and *L. digitata* (Publ. II and III) and describes a more positive prediction on Arctic macroalgal reproductive scenarios for the future than investigated by Müller et al. (2008). In contrast, Wiencke et al. (2006) investigated *in situ A. esculenta* and *L. digitata* zoospores in 0.5-4 m water depths in early and late June with no effects in 2-4 m water depths under longer exposure times (24-45 h) but a decrease in germination in the UVR treatments of 0.5-2 m water depths. This leads to the conclusion that at low temperature (as in the *in situ* experiment of Wiencke et al. 2006), solar radiation effects might be better compensated than at enhanced water temperatures indicating the high impact of rising water temperatures on early macro-algal life stages.

Studies on DNA damage under UVR exposure and under enhanced temperatures have given evidence for a high impact of elevated temperatures and oxidative stress as observed in the present studies. Investigations of temperature related UVR-effects on photosynthesis in the green algae *Ulva bulbosa* and *Ulva clathrata* have given evidence for compensating effects of increased temperatures (10 °C) compared to 0 °C (Rautenberger and Bischof 2006). Lesser (2005) developed a three-step model involving (1) an increase in metabolic rates and an increase in cell division, (2) a phase transition of membrane, PS II functional decrease and an increased production of ROS took place and (3) excess ROS production, damage to membranes, proteins and DNA as well as damage to cell adhesion and proteins, rapid decrease in PS II and Rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase) function and as latest cell apoptosis. Comparing our observations in macroalgal spores and juvenile gametophytes under UVR and high PAR exposure as well as under elevated temperatures to the model of Lesser et (2005), we can conclude that short term experiments follow the model to step 2 while mid-term exposure can lead to step 3. Although it has to be noted that studies on short- and mid term scales cannot provide information about physiological adaptations

(Bischof et al. 1999), developmental cell stages as macroalgal spores and juvenile gametophytes can only be observed on short- and mid-term scales.

4.6. Conclusion

Differences in susceptibility of UVR, PAR and temperature lead to a wide range of damage in organisms and were highly variable between life stages, tissues and species (Vincent and Neale 2000). Their response is dependent on many factors: the efficiency of protection and repair strategies, genetical determinations and pre-acclimation state of the cell, intensity and duration of exposure, spectral irradiance, delayed effects and recovery, as well as interactions with other variables and organisms (Vincent and Neale 2000, Bartsch et al. 2008).

The present study investigated interactive effects of UVR, PAR and temperature during the development of freshly released zoospores (Publ. II and III, V and VI) to 20 days juvenile gametophytes (Publ. I and IV) on phlorotannin levels, fatty acid composition, ultrastructure and germination pattern. The main questions addressed in the beginning (chapter 1.4) can be answered as follows.

Occurrence of ROS in Publ. VI was very likely linked to the occurrence of plasto-globuli within the chloroplasts of juvenile gametophytes (Publ. IV), the decrease in total fatty acid content (Publ. IV), the change in fatty acid composition (Publ. IV) and the decrease in internal and external phlorotannin content (Publ. III) in the present study. While plastoglobuli formation within the chloroplasts could indicate a probable damage of the D1 protein in PS II, a decrease in MUFA and PUFA content of spores and juvenile gametophytes could illustrate lipid peroxidation due to ROS formation upon high PAR and UVR exposure. The decrease in external phlorotannin levels might be related to their antioxidant activity to “buffer” ROS as suggested for mycosporine-like amino acids.

Phlorotannins varied with species, habitat, season, collection depth and developmental stage. Phlorotannin contents of spores and their surrounding medium during release reflected physiological stage, fitness, maturity and adaptations to the habitat of their parental algae and could consequently not be regarded as completely independent. Phlorotannin content during release revealed species-specific differences in intracellular phlorotannin levels. Extracellular levels of phlorotannins were related to the vertical zonation pattern of the parental algae with

highest released levels by species of the upper sublittoral and lowest by species of the lower sublittoral supporting the "transfer"-hypothesis of parental adaptations to their recruits. Nevertheless, during development, intracellular phlorotannin levels were rather small and remained stable (*S. latissima,* Publ. I) or increased only slightly (*A. esculenta*, Publ. IV). In contrast, extracellular phlorotannin levels increased significantly in both species leading to the conclusion that phlorotannins might play a major protective role by UVR-absorption processes outside the cell and by antioxidation in internal cell protection.

An induction via temperature, UVR or PAR could not be supported on short term scales but was shown on mid-term scales of 5-10 days. Sensitivity of phlorotannins to temperature and especially to high PAR could be shown leading to a decrease in internal and external phlorotannin levels while enhanced UVR resulted in no effects on phlorotannin levels.

Fatty acid content and composition was shown to vary with species and developmental stage as well as under low/high PAR and UVR exposure. Under low PAR, MUFA and PUFA were oxidized upon UV-A and UV-B exposure and total FA content decreased dramatically compared to their low light controls. Under high PAR, total FA decreased 50-75% in all treatments illustrating the underestimated effect of high PAR on early developmental stages.

Additionally, it was suggested that storage lipids in spores might fuel phlorotannin synthesis by degrading FA via β-oxidation to Acetyl-CoA.

Consequently, the hypothesis that sensitivity towards visible and UVR of various macroalgal species is determined by their radiation tolerance of their parental tissue, can be supported. Effects of UVR, PAR and water temperature are reflected from the cellular to the species level. As still only few data are available on interactive effects of PAR, enhanced UVR and temperatures, predictions of future consequences for algal recruitment and survival as well as community structures are difficult. While enhanced UVR in combination with low PAR in the laboratory was not affecting algal propagules, field experiments under high PAR showed detrimental effects leading to a decrease of germination. So far, most studies have focused on UVR effects, but the present study showed that high PAR was underestimated and might play a more pronounced role than expected. Nevertheless, different radiation ratios of PAR:UV-A:UV-B applied in the laboratory and in the field experiment should be considered and might lead to different results.

Ozone depletion might be of greater importance to smaller organisms (Karentz et al. 1991; Buma et al. 2000) such as spores and bacteria which are less protected from UVR da-mage, especially in marine environments where concentrations of chromophoric dissolved organic matter (CDOM) are low and UVR transparency is high (Williamson and Zagarese 2003). As suggested by Wiencke et al. (2006), stratospheric ozone depletion over the Arctic in combination with enhanced temperatures might certainly influence the viability of zoospores and hence the macroalgal zonation of Arctic and cold temperate species on Spitsbergen.

4.7. Future perspectives

Although laboratory experiments give the unique opportunity to control environmental factors, they also show artificiality to some extent. Future studies should therefore try to run experiments under more natural conditions, preferably as field experiments, mimicking natural radiation ratios, attenuation and *in situ* temperatures. Nevertheless, field experiments with zoospores are scarce and difficult to conduct (Anderson and North 1966; Reed 1990; Reed et al. 1997; Wiencke et al. 2006). In particular, the circumstances of the pheromone and seasonal based collective release of zoospores and the parental protective mechanisms *in situ* would make an interesting point of future research. So far, several studies have investigated UVR-effects on macroalgae and their developmental stages but especially studies on high PAR effects could shed light on macroalgal recruitment, adaptations and survival in the future.

A screening of UV-protective compounds as phlorotannins in combination with storage compounds as lipids within different species and developmental stages among different habitats and seasons would lead to a comparative approach and help to understand high variability in both compounds. Additional gene expression analysis would reveal adaptation pattern and pathways leading to a broad understanding of the interaction between damage and repair mechanisms.

Qualitative phlorotannin analysis is a highly discussed topic and the first steps to determine phlorotannins among Laminariales quantitatively have been successfully under-taken by Kangasaho and Krock (2010, unpublished data). However, ideas about the factors influencing phlorotannin oxidation (Steinhoff et al. in preparation) and about the metabolic pathway of phlorotannins are still in their infancies and should be considered in future studies

in combination with seminal methods as membrane inlet mass spectrometry MIMS (Rost and Steinhoff 2009, unpublished data).

In the Arctic, macroalgae grow and become mature under challenging conditions. Nonetheless, the mechanisms behind are only investigated basically. Long term monitoring studies are therefore pressing especially in winter where ice coverage and abrasion, low light and nutrient availability and low temperatures demand exceptional survival strategies.

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Lipid content and fatty acid biosynthesis in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential fuel for phlorotannin production

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Lipid content and fatty acid biosynthesis in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential fuel for phlorotannin production

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Abstract

Phlorotannins are considered *inter alia* to act protective to a variety of stressors while lipids in spores are known to fuel various metabolic processes during spore release and settlement. Here, phlorotannin production in zoospores/ juvenile gametophytes in relation to lipid metabolism was investigated for the first time in order to study related metabolic costs. The experiment was carried out in NyÅlesund (Svalbard, Arctic) within the development from spores to juvenile gametophytes of the brown alga *Saccharina latissima* over 20 days. In the release stage, the total phlorotannin content of single zoospores was 1.5 x 10^{-7} µg and 1.9 x 10^{-7} µg in the surrounding medium. Upon release, the total amount of lipids was 1.76 x 10^{-5} µg lipid zoospore^{-1} containing the major fatty acids 16:0 and 18:0, 18:1(n-9), 18:2 (n-6), 18:3(n-3), 20:4(n-6) and 20:5(n-3). During development from spores to gametophytes, a decrease in fatty acids was observed via electron microscopy and a decrease of the fatty acid 18:1(n-9) from 45 to 30 % was measured by gas chromatography in particular. While phlorotannin content within the spores remained stable, phlorotannins accumulated in the surrounding medium of gametophytes to 4.0 x 10^{-7} µg phlorotannins spore^{-1} indicating exudation processes. Results obtained support the key and multifunctional role of lipids in zoospore/gametophyte metabolism and may indicate that gametophytes of *S. latissima* need approximately 10 days to switch to photo-autotrophic strategies, becoming independent of storage lipids. In addition, fatty acids might represent an essential energy source to fuel adaptive responses.

Keywords

Macroalgae, Fatty acid, Phlorotannin, Zoospore, Gametophyte, Ultrastructure

Introduction

The planktonic zoospores represent a sensitive stage in the life-history of brown algae. Upon release, zoospores with a diameter of only 4 µm are exposed to several external factors as e.g. UV radiation and temperature. Before settling, zoospores are able to actively move more than 30 hours (Reed et al. 1992, 1999). In the release stage, most zoospores among the kelp family are equipped with only one or two chloroplasts (Henry and Cole 1982; Loiseaux 1973; Steinhoff et al. 2008) with a photosynthetic capacity too low (Amsler and Neushul 1991) to supply the whole organism with energy for movement, settlement, growth and the synthesis of various metabolic products. Thus, it is likely that neutral lipids of the zoospores (major storage product) serve to fuel these processes (Bartsch et al. 2008). With respect to stratospheric ozone depletion, increasing water temperatures and changing coastal environments, it is important to elucidate acclimation and protective mechanisms of zoospores and estimate energy demands related to adaptive responses and survival. It has been shown that lipid content and composition seem to be highly affected by environmental conditions as by various irradiances (e.g. Sukenik et al. 1990) and low temperatures (Somerville 1995). Although lipids of unicellular algae have been studied extensively (Hitchcock and Nichols 1971; Roughan and Slack 1982; Schlapfer and Eichenberger 1983), hitherto only the adult stages of brown algae have been investigated (e.g. Jamieson and Reid 1972; Harwood 1984; Araki 1987; Jones and Harwood 1992; Gerwick et al. 1993; Graeve et al. 2002).

Another natural product and important secondary metabolite group of brown algae are the phlorotannins. These are polyphenolic substances composed of the monomer phloroglucinol (Ragan and Glombitza 1986). Phlorotannins are proposed to have various cellular functions and induction triggers e.g. a strengthening role in cell walls (Schoenwaelder and Clayton 1998), as protecting substances against short wave UV radiation (Pavia et al.

1997; Targett and Arnold 1998), as herbivore deterrents (Steinberg 1985, 1992) and as substances involved in adhesion processes (Waterman and Mole 1994).

However, there is no information available on the energy demands for phlorotannin synthesis and so far no studies consider lipids as a potential fuel for phlorotannin production during zoospore development into gametophytes. Since the early considerations of phlorotannin metabolism via the acetate-malonate-pathway (e.g. Ragan and Glombitza 1986; Targett and Arnold 1998) there is an uncertainty about the formation of phlorotannins and its relation to lipid consumption from zoospores to young gametophytes. This situation has stimulated us to settle the hypotheses (I) lipid quality and quantity in *S. latissima* gametophytes will change over time and, (II) this pattern will be related to phlorotannin synthesis and induction in the first weeks of gametophyte development.

Material and Methods

Fertile sporophytes of *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl and G.W. Saunders were collected from 4-6 m water depth by SCUBA diving in Kongsfjorden (Ny Ålesund, Spitsbergen, Norway) in late June 2006 and early July 2008. To obtain zoospores, the fertile tissue (sorus) of four individuals was cleaned and kept at 7°C in a moist and dark chamber according to Wiencke et al. (2006). After 1.5 days, the fertile tissue released zoospores upon immersion in 0.2 µm filtered seawater at temperatures 5 °C above the temperature the fertile tissue was stored. Zoospore suspensions from four individuals were mixed and zoospore density was adjusted with 0.2 µm filtered seawater to 28×10^5 for electron microscopy (EM), and to 12×10^5 spores ml^{-1} for lipid and phlorotannin analysis (counted with Neubauer chamber “improved", Brand, Germany). The zoospore suspension was equally distributed in glass Petri-dishes obtaining three replicates per treatment and sampling day for phlorotannin (40 ml) and lipid analysis (10 ml) and two for each sampling

day for EM analysis (12 ml). Petri-dishes containing zoospores were then exposed to 30 µmol photons m^{-2} s^{-1} photosynthetic active radiation (PAR) provided by three white light fluorescent tubes (36 W true light® II Powertwist, USA) and samples were taken after 0, 5, 10, 15 and 20 days. Samples for lipid analysis (10 ml) were filtered on GF/C Filters (Whatman, Maidstone, UK, precombusted at 450 °C for 4 h), covered with chloroform/methanol (2:1 v/v, Merck, Darmstadt, Germany) and frozen at -80 °C before gas chromatographic analysis.

Transmission electron microscopy (TEM)

Samples for transmission electron microscopy were processed after Steinhoff et al. (2008). Pre-fixation was done with 4% glutaraldehyde (w/v), 2% paraformaldehyde and post-fixation with 1% osmium tetroxide (v/v). After rinsing, samples were firstly embedded in agar and later in SPURR's resin. Subsequent to the polymerisation process, sections were cut using an ultramicrotome (EM UC 6, Leica, Wetzlar, Germany) and examined in an EM 109 electron microscope (Zeiss, Oberkochen, Germany) with a slow-scan CCD camera (Proscan, Lagerlechfeld, Germany) and adjusted with Adobe Photoshop CS3.

Phlorotannins

For quantitative phlorotannin analysis, Petri dishes containing spore solution (40 ml each) were filtered on GF/C filters and frozen in liquid nitrogen. After sample transport, filters were defrosted and extracted five times with 2 ml 7:3 acetone/water (analysis grade, Merck, Darmstadt, Germany). Afterwards, the spore-extracts were combined, freeze-dried and re-dissolved in 1 ml MilliQ water. Accordingly, the surrounding medium of the spores (filtrate) obtained during filtration of the spore solution was freeze-dried and re-dissolved in 1 ml MilliQ water. Subsequently, total phlorotannin content of spores and filtrate was measured after Waterman and Mole (1994) and Koivikko et al. (2005) applying the Folin-Ciocalteu

method (Folin-Ciocalteu solution and Sodium carbonate, Merck, Darmstadt, Germany) using phloroglucinol (Sigma-Aldrich, St. Louis, USA) as a standard agent.

Fatty acid analysis

For fatty acid analyses, filters were homogenized and extracted in dichloromethane: methanol (2:1, v/v) following the method described by Folch et al. (1957). Prior to extraction, an internal standard was added (19:0 FAME). For gas liquid chromatography of fatty acids, methyl esters were prepared from aliquots of the extracted zoospores/gametophytes by transesterification with 3% sulfuric acid in absolute methanol for 4 h at 80°C. After extraction with hexane, fatty acid methylesters (FAME) were analyzed with a gas liquid chromatograph (HP 6890, Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m x 0.25 mm I.D.; film thickness: 0.25 µm; liquid phase: DB-FFAP, J&W, Cologne, Germany) using temperature programming (Kattner and Fricke 1986). FAMEs were identified by comparison with known standard mixtures. If necessary, identification of FAMEs was confirmed by gas chromatography-mass spectrometry (GC-MS) measurements. Total lipid concentration referred to the sum of total fatty acid methyl esters.

Statistics

Statistic analysis was made with R (version 2.8.1.), by testing the data set for normal distribution with a Shapiro-Wilk test (Sokal & Rohlf 1995), a 2-way ANOVA and by applying a POST HOC test (Tukey-HSD) of pair-wised comparison to test differences among treatments. Significant differences in fatty acid composition in the course of the experiment were identified by one-way ANOVA. Homogeneity of variances was tested with Levene´s test and normality of residuals with the Shapiro-Wilk test (Sokal and Rohlf 1995). Differences among treatments were tested with a PostHoc test (Tukey-HSD test) and Kruskal-Walis test.

Results

Ultrastructure of germinating zoospores and lipid bodies

Fig. 1a shows a zoospore of *S. latissima* 8 h after release. The spore consisted of one nucleus (N) with nucleolus, one to two chloroplasts (Cp), several black stained lipid vacuoles (L), mitochondria, Golgi bodies, vesicles and other cell compartments. Phlorotannin containing physodes (P, Fig 1a) are located adjacent to the cell walls. The structure of the lipid bodies 8 h after zoospore release (Fig. 1b) and in young gametophytes after 10 days development (Fig. 1c) differed. Right after release the lipid bodies had a globular shape, whereas later on the lipid bodies appeared "corroded" at the margins while the center of the lipid body seems to be unaffected. These results we interpret as consumption of storage lipids. Fig. 1d shows a scheme of this process. Within 10 days, a considerable amount of the entire lipid has been consumed.

Phlorotannin content in developing gametophytes

Within the exposure period of 20 days, the mean phlorotannin content of juvenile gametophytes ranged from 1.23±0.16 to 1.53±0.03 x 10^{-7} µg phlorotannin zoospore/gametophyte^{-1} (Fig. 2), leading to averaged phlorotannin contents of 1.42±0.08 x 10^{-7} µg phlorotannin zoospore/gametophyte^{-1}µg phlorotannin per zoospore or young gametophyte. In the filtrate (Fig. 2) and accordingly in the surrounding media of the zoospores/gametophytes, the phlorotannin content increased from 1.95±0.87 to 3.88±0.17 µg x 10^{-7} phlorotannin zoospore/gametophyte^{-1}. Statistical analyses showed significant differences of phlorotannin values in the filtrate between day-0 and day-15 as well as between day-0 and day-20, where the phlorotannin content had increased significantly with time. In contrast, we could not detect any statistical differences in phlorotannin content per zoospore or gametophyte, respectively (Fig. 2).

Lipid content in developing gametophytes

The initial lipid content of a single zoospore of *S. latissima* (Table 1) was 1.76 x 10^{-5} µg lipid spore^{-1} directly after release. After 10 days, the concentration decreased to 1.25 x 10^{-5} µg lipid spore^{-1}. Thereafter the lipid content increased to 1.38 x 10^{-5} µg spore^{-1} and 1.84 x 10^{-5} µg lipid spore^{-1}, on days 15 and 20, respectively.

Major fatty acids of zoospores at the start of the experiment were: the saturated fatty acids 16:0 and 18:0, the monounsaturated fatty acid 18:1(n-9) and the polyunsaturated fatty acids 18:2 (n-6), 18:3(n-3), 20:4(n-6) and 20:5(n-3), which accounted for about 90% of overall fatty acid content (Table 2). Minor fatty acids were 14:0, 16:1(n-7), 18:4(n-3), 20:3(n-6) and 20:4(n-3), together accounting for 7.7 % of total fatty acids. The major fatty acids showed statistically significant variations in their proportions compared to the initial in the course of the experiment (Table1). The most dominant fatty acid 18:1(n-9) decreased from 44.9 to 29.9 % after 20 days ($p=0.019$) whereas 14:0 and 18:2(n-6) significantly increased ($p= 0.16$ and 0.11) after an initial period of 5 days after the start of the experiment. Their proportions were about 1.3, 2.7 and 2.6% higher, respectively, on the final day of the experiment. The polyunsaturated fatty acids 18:4(n-3), 20:4(n-6) and 20:5(n-3) presented a considerable proportion comprising 10.5 % of the total fatty acids after zoospore release. Their proportions increased significantly to 16.2 % after 15 days of exposure, with a statistically difference of the median (Kruskal Wallis test) found for 18:4(n-3) ($p= 0.24$) and 20:5(n-3) ($p= 0.037$). The proportion of the fatty acid 18:3(n-3) showed, compared to the initial, also a tendency to increase, but the difference was found not to be statistically significant. Only trace amounts of 16:1(n-7), 20:3(n-6) and 20:4(n-3) were detected showing a slight increase from 0.5 to 1.5% and 0.2 to 0.8% for 16:1(n-7) and 20:4(n-3), respectively.

Discussion

The intention of this study was to monitor changes in the phlorotannin content of *S. latissima* zoospores/gametophytes and in their surrounding medium, and to relate these to metabolic

processes estimated as lipid consumption. It was determined for the first time phlorotannin exudation and lipid quality and quantity during development from zoospores to gametophytes over 20 days. We assume that phlorotannin synthesis might be related to consumption processes of the fatty acid 18:1(n-9) within the lipid metabolism of kelp gametophytes.

A summary of the fatty acid composition of various brown algae is outlined in Table 2 to show the variation in fatty acid composition with species or developmental stage. Our results follow the studies on *Laminaria hyperborea* zoospores, both for lipid concentration and composition (Graeve and Bartsch 2002, unpubl. data). Studies on lipid composition of sporophytes of *S. latissima* (Jamieson and Reid 1972) and *Laminaria solidungula* (Graeve et al. 2002) corroborate the occurrence of 18:1(n-9), 16:0 and 18:2(n-6) as the major fatty acids. Despite the fact that macroalgae are known to differ in their fatty acid composition due to latitudinal or habitat-specific adaptations (Khotimchenko and Vaskovsky 1990; Banaimoon 1992; Fleurence et al. 1994; Graeve et al. 2002), our results confirm that there is either a wide intraspecific range within the lipid composition (see Reed et al. 1999, *Macrocystis pyrifera*) or a large difference within different developmental stages of the same species. These observations particularly apply to the fatty acids 16:0, 18:1(n-9), 18:1(n-7), 20:4(n-3) and 20:5(n-3) which considerably differed in their proportions. Graeve et al. (2002) found similar results in *L. solidungula;* with age the unsaturated fatty acid 18:4(n-3) decreased whereas 20:5(n-3) increased (Table 1). In the brown alga *Desmarestia muelleri,* gametophytes contained much lower concentrations of the fatty acid 20:5(n-3) as their older sporophytes (13.2 and 22.8%, respectively). This agrees with our observation in *S. latissima* where zoospores contain only 4.7 %, gametophytes 5.3 % and adult sporophytes 28.4 % of 20:5(n-3) (Jamieson and Reid 1972). Contrary to the findings of Jamieson and Reid (1972) and Graeve et al. (2002), we did not observe a dominance of the fatty acids 18:4(n-3) and 20:5(n-3).

Evidently, after release zoospores actively move for up to more than 30 h and rely on internal energy stores to sustain swimming activity and, thus, begin to consume their lipids (Reed et al. 1992, 1999). Our results indicate that the preferred initial energy source is 18:1(n-1). Within 20 days, this fatty acid decreased by one third of the initial value and may either be used as metabolic energy source or as a precursor for other lipid compounds. The overall lipid content of a single zoospore showed a pronounced decrease until 10 days after release, marking the lowest lipid content measured in this study (Table 1). These findings can be correlated with the EM observations of the lipid body (Fig. 1). Thereafter an increase in the lipid content was observed. These results support hypothesis (I) that the metabolism of young gametophytes is supported by remobilization of stored lipids, especially 18:1(n-9). Later on, the lipid content in young gametophytes increases again, but exhibits a different composition of lipids compared to the first 10 days. Although photosynthesis in zoospores may function directly after release (*Pterygophora californica* and *Macrocystis pyrifera* zoospores, Reed et al. 1992), it does not seem sufficient to maintain the entire spore metabolism. Small differences in lipid reserves composition and consumption within the three investigated zoospore species of Laminariales might therefore reflect differences in zoospores swimming behaviour, photosynthetic efficiency (Reed et al. 1999) and different adaptations to their habitat. Consequently, these results may indicate that zoospores/young gametophytes need approximately 10 days to switch to photo-autotrophic strategies, becoming independent of storage lipids.

As mentioned above phlorotannins are substances with various functions induced by various factors. To explain the phlorotannin production in our study, we will discuss the most probable function/induction processes in more detail.

Due to their bitter tannin taste, phlorotannins are discussed to act as herbivore deterrents (e.g. Hammerstrom et al. 1998) released to prevent possible grazers from ingestion. Here

phlorotannins were released in the absence of grazers. A second potential factor inducing phlorotannins is UV radiation. Phlorotannins are able to absorb in the UV wavelength range and have been discussed to create UV-refugia through exudation processes (Swanson and Druehl 2002). But in our study, zoospores and early gametophytes were only exposed to dim white light. We can therefore support the hypotheses of Henry and Van Alstyne (2004) that "phlorotannin production in embryonic or juvenile stages (in *Fucus gardneri*) is not induced by UV radiation or takes more than three weeks to occur". Phlorotannins are also considered to have a cross-linking role in cell wall strengthening (Schoenwaelder and Clayton 1998). Surprisingly, we could not detect a significant increase of phlorotannin content related to growth inside the spores/ gametophytes as observed by Henry and Van Alstyne (2004) during maturation. This might be due to fairly low intracellular concentrations of phlorotannins which are difficult to extract within the early life stages. Another potential function of phlorotannins is their role in settlement. Phlorotannins are thought to be involved in the adhesion process of zoospores. During the settlement process, phlorotannins are able to form insoluble complexes with proteins and excreted polysaccharides (Waterman and Mole 1994), get adsorbed in the structure of the macro-molecules and being oxidized (H. Pavia, personal communication). Because the Petri-dishes used for exposure were fairly small in size, spores sank and became attached to the glass bottom within the first hours. Thus, the significant phlorotannin excretion observed 10 days after release cannot be linked to a settlement process. However, in addition to their various protective mechanisms, we wish to consider one last explanation in more detail – the stress response. Phlorotannins were induced by absence of the major triggers UV radiation and grazing. Consequently, the only possible explanation for phlorotannin production in our study might be their involvement in other stress responses. The experiment was conducted under low PAR conditions (30 µmol photons m^{-2} s^{-1}), low nutrient concentrations (no enriched medium) and a lack of CO_2 (no aeration).

The combination of these three factors could have lead to a multiple stress response resulting in phlorotannin exudation and should be investigated in more detail.

To what extent lipid consumption and phlorotannin synthesis are related, still remains a matter of debate. Phlorotannins are believed to be formed through the acetate-malonate pathway (Ragan and Glombitza 1986; Waterman and Mole 1994; Arnold and Targett1998). This polyketide secondary metabolite synthesis is based on a two-carbon unit attached to the Coenzyme A (Acetyl-CoA). By polymerization of the acetate units, linear or cyclic molecules are formed. The 18:1(n-9) fatty acid that exhibited a remarkably high amount in *S. latissima* zoospores is one of the most important fatty acid degraded to Acetyl-CoA via β-oxidation (e.g. Harwood 1988), and hence would be an ideal candidate as precursor for phlorotannin production. Currently it is not known if the phlorotannins synthesis from recycled Acetyl-CoA reserves might be controlled by seaweeds or not (Arnold and Targett 1998). In addition, it is unknown if some of the fatty acids of the zoospore/gametophyte storage lipids are used for phlorotannin production and/or maintaining life dependent metabolic functions, or simply produced as a net carbon sink (Arnold and Targett 1998). However, the decrease in 18:1(n-9) fatty acid is correlated with the increase in phlorotannin production and accordingly to phlorotannin exudation (Fig. 3). Consequently, we support hypothesis (II) and suggest that the building blocks used for phlorotannin synthesis might be derived from the storage lipids of the zoospores/gametophytes.

Because of their multitude of vital cellular functions, phlorotannins might play an important role in the early developmental stages. A further characterization of their metabolic pathways in combination with lipid synthesis and consumption under various abiotic factors will shed light on the crucial question of energy demands for developmental but also adaptive processes.

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Figures and tables

Table 1

Lipid content (sum of total fatty acids) and fatty acid composition (*10^{-7} µg and mass % of total fatty acids) of *Saccharina latissima* during the development from zoospores to young gametophytes. (* indicates statistically significant difference compared to the initial day-0 ;Kruskal-Wallis test)

Days	0		5		10		15		20	
Fatty acid per spore (µg x 10^{-7})	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	6.4	4.5	4.9*	0.6	4.5*	0.6	5.9*	2.2	8.6*	0.5
16:0	34.1	21.8	27.5	3.0	24.6	3.9	27.8	10.2	39.0	2.8
16:1(n-7)	0.1	0.6	4.1*	0.5	3.5*	0.2	3.4*	1.3	2.6*	0.7
18:0	9.8	9.0	4.9*	0.2	3.4*	0.4	7.6*	7.5	10.7*	5.6
18:1(n-9)	78.1	43.1	61.7*	8.7	45.4*	6.1	43.4*	14.0	55.0*	4.0
18:2(n-6)	17.4	12.8	13.5	1.8	12.1	1.6	15.0	6.6	21.1	1.6
18:3(n-3)	8.8	5.4	9.6	1.3	8.6	1.0	9.2	2.8	11.1	2.3
18:4(n-3)	3.8	2.5	5.9*	0.7	5.5*	0.5	6.8*	1.5	6.4*	2.3
20:3(n-6)	2.3	1.4	1.7*	0.2	1.5*	0.2	1.5*	0.5	1.6*	0.3
20:4(n-6)	6.4	4.1	5.7	0.8	4.6	0.5	5.0	1.5	5.6	1.2
20:4(n-3)	0.6	0.8	0.3	0.5	1.0	0.0	1.1	0.4	1.3	0.4
20:5(n-3)	8.2	5.0	10.1*	1.3	8.8*	0.7	10.0*	2.9	9.6*	2.9
Total	176.0	111.0	149.9	19.6	123.5	15.7	136.7	51.4	172.6	24.6

Days	0		5		10		15		20	
Fatty acid (%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0*	3.6	0.2	3.2*	0.1	3.6	0.2	4.3*	0.1	4.9*	0.2
16:0	19.5	0.4	18.2	0.4	19.6	0.7	20.0	0.7	22.2	1.0
16:1(n-7)*	0.5	0.0	2.7*	0.2	2.9*	0.3	2.5*	0.4	1.5*	0.4
18:0*	5.0	1.8	3.3*	0.6	2.7*	0.3	4.8*	3.3	6.2*	3.3
18:1(n-9)*	44.9	2.3	40.7*	1.1	36.2*	0.5	31.6*	2.2	31.3*	2.2
18:2(n-6)	9.5	1.0	8.9	0.1	9.7	0.1	10.5	1.3	12.1	1.4
18:3(n-3)	5.0	0.2	6.3	0.1	6.9	0.1	6.8	0.8	6.2	0.8
18:4(n-3)*	2.1	0.0	3.9*	0.2	4.4*	0.1	5.1*	0.8	3.6*	1.0
20:3(n-6)*	1.3	0.1	1.1*	0.1	1.2*	0.1	1.1*	0.1	0.9*	0.1
20:4(n-6)	3.7	0.1	3.7	0.1	3.7	0.1	3.7	0.3	3.2	0.4
20:4(n-3)	0.2	0.3	0.2	0.4	0.8	0.1	0.9	0.0	0.8	0.2
20:5(n-3)*	4.7	0.3	6.7*	0.3	7.1*	0.3	7.4*	0.7	5.4*	1.1

Table 2

Comparison of fatty acids composition in 19 *Phaeophyceae, L. solidungula* sporophytes*, L. hyperborea* zoospores*, S. latissima* sporophytes, zoospores and early gametophytes. Values are weight % of total fatty acids. ns= not specified, * both isomers

Species	***Fatty acids average composition in 19 Phaeophyceae*** [1]	***L. solidungula*** [2]	***L. hyperborea*** [3]	***L. saccharina (=S.latissima)*** [4]	***L. saccharina (=S. latissima)*** [5]	***S. latissima***	***S. latissima***
Developmental stage	ns	young Sporophytes	Zoospores	Sporophytes	Sporophytes	Zoospores Day 0	Gametophytes Day 20
Fatty acid (%)							
14:0	6.5	5.3	1.5	4.0	10.4	3.6	4.9
16:0	14.6	12.4	25.9	10.2	22.9	19.5	22.1
16:1 (n-7)	ns	0.9	0.7	ns	<0.1	0.5	1.5
16:4 (n-3)	ns	ns	ns	0.1	<0.1	ns	ns
18:0	ns	0	2.8	ns	1.0	5.0	6.1
18:1 (n-9)	*18.7	5.3	32.8	8.1	19.3	44.9	30.3
18:1 (n-7)		0	0	ns	0.3	0	1.0
18:2 (n-6)	8.1	7.3	7.7	3.9	6.8	9.5	12.1
18:3 (n-6)	*7.6	ns	0	0.6	1.2	0	0
18:3 (n-3)		9.8	5.7	9.9	3.6	5.0	6.2
18:4 (n-3)	10.8	26.8	4.9	20.3	8.5	2.1	3.6
20:3 (n-6)	ns	ns	ns	ns	0.6	1.3	0.9
20:4 (n-6)	*12.2	10.8	4.5	9.2	13.7	3.7	3.1
20:4 (n-3)		1.1	1.3	ns	0.3	0.2	0.8
20:5 (n-3)	13.7	19.3	10.1	28.4	6.2	4.7	5.4

[1] Pohl and Zurheide, 1979

[2] Graeve et al., 2002

[3] Graeve and Bartsch, 2002, unpubl. data

[4] Jamieson and Reid, 1972

[5] Fleurence et al., 1994

Fig. 1 (a) *Saccharina latissima* zoospore 8 h after release. Cp-chloroplast, L-lipid body, N-nucleus, P-physode containing phlorotannin. (b) Intact lipid body 8 h after release. (c) Lipid body after 10 days development. (d) Schematic drawing of the lipid consumption process. L indicates the lipid.

Fig. 2 Sum of phlorotannin content within zoospores/gametophytes and filtrates in µg x 10^{-7} phlorotannins zoospore/gametophyte^{-1} of *S. latissima* over the exposure period of 20 days. Within the filtrate, significant differences in phlorotannin content were observed between day-0 and day-15 and day-0 and day-20.

Fig. 3 Variation in content of fatty acid 18:1(n-9) and exuded phlorotannins per zoospore/gametophyte over time. The decrease in fatty acid 18:1(n-9) correlated with an increase in phlorotannin production from day-0 to day-20 per zoospore.

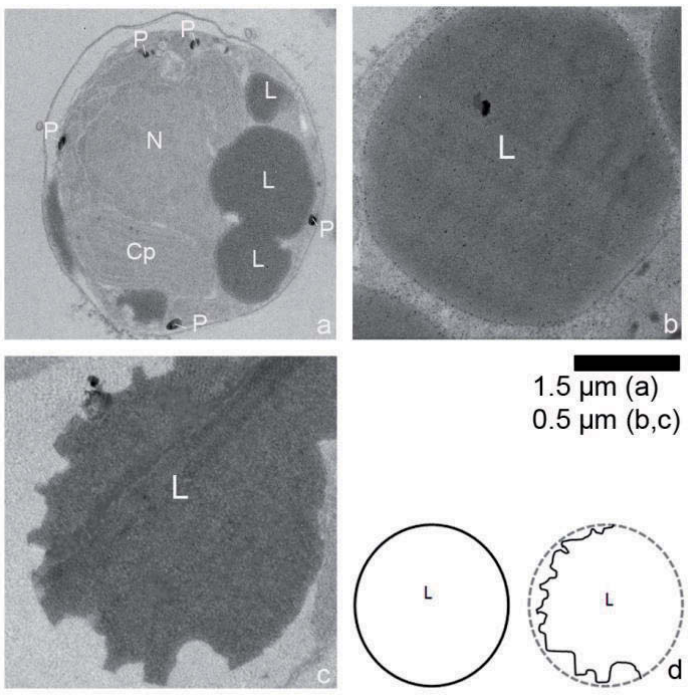

Fig 1

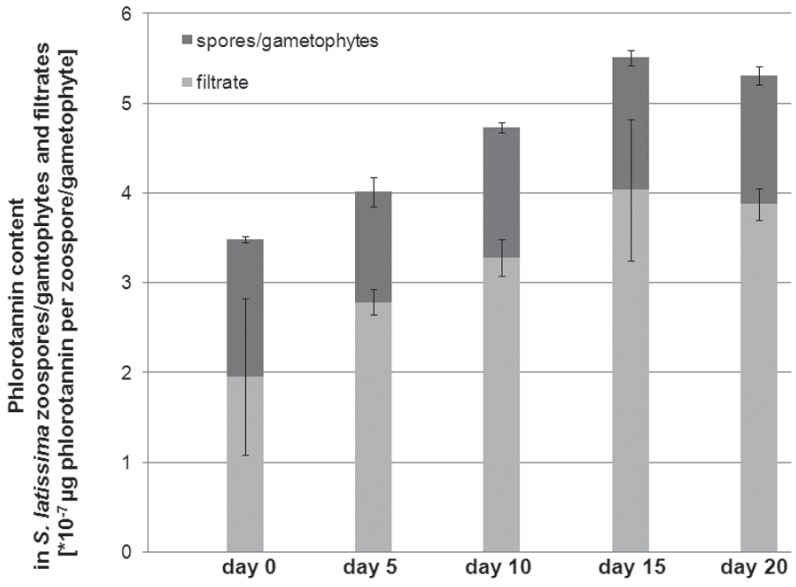
Phlorotannin content
in S. latissima zoospores/gamtophytes and filtrates
[*10-7 µg phlorotannin per zoospore/gametophyte]
spores/gametophytes
filtrate
0
1
2
3
4
5
6
day 0
day 5
day 10
day 15
day 20

Fig 2

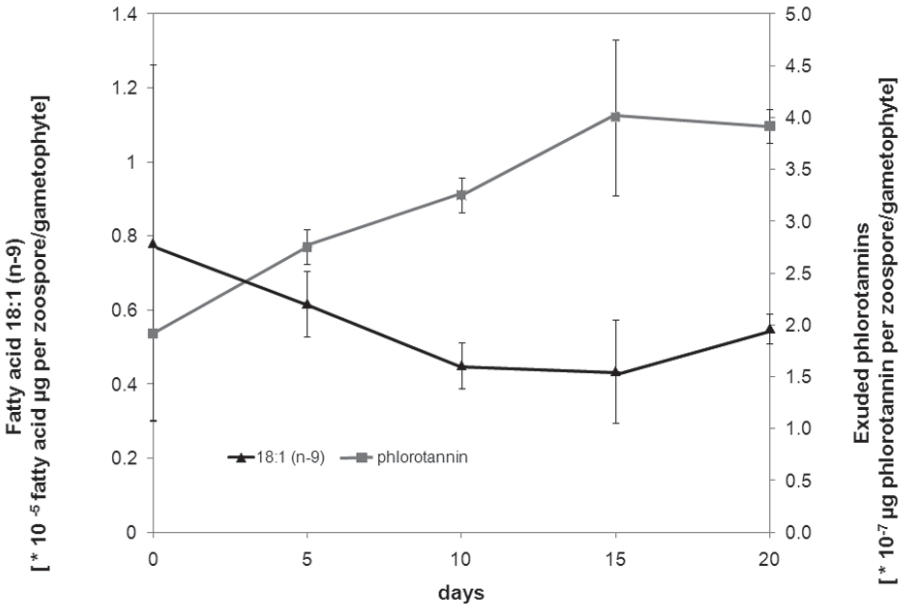
Fatty acid 18:1 (n-9)
[* 10 -5 fatty acid µg per zoospore/gametophyte]
Exuded phlorotannins
[* 10-7 µg phlorotannin per zoospore/gametophyte]
18:1 (n-9)
phlorotannin
days
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
5
10
15
20

Fig 3

Publication II

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Phycologia

Effects of water temperatures, UV radiation and low versus high PAR on phlorotannin content and germination in zoospores of *Saccorhiza dermatodea* (Tilopteridales, Phaeophyceae)

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Christian Wiencke

Sigrid Wuttke

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Effects of water temperatures, UV radiation and low versus high PAR on phlorotannin content and germination in zoospores of *Saccorhiza dermatodea* (Tilopteridales, Phaeophyceae)

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Running title

Phlorotannins in *S. dermatodea*

Key words

Germination, phlorotannins, brown algae, temperature, UV radiation, zoospores

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Abstract

Global climate change will have multiple effects on our environment and might especially change marine coastal ecosystems and their communities due to rising water temperatures and changing light regimes in the water column. Due to the key role of zoospores in the life-history cycle of kelps, we investigated how zoospores of the shallow water species *Saccorhiza dermatodea* (B. de la Pylaie) J. E. Areschoug respond to interactive effects of changing water temperatures, low/high photosynthetically active radiation (PAR) and UV radiation (UVR). Zoospores were examined with respect to germination pattern (germination rates, light micrographs) and phlorotannin content (Folin-Ciocalteu method). In summer 2007, we exposed S. dermatodea zoospores, obtained from fertile thalli in Kongsfjorden (Svalbard, Norway), to low PAR laboratory (UVR:PAR [W m^{-2}] 5.7:4.6; 2-17°C) and high PAR field conditions (UVR:PAR [W m^{-2}] 4.9:86.7; 7°C) for 8 h. Under low PAR+UVR conditions and at 2-12°C, almost 98% of zoospores germinated in all light treatments. The germination rate was reduced under UV-A+UV-B only in the 17°C and 7°C field treatment, to 21.4% and 9.6% respectively. Light microscopic investigations showed differences in germling appearance, growth and pigmentation in the field treatment and at 17°C low PAR conditions, compared to all other treatments, after an exposure time of 8 h. However, phlorotannin content within zoospores remained unaffected by changing UV and low/high PAR conditions. These results suggest that combined effects of present water temperatures, high PAR and UVR can exhibit similar effects on germination as the combined effects of unnatural high temperatures (17°C), low PAR and UVR. With respect to global climate change, the ecological implications of the present study illustrate that zoospores of *S. dermatodea* are able to survive enhanced water temperatures up to 12°C. Moreover, high PAR in combination with UVR might potentially affect zoospores of S. dermatodea stronger than expected in clear waters.

Introduction

Global climate change will have multiple effects on our ecosystems. Rising water temperatures due to global warming (Christensen et al. 2007) and a shift in light regime in the water column due to stratospheric ozone depletion (Bodeker & Waugh 2007) might especially change marine coastal ecosystems and their communities.

The vertical zonation pattern of seaweeds in the eu- and sublittoral depends on a species-specific tolerance to a variety of abiotic and biotic factors. In particular, tolerance to UV radiation (UVR) and the capability to sustain high light stress seem to be major factors (Bischof et al. 2006, Roleda et al. 2007, Karsten et al. 2009) affecting zonation patterns. Along the coastline of Spitsbergen, the brown alga *Saccorhiza dermatodea* (B. de la Pylaie) J. E. Areschoug, grows in the upper-sublittoral, often intermixed with Laminaria digitata and Alaria esculenta (Wiencke et al. 2004a).

The spectral underwater radiation climate is of high importance determining e.g. depth distribution (Lüning 1990, Bischof et al. 2006). Jerlov (1976) divided the water bodies into different types based on spectral transmittance measurements made in the upper portions (0-10m) of the ocean. Clear waters are characterized by a transmission window in the blue while more turbid waters have less attenuation in the blue green. Water masses of our study site (Kongsfjorden, Spitsbergen) in spring were classified as Jerlov coastal water type 1 (Hanelt et al. 2001) representing clear water with highest transmittance at approx. 475 nm (Jerlov 1976). In the more turbid waters present in July/August the water type changes to Jerlov's type 9.

S. dermatodea is distributed in Arctic and sub-Arctic regions and extends into cold-temperate areas (Lüning 1990). The northern distribution limits are determined by summer sea surface temperatures of 3-5 °C, i.e. conditions high enough to enable sufficient growth of the sporophyte (Norton 1977, Müller et al. 2009a). The southern boundary is set by the temperature demands for survival and reproduction with lethal limits in the W-Atlantic at the 17 °C summer sea surface isotherm and a reproduction limit in the E-Atlantic at the 6 °C

winter sea surface isotherm (Müller et al. 2009a). S. dermatodea belongs to the order Tilopteridales and has a morphology and life-history similar to that of kelps.

The various life history stages of seaweeds are known to be differentially tolerant to light stress (Dring et al. 1996, Hanelt et al. 1997, Coelho et al. 2000). The most susceptible stages in the life history of kelps and kelp-like species are their micro-stages, in particular their zoospores relative to juvenile sporophytes as reviewed by Roleda et al. (2007). Before settlement, zoospores can remain in the water column for more than 30 hours (Reed et al. 1992, 1999) and are, due to their small size (3-5 μm), more easily penetrated by solar radiation. Seaweeds have therefore developed various strategies to tolerate radiation stress (Bischof et al. 1998a,b): photolyases to repair DNA damages (Pakker et al. 2000, van de Poll et al. 2002) and synthesis of UV-absorbing compounds to protect against UVR. In brown algae, UV-screening pigments, known as phlorotannins, fulfill a multifunctional role as e.g. anti herbivore deterrents (Steinberg 1988, Steinberg & van Altena 1992), cell wall component (Schoenwaelder & Clayton 1998) and UV-protection (Pavia et al. 1997, Karsten et al. 2009). Until recently, only a few studies have investigated the performance of *S. dermatodea* zoospores and juvenile sporophytes. These includes photoperiodic responses (Henry 1987); germination, growth and DNA damage in juvenile sporophytes (Roleda et al. 2005a), and kinetics of photoinhibition and recovery of photosynthesis (Roleda et al. 2006b). The responses have been related to phenology and depth distribution by Wiencke et al. (2004a, 2006).

So far, our knowledge on the responses to the multiple stress induced by UVR and/or rising temperatures on photosynthesis and germination of zoospores (Hoffman et al. 2003, Véliz et al. 2006, Tala et al. 2007, Müller et al. 2008, Roleda 2009) with respect to global climate changes is limited to a few Arctic and cold temperate kelps. Due to the key role of zoospores in the recruitment of the species, we investigated how zoospores of the kelp-like *S. dermatodea* respond to the simultaneous impacts of enhanced water temperatures, to UVR

and especially to what extent the effects observed are modulated by high and low photosynthetically active radiation (PAR) with respect to the germination process and phlorotannin content.

Materials and Methods

Radiation measurements in the field

In the field, UV-B radiation (290-320 nm) was measured at the primary NDACC (Network for the detection of atmospheric composition change) site operated by the Alfred Wegener Institute at Ny-Ålesund, Svalbard, 78.9°N, 11.9°E. Measurements were conducted by a UV-spectrometer with a 32-multi channel photomultiplier to detect UV-B and an Oriel single monochromator with 256 diode array detector to measure UV-A radiation (320-400 nm) (Wuttke et al. 2007). Spectral irradiances were measured in 5 min intervals. Fig. 1 shows the 5 min dose (units according to Sliney et al. 2007) for August 2nd 2007 with a 50% reduction due to mesh cover of the sensor.

Radiation and temperature experiments

Five fertile individuals of *Saccorhiza dermatodea* (Bachelot de la Pylaie) J. E. Areschoug were collected in Kongsfjorden, Hansneset (Svalbard, Norway, 79° 00'N 012°01'E) by SCUBA divers at the end of July 2007. To obtain zoospores, the fertile tissue (sorus) was cleaned and kept at 7°C in a moist and dark chamber according to Wiencke et al. (2006). After 1.5 days, the fertile tissue released zoospores upon immersion in 0.2 µm filtered seawater at temperatures 5°C above the temperature at which the fertile tissue was stored. Zoospores of all individuals were mixed to obtain a zoospore density of ~1.3 x 10^6 spores ml^{-1} (counted with Neubauer "improved" chamber, Brand, Germany). For germination rate and light microscopy, 5 ml of the zoospore suspension was distributed in three replicates into

small Petri-dishes for all treatments, while for phlorotannin analysis 40 ml of the zoospore suspension were distributed in three replicates into large glass Petri-dishes. Petri-dishes were covered with one of three different cut-off filters allowing the following radiation spectra to pass: only PAR (400 -700 nm, Ultraphan URUV farblos, Digefra, Munich, Germany), PAR and UV-A radiation (PA, 320-700 nm, Folex PR Montage Folie, Dr. Schleussner, Dreieich, Germany) and PAR and UV-A and UV-B radiation (PAB, 290-700 nm, Ultraphan URT 300 foil (Digefra, Munich, Germany).

UVR was generated by three fluorescent tubes (Q-Panel UVA 340, 40 W, Cleveland, OH, USA) and PAR by two daylight fluorescent tubes (36 W true light® II Powertwist, USA). Petri dishes were then exposed for 8 h to the light and temperature conditions in the laboratory (in the following described as low PAR or laboratory) summarized in Tab.1 (measured with a LiCor 250A and a LI-190 Quantum Sensor, Lincoln, NE, USA and a Solar light PMA 2100, Solar light Co., PA, USA). As the sensitivities of the UV-A sensor (PMA 2100) and the UV-B sensor (PMA 2106) overlap, UV-A values were determined by putting an additional glass filter (Schott, Germany) cutting off wavelengths ≤320 nm on top of the sensor. The UV-B values were obtained similarly by subtracting the difference between the readings with and without filter from the readings with filter. This procedure has been applied in numerous parallel studies (e. g. Wiencke et al. 2004b) and is again used here for better comparison. The field experiment was performed 02. August 2007. In this experiment we tried to simulate the clear water light regime of Kongsfjorden in June and of a water depth of 3-5 m (Jerlov coastal water type 1, Hanelt et al. 2001, Brey 2009) by covering Petri-dishes with gauze (50% shielding, Fig. 1). The obtained average radiation conditions during the exposure time of 8 h (described in the following as high PAR or field) are summarized in Tab. 1 (PAR measured in 60 min intervals with a LiCor 250A and a LI-190 Quantum Sensor, Lincoln, NE, USA,). To compare field and laboratory experiments (according to Sliney 2007), mean irradiances and doses of mean UV-A and UV-B radiation under laboratory and field

conditions were calculated over the exposure time of 8 h (Tab. 1). The temperatures were 2, 7, 12 and 17° C in the laboratory experiment and 7° C (measured regularly in the Petri-dishes) in the field experiment.

Germination rate and light microscopy

After exposure, Petri dishes containing samples for observation of the spore germination process were placed under dim white light of 4.6W m^{-2} PAR (21.7±3.8 µmol photons m^{-2} s^{-1}) at the respective temperatures for 6 days. 300 spores per sample were subsequently counted to obtain germination rates. Only spores with a germ tube were considered as germinated. In addition, micrographs of each treatment were taken with an Axiolab microscope and a Plan-Apochromat 63x/1.4 oil objective (Zeiss, Göttingen, Germany).

Phlorotannin measurements

After 8 h exposure, zoospore suspensions were filtered on GF/C Filters to separate spore cells and filtrate, frozen in liquid nitrogen and extracted with 7:3 Acetone/water (analysis grade, Merck, Darmstadt, Germany). The filtrate was freeze-dried and re-dissolved in MilliQ water. Total phlorotannin content of spores and filtrate was measured with the F-C method (Koivikko et al. 2005) by measuring absorbance of supernatants (Folin-Ciocalteu solution and Sodium carbonate, Merck, Darmstadt, Germany). Conversion into concentrations was performed using a standard curve obtained with phloroglucinol (Sigma-Aldrich, St. Louis, USA).

Statistics

Statistical analyses were done with the software “R” (version 2.8.1.) by testing the data set for normal distribution by a Shapiro-Wilk test (Sokal & Rohlf 1995), a 2-way ANOVA and by applying a POST HOC test (Tukey-HSD) of pair-wised comparison to test differences among

treatments. Results were tested firstly if germination is affected by the interactive parameters of temperature and UVR/wavebands (P, PA, PAB) and secondly how irradiance levels (low and high PAR) are affecting germination in interaction with different wavebands (P, PA, PAB) at the same temperature (7 °C). Interactive effects of wavebands (P, PA, PAB) and irradiance levels (low and high PAR) in comparison to the initial phlorotannin content during release were analysed for phlorotannin samples within the zoospores and their filtrates. Significant similarities ($p>0.05$) and differences ($p<0.05$) are indicated by capital letters above the bars in the diagrams (Fig. 2 and Fig. 4).

Results

Germination rate

Under UV-exclusion, over 72% of *S. dermatodea* zoospores germinated in all temperatures in both the laboratory and in the field (Fig. 2). At 7° C, with 98.1% and 98.4% in the low PAR and low PAR+UV-A (PA) treatment, respectively, the highest germination rates were detected. Significantly lower germination rates ($p<0.05$) were observed in the 7° C high PAR (field) treatment compared to the 7°C and 12°C PAR and PA treatments. Here only 82.0% of the spores exposed to PAR and 35.6% of the spores exposed to PAR+UV-A germinated. In the PAR+UV-A+UV-B (PAB) treated samples at 2, 7 and 12°C, we could observe slight, but not significant ($p>0.05$), decreases in germination compared to the PAR and PA treatments (Fig. 2). Only at 17°C and in the 7°C field treatment the germination rate was lowered to 21.4% and 9.6%, respectively. When supplemented with high radiation and approximately comparable UV-A irradiances and lower UV-B irradiances (UVR:PAR= 0.05), high PAR had more detrimental effects on germination capacity than low PAR (UVR:PAR=1.2). Germination rate in the 17°C treatment was significantly different ($p<0.05$) from all other PAR, PA and PAB treatments and the 7°C field treatment from all other PAR and PAB treatments. In the 7° C PA field treatment, no significant differences ($p>0.05$) in germination

rate compared to all other treatments could be detected due to a high standard deviation among the replicates.

The germination process

After six days (Fig. 3), there was no difference in spore development at 2, 7 and 12° C under the three light treatments (P, PA and PAB). All spores had formed a germination tube and the germlings were equal in size (~25-30 μm). When exposed to 17° C PAR and PA, the germlings were shorter with relatively thick germ tubes while under the 17° C PAB condition, spores showed only little signs of germination. Additionally, we observed dark globular pigmented cell structures (probably phlorotannin containing physodes) within the early gametophytes at 2° C PAR and PA, 7° C P, 12° C PAB, at 17 °C PA and at 17° C PAB (Fig. 3 arrows). The germination process in the entire field treatment was different from all other treatments: spores germinated under high PAR (field), however, usually showed thinner germination tubes. In field PA, we observed germinated spores with thin germination tubes in addition to spores that had not germinated, appearing almost destroyed. Barely germinated or "viable" spores were found in the field PAB treatment. Only remains of cell organelles were visible (Fig. 3).

Phlorotannin content in the spores and the surrounding medium

Directly after release, zoospores of S. dermatodea contained 0.25±0.01 μg phlorotannin ml released zoospore solution^{-1} (Fig. 4). After 8 h exposure, no statistically significant differences ($p>0.05$) between the light treatments (P, PA, PAB), the laboratory and field treatment (low and high PAR) or the initial release value within the zoospores were evident (Fig. 4). In contrast, the filtrate measured directly after spore release contained significantly ($p<0.05$) higher amounts of phlorotannins (2.12±0.28 μg phlorotannin ml^{-1}) compared to all

other filtrates in all treatments (light, laboratory and field) after the experiment ranging from 0.82±0.04 to 0.90±0.07 µg phlorotannin mlspore solution^{-1} (Fig. 4).

Discussion

This study addressed for the first time the interactive effects of enhanced water temperatures and UVR on zoospores of the kelp-like species *S. dermatodea*, as well as whether low versus high PAR in combination with UV affects germination pattern and phlorotannin levels in relation to polar environmental change scenarios. During the germination process, we observed a high ability of *S. dermatodea* zoospores to survive germination at temperatures ≤12°C which are predicted to occur until the end of the century (Christensen et al. 2007) in combination with UV-B radiation and low PAR. Under high PAR in combination with UV-B radiation germination decreased to 10%. Phlorotannin content in the surrounding medium decreased by approx. 60% in all treatments 8 h after release. This high oxidation/degradation of phlorotannins might indicate potential extracellular photo-protective mechanisms against harmful UV-B radiation and extracellular oxidation of radicals derived from photo-damage or other metabolic processes. In contrast, intracellular phlorotannin contents of *S. dermatodea* zoospores remained stable upon exposure to high/low PAR and UVR suggesting less vulnerability to photodegradation due to their location within physodes.

Our results on germination rate obtained after six days of incubation under exposure to 21.7 µmol photons m^{-2} s^{-1} PAR (4.6W m^{-2}) differed from germination studies by Roleda et al. (2006c) obtained on the same species after exposure to 8 µmol photons m^{-2} s^{-1} PAR (1.7 W m^{-2}) and at 7°C. In our study, up to 98% of all zoospores germinated, while in the latter study only 23% of the zoospores germinated. Moreover, Roleda et al. (2006c) observed a low germination potential under PAB at 7°C. In their study, only approximately 50% of the spores germinated compared to the control, while in our study almost no difference in germination

pattern was detected under similar conditions. These differences are very likely related to strong seasonal variations in spore vitality caused by different maturation stages of parental algae and environmentally induced acclimation and adaptation processes. Similarly, in sporophytes, different susceptibilities of photosynthesis to UVR depending on the season have previously been observed (Aguilera et al. 2002, Bischof et al. 2002, Brey 2009). The study by Roleda et al. (2006c) was performed between May and June and their experimental individuals were certainly remains from the previous season. Our study, in contrast, was conducted in early August and the material we employed was composed of individuals developed during the actual season, exhibiting a higher vitality, perfectly explaining the results of our laboratory experiments.

In our study, high temperatures (17°C) in combination with low PAR+UV-A+UV-B radiation had strong effects on germination pattern, where numerous cell fragments were visible suggesting irreversible cellular damage and high mortality of zoospores. Similar results were obtained by Müller et al. (2008) who also observed inhibition of germination of zoospores of *Alaria esculenta, Laminaria digitata* and *Saccharina latissima* from Spitsbergen at 18°C. Clearly, temperatures higher than (≥) 12 °C are above the temperatures to be expected on Spitsbergen until the end of this century (Christensen et al. 2007, Müller et al. 2009a). So the upper temperature of 17 °C tested in our study was applied mostly to show the upper temperature limits. The obtained results may, however, also ecologically be important at the southern distributional limit of the species in the West Atlantic, where the temperature of 17 °C is actually limiting the southern distribution of *Saccorhiza dermatodea*. This assumption has to be tested, however, in experiments using specimens from the southern populations.

Light microscopic data of the germination process under low PAR and UVR reflected the results obtained on the germination rate. The occurrence of yellow to greenish and elongated chloroplasts in all treatments was described by Roleda et al. (2006c), while in our

study only non-germinated or delayed zoospores had green chloroplasts. Both in the low PAR (laboratory) and the high PAR (field) treatments (controls), we could observe a change in chloroplast color from green to yellow during germination (micrographs were obtained after 6 d in our study while after 8h and 16 h in Roleda et al. 2006c), indicating that this phenomenon might be related to germination processes (growth and development). Changes in color of the chloroplasts might be related to the accessory pigment composition or to spore density and consequent self-shading and remain open for further investigations. Similar to the results obtained at 17°C, in the high PAR (field) treatment hardly any bulbous tips (see below) were detected, suggesting a delayed or hindered germination, but in this case inhibited by excessive light (Fig. 3).

Germination tubes of kelp zoospores often exhibit a "bulbous structure" at their tip, as shown for *Macrocystis pyrifera* and *A. esculenta* (Pillai et al. 1992, Wiencke et al. 2007). Differences in tube formation might be also related to species-specific ontogeny and unconsidered stress factors. At 17° C and under field conditions (high PAR) actin microfilaments which are primarily responsible for germ tube elongation (Pillai et al. 1992) are most likely irreversibly damaged. Furthermore, microtubules, which are responsible for nuclear division and translocation (Pillai et al. 1992, Huovinen et al. 2000), might also be irreparably harmed. Finally, the replication machinery of the nucleus is most probably affected, especially after exposure to UV-B. This is supported by electron microscopic investigations of the nucleus in the zoospore of *Laminaria hyperborea* (Steinhoff et al. 2008) where nuclei showed a mottled structure after PAB exposure indicating an abnormal condensation of the chromatin.

We could further observe intracellular globular structures in the peripheral protoplasm of the zoospores in 2° C PAR and PA, 7° C PAR, 12° C PAB (not shown), 17° C PA and PAB (Fig. 3), as already reported by Wiencke et al. (2004b) who interpreted these as enlarged physodes due to an enhanced phlorotannin production. This assumption is, however, not

supported in the present study by quantitative phlorotannin measurements (Fig. 4) performed after 8 h of exposure to the different radiation conditions. On the other hand, the induction of phlorotannin production might need longer time scales than 8h (Müller et al. 2008, 2009b; Steinhoff et al. unpublished data) and may show different patterns in different germination stages thereby explaining the differences in phlorotannin measurements to our study. Alternatively, these globular structures could represent an agglomeration of lipid globules which are abundantly present in brown algal spores (Steinhoff et al. 2008). Electron microscopical studies are prompting to solve this question.

Roleda et al. (2006a, 2006c) showed a higher absorption of zoospore suspensions of S. dermatodea and in Arctic kelps in the UV wavelength range after 8 h and 23 h exposure to UV-B, indicating an increase in phlorotannin content. However, in our study we could not detect quantitative differences in phlorotannin content of the zoospores and the filtrate neither between different light (P, PA and PAB) nor different temperature treatments. Similar results were obtained by Müller et al. (2009b) in *S. latissima, A. esculenta* and *L. digitata* under the same exposure time of 8h and similar spore densities compared to our study. Like Müller et al. (2009b) we conclude that the synthesis of cellular phlorotannins may probably need more time than 8 h for induction.

Kelp spores contain only a small amount of phlorotannins (Müller et al. 2009b), and as far as we know, the phlorotannin content in the surrounding medium is much higher. The same applies to spores of *S. dermatodea* (Fig. 4). By releasing zoospores at the same time, parental algae liberate high amounts of phlorotannins into the water column and create micro-environments, the so called “UV refugia” (Swanson & Druehl 2002). Due to the ability of phlorotannins to absorb in the UV wavelength range, UV-protective environments are formed to prevent UV-induced cell damages (Roleda et al. 2006c), an important hypothesis favored also by Müller et al. (2009b). The high phlorotannin concentrations within the filtrate during release demonstrated here support this theory. Due to stable low phlorotannin contents within

the zoospores and the assumption that phlorotannin exudation from the zoospore itself is negligible; it becomes apparent that these UV refugia are a purely parental investment.

Our field experiments differed from results obtained by Wiencke et al. (2006) in an in situ experiment lasting 24-45 h. There, no inhibitory effects of high PAR+UVR were detected in water depths between 0.5-2 m. In contrast, in our study, significant differences in germination rate of spores exposed for 8 h to PAR and PAB were demonstrated. This difference is related to the fact that in our experiment attenuation of the water column could not be simulated perfectly (UV-B:UV-A:PAR; 0.1:5:100). Moreover, the present study was performed in early August but the zoospores were exposed to radiation conditions similar to those typical for the clear water period in June, characterized by the Jerlov coastal water type 1 (Jerlov 1976). The underwater radiation regime in July – August, however, is quite different to that in June due to the inflow of turbid meltwater and the presence of phytoplankton. According to Hanelt et al. (2001) the water body during these conditions is characterized as Jerlov coastal water type 9. Thus, due to decreasing water temperatures and less meltwater discharge in August, turbid meltwater is not distributed equally in the water column but rather as “clouds” carrying attenuating particles. In the absence of these turbidity clouds, clear water stages can occur and would allow high PAR and UVR to penetrate deeply into the water column even in August. To explain the differences in germination patterns between both studies, we hypothesize further that the UV susceptibility of zoospores from *S. dermatodea* changes seasonally similar as the UV susceptibility of photosynthesis in juvenile/adult macrothalli of S. dermatodea and kelps (Aguilera et al. 2002, Roleda et al. 2005b, Brey 2009). Exposure of spores matured in July to the radiation conditions present during the clear water period in June may confer an unbearable stress to the spores and result in the observed strong inhibition of germination. In support of this argumentation, Swanson & Druehl (2000) proposed that meiospores originating from a high UVR environment are more UV tolerant compared to spores from a low UV environment. This might explain the differential

sensitivities of *S. dermatodea* spores towards high PAR and UVR which are reflected in different germination patterns observed in June (Wiencke et al. 2006) and in the present study in August.

We could neither measure differences in phlorotannin content between high and low PAR treatments nor detect any quantitative differences between different light quality treatments (P, PA and PAB), similar as in zoospores of *S. latissima, A. esculenta* and *L. digitata* from Spitsbergen, (Müller et al. 2009b). Nonetheless, an increased phlorotannin production might have occurred under high light conditions, which could not be measured due to high oxidation/degradation rates of phlorotannins during exposure. This is supported by our observations of a ~60% loss of phlorotannins in the filtrate within the first 8 hours (Fig. 4). Swanson & Fahselt (1997) and Swanson & Druehl (2002) proposed that intracellular phlorotannins might be less vulnerable to photodegradation due to their location within physodes leading to stable intracellular phlorotannin contents as obtained in our study. Further investigations should therefore especially focus on phlorotannin oxidation rates under various radiation conditions and their ecological implications.

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Figures and table

Tab. 1 Irradiance [W m^{-2}] and dose [Jm^{-2}] of PAR, UV-A and UV-B radiation during the field and the laboratory experiments (Units according to Sliney et al. 2007). Irradiances are shown as mean irradiance over the exposure period of 8h in the field (under 50% shielding gauze) and in the laboratory. Doses were calculated with mean values over the exposure period of 8 h. Ratios of UV-A:UV-B:PAR are given for better comparison.

	PAR		**UV-A**		**UV-B**	
	Irradiance [W m^{-2}]	**Dose [J m^{-2}]**	**Irradiance [W m^{-2}]**	**Dose [J m^{-2}]**	**Irradiance [W m^{-2}]**	**Dose [J m^{-2}]**
Lab	4.62±0.4	0.13 x 10^6	5.31±0.5	0.15 x 10^6	0.41±0.0	0.11 x10^5
UV-B:UV-A:PAR			8:115:100			
Field (under 50 % gauze)	86.7±19.9	2.40 x 10^6	4.76±2.9	0.13 x 10^6	0.12±0.0	0.03 x 10^5
UV-B:UV-A:PAR			0.1:5:100			

Fig. 1 Diurnal course of atmospheric UV-A and UV-B radiation exposure at Ny-Ålesund on 2 August 2007 (solid lines, grey UV-A, black UV-B) and UV-A and UV-B radiation conditions used in the laboratory (broken dash lines, grey UV-A, black UV-B). Spectral irradiances were measured in 5 min intervals in the field experiments. The 5 min dose is shown after 50% reduction of irradiances by gauze. For a better comparison, 5 min dose of the laboratory conditions are added in dashed lines. Light treatments (UVR and PAR) applied in the field experiment were intended to simulate irradiance in clear water stages in June at water depths of 3-5 m in Kongsfjorden. Exposure time is marked by the grayish background.

Fig. 2 Germination of S. dermatodea zoospores in % under low PAR laboratory conditions (2-17° C, PAR (P), PAR+UV-A (PA), PAR+UV-A+UV-B (PAB)) and high PAR field conditions (7° C, P, PA, PAB). 17° C PAB and field PAB are significantly different ($p<0.05$) from all other treatments. Vertical bars show standard deviations (SD, n=3). Statistical similarities and differences are indicated by the respective capital letters.

Fig. 3 Light microscopy of S. dermatodea zoospores under low PAR laboratory conditions (17° C, PAR (P), PAR+UV-A (PA), PAR+UV-A+UV-B (PAB)) and high PAR field conditions (7° C, P, PA and PAB). Spores are highly affected by high temperatures (17° C) and high PAR and their combined effects (17° C PAB, field PA and PAB). Arrows indicate globular intracellular structures.

Fig. 4 Phlorotannin contents of S. dermatodea as sums of zoospores and their surrounding media (filtrate) after release and after 8 h exposure to low PAR laboratory conditions (7° C, PAR (P), PAR+UV-A (PA), PAR+UV-A+UV-B (PAB)) and high PAR field conditions (7° C, P, PA, PAB). Phlorotannin content within the zoospores and the filtrate remains stable in all treatments. Significant differences in the filtrate were measured between the initial

phlorotannin content and after exposure period of 8h ($p<0.05$). Vertical bars show standard deviations (SD, n=3). Statistical similarities and differences are indicated by capital letters.

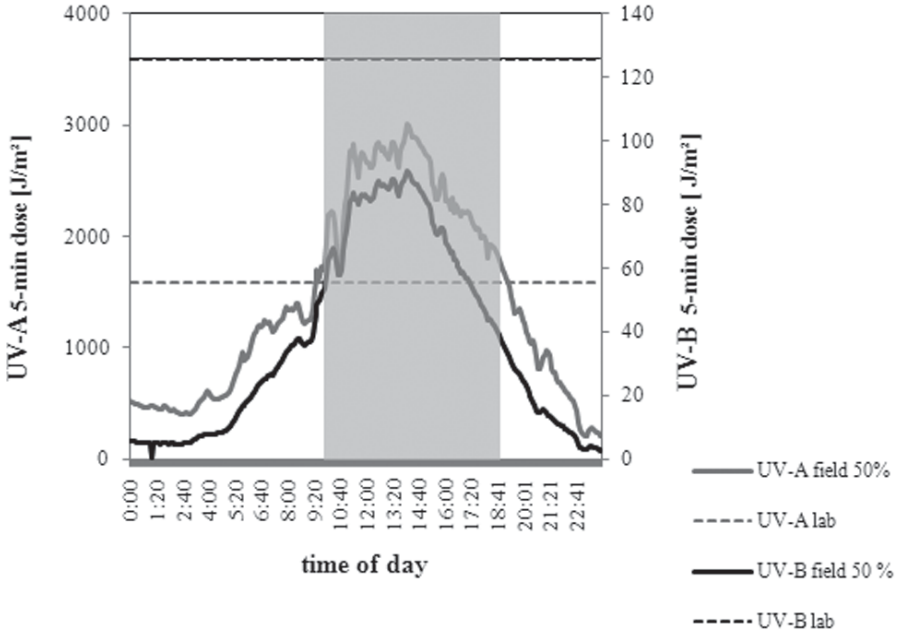

Fig 1

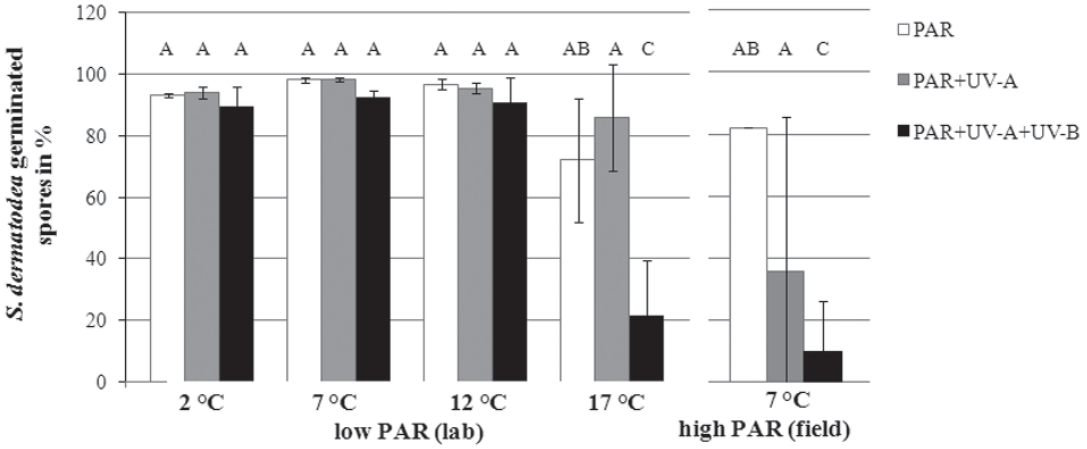

Fig 2

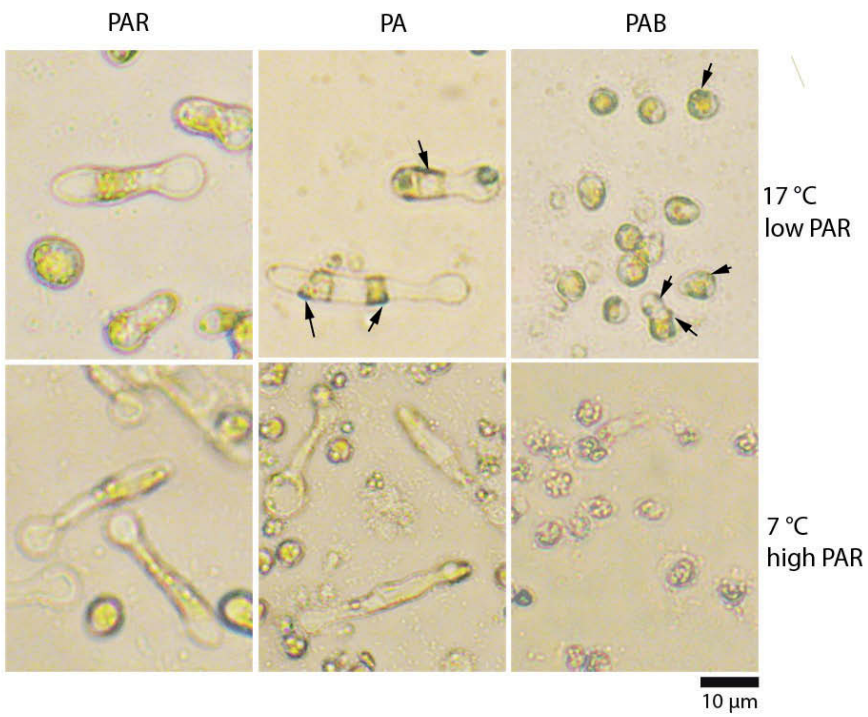
PAR
PA
PAB
17 °C
low PAR
7 °C
high PAR
10 µm

Fig 3

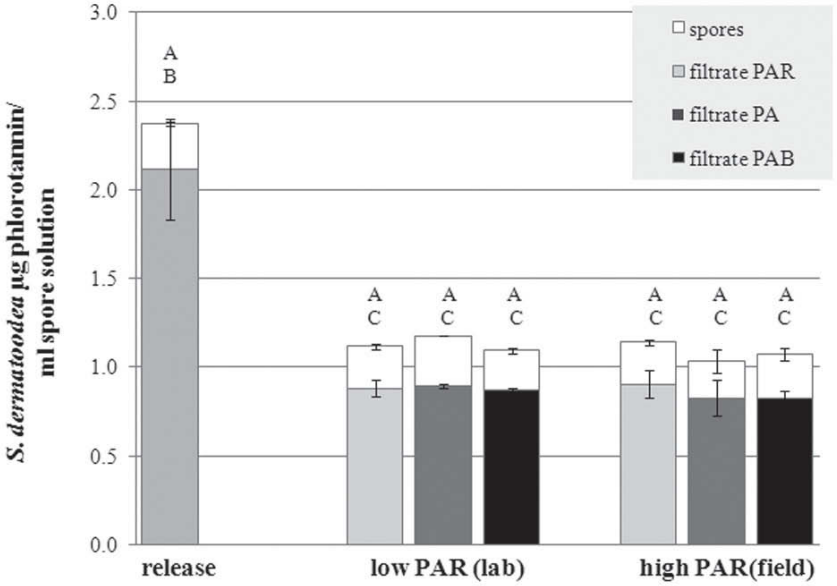
S. dermatoodea µg phlorotannin/ ml spore solution
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
B
A
C
A
C
A
C
A
C
A
C
spores
filtrate PAR
filtrate PA
filtrate PAB
release
low PAR (lab)
high PAR(field)

Fig 4

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Is a changing environment affecting phlorotannin production and germination in Arctic kelp zoospores?

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Running title

Polar kelp zoospores under climate change

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Brown macroalgae, climate change, early life stages, germination, high PAR, kelp zoospores, phlorotannins, Spitsbergen, UV radiation, vertical zonation

Abstract

Global change in Polar regions will lead to changes in the microenvironments of coastal ecosystems affecting especially the sensitive juvenile life-stages of important primary producers including seaweeds. Thus, their reproductive viability and their potential to protect against environmental change are crucial to future ecosystem function. In this study, we exposed kelp zoospores to three global change scenarios to predict consequences for the reproduction and development of three arctic kelps species *Alaria esculenta, Laminaria digitata* and *Saccharina latissima*: (a) various temperatures (2°C, 7°C,12 °C and 17°C) + low PAR (4.9 Wm^{-2}) + low ultraviolet radiation (UV-A 5.4 W m^{-2}, UV-B 0.42 W m^{-2}), (b) present summer temperature (7°C) + low PAR + enhanced UVR (UV-A 9.3 W m^{-2}, UV-B 0.72 W m^{-2}) and (c) present summer temperature+ high PAR (50.3 W m^{-2}) + low UVR. As response variable, phlorotannin content in the spores and the surrounding medium was measured directly after spore release and after 8 h exposure, and germination rate was determined as a general fitness parameter. Results show that (a) zoospores of all species tested sustain water temperatures up to 12°C; (b) upon release, extracellular phlorotannin levels correlated with the vertical zonation pattern and the sensitivity against UVR of the parental algae with highest contents in the filtrates of the upper sublittoral species *A. esculenta.* Enhanced UVR resulted only in *S. latissima* in decreased germination rates while phlorotannin contents in all species remained unaffected. (c) Under exposure to high PAR, phlorotannin content decreased about 60% in the filtrate of *A. esculenta* supporting the high oxidative and consequently very likely cell protecting potential of phlorotannins against photo-induced damage. Global environmental change will consequently have a strong impact on macroalgal spore survivability and distribution dependent on the species specific adaptative and protective potential towards enhanced temperatures, UVR and high PAR.

Introduction

In an era of global climate change, coastal environments will face massive changes. Especially the Polar regions are already and will be dramatically affected by e.g. rising water temperatures and a change in the spectral composition of the sunlight caused by the depletion of the stratospheric ozone layer. For the Arctic, climate simulations as e.g. the A1B scenario predict warming by 4.3 to 11.4°C in winter and between 1.2 to 5.3°C in summer by the end of the century (Christensen *et al.* 2007). Compared to the Antarctic, ozone depletion over the Arctic is much less pronounced but the interannual variability of stratospheric ozone concentration is rather high (Bodeker & Waugh 2007). Due to these factors, changes in ozone are very difficult to detect and chemistry-climate models predict that the first signals of ozone layer recovery in the Arctic are likely to be overseen (Bodeker &Waugh 2007). Global warming and its consequences will lead to changes in the microenvironments of Arctic coastal ecosystems and will especially affect key species and important primary producers such as seaweeds.

There are only few seaweed species endemic to the Arctic region. Most species occurring in the Arctic extend into the temperate regions (Lüning 1985). Northward shifts in northern distribution boundaries of many Arctic species have already been predicted due to the rising water temperatures (Müller *et al.* 2009). Investigated species in the present study were the Arctic cold temperate macroalga *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl et G.W. Saunders, the circumpolar distributed cold temperate species *Alaria esculenta* (Linnaeus) Greville which has the largest reported geographic range of any species in the genus (Widdowson 1971) and the boreal arctic and cold temperate species *Laminaria digitata* (Hudson) J.V. Lamouroux.

Apart from water temperatures, spectral underwater climate is of high importance determining e.g. depth distribution (Bischof *et al*. 2006). After Jerlov (1976), water bodies are divided into different types based on spectral irradiance transmittance measurements made

in the upper water layers (0-10m). The water body of our study site (Kongsfjorden, Spitsbergen) in spring was described as Jerlov coastal water type 1 by Hanelt *et al.* (2001). Coastal water type 1 is characterized by clear water with a transmission window in the blue and highest solar energy transmittance at approx. 475 nm (Jerlov 1976). Under these conditions, high PAR and UV can penetrate deeply into the water column and harm organisms (Hanelt *et al.* 2001). Studies focusing on the interaction of high PAR und UV effects under clear water conditions and potential protective mechanisms are few but necessary (Yakovleva & Titlyanov 2001; Altamirano *et al.* 2004; Wood 1987; Bischof *et al.* 2006; Zacher *et al.* 2007; Cie & Edwards 2008). Considering the fact that especially juvenile gametophytes and sporophytes of kelps are known to be "shade-adapted" due to their position under adult sporophytes (Altamirano *et al.* 2003), high PAR might have strong effects on different developmental stages depending on their thallus structure physiological adaptation and constitution (Altamirano *et al.* 2004).

The polyphenolic phlorotannins, uniquely found in brown algae, are considered to serve among other functions such as antiherbivore deterrents (Amsler & Fairhead 2006) and a strengthening component in cell walls (Schoenwaelder & Clayton 1998) as UV screening substances. Since brown algae are not known to contain other polyphenols (Targett & Arnold 1998; Jormalainen et al 2003), the term phlorotannins is used in the following. The protective mechanism of phlorotannins is debated to be related to their ability to absorb in the UV wavelength range (Pavia *et al.* 1997; Swanson & Druehl 2002; Schoenwaelder *et al.* 2003; Roleda *et al.* 2006a, 2006b; Roleda *et al.* 2007 ; Karsten *et al.* 2009). Absorbance maxima of tissue phlorotannins (260-280 nm) are in the UV-C wavelength range and therefore much shorter than wavelengths algae are exposed to in their natural habitats (Pavia *et al.* 1997; Swanson & Druehl 2002). Nevertheless, the shoulder peak absorbencies of phlorotannins (280-320 nm) observed by Pavia *et al.* (1997) may contribute to the reduction of intracellular exposures of UV-B (Swanson & Druehl 2002).

Although the planktonic zoospores are generally considered to be the most susceptible part of the life-cycle of kelps (Dring *et al.* 1996; Coelho *et al.* 2000; Wiencke *et al.* 2000, 2004, 2006; Roleda *et al.* 2006, 2006a; Véliz et al 2006; Müller *et al.* 2008; Steinhoff *et al.* 2008), most of the seaweed studies so far, have only focused on adult stages. There are studies dealing with kelp zoospores/gametophytes available (e.g. Henry & Cole 1982, 1982a; Reed *et al.* 1992, 1999; Roleda *et al.* 2006, 2006a, 2007, 2009, 2010; Wiencke *et al.* 2000, 2004, 2006; Hoffman *et al.* 2003; Tala *et al.* 2007; Müller *et al.* 2008, 2009, 2009a; Steinhoff *et al.* 2008; Roleda et al 2009, 2010) but research studies on interactive effects of physical stressors as temperature, UV radiation and high PAR are scarce (Hoffman *et al.* 2003).

To understand the effects of temperature and radiation on kelp zoospore survival and distribution pattern, we have drafted three environmental scenarios for three common kelp species growing in 4-10 m water depths on Spitsbergen: (a) enhanced temperatures and low photosynthetic active radiation (PAR) + low UV radiation to distinguish temperature from light effects, (b) present temperature and low PAR + enhanced UV radiation to study impact of enhanced UV radiation due to ozone depletion and (c) present temperature and high PAR + low UV radiation to determine high light effects. These scenarios are addressed by the following hypotheses of this study:

1. Zoospores of the three Arctic kelps *A. esculenta, L. digitata* and *S. latissima* will exhibit species-specific tolerance limits with respect to viability and germination
2. Sensitivity of zoospores against high UV radiation follows a vertical zonation and phlorotannin expression pattern.
3. High PAR causes decreased germination of kelp zoospores and an enhanced production of phlorotannins, especially in shallow water species as *A. esculenta*.

Material and Methods

Radiation measurements in the field

UVB radiation (290-320 nm) was measured in the field at the primary NDACC (Network for the detection of atmospheric composition change) site operated by the Alfred Wegener Institute at Ny-Ålesund, Svalbard, 78.9°N, 11.9°E. UV measurements were conducted using a UV-spectrometer with a 32-multi channel photomultiplier to detect UV-B and an Oriel single monochromator with 256 diode array detector to measure UV-A radiation (320-400 nm) (Wuttke *et al.* 2007). Spectrally resolved irradiances were measured in 5 min intervals.

Radiation and temperature experiments (scenario a-c)

Five fertile individuals of *Alaria esculenta* (Linnaeus) Greville, *Laminaria digitata* (Hudson) J.V. Lamouroux and *Saccharina latissima* (Linnaeus) C.E Lane, C. Mayes, Druehl & G.W. Saunders were collected in Kongsfjorden, Hansneset (Svalbard, Norway, 79° 00'N 012°01'E) and Stuphallet by SCUBA divers between July and August 2007. The fertile tissue (sorus) of one species at a time was cleaned and kept at 7°C for 1.5 days in a moist and dark chamber according to Wiencke *et al.* (2006). Zoospores were released upon immersion in 0.2 µm filtered seawater at temperatures 5°C above the temperature at which the fertile tissue was stored. Zoospore suspensions of all individuals were mixed to obtain a zoospore density of ~$12x10^5$ spores ml^{-1} (counted with Neubauer "improved" chamber, Brand, Germany). For the determination of germination rates, 5 ml of the mixed zoospore suspension was distributed in three replicates into small Petri-dishes for all treatments, while for phlorotannin analysis 40 ml of the zoospore suspension was distributed in three replicates into large glass Petri-dishes. To distinguish UV related effects and controls, Petri-dishes were covered with one of the three different cut-off filters allowing the following radiation ranges to pass: only PAR (400 - 700 nm, Ultraphan URUV farblos, Digefra, Munich, Germany), PAR and UV-A radiation (PA, 320-700 nm, Folex PR Montage Folie , Dr. Schleussner, Dreieich, Germany) and PAR

and UV-A and UV-B radiation (PAB, 220-700 nm, Ultraphan URT 300 foil (Digefra, Munich, Germany).

In the lab, PAR radiation was generated by two daylight fluorescent tubes (36 W true light® II Powertwist, USA) and UV-radiation by three fluorescent tubes (Q-panel UVA 340, 40 W, Cleveland, OH, USA). Petri dishes were then exposed for 8 h to light and temperature conditions in the laboratory (scenario a), under low PAR and high UV radiation (scenario b) as summarized in Tab. 1. PAR was measured with a LiCor, Lincoln, NE, USA and UV with a Solar light PMA 2100, Solar light Co., PA, USA).

In the field experiment (scenario c), we simulated a clear water light regime of June (Jerlov coastal water type I; see Hanelt *et al.* 2001) in 3-5 m water depths of Kongsfjorden (i.e. Brey 2009) ashore by covering Petri-dishes exposed in small tanks with gauze (50% light shielding). Due to changing weather conditions, average light conditions (Tab. 1) during the exposure of 8 h were obtained. PAR was measured in 60 min intervals with a LiCor Quantum meter, LiCor Lincoln, NE, USA). To compare field and laboratory experiments, doses of UV-A / UV-B radiation under laboratory and field conditions were calculated over the exposure time of 8 h (Tab. 1). Additionally, erythemally weighted radiation (UV_{ery}) was calculated according to McKenzie *et al.* (2004) to compare with other studies. The temperatures were 2, 7, 12 and 17°C (scenario a) and 7°C (scenario b) in the laboratory experiments and a constant 7°C (measured regularly in the Petri-dishes) in the field experiments (scenario c).

Determination of germination rates

After experimental exposure, Petri dishes containing samples for observation of spore germination were placed under dim white light of 4.9 W m^{-2} PAR at the respective temperatures for 6 days. 300 spores per sample were subsequently counted. Only spores with a germ tube were considered as germinated.

Phlorotannin measurements

Before and after experimental exposure, zoospore samples for phlorotannin analysis were filtered on GF/C Filters, frozen in liquid nitrogen and extracted with 7:3 Acetone/water (analysis grade, Merck, Darmstadt, Germany). The filtrate was freeze-dried and re-dissolved in MilliQ water. With the F-C method (Koivikko *et al.* 2005), total phlorotannin content within spores and filtrate were obtained by measuring absorbance of supernatants (Folin-Ciocalteu solution and Sodium carbonate, Merck, Darmstadt, Germany). Conversion into concentrations was performed using a standard curve obtained with phloroglucinol (Sigma-Aldrich, St. Louis, USA).

Statistics

Statistical analyses were performed with the freely-available software "R" (version 2.8.1.) by testing the data set for normal distribution with a Shapiro-Wilk test (Sokal & Rohlf 1995). To test differences among treatments, a POST HOC test (Tukey-HSD) of pair-wised comparison was applied.

Results

Phlorotannins

In *A. esculenta*, the initial phlorotannin content in the filtrate (2.52±0.02 µg phlorotannin ml spore solution^{-1}) was significantly higher ($p < 0.05$) compared to all other light and temperature treatments after 8 h exposure (Fig. 1). In contrast, the phlorotannin content within the zoospores of *A. esculenta* did not vary significantly before (0.21±0.02 µg phlorotannin ml spore solution^{-1}) and after exposure (Fig. 1, $p > 0.05$). Significant differences within the light treatments P, PA and PAB were neither observed within the filtrate nor the zoospores ($p > 0.05$; scenario a-c).

Phlorotannin content within the filtrates of *L. digitata* were smaller compared to the filtrates of *A. esculenta*. Merely at the 2 and 12 °C (scenario a) and the high PAR treatment (scenario c), significant differences ($p < 0.05$) to the initial extracellular phlorotannin content of 0.86±0.06 µg phlorotannin ml spore solution^{-1} were observed. Within the zoospores of *L. digitata* (Fig. 2), we could detect significantly lower phlorotannin values ($p < 0.05$) in the temperature treatments of 12°C and 17°C and the high PAR+UV treatment compared to the initial value of 0.21±0.01 µg phlorotannin ml spore solution^{-1} and all other treatments.

In *S. latissima*, phlorotannin contents in the filtrates did not show any significant variation ($p < 0.05$) from the initial value of 0.91±0.03 µg phlorotannin ml spore solution^{-1} (Fig. 3), while within the zoospores (Fig. 3), phlorotannin contents in the temperature treatment of 7°C and 17°C (scenario a) and in the high PAR treatment (scenario c) were significantly lower ($p < 0.05$) compared to initial phlorotannin concentrations and all other treatments. The initial value of 0.34±0.01 µg phlorotannin ml spore solution^{-1} within the zoospores of *S. latissima* was significantly higher versus initial phlorotannin contents of *A. esculenta* and *L. digitata* zoospores ($p < 0.05$; Fig. 4). Filtrates of all investigated species showed higher phlorotannin contents per spore with lower sampling depth ($p < 0.05$; Fig. 4).

Germination rates

Zoospores of *A. esculenta* showed significantly lower ($p<0.05$) germination rates upon PAB exposure (PAR+UV-A+UV-B) in all simulated scenarios (Fig. 1) compared to their controls (PAR) and all PA treatments (PAR+UV-A). At 2°C, significantly ($p<0.05$) less spores of *A. esculenta* germinated in all radiation treatments compared to temperature treatments of 7°C, 12°C and 17°C (scenario a). At 17°C, within the PAB treatment, only 9 % spores germinated.

Additionally, zoospore germination of *A. esculenta* was highly influenced by high PAR (scenario c), where germination dropped significantly ($p < 0.05$) to 31% in the PAR

treatment, 20% in the PA and only 5% in the PAB treatment compared to the respective low PAR treatments at 7°C.

As mentioned already for *A. esculenta* spores, significant lower germination rates within the spectral treatments of PAB compared to PAR and PA could also be observed in *L. digitata* in all temperature treatments of 2-17°C ($p < 0.05$; Fig. 2). In contrast to *A. esculenta*, 57-72% of the *L. digitata* zoospores germinated in the controls (PAR) and 62-68% in the PA treatments of 2°C as wells as in 7°C and 12°C (Fig. 2). At 17 °C, germination rates were significantly lower ($p<0.05$) in all radiation treatments (P, PA, PAB) compared to the temperature and radiation treatments of 2°C, 7°C and 12°C (scenario a).Within the PAB treatments of 2°C, 7°C and 12°C, significantly lower germination rates ($p < 0.05$) with 36-49% compared to their controls (PAR) and UV-A exposed treatments were detected (Fig. 2).

S. latissima spores (Fig. 3) showed in contrast to spores of *A. esculenta* and *L. digitata* significantly lower germination rates with a decreasing trend from the PAR to the PA and to the PAB treatments at 2°C and under high PAR exposure (scenario c). In *S. latissima* (Fig. 3), germination rates under high PAR+UV (field; scenario c; 0-18% germinated spores) differed significantly ($p < 0.05$) from germination rates obtained under low PAR+UV (laboratory; scenario a; 74-75% germination) as well as high PAR+UV (scenario c) from low PAR+high UV (scenario b; 62-88% germinated spores). The cold water treatment of 2°C differed significantly ($p < 0.05$) from all other treatments with only 66% germinated spores in the control (PAR), 39% in the PA and 28% in the PAB treatment (Fig. 3). Enhanced temperatures (scenario a) did not influence spore germination of *S. latissima* (Fig. 3) while enhanced UV radiation decreased germination among the PA and PAB treatment compared to their controls slightly ($p<0.05$).

Discussion

This study intended to test the effects of three climate change scenarios on three kelp key species in Arctic coastal ecosystems. In the following, the three scenarios will be discussed and evaluated in terms of species-dependent differences in susceptibility and acclimation to radiation and temperature stress, potential consequences of global change on kelp zoospore survival and distribution pattern and the future impact on coastal polar communities.

In the first scenario (a) enhanced temperatures and low PAR + low UV radiation were applied to investigate interactive effects of different radiation regimes (PAR, PA and PAB) and temperature. Due to enhanced water temperatures, the glacier and snow input in Polar regions to the water column the salinity, turbidity and optical properties of the water changes (Hanelt *et al.* 2001; Zacher *et al.* 2009). In fact, turbid water prevents UV radiation to penetrate the water column and photosynthetically active radiation is more strongly attenuated (Hanelt *et al.* 2001). Photosynthesizing organisms have to cope in this situation with three main factors- enhanced water temperatures, freshwater discharge and related osmotic changes, and a decreased light availability. Average summer water temperature in Kongsfjorden at present is 4.5°C with a minimum of 2°C in May and a maximum of almost 7°C in July (Brey 2009). In our study, we tested therefore temperatures from 2°C to 17°C, knowing well that a realistic temperature change may maximally rise up to 12°C within the end of the century (Christensen *et al.* 2007). However, we included the temperature treatment of 17°C to test for lethal limits. While germination in zoospores of *A. esculenta* and *L. digitata* was strongly decreased at 17°C (Fig. 1 and 3) in our study and between 15 and 20°C in *Alaria marginata* (Hoffman *et al.* 2003) under the full solar spectrum, zoospores of *S. latissima* did not show any significant decreases in germination under enhanced (12°C) and high (17°C) water temperatures. In contrast, Müller *et al.* (2008) determined very low germination rates of 8-35 % in *S. latissima* zoospores from Spitsbergen at 2-12°C while at 18 °C, less than 10 % germinated spores could be observed at the PA treatment only. Zoospores

of *A. esculenta* showed completely impaired germination at 18°C in Müller *et al.* (2008) while in our study the PAR and PA treatment of *A. esculenta* were similar to germination rates obtained at 7°C and 12°C and the PAB treatment at 17°C showed spore germination less than 10 %. In our study, *A. esculenta* and *S. latissima* zoospores showed significantly lower germination rates at 2°C compared to 7°C and 12°C in all light treatments. Additionally, *A. esculenta* spores in our study germinated much less at 2°C but more at 17°C (PAR and PA treatments) compared to the study of Müller *et al.* (2008). *A. esculenta* and *S. latissima* can be found far south as e.g. France and far north (e.g. Spitsbergen) leading to the conclusion that ecotypic differences and different adaptive processes (Lüning 1985) might exist (Müller *et al.* 2008) and be responsible for differences in germination behaviour. Seasonal patterns (Roleda *et al.* 2006a) and variations in environmental factors as e.g. shown for decreased germination in temperatures of 10°C compared to 20°C in *A. marginata* (Hoffman *et al.* 2003) may influence germination additionally.

Phlorotannins decreased significantly in content at 7°C and 17°C in *S. latissima* zoospores (Fig. 3) probably due to accelerated processes of oxidation (Ragan & Glombitza 1986). In *A. esculenta* filtrates, the phlorotannin content decreased about 60% comparing the initial and the content after 8 h exposure indicating oxidation processes as well. Connan *et al.* (2006) found positive correlations between phenol accumulation in tissues and the antioxidant activity in extracts of *Ascophyllum nodosum* and *Sargassum muticum*. The high antioxidant activity of phenols with increasing levels of phenols correlates well with the observations of enhanced phlorotannin contents in fertile tissues of kelps (van Alstyne 1999) and would proof the parental investment in this protective substances. Nevertheless, Müller *et al.* (2008) could not detect any differences in phlorotannin contents in response to temperature (2-18°C) nor radiation exposure after 8 h but a temperature dependent increase of absorption at higher temperatures in *A. esculenta, L. digitata* and *S. latissima* zoospores. These changes in absorbance are suggested to occur due to photoinduced electron transitions within aromatic

molecules (Swanson & Fahselt 1997; Swanson & Druehl 2002) but absorption patterns are too stable to be explained by this phenomenon which normally occurs within microseconds (Müller et al 2009a).

In the second scenario (b) enhanced UV radiation+low PAR was applied to determine effects of higher UV radiation due to stratospheric ozone depletion. A basis for this scenario is provided by studies of Hanelt *et al.* (2001) who determined the penetration depth of harmful UV radiation in Kongsfjorden (Ny-Ålesund, Norway). In spring, especially in May/ early June (Brey 2009) during the clear water conditions, UV radiation is able to penetrate deeply and had negative effects on plant productivity down to 5-6 m water depths (Hanelt *et al.* 2001). In our study, enhanced UV radiation had surprisingly no additional effects on phlorotannin production, phlorotannin oxidation and on germination rates than low UV radiation. Although our experimental material originated from different water depths, we could not detect species-specific differences in adaptation and sensitivity to UV radiation after 8 h exposure. Even though the UV/PAR ratios emitted by our experimental set-up do not follow ratios obtained under natural conditions (Wiencke *et al.* 2000), the low PAR background avoids possible masking of UV effects by excessive PAR (Molina & Montecino 1996; Nilawati *et al.* 1997). Hoffman *et al.* (2003) used a lower UV irradiance (UV_{ery} of 0.011-0.029 W m^{-2}) than in our study (UV_{ery} of 0.021-0.032 W m^{-2}) but did not investigate germination of spores under high UV levels at 10°C in *A. marginata*. In default of more studies dealing with high UV radiation in combination with low PAR, we can only hypothesize that the duration of exposure might have been too short for a *de novo* synthesis of phlorotannins (Müller *et al.* 2009a).

The third scenario (c) dealt with high PAR radiation + present UV radiation to understand the influences of high PAR in combination with UV radiation. In the Arctic, this scenario

would appear every spring if stratospheric ozone depletion remains to happen as predicted over the Arctic (Bodeker & Waugh 2007). As mentioned above, clear water periods allow high irradiances to penetrate the waterbody deeply (Hanelt *et al.* 2001; Brey 2009). Alterations in temperature might change turnover rates of phytoplankton blooms and would therefore either promote clear water conditions to appear more frequently or would have a shading effect on the organisms in the water column due to high abundances. Thus under first/latter conditions, high PAR and UV could harm the UV sensitive smallest developmental stages of kelps even in deeper habitats and more frequently while in the other case high PAR effects would be negligible. Graham (1996) studied high PAR effects on *Macrocystis pyrifera* zoospores suggesting that prolonged exposure to high PAR can inhibit the recruitment of *M. pyrifera* in shallow water by killing its post-settlement stages. Similar observations were carried out by Cie & Edwards (2008) suggesting strong negative impacts of elevated PAR irradiances over several hours in *Pterygophora californica* and *M. pyrifera* on their subsequent settlement, development and reproductive viability.

As investigated in this study, germination of zoospores of all species was highly affected by high PAR + low UV radiation. In the control, only 30% spores germinated after 8 h exposure to high PAR in the shallow water species *A. esculenta* (Fig 1). In the high PAR control of the sublittoral species *S. latissima*, only 10% germinated spores were determined (Fig 2). Observations by Swanson & Druehl (2000) suggest that UV radiation in combination with excessive solar PAR light may ultimately determine and give rise to the observed differences in meiospore size and UVR tolerance found within natural kelp populations. In contrast, Wiencke *et al.* (2006) investigated *in situ A. esculenta* and *L. digitata* zoospores in 0.5-4 m water depths in early and late June with no effects in 2-4 m water depths under longer exposure times (24-45h) but a decrease in germination in the UV treatments of 0.5-2 m water depths. In contrast, we obtained significant differences in germination rate of spores exposed for 8 h to PAR and PAB within a radiation field simulating the radiation regime of clear water

stages of Kongsfjorden (Jerlov coastal water type 1) in June and 3-5 m water depth (compare Brey 2009).

Differences in results are probably caused by different attenuations in the *in situ* experiment of Wiencke *et al.* (2006) and the light regimes on air of the present study. Under water, photosynthetic pigments of phytoplankton contribute to the attenuation of PAR and the light attenuation in the blue and UVR-region is mainly caused by yellowish dissolved organic substances (Jerlov 1976; Hanelt *et al.* 2001; Brey 2009). In contrast, simulation ashore lacks this attenuations and differences in light colours or light compositions (Jerlov 1976). Although attenuation differences to *in situ* experiments should be considered, PAR and UV could be simulated in compliance with *in situ* measurements of Brey (2009) in our study. Erythemally weighted UV data ($UV_{ery,}$ Tab.1) were calculated to bridge differences in attenuation. It was revealed that UV_{ery} doses of *A. esculenta* and *L. digitata* spores exposed over 24h and in 2 to 4 m water depths (Wiencke *et al.* 2006) were of similar range as UV_{ery} doses over 8 h in our experiment (Tab.1). Considering UV_{ery} doses and long exposure times, PAR was probably low in the *in situ* experiment of Wiencke *et al.* (2006).

Differences in spore sensitivity towards UV radiation are additionally very likely related to strong seasonal variations in spore vitality caused by different maturation stages of parental algae and environmentally induced acclimation and adaptation processes. Depending on the season, different susceptibilities of photosynthesis to UV radiation have previously been observed in sporophytes (Aguilera *et al.* 2002; Bischof *et al.* 2002; Brey 2009). Our experimental material was collected and the studies performed between July and August. Due to turbid water conditions during Arctic summer in Kongsfjorden, the collected parental algae and their zoospores were very likely acclimated to low radiation. In contrast, the experimental conditions the zoospores were exposed to in our study resembled high radiation conditions normally obtained between May and June on Spitsbergen. Discrepancies in germination pattern could therefore be caused by seasonal adaptations and sensitivities towards UV and

visible radiation and should be considered in future studies. Therefore we hypothesize that germination pattern of Arctic zoospores depends strongly both on exposure time, level of irradiance and seasonal adaptations. In fact, longer exposure times to lower PAR and UV exhibited less effects on germination pattern (Wiencke *et al.* 2006) than short exposure times with higher PAR and UV radiation (our study).After exposure to high PAR and low UV radiation, phlorotannin content was significantly lower in *L. digitata* and *S. latissima* zoospores while in *A. esculenta* only the filtrate differed from the initial content. The observations concerning the high temperature and high PAR sensitivity of phlorotannins lead to the question if species as *A. esculenta* might be equipped with a group of phlorotannins that decays or oxidizes faster to enhance the protective potential in shallow waters.

High PAR and UV are known to induce reactive oxygen species and DNA damages (Rijstenbil *et al.* 2000). Ahn *et al.* (2007) investigated antioxidant activities by electron spin resonance spectrometry (ESR) technique for scavenging effects of free radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), alkyl, hydroxyl ($HO^{\bullet}$) and superoxide anion radical ($O_2^{\bullet -}$) and by comet assay for protecting effects of phlorotannins against H_2O_2-mediated DNA damage. Their results show that the investigated phlorotannins phloroglucinol, eckol and dieckol in *Ecklonia cava* have the $DPPH^{-}$, $alkyl^{-}$, $hydroxyl^{-}$ and superoxide radical scavenging activities. In addition, protective effects of the phlorotannins against H_2O_2-mediated DNA damage increased with increased concentrations in the samples (Ahn *et al.* 2006). Meng *et al.* (1989) concluded that polyphenols in general undergo progressive polymerization *in vivo* producing high molecular weight, non-dialyzable polyphloroglucinols (Ragan 1976) which are readily oxidized (Crato 1893) to form low molecular weight phlorotannins (Koch *et al.* 1980). The observations contributes to the hypothesis of different oxidative potentials within the phlorotannin polymers in *L. digitata* and *S. latissima*, both growing in greater water depths and therefore under less light stress conditions. *L. digitata* and *S. latissima zoospores* showed higher oxidation with higher temperatures and higher PAR but less dramatic than

observed in *A. esculenta spores.* Both species might be equipped with a more inert group of phlorotannins related to their deeper habitat. In general, high PAR seems to have tremendous effects on spore development and will determine spore distribution and survival more than hitherto assumed.

The vertical zonation pattern of seaweeds in the coastal zones is reflected by different sensitivities of the early developmental stages to UV radiation (e.g. Lüning 1985; Swanson and Druehl 2000; Roleda *et al.* 2004; Bischof *et al.* 2006; Wiencke *et al.* 2006; Roleda *et al.* 2010). In this context, the concentrations of phlorotannins in the water column are an important parameter for determination of UV susceptibility of brown algal spores. Spores of the upper sublittoral species *A. esculenta* (this study, Fig 4) and *Saccorhiza dermatodea* (Steinhoff *et al.* unpublished data, Fig. 4) are released together with high amounts of phlorotannins from their parental tissue. In contrast, phlorotannin release from the parental tissue of the mid sublittoral species *L. digitata* and *S. latissima* is much lower. In this study, we could for the first time correlate the sampling depths of the parental algae with their phlorotannin amounts in the fertile tissue upon release (Fig. 4). This adds to the hypothesis of Swanson & Druehl (2002) suggesting that algae may be able to formso-called UV refugia by release of UV-screening compounds (phlorotannins) to protect spores from harmful UV radiation (Roleda *et al.* 2010). Nevertheless, future studies should take into consideration the maturity grades of the sori, the sori areas and the amount of seawater, the spores are released in to obtain comparable results.

From the present study we can conclude that 1. Zoospores of the three Arctic kelps *A. esculenta, L. digitata* and *S. latissima* exhibit species-specific tolerance limits with respect to viability and germination, 2. Sensitivity of zoospores against high UV radiation follows the general vertical zonation pattern of the parental algae and is correlated with a high phlorotannin expression within the parental tissue of upper sublittoral species (*A. esculenta*)

and a low exudation in the parental tissue of mid sublittoral species (*L. digitata* and *S. latissima*) and 3. High PAR affects germination pattern and phlorotannin oxidation.

Nevertheless, the investigated factors should not be considered as the only factors but be seen as preliminary identification of possible biological processes (Wiencke *et al*. 2000; Hoffman *et al.* 2003). To understand the complex mechanisms affecting kelp recruitment, dispersal and survival strategies, we need further studies focusing especially on ecological implications.

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Figure captions and Table

Fig. 1 Sum of phlorotannin content [µg phlorotannin ml spore solution^{-1}] in the surrounding medium (lower part of the bars) and the zoospores (upper part of the bars) under scenario a-c in *Alaria esculenta* before and after 8 h exposure. Germination rate [%] of *A. esculenta* zoospores under scenario a-c was obtained 6 d after exposure. White bars indicate control (photosynthetically active radiation, PAR only), dark grey bars PAR+UV-A radiation and black bars PAR+UV-A+UV-B radiation. Capital letters show statistical differences of treatments ($p<0.05$). Vertical bars indicate standard deviation (SD=3).

Fig. 2 Sum of phlorotannin content [µg phlorotannin ml spore solution^{-1}] in the surrounding medium (lower part of the bars) and the zoospores (upper part of the bars) under scenario a-c in *Laminaria digitata* before and after 8 h exposure. Germination rate [%] of *L. digitata* zoospores under scenario a-c was obtained 6 d after exposure. White bars indicate control (photosynthetically active radiation, PAR only), dark grey bars PAR+UV-A radiation and black bars PAR+UV-A+UV-B radiation. Capital letters show statistical differences of treatments ($p<0.05$). Vertical bars indicate standard deviation (SD=3).

Fig. 3 Sum of phlorotannin content [µg phlorotannin ml spore solution^{-1}] in the surrounding medium (lower part of the bars) and the zoospores (upper part of the bars) under scenario a-c in *Saccharina latissima* before and after 8 h exposure. Germination rate [%] of *S. latissima* zoospores under scenario a-c was obtained 6 d after exposure. White bars indicate control (photosynthetically active radiation, PAR only), dark grey bars PAR+UV-A radiation and black bars PAR+UV-A+UV-B radiation. Capital letters show statistical differences of treatments ($p<0.05$). Vertical bars indicate standard deviations (SD=3).

Fig. 4 Collection depth of *Alaria esculenta, Saccorhiza dermatodea*, Laminaria digitata* and *Saccharina latissima* in correlation with the sum of phlorotannin content [µg phlorotannin

spore^{-1}] within the surrounding medium and the zoospores. Vertical zonation of sublittoral Arctic brown algae species reflect parental phlorotannin content liberated upon spore release. Capital letters indicate statistical differences ($p<0.05$) and vertical bars standard deviation (SD=3). *unpublished data Steinhoff *et al.*

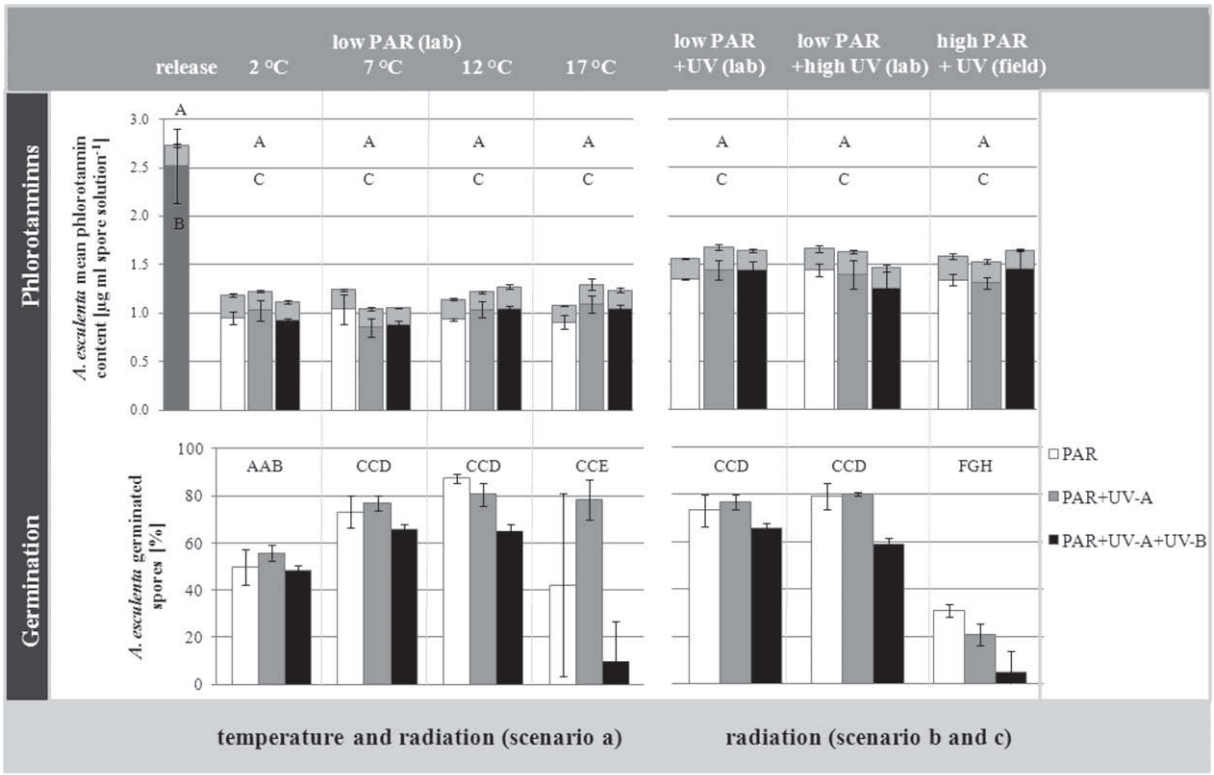
low PAR (lab)
release
2 °C
7 °C
12 °C
17 °C
low PAR +UV (lab)
low PAR +high UV (lab)
high PAR + UV (field)
Phlorotannins
Germination
A. esculenta mean phlorotannin content [µg ml spore solution-1]
A. esculenta germinated spores [%]
A
B
C
AAB
CCD
CCE
FGH
PAR
PAR+UV-A
PAR+UV-A+UV-B
temperature and radiation (scenario a)
radiation (scenario b and c)

Fig 1

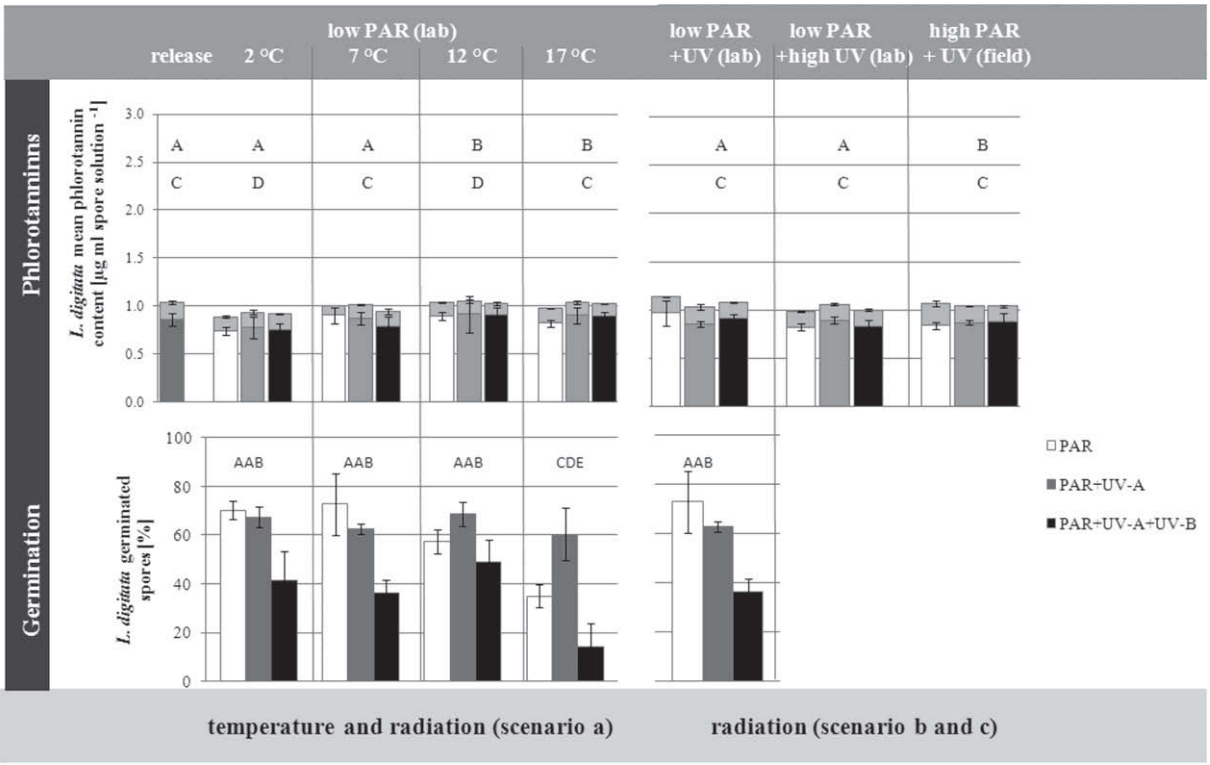
low PAR (lab)
release
2 °C
7 °C
12 °C
17 °C
low PAR +UV (lab)
low PAR +high UV (lab)
high PAR + UV (field)
Phlorotannins
Germination
L. digitata mean phlorotannin content [µg ml spore solution -1]
L. digitata germinated spores [%]
A
B
C
D
AAB
CDE
PAR
PAR+UV-A
PAR+UV-A+UV-B
temperature and radiation (scenario a)
radiation (scenario b and c)

Fig 2

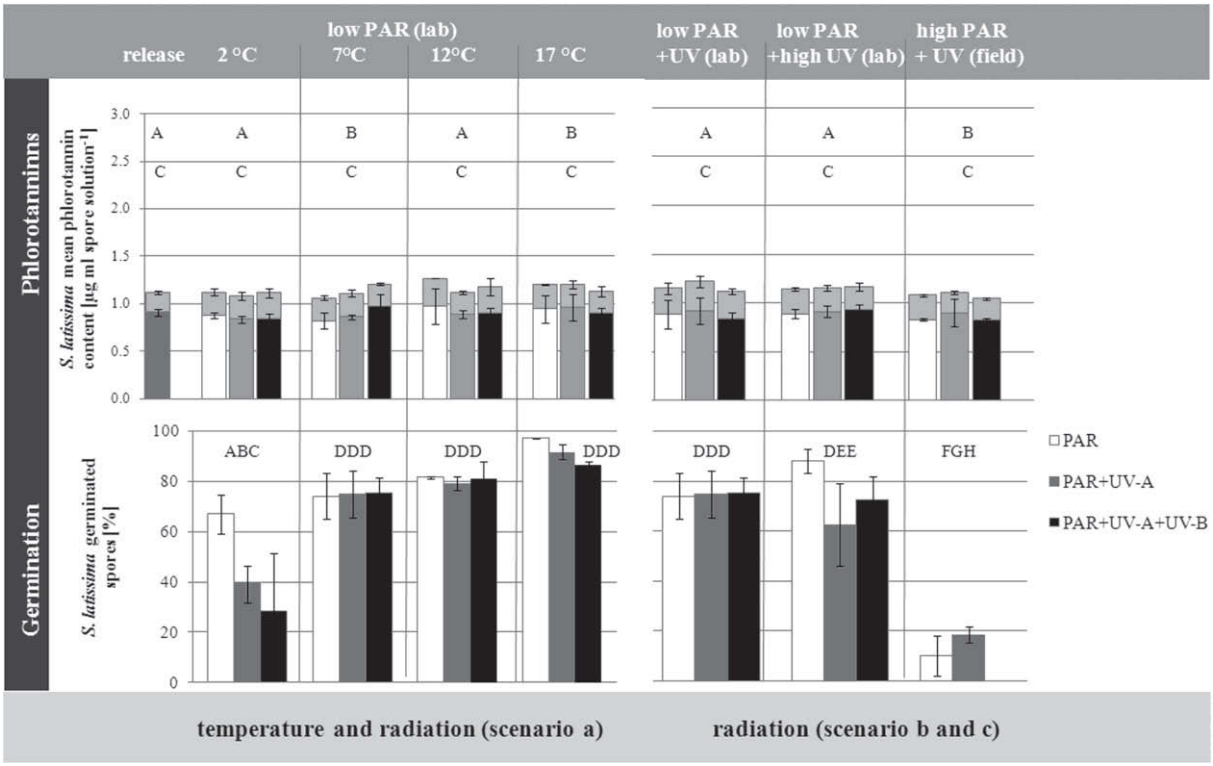
release
2 °C
low PAR (lab)
7°C
12°C
17 °C
low PAR +UV (lab)
low PAR +high UV (lab)
high PAR + UV (field)
Phlorotannins
S. latissima mean phlorotannin content [µg ml spore solution-1]
A A B A B A A B
C C C C C C C C
Germination
S. latissima germinated spores [%]
ABC DDD DDD DDD DDD DEE FGH
PAR
PAR+UV-A
PAR+UV-A+UV-B
temperature and radiation (scenario a)
radiation (scenario b and c)

Fig 3

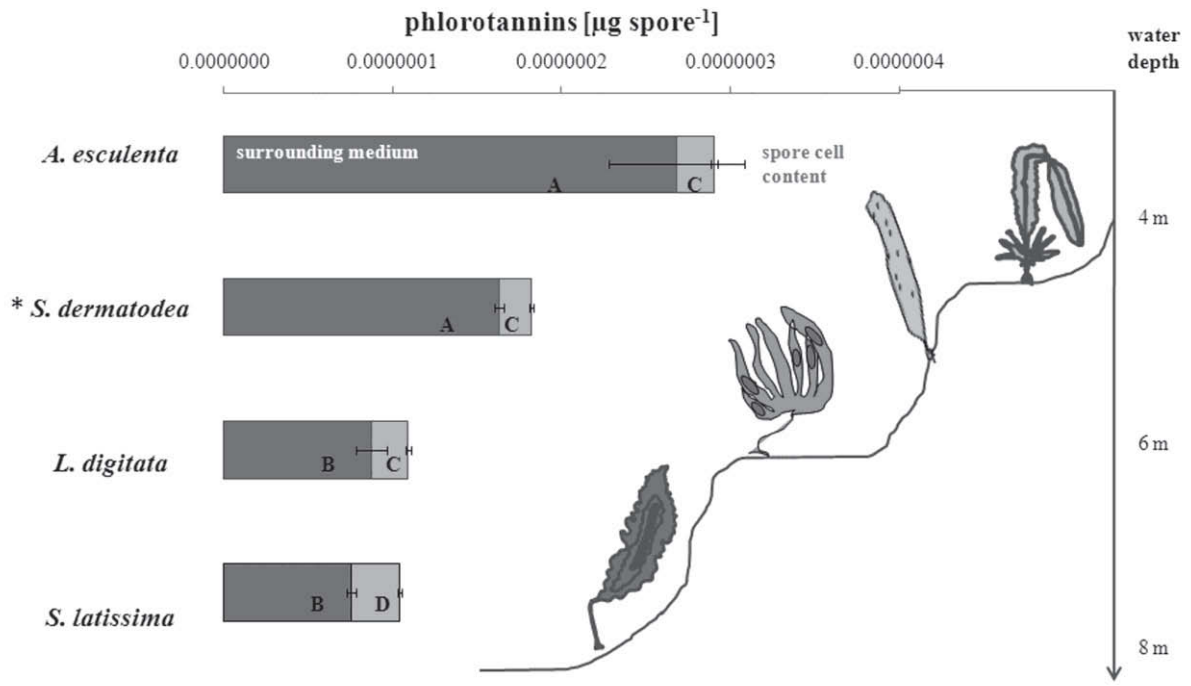
phlorotannins [µg spore-1]
0.0000000
0.0000001
0.0000002
0.0000003
0.0000004
water depth
A. esculenta
surrounding medium
spore cell content
A
C
* S. dermatodea
A
C
L. digitata
B
C
S. latissima
B
D
4 m
6 m
8 m

Fig 4

Tab. 1 Summary of radiation and temperature treatments within all three modelled scenarios and species (*Alaria esculenta, Laminaria digitata* and *Saccharina latissima*). Spectral irradiances in the field (scenario c) were measured in 5 min intervals after shielding with 50% gauze. Irradiances are given as 5-min mean values (W m^{-2}). For a better comparison of data, UV-A, UV-B and UV_{ery} over the exposure period of 8 h are shown in dose [J m^{-2}], additionally.

Species	Light treatment	Temperature [°C]	PAR		UV-A		UV-B		
			Irradiance [W m^{-2}]	Dose [J m^{-2}]	Irradiance [W m^{-2}]	Dose [J m^{-2}]	Irradiance [W m^{-2}]	Dose [J m^{-2}]	UV_{ery} [J m^{-2}]
A esculenta	low PAR + UV (lab) scenario a	2	3.5±0.16	0.10×10^6	5.35±0.33	0.15×10^6	0.40±0.02	0.11×10^5	1.4×10^3
L digitata		7	4.9±0.64	0.14×10^6	5.83±0.70	0.16×10^6	0.45±0.07	0.12×10^5	1.6×10^3
S latissima		12	4.7±0.32	0.13×10^6	4.85±0.42	0.14×10^6	0.43±0.05	0.12×10^5	1.5×10^3
		17	5.4±0.72	0.15×10^6	5.00±0.74	0.14×10^6	0.38±0.06	0.11×10^5	1.4×10^3
A esculenta	lowPAR + high UV (lab) scenario b	7	5.2±0.64	0.15×10^6	9.03±0.61	0.26×10^6	0.72±0.04	0.20×10^5	2.6×10^3
L digitata									
S latissima									
A esculenta	high PAR + UV (field) scneario c	7	33.0±19.6	0.96×10^6	7.07±1.94	0.20×10^6	0.21±0.06	0.06×10^5	8.0×10^2
L digitata			74.6±26.88	1.99×10^6	7.84±0.89	0.23×10^6	0.24±0.02	0.07×10^5	9.2×10^2
S latissima			57.2±18.4	1.66×10^6	4.88±0.10	0.17×10^6	0.16±0.01	0.05×10^5	6.0×10^2

Publication IV

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Phlorotannin production and lipid oxidation as a potential protective function against high visible and UV radiation in gametophytes of *Alaria esculenta* (Alariales, Phaeophyceae)

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Phlorotannin production and lipid oxidation as a potential protective function against high visible and UV radiation in gametophytes of *Alaria esculenta* (Alariales, Phaeophyceae)

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ABSTRACT

Effects of UV-radiation (UVR) and photosynthetically active radiation (PAR) are determined by the sensitivity of species to the various irradiances. In particular sensitive to UVR are unicellular and early developmental stages of brown algae. Polyunsaturated fatty acids, major constituents of cell membranes, are known to be oxidized and produce lipid peroxidation products upon UVR. To prevent photooxidation within the cells, photo-protective substances as phlorotannins are synthesized. In the present study, changes in total fatty acids and fatty acid composition as well as intra- and extracellular phlorotannin contents as an indicator of UVR-protection and acclimation potential towards UVR were determined by gas chromatography and Folin-Ciocalteu, respectively. Zoospores/ juvenile gametophytes of a dominant Arctic brown alga from Spitsbergen (*Alaria esculenta*, Phaeophyceae) were exposed to high and low PAR in combination with UVR in the treatments: UV-B and UV-A (PAB), UV-A (PA) or PAR only (PAR) over a study period of 20 days. While total fatty acid content decreased only under low PAB, total FA contents upon high PAR exposure were generally low in all treatments. Intracellular phlorotannin levels increased under low PAR only in the PAR and PA-treatments after 10 days while extracellular phlorotannin content increased after day 10, 15 and 20 significantly in all treatments. Upon high PAR, spores under PA showed significant higher internal phlorotannin levels after 15 and 20 days than their representatives in the control and PAB treatments. Extracellular phlorotannin levels increased in all treatments (except day 10 PAB) significantly in all treatments after day 10. Our observations suggest that phlorotannins might play a major role in external and internal cell protection by absorption and oxidation processes. Changes in fatty acid content and composition upon UVR and high might be considered as an adaptive mechanism of the *A. esculenta* juveniles subjected to variations in solar irradiance. Hence, global climate change and stratospheric ozone depletion might influence viability and nutritional quality of kelp's early life stages more than assumed.

INTRODUCTION

UV-B radiation and high photosynthetically active radiation (PAR) have been shown to have detrimental effects on marine organisms as summarized by (1- 6). The impact of UV-B radiation on organisms can be manifold. It ranges from the cellular level e.g damages on the DNA (desoxyribonucleic acid), changes in cell wall morphology, cellular stoichiometry, inhibition of photosynthesis (7) and changes in fatty acid content (8, 9) up to the community structure due to different UV-sensitivities of species and their developmental stages (10, 11). In particular sensitive to UVR (UV-radiation) are unicellular stages as e.g. many phytoplankton species (12, 13) and early developmental stages of brown algae (14- 22).

Polyunsaturated fatty acids (PUFA), major constituents of cell membranes, are known to be oxidized and produce lipid peroxidation products upon UVR since early investigations of (23). Some of the lipid oxidation products as e.g malonaldehyde exhibit toxic activities by reacting with biological nucleophiles and crosslink upon UVR to proteins and bind covalently to nucleic acids (24, references therein). Irradiance levels are known to affect ratios of polyunsaturated and saturated fatty acids (25, 26). Consequently, changes in fatty acids driven by UV-B and low/high PAR can influence the entire metabolism of an organism, its protective mechanisms as e.g. membrane fluidity in polar species and formation of UV-protective substances against biotic and abiotic environmental factors, their nutrient uptake as well as the nutritional quality of the organism itself as reviewed by (13).

To prevent photo-oxidation within the cells, photo-protective substances such as phlorotannins are synthesized. Among brown algae, only one group of polyphenolics, the phlorotannins, is present (27-28). Phlorotannins are stored in spherical membrane-bound vesicles, the so-called physodes (29-31) which are distributed throughout the cell and cell walls. Phlorotannins are based on the monomer phloroglucinol (32) and discussed to be formed via the acetate-malonate pathway (33) involving a polyketide synthase-type enzyme complex (34). Phlorotannins have a multifunctional role in ecology and respond to several

environmental parameters, such as salinity, nutrient (35) and light availability as well as UVR and high irradiances (36). Though, the most important property of phlorotannins for this study were their abilities to absorb in the UV wavelength range (6,11,18-19, 37-39) and their radical scavenging (antioxidant) activity (40-43). Phlorotannins can be either integrated into cell walls as supporting substances (44) or released via exudation processes into the surrounding water (32, 38, 45) creating so-called UV-refugia (38) to protect spores from harmful radiation and to deter grazers.Especially polar species are exposed to extraordinary circumstances as low temperatures, ice cover, shifts in light availability (Polar day and night) and a high interannual variability in UVR (46) due to meteorological conditions. Studies investigating fatty acid composition in few-celled marine organisms are scarce (e.g. 9, 12-13).

This study investigated for the first time whether (1) the fatty acid composition and total fatty acids upon UVR and low/high PAR exposure over a study period of 20 days in zoospores of a dominant Arctic brown alga (*Alaria esculenta*, Phaeophyceae) from Spitsbergen (Norway). We further obtained contents of the UV-protective polyphenolic substances phlorotannins (37) as an indicator of UV-protection and acclimation potential towards UVR to spot (2) whether phlorotannin production in zoospores/juvenile gametophytes of *A. esculenta* is affected by low vs high photosynthetically active radiation (PAR) in combination with and without UVR and (3) whether the factor of time is involved in phlorotannin production and exudation processes as well as lipid consumption and change in fatty acid composition and (4) whether storage fatty acids of *A. esculenta* might serve as potential fuel for phlorotannin building blocks in early developmental stages of seaweeds.

MATERIAL AND METHODS

In late May 2009, fertile sporophytes of *Alaria esculenta* (Linnaeus) Greville were collected from 8-9 m water depth by SCUBA diving in Kongsfjorden (Ny Ålesund, Spitsbergen, Norway). To obtain zoospores, the fertile tissue (sorus) of seven individuals was cleaned and

kept at 7°C in a moist and dark chamber according to (16). Upon immersion in 0.2 µm filtered seawater, the fertile tissue released zoospores after 1.5 days and at temperatures 5 °C above the temperature the fertile tissue was stored. Individual zoospore suspensions were mixed and zoospore density was adjusted with 0.2 µm filtered seawater to 12.9 x 10^5 spores per mL for lipid and phlorotannin analysis (counted with Neubauer chamber "improved", Brand, Germany). Obtaining three replicates per treatment and sampling day for phlorotannin (40 ml) and lipid analysis (10 ml), the zoospore suspension was distributed in glass Petri-dishes. Petri-dishes containing zoospores were then exposed to the radiation conditions summarized in Tab. 1 applying a light cycle of 24h:12h (PAR:UV) according to the polar day. Low photosynthetic active radiation (PAR) was provided by three white light fluorescent tubes (36 W true light® II Powertwist, USA) while high PAR was provided by three Osram Biolux (36W/965, München, Germany). PAR was measured with a LiCor 250A and a Li-190 Quantum sensor, LiCor, Lincoln, NE, USA. UVR was generated by three fluorescent tubes (Q-Panel UVA 340, 40 W, Cleveland, OH, USA) and measured with a Solar light PMA 2100 (Solar light Co., PA, USA).

Petri-dishes containing spore solutions were covered with one of three different cut-off filters allowing the following radiation spectra to pass: only PAR (400 -700 nm, Ultraphan URUV farblos, Digefra, Munich, Germany), PAR and UV-A radiation (PA, 320-700 nm, Folex PR Montage Folie , Dr. Schleussner, Dreieich, Germany) and PAR and UV-A and UV-B radiation (PAB, 290-700 nm, Ultraphan URT 300 foil (Digefra, Munich, Germany). For a better comparison of data, doses of PAR, UV-A and UV-B over the exposure period of 24/12h a day were calculated (Tab. 1). Additionally, erythemally weighted radiation was calculated ($UV_{ery,}$ Tab. 1) according to (47). Samples were taken directly after release (day-0) and after 5, 10, 15 and 20 days. To avoid nutrient limitation, spore/ gametophyte suspensions were enriched with an artificial media according to (48).

After exposure, samples for fatty acid analysis (10 ml each) were filtered on GF/C Filters (Whatman, Maidstone, UK, precombusted at 450 °C for 4 h), covered with chloroform/methanol (2:1 v/v, Merck, Darmstadt, Germany) and frozen at -80 °C before gas chromatographic analysis.

Phlorotannin analysis

For quantitative phlorotannin analysis, zoospore solutions (40 ml each) were filtered gently after exposure on GF/C filters and frozen in liquid nitrogen. After extracting spore filters with 7:3 acetone/water (analysis grade, Merck, Darmstadt, Germany), phlorotannin extractions were freeze-dried and re-dissolved in 1 ml MilliQ water for further measurements. Subsequently, total phlorotannin content of spores was measured after (33) and (49) applying the Folin-Ciocalteu method (Folin-Ciocalteu solution and Sodium carbonate, Merck, Darmstadt, Germany) using phloroglucinol (Sigma-Aldrich, St. Louis, USA) as a standard agent.

Fatty acid analysis

For fatty acid analyses, filters were homogenized by ultrasonification and extracted in dichloromethane: methanol (2:1, v/v) following the method described by (50). Prior to extraction, an internal standard was added (19:0 FAME). For gas liquid chromatography of fatty acids, methyl esters were prepared from aliquots of the extracted zoospores/gametophytes by transesterification with 3% sulfuric acid in methanol for 4 h at 80°C. After extraction with hexane, fatty acid methyl esters (FAME) were analyzed with a gas liquid chromatograph (HP 6890, Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m x 0.25 mm I.D.; film thickness: 0.25 µm; liquid phase: DB-FFAP, J&W, Cologne, Germany) using temperature programming (51). FAMEs were identified by comparison with known standard mixtures. If necessary, identification of FAMEs was

confirmed by gas chromatography-mass spectrometry (GC-MS) measurements. Total lipid concentration referred to the sum of total fatty acid methyl esters.

Statistics

Statistic analysis was obtained with R (version 2.8.1.), by testing the phlorotannin data set for normal distribution with a Shapiro-Wilk test according to (52), a 2-way ANOVA and by applying a POST HOC test (Tukey-HSD) of pair-wised comparison to test differences among treatments. Significant differences in fatty acid composition and total fatty acids in the course of the experiment were identified by one-way ANOVA. Homogeneity of variances was tested with Levene´s test and normality of residuals with the Shapiro-Wilk test (Sokal and Rohlf 1995). Differences among treatments were tested with a PostHoc test (Tukey-HSD test) and Kruskal-Walis test.

RESULTS

Phlorotannin content

In Fig 1, phlorotannin contents are shown as sum of phlorotannins per ml spore solution in the spores (upper bars) and the filtrates (lower bars) over the exposure period of 20 days and under different light treatments (P, PA, PAB). Upon release, phlorotannin content within the spores was averaged to 0.31±0.04 µg phlorotannin ml spore solution^{-1} and within the filtrate (surrounding medium) 5.64 ±0.30 µg phlorotannin ml spore solution^{-1} (Fig 1). In the low PAR treatments, spores exhibited significantly higher phlorotannin contents ($p<0.05$) in the 10 PAR and PAR+UV-A, 15 PAR and PAR+UV-A and the 20 PAR and PAR+UV-A treatments compared to the initial and the contents obtained after 5 days (Fig 1). The phlorotannin was particularly high ($p<0.05$; Fig 1) in the 15 PAR and PAR+UV-A (1.25±0.11 µg phlorotannin ml spore solution^{-1}) and the 20 PAR+UV-A treatment (1.60±0.09 µg phlorotannin ml spore solution^{-1}). Phlorotannin contents in all PAR+UV-A+UV-B treatment did not increase over

time. In contrast, within the filtrate all treatments of 10, 15 and 20 days showed significant higher ($p<0.05$) phlorotannin contents compared to the initial and day 5 contents (9.3-12.7 µg phlorotannin ml spore solution^{-1}).

In the high PAR treatments only spores in the 15 PAR+UV-A and the 20 PAR+UV-A treatments showed significant higher phlorotannin contents ($p<0.05$) with 1.24-1.39 µg phlorotannin ml spore solution^{-1} compared to the initial and phlorotannin contents after 5 and 10 days and all PAR+UV-A+UV-B treatments. Within the high PAR filtrates, we could measure significant higher phlorotannin contents ($p<0.05$) in the 10 PAR and PAR+UV-A and all other treatments after 15 and 20 days compared to the initial and the values obtained after 5 days.

By comparison of filtrates within the low light and the high light treatments, significant differences in phlorotannin contents could be observed after 10 days of exposure but not within the light treatments (10.8-12.7 and 9.8-11.9 µg phlorotannin ml spore solution^{-1}, respectively), except the 10d high PAR PAB treatment. In contrast, spores within the low PAR+UV-A treatment had slightly higher phlorotannin contents than their species colleagues under high PAR+UV-A exposure ($p=0.05$).Within the exposure treatments of low PAR+UV-A+UV-B and high PAR+UV-A+UV-B phlorotannin contents (0.15±0.05 µg phlorotannin ml spore solution^{-1}) did not differ.

Fatty acids (FA)

Initial *A. esculenta* spores were dominated by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (both 32%) followed by saturated fatty acids (SAFA) with slightly lower values (up to 31%, largely owing to 18:0 and 16:0 fatty acid; Tab. 2). With high PAR radiation PUFA were remarkably low already after 5 days of PAR+UV-A+UV-B treatments (9.9% to 4.3%; Tab. 2)). Under low PAR treatment PUFA had considerable higher values with up to 60% of total fatty acids (20 PAR and 20 PAR+UV-A; Tab. 2) The

monounsaturated FA 18:1(n-9) exhibited highest amounts in early stages of the high PAR treatment after 5 days (37%) and were remarkably lower towards the end of experiment and ranged between 11% and 5% after 15 and 20days, respectively. During low PAR the initial proportions of 18:1(n-9) in spores were slightly lower compared to the high PAR (about 25%) and reached proportions of 7% to 15% after 20 days of experiment (Tab. 2). The next most abundant were the SAFA due to consistently high levels of the 16:0 fatty acid in all treatments, particularly in high PAR+UV-A+UV-B treatments with percentages of about 37%. The total fatty acid content upon UV-exposure in the present study revealed that under low PAR, total FA content was higher than under high PAR (Tab. 2).

However, low PAR and high PAR treatment showed important differences in total fatty acid (TFA) production. Upon low PAR treatment the TFA content was almost stable during the 20 Days of experiment exhibiting an average amount of 1.08µg/spore. Within the other low light treatments (PA and PAB) a distinct decrease of TFA after 10 days of experiment was obvious with average values of 0.5 and 0.2 µg/spore, for PA and PAB respectively. In contrast, after 15 and 20 days of the high light experiment TFA were almost depleted in all treatments (PAR, PA and PAB). After 15 days a mean TFA amount of 0.27 µg/spore and after 20 days an average value of 0.06 µg/spore was detected (Tab. 2). Only upon PAR radiation after 10 days considerable amounts of TFA were analyzed (1.5µg/spore; Tab. 2).

DISCUSSION

This study involved a comparable analysis of low *vs* high PAR in relation to UVReffects and was carried out for the first time on early life stages of *A. esculenta.* Fatty acid content/ composition and extracellular phlorotannin content were highly affected by both low and high PAR and UV in *A. esculenta* zoospores and juvenile gametophytes. While total FA content decreased only under low PAR+UV-B, total FA contents upon high PAR exposure were

generally low in all treatments. Intracellular phlorotannin levels increased under low PAR only in the P and PA treatments after 10 days while external phlorotannin content increased after day 10, 15 and 20 significantly in all treatments. Upon high PAR, spores under PA showed significant higher internal phlorotannin levels after 15 and 20 days than their representatives in the control and PAB treatments. External phlorotannins increased in all treatments (except 10day PAB) significantly in all treatments after day 10.

Exposure time, level of irradiance, nutrients and developmental stage on fatty acid composition and total fatty acids

Nutrient limitation is known to additionally increase sensitivity towards UV. Although it is suggested that lipid synthesis is not governed by nutrient deficiency in natural aquatic ecosystems but rather by abiotic factors as irradiation and day length (53-54) other studies have revealed that a reduced nutrient uptake changes the C:N:P ratios upon UVR after 2 days (13). A phosphorus deficiency could for instance reduce RNA and slow down the transcription process (13, references therein) and associated delayed or hindered cell division would induce accumulation of various primary and secondary photoproducts (Hessen et al. 1997). To avoid these side effects und to focus entirely on interactive effects of UVR and high/ low PAR in the early developmental macroalgal stages, the spore (gametophytes) suspensions were enriched with Provasoli enriched seawater (48) and nutrient deficiency related effects can therefore be excluded.

FA profiles are more susceptible than overall production parameters like photosynthesis and may be affected by smaller dose rates and shorter exposure times. While short exposure periods to UVR and high visible irradiances might lead to change in FA composition via lipid peroxidation, longer exposure periods might reduce photosynthesis and change in total FA content (13). (12) investigated decreasing UV-B damages with higher

irradiation levels in several microalgae with lowest damage at 200 µmol photons s^{-1} m^{-2} [43 Wm^{-2}] and highest at 15 µmol photons s^{-1} m^{-2} [3 Wm^{-2}]. Although PAR levels were much higher in (12) compared to our study, daily doses of UV-B were calculated to12 kJ m^{-2} while in our study daily doses of UV-B ranged from 19.4-20 kJm^{-2}. In contrast, our low PAR controls showed an increase in total FA over time while high PAR controls showed a strong decrease in total FA compared to the initial value after release indicating an interactive effect of exposure time and irradiance levels (PAR) in *A. esculenta* on total fatty acid content as suggested by (13).

Consequently, high PAR seems to have a similar impact on total FA and FA composition as high energetic short wavebands of UV-B. Additionally, changes in lipid content can be considered as an adaptive and survival mechanism of the juveniles subjected to variations in solar irradiance as suggested by (55) and related to the developmental stage as summarized in Tab. 3. Results obtained propose that interactive effects of high PAR and UV-B on *A. esculenta* juveniles might enhance FA peroxidation leading to feed back stress responses as formation of ROS and antioxidants (e.g. phlorotannin).

Phlorotannins under low vs high PAR and upon UV exposure

As described previously, phlorotannins exhibit a multifunctional protective role against several abiotic and biotic factors due to their chemical characteristics. Nevertheless, the most important properties of phlorotannins for this study were their abilities to absorb in the UV-wavelength range (6, 11, 18-19, 37-39) and to possess antioxidant activity (40, 43). Phlorotannin contents can vary with species, morphology of the algae, habitat and developmental stage as well as with seasonal and environmental parameters. These constraints have to be taken into considerations when interpreting results and distinguish effects of applied experiments.

(37) exposed *A. nodosum* sporophytes to significantly higher radiation conditions with 0.6 Wm^{-2} UV-B for 2 weeks compared to our study leading to a~ 30 % in-crease in mean phlorotannin concentrations. Similar results were obtained by (56) in *F. gardneri* and by *S. muticum* (57) suggesting high photo-protective activity of phlorotannins. Nevertheless, an increase in intracellular phlorotannin levels in the present studies was only detectable after 10 days exposure upon low PAR and UV and non-UV treated juvenile gametophytes *A. esculenta* while low PAR only treatment showed no effects on *S. latissima* juveniles (Steinhoff et al. submitted). Upon high PAR and UV exposure, juvenile gametophytes under PA exhibited significantly higher intracellular phlorotannin levels than their representatives in the high PAR controls and among the PAB treatments. Consequently, it can be assumed that UV-A might be able to either compensate high PAR impact to some extend or that phlorotannin synthesis might be stimulated by wavelengths within the UV-A spectrum.

Different developmental stages are known to exhibit species specific levels of phlorotannin contents. While phlorotannin contents in juveniles of *E. menziesii, Fucus gardneri, H. sessile* and *Lessoniopsis littoralis* were higher relative to adult concentrations, phlorotannin concentrations in *Nereocystis luetkeana* were higher in adult algae (58). Studies of (59) observed less phenolic compounds in juveniles compared to adult stages. Within zoospores, phlorotannins are located in physodes randomly distributed throughout the cell while phlorotannins in adult algae are located in the cell walls (44) and the cytoplasma (31) as well as in the outer epidermal cell layer, cortical cells and in the innermost medullary (60). Due to the small cell size of spores, intracellular phlorotannin contents were therefore rather small and increased in juvenile gametophytes 10 days after release under low PAR and 15 days under high PAR conditions suggesting a coupling either between hindered growth and high PAR or decreased phlorotannin formation/phlorotannin oxidation under high PAR compared to low PAR exposure.

During development, more phlorotannins were exuded both in *A. esculenta* (present study) and *S. latissima* juveniles (Steinhoff et al. submitted) to the surrounding medium than integrated into the cells themselves. Instead, phlorotannins were exuded into the surrounding medium with significantly increasing levels over time but not related to the wavelength range, except the 10 days PAB treatment with significantly lower external phlorotanninin levels.

Total fatty acid content and composition under low vs high PAR and upon UV exposure

Fatty acid profiles are known to change under UV exposure by an increase in lipid peroxidation (61) leading to an increase in short-chained fatty acids and a decrease in poly-unsaturated fatty acids (12-13,62). Nevertheless, UV-related responses are dependent on taxonomy (13), cell-stage (63), nutrient limitation and the UV:PAR-ratio. In contrast, visible light (PAR) influences primarily the content of saturated and polyunsaturated fatty acids (55, references therein, 25-26) but is considered to affect fatty acid composition and content of phytoplankton species specifically (64).

In particular, reactive products found were β-dicarbonyls, α,β-unsaturated aldehydes, 2,4-alkadienals, 4-hydroxy-2 alkenals (65). (24) determined additionally photoproducts derived from arachidonic acid, squalene and linolenic acid: form-aldehyde, acetaldehyde, acrolein, malonaldehyde, n-hexanal, 4-hydroxy-2-nonenal. Some products as e.g malonaldehyde exhibit toxic activities by reacting with biological nucleophiles and crosslinks upon UVR to proteins. The additional covalently binding of FA to nucleic acids (24, references therein) and the decrease in number and synthesis of FA under UV-B exposure (8, 66) may explain the strong decrease of FA content and shift in FA composition upon low PAR and UV-B exposure in our study. (8) observed an overall increase in MUFA and SAFA, especially of the light dependent FA 16:0 (67) while the high susceptible PUFA (16:4(n-1), 18:3(n-3), 20:5(n-3)) decreased about 50%. However, accumulations of short chain SAFA and MUFA as

storage lipid constituents were largely unaffected by UV-B (8) as supported by the present results of *A. esculenta* juveniles.

Decreases in the omega-3 fatty acids 20:5(n-3) and 22:6(n-3) in microalgae upon UV-B exposure were investigated by (12). In each case, the decrease was species dependent and was less in UV tolerant species. In the present study, upon low PAR and UV-B exposure, juveniles of *A. esculenta* showed a decrease of all fatty acids but in particular of 18:1(n-9) 16:0 and 18:0. Upon high PAR and UV-B exposure, the fatty acids 18:0, 18:1(n-9), 18:2(n-6) and 20:5(n-3) decreased dramatically in *A. esculenta* juveniles (Tab. 2). (13) determined an increase in short-chained FA and a decrease in PUFA upon UV exposure. Phospholipids (68) and membrane fatty acids such as 20:5(n-3) and 22:6(n-3) are known to be particularly sensitive to UVR due to a reduced biosynthesis and lipid peroxidation processes (13). A decrease in absolute and relative terms of the membrane FA 20:5(n-3) was especially observed upon high PAR+UV-B in our study. However, total fatty acid content upon UVB-exposure in the present study revealed that under high PAR, total FA content was higher than under low PAR (except sampling day 20). The decrease in total FA from the control to the PA and PAB treatments under low PAR could only be observed on sampling day 5 and 10 under high PAR. Though, additional damaging effects of UV-B + high PAR were surprisingly not observed.

Storage lipids in motile spores seem to fuel swimming after release and germination processes (69). Differences in lipid composition and consumption of various spore species might be related to the swimming behavior, photosynthetic efficiency, and in the light environment inhabited by spores of the various species and can alter the energy budgets of spores and influence the amount of endogenous reserves needed to fuel spore-swimming (69). This is supported by the present study where total FA content in *A. esculenta* spores and gametophytes was several magnitudes higher than in *S. latissima* (Steinhoff et al. unpublished

data). While settlement of *Pterygophora californica* spores was generally reduced under high PAR conditions, settlement decreased in particular with increasing exposure time but not with irradiance (5). No discrepancies in lipid content between algae grown under low PAR and high PAR was observed in *Ulva pertusa* (70). In contrast, our low PAR controls showed an increase in total FA over time while high PAR controls showed a strong decrease in total FA compared to the initial value after release indicating an interactive effect of exposure time and irradiance levels (PAR) in *A. esculenta* as discussed later on. Determination of total FA content and composition in spores and juvenile gametophytes of *S. latissima* obtained under low PAR conditions (Steinhoff et al. 2010, submitted), revealed only minor changes in total FA content but in particular a decrease of 18:1(n-9) over the exposure time of 20 days. In *Ulva fenestrata*, (55) investigated a PAR impact on the ratio of storage lipids (triacylglycerols) to major chloroplast lipids such as glycolipids and phosphatidylglycerol. Relative proportions of FA present in triacylglycerol, monogalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol did not depend on irradiance conditions. Only variations in FA composition of digalactosyldiacylglycerol and phosphatidylglycerol and changes in amount of lipids were responsible for differences in total FA composition among light intensities (55). While the fatty acids 16:0 and 16:4(n-3) showed highest levels under low PAR (24% of incident light), highest levels of 16:0 were measured under high PAR (80% of incident light) as observed also in our study within the first 5 days upon high PAR exposure (without UV treatment).

Our observations suggest that either phlorotannins might play a major role in external rather than in internal cell protection or that intracellular phlorotannin contents might have been diminished immediately after formation due to intracellular oxidation processes. Fatty acid composition and total fatty acid content are strongly affected both by high PAR and

UVR. Changes in lipid content and composition might be considered as an adaptive mechanism of the *A. esculenta* juveniles subjected to variations in solar irradiance. Hence, global climate change and stratospheric ozone depletion might influence viability of early life stages more than assumed. Additionally, the decrease in fatty acid content and composition of kelp juveniles upon UV and high PAR exposure is likely to affect the community level by a decrease in nutritional quality.

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Figure and table legends

Fig. 1 Sum of phlorotannin content in µg phlorotannin ml spore solution^{-1} in the surrounding medium (lower part of the bars) and the zoospores (upper part of the bars) in *Alaria esculenta* at the sampling days 0, 5, 10, 15 and 20 and upon low photosynthetically active radiation (PAR; a), and high PAR (b) exposure. White bars indicate control (PAR only), dark grey bars PAR+UV-A radiation and black bars PAR+UV-A+UV-B radiation. Capital letters show statistical differences of treatments ($p<0.05$). Vertical bars indicate standard deviations (SD=3).

Fig. 2 Trends in total fatty acid content in µg per spore/gametophyte upon low (a) vs high PAR (b) and UV exposure at 5 sampling days (day 0, 5, 10, 15, 20). Trend lines indicate the relation between total fatty acid content and light regimes applied; controls (PAR), PA (PAR+UV-A) and PAB (PAR+UV-A+UV-B).

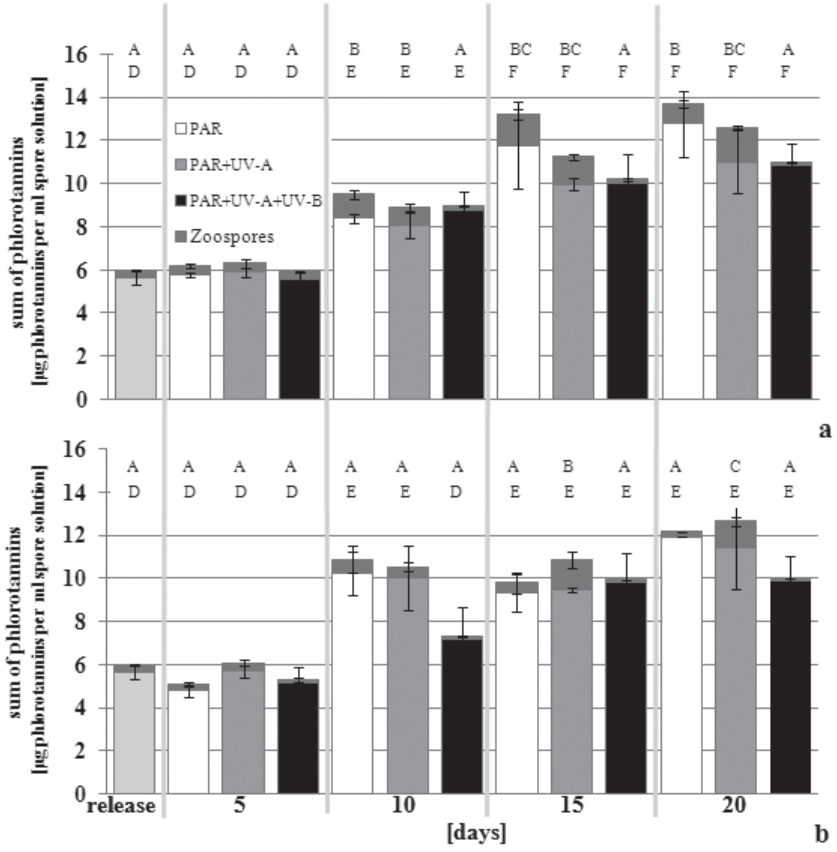
sum of phlorotannins [µg phlorotannins per ml spore solution]
PAR
PAR+UV-A
PAR+UV-A+UV-B
Zoospores
a
b
release
5
10
15
20
[days]

Fig 1

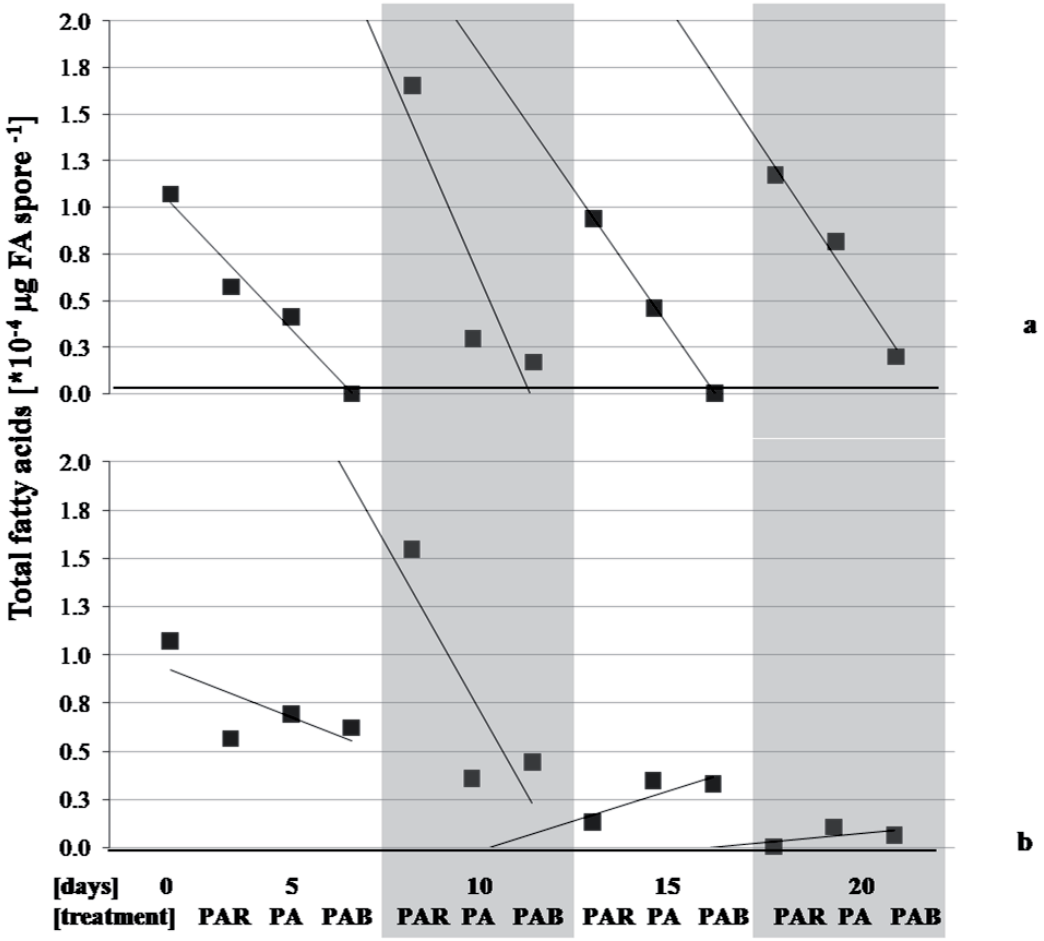
Total fatty acids [*10-4 µg FA spore -1]
2.0
1.8
1.5
1.3
1.0
0.8
0.5
0.3
0.0
a
b
[days] 0 5 10 15 20
[treatment] PAR PA PAB PAR PA PAB PAR PA PAB PAR PA PAB

Fig 2

Tab. 1 Summary of light treatments applied over the exposure period of 20 days. Irradiances are given as mean values (W m^{-2}). For a better comparison of data, UV-A, UV-B and UV_{ery} over the exposure period of 8 h are shown in dose [J m^{-2}], additionally. Ratios of UV:PAR are given for the low PAR laboratory (2.06) and the high PAR field treatment (0.58).

		Irradiances [W m^{-2}]			**Doses [J m^{-2}]**			
	Day	PAR	UV-A	UV-B	PAR	UV-A	UV-B	UV_{ery}
UV:PAR	0							
2.06	5				2.3×10^6	2.3×10^6	9.7×10^4	1.2×10^4
	10	5.5±1.2	10.9±0.4	0.45±0.0	4.7×10^6	4.7×10^6	1.9×10^5	2.4×10^4
	15				7.1×10^6	7.0×10^6	2.9×10^5	3.7×10^4
	20				9.5×10^6	9.4×10^6	3.8×10^5	4.9×10^4
UV:PAR	0							
0.58	5				8.8×10^6	2.5×10^6	1.0×10^5	1.3×10^4
	10	20.6±3.0	11.6±1.9	0.48±0.0	1.7×10^7	5.0×10^6	2.0×10^5	2.6×10^4
	15				2.6×10^7	7.5×10^6	3.1×10^5	3.9×10^4
	20				3.5×10^7	1.0×10^7	4.1×10^5	5.2×10^4

Tab. 2 Total fatty acid (FA) content and proportions of *A. esculenta* spores (day 0) and juvenile gametophytes (day 5-20) under low and high PAR and upon UV exposure (PA and PAB) in µg fatty acids. Total fatty acids (TFA) per spore and relations of SAFA (saturated fatty acids), MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) within the spores and juvenile gametophytes are given in addition.

	Sampling days												
	0	5			10			15			20		
Fatty acid [µg]	initial	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB
LOW PAR													
14:0	834.5	487.1	369.0	-	689.3	268.2	160.4	899.9	481.1	3.7	1012.9	913.3	131.1
16:0	2722.5	1330.0	991.4	-	5082.3	615.6	510.6	1692.4	675.9	11.2	2140.8	1393.6	373.3
16:1(n-7)	77.1	121.9	124.0	-	213.4	118.1	130.8	149.7	106.3	3.0	188.9	282.3	90.4
18:0	1523.1	198.5	274.2	-	6940.4	179.1	188.0	151.0	133.9	5.2	121.8	187.2	102.9
18:1(n-9)	4228.0	1929.6	1362.9	-	2451.8	481.5	327.7	1461.6	541.4	4.3	1576.3	1002.7	192.7
18:2(n-6)	1322.6	755.2	503.5	-	1265.1	140.7	72.5	839.8	272.9	0.8	848.7	469.2	106.9
18:3(n-3)	660.6	554.0	349.8	-	489.5	262.0	99.6	1181.9	675.9	0.4	1518.9	1024.8	257.7
18:4(n-3)	13.8	472.2	310.4	-	722.3	426.5	146.2	2082.2	1119.2	0.4	2857.4	1885.7	448.5
20:4(n-6)	520.1	336.2	194.3	-	339.2	204.4	59.7	1382.0	568.9	0.3	1955.4	1114.7	258.6
20:5(n-3)	1294.2	906.7	546.3	-	519.7	323.1	113.6	1532.8	745.0	0.3	2015.2	1472.0	239.3
TFA per spore $*10^{-4}$	1.07	0.57	0.41	-	1.65	0.29	0.17	0.94	0.46	0.00	1.17	0.82	0.20
SAFA:PUFA	1.33	0.67	0.86	-	3.81	0.78	1.75	0.39	0.38	9.36	0.36	0.42	0.46
SAFA:MUFA	1.18	0.98	1.10	-	4.77	1.77	1.87	1.70	1.99	2.78	1.86	1.94	2.15
MUFA:PUFA	1.13	0.68	0.78	-	0.80	0.44	0.93	0.23	0.19	3.36	0.19	0.22	0.22
HIGH PAR													
14:0	834.5	582.6	288.9	710.0	1632.5	379.7	371.2	140.2	417.8	399.7	8.5	129.4	77.9
16:0	2722.5	1672.6	2182.6	1260.7	2904.1	1088.3	962.7	441.3	619.5	1144.7	26.0	434.7	310.5
16:1(n-7)	77.1	196.6	69.7	143.4	270.5	139.5	160.7	109.0	204.9	156.2	5.1	91.5	24.5
18:0	1523.1	358.9	4415.6	210.3	221.1	269.2	241.8	147.6	142.3	265.3	6.3	181.0	166.7
18:1(n-9)	4228.0	2213.6	894.1	892.6	2364.8	1419.5	694.8	199.8	336.5	1587.5	17.5	155.0	41.7
18:2(n-6)	1322.6	663.6	496.8	553.7	1504.5	273.7	214.7	62.3	160.6	159.8	1.4	39.0	7.1
18:3(n-3)	660.6	546.1	102.9	681.4	1802.7	177.3	413.1	79.1	345.1	53.3	1.0	20.8	6.9
18:4(n-3)	13.8	408.7	93.4	1116.3	3208.0	150.2	426.7	110.3	658.9	38.7	1.6	37.5	5.4
20:4(n-6)	520.1	271.1	43.7	657.9	2109.9	101.0	297.3	60.1	338.6	29.2	0.3	14.2	0.0
20:5(n-3)	1294.2	46.1	79.6	1020.9	2883.0	274.4	579.2	110.4	737.7	76.5	1.5	26.8	16.4
TFA per spore $*10^{-4}$	1.07	0.57	0.69	0.62	1.55	0.36	0.44	0.13	0.35	0.33	0.01	0.11	0.06
SAFA:PUFA	1.33	1.42	8.44	0.54	0.41	1.78	0.82	1.73	0.53	5.06	7.07	5.39	15.51
SAFA:MUFA	1.18	1.08	7.15	2.11	1.81	1.11	1.84	2.36	2.18	1.04	1.80	3.02	8.39
MUFA:PUFA	1.13	1.31	1.18	0.26	0.23	1.60	0.44	0.73	0.24	4.88	3.93	1.78	1.85

Tab. 3 Comparison of fatty acids composition in various developmental stages of *A. esculenta.* Values are given in % of total fatty acids. Fatty acid determination in spores was obtained directly after spore release and in juvenile gametophytes, 20 days after spore release in the present study.

Developmental stage	**Fatty acids in % by weight**									
	14:0	**16:0**	**16:1 (n-7)**	**18:0**	**18:1 (n-9)**	**18:2 (n-c)**	**18:3 (n-3)**	**18:4 (n-3)**	**20:4 (n-6)**	**20:5 (n-3)**
Spores (present study)	6.1	18.0	0.5	6.8	31.8	10.0	5.4	1.4	4.4	11.2
juvenile Gametophytes (present study)	6.6	14.1	1.2	0.8	10.3	5.6	10.0	18.8	12.8	13.2
adult Sporophytes (71)	4.8	10.1	1.5	0.5	6.5	4.2	10.6	27.9	11.9	18.4
adult Sporophytes (72)	4.6	12.7	1.5	1.6	11.3	4.3	9.2	18.6	13.2	10.6

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Physiological responses of polar benthic algae to ultraviolet radiation

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Review

Physiological responses of polar benthic algae to ultraviolet radiation

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Abstract

Stratospheric ozone depletion and the concomitant increase in ultraviolet (UV) B radiation at the earth's surface represent major threats to polar marine ecosystems. Whereas in coastal rocky shore environments macroalgae constitute an assemblage of particular significance to ecosystem function, benthic diatoms dominate microphytobenthic assemblages, which typically grow on shallow-water sediments as highly productive and stabilising phototrophic biofilms. This review summarises present knowledge on how UV radiation affects the physiology of polar benthic algae with an emphasis on cell biological and structural changes, molecular targets and repair mechanisms, induction of reactive oxygen species and antioxidative strategies, photosynthesis and growth, photoprotective mechanisms, interactive effects between UV radiation and other abiotic factors, and finally ecological consequences. Although available data indicate that there are specific characteristics and adaptations in polar benthic micro- and macroalgae that explain their ecological success and limits under environmentally extreme conditions, much more research is needed to understand the underlying mechanisms. In particular, more ecosystem approaches and studies on interactive effects, as well as modern genomic, proteomic and metabolomic approaches could help address all open questions and depict a more holistic picture.

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Keywords: avoidance; DNA repair; growth; interactive effects; life cycle; mycosporine-like amino acids; phlorotannins; photosynthesis; ultrastructure; UV sunscreens.

Introduction

Stratospheric accumulation of ozone is primarily responsible for absorbing parts of the solar ultraviolet radiation (UVR) before it can reach the marine biosphere. Emission of anthropogenic halogenated volatile substances in the past century have – apart from natural sources of these compounds (Laturnus 2001, Laturnus et al. 2002, Gribble 2003) – resulted in a stratospheric enrichment of these compounds, which could persist for many decades. Because of the high chemical reactivity of halogens, they efficiently destroy ozone in the protective layer. This is particularly well reflected in the strong ozone decline over Antarctica each spring, which can amount to more than 75% depletion, a phenomenon known to the public as the 'ozone hole' (Wessel et al. 1998, for details see Whitehead et al. 2000). The Arctic is also currently affected by ozone depletion and consequently increasing UVR (McKenzie et al. 2003, Zacher et al. 2009b).

UVR is differentiated according to the CIE definition (Commission Internationale de l'Eclairage 1935; http://www.cie.co.at) into three wavebands: UVC: 190–280 nm, UVB: 280–315 nm and UVA: 315–400 nm. UVC is strongly mutagenic and lethal to most organisms; however, owing to its complete absorption by the atmospheric ozone layer it does not reach the biosphere. In contrast, UVA is not attenuated by ozone, and hence its fluence will be unaffected by any ozone layer reduction reaching polar organisms. It is the UVB range that increases as a consequence of stratospheric ozone destruction. Although this waveband represents less than 1% of the total solar flux reaching the earth's surface, it is biologically extremely harmful (Franklin and Forster 1997). Calculations indicate that a 10% decline in column ozone would result in an approximate 5% increase in surface irradiance at 320 nm and a 100% increase at 300 nm (Frederick et al. 1989).

Physical and chemical environment in benthic habitats of polar regions

Sea ice is probably a very important factor affecting polar benthic algae, not only with regard to ice-scour (Teixido et al. 2007) but also because benthic algae are stressed during ice break-up in early summer when they become suddenly exposed to very high and stressful photosynthetically active radiation (PAR, 400–700 nm) and UVR

Publication VI

(under revision)

Plant Biology

UVB and temperature-induced reactive oxygen species in gametophytes of three Arctic kelp species (Laminariales, Phaeophyceae)

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UVB and temperature-induced reactive oxygen species in gametophytes of three Arctic kelp species (Laminariales, Phaeophyceae)

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Key words:

Brown algae, life cycle, oxidative burst, reactive oxygen species, temperature, ultraviolet radiation

Abbreviations:

NBT: nitro blue tetrazolium chloride

PAR: photosynthetically active radiation

ROS: reactive oxygen species

SW: seawater

UVR: ultraviolet radiation

Abstract

Stress-induced damages of cell organelles of gametophytes of ecological important, kelp forming brown algae (Laminariales) could have a negative impact on the fitness of following life stages as gametes and sporophytes. This is of special interest since environmental perturbations due to ozone depletion and global warming will strongly increase especially in Arctic regions in the near future. Thus, the present study focuses on gametophytes of three Arctic kelp species *Alaria esculenta, Laminaria digitata* and *Saccharina latissima*, and in particular on their ultrastructural damage and intracellular formation of toxic reactive oxygen species (ROS) after exposure to three radiation conditions (photosynthetically active radiation, UV-A radiation, UV-B radiation). Additionally, extracellular generation of ROS was investigated in gametophytes exposed to three radiation and four temperature (2 – 18 °C) conditions. Ultrastructural damages and the intracellular ROS formation due to UV radiation were minor or negligible in consequently high stress tolerant gametophytes of Laminariales. The observed massive oxidative burst of superoxide anions from gametophytes will be discussed to play an important role in the high cellular stress tolerance of gametophytes. Conclusively, our data indicate that stress tolerant gametophytes of three Arctic kelp species should sustain their crucial function as seed bank for kelp populations even under prospective rising environmental perturbations.

Introduction

Marine brown algae of the order Laminariales (kelps) are distributed along the rocky coastlines of Arctic and cold temperate regions in the Northern Hemisphere (Lüning 1990). They contribute an important part of marine primary production with maximal 1.8 kg carbon fixation m^{-2} $year^{-1}$ by formation of large kelp forests (Thomas 2002). Kelp forests provide e.g. food resources and shelter for various associated marine species, have a substantial impact on wave dampening, and thus avoid shoreline erosion (reviewed in Bischof et al. 2006, Bartsch et al. 2008). Maintenance and renewal of the kelp forests, which are formed by the diploid kelp sporophytes, are based on processes in the haploid microscopic gametophytes. Gametophytes serve as a kind of seed bank because they are able to postpone the formation of gametes until favourable conditions occur (tom Dieck 1993). During the period of endurance, gametophytes can survive high temperatures of more than 20 °C for several weeks and remain fertile under complete darkness for 16 months if tested at a temperature of 8 °C (tom Dieck 1993).

Although gametophytes survive those harsh environmental conditions, a negative impact on the fitness of later developing gametes may be possible. It is known from various marine organisms that environmental perturbations lead to an intra and extracellular increase of reactive oxygen species (ROS) (reviewed in Lesser 2005). Although under sufficient conditions the early quenching of intracellular generated ROS prevents cellular damages, under stress conditions toxic ROS become accumulated in the cells (Lesser 1996, Collen & Davison 2001). If production of intracellular ROS exceeds the antioxidant capacity of cells, ROS become harmful for the genetic material, proteins und membrane lipids (reviewed in Lesser 2005). On the other hand, extracellular generation of ROS plays an important role in stress tolerance of marine macroalgae via cross-linking of cell walls, e,g, in case of pathogen attack (Potin 2008). However, only few data are available about probable ROS formation and

cellular damages in gametophytes of Laminariales under environmental stress (Dring et al. 1996, Küpper et al 2001). Such information is, however, urgently needed to predict the effects of global climate change on kelp forests. Especially in Arctic regions, UVR will highly increase due to a maximal 20 % depletion of the stratospheric ozone layer over the Arctic until 2020 (WMO 2007), whereas the temperature will dramatically raise up about 4 °C in Arctic regions until 2100 (IPCC Report 2007).

Consequently, the main goal of the present study was to determine if gametophytes of Arctic kelp species are affected on the cellular level by conditions mimicking prospective environmental perturbations. Ultrastructural changes and the intracellular formation of toxic ROS were investigated in gametophytes of three key species of Arctic kelp forests (*Alaria esculenta* (L.) Grev., *Laminaria digitata* (Huds.) Lamour., *Saccharina latissima* (L.) Lane, Mayes, Druehl, Saunders) after exposure to three radiation conditions. Additionally, the extracellular ROS production in the medium surrounding gametophytes of Laminariales was studied under three radiation and four temperature conditions.

Material and Methods

Algal material. Gametophytes obtained from freshly released zoospores were used to analyse the ultrastructure of gametophytes and their intracellular production of superoxide anions. Therefore, fertile sporophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* were sampled by SCUBA divers in the Kongsfjorden close to Ny Ålesund (Spitsbergen, Norway, 79 °N) in 4 - 6 m depth in June 2006. Sori of three individuals per species were cleaned with tissue papers and stored in dark, moist chambers for 1 - 2 nights at 2 ± 1 °C. Subsequently sori were stimulated with filtered, 7 °C warm seawater (SW) to release their zoospores. Within ≤ 60 minutes obtained zoospore suspensions were filtered

through 20 µm gauze (Nytal HD 20, Hydro-Bios, Germany). Afterwards zoospore suspensions of three individuals were mixed and adjusted with SW to 2.8 - 4.4 x 10^5 zoospores ml^{-1} whereas zoospore density was assessed under 200 x magnification (Axioplan Neofluar microscope, Zeiss, Germany) with a Neubauer chamber (Brand, Germany). 40 ml of zoospore suspensions were filled into Petri dishes (85 x 10 mm) and placed at 7 °C and 10 ± 3 µmol m^{-2} s^{-1} PAR for 10 days to obtain juvenile gametophytes. Those cultured gametophytes were used for transmission electron microscopic analyses. For light microscopic analysis of intracellular formation of superoxide anions zoospores were allowed to settle down in culture dishes on cover slips to stay there attached until their gametophytic life stage.

In parallel, gametophytes from stock cultures (AWI seaweed collection) of gametophytes of *A. esculenta* (culture number ♀ 3201, ♂ 3202), *L. digitata* (cult. nr. ♀ 3199, ♂ 3200) and *S. latissima* (cult. nr. ♀ 3124, ♂ 3123) from Spitsbergen were used to quantify the extracellular production of superoxide anions as well as to localize ROS in gametophytic cells (only *L. digitata*). Prior their use in experiments male and female vegetative gametophytic tufts were carefully disrupted with mortar and pestle into gametophytic fragments of 1 to 12 cells and suspended in SW. Suspensions with a density of 30 - 50 gametophytic fragments ml^{-1} were dispersed into 24 well Costar® culture plates (100 - 140 settled gametophytes mm^{-2} and 1.5 ml surrounding media per well) and cultivated at 12 °C and 5 ± 2 µmol m^{-2} s^{-1} PAR for two days.

Experimental conditions. The obtained gametophytes were exposed to three different radiation conditions in environmentally controlled chambers at either 7 °C ± 1 °C for microscopic analysis (*L. digitata* and *S. latissima*) or 2, 7, 12 and 18 ± 1.4 °C for extracellular quantification of superoxide anions (*A. esculenta*, *L. digitata* and *S. latissima*) for 8 hours. Three radiation treatments were generated by covering culture dishes with cut-off filter foils admitting wavelengths (1) 400 - 700 nm: photosynthetically active radiation (P, URUV

Ultraphan UV farblos, Difrega, Germany), (2) 320 - 700 nm: P + UV-A radiation (PA, Folanorm SF-AS, Folex GmbH, Germany) or (3) 280 - 700 nm: PA + UV-B radiation (PAB, URT 140 Ultraphan UV farblos, Difrega, Germany). Radiation was generated by fluorescent tubes (36 W true light® II Powertwist, USA, 40 W Q-Panel, USA) whereas PAR amounted 20 µmol m^{-2} s^{-1}, UV-A radiation 5.5 W m^{-2} and UV-B radiation 0.35 W m^{-2} (more detailed in Müller et al. 2008). PAR was measured with a cosine corrected flat-head sensor attached to a LI-COR Li-190 radiometer (LI-COR Biosience, USA) and UVR with a Ramses SAM 80f6 sensor (TriOS Optical Sensors, Germany) linked to a UV-VIS spectroradiometer (IPS 104, TriOS Optical Sensors, Germany).

Transmission electron microscopy. After 8 hours radiation exposure at 7 °C 10 days old gametophytes matured from zoospores were carefully re-suspended and transferred into centrifuge tubes for further treatments. The aldehyde/osmium fixation and the embedding in Spurrs resin followed the method of Steinhoff et al. (2008). Subsequently sections were cut on an ultramicrotome (EM UC 6, Leica, Wetzlar, Germany) with a diamond knife and transferred to 150 mesh copper grids. Samples were stained according to Reynolds (1963) with uranylacetate and lead citrate. 5 - 10 gametophytes per treatment were examined in an EM 109 electron microscope (Zeiss, Oberkochen, Germany). Photographs were taken by a slow scan CCD camera (Proscan, Lagerlechfeld, Germany) and processed with Adobe Photoshop CS2.

Light microscopy. Two hours prior exposure to experimental conditions the 10 days old gametophytes on cover slips were incubated with 40 ml 6 mM NBT- seawater (Nitroblue tetrazolium chloride, Sigma-Aldrich, USA, pH 7.8). Afterwards the medium was replaced by fresh SW, and gametophytes were immediately exposed in duplicates (15 cover slips $replicate^{-1}$, gametophytes from three individuals $cover\ slip^{-1}$) to three radiation conditions at 7 °C. After 8 hours gametophytes on cover slips were kept in an achromatizing solution

(trichloracid 0.15% (w/v) in ethanol/chloroform 4:1 (v/v)) for one hour, in fresh achromatizing solution for extra two hours, and finally stored in glycerine/aqua bidest. (1 : 1 (v/v)). Light microscopic photographs were made from ≥50 gametophytes per treatment under 1000 x magnification (Axioplan 1.4 Oil) using an Axiophot microscope (Zeiss) and the Cell^F program version 2.5 (Build 1163). Generation sites of superoxide anions became apparent in NBT-treated, achromatized gametophytes as blue- purple formazan/diformazan precipitation. Untreated achromatized and pigmented gametophytes of each species were photographed as controls.

Confocal laser scanning microscopy. To determine ROS in gametophytic cells, CM-DCF-DA (Molecular Probes) was dissolved in SW pH 7.2 and added to gametophytes from stock cultures immediately prior exposure (finally 10 μM CM-DCF-DA). After 10 – 20 minutes PAB treatment at 7 °C and 15 minutes incubation in darkness, gametophytes were analysed by confocal laser scanning microscopy (Leica SP2). Excitation wavelength was set to 488 nm while emission was detected between 500 - 570 nm for fluorescence of DCF radicals, and between 650 - 720 nm for chlorophyll fluorescence. The green fluorescent DCF radicals generally develop if diverse ROS react with CM-DCF-DA molecules.

Photometry. Immediately before exposure gametophyte suspensions obtained from stock cultures as well as SW without gametophytes (control) were carefully mixed with 10 mM XTT - SW solution (in SW dissolved XTT (sodium,3′-(1-[phenylamino-carbonyl]-3,4-tetrazolium; Sigma-Aldrich, USA)). After UVR and temperature exposure in eight replicates per treatment, XTT- SW obtained from gametophytes and controls was transferred into 96-well-plates. The absorption of formazan and diformazan as products of superoxide anions and XTT was measured in all specimens at 470 nm using a Spectra Max 190 spectrophotometer (Molecular Devices, USA). Thereby, measured absorption of controls was used as reference. The absolute amount of extracellular superoxide anions produced by gametophytes was

calculated from the referenced absorption and the molar extinction coefficient 2.16 x 10^4 M^{-1} s^{-1} (Sutherland and Learmonth 1997). Statistical analyses of data sets were carried out in accordance with Sokal & Rohlf (1995) using the software Statistica Version 7 (StatSoft, Inc., USA). Effects of irradiation and temperature on oxidative burst of superoxide anions were estimated by a two-factorial, Model I ANOVA (F, $p < 0.05$) subsequent homogeneity of variances (Levene´s test, $p < 0.01$) was proven. In the case of heterogeneous data sets of *L. digitata* and *A. esculenta* square root transformations were accomplished to fulfil the requirements for homogeneity. Finally, multiple mean comparisons were completed with Tukey HSD Post Hoc test ($p < 0.05$).

Results

Ultrastructure of gametophytes

In general, 9 days old primary cells with a length of 8 µm consist of nucleus (N) with nucleolus, chloroplasts (C), vacuoles (V), mitochondria (M) and several lipid globules (L) and are covered by a thin cell wall (Fig. 1). The UV-untreated (P) and the PA-treated gametophytes of *L. digitata* showed an intact chloroplast with some plastoglobules of about 70 nm in size (black dots, arrows Fig. 1 a), whereas the PAB-treated gametophytes contained numerous smaller plastoglobules about 40 - 50 nm in size (Fig. 1 c). Among radiation treatments of *L. digitata*, the difference in the number and size of plastoglobules was, however, not significant. In contrast, plastoglobules of *S. latissima* gametophytes did not show any change in size and amount among the three treatments (Fig. 1 g - i). But they generally exhibited slightly larger plastoglobules (~100 nm) compared to that of *L. digitata* gametophytes. We could not detect disoriented organelle structures neither in *L. digitata* nor in *S. latissima* gametophytes even though the organelles in PAB treatment of *L. digitata*

seemed to be slightly turgid. A general phenomenon especially in *L. digitata* was the appearance of globular cavities in the lipid globules (Fig. 1 a, b).

Intracellular ROS generation

Typical microscopic observations of pigmented, achromatized and NBT-stained gametophytes are displayed in figure 2. In general, intracellular superoxide anions developed mainly in vesicles which were distally or basally located in the primary cells (Fig. 2 g - o). Moreover, a slight NBT-staining of plasma membranes was often detected (Fig. 2 g – o). In addition, the NBT-staining in PAB-treated gametophytes of three species was frequently stronger and appeared mainly in the periphery of the chloroplasts (Fig. 2 m, n, o) if compared to both other radiation treatments (Fig. 2 g - l). Nonetheless, the degree of NBT- staining was not consistent within different radiation treatments since all tiers from non- to relatively strong NBT-staining were observed in each treatment. However, significant DCF radical fluorescence in PAB-treated gametophytes of *L. digitata* revealed the acute intracellular production of ROS (Fig. 3 b). After only 15 minutes PAB-exposure ROS appeared in the cytoplasm, in membranous structures and adjacent to the cell walls and red-fluorescent chloroplasts (Fig. 3 a, b, c).

Oxidative burst

An increase of temperature caused a strong increase of the oxidative burst of superoxide anions from gametophytes of tested species (Fig. 4). The amount of superoxide anions in the medium surrounding gametophytes sharply increased and was highest at 18 °C in *A. esculenta* with 0.1 Mol s^{-1} (slope 0.0024 $x^{2.52}$, $R^2 = 0.99$), followed by *L. digitata* with 0.06 Mol s^{-1} (slope 0.0017 $x^{2.39}$, $R^2 = 0.99$) and *S. latissima* with 0.05 Mol s^{-1} (slope 0.0043 $x^{1.75}$, $R^2 = 0.98$) (Fig. 4). The equations of ascending slopes and their probability (R^2) in brackets are given for the P treatment. The effect of UVR on the oxidative burst of superoxide anions from

gametophytes of three species was minor and interacted strongly with investigated temperatures ($F = 3.1$ (*L. digitata*), $F = 3.9$ (*S. latissima*) or $F = 4.1$ (*A. esculenta*); $df = 6$; $p < 0.01$). At 2 °C a significant higher oxidative burst was elicited from PA- und PAB-treated gametophytes of three species compared to that from P-treated gametophytes as controls ($p \leq 0.04$). In *S. latissima*, however, the oxidative burst of PA-treated gametophytes was not different from both, the control and the PAB treatment at 2 °C ($p > 0.05$). Moreover, the UVR induced oxidative burst of *S. latissima* at 2 °C was as high as all radiation treatments at 7 °C. Nevertheless, the extra impact of irradiation on the quantity of oxidative burst became indistinguishable in the two species *S. latissima* and *A. esculenta* at 7 – 18 °C ($p > 0.05$) (Fig. 4). The oxidative burst from *L. digitata* revealed a more complex pattern with respect to the interactive effects of UV radiation and temperature. At 2 °C the UVR induced increase of extracellular superoxide anions was as high as the control at 7 °C ($p \geq 0.05$). Within the treatments at 7 °C the oxidative burst diverged under P condition from that of the PAB treatment ($p < 0.02$), whilst oxidative burst of both of these treatments differed not from that after PA treatment ($p > 0.05$). At 12 °C the irradiation of gametophytes with PA and PAB radiation resulted in an equally elevated oxidative burst, which was higher than that of the control at 12 °C ($p < 0.03$). But oxidative burst was equivalent in three radiation treatments at 18 °C ($p > 0.05$). In conclusion, significant PA and PAB effects on the oxidative burst from gametophytes revealed at lower temperatures were species-specific masked at elevated temperatures, at least at 18 °C in all of three determined species (Fig. 4). Moreover, if above not explicitly mentioned, for the most part the response to radiation treatments differed amongst different temperatures.

Discussion

Our data reveal a high cellular stress tolerance of gametophytes of Laminariales against UVR since significant ultrastructural changes were not detected in gametophytes of *Laminaria*

digitata and *Saccharina latissima*. Moreover, the formation of intracellular superoxide anions was generally minor in gametophytes of three Arctic kelp species if compared to a study investigated sporophytes of *L. digitata* (Küpper et al. 2001). On the other hand, a massive oxidative burst of superoxide anions from gametophytes was moderately induced by UVR and strongly by increasing temperatures. That observed oxidative burst reaction is supposed to be at least partly responsible for the high stress tolerance of gametophytes of Laminariales against abiotic stress.

Cellular stress tolerance of gametophytic cells against UV radiation

The ultrastructure of the nucleoplasm and mitochondria of the zoospores of *Laminaria hyperborea* was strongly damaged after an exposure to UV-B radiation (Steinhoff et al. 2008). Moreover, plastoglobules emerged in their chloroplast after PAB treatment (Steinhoff et al. 2008). However, in present study scrutinized gametophytes being the following life stage of zoospores of Laminariales did not display any ultrastructural changes, whereas plastoglobules were evident after all radiation treatments (Fig. 1). Plastoglobules are lipoprotein particles inside chloroplasts and may fulfil a major role in the recycling of lipophilic products arising from oxidative metabolism during stress (del Rio et al. 1998, Olmos et al. 2007). An increase in the number of plastoglobules is correlated with plastid development and the "upregulation of plastid lipid metabolism in response to oxidative stress" (Austin II et al. 2006, Lichtenthaler & Tevini 1970).

High lipid content is a particular feature of meiospores, probably fuelling spore movements and spore germination (cf. Reed et al. 1999). The cavities in the lipid globules point to a consumption of this storage compound during germination.

Nevertheless, we could not observe a significantly enhanced plastoglobuli production or its enlargement in UVR-treated gametophytes of *L. digitata* and *S. latissima* (Fig. 1). Directly

associated was the weak NBT-staining and hence low concentrations of intracellular superoxide anions in gametophytes of three Laminariales species at all radiation conditions (Fig. 2) if compared to the intense NBT-staining of epidermal and outer cortical cells of sporophytes of *L. digitata* in response to oligoguluronates (Küpper et al. 2001). Therefore, a low intracellular production of superoxide anions in gametophytes is related to negligible ultrastructural changes in the apparently high stress tolerant gametophytes of the three Arctic Laminariales tested (Fig. 1, 2).

In contrast, not specified ROS were significantly $DCF^{\cdot}$-labelled in the cytoplasm and in the periphery of chloroplasts in gametophytes of *L. digitata* after an exposure to PAB (Fig. 3). A UV-B induced increase of $DCF^{\cdot}$-labelled ROS was correlated with a UV-B-induced chronic photoinhibition in *Fucus spiralis* embryos and in the plankton diatom *Thalassiosira pseudonana* (Coelho et al. 2001, Rijstenbil 2002). Correspondingly, Dring et al. (1996) demonstrated a reduced maximum quantum yield (Fv/Fm) of gametophytes of *L. digitata, S. latissima* and *L. hyperborea* from Helgoland to ≤10 % after eight hours PAB treatment if compared to UVR-untreated controls. Though a recovery from PAB treatment for one day allowed gametophytes to recover their Fv/Fm to 70 % (*S. latissima*), 60 % (*L. hyperborea*) or only 10 % (*L. digitata*) if compared to controls (Dring et al. 1996). Thus, the general linkage of ROS production and chronic photoinhibition may also take place in PAB-treated gametophytes of Laminariales from Spitsbergen, at least in gametophytes of *L. digitata* exhibiting a strong DCF radical fluorescence (Fig. 3) and the most UV-B impairment of photosynthesis after one day recovery (Dring et al. 1996).

Role of oxidative burst for stress tolerance of gametophytes

An oxidative burst of ROS is a crucial element in algal immunity against pathogens and initiates oxidative cross-linking of cell wall material, and thus strengthens more fluid membranes under stress conditions (Potin 2008 and references therein). Further on, the extent

of oxidative burst reactions of the red algae *Chondrus crispus* was related to the inherent stress tolerance of different life stages (Bouarab et al. 1999). Tolerant gametophytes of *C. crispus* responded to sulphated oligosaccharides with a massive oxidative burst, but more vulnerable tetrasporophytes of *C. crispus* with an only minor burst reaction in the same experiment (Bouarab et al. 1999). However, the natural resistance of gametophytes of *C. crispus* vanished if oxidative burst- induced lipooxygenases were inhibited (Bouarab et al. 1999). Hence, the extraordinary cellular stress tolerance of gametophytes of Laminariales against UVR at 7 °C (Fig. 1) might be related to the oxidative burst of superoxide anions from the gametophytic cells (Fig. 4). But conversely to *C. crispus*, only the sporophytes of *L. digitata* elicited a significant oxidative burst in response to oligoguluronates, but not their gametophytes (Küpper et al. 2001). If the relation of a significant oxidative burst and a high stress tolerance is in general true, the results of Küpper et al. (2001) would consequently indicate that sporophytes of *L. digitata* are more tolerant than its gametophytes. Accordingly, Dring et al. (1996) and Véliz et al. (2006) reported for Laminariales a decreasing UV-B tolerance from adult sporophytes to juveniles and gametophytes.

In contrast, the massive temperature- induced oxidative burst of gametophytes of three Arctic Laminariales (Fig. 4) correlates with a high thermal tolerance of gametophytes whose upper survival limit exceeds that of sporophytes to 1 - 7 °C (tom Dieck 1993). Moreover, the increase of oxidative burst correlates with increased growth rates of gametophytes of Laminariales from Helgoland (Lüning 1980, Lee and Brinkhuis 1988), all evaluated under rising temperatures up to 17 – 18 °C. An improved vegetative growth of gametophytes points to an improved stress tolerance since multicellular gametophytic tufts are typically formed in laboratory cultures to continue their existence under suboptimal conditions for many years (Kain 1964). Besides, male and female fertility of (only) PA-treated gametophytes of *L. digitata* from Helgoland increased up to 18 °C (Müller et al. 2008) comparable to the general

increase of oxidative burst in our study. The positive correlation of enhanced vegetative growth as well reproduction with the increasing oxidative burst of ROS of gametophytes up to 17 – 18 °C implies that the oxidative burst reaction may be an important tool for the thermal stress tolerance of gametophytes of Laminariales.

Along these lines, our data indicate that the highly stress tolerant gametophytes of Arctic kelp populations may successfully continue with their functions during the algal life cycle, and thereby supporting the persistence of kelp forests under increasing environmental pressures due to global climate changes. Nonetheless, our laboratory study on the ROS formation in microstages of Laminariales has a pioneering character. The research of ROS formation as well as its elimination in various life stages of ecologically important Laminariales, and particularly under the impact of two or multiple stress factors under field conditions merits definitely further attention.

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Figure descriptions

Fig. 1. Electron- microscopic photographs of 10 days old gametophytes of *L. digitata* (**a - f**) and *S. latissima* (**g - l**) after irradiation with P (400 - 700 nm, **a, d, g, j**), PA (320 - 700 nm, **b, e, h, k**) or PAB (280 - 700 nm, **c, f, i, l**) for 8 hours. **A - c** and **g - i** show the entire gametophytes whether **d - f** and **j - l** focus on one chloroplast exemplarily. Plastoglobuli in the chloroplasts are highlighted by black arrows. C = chloroplast, L = lipid globule, N = nucleus, V = vacuole, scale bar 2 μm (a-c and g-i) or 500 nm (d-f and j-l).

Fig. 2. Typical light- microscopic observations of pigmented, achromatized and NBT-stained/achromatized, 10 days old gametophytes of *Alaria esculenta* (**a, d, g, j, m**), *Laminaria digitata* (**b, e, h, k, n**), and *Saccharina latissima* (**c, f, i, l, o**). Blue/purple staining in de-pigmented gametophytic cells (see i. a. arrows in **g - o**) illustrates the precipitation of formazan/diformazan by the reaction of NBT with superoxide anions after 8 hours irradiation with P (400 - 700 nm, **g – i**), PA (320 - 700 nm, **j – l**) or PAB (280 - 700 nm, **m – o**) treatment. Naturally pigmented (**a – c**) and non-stained, achromatized gametophytes (**d – o**) are presented as references. Scale bar 10 μm

Fig. 3. Typical confocal laser scanning- microscopic photograph of 8 hours PAB exposed gametophyte of *Laminaria digitata* from stock culture. **a)** Red colour illustrates the auto-fluorescence of the chloroplasts. **b)** Green colour developed if non-fluorescent DCF-DH reacted with gametophytic produced hydrogen peroxide towards fluorescent DCF radicals. **c)** Overlay of auto- and $DCF^{\cdot}$-fluorescence pattern of gametophytes. Scale bar 20 μm

Fig. 4. Absorbance of (di-) formazan at 470 nm represents the oxidative burst of superoxide anions from gametophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* (n = 8). Absorbance of (di-) formazan measured in the medium of UVR- and temperature-treated gametophytes was referenced by UVR and temperature exposed seawater (control).

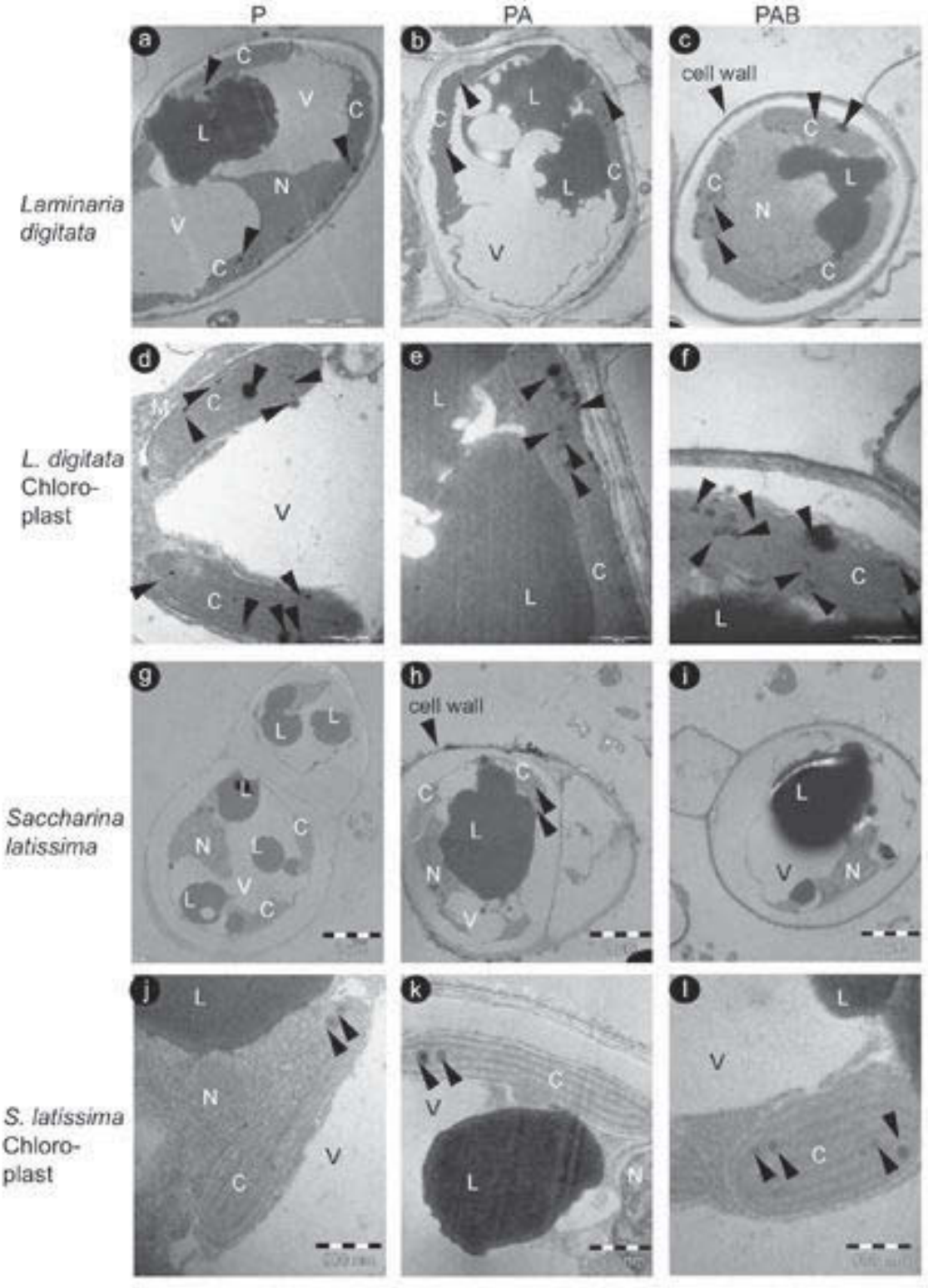
P
PA
PAB
Laminaria digitata
L. digitata Chloro-plast
Saccharina latissima
S. latissima Chloro-plast
cell wall
cell wall
a
b
c
d
e
f
g
h
i
j
k
l

Fig. 1

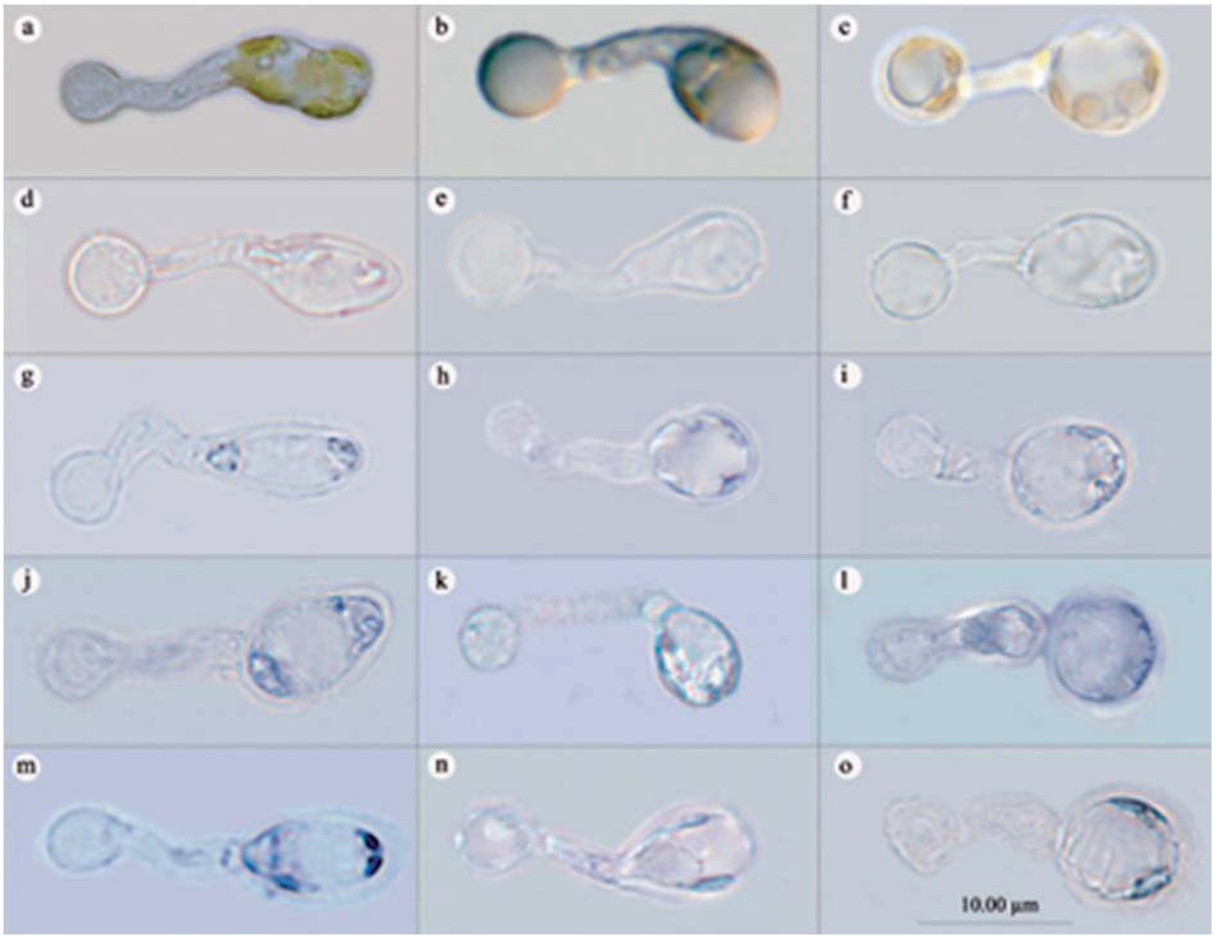

Fig. 2

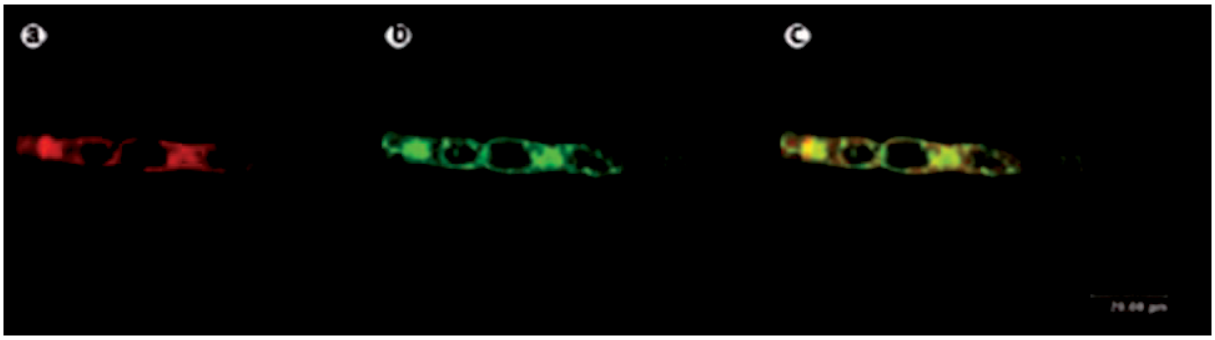

Fig. 3

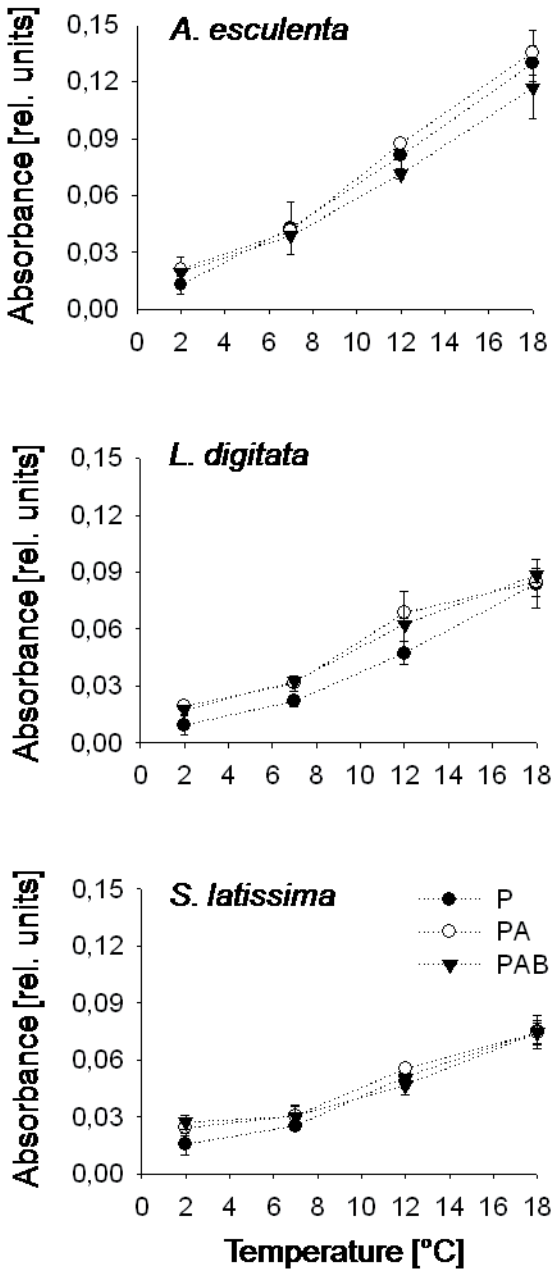
A. esculenta
L. digitata
S. latissima
P
PA
PAB
Absorbance [rel. units]
Temperature [°C]
0,00
0,03
0,06
0,09
0,12
0,15
0 2 4 6 8 10 12 14 16 18

Fig. 4

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Erklärung gemäß § 6 (5) der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007

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