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Phlorotannins as UV-protective Substances in early developmental Stages of Brown Algae

Phlorotannine als UV-Schutzsubstanzen in frühen Entwicklungsstadien von Braunalgen

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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

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This publication is a slightly altered version of a cumulative dissertation conducted at the Alfred-Wegener-Institute for Polar and Marine Research, at the University of Bremen and at the Bremen International Graduate School for marine Sciences (GLOMAR) and was submitted 2010 to the Department of Biology and Chemistry at the University of Bremen.

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

Abbreviations

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 temperierten und arktischen Regionen. Mikro- und Makroalgengemeinschaften stellen eine dominante Gruppe der benthischen Küstenökosysteme 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 stratosphärische Ozonschicht absorbiert. Jedoch hat der Ozongehalt der Atmosphäre aufgrund des Ausstoßes von synthetischen Fluorchlorkohlenwasserstoffmolekülen in den letzten 50 Jahren signifikant abgenommen. Im gleichen Zeitraum erhöhte sich die mittlere globale Temperatur durch den sogenannten Treibhauseffekt. Diese globalen Umweltveränderungen könnten Ökosysteme in einem bisher unbekannten Ausmaß beinträchtigen. Polare Ökosysteme zeichnen sich durch eine besondere Sensitivität 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 insbesondere auf Makroalgengemeinschaften hervorrufen. Effekte auf zellulärer Ebene können mit Effekten auf der Gemeinschaftsebene verknüpft sein. Bei einer weiteren Zunahme von UV-B Strahlung, könnte sich die Artzusammensetzung innerhalb der

Gemeinschaftsebene und die artbezogene Tiefenverteilung (vertikale Zonierungsmuster) im 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 Strahlungsregimes und Strahlungsstärken, Temperaturexperimente und Expositionszeiten miteinander zu vereinen. Für ein umfassendes Verständnis der Effekte auf einige juvenile arktische Makroalgen von Spitzbergen (Kongsfjorden), wurde 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* Sporen. 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 und 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 Starklichtexposition sank der Gesamtfettsäuregehalt um 50-75% in allen Behandlungen und veranschaulicht somit die unter-schätzten Effekte von Starklicht auf junge Entwicklungsstadien.

Zusammenfassung

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 Lipidperoxidation, 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 verfügbar 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 Milieu von größerer Bedeutung sein, da sie physikalisch gesehen weniger geschützt gegenüber UV-Schäden sind und die Konzentrationen von chromophorischem gelöstem organischem Material gering und die UV-Durchlässigkeit hoch sind. 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 (2012). Lipid content and fatty acid biosynthesis in zoospores/ developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential fuel for phlorotannin production. *Polar Biology* 34 (7): 1011-1018
II	**Steinhoff FS**, Wiencke C, Wuttke S and Bischof K (2011). Effects of water temperatures, UV radiation and low versus high PAR on phlorotannin content and germination in zoospores of *Saccorhiza dermatodea* (Tilopteridales, Phaeophyceae). *Phycologia* 50 (3): 256-263
III	**Steinhoff FS**, Bischof K, Wuttke S and Wiencke C. Is a changing environment affecting phlorotannin production and germination in Arctic kelp zoospores? Manuscript
IV	**Steinhoff FS**, Graeve M, Bartoszek K, Bischof K and Wiencke C (2012). Phlorotannin production and lipid oxidation as a potential protective function against high photosynthetically active and UV radiation in gametophytes of *Alaria esculenta* (Alariales, Phaeophyceae). *Photochemistry and Photobiology* 88 (1): 46-57
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 (2012). UVB and temperature-induced reactive oxygen species in gametophytes of three Arctic kelp species (Laminariales, Phaeophyceae). *Phycological Research* 60: 27-36

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).

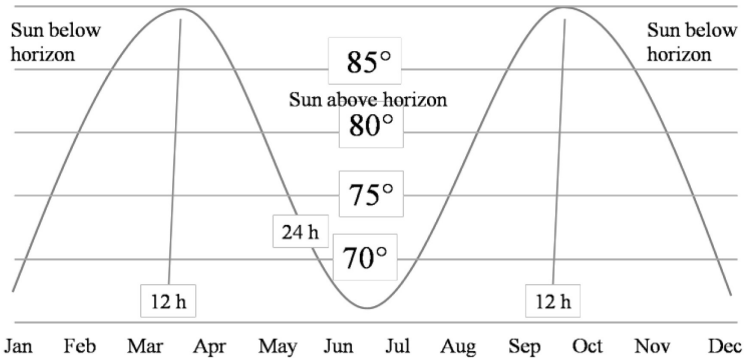


Fig 1 Daylength north of the Polar circle in relation to latitude (modified after Lüning 1990). Polar day at 80°N last from mid-April to end of August while Polar night lasts from mid-October to mid February. Between mid-February and mid-April and early-September and mid-October twilight conditions occur.

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 led 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).

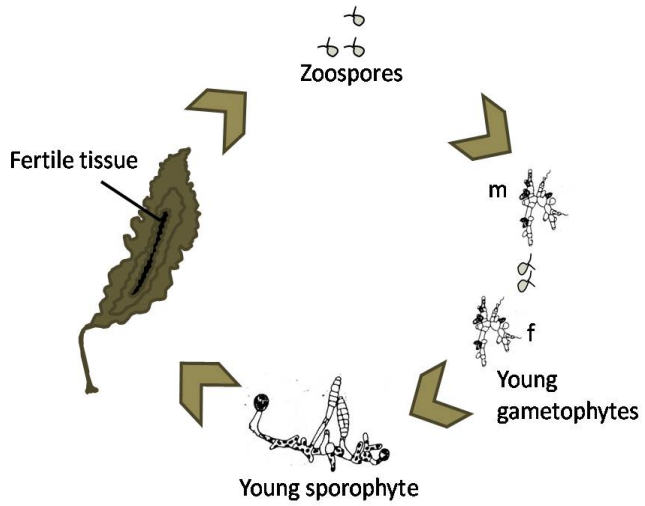


Fig 2 Developmental cycle of the brown alga *Saccharina latissima*. Microscopic zoospores are released by the fertile tissue of the parental algae into the water, settle and grow into juvenile female (f) and male (m) gametophytes. Gametophytes release gametes, become fertilized and the zygote develops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

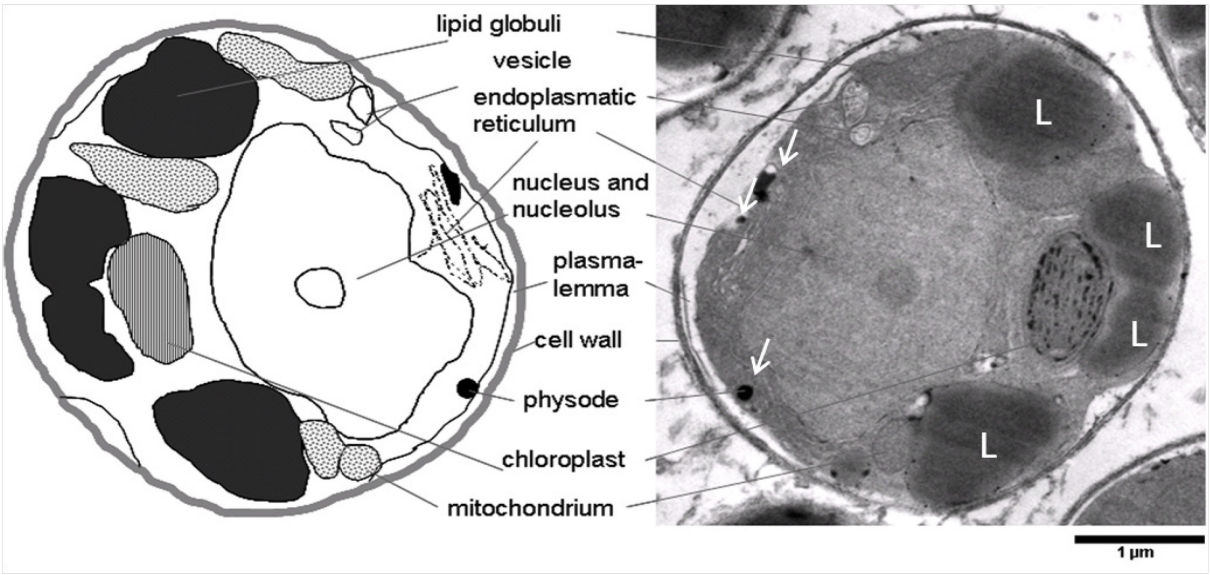


Fig 3 Electron micrograph of a *Laminaria hyperborea* zoospore 8 h after release (modified after Steinhoff et al. 2008). White arrows indicate phlorotannin containing physodes and white L lipid globules.

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).

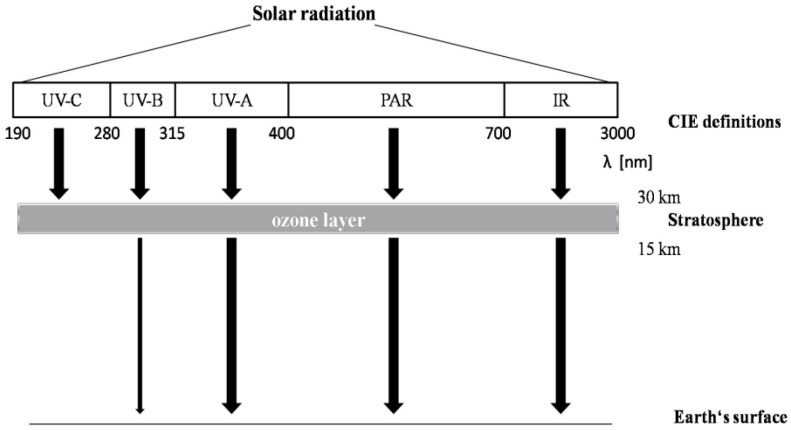


Fig 4 Solar radiation spectrum reaching the Earth's surface. By the ozone layer, short and high energetic wavelengths are filtered out of the solar spectrum. Wavelengths (λ) are given according to CIE

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.

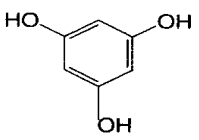


Fig 5 The phlorotannin monomer phloroglucinol (1,3,5-trihydroxybenzene).

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*	Connan et al. 2006, 2007
	-/v	*Dictyota cervicornis, Dictyota ciliolata, 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
		Alaria esculenta, Laminaria digitata,	Roleda et al. 2006a
Screening capacity	+	*Saccorhiza dermatodea*	
		Alaria esculenta, Laminaria digitata,	Müller et al. 2009
	+	*Saccharina latissima*	
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*	
	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 eco-system. 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).

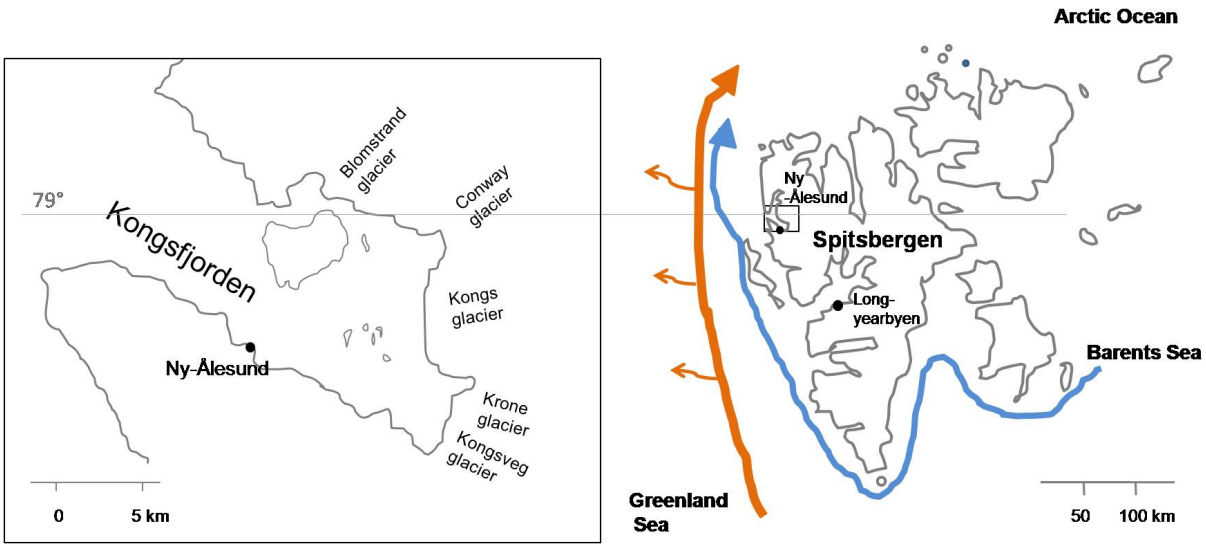


Fig 6 Study area Kongsfjorden in the North-West of Spitsbergen. Kongsfjorden is influenced by the West Spitsbergen Current (WSC, red arrow) from the West, the Arctic-type coastal water (blue arrow) and from glacier ice and their melt water from easterly directions.

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).

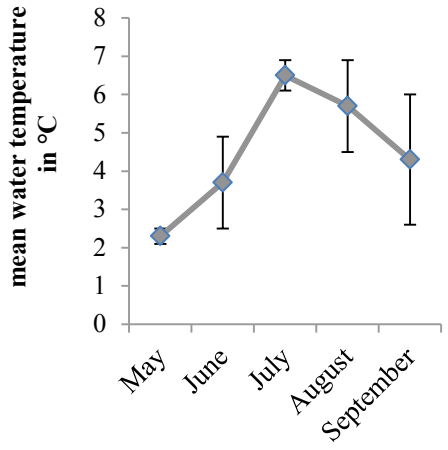


Fig 7 Mean water temperature [°C] in Kongsfjorden during the summer season (Ny-Ålesund, Spitsbergen, Norway). Data modified after Brey (2009).

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

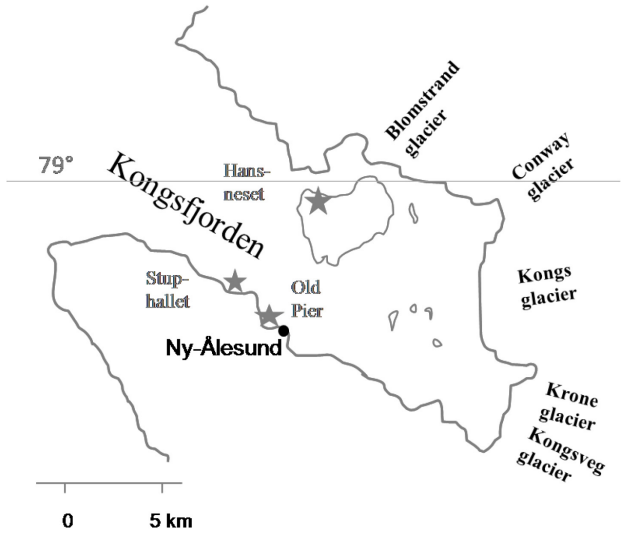


Fig 8 Algae collection sites in Kongsfjorden. Fertile algae were collected at Hansneset, Stuphallet and the Old Pier as indicated by grey stars.

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

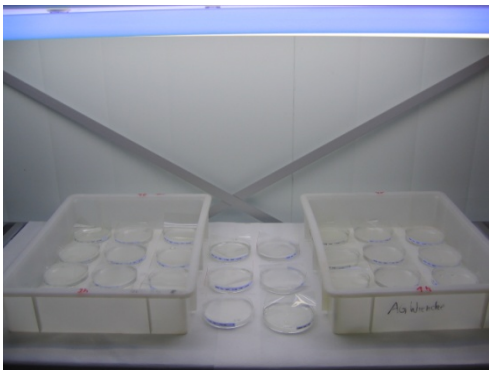
2.2. Experimental design, set-up and investigated parameters

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	Review Polar benthic algae	8 h/10 d
Temperature tested	7°C	2,7,12,17°C	2,7,12,17°C	7°C		2,7,12,18°C
PAR low	•	•	•	•		•
PAR high		•	•	•		
UV-A		•	•	•		•
UV-B		•	•	•		•
Phlorotannins Spores+ Filtrate	•	•	•	•		
Fatty acid composition	•			•		
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.

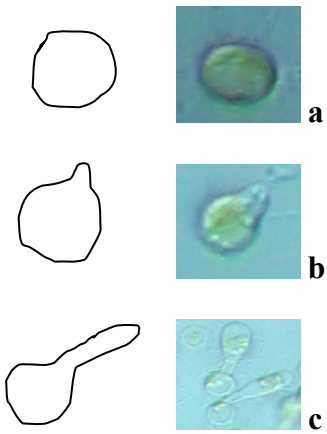


Fig 10 Determination of non-germinated (a) and germinated (b,c) spores. Spores exhibiting a bulge structure or a germ tube (b) were considered as germinated. Modified after Steinhoff (2006).

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

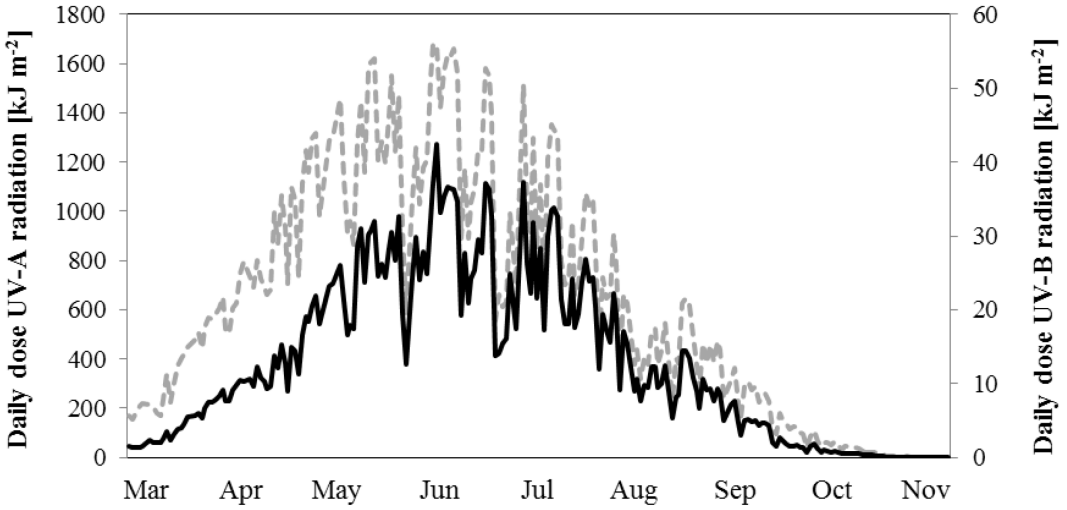


Fig. 11 UVR measurements (in air) during the Arctic summer season 2007 in Ny-Ålesund. Daily UV-A (dashed grey line) and UV-B dose in kJ m^{-2} (black solid line, secondary axis).

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).

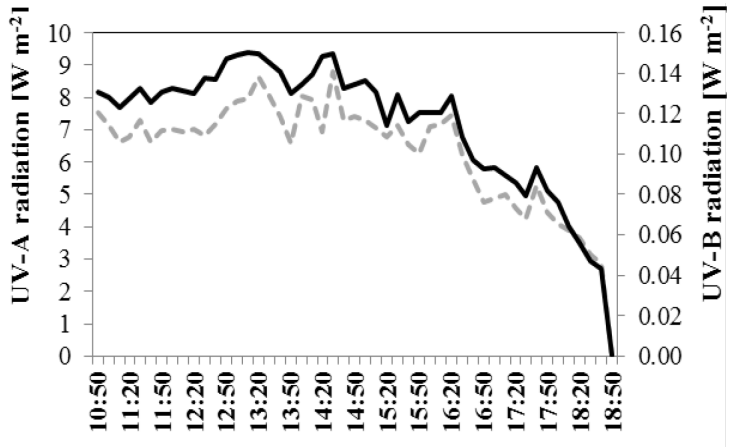


Fig. 12 Underwater UV irradiances [W m^{-2}] measured with an UVR Logger on the 10th June 2009 exemplarily for a 8 h exposure. UV-A (dashed grey line) and UV-B (black solid line) were measured in 3 m water depth of Kongsfjorden (in front of the harbor, Ny-Ålesund). Attenuation of UVR can be explained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

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.

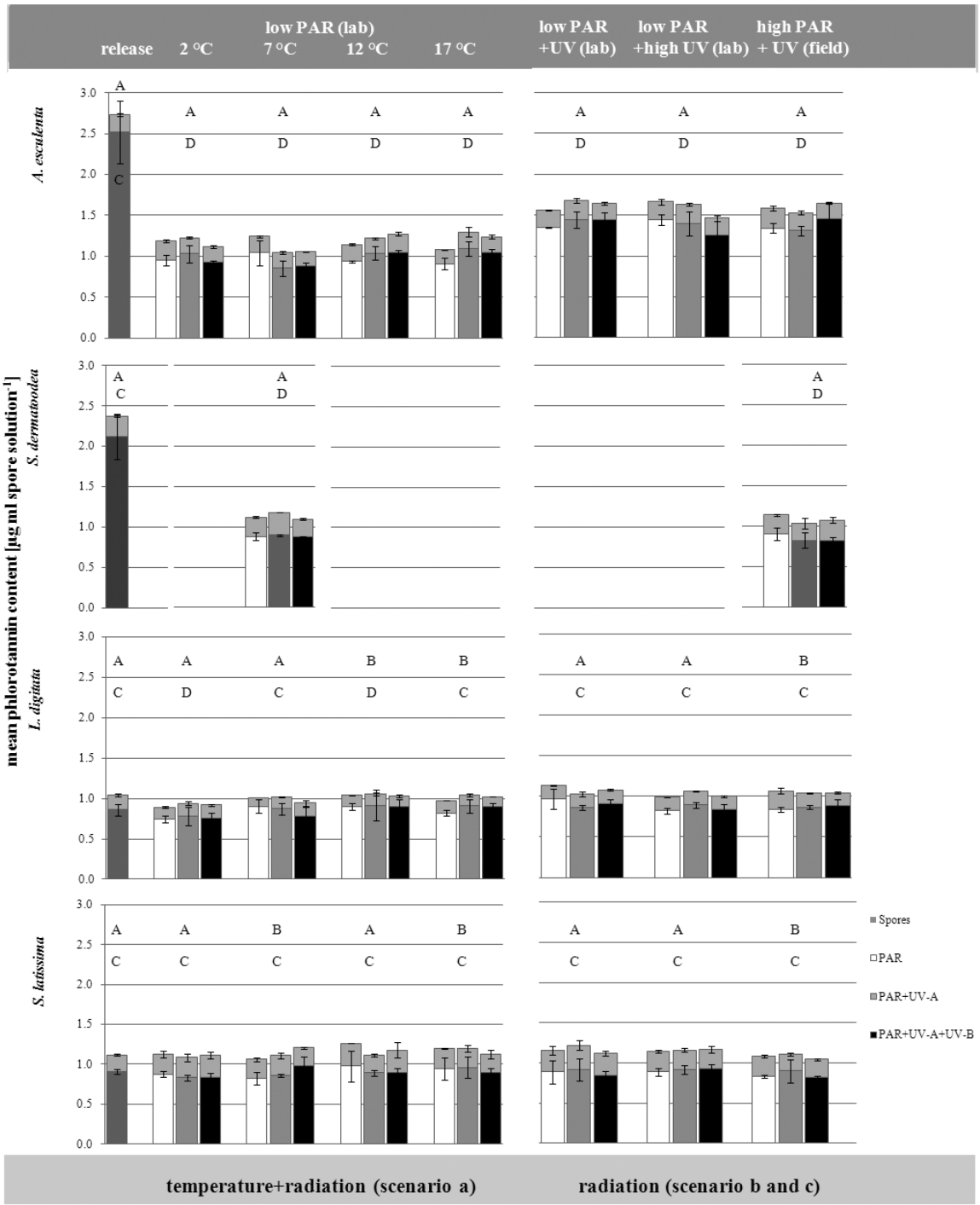


Fig. 13 Sum of phlorotannins in zoospores (upper part of the bars) and filtrates (lower part of the bars) in 4 investi-gated zoospore species: (1) *A. esculenta*, (2) *S. dermatodea*, (3) *L. digitata* and (4) *S. latissima* displayed as an inter-active parameter of various temperatures (2-17°C) and 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.

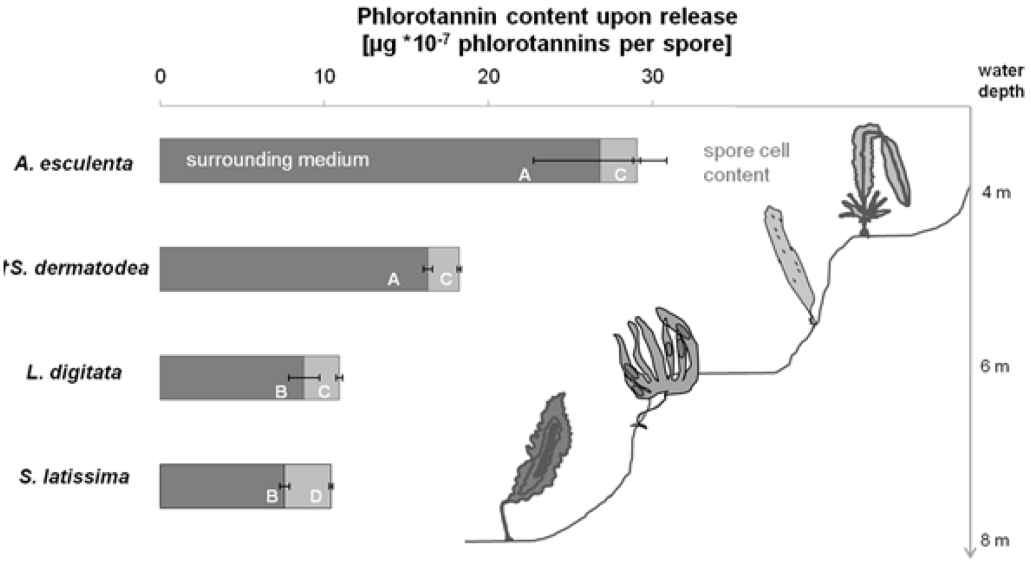


Fig. 14 Summary of phlorotannin content during release within zoospores and the surrounding medium (filtrate) in correlation with collection depth of the parental algae (Publ. II and III; Figure modified after Publ. III). The shallow water species *A. esculenta* and *S. dermatodea* released significantly 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 (FA) composition patterns. In Fig. 15, a summary of relative major 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*.

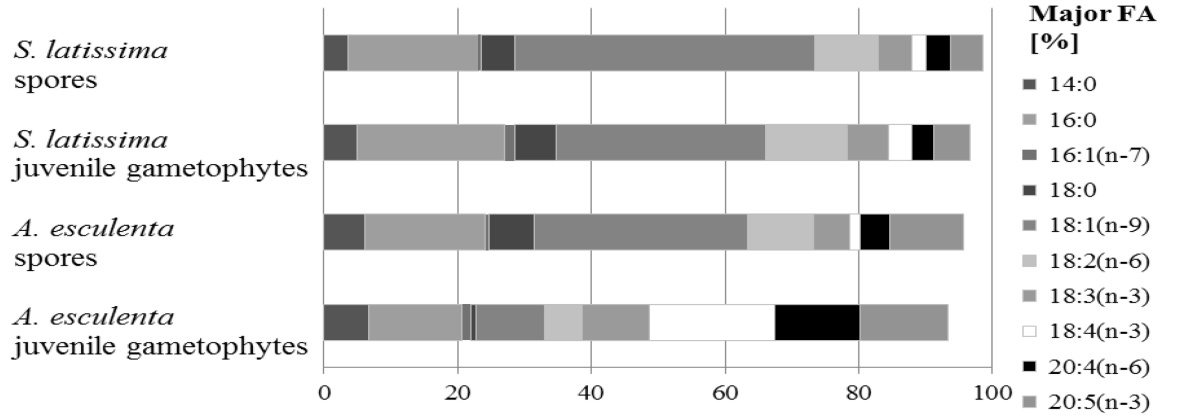


Fig. 15 Summary of major fatty acid (FA) composition in % of *S. latissima* and *A. esculenta* spores and juvenile gametophytes (Publ. I and IV) displayed as sums of FA.

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.

Tab. 5 Summary of fatty acid ratios: saturated to polyunsaturated FA (SAFA:PUFA), saturated to monoun-saturated FA (SAFA:MUFA) and monounsaturated fatty acids to poly-unsaturated 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

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*.

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).

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.

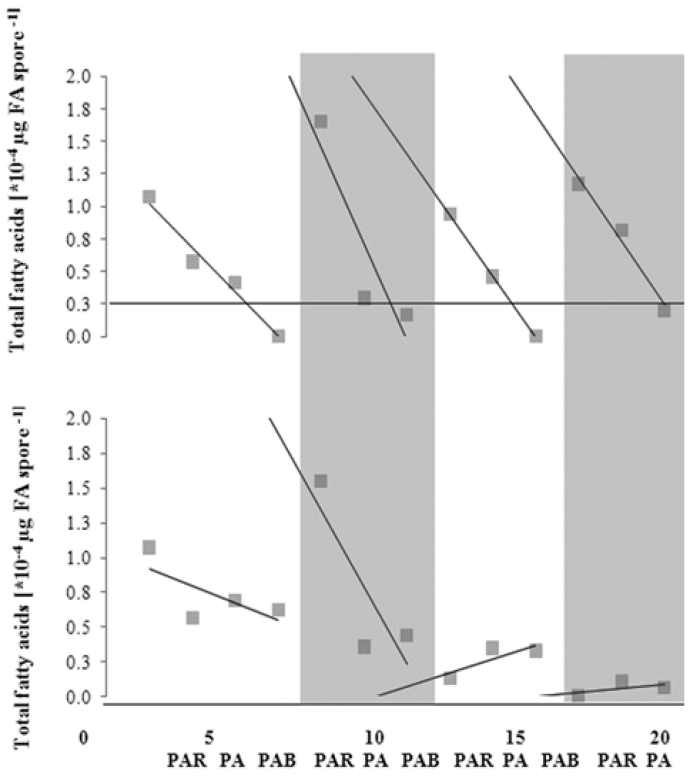


Fig. 16 Total fatty acids per *A. esculenta* spore upon UVR exposure and low PAR (a) vs high PAR (b) treatment. Black lines indicate trend lines.

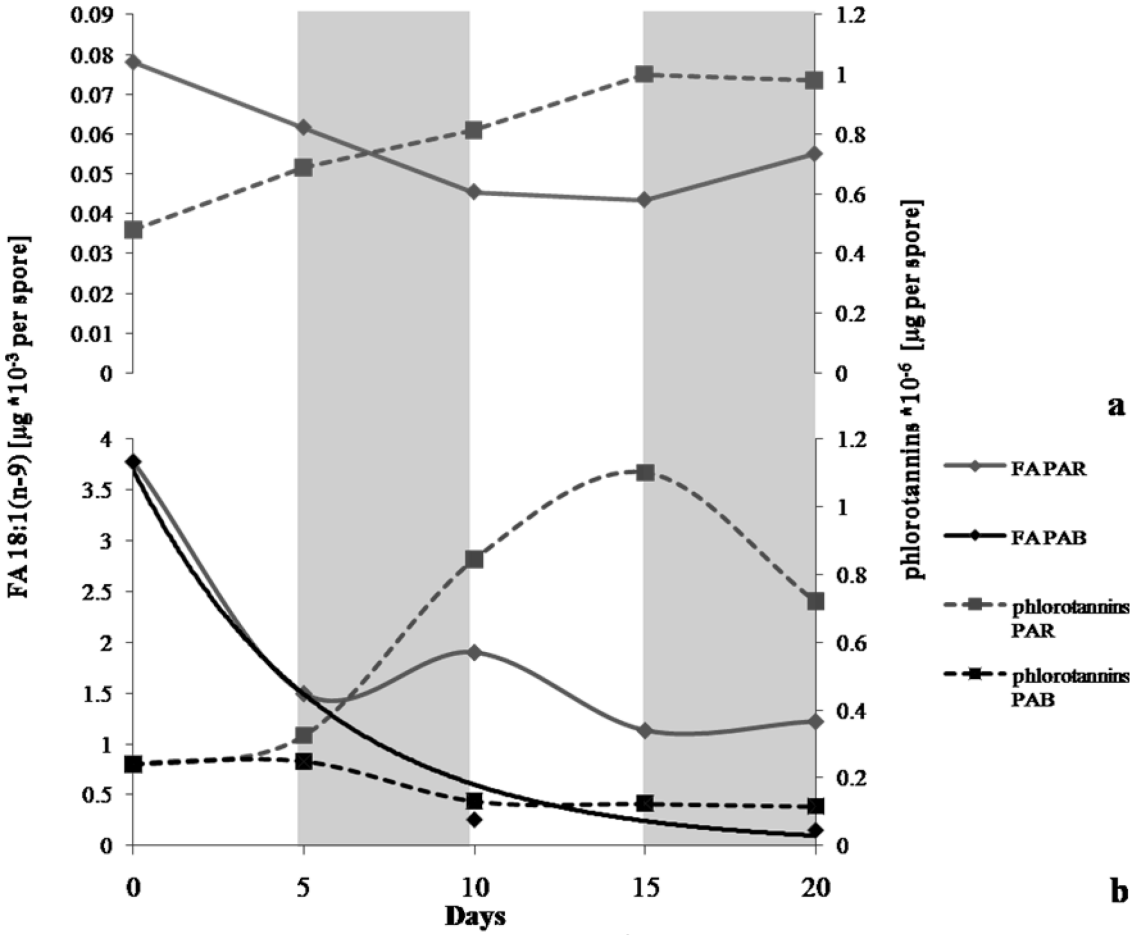


Fig. 17 Correlation between fatty acid 18:1(n-9) [µg FA*10^{-3} per spore] and exuded phlorotannins [µg phlorotannin *10^{-6} per spore] over 20 days in (a) *S. latissima* – Publ. I and (b) *A. esculenta*- Publ. IV. FA 18:1(n-9) is indicated by grey (PAR treatment) and black (PAB treatment) solid lines 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.

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).

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

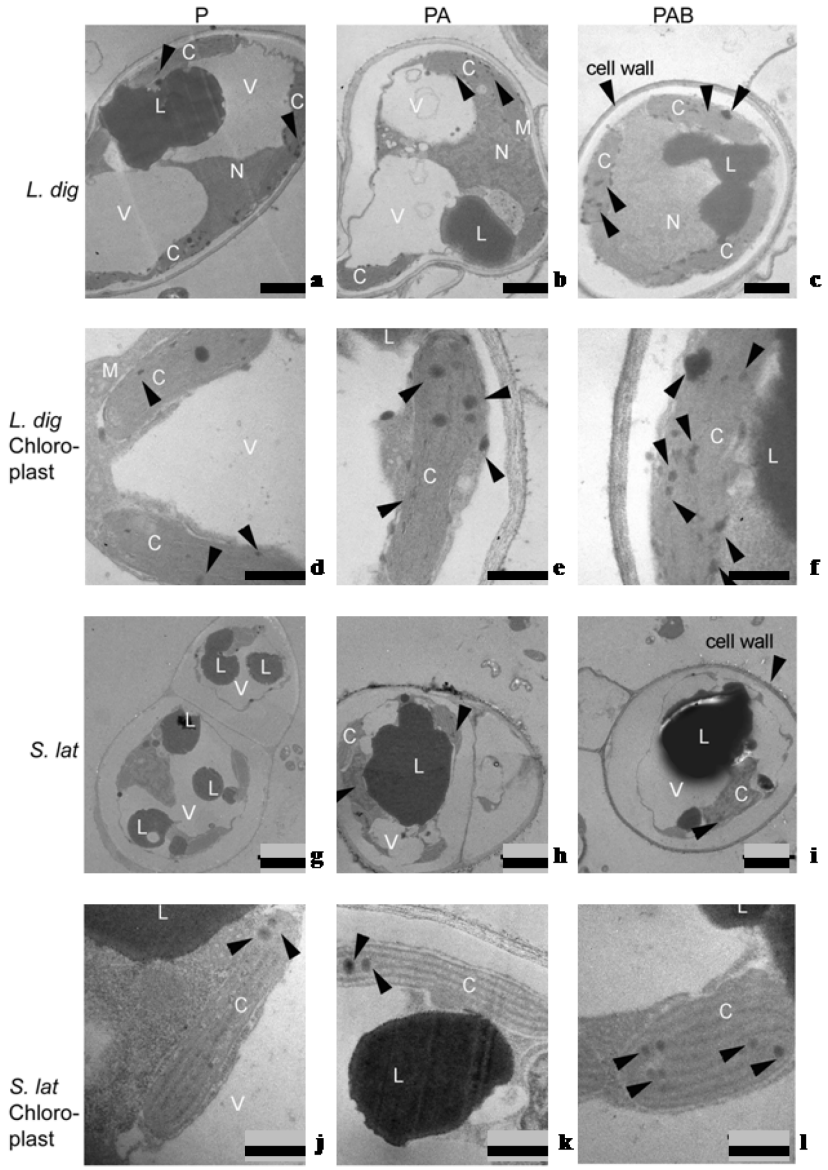


Fig. 18 Electron- micrographs of 10 days old gametophytes of *L. digitata* (a - f) and *S. latissima* (g - l) after irradiation with PAR (a, d, g, j), PA (b, e, h, k) or PAB (c, f, I, l) for 8 hours. A - c and g - i show the entire gametophytes (scale 2 µm) while d - f and j - l focus on one chloroplast 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.

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).

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 distinguish 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 therefore 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 xanthophyll-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). Nevertheless, 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 de-

velopmental 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 feedback stress responses as formation of ROS (Bischof et al. 2002) and anti-oxidants (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 undertaken 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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Original Publications

Publication I

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Lipid content and fatty acid consumption in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential precursors for secondary metabolites as phlorotannins

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ORIGINAL PAPER

Lipid content and fatty acid consumption in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential precursors for secondary metabolites as phlorotannins

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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 Ny-Ålesund (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 1.5×10^{-7} µg and 1.9×10^{-7} µg in the surrounding medium. Upon release, the total amount of lipids was 1.76×10^{-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 in 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 4.0×10^{-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 h (Reed et al. 1992, 1999). In the release stage, most zoospores among the kelp family are equipped with only one or two chloroplasts (Loiseaux 1973; Henry and Cole 1982a; 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 (Brzezinski et al. 1993; Bartsch et al. 2008). Nevertheless, "the ultimate fate

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of lipid reserves in macroalgal spores and gametes is unknown" (Brzezinski et al. 1993).

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 and Carmeli 1990; Napolitano 1994) 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; Brzezinski et al. 1993), hitherto only the adult stages of brown algae have been investigated (e.g. Jamieson and Reid 1972; Harwood 1984; Smith and Harwood 1984; Araki et al. 1987; Jones and Harwood 1992; Harwood 1998; Gerwick et al. 1993; Thompson 1996; 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; Steinberg and van Altena 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) changes in certain fatty acids and/or fatty acid composition might indicate the fate of fatty acids in the spore metabolism especially as secondary metabolites.

Materials 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, pre-combusted 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

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 first embedded in agar and later in SPURR's resin. Subsequent to the polymerization 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). Afterward, the spore extracts were combined, freeze-dried, and re-dissolved in 1 ml of 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 of 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 FAMEs). 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 × 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 and Rohlf 1995), a two-way ANOVA, and by applying a Post hoc test (Tukey-HSD) of pairwise comparison to test differences between 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 between treatments were tested with a Post hoc test (Tukey–HSD test) and Kruskal–Walis test.

Results

Ultrastructure of germinating zoospores and lipid bodies

A zoospore of *S. latissima* 8 h after release is shown in Fig. 1a. 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 (after 10 days). In Fig. 1d, a scheme of this process is shown.

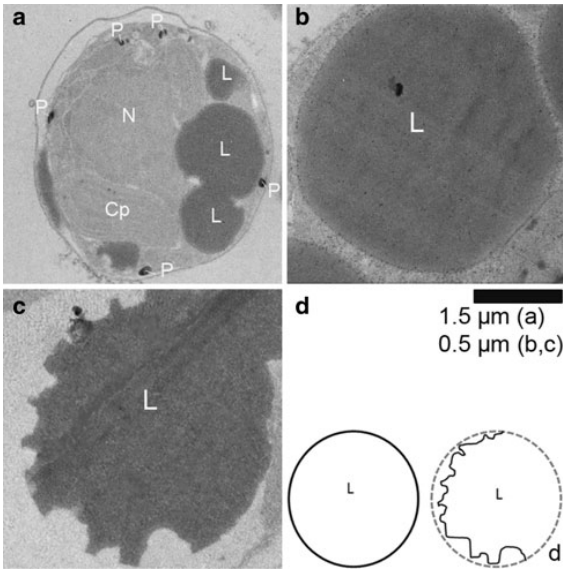

Fig. 1 **a** *Saccharina latissima* zoospore 8 h after release. *Cp* chloroplast, *L* lipid body, *N* nucleus, *P* physode containing phlorotannin. **b** Intact lipid body 8 h after release. **c** Lipid body after 10 days of development. **d** Schematic drawing of the lipid consumption process. *L* indicates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

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 $1.53 \pm 0.03 \times 10^{-7}$ µg phlorotannin zoospore/gametophyte^{-1} (Fig. 2), leading to averaged phlorotannin contents of $1.42 \pm 0.08 \times 10^{-7}$ 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 $\times 10^{-7}$ phlorotannin zoospore/gametophyte^{-1}. Statistical analyses showed significant differences in 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

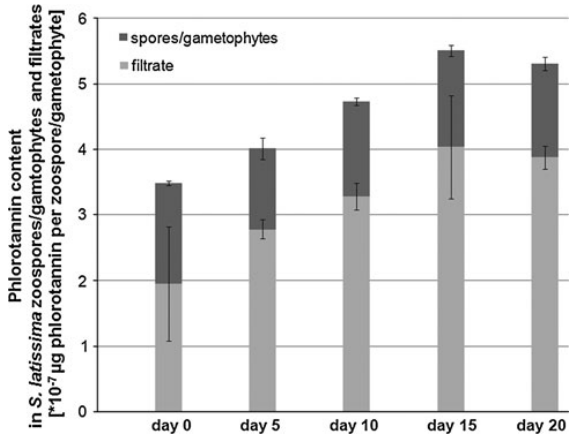

Fig. 2 Sum of phlorotannin content within zoospores/gametophytes and filtrates in µg × 10^{-7} phlorotannins zoospore/gametophyte^{-1} of *S. latissima* over the exposure period of 20 days. Within the filtrate, significant differences in phlorotannin content were observed between day 0 and day 15 and day 0 and day 20. Vertical bars indicate standard deviations (SD = 3)

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 × 10^{-5} µg lipid spore^{-1} directly after release. After 10 days, the concentration decreased to 1.25 × 10^{-5} µg lipid spore^{-1}. Thereafter the lipid content increased to 1.38 × 10^{-5} µg spore^{-1} and 1.84 × 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 (Table 1). 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 ($P = 0.016$ and 0.04 (F-test)) 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 in the median (Kruskal–Wallis test) found for 18:4(n-3) ($P = 0.024$) 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 content of the secondary metabolite phlorotannin in *S. latissima* zoospores/gametophytes and in their surrounding medium and to investigate whether a relation between these metabolic processes estimated as lipid consumption can be drawn. It was determined for the first time phlorotannin exudation and lipid quality and quantity during development from zoospores to gametophytes over 20 days. Due to the decrease in total fatty acids and the decrease in the fatty acid 18:1(n-9), we assume that this fatty acid might play a major role in spore/early gametophyte metabolism. By fueling essential life-maintaining processes, it might potentially act among other functions as a precursor for the production of secondary metabolites, e.g. phlorotannins.

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, for both lipid concentration and composition (Graeve and Bartsch, unpublished 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 Vaskowsky 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) that 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

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

Days	0		5		10		15		20	
Fatty acid per spore (µg 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

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 (Brzezinski et al. 1993; 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 be used either 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 interpreting their corroded margins after 10 days as lipid consumption compared to the initial electron micrograph (Fig. 1). After 15 days, 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

Table 2 Comparison of fatty acids composition in 19 Phaeophyceae, *L. solidungula* sporophytes, *L. hyperborea* zoospores, *S. latissima* sporophytes, zoospores, and early gametophytes

Species	Fatty acids average composition in 19 Phaeophyceae[a]	*L. solidungula*[b]	*L. hyperborea*[c]	*L. saccharina* (= *S.latissima*)[d]	*L. saccharina* (= *S. latissima*)[e]	*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)		5.3	32.8	8.1	19.3	44.9	30.3
18:1(n-7)	18.7*	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)		ns	0	0.6	1.2	0	0
18:3(n-3)	7.6*	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)		10.8	4.5	9.2	13.7	3.7	3.1
20:4(n-3)	12.2*	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

Due to various authors and brown algal species, the origin of the collected species/developmental stages might differ geographically. Values are weight % of total fatty acids

ns not specified, * both isomers

[a] Pohl and Zurheide 1979

[b] Graeve et al. 2002

[c] Graeve and Bartsch, unpublished data

[d] Jamieson and Reid 1972

[e] Fleurence et al. 1994

consumption within the three investigated zoospore species of Laminariales might therefore reflect species-specific differences in zoospores swimming behavior, photosynthetic efficiency (Brzezinski et al. 1993; Reed et al. 1999) and different adaptations to their habitat. Additionally, the differences might be related to different sensitivities of the methods applied. 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 earlier, 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). In addition, phlorotannin exudation was shown to be correlated with an increase in irradiation dose (Abdala-Díaz et al. 2005). 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 in 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 that are difficult to extract within the early life stages or differences in phlorotannin

content in different developmental stages (Jennings and Steinberg 1997; Alstyne et al. 2001). 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 macromolecules 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 two last explanations in more detail—the constitutive production and the stress response. Phlorotannins were induced by absence of the major, so far assumed triggers, UV radiation and grazing (e.g. Fairhead et al. 2006).Consequently, phlorotannin production in our study might indicate a more constitutive phlorotannin production than previously assumed or the involvement of phlorotannins 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 and whether 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 Targett 1998). 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 acids degraded to Acetyl-CoA via β-oxidation (e.g. Harwood 1988) and hence would be an ideal candidate as precursor for secondary metabolites as the investigated phlorotannin. Currently, it is not known whether the phlorotannins synthesis from recycled Acetyl-CoA reserves might be controlled by seaweeds or not (Arnold and Targett 1998). In addition, it is unknown whether 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). Consequently, we support hypothesis (II) and suggest that especially the fatty acid 18:1(n-9) plays a major role in spore/early gametophyte metabolisms and might potentially act among other functions as a precursor for secondary metabolites as e.g. phlorotannins.

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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Publication II

Steinhoff FS, Wiencke C, Wuttke S, Bischof K

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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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 [UV-B:UV-A:PAR (W m^{-2}) 8:114:100; 2–17ºC] and high PAR outdoor experimental conditions [UV-B:UV-A:PAR (W m^{-2}) 0.1:5:100; 7ºC] for 8 h. Under low PAR+UVR conditions and at 2–12ºC, almost 98% of zoospores germinated in all light treatments. The germination rate was reduced under PAR+UV-A+UV-B only in the 17ºC and 7ºC outdoor treatment, to 21.4% and 9.6%, respectively. Light microscopic investigations showed differences in germling appearance, growth and pigmentation in the outdoor treatment and at 17ºC low PAR conditions, compared with 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 (17ºC), 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 12ºC. Moreover, high PAR in combination with UVR might potentially affect zoospores of *S. dermatodea* stronger than expected in clear waters.

Publication III

Steinhoff FS, Bischof K, Wuttke S, Wiencke C

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

Manuscript (submitted)

Abstract

Changing environmental parameters 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 spores of the arctic kelp species *Alaria esculenta, Laminaria digitata* and *Saccharina latissima* under three setups (a-c) in order to investigate effects of temperature, low/high UV radiation (UVR) and low/high photosynthetic active radiation (PAR) on potential photo-protective substances phlorotannins. As a response variable, phlorotannins were obtained within spores (intracellular) and the surrounding medium (extracellular content) and germination was determined as a general fitness parameter for the various scenarios. 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 littoral species *A. esculenta* and lowest in the lower sublittoral species *S. latissima*. The high extracellular phlorotannin contents of *A. esculenta* decreased within the exposure time to values exhibited by *S. latissima* and *L. digitata* indicating the high oxidative and consequently very likely cell protecting potential of phlorotannins against photo-induced damage. Despite the correlation between high PAR and intracellular phlorotannin contents, spores of all species were affected by high PAR more than assumed. Macroalgal spore survivability and distribution depends therefore on the species-specific adaptive and protective potential towards enhanced temperatures, UVR and high PAR which seems pre-determined by the parental tissue.

Publication IV

Steinhoff FS, Graeve M, Bartoszek K, Bischof K, Wiencke C

Phlorotannin production and lipid oxidation as a potential protective function against high photosynthetically active 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 Photosynthetically Active and UV Radiation in Gametophytes of *Alaria esculenta* (Alariales, Phaeophyceae)

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ABSTRACT

Radiation damage can *inter alia* result in lipid peroxidation of macroalgal cell membranes. To prevent photo-oxidation within the cells, photoprotective substances such as phlorotannins are synthesized. In the present study, changes in total fatty acids (FA), FA composition and intra/extracellular phlorotannin contents were determined by gas chromatography and the Folin-Ciocalteu method to investigate the photoprotective potential of phlorotannins to prevent lipid peroxidation. *Alaria esculenta* juveniles (Phaeophyceae) were exposed over 20 days to high/low photosynthetically active radiation (PAR) in combination with UV radiation (UVR) in the treatments: PAB (low/high PAR + UV-B + UV-A), PA (low/high PAR + UV-A) or low/high PAR only. While extracellular phlorotannins increased after 10 days, intracellular phlorotannins increased with exposure time and PA and decreased under PAB. Interactive effects of time:radiation wavebands, time:PAR dose as well as radiation wavebands:PAR dose were observed. Low FA contents were detected in the PA and PAB treatments; interactive effects were observed between time:high PAR and PAB:high PAR. Total FA contents were correlated to extra/intracellular phlorotannin contents. Our results suggest that phlorotannins might play a role in intra/extracellular protection by absorption and oxidation processes. Changes in FA content/composition upon UVR and high PAR might be considered as an adaptive mechanism of the *A. esculenta* juveniles subjected to variations in solar irradiance.

INTRODUCTION

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

Studies investigating FA composition in few-celled marine organisms are scarce (9,12,13). Polyunsaturated FA (PUFA), major constituents of cell membranes, are known to be oxidized and produce lipid peroxidation products upon UVR (23). Some of the lipid oxidation products such as malonaldehyde exhibit toxic activities by reacting with biological nucleophiles and crosslink upon UVR to proteins and bind covalently to nucleic acids (24). Irradiance levels are known to affect ratios of polyunsaturated and saturated FA (25,26). Consequently, changes in FA, driven by UV-B and low/high PAR, can influence the entire metabolism of an organism, its protective mechanisms such as 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 (13).

To prevent photo-oxidation within the cells, photoprotective substances such as phlorotannins are synthesized. Among brown algae, only one group of polyphenolics, the phlorotannins, is present (27,28) which might serve as an indicator of UV-protection and acclimation potential toward UVR. Within spores, phlorotannins are located in spherical membrane-bound vesicles (physodes) (29–31) which are randomly distributed throughout the cell. Phlorotannins in adult algae are located in the cell walls (32) and the cytoplasma (31) as well as in the outer epidermal cell layer, cortical cells and in the innermost medullary (33). Phlorotannins are based on the monomer phloroglucinol (34) and discussed to be formed *via* the acetate–malonate pathway (35) involving a polyketide synthase-type enzyme complex (36). It was suggested for the brown alga *Saccharina latissima* that phlorotannin building blocks are derived from storage lipids within the early developmental stages linking lipid metabolism with potential

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photoprotective mechanisms (37). Phlorotannins have a multifunctional role in ecology and respond to several environmental parameters, such as salinity, nutrient (38) and light availability as well as UVR and high irradiances (39). However, the most important property of phlorotannins for this study was their ability to absorb in the UV wavelength range (6,11,18,19,40–42) and their radical scavenging (antioxidant) activity (43–46). Phlorotannins can be either integrated into cell walls as supporting substances (32) or released *via* exudation processes into the surrounding water (34,41,47) creating so-called UV refugia (41) to protect spores from harmful radiation and to deter grazers. Especially polar species are exposed to extraordinary circumstances such as low temperatures, ice cover, shifts in light availability (polar day and night) and a high interannual variability in UVR (48) and can therefore serve as a role model.

This study investigates for the first time FA and phlorotannins in spores of a dominant Arctic brown alga (*Alaria esculenta*, Phaeophyceae) from Spitsbergen (Norway) upon UVR and low/high PAR exposure over a study period of 20 days. The major aim was to gain insights whether:

1. The FA composition and total FA change in early life stages.
2. Phlorotannin production in spores/juvenile gametophytes of *A. esculenta* is affected by low *vs* high PAR in combination with and without UVR.
3. The factor of time is involved in phlorotannin production and exudation processes as well as lipid consumption and change in FA composition.
4. Storage FA of *A. esculenta* might correlate and serve as potential fuel for phlorotannin building blocks in early developmental stages of seaweeds.

MATERIAL AND METHODS

In late May 2009, fertile sporophytes of *A. esculenta* (Linnaeus) Greville were collected from 8 to 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 (16). Upon immersion in 0.2 μm filtered seawater, the fertile tissue released zoospores after 1.5 days at temperatures 5°C above the temperature the fertile tissue was stored. Individual spore suspensions were mixed and spore density was adjusted with 0.2 μm filtered seawater to 12.9×10^5 spores mL^{-1} 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 spore suspension was distributed over glass Petri-dishes. Petri-dishes containing spores were then exposed to the radiation conditions summarized in Table 1, applying a light cycle of 24 h:12 h (PAR:UVR) according to the polar day. In an orthogonal experimental set-up, both high and low PAR in combination with and without UVR were applied to distinguish between radiation effects caused by UVR only (low PAR + UVR) or high PAR (high PAR-UVR) or in their respective combinations (low PAR-UVR and high PAR + UVR). Low PAR was provided by three white light fluorescent tubes (36 W true light® II Powertwist) while high PAR was provided by three Osram Biolux (36 W/965; München, Germany). PAR was measured with a LiCor 250A and a Li-190 Quantum sensor (LiCor, Lincoln, NE). UVR was generated by three fluorescent tubes (Q-Panel UVA 340, 40 W; Cleveland, OH) and measured with a Solar light PMA 2100 (Solar Light Co., PA).

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). For a better comparison of data, doses of PAR, UV-A and UV-B over the exposure period of 24/12 h a day were calculated (Table 1). Additionally, erythemally weighted radiation (49) was calculated (UV_{ery}, Table 1). 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 medium (50).

After exposure, samples for FA 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 vol/vol, Merck, Darmstadt, Germany) and frozen at −80°C before further extraction and gas chromatographic analysis.

Phlorotannin analysis

For quantitative phlorotannin analysis, spores/juvenile gametophytes were detached gently from the Petri-dish bottom after exposure. Then, spore/juvenile gametophyte solutions (40 mL each) were filtered under low pressure on GF/C filters and frozen in liquid nitrogen. After extracting spore filters with 7:3 acetone/water (analysis grade, Merck), phlorotannin extractions were freeze-dried and redissolved in 1 mL MilliQ water for further measurements (37). Subsequently, total phlorotannin content of spores was measured (35,51) applying the Folin-Ciocalteu method (Folin-Ciocalteu solution and sodium carbonate, Merck) using phloroglucinol (Sigma-Aldrich, St. Louis) as a standard agent.

FA analysis

For FA analyses, filters of the three replicates per treatment were pooled due to the extreme low lipid concentrations. The filters were homogenized by ultrasonification and extracted in dichloromethane: methanol (2:1, vol/vol) following the method after Folch *et al.* (52). Prior to extraction, an internal standard was added (19:0 FA methyl esters [FAME]). For gas liquid chromatography of FA, methyl esters were prepared from aliquots of the extracted spores/gametophytes by transesterification with 3% sulfuric acid in methanol for 4 h at 80°C. After extraction with hexane, FAME were analyzed with a gas liquid chromatograph (HP 6890; Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m × 0.25 mm I.D.; film thickness: 0.25 μm; liquid phase: DB-FFAP; J&W, Cologne, Germany) using temperature programming (53). 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 FAME. During sample processing, we unfortunately lost the FA treatment low PAR + UV-A + UV-B as indicated in Table 3 and Fig. 2 by dashes.

Statistical analysis and multivariate regression model

Statistical analysis was done with R (version 2.11.0) (54). First we did a two-way ANOVA, applying a post hoc test (Tukey HSD) of pair-wise comparison to test for differences among treatments for the phlorotannin measurements. Obtained *P*-values are listed for the phlorotannin data in a separate table. Significant differences among phlorotannin treatments ($P < 0.05$) are highlighted in gray (Table 2a,b). This however did not allow us to model the effects that the treatments have on the phlorotannin and total FA or whether there might be a correlation between them. We therefore studied them under a multivariate regression setting as a trivariate response to the treatments with correlated residual structure. Additionally, all 10 FA (Fig. 3) were tested separately to obtain the individual responses to the applied environmental conditions. For each FA, an ANOVA comparison between two models, a model without any interaction in the predictors and one model with all predictors interacting, was carried out. The treatments are time (considered continuous), PAR dose (discrete two levels low and high PAR) and radiation wavebands (discrete three levels PAR, PA, PAB). In the following, the model is described in more detail to trace the mathematical analysis if required. The model can be written as

Table 1. Summary of radiation treatments applied over the exposure time 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 UVR: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})			
	Time (days)	PAR	UV-A	UV-B	PAR	UV-A	UV-B	UV_{ery}
UVR:PAR 2.06	0	5.5 ± 1.2	10.9 ± 0.4	0.45 ± 0.0				
	5				2.3×10^6	2.3×10^6	9.7×10^4	1.2×10^4
	10				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
UVR:PAR 0.58	0	20.6 ± 3.0	11.6 ± 1.9	0.48 ± 0.0				
	5				8.8×10^6	2.5×10^6	1.0×10^5	1.3×10^4
	10				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

$$[y_1 y_2 y_3] = \mathbf{x}^T\mathbf{B} + [\varepsilon_1 \varepsilon_2 \varepsilon_3],$$

where $[y_1\ y_2\ y_3]$ is the response vector (phlorotannin in spore solution, phlorotannin in spore and total FA [TFA]), $\mathbf{x}$ is the design vector of length depending on the considered model (*e.g.* if we allow for interactions in predictors or not), $\mathbf{B}$ is the unknown effect matrix to be estimated with three columns and number of rows equal to the length of $\mathbf{x}$ and $[\varepsilon_1\ \varepsilon_2\ \varepsilon_3]$ are the residuals jointly distributed as Normal($\mathbf{0}$, Σ), where Σ is the 3 by 3 covariance matrix, with entries $\Sigma_{ii} = \mathrm{Var}(\varepsilon_i) = \sigma_{ii}^2$ and $\Sigma_{ij} = \mathrm{Cov}(\varepsilon_i,\varepsilon_j) = \sigma_{ij}$. Significantly nonzero entries in $\mathbf{B}$ will tell us how the predictors influence the responses and nonzero off-diagonal entries of Σ will tell about the interactions in the joint response. As we assumed the residuals are normally distributed the joint distribution of all of the data is multivariate normal. Due to the missing values and the pooling of the TFA data we could not estimate $\mathbf{B}$ by the least-squares formula and Σ as the covariance of the residuals. However as we assumed normality we can write the likelihood function (ignoring missing measurements and repeated TFA measurements). To get a measure of a model's relative goodness of fit, we use the Akaike information criterion (AIC) for model selection (interactions in predictors and nondiagonal elements in Σ). To obtain parameter estimates we use an iterated generalized least squares approach (GLS) maximizing the log-likelihood function using R's built-in optim() function over the parameters describing Σ. At each candidate for Σ we estimate $\mathbf{B}$ *via* the GLS formula

$$\mathrm{vec}\,\hat{\mathbf{B}} = (\mathbf{X}^T\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}^T\mathbf{V}^{-1}\mathbf{Y},$$

where vec is the vectorization (stacking of columns) operator, $\mathbf{X}$ is the model appropriate design matrix, $\mathbf{V}$ the model appropriate residual covariance structure and $\mathbf{Y}$ is the vector of measurements of all three responses. The significance of the estimated element $\mathbf{B}_{ij}$ was assessed by approximate *P*-values coming from the *t* distribution with *n*-*k* degrees of freedom of the statistic $\mathbf{B}_{ij}/\sqrt{(\mathbf{X}^T\mathbf{V}^{-1}\mathrm{ij,\ ij})}$ where *n* is the length of $\mathbf{Y}$ and *k* is the number of elements in $\mathbf{B}$. The mathematical theory behind such iterated GLS procedures is complex and can be deepened in Ref. 55.

RESULTS

Phlorotannin content

In Fig. 1, the sum of phlorotannin content in the surrounding medium (lower part of the bars) and the spores/gametophytes (upper part of the bars) in *A. esculenta* at the sampling days 0, 5, 10, 15 and 20 and upon low PAR (a), and high PAR (b) exposure is presented. 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/gametophyte solution)$^{-1}$ (Fig. 1). In the low PAR treatments (Fig. 1a), spores exhibited significantly higher phlorotannin contents 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 (Table 2a). The phlorotannin content was particularly high (Fig. 1) in the 15 PAR and PAR + UV-A (1.25 ± 0.11 μg phlorotannin (mL spore/gametophyte solution)$^{-1}$) and the 20 PAR + UV-A treatment (1.60 ± 0.09 μg phlorotannin (mL spore/gametophyte solution)$^{-1}$). Phlorotannin contents in all PAR + UV-A + UV-B treatment did not increase over time ($P > 0.05$) compared to the initial (except 10 PAR + UV-A). In contrast, within the filtrate all treatments of 10, 15 and 20 days showed significant higher (Table 2a) phlorotannin contents compared to the initial and day 5 contents (9.3–12.7 μg phlorotannin (mL spore/gametophyte solution)$^{-1}$).

In the high PAR treatments (Fig. 1b) only gametophytes in the 15 PAR + UV-A and the 20 PAR + UV-A treatments showed significant higher phlorotannin contents with 1.24–1.39 μg phlorotannin (mL spore/gametophyte solution)$^{-1}$ compared to the initial and phlorotannin contents after 5 and 10 days and all PAR + UV-A + UV-B treatments (Table 2b). Within the high PAR filtrates, we could measure significant higher phlorotannin contents in the 10 PAR and PAR + UV-A and all other treatments of day 15 and 20 compared to the initial and the values obtained after 5 days (Table 2a,b).

In contrast, gametophytes within the low PAR + UV-A treatment had slightly higher phlorotannin contents than their congeners under high PAR + UV-A exposure. 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/gametophyte solution]$^{-1}$) did not differ.

Fatty acids

Throughout the text, the saturated FA 14:0, 16:0 and 18:0 are indicated as SAFA, 16:1(n-7) and 18:1(n-9) as monounsaturated FA (MUFA) and 18:2(n-6), 18:3(n-3), 18:4(n-3), 20:4 (n-6) and 20:5(n-3) as PUFA. Initial *A. esculenta* spores were dominated by MUFA and PUFA (both 32%) followed by

Table 2. *P*-values derived from Tukey HSD post hoc test of the phlorotannin analysis within spores/early gametophytes and their filtrates for low PAR (a) and high PAR (b). Gray colored boxes describe significant deviation between treatments tested ($P \leq 0.05$).

(a) Phlorotannins p-values — LOW PAR

			0			5			10			15			20		
				PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB
0		Spore		0.99	0.99	1.00	0.00	0.00	0.98	0.00	0.00	0.96	0.00	0.00	0.94
		Filtrate		1.00	1.00	1.00	0.05	0.13	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	PAR	Spore			1.00	0.99	0.00	0.10	0.69	0.00	0.00	0.60	0.02	0.00	0.54
		Filtrate			1.00	1.00	0.15	0.30	0.06	0.00	0.00	0.00	0.00	0.00	0.00
5	PA	Spore				0.99	0.00	0.05	0.52	0.00	0.00	0.42	0.01	0.00	0.37
		Filtrate				1.00	0.10	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
	PAB	Spore					0.00	0.00	0.97	0.00	0.00	0.94	0.00	0.00	0.91
		Filtrate					0.04	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	PAR	Spore						0.32	0.00	0.29	0.94	0.00	0.98	0.01	0.00
		Filtrate						1.00	0.99	0.02	0.70	0.65	0.00	0.08	0.13
10	PA	Spore							0.00	0.00	0.01	0.00	0.99	0.00	0.00
		Filtrate							0.99	0.01	0.44	0.40	0.00	0.03	0.05
	PAB	Spore								0.00	0.00	1.00	0.00	0.00	1.00
		Filtrate								0.07	0.94	0.91	0.00	0.22	0.32
	PAR	Spore									0.97	0.00	0.04	0.95	0.00
		Filtrate									0.69	0.73	0.99	0.99	0.99
15	PA	Spore										0.00	0.32	0.19	0.00
		Filtrate										1.00	0.05	0.98	0.99
	PAB	Spore											0.00	0.00	1.00
		Filtrate											0.06	0.98	0.99
	PAR	Spore												0.00	0.00
		Filtrate												0.56	0.42
20	PA	Spore													0.00
		Filtrate													1.00
	PAB	Spore													
		Filtrate													

(b) Phlorotannins p-values — HIGH PAR

			0			5			10			15			20		
				PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB
0		Spore		1.00	1.00	0.99	0.97	0.99	0.99	0.99	0.00	0.99	1.00	0.00	0.99
		Filtrate		0.99	1.00	0.99	0.00	0.00	0.80	0.05	0.00	0.00	0.00	0.00	0.00
	PAR	Spore			0.99	0.99	0.96	0.99	0.99	0.99	0.00	0.99	1.00	0.00	0.99
		Filtrate			0.99	1.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00
5	PA	Spore				0.99	0.99	0.99	0.99	1.00	0.00	0.99	0.99	0.01	0.99
		Filtrate				0.99	0.00	0.00	0.83	0.05	0.00	0.00	0.00	0.00	0.00
	PAB	Spore					0.77	0.92	1.00	0.98	0.00	1.00	1.00	0.00	1.00
		Filtrate					0.00	0.00	0.38	0.01	0.00	0.00	0.00	0.00	0.00
	PAR	Spore						0.99	0.68	0.99	0.08	0.74	0.93	0.30	0.79
		Filtrate						1.00	0.01	0.90	0.99	0.99	0.76	0.94	0.99
10	PA	Spore							0.86	1.00	0.01	0.89	0.99	0.05	0.92
		Filtrate							0.04	0.98	0.99	1.00	0.66	0.87	1.00
	PAB	Spore								0.96	0.00	1.00	1.00	0.00	1.00
		Filtrate								0.78	0.21	0.08	0.00	0.00	0.08
	PAR	Spore									0.00	0.97	0.99	0.02	0.98
		Filtrate									0.99	0.99	0.13	0.22	0.99
15	PA	Spore										0.00	0.00	0.99	0.00
		Filtrate										0.99	0.28	0.45	0.99
	PAB	Spore											1.00	0.00	1.00
		Filtrate											0.50	0.73	1.00
	PAR	Spore												0.00	1.00
		Filtrate												0.99	0.53
20	PA	Spore													0.00
		Filtrate													0.76
	PAB	Spore													
		Filtrate													

SAFA with slightly lower values (up to 31%, largely owing to 18:0 and 16:0 FA; Fig. 2). Under high PAR, PUFA were remarkably low already after 5 days of PAR + UV-A + UV-B treatments (9.9–4.3%; Fig. 2). Under low PAR treatment, PUFA had considerable higher values with up to 60% of total FA (20 PAR and 20 PAR + UV-A; Fig. 2). The monounsaturated FA 18:1(n-9) exhibited highest amounts within the high PAR treatment after 5 days (37%) and were remarkably lower toward the end of experiment and ranged between 11% and 5% after 15 and 20 days, respectively. During low PAR the initial proportions of 18:1(n-9) in gametophytes were slightly lower compared to the high PAR (about 25%) and reached proportions of 7–15% after 20 days of experiment (Table 3, Fig. 3). The next most abundant were

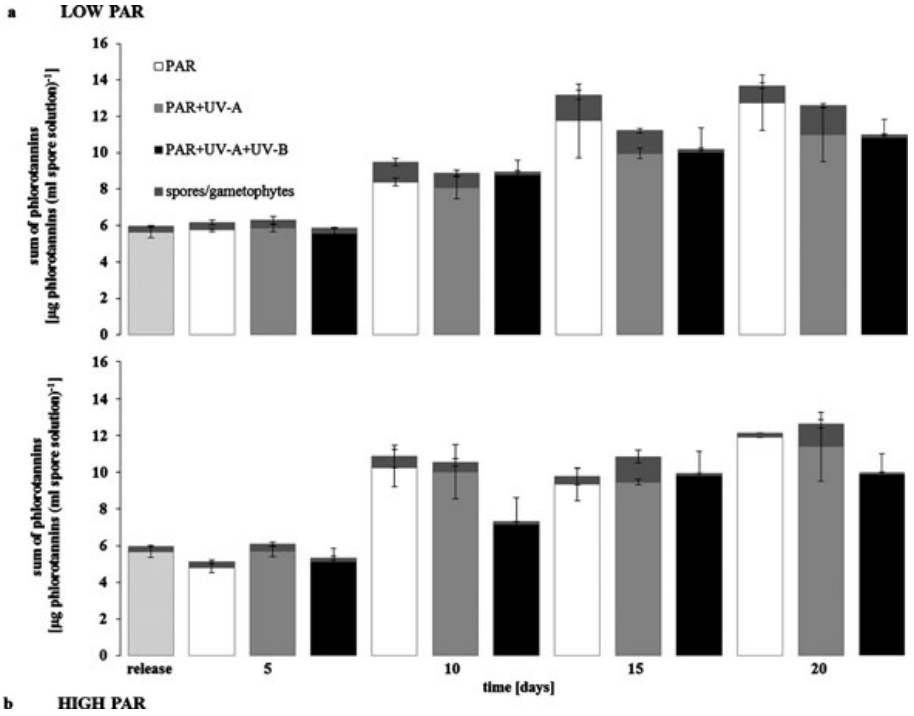

Figure 1. Sum of phlorotannin content (± SD) in the surrounding medium and the spores/gametophytes in *Alaria esculenta* during the experiment (0–20 days) upon low PAR (photosynthetically active radiation, upper panel [a]) and high PAR, (lower panel [b]). The corresponding statistical analysis of treatments is given in Table 2a,b.

the SAFA due to consistently high levels of the 16:0 FA in all treatments, particularly in high PAR + UV-A + UV-B treatments with percentages of about 37% (Table 3; Fig. 3). The TFA content upon UVR exposure in the present study revealed that under low PAR, total FA content was higher than high PAR (Fig. 4).

However, the low PAR and high PAR treatment showed important differences in TFA increase and decrease. In the low PAR treatment, the TFA content of the control (PAR only) was varying during the 20 days of the experiment exhibiting an average amount of 1410.6 μg (mL spore/gametophyte)$^{-1}$, which is comparable to the initial value (Fig. 4).

Within the other low PAR dose treatments (PA and PAB), a distinct decrease of TFA after 10 days of experiment occurred with average values of 382.9 and 219.1 μg (mL spore/gametophyte solution)$^{-1}$, for PA and PAB respectively (compare Fig. 4). In contrast, after 15 and 20 days of the high PAR dose experiment, TFA were almost depleted in all treatments (PAR, PA and PAB; Fig. 4).

After 15 days a mean TFA content of 1222.4 μg (mL spore/gametophyte solution)$^{-1}$ and after 20 days an average value of 1523.3 μg (mL spore/gametophyte solution)$^{-1}$ was detected (Fig. 4) upon low PAR. Total FA were significantly affected by UV-A and UV-B as well as by the interaction of time and high PAR (Table 5). The results of the regression analysis of the FAs are summarized in Table 4. For each FA, the model comparison procedure resulted in no significant (at the 0.05 level) improvement when considering the model with interactions between the different predictors. This could be due to the fact that the interaction effects might be too weak to be noticed within the small sample size available. The majority of the different FAs showed only minor dependencies on PA (18:1(n-9) and 18:2(n-6)) but major dependencies on PAB (14:0, 16:0, 18:1(n-9), 18:2(n-6), 18:3(n-3)). However, the FA 18:1(n-9) and 18:2(n-6) strongly depended on time and suggest a decrease over time, especially within the high PAR treatments (Fig. 3, Table 4).

Analysis of interactions between phlorotannins and TFA data

The AIC model selection criterion indicated the model where all of the predictors (time, PAR dose, radiation wavebands) were interacting, with residual covariance structure as $\sigma_{12} = 0$ (phlorotannins in filtrates responding independently of phlorotannins in spores/gametophytes) and $\sigma_{13} \neq 0$, $\sigma_{23} \neq 0$ (phlorotannin response dependent on the response of TFA). In Table 5, the GLS estimates of the elements of **B** under the best found estimate of $\Sigma = \begin{bmatrix} 1.392 & 0 & 136.211 \\ 0 & 0.057 & 24.576 \\ 136.211 & 24.576 & 121403 \end{bmatrix}$ are presented. The estimate of $\sum$ gives a correlation of 0.2563 between TFA and phlorotannin in the filtrates and 0.2567 between TFA and phlorotannin in spores/gametophytes. Consequently, the phlorotannin content within the spore/gametophyte media only depends on time (Fig. 5; Table 5). The phlorotannin content within the spores/gametophytes depends on all of the predictors with all interaction effects apart from high PAR. Consequently, in the absence of UV-A or UV-B radiation, the phlorotannin content within the spores/gametophytes is not

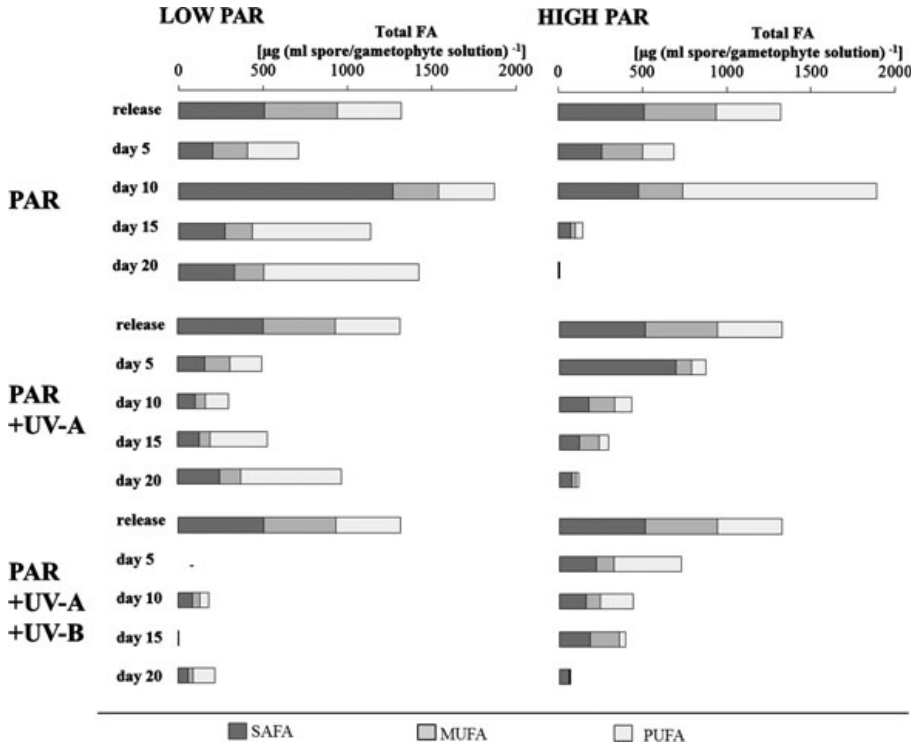

Figure 2. Sum of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) upon low *vs* high PAR and UVR exposure during the experiment.

Table 3. Total fatty acid (FA) proportions of *Alaria esculenta* spores (day 0) and juvenile gametophytes (day 5–20) under low and high PAR and upon UVR exposure (PA and PAB) in mass % of total FA for a spore/gametophyte density of 12.9×10^5 spores/gametophytes mL^{-1}.

	Sampling time (days)												
	0	5			10			15			20		
Fatty acid (%)	Initial	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB	PAR	PA	PAB
Low Par													
14:0	6.1	6.5	6.9	–	3.2	7.0	7.3	7.4	8.1	10.5	6.6	8.6	5.1
16:0	18.0	17.8	18.5	–	23.6	16.1	23.3	13.8	11.3	31.8	14.1	13.1	14.5
16:1(n-7)	0.5	1.6	2.3	–	1.0	3.1	6.0	1.2	1.8	8.5	1.2	2.7	3.5
18:0	6.8	2.7	5.1	–	32.3	4.7	8.6	1.2	2.2	14.7	0.8	1.8	4.0
18:1(n-9)	31.8	25.9	25.4	–	11.4	12.6	15.0	12.0	9.1	12.0	10.3	9.5	7.5
18:2(n-6)	10.0	10.1	9.4	–	5.9	3.7	3.3	6.9	0.0	2.2	5.6	4.4	4.2
18:3(n-3)	5.4	7.4	6.5	–	2.3	6.8	4.5	9.7	11.3	1.0	10.0	9.7	10.0
18:4(n-3)	1.4	6.3	5.8	–	3.4	11.1	6.7	17.0	18.8	1.1	18.8	17.8	17.4
20:4(n-6)	4.4	4.5	3.6	–	1.6	5.3	2.7	11.3	9.5	1.0	12.8	10.5	10.0
20:5(n-3)	11.2	12.1	10.2	–	2.4	8.4	5.2	12.5	12.5	0.8	13.2	13.9	9.3
High Par													
14:0	6.1	7.9	8.2	9.3	3.2	6.4	11.2	8.8	8.1	9.2	8.1	9.2	9.3
16:0	18.0	22.7	23.4	26.6	24.2	16.7	34.2	15.6	25.4	31.0	14.4	13.6	37.2
16:1(n-7)	0.5	2.7	3.0	3.6	0.8	2.8	6.8	1.8	6.3	6.5	1.3	4.5	2.9
18:0	6.8	4.9	5.8	6.2	48.9	4.2	8.3	2.6	8.5	12.9	1.1	3.1	20.0
18:1(n-9)	31.8	30.1	30.5	36.9	9.9	12.0	23.1	11.0	11.5	11.1	11.7	7.4	5.0
18:2(n-6)	10.0	9.0	5.9	3.7	5.5	3.7	1.9	0.0	3.6	2.8	7.5	3.5	0.9
18:3(n-3)	5.4	6.2	3.8	1.2	1.1	7.2	1.3	8.4	4.6	1.5	9.0	7.6	0.8
18:4(n-3)	1.4	5.6	3.2	0.9	1.0	7.4	2.0	13.8	6.3	2.7	15.9	14.5	0.6
20:4(n-6)	4.4	3.7	2.2	0.7	0.0	5.2	0.5	8.1	3.5	1.0	10.5	7.5	0.0
20:5(n-3)	11.2	0.6	5.9	1.8	0.9	10.0	1.9	12.6	6.4	1.9	14.3	16.2	2.0

– indicates missing value.

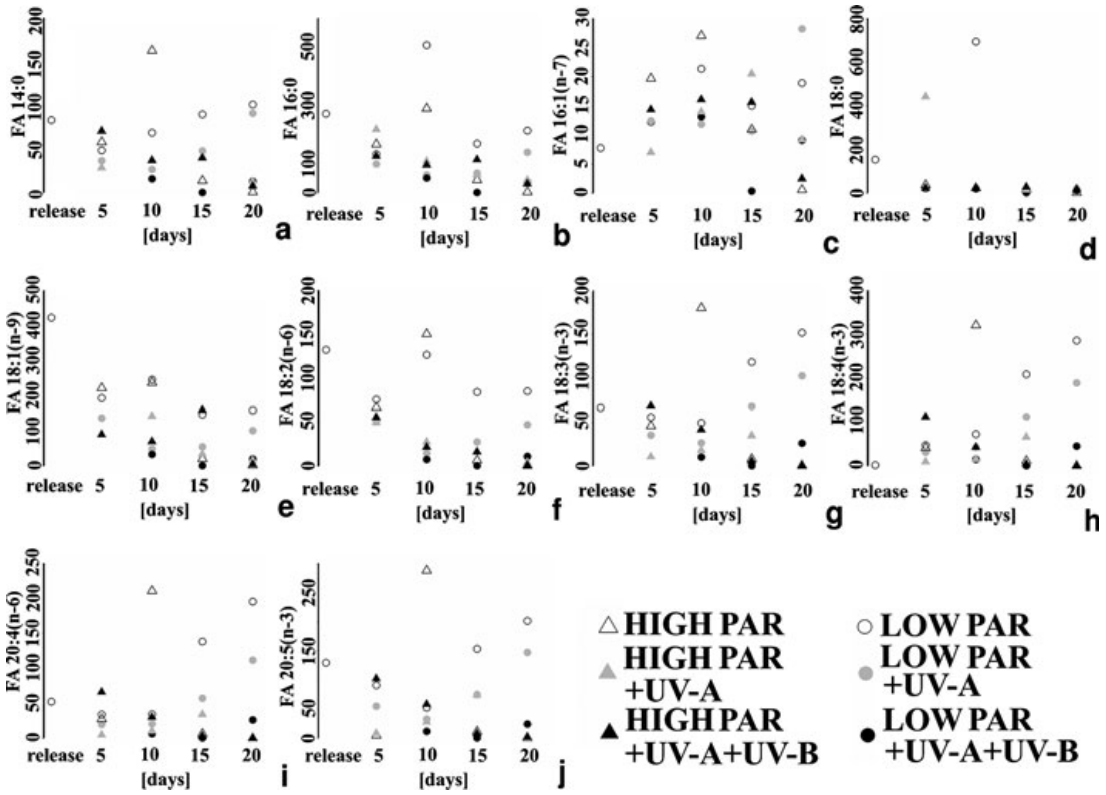

Figure 3. (a–j) Individual fatty acids during exposure time. Different treatment combinations of low and high PAR and PAR, PA, PAB treatments are indicated for the measurements. (a) 14:0, (b) 16:0, (c) 16:1(n-7), (d) 18:0, (e) 18:1(n-9), (f) 18:2(n-6), (g) 18:3(n-3), (h) 18:4 (n-3), (i) 20:4(n-6) and (j) 20:5(n-3). The statistical evaluation of each fatty acid is presented in Table 4.

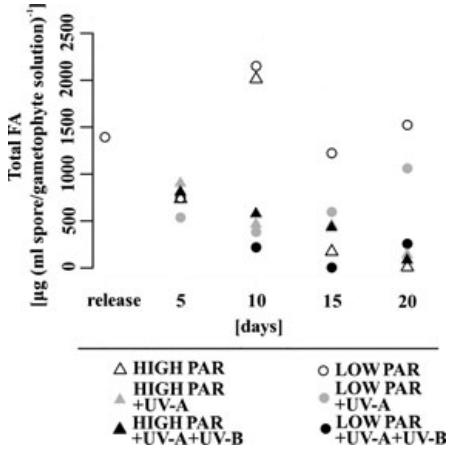

Figure 4. Total fatty acids (TFA) during exposure time. Different treatment combinations of low and high radiation as well as PAR, PA, PAB treatments are indicated for the measurements. The statistical evaluation of TFA content within the various treatments is displayed in Table 5.

affected by the PAR dose. TFA is affected by changes in radiation wavebands and PAR dose. The effects of PAR dose and time are only present upon interaction of these two variables and when UV-B levels are high.

DISCUSSION

This study involved a comparable analysis of low *vs* high PAR in relation to UVR effects and was carried out for the first time on early life stages of *A. esculenta*. Fatty acid content/composition and intra/extracellular phlorotannin content were highly affected by both low and high PAR and UVR in *A. esculenta* spores and juvenile gametophytes.

Phlorotannins under low *vs* high PAR and upon UVR exposure

The variation of phlorotannin content with species, morphology of the algae, habitat and developmental stage as well as with seasonal and environmental parameters have to be taken into consideration when interpreting results and distinguishing effects of applied experiments.

Compared to our study, *A. nodosum* sporophytes were exposed to significantly higher radiation conditions with 0.6 W m^{-2} UV-B for 2 weeks leading to a 30% increase in mean phlorotannin concentrations (40). Similar results were obtained in *Fucus gardneri* (56) and in *S. muticum* (57) suggesting a response-induced production of phlorotannins. Nevertheless, an increase in intracellular phlorotannin levels in the present studies was only detectable after 10 days exposure upon low PAR and UVR and non-UV treated juvenile gametophytes *A. esculenta*, while low PAR only treatment showed no effects on *S. latissima* juveniles (37). Upon combined high PAR and UVR exposure, juvenile gametophytes

Table 4. Analysis of interactive effects between radiation wavebands (PAR, PA and PAB) and PAR dose (low and high PAR) on the individual fatty acids. Estimates of regression coefficients for independent regressions of the individual FAs on the different days, radiation and treatment predictors. Significance codes are 0 ‘‘***’’ 0.001 ‘‘**’’ 0.01 ‘‘*’’ 0.05 ‘‘ ’’ 1.

	14:0	16:0	16:1(n-7)	18:0	18:1(n-9)	18:2(n-6)	18:3(n-3)	18:4(n-3)	20:4(n-6)	20:5(n-3)
(Intercept)	82.7485***	279.673***	15.3361**	196.580*	295.812***	119.077***	80.4240**	82.320	67.825*	115.156**
Time	−0.9394	−6.152	−0.0525	−7.252	−9.095**	−2.867*	0.3176	3.659	1.700	−0.005532
High PAR	−5.5124	−25.164	0.1188	−19.282	−27.117	−14.657	−20.2999	−29.785	−20.761	−26.223108
PA	−27.6577	−90.171	−0.5355	−24.005	−91.141*	−46.450**	−37.2620	−54.644	−46.453	−49.414228
PAB	−40.3643*	−116.463*	−4.5629	−70.279	−103.432**	−55.850**	−51.5291*	−83.785	−60.425	−70.87

†The linear model for investigating the effects of radiation wavebands and PAR dose on the individual fatty acids is described as fatty acids = intercept + time + PAR dose + radiation wavebands + ε, with PAR being the baseline for radiation wavebands and low PAR the baseline for PAR dose.

Table 5. The linear model refers to the model presented in the section ‘‘statistical analysis and multivariate regression model.’’ It investigates correlations between phlorotannins within the spores/gametophytes and their medium (filtrate) and total fatty acids with PAR being the baseline for radiation wavebands (PAR, PA and PAB) and low PAR the baseline for PAR dose. The generalized least squares approach estimates for **B** under the numerically found maximum likelihood estimate of Σ. Significance codes: 0 ‘‘***’’ 0.001 ‘‘**’’ 0.01 ‘‘*’’ 0.05 ‘‘ ’’ 1.

	Phlorotannins within the media	Pr(>|t|)	Phlorotannins within the gametophytes	Pr(>|t|)	TFA	Pr(>|t|)
(Intercept)	4.831	$<2*10^{-16}$***	0.503	$1.39*10^{-6}$***	1.309	$2.68*10^{-8}$***
Time	0.392	$<2*10^{-16}$***	0.032	$1.47*10^{-4}$***	0.010	0.595
PA	−0.283	0.724	−0.536	0.001**	−1.104	0.004**
PAB	−0.227	0.777	−0.393	0.017*	−1.495	0.001**
High PAR	−0.729	0.310	−0.277	0.068	0.307	0.383
Time:PA	−0.058	0.307	0.053	$8.93*10^{-6}$***	0.025	0.344
Time:high PAR	0.007	0.876	−0.020	0.044*	−0.081	$6.04*10^{-4}$***
PA:high PAR	1.048	0.122	0.376	0.009**	0.546	0.088
PAB:high PAR	−0.186	0.784	0.515	$3.31*10^{-4}$***	1.064	0.001**

under PA exhibited significantly higher intracellular phlorotannin levels than their representatives in the high PAR controls and among the PAB treatments (Tables 2a,b and 5). Consequently, it can be assumed that UV-A might be able to either compensate high PAR impact to some extent 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, F. gardneri, H. sessile* and *Lessoniopsis littoralis* were higher relative to adult concentrations, phlorotannin concentrations in *Nereocystis luetkeana* were higher in adult algae (58). However, less phenolic compounds in juveniles compared to adult stages were observed in two tropical brown algae species (59). Due to the small cell size of spores, intracellular phlorotannin contents were 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 mortality/hindered growth and high PAR or decreased phlorotannin formation/phlorotannin oxidation under high PAR compared to low PAR exposure (significant interactive effect of time and high PAR, Table 5).

Exposure time, level of irradiance, nutrients and developmental stage on FA composition and total FA

Nutrient limitation is known to additionally increase sensitivity toward UVR. 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 (60,61) 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 and associated delayed or hindered cell division would induce accumulation of various primary and secondary photoproducts (13). To avoid these side effects and to focus entirely on interactive effects of UVR and high/low PAR in the early developmental macroalgal stages, the spore (gametophyte) suspensions were enriched with Provasoli enriched seawater (50) and nutrient deficiency related effects can therefore be excluded.

FA profiles are more susceptible than overall production parameters as 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 in phytoplankton (13) but also in early developmental stages of brown algae (this study, Table 6). Decreasing UV-B damages with higher irradiation levels in several microalgae were investigated (12) with lowest damage at 200 μmol photons s^{-1} m^{-2} [43 W m^{-2}] and highest at 15 μmol photons s^{-1} m^{-2} [3 W m^{-2}]. Although PAR levels were much higher, daily doses of UV-B were calculated to 12 kJ m^{-2} (12) while in our study, daily doses of UV-B ranged from 19.4 to 20 kJ m^{-2}. In contrast, our low PAR

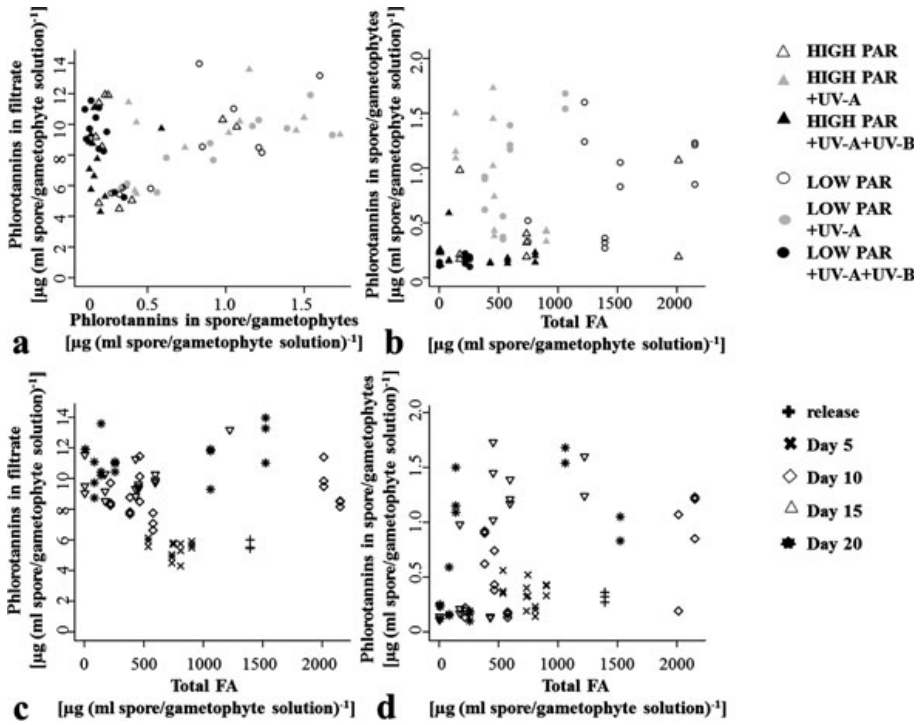

Figure 5. (a–d) Interactive effects (a) Phlorotannin content within filtrates *vs* phlorotannin content in spores/gametophytes and (b) total fatty acids *vs* phlorotannin content in spores/gametophytes with different treatment combinations marked. (c) Total fatty acids (TFA) *vs* phlorotannin content within the filtrates and (d) TFA *vs* phlorotannin content within spores/gametophytes over time. The corresponding statistical analysis of interactive effects is displayed in Table 5.

Table 6. Comparison of fatty acids composition in various developmental stages of *Alaria 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.

	Fatty acids in % by weight									
Developmental stage	14:0	16:0	16:1(n-7)	18:0	18:1(n-9)	18:2(n-6)	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 (73)	4.8	10.1	1.5	0.5	6.5	4.2	10.6	27.9	11.9	18.4
Adult Sporophytes (74)	4.6	12.7	1.5	1.6	11.3	4.3	9.2	18.6	13.2	10.6

controls (low PAR only) showed varying total FA contents over time while high PAR controls (high PAR only) showed a strong decrease in total FA compared to the initial value. Since a methodological problem can be excluded, the high variations in FA content both in the high and low PAR only treatments (day 10) remain a matter of debate. However, we can support a positive UV-A effect only upon low PAR + UV-A exposure on PUFA while upon high PAR + UV-A exposure, the SAFA and MUFA was much higher and the total FA content decreased dramatically over the exposure period.

Consequently, high PAR over time seems to have a similar impact on total FA and FA composition as high energetic short wavebands of UV-B (Table 5). Changes in lipid content can be considered as an adaptive and survival mechanism of the juveniles subjected to variations in solar irradiance (62) and related to the developmental stage as summarized in Table 6. Results obtained suggest that interactive effects of high PAR and UV-B on *A. esculenta* juveniles might enhance FA peroxidation leading to feedback stress responses as formation of ROS and antioxidants (*e.g.* phlorotannin).

TFA content and composition under low *vs* high PAR and upon UVR exposure

Fatty acid profiles are known to change under UVR exposure by an increase in lipid peroxidation (63) leading to an increase in short-chained FA and a decrease in PUFA (12,13,24). Nevertheless, UV-related responses are dependent on taxonomy (13), cell-stage (64), nutrient limitation and the UVR: PAR-ratio. In contrast, visible light (PAR) influences primarily the content of saturated and PUFA (25,26,62) but is considered to affect FA composition and content of phytoplankton species specifically (65).

In particular, reactive products found were β-dicarbonyls, α,β-unsaturated aldehydes, 2,4-alkadienals, 4-hydroxy-2 alkenals (66). Additionally, photoproducts derived from arachidonic acid, squalene and linolenic acid: formaldehyde, acetaldehyde, acrolein, malonaldehyde, *n*-hexanal, 4-hydroxy-2-nonenal were detected (24). Some products such as 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) and the decrease in number and synthesis of FA under UV-B exposure (8,67) may explain the strong decrease of FA content and shift in FA composition upon low PAR and UV-B exposure in our study. An overall increase in MUFA and SAFA was described in earlier studies (8,68), especially of the radiation dependent FA 16:0 (68), while the high susceptible PUFA (16:4(n-1), 18:3(n-3), 20:5(n-3)) decreased about 50%. Although accumulations of short chain SAFA and MUFA as storage lipid constituents were largely unaffected by UV-B in phytoplankton (8), our results suggest a significant impact of UV-B on SAFA, MUFA and PUFA in *A. esculenta* juveniles.

Upon UV-B exposure, decreases in the omega-3 FA 20:5 (n-3) and 22:6(n-3) were observed in microalgae (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 FA but in particular of 14:0, 16:0, 18:1(n-9), 18:2(n-6) and 18:3(n-3). An increase in short-chained FA and a decrease in PUFA upon UVR exposure were observed in phytoplankton cells (13). Phospholipids (69) and membrane FA 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, TFA content upon UVB-exposure in the present study revealed that under high PAR, total FA content was significantly ($P = 0.001$) higher than low PAR (except sampling day 20). Despite decreasing total FA contents upon both low and high PAR and UVR exposure, distinct lipid peroxidation processes with increasing SAFA contents and decreasing MUFA/PUFA contents could be observed in all PA and PAB treatments (Fig. 2).

Storage lipids in motile spores seem to fuel swimming after release and germination processes (70,71). 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* (37). 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 were observed in *Ulva pertusa* (72). In contrast, our low PAR controls showed variations in total FA over time, while high PAR controls showed a strong decrease in total FA compared to the initial value after release with Table 5 showing an interactive effect of exposure time and irradiance levels (PAR) in *A. esculenta* as discussed previously.

Determination of total FA content and composition in spores and juvenile gametophytes of *S. latissima* obtained under low PAR conditions (37), 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. Since a similar decrease of 18:1(n-9) was observed in the present study, the previously suggested hypothesis (37) that building blocks for secondary metabolites as phlorotannins might be mainly fueled by this FA can be supported for *A. esculenta* juveniles as well. In *Ulva fenestrata*, a PAR impact on the ratio of storage lipids (triacylglycerols) to major chloroplast lipids such as glycolipids and phosphatidylglycerol was investigated (62). 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 radiation doses in *U. fenestrata* (62). While the PUFA 16:4(n-3) showed highest levels under low PAR (24% of incident radiation), highest levels of 16:0 were measured under high PAR (80% of incident radiation; 62). The shift in dominance of PUFA under low PAR to SAFA under high PAR was observed also in the present study within the first 5 days upon high PAR exposure (without UV treatment).

However, these observations were obtained among different divisions (Chlorophyta and Phaeophyta) and different developmental stages (sporophytes, spores/early gametophytes), the impact on FA composition seem to be rather dependent on the interaction of environmental parameters than on single factors.

TFA content interacting with phlorotannins

One of the issues we considered in the regression analysis was whether TFA and phlorotannin respond independently to the predictors or do they interact with each other. The AIC criterion indicated a significant correlation between TFA and phlorotannins. Since high TFA were correlated with high phlorotannin contents, the photoprotective role of phlorotannins with *A. esculenta* juveniles can be supported indicating a feedback response to environmental radiation conditions. Toward intuitive conclusions, the phlorotannin contents within the juvenile *A. esculenta* seem to be affected independently from the phlorotannin content in their filtrates (surrounding medium) and should be addressed in further studies.

Our observations suggest that (1) FA composition and TFA content are strongly affected 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. (2) Intracellular phlorotannins were more affected by the environmental parameters given than extracellular phlorotannin contents indicating an independent impact and different protective roles. (3) Phlorotannins were induced after 10 days both upon PAR only and PAR + UVR exposure in contrast to previous hypotheses of immediate induction mechanisms and induction by UVR only. (4) TFA could be correlated to phlorotannin contents indicating that building blocks for secondary metabolites as phlorotannins might be derived from FA as 18:1(n-9) but should be investigated in more detail in further studies.

Hence, global climate change and stratospheric ozone depletion might influence viability of early life stages more

than assumed. Additionally, the decrease in FA content and composition of kelp juveniles upon UVR and high PAR exposure is likely to affect the community level by a decrease in nutritional quality.

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Publication V

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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

(Aguilera et al. 2002a, Bischof et al. 2002a). Although irradiance decreases logarithmically with increasing water depth, the 1% penetration depth of UVB was recorded down to 19 m in Potter Cove, King George Island (Richter et al. 2008). For benthic microalgae, the sediment has been proposed as a refuge to escape harmful radiation by migration (Wulff et al. 2008a), but UVR has been shown to penetrate approximately 0.6 mm (UVB) and 1 mm (UVA) into a sandy sediment (Wulff et al. 1999). In the undergrowth of seaweed forests, UVR levels are lower, but the canopy algae are exposed to full solar radiation (Hanelt 2004).

Structure and function of benthic micro- and macroalgal assemblages

The shallow water zone of polar regions is dominated by two types of algal assemblages. Whereas macroalgal species of the Chlorophyta, Rhodophyta and Phaeophyceae primarily settle on hard bottom substrata, such as rocky shorelines, the microscopic forms preferentially cover extensive sediment areas. At their growth sites, seaweeds often form submerged, complexly structured underwater forests characterised by high primary productivity, thereby providing food, shelter and habitat to many marine invertebrates and fish (Bischof et al. 2006). Macroalgal assemblages typically show distinct vertical zonation patterns, exhibiting a characteristic sequence of species with increasing depth, which is related to the vertically changing environmental parameters (Bischof et al. 2006). Being at the basis of marine food webs, macroalgae are directly consumed by a diversified suite of micro- and macrograzers (Duffy and Hay 2000), whereas algal exudates might fuel the microbial loop if they are used by free-living and alga-associated bacteria. In addition, seaweeds serve many species as habitat, to which sessile forms attach directly, and can host motile animals by provision of shelter from predators.

Microphytobenthic biofilms in shallow waters are typically dominated by Bacillariophyta. This phototrophic assemblage is best known from temperate marine regions as highly productive, providing a major food source for benthic suspension- or deposit-feeders (Cahoon 1999), a control barrier for oxygen fluxes at the sediment/water interface (Risgaard-Petersen et al. 1994), and a stabiliser of sediment surfaces against erosion by the excretion of extracellular polymeric substances (De Brouwer et al. 2005). Consequently, the microphytobenthos represents a key component in the functioning of trophic webs in coastal zones that are characterised by sediments (Cahoon 1999). However, in contrast to temperate and tropical regions, structure and function of microphytobenthic assemblages are poorly studied in polar waters (Glud et al. 2009).

UVR effects on benthic micro- and macroalgae

Solar radiation is essential for life in polar regions. In the process of photosynthesis, the micro- and macrophytobenthic assemblages absorb visible light energy with their respective photosynthetic antennae to chemically fix inorganic carbon into energy-rich organic compounds. However, an increase in UVR can inhibit many biological processes. The major cellular targets of UVB are various biomolecules, which directly absorb this radiation, or which are indirectly affected by various UV-induced photochemical reactions. The biological and, ultimately, ecological consequences are numerous.

Cell biological and ultrastructural changes

The cell biological and ultrastructural changes due to UVR exposure depend on the general physiological constitution, the life history and developmental stage of the organism under study. The most susceptible stages in the life histories of macroalgae are their spores, gametes and zygotes. This has been demonstrated in species of the genus *Fucus*, and in various Laminariales and Gigartinales from Spitsbergen, King George Island (Antarctica) and the North Sea (Schoenwaelder et al. 2003, Roleda et al. 2007b, 2008a, Müller et al. 2008, Zacher et al. 2009a). As discussed below, multiple cellular processes are negatively affected by UVR, in particular photosynthesis (Roleda et al. 2006d), nuclear division (Huovinen et al. 2000) and motility (Makarov and Voskoboinikov 2001). By contrast, there are repair mechanisms operating which mitigate damage. This is shown by the effective recovery of photosynthesis and the repair of DNA damage (Roleda et al. 2004, 2006a, 2008a, Zacher et al. 2008). Damage can also be prevented by UV-absorbing phlorotannins in brown algae (Swanson and Druehl 2002, Roleda et al. 2005, 2006c) and mycosporine-like amino acids in red algae (Roleda et al. 2008a, Zacher et al. 2008). The balance between the damaging effects of UVR and the repair and protective mechanisms can be measured by the integrative parameter 'germination'. If spores or zygotes germinate after UVR exposure, the repair and protective mechanisms are strong enough to cope with the damaging effects of UVR.

Thus, the effects on the ultrastructure of an algal cell can be quite diverse, ranging from none to very strong damage, the latter usually indicating cell death. But one should always keep in mind that an electron micrograph shows a snapshot of only the moment when the organism was fixed, and hence provides no information about physiological processes, such as UVR acclimation. Ongoing repair or degradation processes, for instance, can be easily overlooked or misinterpreted. Therefore, when studying UVR-induced ultrastructural effects, additional physiological information should always be taken into account.

UVR affects all cellular components, especially the chloroplasts, the mitochondria, the nucleus and also the cytoplasm. Table 1 summarises present knowledge of UVR effects on the fine structure of marine algal cells of different systematic positions from polar to cold temperate regions. When comparing the results, it must be noted that the data were obtained from different life history and developmental stages and that different exposure times and radiation conditions were used in the various studies.

Table 1 Summary of present knowledge of UVR effects on the ultrastructure of marine algal cells of different systematic position from polar to cold-temperate regions.

Phylum/Class	Species	Developmental stage	UV exposure	Chloroplast	Nucleus	Mitochondria	Others	Reference
Rhodophyta	*Palmaria decipiens*	Male gametophytes	2–23 h 0.37 W m^{-2} UVB 6.84 W m^{-2} UVA 3.35 W m^{-2} PAR	After 2 h: chloroplast thylakoids dilated, vesicle-like formations at the chloroplasts margins; after 6–8 h: thylakoids disrupted and connected to vesicles; after 12–23 h: changes disappeared, thylakoids irregular in shape	–	After 4 h: tubuli swollen, sacculus appearance; after 23 h: changes disappeared	–	Poppe et al. 2002, 2003
	Palmaria palmata	Vegetative thalli	6/24 h 0.20 W m^{-2} UVB 4.7 W m^{-2} UVA 25–30 μmol photons m^{-2} s^{-1}	After both exposure times: thylakoid lumen enlarged, thylakoid membranes wrinkled, outer chloroplast envelope with protrusions into the cytoplasm	–	Cristae visible, swollen	–	Holzinger et al. 2004
	Palmaria palmata	Vegetative thalli	2–48 h 0.6–0.8 W m^{-2} UVB 6.24–8.41 W m^{-2} UVA 3.12–4.66 W m^{-2} PAR	Vesiculation of thylakoids after 2 h, after 6 h increased vesiculation, after 24 h growth of vesicles and expansion of intrathylakoidal space	–	After 2 h: tubuli swollen, inner mitochondrial membrane sacculus type	–	Poppe et al. 2003
	Phycodrys austrogeorgica	Gametophytes	2–12 h 0.6–0.8 W m^{-2} UVB 6.24–8.41 W m^{-2} UVA 3.12–4.66 W m^{-2} PAR	After 2–7 h: dilated thylakoids, formation of inverted thylakoid vesicles; after 12 h: detached phycobilisomes, thylakoid and chloroplast envelope disintegrated	–	–	Crystalline inclusions in cyto-plasm	Poppe et al. 2003
	Bangia atropurpurea	Gametophytes	2–96 h 0.6–0.8 W m^{-2} UVB 6.24–8.41 W m^{-2} UVA 3.12–4.66 W m^{-2} PAR	Thylakoids change from parallel/central organisation to vesicle-like appearance adjacent to the pyrenoid, tubularly arranged thylakoids	–	–	–	Poppe et al. 2003
	Odonthalia dentata	Vegetative thalli	6/24 h 0.20 W m^{-2} UVB 4.7 W m^{-2} UVA 25–30 μmol photons m^{-2} s^{-1}	After 6 h: irregularities in thylakoid membranes, granular structures and interrupted appearance of thylakoid membranes; after 24 h: reduction and branching of thylakoid membranes; small protrusions at outer chloroplast membrane	–	–	–	Holzinger et al. 2004
Phaeophyceae	*Laminaria hyperborea*	Zoospores	8 h 0.31±0.05 W m^{-2} UVB 5.7±0.7 W m^{-2} UVA 18.9±1.2 μmol photons m^{-2} s^{-1}	Formation of plastoglobuli, disrupted and fragmented thylakoids at 17°C, vesicle-like structures	Mottled nucleo-plasm, nucleopores varied in size	Tubulus/sacculus type, swollen cristae	–	Steinhoff et al. 2008

(Table 1 continued)

Phylum/Class	Species	Developmental stage	UV exposure	Chloroplast	Nucleus	Mitochondria	Others	Reference
	Saccharina latissima	Gametophytes	8 h 0.35 W m^{-2} UVB 5.5 W m^{-2} UVA 20 µmol photons m^{-2} s^{-1}	Formation of plastoglobuli	–	–	–	R. Müller, unpublished results
	Laminaria digitata	Gametophytes	8 h 0.35 W m^{-2} UVB 5.5 W m^{-2} UVA 20 µmol photons m^{-2} s^{-1}	Formation of plastoglobuli	–	–	–	R. Müller, unpublished results
Chlorophyta	*Prasiola crispa*	Vegetative thalli	6/24 h 0.20 W m^{-2} UVB 4.7 W m^{-2} UVA 25–30 µmol photons m^{-2} s^{-1}	No effects after 6 h; after 24 h: slight alterations, reduced number of plastoglobuli, dilatations of thylakoids	–	After 24 h: slight damage		Holzinger et al. 2006
	Urospora penicilliformis	Vegetative fertile gametophytes	16 h 0.40 W m^{-2} UVB 4.34 W m^{-2} UVA 22 µmol photons m^{-2} s^{-1}	No significant effects in chloroplasts	–	–		Roleda et al. 2009a

Numerous investigations have been made within the Rhodophyta. Poppe et al. (2002, 2003) investigated laboratory-grown *Palmaria decipiens* (Reinsch) R.W. Ricker, *Palmaria palmata* (Linnaeus) Kuntze, *Phycodrys austrogeorgica* Skottsberg and *Bangia atropurpurea* (Roth) C. Agardh, whereas Holzinger et al. (2004) studied field-grown *Odonthalia dentata* (Linnaeus) Lyngbye and *P. palmata*. The most striking effect of UVR in the species studied was the disturbance of chloroplast fine structure. The intrathylakoidal space was generally enlarged and the thylakoid membranes became wrinkled. In UVR-sensitive species or in less sensitive species after strong UVR exposure, the thylakoids became tubular, e.g., in *B. atropurpurea* (Poppe et al. 2003) or disintegrated into "inverted" vesicles, obviously a general phenomenon in red algal chloroplasts. In these vesicles, the phycobilisomes, which are normally attached to the exterior of the thylakoids, were exposed to the inside. In such cases, the photosynthetic apparatus was strongly damaged and photosynthesis was impaired if not fully inhibited. In some cases, these fine-structural changes were, however, partly reversible, reflecting acclimation of photosynthesis to UVR, as shown in *P. decipiens* (Poppe et al. 2002). Another effect of UVR was formation of protrusions of the chloroplast envelope, as shown in *P. palmata* and *O. dentata* (Holzinger et al. 2004), or disintegration of both envelope membranes, as shown in *P. austrogeorgica* (Poppe et al. 2003). In the latter species, the protein crystals occurring generally in the cytoplasm became corroded after UVR treatment, indicating either damage or remobilisation of the stored protein for repair processes (Poppe et al. 2003). Within the mitochondria, the cristae appeared swollen under UVR and were transformed into sacculi (Poppe et al. 2002, Holzinger et al. 2004).

In contrast, foliose thalli of the green algae *Prasiola crispa* (Lightfoot) Kützing (Holzinger et al. 2006) and *Urospora penicilliformis* (Roth) J.E. Areschoug (Roleda et al. 2009a) showed no effect after mild UVR exposure. After stronger exposure, only slight alterations appeared within the chloroplasts, such as dilatations of thylakoids and a reduced number of plastoglobuli (Holzinger et al. 2006). Under these conditions, photosynthetic efficiency decreased. The mitochondria showed slight damages and cytoplasmic globules increased in size and became more abundant. Thus, in contrast to most of the red algae discussed above, *P. crispa* and *U. penicilliformis* can cope with UV stress relatively well.

In brown algae, two different life history stages have been examined by electron microscopy, namely, zoospores and filamentous gametophytes. After UVB exposure, the zoospores of *Laminaria hyperborea* (Gunnerus) Foslie showed an enhanced formation of plastoglobuli in the chloroplast, the nucleoplasma became mottled and the structure of the mitochondria changed from the tubulus type to the sacculus type (Steinhoff et al. 2008). Phlorotannin-containing physodes were present but did not show any change after UVR exposure, and thus their contribution to UV protection is doubtful. The fine structure of gametophytes of *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl *et* G.W. Saunders and *Laminaria digitata* (Hudson) J.V. Lamouroux changed little under UVR. The only change was increase in size of plastoglobuli in the chloroplast (R. Müller, unpublished results), generally regarded as an indication for the upregulation of plastid lipid metabolism in response to oxidative stress.

Because no data on these UVR effects on benthic diatoms are available, here we summarise results obtained on the three pelagic diatom species *Cyclotella* sp., *Nitzschia closterium* W. Smith (*Cylindrotheca closterium*) and *Thalassiosira nordenskjoldii* Cleve (Buma et al. 1996). Exposure of these microalgae to UVB resulted in increased cell size. Moreover, although chloroplast number increased, this organelle became disoriented and smaller. The nucleus appeared enlarged and the contrast between nucleolus, chromatin and nucleoplasm faded with increasing UVB exposure. These ultrastructural changes mirror the fact that cell division was inhibited, whereas growth still continued as the general cell metabolism was not or only little affected by UVR. Similar results were obtained with the coccolithophore *Emiliana huxleyi* (Lohm.) Hay *et* Mohler (Buma et al. 2000). On the basis of the observed phenomenon, there probably is a disruption of processes in microtubule dynamics. Similarly, in *Macrocystis pyrifera* (Linnaeus) C. Agardh gametophytes, nuclear division and translocation were inhibited after UVR exposure (Huovinen et al. 2000). An often observed response to UVR, which presumably is also related to microtubule function, is the inactivation and/or loss of flagella as shown, e.g., in *Chlamydomonas reinhardtii* P.A. Dangeard (Hessen et al. 1995) and in sperm of the Japanese scallop (Li et al. 2000). Cell motility is affected, as has also been demonstrated in zoospores of *Saccharina latissima* [as *Laminaria saccharina* (L.) J.V. Lamour.; Makarov and Voskovoinikov 2001].

Changes in chloroplast ultrastructure after UV stress also seem to be a general phenomenon in freshwater algae, e.g., *Micrasterias denticulata* Brébisson *ex* Ralfs (Lütz et al. 1997) and in endosymbiotic dinoflagellates (Hannack et al. 1998). Other features include the formation of large endoplasmic reticulum cisternae in *M. denticulata* (Meindl and Lütz 1996) and of lipid globules containing secondary carotenoids in snow algae, e.g., *Chlamydomonas nivalis* (F.A. Bauer) Wille (Remias et al. 2005). For further information on these algal groups, the reader is directed to the review by Holzinger and Lütz (2006).

Molecular targets and repair mechanisms

DNA is one of the most UV-sensitive biomolecules, and UV-induced damage occurs directly through absorption of UVB quanta by aromatic residues. Absorbed energy can be dissipated by various mechanisms involving single bases (e.g., single-strand breaks) or interactions between adjacent bases (e.g., dimerisation) and between non-adjacent bases (i.e., inter- or intrastrand crosslinks) (Karentz et al. 1991). The structural consequences are conformational alterations, such as the frequently observed formation of cyclobutane dimers and pyrimidine (6-4)-pyrimidone (6-4)-photoproducts (CPDs) (Lois and Buchanan 1994). Such UV-induced DNA damage can significantly affect transcription, causing erroneous

replication and promoting mutations, which finally increase mortality of algal cells.

Life stage-dependent susceptibility to UVB-induced DNA damage has been observed in several polar macroalgae. Zoospores of the sublittoral kelps from Spitsbergen [*Alaria esculenta* (Linnaeus) Greville, Wiencke et al. 2007; *Laminaria digitata*, U. Lüder, M. Roleda and C. Wiencke, unpublished results; *Saccorhiza dermatodea* Bachelot de la Pylaie, Roleda et al. 2006a] are more susceptible to UVB-induced DNA damage than their respective young sporophytes (Roleda et al. 2005, 2006b). Recently, filamentous *Urospora penicilliformis* gametophytes from Antarctica were also reported to sustain significantly less DNA damage compared with propagules exposed to the same UVB dose (Roleda et al. 2009b). In multicellular life stages, the relatively thick cell wall might be able to selectively filter short UV-wavelengths from reaching the UV-sensitive targets (i.e., nucleus) compared with the thin-walled or "naked" propagules. By contrast, an inverse relationship between thallus thickness and remaining DNA damage also occurs in young sporophytes of different kelp species (Roleda et al. 2005, 2006b, 2007a). Aside from UV screening by cell walls, intracellular mechanisms, such as synthesis of UV-absorbing compounds (see below), are also important for UV protection against DNA damage. Moreover, release of a cloud of spores (e.g., kelp zoospores with phlorotannin-containing physodes) can self-shade, thereby forming a type of "biofilter" against harmful UVR (Roleda et al. 2006c).

Sensitivity of propagules to UVR-induced DNA damage is related to size of the propagules, ploidy levels and depth distribution of the adult plants (Roleda et al. 2007a, 2008a, Zacher et al. 2007a). Propagules of eulittoral *Urospora penicilliformis* (Roleda et al. 2009b) exposed to a comparable UVB dose showed significantly lower DNA damage than gametes of the sublittoral *Ascoseira mirabilis* Skottsberg (Roleda et al. 2007b). Among kelps, the reported prevalence of larger, more UV-tolerant meiospores originating from species or populations from sites exposed to high UV radiation suggest that kelp meiospores are pre-adapted to the UV conditions of the parent plant (Swanson and Druehl 2000, Wiencke et al. 2006). Comparison between spores of different ploidy levels in Antarctic *Gigartina skottsbergii* Setchell *et* N.L. Gardner showed less DNA damage in diploid carpospores compared with haploid tetraspores (Roleda et al. 2008a). Haploid cells are reported to be efficient replicators, whereas diploid cells are resistant to damage (Long and Michod 1995). The higher tolerance of carpospores to UVR confirms the genetic buffering hypothesis suggested for vascular plants which proposes that diploids benefit from better cellular regulation and are, therefore, more vigorous and tolerant to radiation stress (Raper and Flexer 1970). During the diploid state, DNA damage can be repaired, because there are two copies of this biomolecule in each cell and one copy can be presumed to be undamaged.

Repair of UV-induced DNA photoproducts can take place by various mechanisms that include photoreactivation (light-mediated repair) or nucleotide and base excision repair (Britt 1996). Light-induced DNA repair is based on the activity of the key enzyme photolyase, which binds to complementary DNA strands and efficiently breaks pyrimidine dimers. However, this enzyme only functions as a DNA repair mechanism when blue light is available for activation. Significant removal of CPDs, indicating repair of DNA damage, was observed in Arctic seaweeds after recovery in low white light. DNA damage repair could be mediated either by light-dependent photolyases or light-independent nucleotide excision repair (Pakker et al. 2000, van de Poll et al. 2002a).

In the planktonic Antarctic diatom *Chaetoceros dichaeta* L.A. Mangin, no CPD accumulation was detected in high light-acclimated cells (Buma et al. 2006). For polar benthic microalgae, only one study has addressed UV effects on DNA damage (Wulff et al. 2008a). In this study, only minimal CPD accumulation was observed under unnaturally high intensities of UVB, and the effect was completely reversed after recovery under photoreactivating radiation (Wulff et al. 2008a).

Besides UV-induced DNA photoproducts, damage to protein molecules represents the second major direct effect of UVB in algae. Typical target proteins are those associated with the plasmalemma or involved in photosynthesis, such as the D1 protein of photosystem II and the enzyme Rubisco in the Calvin cycle (Bischof et al. 2000). The native three-dimensional structure of proteins is a prerequisite for any specific function, and hence any structural damage can be reflected in loss of cell vitality because these molecules have multiple roles as enzymes, hormones and structural components. However, because proteins typically occur as numerous copies inside the algal cell, any UV-induced damage is less severe than DNA damage. By contrast, degradation and replacement of damaged protein molecules require energy, which might otherwise support more essential processes, such as DNA repair.

The replacement of any UV-damaged protein by *de novo* biosynthesis contributes to counteracting UV damage. The molecular mechanisms are well studied in Cyanobacteria and might be similar in polar micro- and macroalgae. Exposure to moderate doses of UVB results in an increased turnover rate of the D1 and D2 reaction centre subunits of photosystem II, thus rapidly replacing damaged proteins by newly synthesised polypeptides (Campbell et al. 1998, Máté et al. 1998). A key step in this repair process is the UVB-induced differential transcription of reaction centre protein encoding genes.

Other biomolecules, such as photosynthetic pigments, can also be destroyed under UVR, with the phycobilins (main pigments of Rhodophyta) being the most sensitive, and carotenoids generally being less affected than chlorophylls. In contrast, lipids, which are major compounds of all biological membranes, cannot absorb UVR, but are easily peroxidised through UV-induced reactive oxygen species (ROS, see below) (Bischof et al. 2006).

Induction of reactive oxygen species (ROS) and antioxidative strategies

Polar micro- and macroalgae perform oxygenic photosynthesis using water as an electron donor thereby releasing molecular oxygen, which can be accumulated and easily chemically converted to potentially damaging

ROS. Photooxidative stress, including UVB, stimulates various cellular processes leading to the production of superoxide radicals (O_2^-), as well as singlet-oxygen ($^1O_2$) and hydroxyl radicals (OH^-). The sources and production sites of ROS are mainly related to photosynthetic activities, such as pseudocyclic photophosphorylation and the Mehler reaction, which stimulate the accumulation of hydrogen peroxide (Elstner 1990, Asada 1994).

Besides these internal processes, formation of ROS might also take place in the environment by UV-induced photoactivation of dissolved organic matter, photochemical degradation and liberation of excited electrons that initiate the reduction of molecular oxygen resulting in superoxide anion radicals (Cooper and Zika 1983). A second reduction step of superoxide radicals followed by protonation yields hydrogen peroxide, which is a powerful oxidant because of its relatively long half-life that allows long distance diffusion (Asada 1994). There are indications that UVB in Antarctic summer can stimulate the input of hydrogen peroxide from photochemical reactions and atmospheric wet deposition into shallow waters increasing its concentration from typical 20–300 nM up to 5000 nM (Abele et al. 1999).

ROS can cause extensive damage to DNA, proteins, and other biological molecules and structures (Bischof et al. 2006). Polar micro- and macroalgae have evolved a complex defence system against ROS including non-enzymatic antioxidants, such as ascorbate (vitamin C), tocopherol (vitamin E), carotenoids and reduced glutathione. However, these low-molecular weight antioxidants are chemically consumed and hence not considered the most efficient detoxifying agents. In contrast, antioxidant enzymes can efficiently counteract all UV-induced ROS and include superoxide dismutase, catalase, glutathione peroxidase and the enzymes involved in ascorbate-glutathione cycle like ascorbate peroxidase, mono-dehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase (Schriek 2000, Aguilera et al. 2002b, Dummermuth et al. 2003). Whereas Antarctic diatoms have high enzymatic antioxidant activities even at low temperatures due to low activation energies (Schriek 2000), Arctic macroalgae are capable of upregulating some of their antioxidative enzymes under UVR, thereby allowing fast acclimation to changes in environmental radiation conditions (Aguilera et al. 2002b). In addition, some polar seaweeds might even synthesise and accumulate specific antioxidants, such as bromophenolic compounds (Dummermuth et al. 2003).

Physiological processes and acclimation

The effects of UVB exposure on biological systems are manifold, and any damage or disturbance of biomolecules will strongly affect physiological processes, such as photosynthesis, growth and reproduction.

Photosynthesis Photosynthesis is probably the most intensively studied process under UVR stress in polar algae. Owing to biochemical complexity of this process, numerous sites can be affected by the UV-waveband. These include, e.g., inhibition of energy transfer within the photosystem II reaction centre, the water splitting complex, or the light-harvesting complex, as well as pigment changes. In addition, key enzymes of the Calvin cycle, such as Rubisco, are typical molecular targets. The response of polar micro- and macroalgae towards UVB exposure is determined by the interplay of genetically fixed adaptation and physiological acclimation (Bischof et al. 2006).

Many micro- and macroalgae from the intertidal and supralittoral zone, such as the chlorophyte *Urospora penicilliformis* and *Prasiola crispa*, and the phaeophycean *Fucus distichus* Linnaeus, are only mildly affected by UVR, indicating a broad photophysiological tolerance (Hanelt et al. 1997, Holzinger et al. 2006, Roleda et al. 2009a,b). In particular, their ability for dynamic photoinhibition, a photoprotective mechanism by which excessive radiation energy absorbed is dissipated harmlessly as heat (Krause and Weis 1991), contributes to this radiation stress tolerance. Dynamic photoinhibition is characterised by a pronounced decrease in photosynthetic activity under high photon fluence rates especially at noon, followed by a fast and full recovery during subsequent exposure to low radiation conditions, e.g., in late afternoon, as documented for Arctic seaweeds in the field (Hanelt et al. 1997). In most polar seaweed species studied so far, UVB contributes significantly to some degree to photoinhibition of photosynthesis, whereas recovery of photosynthetic efficiency, regardless of life history phase, is always lower in deep-water species compared with species from shallow waters. However, the underlying photoprotective mechanisms involved need to be elucidated.

In contrast to intertidal seaweeds, all deep-water and understory algae from the sublittoral recover only partly and slowly, or even bleach, indicating chronic photoinhibition (Karsten et al. 2001). Whereas dynamic photoinhibition is a reversible physiological mechanism, chronic photoinhibition is reflected by photodamage to components of the photosynthetic apparatus, such as the D1 protein (Bischof et al. 2006) and requires *de novo* synthesis of proteins. Nevertheless, at least some macroalgal species are able to acclimate their photosynthetic performance to UVR, as documented in sporophytes of Arctic Laminariales collected at different depths (Bischof et al. 1998, 1999). The acclimation response included a reduction in the degree of photoinhibition, an effect that might be explained either by the activation of the antioxidative capacity, increased activity of repair and recovery mechanisms counteracting the inhibitory effects or by the formation of UV-screening compounds.

The sensitivity of photosynthesis in reproductive cells of Antarctic macroalgae to PAR and UVR is related to the observed vertical zonation pattern of the adult plants (Zacher et al. 2007a, Roleda et al. 2008b). This response was also reported in the early life stages of Arctic macroalgae (Roleda et al. 2006d). It seems that low light adaptation of photosynthesis is a typical feature of reproductive cells of macroalgae (Roleda et al. 2006a,d, Wiencke et al. 2007, Zacher et al. 2007a), which can be explained by differences in chlorophyll antenna size and number of chloroplasts present in reproductive cells compared with multicellular macroscopic stages. *In situ* solar radiation can, therefore, exert a significant effect on survival of spores in the field (Wiencke et al. 2006).

Consequently, survival of propagules will be dependent on their immediate settlement on substrata at depths in between rock crevices, under boulders or under algal canopies where the prevailing low-light microenvironment is suitable for their germination.

The few studies of UVR effects on photosynthesis in benthic diatoms from polar waters indicate only temporary, low level photoinhibition facilitated by dynamic recovery and efficient DNA damage repair (Wulff et al. 2008a,b). In benthic microalgae, photosynthetic performance measured as maximum quantum yield of photosystem II (F_v/F_m) seems to be affected by UVA and UVB, but observed effects are mostly transient and disappear after 1 or 2 days of treatment (Wulff et al. 2008a,b,c).

The ability to cope with UVR after a period of darkness was tested in an Antarctic benthic diatom assemblage where the diatoms were kept in total darkness for 15 and 64 days, respectively, and then exposed to relatively high intensities of UVR (Wulff et al. 2008c). The diatom assemblage was dominated by the large species *Gyrosigma fasciola* (Ehrenberg) J.W. Griffith *et* Henfrey and *Pleurosigma obscurum* W. Smith, and these cells were able to resume photosynthetic activity after 64 days in darkness and coped with relatively high intensities of UVR compared with their natural habitat. However, although the cells surviving darkness could cope with the applied UVR, not all individual cells in the population survived the long dark period.

Growth Because the photosynthetic process in many species of polar micro- and macroalgae can acclimate to variable radiation stress, long-term effects of increasing UVR should be measured in growth and reproduction rather than photosynthesis alone. Growth and reproduction are typically related to ecological success (fitness) of individual algal species under given environmental conditions. In contrast to all other cellular processes, such as photosynthesis, respiration, anabolic pathways, etc., growth integrates well the impacts of all positive and negative abiotic factors and, thus, represents the most important physiological key parameter to describe the performance of algae in their polar ecosystem.

A simple growth model: $G=P-R-L$, proposed by Carr et al. (1997), follows a growth differentiation balance, where growth increment over time (G) is a function of the rate of biomass production through gross photosynthesis (P) and loss due to respiration (R) and tissue loss or decay (L). Under radiation stress (high PAR+UVR), photoinhibition of photosynthesis decreases potential carbon accretion (P) into plant dry matter (G) (Long et al. 1994). Dark respiration (R) represents the energy used to synthesise new biomass (growth respiration) and to maintain metabolic activity (maintenance respiration). Exposure to UVR causes molecular and cellular damage which might further increase loss due to respiration (R) by diverting more photosynthates to repair and/or protection (e.g., production of UV-sunscreen compounds), all of which results in decreasing growth.

Similar to photosynthetic performance, growth sensitivity of polar seaweeds is also strongly correlated to the vertical distribution (Aguilera et al. 1999, Michler et al. 2002, Roleda et al. 2007a). Moreover, growth rates of juvenile life stages of macroalgae are more susceptible to UVR exposure compared with adult plants, which is well documented for young kelp sporophytes (Roleda et al. 2005, 2006b). Because many growth studies on seaweeds were performed for only short periods on intact young plants or on discs excised from adult macroalgal thalli, UV-induced morphological deformation and tissue damage might easily have been overlooked (Aguilera et al. 1999). However, pronounced tissue necrosis and loss of parts of the thalli was reported in the UV-sensitive Arctic *Laminaria solidungula* J. Agardh after 1 week of daily exposure to 18 h UVR (Michler et al. 2002), indicating that such changes have a strong effect on growth performance.

In contrast to polar seaweeds and planktonic microalgae, studies of UVR effects on growth of benthic diatoms from Antarctic habitats are rare (Wulff et al. 2008a,b) and for the Arctic region virtually lacking. On the Antarctic Peninsula, Zacher et al. (2007c) studied UVA and UVB effects in a 15-week field experiment on an intertidal hard bottom platform. The growth of the microalgal assemblage dominated by the diatom genera *Cocconeis* and *Navicula* was unaffected by both UVA and UVB radiation. In a similar subtidal experiment, Campana et al. (2008) reached the same conclusion, the subtidal assemblage being dominated by the diatom species *Fragilaria striatula* Lyngbye, *Navicula* cf. *perminuta* Grunow and *Navicula* cf. *hanseni* Möller. In laboratory studies, growth of Antarctic benthic diatoms also seems to be unaffected by UVR. There are basically two strategies by which these microalgae respond to enhanced UVR: acclimation or avoidance. The first process includes repair and protection mechanisms, and, as recently shown for Antarctic benthic diatoms, they have only minimal DNA damage under UVB treatment and probably an elevated and efficient repair capacity (Wulff et al. 2008a). The second process includes migration and vertical movement (see below). In addition, because growth was always unaffected, these authors concluded that UVR does not seem to be a threat to benthic marine Antarctic diatoms.

Photoprotective mechanisms

Avoidance In contrast to sessile seaweeds, benthic diatoms can physically move away from harmful UVB, which represents one of the most effective avoidance strategies (Wulff et al. 2008a). However, it always requires an ability to detect this waveband. Furthermore, all algae face a dilemma with regard to solar radiation. Whereas sufficient PAR is an essential prerequisite for photosynthesis, too much UVR can be harmful. Migration and vertical movement in sediments in combination with self-shading definitely seems to be an efficient way for motile diatoms to receive the optimum light requirements and avoid long-term exposure to high UVR, thereby saving energy for photoprotective acclimation.

In contrast to motile benthic diatoms, most macroalgae must cope with prevailing radiation conditions. If these algae grow deep in the water column, they are never exposed to UVR (Karsten et al. 2001). In contrast, in the intertidal zone (eulittoral) or in sheltered coastal lagoons, macroalgae must cope with high radiation. Here, green algae of the genera *Ulva*, *Acrosiphonia* or

Urospora often form mat- or turf-like assemblages, which exhibit steep gradients of UVB, but also of physiological responses. Whereas top layers are exposed to high surface irradiance and hence often bleach, bottom layers permanently experience very low radiation conditions or even remain in darkness (Bischof et al. 2002b, 2006). Another strategy for small macroalgae to avoid high radiation in shallower water is to grow under the protecting canopy of larger macroalgal taxa, such as kelps. Indeed, recruitment of juvenile kelps under the canopy of adult plants is considered to be an adaptive behaviour, which effectively protects these early life stages from radiation stress, therefore minimising ecological cost for protection and hence enabling allocation of more photosynthate for growth (Herms and Mattson 1992).

Another mechanism that might be involved in UVR protection and acclimation is the establishment of a physical barrier that shields the photosynthetic apparatus against damaging radiation (Karentz 1994). For example, increasing thallus thickness was observed to minimise UVB-induced DNA damage as outer cell layers can shade inner cells and present a longer pathlength for UVR absorption (Franklin and Forster 1997, Roleda et al. 2007a). Thallus translucence or opacity can also influence reflection, attenuation, scattering, absorption or transmittance of UV radiation (Caldwell et al. 1983). Among kelps, optically dark pigmented juvenile sporophytes show strong absorbance in the UV waveband (Roleda et al. 2005, 2006b), characteristic of the UV-absorbing phlorotannins accumulated within the outer cortical layer of the thalli of Laminariales (Lüder and Clayton 2004, Shibata et al. 2004). In *Hormosira banksii* (Turner) Decaisne (Fucales), a brown layer consisting of oxidised phenolic compounds released from the peripheral cells forms a protective lamina for the photosynthetic tissue beneath (Schoenwaelder 2002). UVR can, therefore, be attenuated by cellular UV-absorbing compounds and cell walls of the epidermal tissue, effectively reducing UV fluence before it reaches important physiological targets. UV-absorbing compounds located in the epidermis are reported to protect Arctic vascular plants from UVB radiation (Nybakken et al. 2004). The search for flavonoid-like compounds at least in *Urospora penicilliformis* gave a negative result (Roleda et al. 2009b).

Photoprotective substances

One of the most important physiochemical acclimation mechanisms against UVR is the biosynthesis and accumulation of UV-sunscreen substances. Typically absorbing in the UVA and UVB range, these biomolecules have been invoked to function as passive shielding solutes by dissipating the absorbed short wavelength radiation energy in the form of harmless heat without generating photochemical reactions (Bandaranayake 1998). The most common photoprotective sunscreens in many polar micro- and macroalgae are the mycosporine-like amino acids (MAAs), a suite of chemically closely related, colourless, water-soluble, polar and, at cellular pH, uncharged or zwitterionic amino acid derivatives. MAAs exhibit a high molar absorptivity for UVA and UVB, and have been reported as photochemically stable molecules, which are prerequisites for their sunscreen function (Conde et al. 2000). Whereas MAAs have been mainly observed in numerous Rhodophyta (Karentz et al. 1991, Hoyer et al. 2001), Phaeophyceae and most Chlorophyta typically lack these compounds, except the green algal genus *Prasiola* which contains high concentrations of a unique MAA with an absorption maximum at 324 nm (Hoyer et al. 2001, Karsten et al. 2005). Most Phaeophyceae synthesise photoprotective phlorotannins under UV exposure (Pavia et al. 1997), these compounds will be reviewed in detail below. Whereas planktonic centric diatoms from Antarctica also accumulate MAAs under UVR (Buma et al. 2006), benthic pennate taxa are surprisingly poor in these compounds or even lack them (Wulff et al. 2008a).

Field and laboratory experiments in the Arctic with the intertidal red alga *Devaleraea ramentacea* (Linnaeus) Guiry showed a continuous decrease in photosynthetic performance under UVR with increasing collecting depths between 1 and 5 m. Total MAA concentration was also correlated with original sampling depth, i.e., shallow water isolates contained much higher amounts than algae from deeper waters, also indicating a strong correlation between MAA contents and degree of sensitivity of photosynthetic activity in *D. ramentacea* (Karsten et al. 1999). The sunscreen function of MAAs has been further inferred in other polar red algae from a decrease in concentration with increasing depth (Hoyer et al. 2001, 2003). Supra- and eulittoral Rhodophyta typically experience the strongest insolation, and consequently synthesise and accumulate highest MAA values, which are positively correlated with the natural UV doses (Karsten et al. 1998). In contrast, many red algal taxa growing in deep waters are biochemically incapable of producing MAAs (Hoyer et al. 2001, 2002; Karsten et al. 2001), which explains their strong sensitivity to high ambient solar radiation. These species do not experience UV exposure in nature, and, hence, there is no physiological need to synthesise and accumulate metabolically expensive nitrogen-containing MAAs. This in turn saves energy to better support other essential pathways, such as the biosynthesis of light-harvesting phycobilisomes to guarantee sufficient PAR absorption under the prevailing low-light conditions.

In the Antarctic red alga *Palmaria decipiens*, juveniles collected in the upper to mid sublittoral during winter contained low MAA concentrations, whereas mature plants collected in late spring and summer exhibited significantly higher values, indicating strong seasonal effects, which are related to the changing daylengths and radiation conditions (Post and Larkum 1993). Based on the MAA concentrations and the induction patterns after exposure to different defined radiation conditions, red algae can be physiologically classified into three categories (Hoyer et al. 2001): Type I – no MAAs at all, Type II – MAAs inducible in variable concentrations, and Type III – permanently high MAA values. Whereas Type I represents deep-water red algae of the lower sublittoral, Types II and III species grow from the supra- and eulittoral to the upper and mid-sublittoral zone. Experiments under defined radiation conditions demonstrated that the induction, biosynthesis and accumulation of MAAs are very flexible and species-specific processes. Whereas

some polar Rhodophyta synthesise MAAs particularly under UVB, others do so under UVA or higher PAR (Hoyer et al. 2003). Although experimental evidence for a particular trigger mechanism is still missing, it is reasonable to assume that a signal transduction pathway is involved in the overall process leading to high MAA concentrations. Based on the different types of MAA induction patterns in polar red algae, the presence of various photoreceptors, most probably between the blue light and UVB range, must be considered (Kräbs et al. 2002).

The entire algal thallus does not respond uniformly to ambient UVR. Young apical or marginal zones, i.e., growing cells, synthesise and preferentially accumulate MAAs, leading to cross-sectional and longitudinal concentration gradients (Hoyer et al. 2001). Populations of *Devaleraea ramentacea* collected from very shallow water typically exhibit green apices and red basal parts. The more exposed green tips contain 5-fold higher MAA contents than the red bases (Karsten et al. 1999). Older tissue regions usually exhibit thicker cell walls and a leathery texture and are therefore optically opaque. The higher MAA concentrations in the most exposed thallus regions are essential to guarantee protection of the more delicate meristematic cells.

From an ecological standpoint, the ephemeral, tufty *Prasiola* is intriguing because of its ability to grow in polar regions subaerially on soil and rocks underneath or near seagull or penguin colonies (Holzinger et al. 2006), i.e., habitats rich in nitrogen-containing faeces. Considering a relation between MAA concentration and nitrogen availability in different species of the red alga *Porphyra* (Korbee et al. 2005), it seems that this nutrient might be a critical factor for the broad photophysiological tolerance of *Prasiola* under subaerial conditions (Holzinger et al. 2006). Seasonal studies on *Prasiola* in Antarctica indicate some variation in MAA concentrations but always with high minimum steady-state amounts (Jackson and Seppelt 1997). Consequently, it appears that the 324-nm MAA in *Prasiola* is expressed constitutively.

As well as functioning as natural UV-protective compounds, some MAAs, such as mycosporine-glycine, also have moderate antioxidant activity (Dunlap and Yamamoto 1995). In addition, the presumed biochemical precursor of MAAs, 4-deoxygadusol, exhibits strong antioxidant activity (Dunlap et al. 1998). The photophysicochemical properties of MAAs guarantee both a high UV-protective effectiveness in combination with antioxidant capabilities.

Phlorotannins are exclusively produced by brown algae and include hundreds of phenolic compounds (126–650 kDa) composed of the monomer phloroglucinol (Amsler and Fairhead 2005). The primary biological function of phlorotannins is the strengthening of cell walls, and one of the several suggested secondary functions is the protection of algae against harmful UVB radiation (Ragan and Glombitza 1986, Swanson and Druehl 2002). This role is based on the absorbance of phlorotannins in the UV region, with peaks at approximately 195 nm and 270 nm, whereas the absorbance shoulders extend from $<$200 nm to 400 nm (Ragan and Glombitza 1986, Pavia et al. 1997).

Experimental evidence showed that intra- and/or extracellular phlorotannins within very dense kelp spore suspensions used as biofilters shielded underlying kelp spores from UVB thereby allowing germination (Roleda et al. 2006c, Swanson and Druehl 2002). Such UVB protection of kelp spores by exuded phlorotannins might also occur in dense kelp beds (Swanson and Druehl 2002), although the sunscreen capability of phlorotannins diminishes with decreasing concentrations (Roleda et al. 2006c). Thus, different UVR responses of spore germination of the Arctic *Alaria esculenta* and *Laminaria digitata* at different dates within 2 weeks (Wiencke et al. 2006) are probably attributed to changing phlorotannin contents in the water column.

Whether UVR exposure can induce the formation and accumulation of phlorotannins in Phaeophyceae is still an open question because of contradictory results in the literature. Light-microscopic analysis of zoospores of the Arctic kelps *Alaria esculenta*, *Saccorhiza dermatodea* and *Laminaria digitata* indicated that UVB treatment led to an enlargement of phlorotannin-containing physodes, an aggregation of several small physodes to form larger ones, as well as to an exudation of physodes into the surrounding seawater (Wiencke et al. 2004, 2007, Roleda et al. 2006c). These observations were, however, not confirmed by an ultrastructural analysis of UVB-exposed zoospores of the UVR sensitive, cold-temperate *Laminaria hyperborea* (Steinhoff et al. 2008).

Moreover, zoospore suspensions of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima*, juveniles and embryos of *Fucus gardneri* P.C. Silva, and sporophytes of *Desmarestia anceps* Montagne and *D. menziesii* J. Agardh did not show any concentration increase after UVB treatment (Henry and van Alstyne 2004, Fairhead et al. 2006, Müller et al. 2009). An accumulation of phlorotannin contents after treatment with UVB was also not detected in the seawater surrounding blades of *Macrocystis integrifolia* and zoospores of *A. esculenta*, *L. digitata* and *S. latissima* (Swanson and Druehl 2002, Müller et al. 2009). Otherwise, an induction of phlorotannins by UVR was observed in the blades of the giant kelp *M. integrifolia* (Swanson and Druehl 2002) and in sporophytic tissues of *Ascophyllum nodosum* (Linnaeus) Le Jolis, *S. latissima* and *Nereocystis luetkeana* (K. Mertens) Postels *et* Ruprecht (Pavia et al. 1997, Pavia and Brock 2000, Swanson and Fox 2007).

Generally, the often observed discrepancies between studies on UVR-sunscreen function of phlorotannins might be attributed to low replicate numbers, different exposure times and irradiation intensities, analytical methods and/or selected species/life stages studied, although ontogenetic, intra- and interspecific phlorotannin levels are known to be highly variable (Amsler and Fairhead 2005). In particular, the standard measurement of hundreds of compounds pooled as phlorotannins could lead to false negative or positive results, whereas the analysis of single phlorotannin compounds after UVB treatment might help to better understand the UV protective role of phlorotannins. Therefore, future studies should focus more attention on the simultaneous analysis of UVR responses of different algal species in the field, of their intra- and extracellular phlorotannin levels and compositions, and of their absorption properties to substantiate the role of phlorotannins as UV protectants in brown algae from polar environments.

Interactive effects of UVR with other abiotic and biotic factors

In nature, plants are subject to the influence of a multitude of abiotic and biotic factors (Alexieva et al. 2003). Stress factors do not operate individually or independently, so that spatial or temporal variations and interactions between and co-variation of stresses are the norm in the natural environment (Jones et al. 1993, Vinebrooke et al. 2004). Stressors act synergistically when their combined effect on biological components is larger than the responses to each single stressor, and antagonistically when the impact is smaller (Folt et al. 1999). It is also possible that plants exposed to a single stress agent are capable of increasing their resistance to subsequent unfavourable impacts (cross-adaptation; Alexieva et al. 2003).

In polar regions, several environmental factors have changed and are still changing in parallel, such as ozone depletion and the concomitant rise in UVB, as well as the increase in greenhouse gases (e.g., CO_2) and the resulting global warming and acidification of the oceans. Consequently, any increase in UVB does not have an independent effect on marine benthic micro- and macroalgae because changes in the other environmental factors have to be considered as well (Zacher et al. 2009b).

Bothwell et al. (1994) reported that benthic diatoms benefit from UVR because their grazers were more susceptible to applied radiation. Also, in the field, grazers had a larger impact than UVR, although no negative effect of UVR on the grazers was apparent (Zacher et al. 2007c).

Hoffman et al. (2003) supported the hypothesis that temperature mitigates the net biological effect of UVR on macroalgae and *vice versa*. Correspondingly, the photosynthetic performances of sporophytes of the Antarctic green alga *Enteromorpha bulbosa* (Suhr) Montagne, the subantarctic *Ulva clathrata* (Roth) C. Agardh, the Arctic kelp *Alaria esculenta*, and the Arctic red algae *Palmaria palmata*, *Coccotylus truncatus* (Pallas) M.J. Wynne *et* J.N. Heine and *Phycodrys rubens* (Linnaeus) Batters were less impaired by UVB at higher temperatures compared with lower temperatures (van de Poll et al. 2002b, Rautenberger and Bischof 2006, Fredersdorf et al. 2009). In the latter three red algae, the recovery of photosynthesis after UVB stress was likewise improved at higher temperatures, although the influence of UVB acted predominantly on relative growth rate and CPD accumulation as a marker of DNA damage (van de Poll et al. 2002b). In contrast, the germination of zoospores and photosynthetic performance of sporophytes of the UVB-tolerant *A. esculenta* were mainly determined by temperature when thalli were exposed to both UVR and stressful temperature (Müller et al. 2008, Fredersdorf et al. 2009). Germination of highly UVB-vulnerable zoospores of the Arctic kelps *Saccharina latissima* and *Laminaria digitata* were more UVB affected at unfavourable lower and higher temperatures rather than at the ambient temperature of 7°C (Müller et al. 2008). Germination of zoospores of the Helgoland ecotype of *L. digitata* was, however, less harmed by UVB at temperatures higher than 2°C (Müller et al. 2008). In contrast, the sensitivity of germlings of three *Fucus* species from Helgoland to UVB radiation was enhanced with increasing temperature (Altamirano et al. 2003). Thus, the temperature dependence of UVR effects on seaweeds varies highly within and among species and genera.

There are only few ecophysiological studies on interactive effects of UVR and/or temperature with salinity in polar seaweeds, although significant amounts of freshwater regularly flow into polar waters due to rivers and melting of glaciers and snow (Karsten et al. 2003, Fredersdorf et al. 2009). These studies also reveal life stage-, intra- and interspecies-specific responses to interactive stress. In a study by Karsten et al. (2003), interactive effects of UVR and salinity on two shallow water Arctic red macroalgae, *Devaleraea ramentacea* and *Palmaria palmata*, were investigated. Whereas *D. ramentacea* exhibited euryhaline features and acclimated well to the UVR applied, *P. palmata* can be characterised as a stenohaline plant because of its high mortality already under mild hyposaline conditions. In addition, the latter species had a limited ability to acclimate to changing PAR/UV radiation pointing to a relatively low physiological plasticity.

At the assemblage level, species-specific UVB responses of algae and distinct food preferences of grazers result in complex changes (Zacher et al. 2007b, Zacher and Campana 2008). For instance, the preference of snails for feeding on UVB-sensitive green algal recruits favoured the recruitment of leathery red algal recruits in the Antarctic intertidal phytobenthos (Zacher et al. 2007b). Although UVR effects on diversity and species composition of macroalgal assemblages were less pronounced, the biomass and recruit density of the assemblage was over the long-term more substantially affected by grazing (Zacher et al. 2007b, Zacher and Campana 2008). The effects of UVB and temperature on the recruitment and succession of the green alga *Ulva intestinalis* Linnaeus from Nova Scotia (Canada) were likewise mitigated by consumers, whereas nutrient supplements enhanced grazing pressure (Lotze et al. 2002). High levels of nutrients also abated detrimental UVB effects on a diatom-dominated, soft-bottom assemblage off the Swedish west coast (Wulff et al. 2000). In Antarctic waters, which are characterised by generally high nutrient levels (Korb and Gerard 2000), a diatom-dominated, hard-bottom assemblage was almost unaffected by UVB, although grazing structured the assemblage almost entirely (Zacher et al. 2007c). These few studies on interactive effects on micro- and macrophytobenthos of polar latitudes indicate that grazing pressure might have a generally stronger impact on the assemblage than UVB, whereas nutrients might influence the impact of grazing on algae. Comparable observations were made in the Arctic microbial food web, where negative UVB effects on the growth of nano- and picoautotrophs as well as heterotrophic nanoflagellates were much less substantial than mortality imposed by grazing (Wickham and Carstens 1998).

Nevertheless, there are numerous fundamental and still unanswered questions about the tolerance of benthic micro- and macroalgae to combined stress, especially at

the respective ratio of PAR/UVR. One unsolved problem is related to the comparability of experiments undertaken in the field and in the laboratory because natural solar radiation (waveband ratios, intensities) is very difficult to artificially mimic. Consequently, PAR/UVR ratios in both approaches can be quite different and hence in the laboratory often so unnatural that the resulting data have to be interpreted with caution. Clearly, many more ecologically relevant studies on interactive effects, especially on the most sensitive developmental stages and on the algal assemblages are required for predictions of the effects of global change on polar marine ecosystems.

Ecological consequences

To determine ecological effects resulting from increased UVR is a difficult task. Probably, effects on the community or even ecosystem level had already taken place before the official discovery of the ozone hole over Antarctica in the 1970s. From field experiments in 10 different coastal regions ranging from tropical to polar waters, Wahl et al. (2004) concluded that any observed UV effect disappeared during species succession over 2–3 months. The Antarctic assemblage studied was dominated by benthic diatoms (Wahl et al. 2004). Although the few existing studies on benthic microalgae point to a capacity to acclimate to ambient and increasing UVR, much more ecological research is needed to identify the underlying mechanisms and interactions, and particularly to include several trophic levels under natural conditions. Considering only one trophic level could lead to misinterpretations of possible UV effects, e.g., the observed negative UV effects on grazers might be interpreted as a positive UV effect on benthic diatoms (Bothwell et al. 1994). Also, Xue et al. (2005) and Bancroft et al. (2007) stress the importance of including several trophic levels, as well as multiple stressors because UVB effects on one trophic level can lead to instabilities of a whole ecosystem favouring a few hardy species.

For macroalgae, colonisation stages and spores seem to be more sensitive to UVR compared with respective adult thalli. Consequently, in polar environments influenced by ice and scouring (frequent colonisation events) UVR could be an important controlling factor. In addition, a changed zonation pattern has been suggested (e.g., Bischof et al. 2006) with possible cascade effects at the assemblage level, including epiphytes and grazers. From temperate regions, Lotze et al. (2002) reported that although UVR could be a structuring force during early succession (early colonising species and microscopic recruits), grazers could mediate this effect over time. Including several abiotic factors (UV, temperature and nutrients) led to complex interactions of ecological controls on macroalgal recruitment, and various synergistic effects of abiotic and biotic (e.g., grazers) factors were described (Lotze and Worm 2002).

Although the number of field studies on benthic algae in polar areas has increased over the recent years, still very few take the ecosystem approach (Wahl et al. 2004, Zacher et al. 2007b). For future studies and to better understand ecosystem consequences, this is clearly one of the greatest challenges to elucidate, e.g., UV effects on standing stock biomass, benthic primary production, biodiversity, food quality, trophic webs and interactions, etc.

Conclusion

Although considerable progress has been achieved in recent years on UVR effects, our present knowledge about the physiological capabilities of benthic micro- and macroalgae from polar regions is still fragmentary. Owing to the extreme remoteness of the polar regions and the infrequency of scientific studies, many open questions still have to be addressed. In particular, more experimental evidence is needed from both field and controlled laboratory studies to precisely document susceptibility, tolerances, acclimation and adaptation with an emphasis on the underlying mechanisms, which can only be elucidated by modern genomic, proteomic and metabolomic approaches. In addition, and as already mentioned, more ecosystem approaches and studies on interactive effects are needed to depict a more holistic picture.

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Publication VI

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UV-radiation and elevated temperatures induce formation of reactive oxygen species in gametophytes of cold-temperate/Arctic kelps (Laminariales, Phaeophyceae)

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UV-radiation and elevated temperatures induce formation of reactive oxygen species in gametophytes of cold-temperate/Arctic kelps (Laminariales, Phaeophyceae)

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SUMMARY

Enhanced UV-radiation (UVR) through stratospheric ozone depletion and global warming are crucial stressors to marine macroalgae. Damages may arise through formation of reactive oxygen species (ROS) in gametophytes of ecologically important kelps, brown algae of the order Laminariales, Such stress-induced damages may have a negative impact on their fitness and further impact their following life stages. In our study, gametophytes of three kelp species *Alaria esculenta* (L.) Grev., *Laminaria digitata* (Huds.) Lamour., *Saccharina latissima* (L.) Lane, Mayes, Druehl, Saunders from the Arctic, and of *L. hyperborea* (Gunnerus) Foslie from the North Sea were exposed to photosynthetically active radiation, UV-A, and UV-B radiation and four temperatures (2–18°C). ROS are formed predominantly in the peripheral cytoplasm and in chloroplasts especially after exposure to UVR. Superoxide (O_2^{*-}) is additionally formed in small, globular cytoplasmic structures, possibly mitochondria. In the surrounding medium O_2^{*-}-concentration increased markedly at elevated temperatures and under UV stress in some cases. Ultrastructural damage was negligible pointing to a high stress tolerance of this developmental stage. 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.

Key words: brown algae, confocal laser scanning microscopy, electron microscopy, gametophytes, kelps, light microscopy, reactive oxygen species, temperature, ultraviolet radiation.

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 are important marine primary producers fixing up to 1.8 kg carbon m^{-2} $year^{-1}$ and forming large kelp forests (Thomas 2002). Kelp forests provides, 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 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 favorable 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 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 formation of reactive oxygen species (ROS) (Lesser 2005; Dring 2006). Moderate amounts of ROS act as signals and can induce an endorsement of the antioxidative capacity thereby increasing stress tolerance and viability of the cells. However, a high level of ROS that could not be compensated by the cell will cause cellular damage, apoptosis or necrosis. Exceeding production of intracellular ROS becomes harmful for the DNA, proteins and membrane lipids (Lesser 2005). On the other hand, extracellular generation and release of ROS is a powerful measure in marine macroalgae to resist infections in case of pathogen attacks (Potin 2008).

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So far, only few data are available about ROS formation and possible cellular damages in gametophytes of ecologically important 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 the seed bank of kelp forests. Especially in Arctic regions, the impact of UVR will remain high as recent results indicate that the springtime polar ozone depletion will continue to be severe (Weatherhead & Anderson 2006; WMO 2007). Moreover, temperatures in the Arctic will dramatically rise by about 4°C until 2100 (IPCC 2007; Müller *et al*. 2011).

The main goal of the present study was to determine how kelp gametophytes with a cold-temperate to Arctic distribution will react under conditions of environmental perturbations. Therefore, the intracellular formation of ROS and of ultrastructural changes were comparably investigated in gametophytes of three key species within Arctic kelp forests (*Alaria esculenta* (L.) Grev., *Laminaria digitata* (Huds.) Lamour., *Saccharina latissima* (L.) Lane, Mayes, Druehl, Saunders) and of *L. hyperborea* (Gunnerus) Foslie from Helgoland (North Sea) after exposure to different radiation conditions. Extracellular ROS production was also studied under three radiation and four temperature conditions in gametophytes of the three species from the Arctic.

MATERIALS AND METHODS

Algal material

Gametophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* obtained from freshly released zoospores were used to analyze their ultrastructure and the intracellular production of superoxide anions through Nitro blue tetrazolium chloride (NBT)-staining (see below). Therefore, fertile sporophytes of the three species 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 one to two nights at 2 ± 1°C. Subsequently sori were stimulated with filtered, 7°C warm seawater (SW) to release their zoospores. Zoospore suspensions obtained within ≤60 min were filtered through 20 μm gauze (Nytal HD 20, Hydro-Bios, Kiel, Germany). Zoospore density was assessed under 200× magnification (Axioplan Neofluar microscope, Zeiss, Göttingen, Germany) with a Neubauer chamber (Brand, Wertheim, Germany). Afterwards, zoospore suspensions of three individuals were mixed and adjusted with SW to 2.8–4.4 × 10^5 zoospores mL^{-1}. 40 mL of zoospore suspensions were filled into Petri dishes (85 × 10 mm) and placed at 7°C and 10 ± 3 μmol m^{-2} s^{-1} photosynthetically active radiation (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 anion radicals (O_2^{*-}) zoospores were allowed to settle and germinate on cover slips in culture dishes.

In parallel, gametophytes from unialgal stock cultures (AWI seaweed collection) 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 O_2^{*-} through formation of diformazan (see below). Additionally, *in vivo* ROS formation was determined in *L. hyperborea* from Helgoland (cult. nr. ♀ 3091, ♂ 3090) by NBT-staining and confocal laser scanning microscopy using the ROS indicator CM-H_2DCF-DA (see below). Prior to 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 35–50 gametophytic fragments mL^{-1} were dispersed into 24 well Costar® culture plates (50–75 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 climate chambers at either 7 ± 1°C for microscopic analysis or 2, 7, 12 and 18 ± 1.4°C for extracellular semi-quantification of superoxide (O_2^{*-}; *A. esculenta*, *L. digitata* and *S. latissima*) for 8 h. Three radiation treatments were generated by covering culture dishes with cut-off filter foils admitting wavelengths (i) 400–700 nm: photosynthetically active radiation (P, URUV Ultraphan UV farblos, Difrega, Munich, Germany) (ii) 320–700 nm: P + UV-A radiation (PA, Folanorm SF-AS, Dreieich, Germany) or (iii) 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, Durofest Lightning, Philadelphia, USA, 40 W Q-Panel, PA, Cleveland, OH, 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, Lincoln, NE, USA) and UVR with a Ramses SAM 80f6 sensor (TriOS Optical Sensors, Germany) linked to a UV-VIS spectroradiometer (IPS 104, TriOS Optical Sensors, Rastede, Germany).

Light microscopy

Two hours prior to exposure to experimental conditions, 10-day-old gametophytes of *A. esculenta*, *L. digitata*

and *S. latissima* (pooled from three individuals) as well as gametophytic fragments of *L. hyperborea* (pooled from three male and female gametophytic tufts) were incubated with 40 mL 6 mM NBT Sigma-Aldrich, St. Louis, MO, USA) diluted in SW (pH 7.8; NBT-SW) for 2 h. After that, NBT-SW was replaced by fresh SW, and gametophytes were immediately exposed to three radiation conditions at 7°C. After 8 h exposure, gametophytes were kept in an achromatizing solution (trichloracid 0.15% (w/v) in ethanol/chloroform 4:1 (v/v)) for 1 h, in fresh achromatizing solution for an extra 2 h, and finally stored in glycerine/destilled water (1:1 (v/v)). Light microscopic photographs were made from ≥50 gametophytes per treatment under 1000 × magnification (Axioplan 1.4 Oil) using an Axiophot microscope (Zeiss) and the Cell^F program version 2.5 (Build 1163). Generation sites of O_2^{*-} 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 of *L. hyperborea*, 5-(and-6) chloromethyl-2′7′-dichlordihydroflourescein diacetate (CM-H_2DCF-DA; Molecular Probes, Life Technologies, Darmstadt, Germany) was dissolved in SW pH 7.2 and added to gametophytes from stock cultures immediately prior exposure (final concentration: 10 μM CM-H_2DCF-DA). CM-H_2DCF-DA is a derivate of the commonly used ROS indicator H_2DCF-DA and permits longer retention within the cell and shows less non-specific fluorescence under illumination (personal information from the company: Molecular Probes). It has successfully been used for *in vivo* ROS detection in plants (Shin and Schachman 2004; Yao & Greenberg 2006; Kristiansen *et al*. 2009) and seaweeds, e.g. embryos of *Fucus spiralis* (Rijstenbil *et al*. 2000). After 15 min exposure to P or PAB at 7°C and 15 min incubation in darkness, gametophytes were analyzed 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-H_2DCF-DA molecules. At least 40 cells were examined from each condition. Relative intensity of DCF fluorescence was determined for semi-quantification ($n = 6$).

Photometry

Immediately before exposure, gametophyte suspensions containing 280 ± 50 cells mL^{-1} 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). 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 O_2^{*-} and XTT was measured in all specimens at 470 nm using a Spectra Max 190 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Thereby, measured absorption of controls was used as reference. The absolute amount of extracellular O_2^{*-} produced by gametophytes was calculated from the referenced absorption and the molar extinction coefficient 2.16×10^4 M^{-1} s^{-1} (Sutherland & Learmonth 1997). Statistical analyses of datasets were carried out in accordance with Sokal and Rohlf (1995) using the software Statistica Version 7 (StatSoft, Inc., Tulsa, OK, USA). Effects of irradiation and temperature on the production of O_2^{*-} were estimated by a two-factorial, Model I analysis of variance (ANOVA) (F, $P < 0.05$) subsequent homogeneity of variances (Levene's test, $P < 0.01$) was proven. In the case of heterogeneous datasets of *L. digitata* and *A. esculenta*, square root transformations were accomplished to fulfill the requirements for homogeneity. Finally, multiple mean comparisons were completed with Tukey Honestly Significant Differences (HSD) Post Hoc test ($P < 0.05$).

Transmission electron microscopy

After 8 h radiation exposure at 7°C, 10-day-old gametophytes of *A. esculenta*, *L. digitata* and *S. latissima* were carefully resuspended by jetting the medium against the bottom of the Petri-dish using a pipette 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, ultrathin 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. Five to ten 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.

RESULTS

Intracellular localization of ROS generation

Typical microscopic observations of pigmented, achromatized and NBT-stained 10-day-old gametophytes of

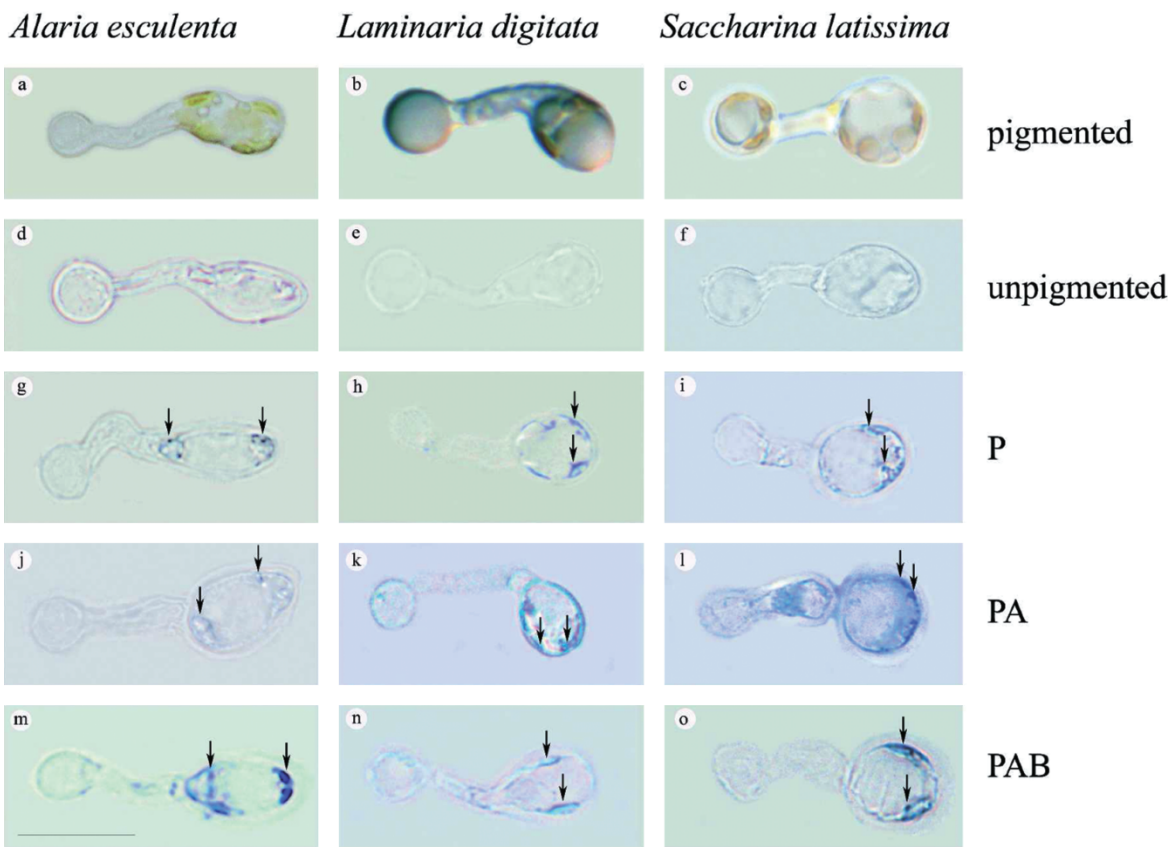

Fig. 1. Typical light-microscopic observations of pigmented, achromatized and 4-nitroblue tetrazolium chloride (NBT)-stained/achromatized, 10-day-old gametophytes of *Alaria esculenta* (a, d, g, j, m), *Laminaria digitata* (b, e, h, k, n), and *Saccharina latissima* (c, f, i, l, o). Blue/purple staining 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 h 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. P, photosynthetically active radiation; PA, P + UV-A radiation; PAB, PA + UV-B radiation.

Alaria esculenta, *Laminaria digitata* and *Saccharina latissima* are displayed in Fig. 1. In general, intracellular O_2^{*-} developed mainly in globular structures, which were distally or basally located in the primary cells (Fig. 1g–o). Moreover, a slight NBT-staining of the peripheral cytoplasm was often detected (Fig. 1g–o). In addition, the NBT-staining in PAB-treated gametophytes was frequently stronger and appeared mainly in the periphery of the chloroplasts (Fig. 1m, n, o) if compared to both other radiation treatments (Fig. 1g–l). Nonetheless, the degree of NBT-staining was not consistent within different radiation treatments since all responses from non- to relatively strong NBT-staining were observed in each treatment. No O_2^{*-} formation was observed in the empty embryospore (Fig. 1).

In contrast to the results explained above on young 10-day-old gametophytes, formation of O_2^{*-} in gametophytes from stock cultures of *L. hyperborea* after NBT-staining could only be demonstrated after exposure to PAB, not after exposure to P (Fig. 2). After PAB exposure the staining of the peripheral cytoplasm and cytoplasmic strings was stronger (Fig. 2b) than in the juvenile gametophytes of the other three species studied (Fig. 1). Moreover, O_2^{*-} were detected in numerous globular cytoplasmic structures, possibly in mitochondria (Fig. 2b, small arrows) and in plastids (Fig. 2b, large arrow).

Furthermore, significant CM-DCF radical fluorescence in gametophytes of *L. hyperborea* exposed to P and PAB revealed the intracellular production of high amounts of reactive oxygen species (Fig. 3). After only 15 min exposure, high amounts of ROS were detected in the chloroplasts and the peripheral cytoplasm in particular in the PAB-treated gametophytes. Semi-

quantification of relative fluorescence intensity of DCF-Signal (asterisks in Fig. 3) indicates also a clear increase of ROS concentration (39.5%) after PAB compared to P exposure.

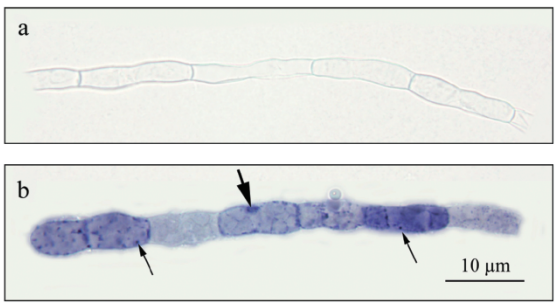

Fig. 2. Gametophytes of *Laminaria hyperborea* from stock culture. Typical light-microscopic observation of 4-nitroblue tetrazolium chloride (NBT)-stained and achromatized cells after 8 h irradiation with (a) P (400–700 nm) or (b) PAB (280–700 nm) treatment showing the generation of superoxide anion radials (O_2^{*-}). Arrows point to sites of increased O_2^{*-}-formation (Small arrows, presumably mitochondria; Large arrow, presumably a chloroplast).

Production of O_2^{*-} in the surrounding medium

An increase of temperature caused a rise of superoxide anion radical (O_2^{*-}) production by gametophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* (Fig. 4). The amount of O_2^{*-} in the medium surrounding 280 ± 50 gametophytic cells 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) are given for the P treatment. The effect of UV-R on the O_2^{*-} production 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*); d.f. = 6; $P < 0.01$). At 2°C a significant higher O_2^{*-} release 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 O_2^{*-} production 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 generation of O_2^{*-} in *S. latissima* was at 2°C similarly high as in all radiation treatments at 7°C.

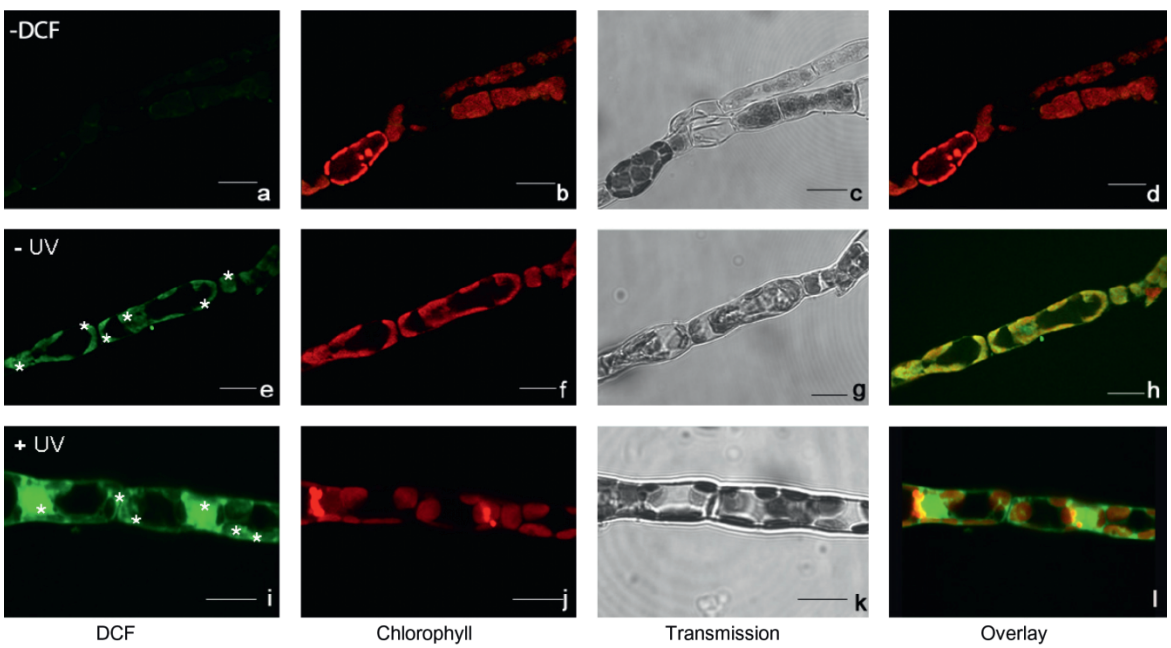

Fig. 3. Typical confocal laser scanning micrographs of gametophytes of *Laminaria hyperborea* from stock culture a–d. Controls were taken under identical settings without CM-H2DCF-DA staining. Cultures were exposed for 15 min to P (400–700 nm; c–h) or PAB (280–700 nm; i–l), respectively. Green color in a, c and i indicates the presence of reactive oxygen species (ROS) after reaction with CM-H2 DCF-DA. Red color in b, f and j illustrates the auto-fluorescence of the chloroplasts. c, g and k show transmission images. Overlays of green and red emission are presented in d, h and l. Regions where fluorescence intensities were measured are marked by asterisks. Scale bar 9 µm.

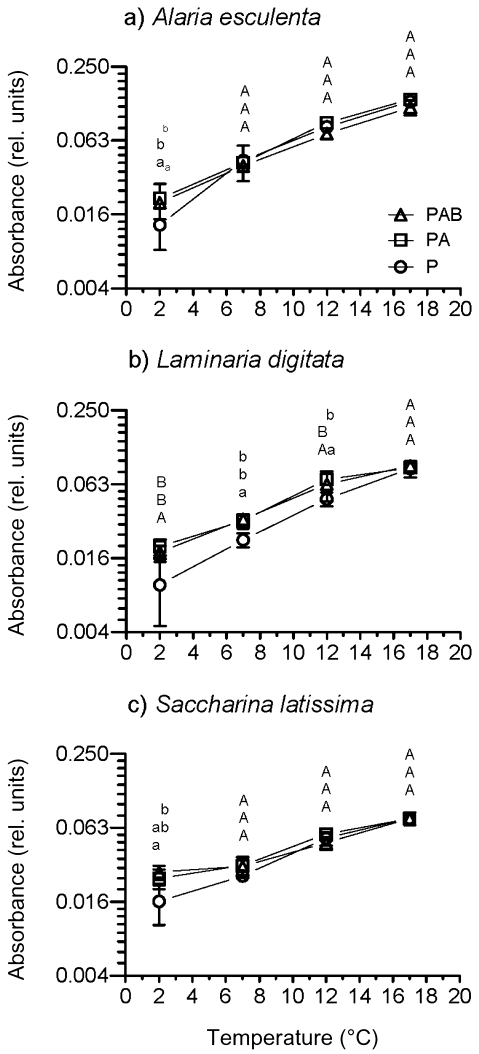

Fig. 4. Absorbance of (di-)formazan at 470 nm (log 2) as a function of temperature, representing the release of O_2^{*-} from gametophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* ($n = 8$) after an exposure of 8 h to the different radiation conditions (further explanation in Fig. 1). Absorbance of (di-) formazan measured in the medium of UVR- and temperature-treated gametophytes was referenced by UVR and temperature exposed seawater (control). Significant differences among irradiation treatments within a given temperature are marked with uppercase letters at a significance level of $P \leq 0.001$, with lowercase letters at a significance level of $P \leq 0.01$ and with subscript lowercase letters at a significance level of $P \leq 0.05$, respectively.

Nevertheless, the extra impact of irradiation on the quantity of the O_2^{*-} release became indistinguishable in the two species *S. latissima* and *A. esculenta* at 7–18°C ($P > 0.05$) (Fig. 4). The O_2^{*-} production in *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 O_2^{*-} was as high as the control at 7°C ($P \geq 0.05$). Within the treatments at 7°C the O_2^{*-} generation diverged under P condition from that of the PA and PAB treatment ($P < 0.02$), whilst the O_2^{*-} release of PA treatment differed not from that after PAB treatment ($P > 0.05$). At 12°C the irradiation of gametophytes with PA and PAB radiation resulted in an equally elevated O_2^{*-} release, which was higher than that of the control at 12°C ($P < 0.03$). But the O_2^{*-} generation was equivalent in three radiation treatments at 18°C ($P > 0.05$). In conclusion, significant PA and PAB effects on the O_2^{*-} production in gametophytes revealed at lower temperatures were species-specifically masked at elevated temperatures, at least at 18°C in all three determined species (Fig. 4). Moreover, if not explicitly mentioned above, for the most part the response to radiation treatments differed among different temperatures.

Ultrastructure of gametophytes

In general, 9-day-old primary cells with a length of 8 μm consist of a nucleus (N) with nucleolus, chloroplasts (C), vacuoles (V), mitochondria (M) and several lipid globules (L) and are covered by a thin cell wall (Fig. 5). Physodes were rare. 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. 5a), whereas the PAB-treated gametophytes contained numerous smaller plastoglobules about 40–50 nm in size (Fig. 5c). 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. 5g–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 giving them a 'corroded' appearance (Fig. 5a,b).

DISCUSSION

This is the first study showing the formation of ROS, in particular of O_2^{*-}, in kelp gametophytes after exposure for short time periods to radiation and temperature stress. ROS are formed predominantly in the peripheral cytoplasm and in chloroplasts, O_2^{*-} is probably additionally formed in mitochondria. Intracellular ROS

formation was enhanced by exposure to PAB. The concentration of O_2^{*-} in the surrounding medium was amplified by temperature and slightly enhanced by UV-radiation in some cases.

In our study, non-specified ROS were detected by CM-H_2DCF-DA in the cytoplasm and in the periphery of chloroplasts in gametophytes of *L. hyperborea* after an exposure to PAB (Fig. 3). UV-B induced increase of DCF-labeled ROS similarly detected in *Fucus spiralis* embryos was attended by a chronic photoinhibition (Coelho *et al*. 2001). Correspondingly, Dring *et al*. (1996) demonstrated a reduced maximum quantum yield (Fv/Fm) of gametophytes of *L. hyperborea*, *L. digitata* and *S. latissima* off Helgoland to ≤10% after 8 h PAB treatment if compared to UVR-untreated controls. Taking these results together we hypothesize that the linkage of ROS production and chronic photoinhibition may be a general response not only in UV-exposed *Fucus* embryos but also in PAB-treated kelp gametophytes. This linkage is apparent at least in gametophytes of *L. hyperborea* off Helgoland shown to exhibit a strong DCF radical fluorescence (Fig. 3) and a very strong UV-B impairment of photosynthesis (Dring *et al*. 1996).

NBT-staining indicative of O_2^{*-} production was demonstrated in juvenile gametophytes of *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* after exposure to PAB, PA and P (Fig. 1) as well as in stock cultures of *L. hyperborea* after exposure to PAB (Fig. 2). The response was most pronounced in the latter species after PAB-exposure. From the cytological point of view the formation of O_2^{*-} in the 10-day-old juvenile gametophytes irradiated for 8 h is comparable to the formation of ROS in 1–4-day-old embryos of *Fucus spiralis* exposed for 30 min to PAB (sun conditions, see Coelho *et al*. 2001). In all cases ROS and O_2^{*-} are produced in the periphery of the cells. In *Fucus spiralis* there is a more pronounced basal ROS production, i.e. in the rhizoid, whereas O_2^{*-} production in the kelp gametophytes has been demonstrated both, basally and distally (Fig. 1). Unfortunately, there are no data about the time course of ROS production in both cases. Such data are present, however, for *Fucus evanescens*. In this species ROS concentration started to increase immediately after exposure to excessive light and increased linearly up to a maximum at the end of the study period of 3 h (Collen & Davison 1997). The authors suggest that ROS are formed by restricted photosynthetic (and respiratory) electron transport impacted by abiotic stress.

However, the responses in the kelp gametophytes studied here are much less strong than in sporophytes of *Laminaria digitata*. In this species, Küpper *et al*. (2001) demonstrated O_2^{*-} production through NBT-staining after addition of oligoguloronates. O_2^{*-} accumulated predominantly adjacent to the epidermis and to a lesser degree around the outer cortical cells but not in the medulla. Obviously, O_2^{*-} production is, like in the kelp gametophytes, bound to photosynthetically active cells. This conclusion is also supported by the finding by Küpper *et al*. (2001) that young blade tissues exhibited the highest response and older blade tissues were less reactive. However, the process of O_2^{*-} production described here for the kelp gametophytes is quite different compared to that in sporophytes of *L. digitata* (Küpper *et al*. 2001). In the gametophytes it is a response to the radiation conditions, whereas in the sporophytes it is a response to the addition of oligoguloronates. These constituents of alginate, the main brown algal cell wall polysaccharide, are hypothesized to be formed by alginate depolymerizing enzymes from, for example, molluscs and brown algal endophytes. Oligoguloronates elicit an oxidative burst, which is regarded as an immediate and efficient defense against these organisms. It is a rapid, transient production of enormous amounts of ROS, one of the earliest plant responses to pathogen infection (Wojtaszek 1997). It initiates oxidative cross-linking of cell wall material, and thus strengthens cell walls under stress conditions (Potin 2008 and references therein).

A similarly strong oxidative burst has been detected in the red alga *Chondrus crispus* (Bouarab *et al*. 1999). The infection resistant gametophytes of *C. crispus* responded to sulfated oligosaccharides with a remarkable increase of the ROS production, while the infection susceptible tetrasporophytes of *C. crispus* showing only a moderate ROS generation under identical conditions (Bouarab *et al*. 1999). The natural resistance of gametophytes of *C. crispus* vanished if the oxidative burst was inhibited by diphenyleneiodonium, a suicide inhibitor of NADPH oxidase (Bouarab *et al*. 1999). These results point to the importance to investigate different stages in the life history of species, which might differ in their infection and stress tolerance.

Although our results show that ROS are formed in kelp gametophytes, they obviously do not induce cellular damage. This high stress tolerance is demonstrated by our electron microscopic investigations. UVR has been shown in various brown, red and green algae to affect various cellular components, especially chloroplasts, mitochondria and the nucleus (Karsten *et al*. 2011). A previous study on the ultrastructure of zoospores from *Laminaria hyperborea* demonstrated strong damage of the nucleoplasm and the mitochondria after PAB exposure (Steinhoff *et al*. 2008). Further, it had been shown that plastoglobules emerged after this treatment in their chloroplast (Steinhoff *et al*. 2008). Plastoglobules are lipoprotein particles inside chloroplasts and were invoked to fulfill a major role in the recycling of lipophilic products arising from oxidative metabolism during stress (del Río *et al*. 1998, Olmos *et al*. 2007). An increase in the number of plastoglobules has been correlated with

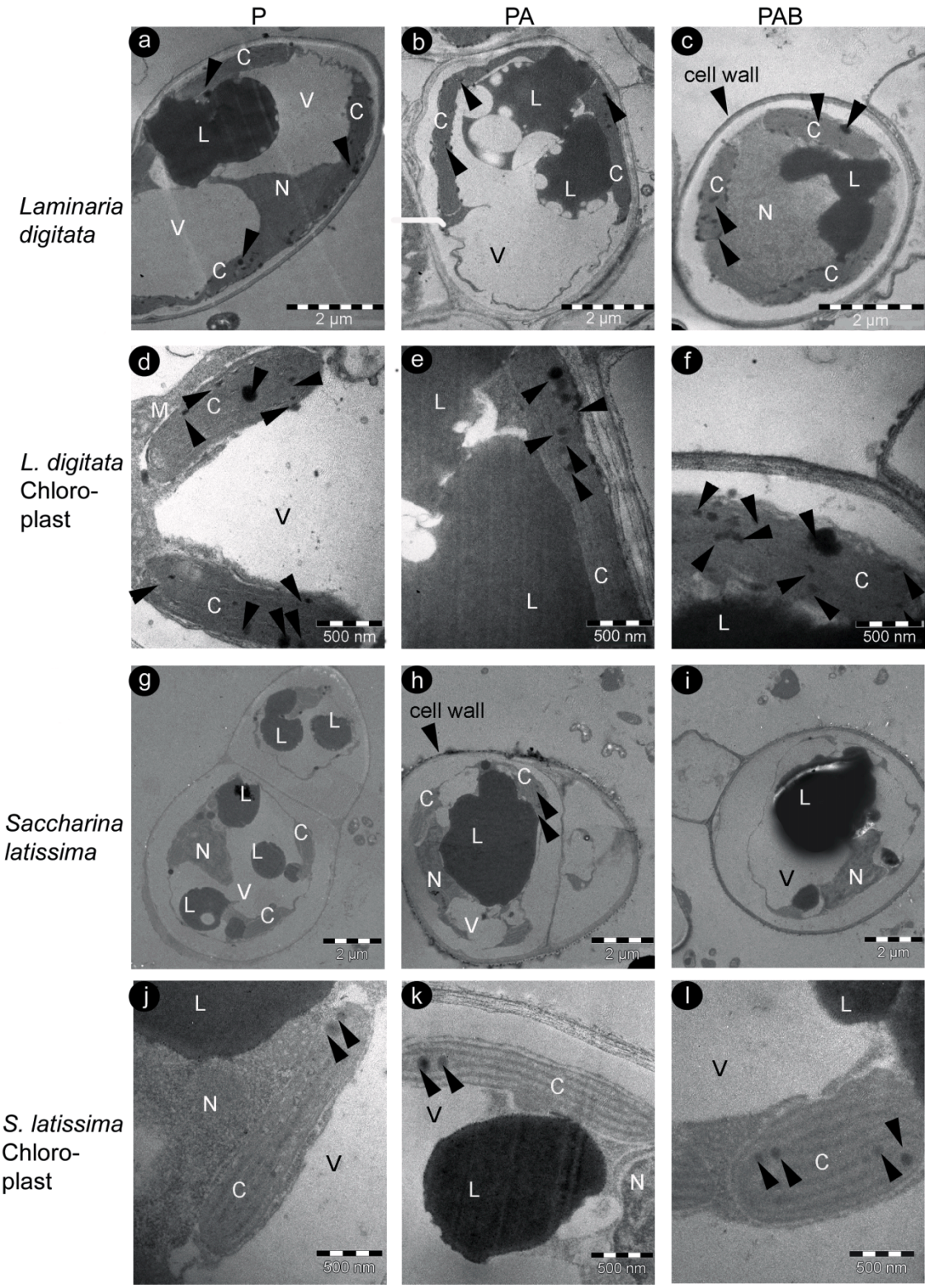

Fig. 5. Transmission electron-micrographs of 10-day-old gametophytes of *L. digitata* (a–f) and *S. latissima* (g–l) after 8 h irradiation with P (400–700 nm, a, d, g, j), PA (320–700 nm, b, e, h, k) or PAB (280–700 nm, c, f, i, l) for 8 h. a–c and g–i show the entire gametophytes whether d–f and j–l focus on one chloroplast exemplarily. Plastoglobuli in the chloroplasts are highlighted by black arrows. C, chloroplast; L, lipid globule; N, nucleus; P, photosynthetically active radiation; PA, P + UV-A radiation; PAB, PA + UV-B radiation; V, vacuole, scale bar 2 μm (a–c and g–i) or 500 nm (d–f and j–l).

plastid development and the 'upregulation of plastid lipid metabolism in response to oxidative stress' (Lichtenthaler & Tevini 1970; Austin *et al*. 2006). In contrast, gametophytes did not show any of these features in our study. A reason for the high stress tolerance of the studied kelp gametophytes may be seen in their content of phlorotannins. Phlorotannins of *Ecklonia cava* have recently been shown to exhibit free radical scavenging activities (Ahn *et al*. 2007). A high antiradical power was also demonstrated in soral tissue of *Saccharina latissima* and related to the numerous phlorotannin containing physodes (Holzinger *et al*. 2011). Compared to the density of physodes in sporangial parent cells of *Saccharina latissima*, the density of physodes in the examined juvenile kelp gametophytes is, however, extremely low. So in the present case phlorotannins cannot be invoked for the high stress tolerance in these developmental stages. Another feature of our samples was the appearance of 'corroded' lipid globules (Fig. 1a,b) interpreted as a consumption of lipids, the major storage compounds of meiospores (Reed *et al*. 1999) during the germination process (Steinhoff *et al*. 2011).

In conclusion, our data evidence a high cellular stress tolerance of gametophytes of Laminariales against UVR as significant ultrastructural changes were not detected in gametophytes of *Laminaria digitata* and *Saccharina latissima*. This finding also corresponds to the known higher temperature tolerance of about 3–4°C in kelp gametophytes compared to their sporophytes (Wiencke *et al*. 1994). Therefore these developmental stages will most probably not be affected by global climate changes and can function also in future as a seed bank even under increasing environmental pressures. 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, definitely merits further attention.

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