Effects of ultraviolet radiation on early life stages of cold temperate and Arctic macroalgae: Implications for recruitment and vertical depth distribution

Effekte von UV-Strahlung auf frühe Lebensstadien kaltgemäßigter und arktischer Makroalgen: Auswirkungen auf Rekrutierung und Vertikalverteilung

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Michael Y. Roleda

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Michael Y. Roleda
Biologische Anstalt Helgoland in der Stiftung Alfred Wegener Institut für Polar- und Meeresforschung, Meeresstation
D-27498 Helgoland

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BED	biological effective dose
BED_{50}	BED to achieve 50% inhibition of germination
BED_{DNA}	biological effective dose using weighing function for DNA damage of *Escherichia coli*
Chl *a*	chlorophyll *a*
CPD(s)	cyclobutane pyrimidine dimer(s)
CPD Mb^{-1}	amount of cyclobutane pyrimidine dimers per million bases
DNA	deoxyribonucleic acid
ETR_{max}	maximal relative electron transport rate
F_v/F_m	optimal quantum yield of PSII in dark-acclimated state
$\Delta F/F_m'$	effective quantum yield of PSII in light-acclimated state
HPLC	high performance liquid chromatography
J	Joules
LD	light:dark photoperiod
MAA	mycosporine-like amino acid
nm	nanometer
PAR	photosynthetically active radiation (400- 700 nm)
P	PAR
PA	PAR + UV-A
PAB	PAR + UV-A + UV-B
PAM	pulse-amplitude modulated fluorometer
PC	personal computer
PFD	photon flux density
PS I	photosystem I
PSII	photosystem II
PVC	Polyvinyl Chloride
SD	standard deviation
spp.	species
UVR	ultraviolet radiation
UV-A	ultraviolet-A (315 nm < λ < 400 nm)
UV-B	ultraviolet-B (280 nm < λ < 315 nm)

UV-C	ultraviolet-C (100 nm < λ < 280 nm)
UV_{ery}	Erythema weighted UV dose
µm	micrometer
µmol	micromoles
XE-PAM	Xenon pulse-amplitude modulated fluorometer
W	Watt
λ	wavelength
±	standard deviation

DEFINITION OF TERMS

Auxiliary cell	Vegetative cell which receives the 2N zygote nucleus after fertilization
Carpogonium	A female sexual cell consisting of a basal portion, the contents of which function as a gamete, and an elongated, receptive portion, the trichogyne; female gametangium = egg container (oogamous life cycle only)
Carpospore	2N spore resulting from fertilization; spore formed in a carposporangium
Carposporophyte	2N generation where the 2N zygote is cloned to form the carpospores
Cystocarp	Pericarp (1N) + carposporophyte (2N)
Connecting filaments	Cell that carries the zygote nucleus from fertilized egg to internal auxiliary cell where it is cloned and differentiates into carposporophyte
Gametophyte	The haploid sexual phase that exhibits an alternation of generations from which gametes are produced by mitosis
Gonimoblast	Tissue which develops from the carpogonium or an auxiliary cell and which ultimately produces carposporangia or carpotetrasporangia
Holdfast	An attachment structure that anchors macroalgae to the substratum, which resembles a collection of roots but has no nutrient gathering role
Juvenile	The life stage between the embryonic stage and the adult stage, characterized by the absence of reproductive ability
Ooblastema	Also known as connecting filaments
Oogamous	Having large, non-motile eggs and small motile sperm
Pericarp	1N vegetative tissue surrounding the carposporophyte
Sorus	Cluster of sprongia in the epidermal tissue of a fertile frond
Spermatium	Non-flagellated male gamete
Sporangia	Structure producing and containing spores or gametes

Sporophyte	2N, spore producing, asexual generation of the life cycle; typically formed by the fusion of haploid gametes
Syngamy	Fusion of gametes
Tetrasporangium	container with 2N cell which undergoes meiosis to form 4 tetraspores
Tetraspore	Meiospore formed by meiosis from 2N tetrasporophyte cell
Tetrasporophyte	2N generation from germinating carpospores
Thallus	A relatively undifferentiated plant body lacking true leaves, stems and roots
Trichoblast	Usually a uniseriate filament of cells with little pigmentation; either remains sterile or undergoes division to form spermatangia or carpogonia
Trichogyne	Extension of egg to which spermatium attaches
Zoospore	A motile asexual spore using flagellum for locomotion; Also called swarmer

SUMMARY

Mastocarpus stellatus and *Chondrus crispus* (Gigartinales) are morphologically comparable red algae distributed along the North Atlantic coast and co-inhabit the rocky eulittoral and upper sublittoral zone. In Helgoland, *M. stellatus* was accidentally introduced and has successfully colonized all natural substrates out-competing *C. crispus* effectively changing the appearance of the intertidal biotope of the island. Members of the order Laminariales form extensive sublittoral kelp beds mainly in cold temperate waters of the N-Pacific, S and N Atlantic extending to the Arctic region. Their depth distribution limit varies among species. *Laminaria ochroleuca* can be found in the upper sublittoral down to a depth of 100 m. Depth distribution of kelp species in Helgoland is characterized by occurrence of *Laminaria digitata* in the upper sublittoral whereas *L. saccharina* and *L. hyperborea* dominate the mid and lower sublittoral. The kelp forest of Kongsfjorden, Spitsbergen (Svalbard) is structured by the annual *Saccorhiza dermatodea* and the perennial canopy species *Alaria esculenta*, *L. digitata* and *L. saccharina* growing in this sequence down to about 10- 20 m. The endemic Arctic species *L. solidungula* occurs predominantly in the inner zone of the fjord at great depths.

In this study, the susceptibility of the early life stages (spores and juveniles) of Gigartinales and Laminariales to ultraviolet radiation was investigated in the laboratory and *in situ* (Arctic Laminariales only). Experimental units were exposed to radiation consisting either of photosynthetically active radiation (PAR; 400- 700 nm), PAR + UV-A radiation (UV-A; 320- 400 nm) and PAR + UV-A + UV-B radiation (UV-B; 280- 320 nm) using cut-off filters. Optimum (F_v/F_m) and effective quantum yields of PSII ($\Delta F/F_m'$), pigment content, DNA damage and repair, germination and growth were measured. Responses were related to the thallus thickness, optical characteristics, UV-screening compounds of the control specimen maintained in culture and collected *in situ*. Ecological implication was related to seasonal reproduction, recruitment success, and upper depth distribution observed in the field.

Photosynthetic efficiency of spores of Laminariales and Gigartinales is already inhibited at 20 and 60 µmol photons m^{-2} s^{-1} PAR respectively. UV radiation (UVR) contributes significant additional effect on photoinhibition. Recovery of the PSII function after exposure to dim white light was observed but not in zoospores exposed to irradiation longer than 4 hours of PAR + UV-A + UV-B and 8 hours of PAR + UV-A. Photosynthetic

efficiency of carpospores was able to recover in all treatments. Juveniles of *Mastocarpus* and *Chondrus* repeatedly exposed to 15- 28 days of UVR showed complete acclimation of photosynthetic efficiency after 3 days while $\Delta F/F_m$' of *Laminaria ochroleuca* was only partially able to acclimate to UVR. Chlorophyll *a* (Chl a) contents in young gametophytes of both *Mastocarpus* and *Chondrus* were not affected by repeated UV exposures. A higher total carotenoid content was measured in plants exposed to UVR indicating a photoprotective role of the pigment enhancing acclimation of photosynthesis. Chlorophyll *a* and accessory pigments were significantly affected under UVR in *L. ochroleuca* but not in other Laminariales from Helgoland and Spitsbergen.

Reproductive cells were more susceptible to UVR compared to young and adult gametophytes and sporophytes. Differences in DNA damage between life stages were observed in both groups of macroalgae. DNA damage measured through formation of Cyclobutane pyrimidine dimers (CPDs) was observed in carpospores of *Mastocarpus* and *Chondrus* but not in foliose gametophytic stages. In Laminariales, haploid zoospores were more sensitive to DNA damage compared to diploid young sporophytes. Removal of CPDs indicating repair of DNA damage was observed in all species but minimal in the lower sublittoral *L. hyperborea*. The effective DNA damage repair mechanism in carpospores of *Mastocarpus* and zoospores of *L. digitata* will enhance their recruitment success. Diploid carpospores were found to incur less genetic damage compared to the haploid zoospores. Haploid zoospores were, however, more efficient in DNA damage repair. Diploid cells are, conversely, more resistant to damage. During the diploid state DNA damage can be repaired, since there are two copies of the gene in the cell and one copy is presumed to be undamaged.

Laboratory spore germination experiments showed that sublittoral Laminariales species are more UV-sensitive than the eulittoral Gigartinales species. Dose response relationship showed higher dose is needed to achieve 50% germination inhibition (BED_{50}) in *Mastocarpus* and *Chondrus* (762 and 248 J m^{-2} respectively) compared to the Laminariales (52- 86 J m^{-2}) at Helgoland. *In situ* field experiments in the Arctic, however, required up to ten-fold higher BED_{50} (418- 1124 J m^{-2}) to inhibit germination in *Saccorhiza dermatodea*, *Alaria esculenta* and *Laminaria digitata*. Higher ambient PAR in the field was observed to enhance UV tolerance of zoospores. The sensitivity of Gigartinales carpospores and Laminariales zoospores were observed to be related to the vertical and depth distribution of the foliose gametophytes and adult sporophytes, respectively.

In contrast, the sensitivity of young Laminariales sporophytes to DNA damage is not related to the upper depth distribution limits of their respective adult sporophytes. DNA damage repair entail energy loss at the expense of growth. After 18- 21 days of repeated daily 6 hours UVR exposure and 18 hours recovery, the lower sublittoral but thick species *L. hyperborea* and *L. solidungula* as well as the cold-temperate upper sublittoral *L. digitata* have lower remaining tissue DNA damage. This is attributed either to a better repair mechanism, protection by thallus thickness and optical characteristics, or screening by UV-absorbing compounds but at the expense of energy which is not available for growth. The combined protective mechanism due to thallus thickness and higher UV-absorbing compounds caused a reduced growth rate in *L. hyperborea* and *L. solidungula*.

Growth rates of young *Mastocarpus* and *Chondrus* gametophytes exposed to UVR were not affected while growth rates of all young Laminariales sporophytes exposed to UVR were significantly lowered. Furthermore, morphological damage was observed in *Laminaria ochroleuca* which include tissue deformation, lesion, blistering and thickening of the meristematic part of the lamina. Growth as an integrative parameter of all physiological processes in juvenile plants was observed to be related to the upper depth distribution of the adult sporophytes.

Aside from UV tolerance and efficient DNA repair mechanism, the apomictic reproduction (direct-type life history) of Helgolandian *Mastocarpus* could also have conferred ecological advantage to its successful dispersal and colonization of open spaces in the upper eulittoral. This is because the direct development female gametophyte requires only an open space and a single establishment event; whereas sexual generation must first pass through the crustose, tetrasporophytic generation, requiring two open spaces and two establishment events. Moreover, spores are produced year-round, not seasonally as in sexual populations. On the other hand, the distinct reproductive seasons of Helgolandic *Laminaria* spp. is remarkable. To ensure reproductive success, formation of propagules is synchronized with the onset of favorable environmental condition. The summer reproductive season of the upper sublittoral *L. digitata* suggests that sporogenic tissues as well as zoospores of this species could tolerate or possess effective protective mechanism against high solar radiation. On the other hand, winter reproduction in the lower sublittoral *L. hyperborea* is thought to be a strategy to avoid reproductive failure due to the relative sensitivity of their zoospores to high PAR and UVR.

In conclusion, the susceptibility of the reproductive cells to UVR is one important factor for determination of spore viability, germination success and recruitment capacity of germlings to the upper distribution limit of the adult life history stages. Juveniles are observed to possess several mechanisms to minimize UVR damage and, hence, are less sensitive but at the expense of growth.

ZUSAMMENFASSUNG

Die sich morphologisch ähnelnden *Rotalgen Mastocarpus stellatus* und *Chondrus crispus* (Gigartinales) kommen entlang der Nordatlantischen Küste vor und besiedeln gemeinsam die Felsküsten im Eu- und oberen Sublitoral. Nachdem *M. stellatus* auf Helgoland eingeschleppt wurde, verdrängte sie wahrscheinlich *C. crispus* und kolonisierte alle noch unbesiedelten Substrate, wodurch das Erscheinungsbild des Eulitorals der Insel verändert wurde. Angehörige der Ordnung Laminariales bilden im Sublitoral großflächige Seetangwälder in den kaltgemäßigten Zonen des Nord-Pazifik, des Nord- und Süd-Atlantiks und der Arktis. Die Arten der Gattung *Laminaria* kommen in verschiedenen Tiefen der Wassersäule vor, wobei man *Laminaria ochroleuca* vom oberen Sublitoral bis zu einer Tiefe von 100m finden kann. Auf Helgoland wächst *Laminaria digitata* im oberen Sublitoral, während *L. saccharina* und *L. hyperborea* das mittlere und untere Sublitoral dominieren. Im Kongsfjord auf Spitzbergen wachsen die einjährige Braunalge *Saccorhiza dermatodea* und die mehrjährigen Arten *Alaria esculenta*, *L. digitata* und *L. saccharina* in dieser Reihenfolge bis in 10- 20 m Wassertiefe. Die endemische arktische Art *L. solidungula* tritt dabei bevorzugt im inneren Bereich des Fjords in größerer Tiefe auf.

In dieser Arbeit wurde die Empfindlichkeit der frühen Entwicklungsstadien (Sporen und juvenile Stadien) der Gattungen Gigartinales und Laminariales gegenüber ultravioletter Strahlung im Labor und *in situ* (nur Laminariales) gemessen. In der Versuchsanordnung wurde die Algen photosynthetisch aktiver Strahlung (PAR; 400- 700 nm), PAR + UV-A-Strahlung (UVBR; 280- 320nm), als auch PAR + UV-A + UV-B-Strahlung mittels UV-absorbierender Filter ausgesetzt. Gemessen wurde die optimale (F_v/F_m) und effektive Quantenausbeute von PSII ($\Delta F/F_m'$), der Pigmentgehalt, DNA-Schäden, DNA-Reparatur, Keimung und Wachstum. Die Ergebnisse wurden mit der Dicke der Thalli, optischen Eigenschaften und UV-absorbierenden Substanzen von Kontrollalgen, die *in situ* gesammelt und kultiviert wurden, verglichen. Ökologische Auswirkungen wurden mit saisonalen Reproduktions- und Besiedlungsraten und dem Vorkommen in der oberen Wassersäule korreliert.

Die photosynthetische Aktivität ist bei den Laminariales und auch den Gigartinales schon bei einer Bestrahlungsstärke von 20 bis 60 µmol Photonen m^{-2} s^{-1} PAR und unter UV-

Strahlung (UVR) sogar noch stärker inhibiert. Eine Wiederherstellung der PSII-Funktion in Schwachlicht Exposition konnte außer bei Zoosporen, die länger als 4 Stunden einer Bestrahlung mit PAR + UV-A + UV-B bzw. 8 Stunden einer Bestrahlung mit PAR + UV-A ausgesetzt waren, beobachtet werden. Die photosynthetische Aktivität der Carposporen von Rotalgen erholte sich nach allen Bestrahlungsmodi. Juvenile Sporen von *Mastocarpus* und *Chondrus*, die wiederholt während eines Zeitraumes von 15- 28 Tagen einer Bestrahlung mit UVR ausgesetzt waren, zeigten nach drei Tagen eine vollständige Akklimatisation der photosynthetischen Aktivität an UVR, während $\Delta F/F_m$' bei *Laminaria ochroleuca* sich nur teilweise anpassen konnte. Der Chlorophyll *a*-Gehalt (Chl *a*) von jungen Gametophyten bei beiden Rotalgenarten (*Mastocarpus* und *Chondrus*) wurde durch wiederholte UV-Bestrahlung nicht beeinflusst. Dagegen wurde ein höherer Carotenoidgehalt in Pflanzen, die mit UVR bestrahlt wurden, festgestellt. Dieses Ergebnis deutet an, dass diese Pigmentgruppe eine Rolle bei der Photoprotektion durch eine verbesserte Akklimatisierung der Photosynthese unter ultravioletter Bestrahlung spielt. Chlorophyll *a* und die akzessorischen Pigmente wurden innerhalb der Gattung Laminariales nur bei der Art *L. ochroleuca* durch UV-Strahlung signifikant beeinträchtigt.

Fortpflanzungszellen sind anfälliger gegenüber UV-Strahlung als bereits differenzierte junge und adulte Gametophyten und Sporophyten. Ontogenetische Unterschiede bei der Schädigung der DNA konnten in beiden Makroalgengruppen (Gigartinales und Laminariales) festgestellt werden. Einen Schaden der DNA wurde nur bei Carposporen, nicht aber bei den Gametophyten der Gattungen *Mastocarpus* und *Chondrus* durch Nachweis der Bildung von Cyclobutyl-Thymindimere (CPDs) festgestellt. Bei der Ordnung Laminariales reagierten haploide Zoosporen sensitiver mit DNA-Schaden als junge diploide Sporophyten. Die Abnahme des Gehalts an CPDs, weist auf eine Reparatur der geschädigten DNA bei allen Arten hin. Dies war aber am wenigsten ausgeprägt bei der im unteren Sublitoral ansässigen *L. hyperborea*. Die effektive DNA-Reparatur bei den Carposporen der Gattung *Mastocarpus* und bei den Zoosporen von *L. digitata* erhöht die Möglichkeit zur Besiedlung des oberen Eulitoral. Außerdem wurde beobachtet, dass sich die diploiden Carposporen geringere DNA-Schäden zuzogen als die haploiden Zoosporen. Dennoch können haploide Zoosporen effizienter ihre DNA reparieren. Andererseits sind diploide Zellen widerstandsfähiger gegenüber einer Schädigung der DNA. Denn in der diploiden Phase eines Organismus kann ein auftretender Schaden repariert werden bzw. ohne Folge bleiben, wenn eine Kopie des Gens unbeschädigt bleibt.

Die Auskeimversuche von Sporen im Labor zeigten, dass die sublitoralen Laminariales UV-sensitiver als die eulitoralen Gigartinales waren. Bei einer Analyse der Dosiswirkung auf die Versuchsalgen von Helgoland wurde festgestellt, dass eine höhere Dosis bei den Gattungen *Mastocarpus* and *Chondrus* (762 bzw. 248 J m^{-2}) nötig war, um das Keimen von 50% der Sporen zu verhindern (BED_{50}) als bei Angehörigen der Gattung Laminariales (52- 86 J m^{-2}). *In situ* Feld-Experimente in der Arktis erforderten einen zehnfach höheren BED_{50} (418- 1124 J m^{-2}), um die Keimung der Sporen der Arten *Saccorhiza dermatodea*, *Alaria esculenta* and *Laminaria digitata* zu inhibieren. Eine höhere PAR im Feld steigerte also die UV-Toleranz von Zoosporen. Es wurde festgestellt, dass ein Zusammenhang zwischen der Sensitivität von Carposporen der Gattung Gigartinales und Zoosporen der Gattung Laminariales und der Vertikalverteilung in der Wassersäule der foliosen Gametophyten bzw. adulten Sporophyten besteht.

Die Empfindlichkeit von jungen Sporophyten der Gattung Laminariales gegenüber DNA-Schäden hingegen hängt nicht mit der Verteilung ihrer adulten Sporophyten in der Tiefe zusammen. Eine Reparatur der DNA hat einen Energieverbrauch zur Folge, der sich negativ auf das Wachstum der Algen auswirkt. Nach 18 bis 21 Tagen wiederholter, täglich 6stündiger Bestrahlung mit UV-Strahlung und einer anschließenden 18stündigen Erholungsphase, hatten sowohl die im unteren Sublitoral vorkommenden, aber mit dickeren Thalli ausgestatteten Arten *L. hyperborea* und *L. solidungula* als auch die im oberen Sublitoral Helgolands heimische Art *L. digitata* einen geringeren, bleibenden Schaden in der DNA. Dies hängt entweder mit besseren Reparaturmechanismen, der Dicke des Thallus und seiner optischen Charakteristik oder mit der Abschirmung durch UV-absorbierende Substanzen zusammen. Dies ist aber nur durch einen höheren Energieverbrauch, der zu Lasten des Wachstums geht, möglich. Größere Thallusdicke und höhere Konzentration von UV-absorbierenden Substanzen war bei *L. hyperborea* und *L. solidungula* mit einem verringerten Wachstum verbunden.

Während die Wachstumsraten von jungen Gametophyten der Gattungen *Mastocarpus* und *Chondrus* durch eine Bestrahlung mit ultraviolettem Licht sich nicht veränderten, wurde ein signifikant verringertes Wachstum bei allen jungen Sporophyten der Gattung Laminariales festgestellt. Des Weiteren wurden morphologische Schäden bei *L. ochroleuca* beobachtet. Diese waren Missbildungen, Verletzungen, Aufplatzen und Verdicken des meristematischen Gewebes. Das Wachstum von juvenilen Pflanzen, ein integrativer Parameter aller physiolo-

gischen Prozesse, hängt mit der Verteilung der adulten Sporophyten in flacher Tiefe zusammen.

Neben UV-Toleranz und effizienten DNA-Reparaturmechanismen könnte auch die apomiktische Reproduktion der auf Helgoland eingeführten Gattung *Mastocarpus*, dieser einen ökologischen Vorteil bei der Besiedlung und Kolonisierung von freiem Raum im oberen Eulitoral verschafft haben. Die sich direkt entwickelnden weiblichen Gametophyten brauchen nur freien Raum und ein einziges Besiedlungsereignis, wohingegen die sexuelle Generation zuerst die krustenförmige, tetrasporophytische Generation durchleben muss. Dazu sind zweimal ein freier Raum und zwei Besiedlungsereignisse notwendig. Außerdem werden Sporen das ganze Jahr über produziert und nicht nur saisonal wie bei der sexuellen Vermehrung. Auf der anderen Seite sind die verschiedenen reproduktiven Jahreszeiten der Gattung *Laminaria* spp. auf Helgoland bemerkenswert. Um einen reproduktiven Erfolg sicherzustellen, sollte die Produktion von Fortpflanzungsorganen mit dem Auftreten von günstigen Umweltbedingungen synchronisiert werden. Die reproduktive Sommerzeit der sublitoralen Art *L. digitata* würde darauf hinweisen, dass sowohl Sorusgewebe als auch Zoosporen dieser Art entweder eine hohe Toleranz bzw. effektiv schützender Mechanismen gegenüber hoher Sonneneinstrahlung besitzen. Andererseits wird die Winterreproduktion der tiefer zonierten Art *L. hyperborea* dahingehend interpretiert einen Reproduktionsausfall zu verhindern, da ihre Zoosporen eine höhere Empfindlichkeit gegenüber PAR und UVR besitzen.

Zusammenfassend kann man sagen, dass die Empfindlichkeit von reproduktiven Zellen gegenüber ultravioletter Strahlung ein wichtiger Faktor für die Entwicklungsfähigkeit der Sporen, für den Keimungserfolg und für das Wachstum der Keimlinge in der obere Verbreitungszone der adulten Lebensstadien ist. Es wurde festgestellt, dass Jugendstadien mehrere Mechanismen zur Reduzierung der Schäden durch ultraviolette Strahlung besitzen und damit weniger empfindlich sind, was aber zu Lasten ihrer Wachstumsrate geht.

1 INTRODUCTION

1.1 Marine macrophytes and communities

The total annual primary production of aquatic freshwater and marine ecosystems is estimated at 45.8 x 10^{15} g C. Of which, the phytoplankton fraction is 95% while the small areas of coastal macrophytes, salt marshes, and estuaries contribute 3.18% of the global aquatic primary production (Mann 1973; de Vooys 1977). Marine macrophytes or seaweeds occupy different habitats in the rocky intertidal and subtidal zones and form the major components of these habitats. They play important ecological roles as nutritional base in marine communities by providing food for many marine herbivores (Lüning 1985). In the temperate and polar regions, seaweeds can form dense underwater forests called kelp forest. The lush blades form canopies and the labyrinth holdfast support different marine communities by providing a physical structure for shelter, protection from predators and nurseries for many marine animals. The kelp forest also serves several species of fish foraging area for the many invertebrates associated with the kelp.

The benthic marine flora is dominated by multicellular Chlorophyta, Rhodophyta, and Phaeophyta. Seaweed morphologies include crustose, filamentous, pseudoparenchymatous and parenchymatous while their anatomy ranges from almost no differentiation between cells to the complex tissues of kelp and fucoids (Lobban & Harrison 1994). Seaweeds have also been classified into functional-form groups, such as calcareous, encrusting, thin sheets and thick leathery, which have characteristics levels of productivity (Littler & Littler 1980). The island of Helgoland (North Sea) has a diverse marine macroalgal flora (Bartsch & Kuhlenkamp 2000). The rocky intertidal zone supports prominent and extensive pure *Mastocarpus stellatus* Stackhouse (Guiry) biotope or a mixture of *M. stellatus* and *Chondrus crispus* Stackhouse community (Bartsch & Tittley 2004). Community structure in the subtidal down to 15 m depth has been described by Lüning (1970) where kelps constitute the major biomass of the primary producers. *Laminaria digitata* (Hudson) Lamouroux inhabits the uppermost part of the sublittoral while *L. saccharina* (Linnaeus) Lamouroux and *L. hyperborea* (Gunnerus) Foslie dominates the middle and lower sublittoral of the kelp zone, respectively (Lüning 1979). *Laminaria ochroleuca* de la Pylaie is distributed along the coast of S and N Atlantic (Benhissoune *et al*. 2002; Izquierdo *et al*. 2002), the Mediterranean

(Ribera *et al*. 1992) and an isolated population in the Strait of Messina down to depths in excess of 100 m (Drew 1972).

In the Arctic, marine seaweeds have a circumpolar distribution and are also found throughout the temperate North Atlantic (Lüning 1985). In Kongsfjorden, Spitsbergen (Svalbard) the kelp forest is structured by the annual *Saccorhiza dermatodea* (Bachelot de la Pylaie) J. Agardh in the upper sublittoral and the perennial canopy species *Alaria esculenta* (Linnaeus) Greville, *Laminaria digitata* and *L. saccharina* in the mid sublittoral (Wiencke *et al*. 2004a). The endemic Arctic species *L. solidungula* J. Agardh occurs predominantly in the inner zones of the fjord at great depths (Hop *et al*. 2002).

1.2 Factors determining algal zonation and upper distribution limit

Propagules of marine macroalgae (spores, gametes and zygotes) can be dispersed throughout the intertidal and sublittoral zones (Hoffmann & Ugarte 1985). Macroalgal recruitment in coastal environments can however be influenced, beside other factors, either by temperature, ultraviolet radiation, nutrients and their synergistic effects (Lotze & Worm 2002). The survival of juvenile plants is further controlled by demographic factors, competitors, and grazers (Dean *et al*. 1989) in shaping up algal zonation pattern and distribution limits.

Intertidal seaweeds are periodically exposed to air during low tides where they experience a variety of stressful environmental conditions (see review, Davison & Pearson 1996). To be able to inhabit the intertidal zone, an alga should be able to withstand emersion. Davison and Pearson (1996) classified stresses in the intertidal zone as disruptive or limitation stress. Stresses that are disruptive include exposure to high light, high temperature, freezing, desiccation and osmotic shock. Limitation stress, on the other hand, is the temporary isolation from essential nutrients such as nitrogen and phosphorous during tidal emersion. Moreover, the algae are exposed to a very different environment for photosynthesis in the air. Inter-specific differences in stress tolerance among intertidal macroalgal species are therefore important in shaping up the community structure and zonation pattern.

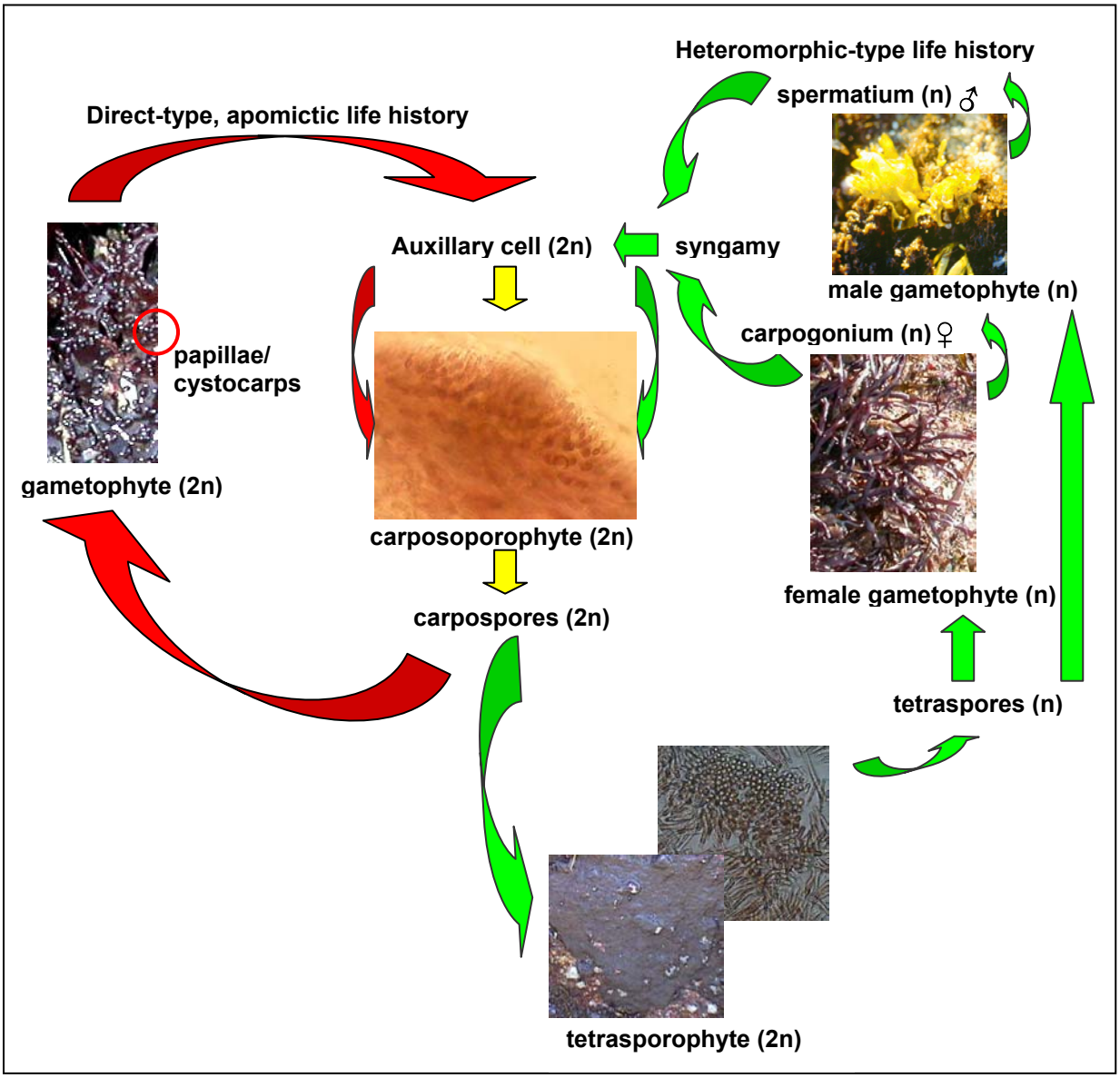

Fig 1. Summary of the principal features of the direct-type (apomictic) and heteromorphic life histories in *Mastocarpus stellatus*. The heteromorphic life history involves a change in ploidy level between the diploid sporophyte and haploid gametophyte, whereas the direct-type life history is diploid throughout. Female gametophyte and tetrasporophyte are typically reddish-black to olive brown in color while male gametophyte is bright yellow to light pink. Modified from http://www.mbari.org/staff/conn/botany/reds/mastocar/lifehist.htm and Maggs 1988.

Sublittoral algae have a very limited resistance to desiccation and freezing when compared to intertidal species. In the subtidal zone, irradiance is the major factor which may determine the upper and lower distribution limit of macroalgal species (Lüning 1985). The light compensation point sets the physiological constraint for the depth limit of macroalgae and determines the ability of macroalgae to grow in shaded habitats, below rocks or canopies of other algae (Markager & Sand-Jensen 1992). This explains why adult sporophytes of *Laminaria digitata* are excluded from the deeper kelp zones (Lüning 1979) while *L.*

ochroleuca are able to produce enough photosynthate for growth at depths down to 100 m, where water clarity is similar to Jerlov's (1976) type IB Oceanic water, and 5% of surface PAR (18 W m^{-2}) reaches the kelp community at 50 m (Drew *et al*. 1982).

Aside from compensatory light requirements, sublittoral macroalgae are also subjected to mechanical stress due to constant water motion. Seaweeds cope with waves and currents by having strong holdfasts, flexible stipes and blades. A significant positive relationship between wave intensity and depth of the upper limit of giant kelp *Macrocystis pyrifera* Agardh was observed in the Pacific coast of USA (Graham 1997). On the other hand, density of *Laminaria ochroleuca* was observed to be higher in an estuarine and sheltered site (8- 30 plants m^{-2}) compared to sites exposed to wave and current surge (2- 3 plants m^{-2}) (John 1971; Sheppard *et al*. 1978).

1.3 Seaweed life histories

Seaweed life histories follow different patterns. In some species the thalli are haploid and the only diploid stage is the zygote (e.g. in the green alga *Ulothrix*). In other species the thalli are diploid and the only haploid stages are the gametes (e.g. in the brown alga *Fucus*). An alternation between haploid gametophyte and diploid sporophyte is common, but many variations exist. Some seaweeds with such a heteromorphic life history have heteromorphic sporophytes and gametophytes. Sexual reproduction may be isogamous, anisogamous, or oogamous. Gamete fusion or syngamy is regulated by cell recognition mechanisms on cell surfaces. In brown algae, motile gametes may be attracted to each other or to a stationary egg by volatile pheromones (Müller 1989). In red algae, sexual reproduction often involves complex post-fertilization development of carposporophyte for zygote amplification (Lobban & Harrison 1994).

The red alga *Mastocarpus stellatus* has principally a heteromorphic life history where carpospore produces crustose plants previously referred to the genus *Petrocelis* (**Fig. 1**). Some populations, however, have a direct-type life history where carpospores produce basal disc initiating uprights which further develop into foliose plants, again producing carpospores, suggesting a complete apomictic life history (Chen *et al*. 1974; West *et al*. 1977; Rueness 1978; Guiry *et al*. 1984). The heteromorphic life history involves a change in ploidy level

between the diploid sporophyte and haploid gametophyte, whereas the direct-type life history is diploid throughout (Maggs 1988). Female gametophytes and tetrasporophytes are typically reddish-black to olive brown in color while male gametophytes are bright yellow to light pink. The previously *Petrocelis*-classified tetrasporophytic crust of *Mastocarpus* are usually 5 cm but can get up to 1 meter in diameter and are typically 2- 2.5 mm thick. Molecular evidence confirms the relationship between *Petrocelis sp*. and *Mastocarpus* (Bird *et al*. 1994).

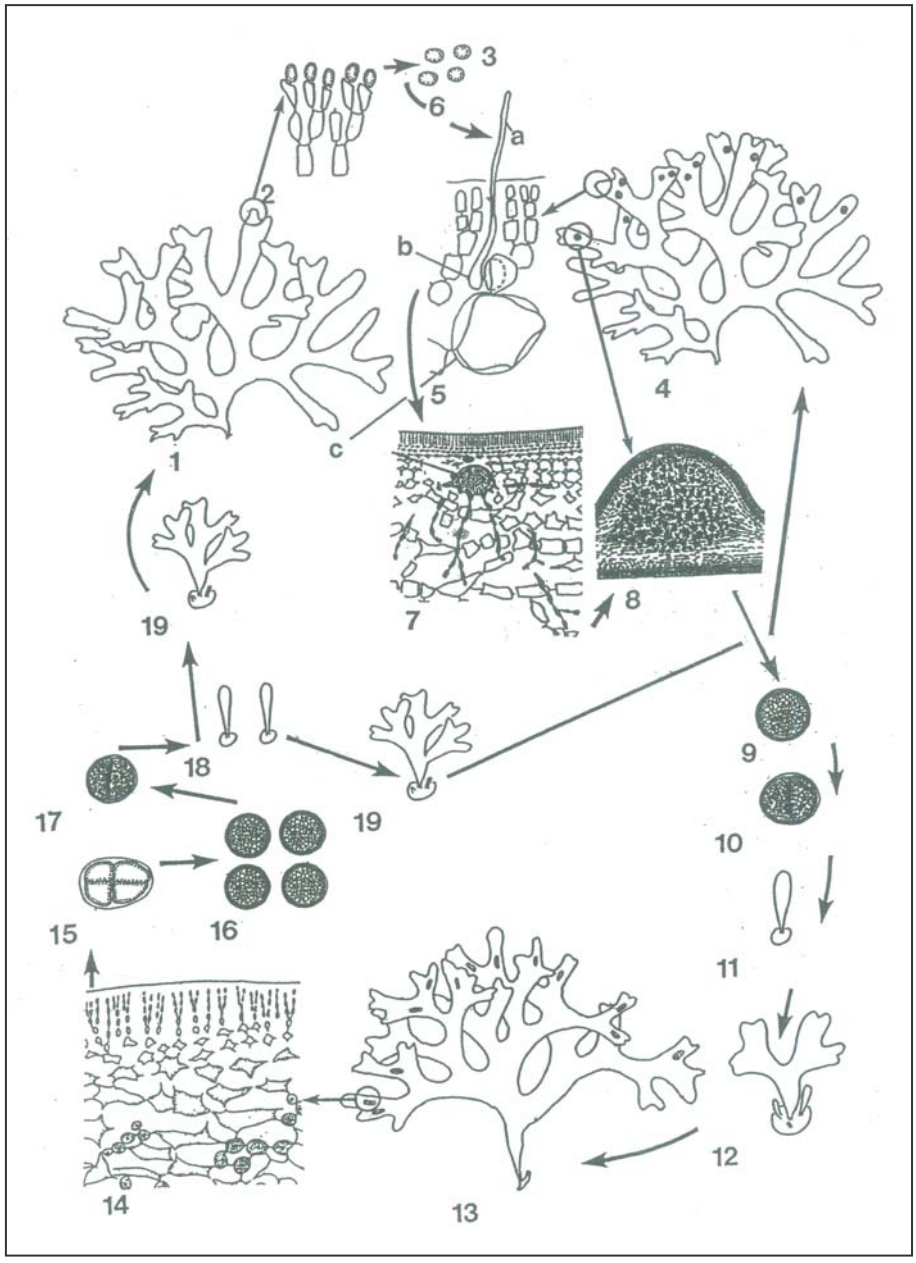

Fig. 2. Schematic diagram of the life history of *Chondrus* showing (1) male gametophyte, (2) transverse section of spermatangial sorus, (3) spermatia, (4) female gametophyte, (5) procarp, (6) fertilization, (7) young gonimoblast from supporting cell, (8) carposporangia, (9) carpospore (10) carposporeling, (11, 12) young tetrasporophytes, (13) tetrasporophyte, (14) young tetrasporangial sorus, (15) tetrasporangium, (16) tetraspores, (17) tetrasporeling, (18, 19) young gametophytes, (a) trichogyne, (b) carpogonium, and (c) supporting cell. Adapted from Masuda & Hashimoto 1993.

Mastocarpus stellatus was recently introduced in Helgoland (Kornmann & Sahling 1994). Since its introduction, only female gametophytes are observed in the field which are fertile all year round. A study on different isolates of *M. stellatus* across North Atlantic showed that in plants from Denmark and Iceland, only the direct-type life history is found (Guiry & West 1983) from where the Helgolandian population originated.

In *Chondrus crispus*, the tetrasporophytic and gametophytic generations are isomorphic (**Fig. 2**). The gametophytes are similar in morphology to *Mastocarpus*. However, the types of carrageenan composing their cell walls are distinct making it easy to identify life history stages in the vegetative state (Garbary & DeWreede 1988). A typical alternation of a haploid gametophyte with a diploid carposporophyte, and a diploid tetrasporophyte is indicated in this species (Chen & McLachlan 1972). Demographic studies on *Chondrus* fronds, however, showed that many populations are found to be overwhelmingly gametophytic (approximately 80%; Bhattacharya 1985; Chopin 1986; Dudgeon & Johnson 1992; Lindgren & Åberg 1996; Carrington *et al*. 2001), although tetrasporophyte dominated populations have also been infrequently observed (Lazo *et al*. 1989).

The brown algal genus *Laminaria* J.V. Lamouroux has a heteromorphic life history with an alternation of macroscopic sporophytes and microscopic gametophytes (**Fig. 3**). The sporophyte develops sporangia which divide meiotically producing flagellated haploid zoospores. Zoospores are released and upon settlement develop into microthalli which bear the gametangia. Sexual reproduction is oogamous. Male gametophytes produce sperm (male gametes) in antheridia and female gametophyte produces eggs in oogonia. Fertilization takes place with the egg partially extruded from the oogonium and the zygote often develops *in situ* to form a sporophyte. Sporophytes of Laminariales are differentiated into holdfast, stipe and lamina while gametophytes are undifferentiated and are filamentous and creeping.

Enormous amounts of propagules are produced by seaweeds, but only a small fraction survives to become mature plants. Competition takes place within and between species, and grazing and physical factors account for much of the mortality among juveniles. Marine communities are dynamic where recruitment and succession take place on available substrates across the vertical gradient of coastal environments (Lobban & Harrison 1994).

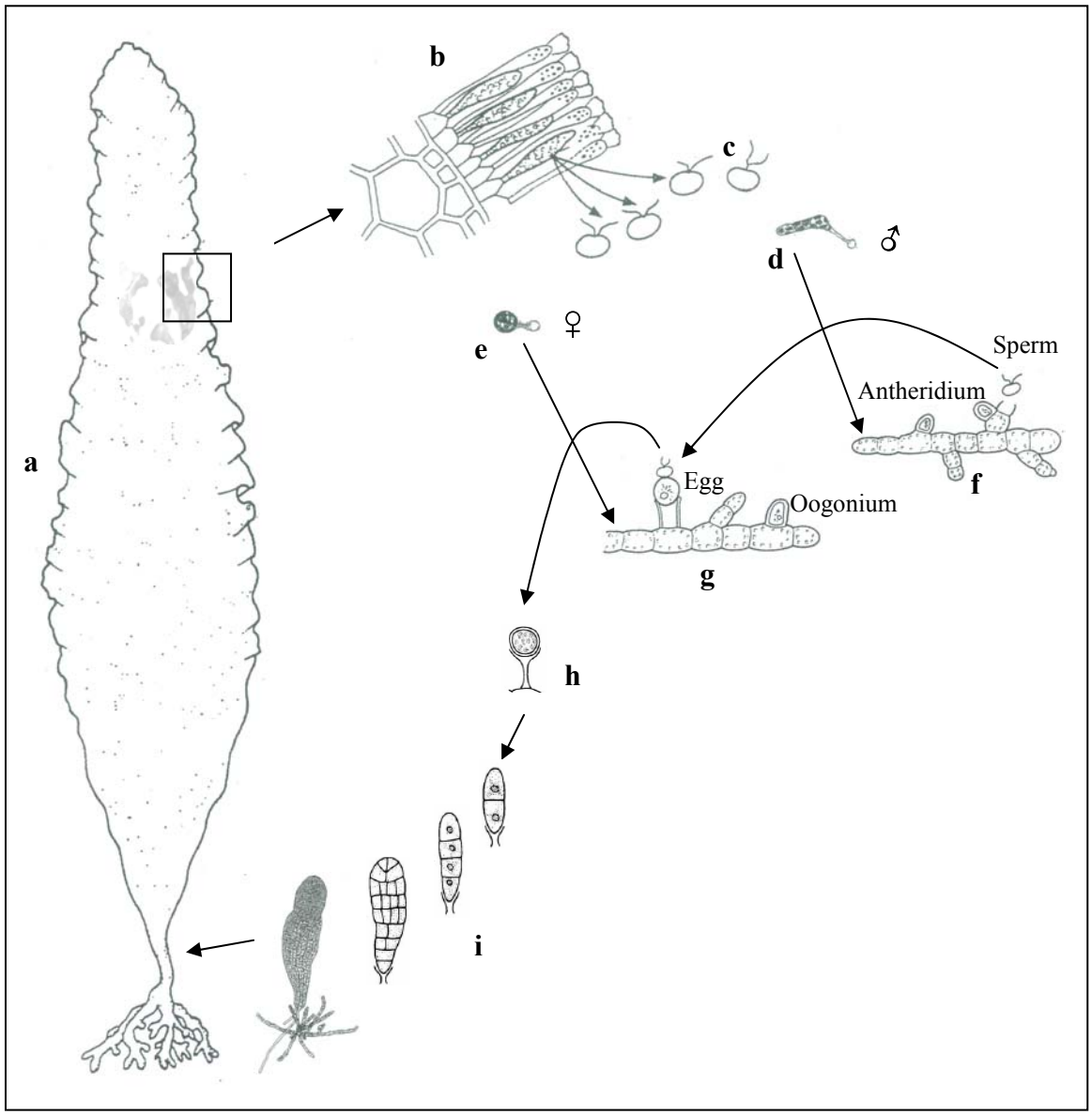

Figure 3. Schematic diagram of the life cycle of *Laminaria* showing (a) macroscopic sporophyte, (b) section of sorus with sporangia, (c) haploid zoospores, (d, e) germinating spores, (f) microscopic male gametophyte with sperm-producing antheridia, (g) microscopic female gametophytes with egg-producing oogonia, (h) zygote, and (i) developing young sporophyte. Modified from Bold 1973.

1.4 Photosynthetically active and ultraviolet radiation

The sun radiates energy over a broad spectrum of wavelengths. Light differs in its wavelength, frequency and energy. High energy wavelengths are characterized by high frequencies and short wavelengths. Wavelengths between about 400 nm - 700 nm are absorbed by the pigments inside the retina of our eyes and are collectively referred to as 'visible light' or known as photosynthetically active radiation (PAR). The amount of light harvested by a thallus depends on both pigment concentration and thallus morphology. The

rate of photosynthesis is strongly dependent on irradiance level. At the compensation point, photosynthesis equals respiration. Photosynthesis is maximal at saturating irradiance while at very high irradiance; the photosynthetic rate may be declined because of photoinhibition. Seaweeds can acclimate to differences in light quality and quantity by increasing the quantity of pigment or the density of photosynthetic units, and by changing the ratio of accessory pigments to chlorophyll *a* (Lobban & Harrison 1994).

UV radiation is defined by the Commission Internationale de l'Éclairage, International Commission on Illumination (CIE) as UV-C (100 nm < λ < 280 nm), UV-B range (280 nm < λ < 315 nm), and UV-A (315 nm < λ < 400 nm). The level of UV radiation (UVR) that reaches the Earth's surface can vary depending on a number of factors. The ozone layer absorbs most of the sun's UV-B radiation, but the absorption has decreased due to the thinning and destruction of ozone layer from the release of ozone-depleting substances that have been widely used in industry (Solomon 1990). Solar UV irradiance is highest when the sun is in zenith i.e. around noon and during the summer months. UV irradiance increases with altitude because the atmosphere is thinner and less capable of absorbing the UV rays. Cloud cover reduces UV levels while some surfaces, such as snow, sand, grass, or water can also reflect much of the UVR that reaches them.

Most stratospheric ozone is produced in low latitudes and transported to high latitudes. In the tropics, the ozone layer is thin compared to the mid- and high-latitudes. The sun's irradiance is highest at the equator, where the sun is directly in zenith and UV rays travel the least distance through the atmosphere. In contrast to the Polar Regions, the sun’s angle is lower in the sky. Sunlight passes over long distances through ozone-rich portions of the atmosphere, so more of the UV-B is absorbed. Average UV-B exposure at the poles is therefore lower than at the equator (Wängberg *et al*. 1996).

Stratospheric ozone depletion over Antarctica was first observed and reported in the early 1980s (Farman *et al*. 1985). Net springtime stratospheric ozone loss of up to 60-70% was since observed a yearly recurring phenomenon over Antarctica that intensifies ambient UV-B radiation (Crutzen 1992; Herman *et al*. 1996). Moreover, the area affected by ozone depletion has expanded to 5 fold over the past decades in the continental Antarctica. In the Arctic, springtime stratospheric ozone depletion was also detected recently at a less severe loss of up to ~ 20-25% (Müller *et al*. 1997; Dahlback 2002). Ozone loss of ~ 6% has also

been reported in the mid latitudes (WMO 1998). The increasing UVR on the earth's surface caused by stratospheric ozone depletion has been documented in the polar and temperate regions (Smith *et al*. 1992; Pearce 1996; Solomon 1999; Staehelin *et al*. 2001).

1.5 The damaging effects of high light stress and UV radiation

Excessive amounts of photosynthetically active radiation (PAR) and UV radiation (UVR) cause a broad spectrum of photochemical, genetic and other damaging effects in aquatic organisms. On the other hand, there are also repair and protective mechanism. The balance between damage, repair and the energetic costs of protection can be manifested in terms of increase energy demand, changes in cell composition, and decreased growth and survival rates (Vincent & Neale 2000).

When exposed to irradiances exceeding the energy requirement for photosynthesis, a strong degradation of the reaction center protein (D1) of Photosystem II (PS II) can occur (Ohad *et al*. 1984; Mattoo *et al*. 1984; Hanelt *et al*. 2003). This process is called chronic photoinhibition to distinguish it from dynamic photoinhibition through the xanthophyll cycle, which regulates quantum yield of photosynthesis (Demmig-Adams & Adams 1992). This involves a fast reversible process during which the quantum yields of PS II is diminished by increasing thermal energy dissipation is probably controlled by carotenoids (Osmond 1994). However, thermal energy dissipation by the xanthophyll cycle might not effectively protect the algae against harmful UV effects (Bischof *et al*. 1999).

Enhanced ultraviolet-B (UV-B) radiation due to stratospheric ozone depletion has several effects on the physiology and productivity of marine macrophytes. The negative impact of exposure to UVR includes (1) photoinhibition and eventual photodamage to the photosynthetic apparatus (Hanelt *et al*. 1997a); (2) protein breakdown and the loss of specific enzymatic or biological function (Lao & Glazer 1996); (3) formation of cyclobutane pyrimidine dimers (CPDs) in the DNA, inhibiting genome replication and expression (Buma *et al*. 1995, 2000; van de Poll *et al*. 2001, 2002); (4) absorption by aromatic sulfhydryl-containing biomolecules causing direct molecular damage (Vass 1997); and (5) production of reactive oxygen species responsible for oxidative damage within the cell (Rijstenbil *et al*. 2000). Recent studies have implicated the role of solar and especially UV radiation (UVR) in

determining macroalgal zonation patterns from polar to temperate regions (Dring *et al*. 1996; Hanelt *et al*. 1997b; Bischof *et al*. 1998).

1.6 Protection against and repair of UV-induced damage

Responses to UV damage are counterbalanced by protection strategies such as avoidance, screening, photochemical quenching and repair. The physiological balance to counteract the negative effects can, however, increase energy demands for protection and repair at the expense of growth.

Avoidance strategies include habitat selection by recruiting under the canopy of adult sporophytes and circadian rhythms by phasing cellular activities sensitive to light at night (Suzuki & Johnson 2001). Reproductive seasonality and diel periodicity in spore release (Reed *et al*. 1988; Amsler & Neushul 1989) could also ensure reproductive success by synchronizing propagules production and release with the onset of favourable environmental condition (Santelices 1990; Kinlan *et al*. 2003).

Screening includes extracellular (cell walls, thallus thickness) and intracellular mechanisms (UV-absorbing compounds). Species morphology is an important trait in algal ecology. Littler and Littler (1980) defined functional-form groups in marine macroalgae and grouped the genus *Laminaria* in the thick leathery functional form. However, different thallus thickness is observed in different species of the genus whereby *Laminaria hyperborea* (Gunnerus) Foslie is considered the thickest leathery species (Johansson & Snoeijs 2002). The optical effect of the outer cell layers can influence reflection, attenuation, scattering, absorption or transmittance of UV radiation to the inner cells (Caldwell *et al*. 1983) enhancing UVR tolerance in species with increasing thallus thickness (Franklin & Forster 1997).

The pronounced wavelength selectivity of absorption in leaf epidermis of terrestrial plants is often attributed to flavonoids and other related UV-absorbing compounds (Robberecht & Caldwell 1978). Flavonoids absorb UVR with maximum effectiveness around 295 nm (Stapleton & Walbot 1994; Landry *et al*. 1995). Among Laminariales phlorotannins are accumulated within the outer cortical layer of the thalli (Lüder & Clayton 2004; Shibata *et al*. 2004). UVR can therefore be attenuated by cellular UV-absorbing compounds and cell

walls of the epidermal tissue effectively reducing UV fluence from reaching physiological targets.

Several functions of phlorotannins have been reported in brown algae such as herbivore deterrents, digestion inhibitors, antibacterial agents, and UV screens (Schoenwaelder 2002). The production of these compounds involves a substantial cost in terms of individual growth (Pavia *et al*. 1999). However, synthesis of moderate levels of phlorotannins and growth could be maintained simultaneously (Steinberg 1995). The synthesis and production of this compound in nature is not strictly inducible as a secondary metabolite but also serve some primary and secondary roles in reproduction, fertilization, spore attachment and cell wall construction (Arnold 2003). Its synthesis could also be in response to specific environmental cues or stress factors and has different functions in different kelp species.

UV-B induced DNA damage can be repaired under photo-reactivating light (van de Poll *et al*. 2002), nucleotide and base excision repair, and recombination repair (Roy 2000). Stress proteins specific for light-induced stresses in photosynthetic organisms (early light-induced proteins, ELIPs) play a role in photoprotection (Adamska 1997). Antioxidant enzymes, lipid-soluble antioxidants inside cellular membranes (e.g. carotenoids), and water-soluble reductants found in the cytosol are defences against forms of reactive oxygen (Dunlap & Yamamoto 1995).

Physiological adjustments enable the organism to undergo acclimation in order to optimise growth in a given environment. Photosynthesis is a dynamic process which can acclimate to variations in light intensity and spectral quality (reviewed by Senger & Bauer 1987; Falkowski & LaRoche 1991). Short term light fluctuations elicit fast and reversible reactions such as: fluorescence or heat dissipation via the xanthophyll cycle which is considered a major photoprotective process, or energy redistribution between the two photosystems (Hall & Rao 1994). Some photoadaptive mechanisms that might have been involved in UVR acclimation is the establishment of a physical barrier which shields the photosynthetic apparatus against damaging radiation (Karentz 1994). Acclimation to high PAR irradiance significantly increases UV-B tolerance through photoinduction of screening compounds, increased activity of photorepair enzymes and other physiological changes related to life under high irradiance (Warner & Caldwell 1983).

1.7 Statement of the research questions

Physiological studies on the early life stages of macroalgae in response to environmental stress are wanting. The question whether early developmental stages of macroalgae, specially algal spores which are devoid of cell wall, are more susceptible to UVR than the large developmental stages is addressed in this study. Among the few available studies, the effect of UVR on photosynthesis had been studied only on the gametophytes and zoospores of Laminariales (Dring *et al*. 1996; Wiencke *et al* 2000) and unicells of Ulvales (Cordi *et al*. 2001). Other studies on impact of UVR on the early life stages of Ulvales, Corallinales, Laminariales, Fucales and Ectocarpales are focused either on the (1) sporulation of fertile thalli, (2) germination and photomovement of spores, (3) growth of germlings and (4) DNA synthesis in gametophytes (Houvinen *et al*. 2000, Swanson & Druehl 2000, Cordi *et al*. 2001, Makarov & Voskoboinikov 2001, Bañares *et al*. 2002, Flores-Moya *et al*. 2002, Altamirano *et al*. 2003, Han *et al*. 2003). The negative physiological response to UVR includes: photoinhibition of photosynthesis, lower spore release and motility, lower DNA synthesis and nuclear translocation, higher spore mortality and lower germling growth.

Spores and germlings of kelps can be found to remain viable in plankton for extended periods of time (Reed *et al*. 1992). Surviving spores are therefore capable of dispersal, settlement, attachment, and initiation of new individuals across the expanse of the vertical tidal zones, especially in crevices and sheltered tide pools. However, they can be exposed to air during low tides and the whole spectrum of solar radiation which may contribute to the post-recruitment mortality and exclude sensitive species from higher positions on the shore.

Survival of early transitional life history stages (e.g. spores, sporelings and germlings) is the most critical phase leading to the successful formation of benthic populations. In Helgoland, *Mastocarpus stellatus* was able to successfully colonize open spaces in the upper eulittoral while the distribution of *Chondrus crispus* is limited under canopies of *Fucus spp*. in the eulittoral, in tidepools and upper sublittoral. Differential susceptibility between *M. stellatus* and *C. crispus* carpospores and young gametophytes to UVR could therefore influence spore viability and establishment of early post-settlement stages and growth of young juveniles.

Although interference competition, demographic factors and grazing pressure on the early life stages also play important role in recruitment and subsequent community structure (Lubchenco & Menge 1978; Lubchenco 1980; Dean *et al*. 1989; Reed 1990; Worm & Chapman 1996, 1998), this study is focused on the physiological constraints that lead to the presently observed zonation pattern in Helgoland and Spitsbergen shoreline and susceptibility of early life stages of different Laminariales across a latitudinal gradient.

The present study is the first to investigate the impact of UV-B-induced DNA damage and repair capacity on the photosynthesis, germination and growth of the early life stages of Gigartinales from Helgoland as well as Laminariales from the temperate Atlantic coast of Spain and the North Sea, and the Arctic population in Kongsfjorden, Spitsbergen. One goal of the thesis is to test the hypothesis that susceptibility of early life history stages to UVR determines the upper depth distribution limit of the mature sporophytes.

1.8 Thesis outline

The susceptibility of spores and juvenile stages of ecologically important red and brown macroalgae to UVR was investigated in the laboratory. Field germination experiment was also conducted for the first time. This thesis is divided into 7 publications.

Publication 1 examines the relative susceptibility between spores and gametophytes of the two coexisting Gigartinales (*Mastocarpus* and *Chondrus*) in the rocky littoral zone of Helgoland. A higher tolerance against UV-B-induced DNA damage and effective repair mechanism in carpospores of the introduced *Mastocarpus* is speculated to be responsible for its successful recruitment and colonization of the eulittoral area effectively changing the appearance of the intertidal biotope of the island.

Publication 2 describes the effect of UVR on growth, photosynthesis and pigments in the temperate *Laminaria ochroleuca*. Continuous growth measurement using growth chambers with online measuring technique was first conducted and reported in this study. This study is also the first to report morphological damage, which includes tissue deformation, lesion, blistering and thickening of meristematic lamina, on young sporophytes exposed to UVR.

Publications 3, 4 and 5 follow-up growth experiments on different Laminariales species from Helgoland and Spitsbergen using the growth chambers with online video measuring technique. Tissue morphology, optical characteristics and absorption spectra, as well as remaining tissue DNA damage, were found to contribute on the sensitivity of growth to UVR.

Publication 6 compares the relative sensitivity of the three *Laminaria* species from Helgoland to UVR in the laboratory. Efficient DNA damage repair and recovery of PSII damage contributed to germination success in different species. UVR sensitivity was found to be related to the reproductive seasonality of kelps and on the upper depth distribution of the adult sporophytes around the island.

Publication 7 is the first field study on spores' germination capacity of brown algal species exposed to ambient solar radiation. Higher ambient PAR in the field was observed to enhance UV tolerance of zoospores. Relative susceptibility was found to be related to the upper depth distribution limit of the adult sporophytes.

2 MATERIAL AND METHODS

2.1 Algal materials

2.1.1 Gigartinales

2.1.1.1 Carpospores

Carpospores were obtained from unialgal cultures of vegetative *Mastocarpus stellatus* Stackhouse (Guiry) and *Chondrus crispus* Stackhouse fronds which are maintained in the Biologische Anstalt Helgoland (BAH). The culture medium of the gametophytes with fertile carposporophytes was exchanged with fresh Provasoli-enriched seawater (Starr & Zeikus 1993) and after 3 days the spore containing medium was collected. The spores were allowed to sink for 4 hours. Then the supernatant water was slowly sucked out by a vacuum pump to obtain a concentrated spore suspension. Then, it was slowly and continuously agitated using a magnetic stirrer to stop the spores from settling and derive a homogenous spore suspension. A working spore suspension with a density of approximately 4 x 10^3 spores ml^{-1} was adjusted after counting the number of spores in 20 µl of suspension on a cavity slide using an inverse microscope.

2.1.1.2 Young gametophytes

Following the direct-type life history, carpospores of *Mastocarpus* develop directly into female gametophytes (Maggs 1988). The young vegetative fronds of *Chondrus* used for the study was found to be gametophytic after acetal-resorcinol test (Garbary & DeWreede 1988). The young fronds of both species were maintained in Provasoli-enriched culture medium (Starr & Zeikus 1993).

2.1.2 Laminariales

2.1.2.1 Zoospores

Fertile specimens of the Helgoland species of *Laminaria digitata* (Hudson) Lamouroux were collected by hand in the upper sublittoral (0.5- 1 m) during low tide, while *L. saccharina*

(Linnaeus) Lamouroux and *L. hyperborea* (Gunnerus) Foslie were collected by SCUBA diving in the mid (2- 4 m) and lower (5- 7 m) sublittoral respectively around the island of Helgoland. Sporophytes with sori were collected at different times during the peak fertile season of *L. digitata* (May- July), *L. saccharina* (September- November) and *L. hyperborea* (December- February). Fertile specimens of *Saccorhiza dermatodea* (Bachelot de la Pylaie) J. Agardh, *Alaria esculenta* (Linnaeus) Greville and *Laminaria digitata* were collected between May and June 2004 by SCUBA divers in Kongsfjorden at Prins Heinrichøya or Blomstrandhalvøya close to Ny Ålesund (Spitsbergen, 78°55'N, 11°56'E).

Thallus parts with sori were cleaned and blotted dry with tissue paper and kept for 2 days in a wet chamber in dim light at 0 °C (Spitsbergen isolates) or at 5 ± 1 °C (Helgoland isolates). Spores were released from 5 individual sporophytes per species by flooding Provasoli enriched seawater (Starr & Zeikus 1993) to the thallus in separate Petri-dishes. Spore density released from individual sporophytes was adjusted to 2.0 x 10^5 – 4.0 x 10^5 spore ml^{-1} after counting using a Neubauer chamber (Brand, Germany).

2.1.2.2 Young sporophytes

Stock gametophyte cultures of different species of Laminariales, originally established from zoospores of fertile sporophytes collected from La Coruña (Spain), Helgoland (North Sea, Germany) and Kongsfjorden, Spitsbergen (Svalbard, Norway) were used to obtain young sporophytes. Filaments of male and female gametophytes were fragmented together using mortar and pestle, and transferred to culture dishes filled with Provasoli-enriched seawater (Starr and Zeikus 1993). Cultures were maintained inside a temperature-controlled room at 12 ± 2 °C (temperate species) or at 5 ± 2 °C (Arctic species) and 10 μmol photons m^{-2} s^{-1} white light with different light: dark (LD) photoperiod to initiate gametangia formation (12:12 LD for *Laminaria ochroleuca*, *L. digitata*, *L. saccharina* and *L. hyperborea*; 8:16 LD for *L. solidungula* J. Agardh, *Saccorhiza dermatodea* and *Alaria esculenta*). After 6- 10 weeks, young sporophytes (approximately 3 mm length) were taken from the bottom of the culture dishes and transferred to aerated 5-liter culture bottles until sporophyte size was large enough for experimentation. At this time, all young sporophyte cultures were maintained at 12:12 LD photoperiod.

2.2 Laboratory irradiation treatments

Photosynthetically active radiation (PAR) was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany) and ultraviolet radiation (UVR) was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland,OH, USA), emitting a spectrum similar to solar radiation in the range 295 to 340 nm. Three kinds of glass filters and filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes. Therefore, experimental units were covered with the following filters: Quartz glass, Ultraphan transparent (Digefra GmbH, Germany), WG 320 (Schott GmbH, Germany) or Folanorm (Folex GmbH, Germany), GG400 (Schott GmbH, Germany) or Ultraphan URUV farblos (Digefra GmbH, Germany) corresponding to the PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) and PAR (P) treatments respectively. Irradiation was measured using a cosine sensor connected to a UV-VIS Spectroradiometer (Marcel Kruse, Bremerhaven, Germany) below the cut-off filters. The biologically effective doses (BED) between 280 and 320 nm were calculated using 2 action spectra for well-known biological responses: the generalized plant damage (280- 312 nm, Caldwell 1971) and DNA damage for *Escherichia coli* (280- 320 nm, Setlow 1974). Erythema-weighted UV dose (UV_{ery}) below the cut-off filters was also measured using an ELUV-14 UV-Data logger (ESYS GmbH, Berlin, Germany; El Naggar *et al*. 1995). Weighted and unweighted irradiances for each experiment are presented in tables in each chapter (see different publications). The UV (total UVR): PAR ratio of 0.6- 1.26 used in our treatments is within the highly variable ratio of UV (305, 320, 340, and 380): PAR ratio measured in Helgoland which ranges between 0.002- 1.4 depending on cloud cover and season (Dring *et al*. 2001).

2.3 Laboratory experimental set-up for growth measurements

2.3.1 Basin flow through culture system

Thalli of young Gigartinales gametophytes and Laminariales sporophytes were exposed to experimental irradiances in a large flow-through basin (600 x 400 x 120 mm). Inside the basin, upright standing PVC rings (120 mm diameter x 70 mm height) served as enclosure for the algae. The PVC rings had a 5 mm diameter hole at the bottom for water inflow through silicon tubes and water flowed out into the basin through four equidistant 10 mm diameter holes around the PVC rings covered with mesh. From a reservoir, 80 l of filtered and

pasteurized seawater flow circulating into the basin through the PVC pipes using submersible water pump (Eheim; Typ 1060, 38 L min^{-1}, Deizisau, Germany) which also provided water movement inside the PVC rings. Water level in the basin was maintained at 60 mm, to simulate low tides. During the experiment, water temperature was maintained at 10 ± 1 °C. Seawater was changed weekly to prevent depletion of nutrients.

2.3.2 Online video scanning system

Three growth chambers (ISITEC GmbH, Bremerhaven, Germany) with online video measuring technique and a circulating water system were used for the experiment (see Figure 1 of publication 3). Light sources were mounted 15 cm above the platform consisting of 2 white fluorescent lamps (Philips, TL 8W/965, Holland) and 2 UV lamps (Q-Panel UVA-340, 40 Watt, Cleveland, USA; modified from the figure). Infrared diodes (maximum at 930 nm) mounted at the sides of the chamber produced infrared images of the object for the video camera, also during dark periods. The image captured by a CCD camera was analysed by a MedeaLAB Count and Classify software (Multimedia and Software GmbH, Erlangen, Germany) which calculates growth of the algae in terms of increased number of pixel. The algae were fixed between the top and bottom of Plexiglas chambers and acclimated for 3 days to 16:8 light:dark (LD) photoperiod without UVR. After acclimation, UVR was supplemented in the middle of the light phase (09:00- 15:00 hours). Two types of glass filters: Schott-GG 400 (Schott, Germany) and Quartz glass were placed over the top of the Plexiglas chamber covering the algae corresponding to the photosynthetically active radiation (PAR= P) and PAR + UV-A + UV-B (PAB) treatments respectively. Three growth chambers, each unit containing a replicate for P and PAB treatments, were operated simultaneously per species. Seawater was changed weekly to ensure enough nutrient supply within the medium.

2.4 Measurement of photosynthetic performance

2.4.1 Thalli

Young thalli were acclimated to 46 µmol photons m^{-2} s^{-1} (≈ 10 W m^{-2}) white light at ambient temperatures for 3 days under 16:8 h light: dark (LD) cycles. To determine the effects of different light treatments, 6 hours UV exposure was supplemented in the middle of the light

phase. Photosynthetic activity was determined by measuring the variable chlorophyll fluorescence of PSII with a Diving PAM device (Heinz Walz GmbH, Germany). Effective quantum yield ($\Delta F/F_m$') was measured 1 hour before UV exposure, during UV exposure and after UV exposure in the course of several days (15 days for gametophytes of Gigartinales and 28 days for sporophytes of Laminariales).

2.4.2 Spores

Photosynthetic efficiency measured as variable fluorescence of photosystem II (PSII), was determined using a Xenon Pulse Amplitude Modulation fluorometer (XE-PAM) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of spore density, spore suspension was filled into 5 ml Quartz cuvettes and the maximum quantum yield (F_v/F_m) was measured to determine initial photosynthetic efficiency (control at T_0, n=5) as described by Hanelt (1998). To evaluate the effect of different radiation and exposure time treatments, 5 ml of fresh spore suspension were filled into each 35 mm x 10 mm cell culture dish and exposed to the 3 radiation conditions (P, PA, PAB) in a series of time treatments (n=5, per treatment combination) at 10 ± 1°C. After treatments, F_v/F_m was measured and spore suspension was returned to the same culture dish and cultivated under dim white light (10 µmol photons m^{-2} s^{-1}) for recovery. Spore suspension measured at T_0 was also maintained at the same condition. After 2 days, measurements of photosynthetic efficiency were repeated to determine recovery and handling effect on untreated T_0 samples (now designated control at T_2 measurement), which were eventually used as control. Settled and germinating spores were slowly resuspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes. F_v/F_m after exposure and after recovery was expressed as percent of T_0 and T_2 control, respectively.

2.5 Measurement of thallus growth

In the basin flow through culture system, thallus growth was measured every 3 days in terms of weight (g) and surface area (mm^2) increase using a weighing scale (Sartorius CP225D, Germany) and a scanner with an image analysis software (Winfolia™ 5.0, Regent Instrument Inc., Canada) respectively. In the growth chambers with online video scanning system,

growth was continuously measured as increase in pixel sizes every 10 min for 18- 21 days. Growth rates were computed by plotting all data points (entire experiment period) of each replicate per treatment. They were individually fitted to an exponential equation $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase.

2.6 Spore mortality and germination rate

2.6.1 Gigartinales

For this experiment, 20 ml of working spore suspension and 20 ml of Provasoli enriched seawater were filled into 85 mm x 15 mm culture dishes laid with cover slips. The dishes were then exposed to the same combination of treatments used for photosynthesis and an additional treatment of 16 h exposure at four varying levels of radiation, in triplicates. After the treatment, spores were allowed to germinate in low white light (10 µmol photons m^{-2} s^{-1}) for 6 days. Carpospores settled on the cover slip were scored as dead or alive by counting 300 cells per replicate using light microscope (Olympus CH-2, Japan) equipped with 20x seawater immersion objective. Dead cells are easily recognizable (no pigment and ghost like cell) from the live cells (pigmented and dividing cells, sometimes with germinating tubes, see Figure 1 of publication 1). Because dead cells were also observed under P treatment, the percentage dead cells for PA and PAB was calculated by using the average number of dead cells in P as a control value as described by Wiencke *et al*. (2000):

$$\text{Dead spores (\%)} = 100 \times (S_{dead} - D_C) \times L_C^{-1}$$

Where S_{dead} is the number of dead spores under UV-exposure, and D_C and L_C are the numbers of dead and living spores under P respectively. Mortality data and BED were used to create a dose-response relationship and to calculate the BED_{50}, which is the UV dose needed to achieve 50% inhibition in germination.

2.6.2 Laminariales

Cover slips were put inside the 85mm x 15mm culture dishes and filled with 40 ml Provasoli enriched seawater. To ensure that the density of spores per unit area is similar throughout the experiments, 2- 4 drops of the working spore suspension were put into each dish. The dishes were then exposed to the same experimental treatments, in triplicates. After treatment, spores

were allowed to germinate in low white light (10 µmol photons m^{-2} s^{-1}) for 3 days. Triplicate of untreated samples (control) were also allowed to grow at the same low light condition. Spores settled on the cover slip were scored as germinated or not germinated by counting 300 cells per replicate using a light microscope (Olympus CH-2, Japan) equipped with 20x seawater immersion objective. A spore was classified as germinated when at least a germ-tube was formed. Dead and living cells were not differentiated. Since non-germinated cells were also observed under control, germination rate under P, PA and PAB treatments was expressed as percent of control. Dose response relationship was also calculated to determine biological effective dose needed to inhibit 50% germination (BED_{50}).

2.7 Experimental set-up and sampling for spore DNA damage and repair

Spore suspensions were exposed to the whole light spectrum (PAB) under different exposure times (1, 2, 4, 8, 16 hours) and at 16 hours of 4 irradiation levels. For each treatment, 6 experimental units were prepared. After the irradiation treatment, 3 experimental units (as replicates) were processed immediately while the other 3 were allowed to recover in low white light for 6 days in Gigartinales carpospores and 2 days in Laminariales zoospores before processing. Settled and germinating spores were resuspended from the bottom of the Petri dishes by jetting pressurized seawater from a wash bottle. The spore samples were filtered through 44 mm diameter 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK). Filters were individually filled into 2 ml Eppendorf tubes and frozen at -80 °C for further DNA extraction and analysis of CPDs.

2.7.1 Extraction of algal DNA

DNA was isolated following the CTAB extraction procedure described by van de Poll *et al*. (2001). Spore samples were immediately treated with extraction buffer while vegetative fronds were first homogenized in liquid nitrogen. After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 µl 10 mg ml^{-1}, 30 min, 37 °C; Sigma, MO) and stored at –20 °C. The DNA concentration was quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, CA). A

dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

2.7.2 Assay for CPD detection

The immunoassay for CPDs was modified after Vink *et al*. (1994) and van de Poll *et al*. (2001). Heat denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 µm, Schleicher & Schuell, Keene, NH) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a two step antibody assay, the membrane was treated with ECL Western blotting detection reagent (Amersham Buckinghamshire, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) at different exposure times. The films were developed using X-ray film developer. Developed films were scanned using Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 µg ml^{-1} DNA for each calibration point. The UV-irradiated DNA (45 min exposure to 2 TL 20W/12 lamps, Philips, Eindhoven, Netherlands) was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

2.8 Thallus morphology

Wet weight: surface area (mg: mm^2) ratio was used to determine thallus thickness. Surface area of sporophytes taken directly from the unialgal cultures was measured using a scanner and WinFolia 5.0 image analysis software (Regent Instrument, Quebec City, Canada) and the corresponding wet weight of the blotted tissue was weighed using Sartorius (CP225D, Germany) weighing scale (n = 10).

2.9 Spectrophotometric measurements

2.9.1 Zoospores suspension

To determine the presence of UV absorbing compounds in the zoospore suspension, untreated samples were filled into quartz cuvettes and scanned in the 250- 700 nm waveband using Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere. Absorbance spectra of the zoospore suspension, the medium (filtrate) and zoospores were measured from: (1) zoospore suspensions with seawater as reference (2) filtrates with seawater as reference and (3) zoospore suspensions and filtrates as reference, respectively. The filtrate was obtained by filtering the zoospores out of the suspension through 44 mm diameter 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK) using a vacuum pump at 400- 600 millibars to minimize damage to the cells.

2.9.2 Tissue of young sporophytes

To determine the presence of UV absorbing compounds in the tissue, young sporophytes obtained from culture were inserted into seawater filled quartz cuvettes and scanned with seawater as reference in the 250- 750 nm waveband using a Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere. For comparison, young sporophytes collected in the field in Spitsbergen were also scanned.

2.10 Pigment analysis

Frozen samples at –80 °C from previous growth experiments were treated with 100 µl of 100% N-N-dimethylformamide and stored in darkness for approximately 16 h. Extracts were centrifuged at 5000 rpm. Diethyl ether and water added to the supernatant to precipitate the pigments. The volatile solvent was then evaporated under a stream of gaseous nitrogen and the precipitated pigments were re-dissolved in 100 µl of acetonitrile/methanol/tetrahydrofuran (75:15:10 by vol.; solvent A). Sample volume of 50 µl were injected into an HPLC set up (Waters, Eschborn, Germany), equipped with a 600E gradient module with system controller and a Model 996 photodiode array detector. Separations were performed on a stainless-steel Merck LiChrosphere RP 18 cartridge (5 µm: 125 mm long, 4 mm inner diameter) filled with

material identical to the main cartridge. Pigments were eluted by a binary gradient system of helium-degassed solvents and monitored at 436 nm. Each second, absorption spectra in the range 300- 800 nm were recorded by the diode array detector. For optimum resolution, the flow rate of the mobile phase of solvent A and solvent B (0.13 M ammonium acetate, 0.05 M tetrabutyl ammonium acetate) was adjusted according to the protocol of Bischof *et al*. (2002). Pigments were identified by co-chromatography with standards obtained from DHI Water and Environment (Hørsholm, Denmark).

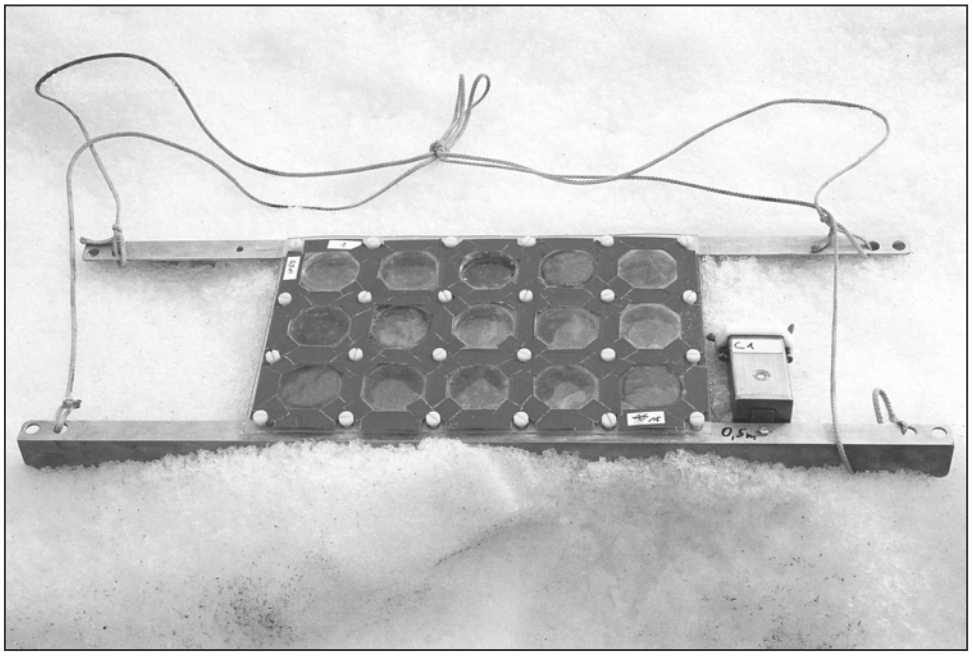

Fig. 4. Experimental field set-up used in Kongsfjorden, Spitsbergen. Stainless steel frame holding 15 petri dishes (53 x 12 mm) covered with different filter foils corresponding to P, PA, PAB treatments. Treatment was assigned randomly. An ELUV 14 datalogger is fastened to determine UV-B dose at different depths.

2.11 Field experiment

Sample holders made of stainless steel frame (0.25 x 0.40 m) with bottom and top UV-transparent Plexiglas 'GS 2458' (Röhm, Darmstadt, Germany) platforms accommodating 15 Petri-dishes (53 x 12 mm) arranged in 3 rows and 5 columns are used for the field experiments (**Fig. 4**). To determine the effect of different radiation treatments, the top Plexiglas area representing each Petri-dish was covered with different filter foils corresponding to P, PA and PAB treatments. Assignment of treatment was done randomly. The Petri-dishes were then completely filled with filtered seawater and 2- 5 drops of zoospore

suspension from different sporophytes. Then it was covered, air-bubble free, with the top UV-transparent Plexiglas. Four sample holders were prepared and deployed at different depths in the fjord using anchors and floats. They were exposed to ± 24 hours in the field (one set-up was exposed to 45 hours).

In each sample holder, an ELUV 14 datalogger was fastened to determine UV-B dose (recorded as erythema-weighted dose, UV_{ery}; El Naggar *et al.* 1995) at different depths (0.25 m, 0.5 m, 1 m, 2 m and 4 m) where the set-up was deployed. Surface PAR was measured through out the scientific campaign using a cosine quantum sensor attached to a LI-COR data logger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska, USA). Diffuse vertical attenuation coefficients of downward irradiance of UV-B radiation were determined after Kirk (1994).

After treatment exposure, the sample holders were recovered from the fjord and the individual Petri-dishes were covered with corresponding lids and exposed to dim white light (10 µmol photons $^{-2}$ s^{-1}) using daylight fluorescent tubes (Osram Daylight Lumilux De Luxe L36W/12-950) at a temperature of 10 °C for 3 days to determine germination rates. Germination was determined microscopically by use of an Axioplan microscope (Zeiss, Göttingen, Germany) equipped with a 25x seawater immersion objective. A spore was classified as germinated if at least a germ-tube was formed. Dead and living, but not germinated spores were not differentiated. In each sample, approximately 300 spores were counted and the percentage of germinated and non-germinated spores determined. Dead spores and their remains were readily identified.

2.12 Statistical analyses

Data were tested for homogeneity of variances (Levene Statistics) and normality (Kolmogorov-Smirnov test). Transformations were done to heteroskedastic and non-normal data. Corresponding statistical analyses were performed on different data set in each chapter (see different publications). Statistical analyses were done using SPSS program (SPSS, Chicago, IL, USA).

3 PUBLICATIONS

3.1 List of publications

Publication 1: **Roleda MY**, van de Poll WH, Hanelt D, Wiencke C (2004) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. *Marine Ecology Progress Series* 281: 37-50

Publication 2: **Roleda MY**, Hanelt D, Kräbs G, Wiencke C (2004) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. *Phycologia* 43: 603-613

Publication 3: **Roleda MY**, Hanelt D, Wiencke C (2005) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. *Polar Biology* 28: 539-549

Publication 4: **Roleda MY**, Wiencke C, Hanelt D (2006) Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic *Laminaria* sporophytes. *Planta* 223: 407-417

Publication 5: **Roleda MY**, Hanelt D, Wiencke C (2006) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). *Marine Biology* 148: 1201-1211

Publication 6: **Roleda MY**, Wiencke C, Hanelt D, van de Poll WH, Gruber A (2005) Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction. *Plant, Cell and Environment* 28: 466-479

Publication 7: Wiencke C, **Roleda MY**, Gruber A, Clayton MN, Bischof K (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments. *Journal of Ecology* 94: 455-463

3.2 Declaration of contributions to the publications

Conceptualization and implementation of the experimental designs, data gathering and analyses, and manuscript writing of publications 1, 2, 3, 4, 5 and 6 were initiated and conducted by M.Y. Roleda. Co-authors for each publication have contributed in parts on experimental works, laboratory analysis and manuscript editing. Prof. Christian Wiencke and Prof. Dieter Hanelt are also responsible for the research theme and supervision of this dissertation. The second author (M.Y. Roleda) of publication 7 has contributed on the experimental works, data collection and management, statistical analyses and manuscript writing and editing.

Publication 1

Roleda MY, van de Poll WH, Hanelt D, Wiencke C

PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern

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PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern

Michael Y. Roleda[1,4,*], Willem H. van de Poll[2], Dieter Hanelt[1,5], Christian Wiencke[3]

[1]Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Postfach 180, 27483 Helgoland, Germany

[2]Department of Marine Biology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

[3]Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

[4]Biology Department, De La Salle University, 2401 Taft Avenue, 1004 Manila, Philippines

[5]*Present address:* Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-Str. 18, 22609 Hamburg, Germany

ABSTRACT: The effects that ultraviolet radiation (UVR, 280 to 400 nm) and photosynthetically active radiation (PAR, 400 to 700 nm) had on early life stages of *Mastocarpus stellatus* and *Chondrus crispus* were studied to determine if differences in UVR tolerance could influence their recruitment success on the upper eulittoral shores of Helgoland (North Sea). Photosynthesis, germination capacity, DNA damage and carpospore repair were measured after exposures to different time lengths and intensities of PAR+UV-A+UV-B, PAR+UV-A or PAR alone, and also after recovery in low white light. Germination and photosynthesis of the low light adapted carpospores of both species were inhibited as PAR was increased. Supplemental UV-A and UV-B had a small additional effect on the F_v/F_m of *M. stellatus* but this effect was more pronounced in *C. crispus*. However, photosynthesis of both species significantly recovered after 48 h. Carpospore viability in *C. crispus* was more sensitive than in *M. stellatus* to UVR, while a higher dose was needed to achieve 50% germination inhibition in *M. stellatus*. Furthermore, UV-B-induced DNA damage, measured as cyclobutane-pyrimidine dimers (CPDs), was less in *M. stellatus* spores, which also exhibited an efficient DNA repair mechanism compared with *C. crispus*. In contrast, growth and chlorophyll *a* contents in young gametophytes of both species were not affected by repeated UV exposures. Higher total carotenoid was measured in plants exposed to UVR, indicating a photoprotection role, because photosynthesis completely acclimated to UVR after 3 d. Furthermore, DNA damage was not detected on mature fronds of both species when exposed to the full solar spectrum. Therefore, the susceptibility of carpospores to UVR could influence species recruitment to the upper eulittoral zone.

KEY WORDS: Carpospores · Gametophytes · *Mastocarpus stellatus* · *Chondrus crispus* · Germination · Cyclobutane-pyrimidine dimers · DNA repair · Pigments

INTRODUCTION

Mastocarpus stellatus Stackhouse (Guiry) and *Chondrus crispus* Stackhouse (Gigartinales) (hereafter called '*Mastocarpus*' and '*Chondrus*' respectively) are morphologically similar red algae abundantly distributed along the North Atlantic coasts and co-inhabit the rocky littoral zone (Lüning 1990). *Chondrus* has always been regarded as an abundant species within the lower eulittoral and upper sublittoral of the island of Helgoland (North Sea) (Kornmann & Sahling 1977), while *Mastocarpus* was not recorded there before 1983, when material from Iceland was accidentally introduced during a scientific campaign (Kornmann &

*Email: mroleda@awi-bremerhaven.de

Sahling 1994). Now it has successfully established and colonized all natural and man-made hard substrates, forming extensive stands that have changed the appearance of the intertidal biotopes of the island (Bartsch & Kuhlenkamp 2000).

Since the introduction of Icelandic *Mastocarpus* to Helgoland, only presumptive female gametophytes have been observed in the field and fertile plants are found all year round. *Mastocarpus* is heteromorphic and has 2 basic types of life history (Chen et al. 1974, Guiry et al. 1984). However, studies on different isolates of *Mastocarpus* showed that only apomictic or the direct-type life history is found in plants from Denmark and Iceland (Guiry & West 1983), from where the Helgoland population originated. *Chondrus* is isomorphic (Chen & McLachlan 1972) but a demographic study showed that most populations are overwhelmingly gametophytic (approximately 80%; Bhattacharya 1985, Chopin 1986, Dudgeon & Johnson 1992), although tetrasporophyte dominated populations have been also infrequently observed (Lazo et al. 1989).

Intertidal algae experience drastic environmental fluctuations on a daily basis. During low tide they can be exposed to desiccation, hyper- or hypo-osmotic shock, high or freezing temperatures and full solar radiation, depending on the season and latitude. Furthermore, stratospheric ozone depletion increases the ultraviolet-B radiation (UV-BR) component of the solar spectrum on the earth's surface (Smith et al. 1992). Recent studies have implicated the role of solar and especially ultraviolet radiation (UVR) on macroalgal zonation patterns from polar to temperate regions (Dring et al. 1996a, Hanelt et al. 1997, Bischof et al. 1998). Aside from photoinhibition of Photosystem II (PSII), UV-B damages DNA by forming cyclobutane-pyrimidine dimers (CPDs). These photoproducts inhibit transcription and replication of DNA and consequently disrupt cell metabolism and division (Buma et al. 1995, 2000), which could directly inhibit growth and survival.

Comparative ecophysiological studies on *Mastocarpus* and *Chondrus* showed that *Mastocarpus* is more tolerant of environmental stress such as temperature, desiccation and freezing (Dudgeon et al. 1989, 1990, 1995). Moreover, *Mastocarpus* was also found to be more efficient in scavenging reactive oxygen and more resistant to oxidative stress (Collén & Davison 1999), which may explain the extensive distribution of *Mastocarpus* from the upper eulittoral to the upper sublittoral zone. Recently, Bischof et al. (2000) also reported the sensitivity of *Chondrus* photosynthesis to UVR while the relative tolerance of *Mastocarpus* photosynthesis to UVR was determined to be due to its 6-fold higher total mycosporine-like amino acid (MAA) content. All previous comparative studies on the physiological response to environmental variations between these species were conducted on mature fronds (and crusts in the case of *Mastocarpus*).

Early developmental stages of macroalgae, especially 'naked' algal spores, are more susceptible to UVR than are the large developmental stages (as shown for zoospores of various brown algae by Wiencke et al. 2000). Physiological studies on the early life stages of macroalgae in response to environmental stress are lacking. Effects of UVR on photosynthesis have been studied on gametophytes and zoospores of laminariales (Dring et al. 1996b, Wiencke et al. 2000) and unicells of ulvales (Cordi et al. 2001). Other studies on the impact of UVR on the early life stages of green, red and brown macroalgae have focused either on (1) the sporulation of fertile thalli, (2) germination and photomovement of spores, (3) growth of germlings or (4) DNA synthesis in gametophytes (Huovinen et al. 2000, Swanson & Druehl 2000, Cordi et al. 2001, Makarov & Voskoboinikov 2001, Bañares et al. 2002, Flores-Moya et al. 2002, Altamirano et al. 2003a,b, Han et al. 2003). All these studies indicated high sensitivity of the early life stages of macroalgae to UV-B. In contrast to the relatively abundant information on UV-B-dependent DNA damage in higher plants and phytoplankton, information on this subject with regards to macroalgae is still limited (e.g. Pakker et al. 2000a,b, van de Poll et al. 2001, 2002a,b, Bischof et al. 2002a). Only 1 study has investigated DNA damage on macroalgal spores (Wiencke et al. 2000).

Our study focuses on UVR effects on carpospores and young gametophytes of *Mastocarpus* and *Chondrus*, in order to test if their sensitivity plays a role in the establishment of the zonation pattern on the Helgoland shoreline. This study is the first to investigate UV-B-induced DNA damage and photoinhibition on germination and growth of these early life stages. The survival of these transitional life history stages is the most critical phase leading to the successful formation of a benthic population. In the field, *Mastocarpus* is able to colonize open spaces in the upper eulittoral, while the distribution of *Chondrus* is limited to sites under canopies of *Fucus* spp. in the eulittoral, in tidepools and in the upper sublittoral. We hypothesize that differences in UVR sensitivity between *Mastocarpus* and *Chondrus* carpospores and young gametophytes affect the establishment of early post-settlement stages and growth of young juveniles in the upper eulittoral.

MATERIALS AND METHODS

Algal material. Unialgal cultures of *Mastocarpus* and *Chondrus* fronds are maintained in the Biologische Anstalt Helgoland (BAH). Carpospores from *Mastocarpus* cultures gave rise to discoid germlings with

upright fronds following the direct-type life history. The gametophytic fronds produced from the basal discs were scraped from the culture dish and maintained in aerated culture vessels. Young vegetative fronds of *Chondrus* were found to be gametophytic after acetal-resorcinol testing (Garbary & DeWreede 1988). The young fronds in culture were also observed to become fertile and to release spores. Histological cross sections of the sporulating fronds showed single-spored carposporangia. For both species, Provasoli enriched culture medium (Provasoli 1968) was used.

To obtain carpospores from both species, the culture medium of the stock gametophytes was changed and after 3 d the spore-containing medium was collected. The spores were allowed to settle for 4 h. The supernatant water was slowly sucked out by vacuum pump to obtain a concentrated stock spore suspension. The latter was then slowly and continuously agitated using a magnetic stirrer to stop the spores from settling and to derive a homogenous spore suspension. The density of the suspension was adjusted to approximately 4×10^3 spores ml^{-1} after counting the number of spores in 20 µl of suspension on a cavity slide using an inverse microscope.

Experimental light conditions. Light was provided by 3 white fluorescent lamps (Osram, L65 Watt/25S, Germany), emitting background photosynthetically active radiation (PAR) of 400 to 700 nm and 3 UV lamps (Q-Panel UVA-340, 40 Watt, Cleveland), emitting a spectrum similar to solar radiation in the range of 295 to 340 nm. Three kinds of filter foils and glass filters were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes. Experimental units were covered with the following filters: Ultraphan 280 (Digefra GmbH, Germany), Quartz glass; Folanorm 320 (Folex GmbH, Germany), Schott-WG 320 or Schott-GG 400 (Schott, Germany) corresponding to the PAR+UV-A+UV-B (PAB), PAR+UV-A (PA) and PAR (P) treatments respectively. Furthermore, 4 irradiation levels were achieved by varying the distance between the experimental units and the light source (where level 1 = highest radiation and level 4 = lowest radiation; Table 1). Irradiation was measured using a cosine sensor connected to a UV-VIS spectrometer (Marcel Kruse, Bremerhaven, Germany) below the cut-off filters. The biologically effective doses (BED) applied in each treatment were calculated using 2 action spectra for well-known biological responses: DNA damage for *Escherichia coli* (280 to 320 nm, Setlow 1974) and the generalized plant damage (280 to 312 nm) described by Caldwell (1971). Both unweighted and weighted irradiances for each treatment are compiled in Table 1.

Spore photosynthesis. From the working suspension, 5 ml were put into each 35 × 10 mm cell culture dish (5 replicates per treatment). To evaluate the effect of different radiation and exposure time treatments, carpospores of both species were exposed to the 3 radi-

Table 1. Experimental treatments applied with different irradiance levels (1 = highest, 4 = lowest) and the corresponding weighted irradiances using the biologically effective weighing function for the DNA damage of *Escherichia coli* (Setlow 1974) and general plant damage (Caldwell 1971)

Treatment		Experimental irradiance ($W m^{-2}$) PAR (400–700 nm)	UVA (320–400 nm)	UVB (280–320 nm)	Weighted irradiance ($W m^{-2}$) DNA damage (Setlow 1974)	Generalized plant damage (Caldwell 1971)
Carpospore experiment						
PAB	1	28	15.87	1.00	0.013	0.050
Ultraphan 280	2	14	10.20	0.55	0.005	0.017
(PAR+UVA+UVB)	3	14	7.48	0.35	0.002	0.007
	4	13	6.8	0.30	0.002	0.006
PA	1	27	13.77	0.04	1.41×10^{-4}	0
Folanorm 320	2	14	8.80	0.01	5.89×10^{-5}	0
(PAR+UVA)	3	13	6.10	0.01	2.41×10^{-5}	0
	4	12	6.00	0.01	1.47×10^{-5}	0
P	1	26	0.07	0	0	0
Schott-GG 400	2	14	0.04	0	0	0
(PAR alone)	3	13	0.03	0	0	0
	4	12	0.03	0	0	0
Gametophyte experiment						
Quartz (PAR+UVA+UVB)		12	6.24	0.50	0.021	0.050
WG 320 (PAR+UVA)		11	6.08	0.16	0.001	0.002
GG 400 (PAR alone)		8	0.01	0	0	0

ation conditions for 1, 4 and 8 h at 10 ± 1°C. As control, initial photosynthesis of another 5 replicates was measured without treatment. Photosynthetic efficiency, measured as variable fluorescence of PSII, was determined using a Xenon Pulse Amplitude Modulation fluorometer (XE-PAM, Heinz Walz, Germany), coupled to a PC with WinControl software (Heinz Walz GmbH, Germany). After treatments, spore suspension was poured into 5 ml quartz cuvettes and the optimum quantum yield (F_v/F_m) was measured as described by Hanelt (1998). After measurements, the spore suspension was exposed for 2 d under low white light (10 µmol photons m^{-2} s^{-1}) to recover. The control was also maintained under the same conditions.

Determination of spore mortality. For this experiment, 20 ml of working spore suspension and 20 ml of Provasoli enriched seawater were filled into 85 × 15 mm culture dishes with cover slips. The dishes were then exposed to the same combination of treatments used for photosynthesis and also to an additional treatment of 16 h exposure at 4 varying levels of radiation, (3 replicates per treatment). After the treatment, spores were allowed to germinate in low white light (10 µmol photons m^{-2} s^{-1}) for 6 d. Carpospores settled on the cover slip were scored as dead or alive by counting 300 cells per replicate using a light microscope (Olympus CH-2, Japan) equipped with 20× seawater immersion objective. Dead cells were easily distinguishable (no pigment and hollow ghost-like cells) from the live cells (pigmented and dividing cells, sometimes with germinating tubes; Fig. 1). Because dead cells were also observed in the P treatment, the percentage of dead cells for PA and PAB treatments due to the effect of PAR was calculated by using the average number of dead cells as a control value as described by Wiencke et al. (2000):

$$\text{Dead spores (\%)} = 100 \times (S_{dead} - D_C) \times L_C^{-1}$$

where S_{dead} is the number of dead spores under UV exposure, and D_C and L_C are the number of dead and living spores under white light respectively. Mortality data and BED were used to create a dose-response relationship and to calculate the BED_{50}, which is the UV dose needed to achieve 50% inhibition in germination.

Spore DNA damage and repair. In parallel with the mortality experiment, DNA damage and subsequent repair of this damage was determined after 16 h exposure to the 4 irradiation levels. From the working spore suspension, 40 ml was used for each experimental unit. For each treatment, 6 experimental units were prepared. After the irradiation treatment, 3 experimental units (as replicates) were processed immediately while the other 3 were allowed to recover for 6 d in low white light before processing. The spore samples were filtered through 44 mm 1.0 µm pore size Nuclepore® polycarbonate membrane (Whatman, UK) and frozen at −80°C in 2 ml eppendorf tubes for further DNA extraction and analysis of CPDs.

Young gametophyte photosynthesis and growth. In a temperature controlled room, a large flow-through basin (600 × 400 × 120 mm) was placed on a shelf along with lamps as previously described. Inside the basin were cut-out PVC pipes (120 mm diameter) standing upright (70 mm height), which held the algae. The PVC pipes had a 5 mm diameter hole at the bottom for water inflow through silicon tubes and water flowed out into the basin through 4 equidistant, screen covered 10 mm diameter holes in the middle of the PVC pipes. From a reservoir, 80 l of filtered and pasteurized seawater was circulated into the basin through the PVC pipes using a submersible water pump (Eheim; Typ 1060, 38 l min^{-1}, Deizisau, Germany) which also provided water movement inside the PVC pipes. Water level in the basin was maintained at 60 mm, to simulate low tide water. During the experiment, water temperature was maintained at 10 ± 1°C. Water was changed weekly to prevent depletion of nutrients.

To determine the effects of different light treatments, glass filters corresponding to PAB, PA and P treatments

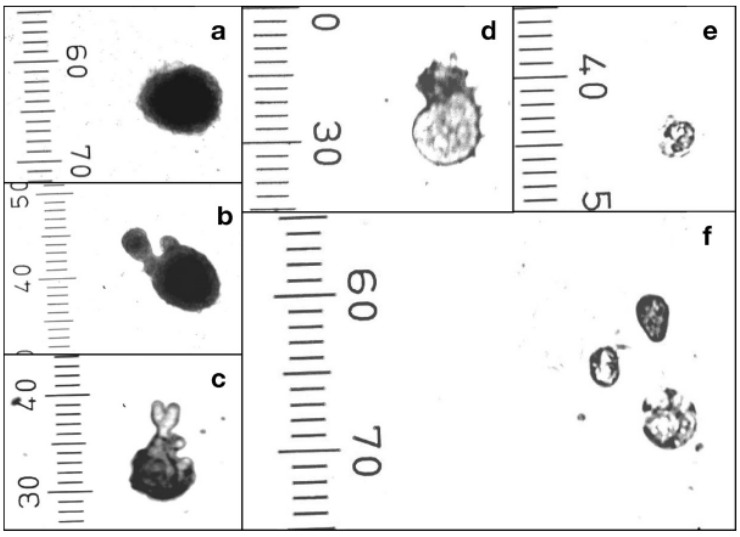

Fig. 1. Carpospores of *Mastocarpus stellatus* (a & b = germinating, d & e = dead) and *Chondrus crispus* (c = germinating, f = dead and alive). Scales are in µm

were laid on top of the standing PVC pipes. Young *Chondrus* and *Mastocarpus* thalli (average size = 20 to 27 mm^2, n = 5), previously acclimated to 46 µmol photons m^{-2} s^{-1} (≈10 W m^{-2}) white light at 10°C for 3 d, were grown for 15 d under 16:8 h light:dark (LD) cycles (05:00 to 21:00 h) with 6 h UV exposure in the middle of the light phase (09:00 to 15:00 h). Photosynthetic activity was determined by measuring the variable chlorophyll fluorescence of PSII with a Diving PAM device (Heinz Walz GmbH, Germany). Effective quantum yield ($\Delta F/F_m'$) was measured every 3 d: 1 h before UV exposure (08:00 h), after 6 h of cumulative UV exposure (15:00 h), and 2 h after UV exposure (17:00 h). Growth, in terms of weight (g) and surface area increase (mm^2), was also measured every 3 d using a weighing scale (Sartorius CP225D, Germany) and a scanner with image analysis software (Winfolia™ 5.0, Regent Instrument Inc., Canada) respectively. Growth rates were computed by plotting all data points (entire experiment period) of each replicate per treatment. They were individually fitted to an exponential equation, $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase. After the experiment, the fronds were immediately frozen in liquid nitrogen and stored at –80°C for further pigment analysis.

Outdoor exposure experiments on mature gametophytes. Vegetative gametophytic fronds of *Mastocarpus* and *Chondrus* (average size = 76 ± 22 mm^2) were collected in the Northwestern intertidal flat of Helgoland. They were cleaned of epiphytes and the meristematic tips (size = 51 to 99 mm^2) were used for the experiment. They were spread out under UV transparent (Quartz) and non-transparent (GG 400) filter glasses, incubated directly below the water surface inside a basin and exposed to the full solar radiation for 4 h (10:00 to 14:00 h) on the roof of the institute (BAH). During the experiment (18 March 2003), 11 h sunshine and 4 to 9°C air temperature was recorded in Helgoland. This is equivalent to a maximum UV radiation of 29.4 W m^{-2} UVA (= 1.2×10^6 J m^{-2}) and 1.6 W m^{-2} UVB (= 4.95×10^4 J m^{-2}) as measured by a multichannel UV-spectroradiometer (Isitec GmbH, Bremerhaven) installed on the roof of the institute. After exposure, 8 replicates per treatment were harvested and the rest of the fronds were allowed to recover overnight with screen cover under low light condition. Seawater was continuously replaced during the course of the experiment. Samples were immediately frozen in liquid nitrogen and stored at –80°C after treatment and recovery for further experiments. Three replicates were used for pigment analysis and 5 replicates for DNA extraction and CPDs analysis.

Pigment extraction and characterisation. Frozen samples were treated with 100 µl of 100% N-N-dimethylformamide and stored in darkness for approximately 16 h. Subsequent analyses for chlorophyll *a* and α- and β-carotene using HPLC were performed as described by Bischof et al. (2002b).

DNA extraction. DNA was isolated following the CTAB extraction procedure described by van de Poll et al. (2001). Spore samples were immediately treated with extraction buffer while vegetative fronds were first homogenized in liquid nitrogen. After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 µl 10 mg ml^{-1}, 30 min, 37°C; Sigma, MO, USA) and stored at –20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence spectrophotometer (Variance Scientific Instrument, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection. The immunoassay for CPDs was modified after Vink et al. (1994) and van de Poll et al. (2001). Heat denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 µm, Schleicher & Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a 2 step anti-body assay, the membrane was treated with ECL Western blotting detection reagent (Amersham, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) for different exposure times. The films were developed using X-ray film developer. Developed films were scanned using Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray-scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 µg ml^{-1} DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis. All data were tested for homogeneity of variances (Levene's test) and normality (Kolmogorov-Smirnov test). Corresponding transformations were done to heteroskedastic (unequal variances) and non-normal data. Carpospore data were subjected to multiple analysis of variance (MANOVA) to determine the main effects and interactions of species, irradiance exposure time and radiation levels on the photosynthesis, spore viability, DNA damage and repair in *Mastocarpus* and *Chondrus*. Biologically effective UV-B doses ($BED_{DNA300\ nm}$ and $BED_{plant\ damage313\ nm}$) resulting in a 50% inhibition in germination were determined

from all germination data (expressed as % of P) using linear ($y = a + bx$) and non linear ($y = a + bx + cx^2$) regressions, corresponding to the best fit curve for *Mastocarpus* and *Chondrus* respectively.

Time series measurements on the photosynthetic yield ($\Delta F/F_m'$) of the young gametophytes were subjected to repeated measures analysis of variance (RMANOVA) to determine the effects of light treatments across the sampling days. Differences in growth rates and pigment contents were tested for statistical significance using analysis of variance (ANOVA, $p = 0.05$). All test were followed by Duncan's multiple range test (DMRT, $p = 0.05$), when appropriate. Statistical analyses were done using SPSS® software.

RESULTS

Spore photosynthesis

Initial measurement of the controls showed that *Mastocarpus* spores had a higher mean (±SD) optimum quantum yield ($F_v/F_m = 0.533 \pm 0.01$) compared with *Chondrus* spores (0.386 ± 0.04). However, after 2 d in low white light (10 µmol photons m^{-2} s^{-1}), photosynthesis of the *Mastocarpus* controls had reduced to 0.453 ± 0.02 but did not vary much in *Chondrus controls* (0.394 ± 0.04). Under P treatment of around 56 µmol photons m^{-2} s^{-1} (= 12 ± 1 W m^{-2}), carpospores were photoinhibited after all exposure times (Fig. 2a,b). However, the effect in *Chondrus* was less than that in *Mastocarpus*. Additional UV-A did not further reduce the F_v/F_m of *Mastocarpus* regardless of exposure time, but significantly reduced the F_v/F_m of *Chondrus*. The combined effect of UV-A and UV-B lowered the photosynthesis of both species with increasing time, with a more pronounced effect in *Chondrus*.

After 48 h in low white light, photosynthesis of both species in all treatments was able to recover. However, the effect of long exposure times (4 and 8 h) to relatively high PAR on *Mastocarpus* and *Chondrus* carpospores of was still persistent, but better recovery was observed in *Chondrus* compared to *Mastocarpus* (Fig. 2c,d). Conversely, higher recovery was observed in carpospores of *Mastocarpus* exposed to UVR compared with those of *Chondrus*. Multiple analysis of variance (MANOVA) showed significant effect of the main factors (species, irradiance and exposure time) on carpospore photosynthesis after treatment and recovery ($p < 0.001$, Table 2). Moreover, 2-way interactions between species and irradiance as well as between species and exposure were also found to be significant in photosynthesis after treatment but only the interaction between species and irradiance was found to be significant ($p = 0.005$) after recovery. No 3-way interaction was found to be significant for carpospore photosynthesis. Duncan's multiple range test (DMRT, $p = 0.05$) also showed no homogenous subsets among the variables, such that the effect of irradiance (P, PA and PAB) and exposure time (1, 4 and 8 h) were significantly different from each other.

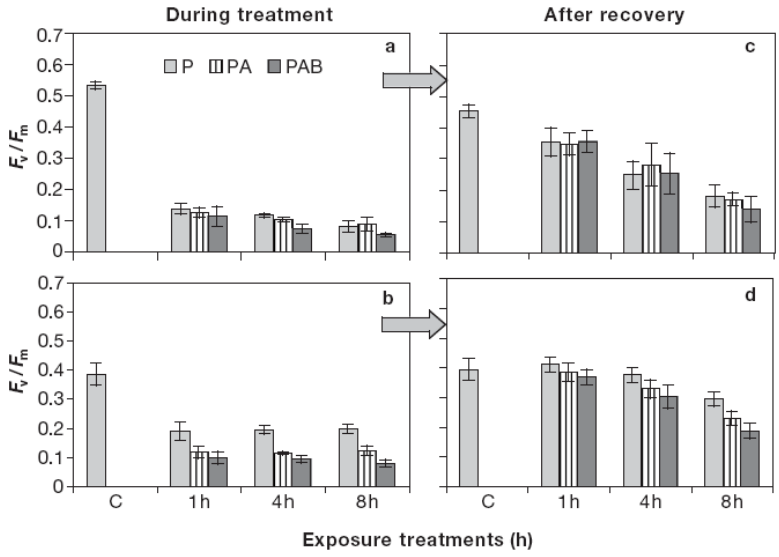

Fig. 2. (a,c) *Mastocarpus stellatus* and (b,d) *Chondrus crispus*. (a,b) Mean optimum quantum yield (F_v/F_m) of carpospores during treatment of photosynthetically active radiation, PAR = P; P+UVA = PA; PA+UVB = PAB and exposure time. PFD is 56 µmol photon m^{-2} s^{-1}. (c,d) Corresponding photosynthetic recovery after 48 h post culture in low white light (10 µmol photon m^{-2} s^{-1}). Control (= C) is without treatment and continuously maintained at 10 µmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD), n = 5

Spore mortality

Spore viability and germination capacity were also found to be low light adapted. Exposure of 1 to 8 h of high P (50 ± 5 µmol photons m^{-2} s^{-1}) was sufficient to induce significantly higher mortality in *Chondrus* (Fig. 3b) compared with *Mastocarpus* spores (Fig. 3a). After 16 h exposure to varying radiation level of P (50 to 130 µmol photons m^{-2} s^{-1}), mortality due to high PFD (photon flux density) of PAR doubled in *Mastocarpus* (Fig. 3c) and slowly increased in *Chondrus* (Fig. 3d). The spore mortality under UVR (PA, PAB) was higher in

Table 2. *Mastocarpus stellatus* and *Chondrus crispus*. Multiple analysis of variance (MANOVA) and significance values for main effects and interactions of species, irradiance, exposure time and radiation levels on the photosynthesis, spore viability, DNA damage and repair of carpospores of both algae species. *Significant; ns: not significant

Variable	Source of variation	df	*F*	p
Spore photosynthesis and viability experiment				
F_v/F_m (after treatment)	Species (A)	1	99.47	<0.001*
	Irradiance (B)	2	116.25	<0.001*
	Exposure time (C)	2	17.43	<0.001*
	A × B	2	42.53	<0.001*
	A × C	2	15.62	<0.001*
	B × C	4	1.47	0.219^{ns}
	A × B × C	4	0.71	0.585^{ns}
F_v/F_m (after recovery)	Species (A)	1	62.08	<0.001*
	Irradiance (B)	2	9.83	<0.001*
	Exposure time (C)	2	154.49	<0.001*
	A × B	2	5.81	0.005*
	A × C	2	2.61	0.081^{ns}
	B × C	4	1.57	0.191^{ns}
	A × B × C	4	0.41	0.804^{ns}
Spore mortality	Species (A)	1	692.56	<0.001*
	Irradiance (B)	2	38.67	<0.001*
	Exposure time (C)	2	45.50	<0.001*
	A × B	2	1.34	0.276^{ns}
	A × C	2	3.87	0.030*
	B × C	4	2.73	0.044*
	A × B × C	4	2.20	0.088^{ns}
Spore viability, DNA damage and repair experiment				
Spore mortality	Species (A)	1	53.39	<0.001*
	Irradiance (B)	2	101.06	<0.001*
	Radiation level (C)	3	98.75	<0.001*
	A × B	2	8.48	0.001*
	A × C	3	4.21	0.010*
	B × C	6	3.58	0.009*
	A × B × C	6	0.91	0.499^{ns}
DNA damage	Species (A)	1	10.72	0.005*
	UVB level (B)	3	45.83	<0.001*
	A × B	3	1.63	0.221^{ns}
DNA repair	Species (A)	1	51.11	<0.001*
	UVB level (B)	3	34.98	<0.001*
	A × B	3	4.81	0.014*

Chondrus compared with *Mastocarpus* across all treatments. In the first spore mortality experiment, the main factors and 2-way interactions (species × exposure time and irradiance × exposure time) were found to significantly affect spore mortality (Table 2). DMRT showed that the effect of irradiance (P vs PA vs PAB) differed significantly among treatments, while exposure times of 1 and 4 h were homogenous but significantly different from 8 h. In the second spore mortality experiment, the main effects and all 2-way interactions between species, irradiance and radiation level significantly affected carpospore mortality; no 3-way interaction was observed. Moreover, DMRT ($p = 0.05$) showed that P ≠ PA ≠ PAB and radiation level (light quantity) was also significantly different from each other.

Dose-response relationship (BED_{50}) for both *Mastocarpus* ($R^2 = 0.802$ [linear regression]; 0.776 [non linear regression]) and *Chondrus* ($R^2 = 0.780$ [linear regression]; 0.930 [non linear regression]) were highly significant ($p < 0.05$), indicating clear relationship between UV dose and inhibition of germination. A higher dose is needed to achieve a 50% inhibition of germination ($BED_{DNA\ 300\ nm}$ and $BED_{plant\ damage\ 313\ nm}$) in *Mastocarpus* (Fig. 4).

DNA damage and repair

After 16 h exposure to increasing radiation, higher DNA damage was observed in the carpospores of *Chondrus* compared with *Mastocarpus* (Fig. 5a). CPD induction in both species increased with radiation intensity. Analysis of variance (ANOVA, $p < 0.05$) showed significant differences between species and UV-B level on DNA damage (Table 3). DMRT showed that accumulation of DNA damage in UV-B level 1 = level 2 ≠ level 3 ≠ level 4. After 6 d post-culture in low white light, the amount of remaining CPDs was also significantly higher in *Chondrus* ($p < 0.001$) and in samples exposed to higher UV-BR ($p < 0.001$; Fig. 5b, Table 2). DMRT showed that DNA repair mechanism in UV-B level 1 ≠ level 2 ≠ level 3 = level 4.

The rooftop experiment simulating a low tide field condition showed no accumulation of DNA damage in exposed plants compared to non-exposed plants (data not shown).

Gametophyte photosynthesis, pigment contents and growth

Initial values of the mean effective yields ($\Delta F/F_m'$) of *Mastocarpus* and *Chondrus* gametophytes showed similar photosynthetic efficiencies with that of the carpospores, where higher $\Delta F/F_m'$ were observed in *Mastocarpus* (0.617 ± 0.03) compared with *Chondrus* (0.497 ± 0.07) (Fig. 6). Adverse reductions in the mean $\Delta F/F_m'$ of the cultured young *Mastocarpus* and *Chondrus* gametophytes were observed only during the first day of UV exposure. After 6 h of PA exposure, a comparable reduction of ~22 and 23% in the $\Delta F/F_m'$ was observed in *Mastocarpus* and *Chondrus* respectively. In the PAB treatment, a higher reduction in the $\Delta F/F_m'$ was observed in *Chondrus* (43%) than in *Mastocarpus* (30%). Two hours after the end of the first UV treatment, photosynthesis was able to recover to values comparable to pre-UV treatment. Time series measurements showed that both algae were able to acclimate to

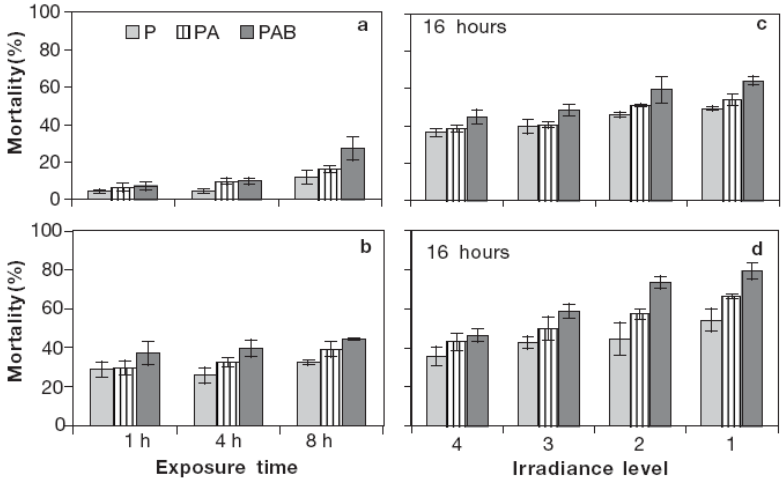

Fig. 3. (a,c) *Mastocarpus stellatus* and (b,d) *Chondrus crispus*. Spore mortality (a,b) under varying exposure time of P, PA and PAB and (c,d) under 16 h of varying irradiation levels of P, PA and PAB. Radiation level 1 = highest and 4 = lowest. PDF ranges from 50 to 130 µmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD), n = 3

UVR (Fig. 6). Statistical analysis showed that changes in the $\Delta F/F_m'$ were not significantly different between species (RMANOVA, $p > 0.05$; Table 3) across all sampling times. However, a significant effect of irradiance was observed after 6 h UVR treatment ($p < 0.001$) but complete recovery was observed after 2 h recovery in white light where $\Delta F/F_m'$ were not significantly different between treatments (Table 3).

In both the laboratory and rooftop experiments, higher chlorophyll *a* levels were measured in plants exposed to PA and PAB (Fig. 7a,b). However, the effect of irradiance was not significantly different between treatments (Table 3). Carotenoids were only determined in *Chondrus*, where different reactions to UVR were observed between young gametophytes and mature wild plants. UVR significantly reduced α-carotene

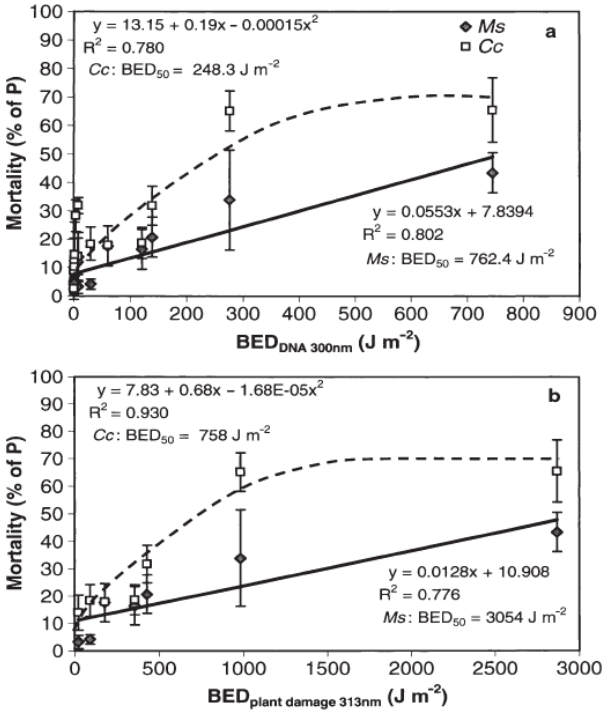

Fig. 4. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). Empirical relationship between biologically effective UVB doses and germination using action spectra for DNA damage $BED_{DNA\,300\,nm}$ (a) and generalized plant damage $BED_{plant\,damage\,313\,nm}$ (b) with corresponding BED_{50} derived from the regression equations. Vertical bars are standard deviations (SD), n = 3

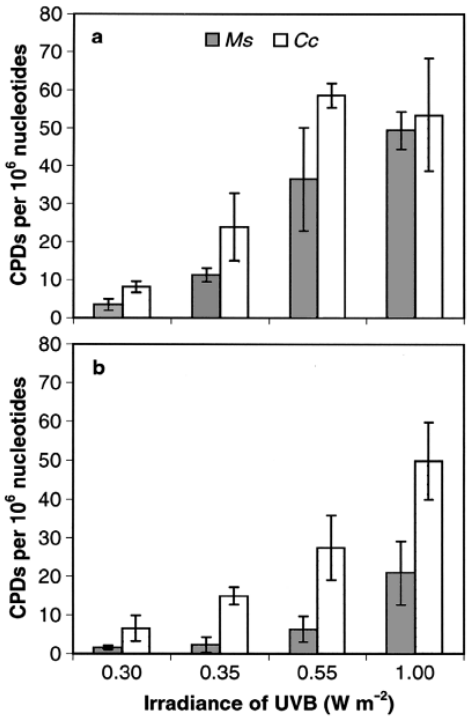

Fig. 5. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). UVB induced DNA damage (induced CPD concentrations per million nucleotides) after (a) exposure to varying levels of UVB-radiation and (b) corresponding CPD repair after 6 d recovery in low white light (10 µmol photon m^{-2} s^{-1}). Vertical bars are standard deviations (SD), n = 3

Table 3. *Mastocarpus stellatus* and *Chondrus crispus*. Repeated measures analysis of variance (RMANOVA) on the photosynthetic yields ($\Delta F/F_m'$) and analysis of variance (ANOVA) on pigments and growth of young gametophytes of both algae species. *Significant; ns: not significant

Dependent variable	Independent variable: Sampling time	Source of variation	df	*F*	p
Photosynthesis					
$\Delta F/F_m'$	08:00 (before UV)	Species (A)	1	1.72	0.201^{ns}
		Irradiance (B)	2	2.32	0.120^{ns}
		A × B	2	0.65	0.532^{ns}
	15:00 (after 6 h UV exposure)	Species (A)	1	0.83	0.372^{ns}
		Irradiance (B)	2	12.07	0.000*
		A × B	2	0.44	0.649^{ns}
	17:00 (after 2 h recovery in white light)	Species (A)	1	0.05	0.823^{ns}
		Irradiance (B)	2	2.57	0.098^{ns}
		A × B	2	0.31	0.736^{ns}
Pigments					
Chlorophyll *a*		Species (A)	1	8.03	0.015*
		Irradiance (B)	2	2.38	0.135^{ns}
		A × B	2	0.24	0.791^{ns}
C. crispus					
α-carotene		Irradiance	2	10.50	0.011*
β-carotene		Irradiance	2	7.12	0.026*
Growth					
Area		Species (A)	1	110.82	<0.001*
		Irradiance (B)	2	0.32	0.730^{ns}
		A × B	2	2.13	0.141^{ns}
Weight		Species (A)	1	95.91	<0.001*
		Irradiance (B)	2	0.28	0.758^{ns}
		A × B	2	1.95	0.164^{ns}

but enhanced β-carotene content in young gametophytes (Fig. 7c,d, Table 3). In mature thalli, α-carotene was not affected while β-carotene reacted similarly to UVR, but the effect of irradiance was insignificant. Overnight recovery of plants exposed to the full solar spectrum showed no significant change in all of the pigments examined.

Increases in surface area and wet weight as growth parameters were positively correlated in *Mastocarpus* ($r = 0.937$, $p < 0.001$) and *Chondrus* ($r = 0.980$, $p < 0.001$) (Fig. 8). Regardless of treatment, the rate of increase in surface area was faster in *Chondrus* (slope, $r = 0.077$) than in *Mastocarpus* ($r = 0.044$) (Fig. 9). Consequently, growth rate under all treatment was higher in *Chondrus* (7.0 to 8.4% d^{-1}) than in *Mastocarpus* (4.4 to 5.2% d^{-1}) (Fig. 9, inset a). Growth rates of UVR treated plants expressed as a percentage of PAR (Fig. 9, inset b) showed that UVR had a relatively low impact on the growth rate of both species. Under PA, growth rate was similar between *Mastocarpus* and *Chondrus*, but higher growth rate was observed in *Mastocarpus* under PAB treatment. However, statistical analysis showed insignificant effect of irradiance on the growth rate of both species (Table 3).

DISCUSSION

Carpospores of *Mastocarpus* and *Chondrus* are more susceptible to the detrimental effects of UVR than were the conspecific young gametophytes. Optimum quantum yields (F_v/F_m) of carpospores were able to recover after pulse UV exposure while effective quantum yields ($\Delta F/F_m'$) of young gametophytes were able to acclimate to chronic UV exposure. The carpospores used in our experiments were low light adapted since sporulation occurred in a low light environment and were maintained under this condition to prevent germination. In a pilot experiment, we observed a maximum germination of 95 ± 5% under 10 µmol photons m^{-2} s^{-1} of PAR (data not shown). For *Chondrus* spores, a wide range of optimum photon flux densities (PFD) of PAR (20 to 80 µmol photons m^{-2} s^{-1} = 4.3 to 17.2 W m^{-2}) has been reported (Tasende & Fraga 1992 and references therein). However, we found that exposure of 1 to 16 h of 12 W m^{-2} PAR (50 ± 5 µmol photons m^{-2} s^{-1}) induced ~30% mortality in *Chondrus* and photosynthesis was also reduced under these conditions in both species. Therefore, it appears that low PFD of PAR is optimal for spore germination. Our data also indicate a large impact of the PAR waveband on carpospore survival, because in the field, the UVB radiation applied in our experiments would be accompanied by a 10- to 20-fold higher PAR. Whether carpospores are capable of acclimating to higher PAR is still unknown. Physiological acclimation to high PAR can increase UVB tolerance. In higher plants, this was facilitated through photoinduction of screening compounds, increased activity of photorepair enzymes and other changes related to life under high irradiance (Warner & Caldwell 1983).

Carpospores of *Mastocarpus* were found to be more tolerant of the deleterious effect of UVR compared with *Chondrus* carpospores. Apart from the BED_{50}, differences in tolerance were also reflected in UVB induced photoinhibition and DNA damage. F_v/F_m of *Mastocarpus* spores were relatively less affected under UVR, and UV-B induced DNA damage was also lower compared with *Chondrus*. Moreover, DNA damage was also repaired faster by *Mastocarpus*. However, these differences were not observed in the growth, $\Delta F/F_m'$ and DNA damage of the gametophytic life stages of these algae. Therefore, differences in the sensitivity between *Mastocarpus* and *Chondrus* carpospores to high light and UVB radiation elucidate the

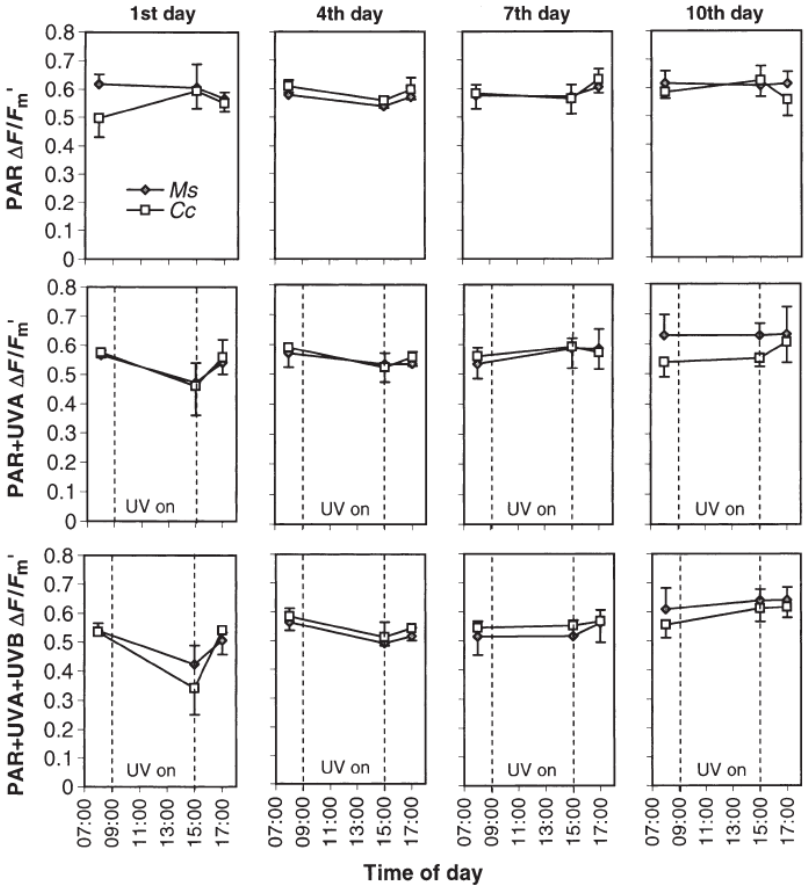

Fig. 6. *Mastocarpus stellatus* (*Ms*) and *Chondrus crispus* (*Cc*). Time series of the circadian pattern of the mean effective quantum yield ($\Delta F/F_m'$) of young gametophytes during the light phase of the 16:8 h light:dark photoperiod. Vertical bars are standard deviations (SD), n = 5

differences in their zonation. A lower sensitivity to high light and UVR would allow *Mastocarpus* to recruit at higher shore levels. Conversely, the higher sensitivity of *Chondrus* would limit recruitment at lower shore levels or under the canopy of *Fucus* spp. and *Mastocarpus*. Survival of the carpospores and recruitment of young juveniles is critical in structuring the community because once established, growth rates of young gametophytes are higher in *Chondrus* compared with *Mastocarpus*, regardless of irradiance condition. Our data concur with a previous *in situ* growth study, where 2-fold productivity was also reported in *Chondrus* (2.65 g dry wt m^{-2} d^{-1}) compared to *Mastocarpus* (1.04 g dry wt m^{-2} d^{-1}) (Dudgeon et al. 1995).

Bischof et al. (2000) reported a 6-fold higher concentration of MAAs in *Mastocarpus* when compared with *Chondrus*, and related this to the higher UV photosynthetic tolerance of *Mastocarpus*. Although MAAs were not determined in this study, we observed that photosynthesis and growth of gametophytes of both species were equally resistant to a daily repeated UV treatment. Whether carpospores of these species produce sufficient MAAs to achieve the same degree of protection to that of the gametophytes remains to be studied. The presence of UV screening compounds such as MAAs reduces the effective UVR that penetrates to UV sensitive targets in the cell. Therefore, the absence of passive protection increases the sensitivity of carpospores to UVB induced photoinhibition and DNA damage.

The higher photosynthetic rate of *Mastocarpus* compared with *Chondrus* (F_v/F_m of carpospores and $\Delta F/F_m'$ of young gametophytes) under control conditions, is attributed to *Mastocarpus'* efficient use of the available photon flux density. The initial values of the mean quantum efficiency of cultured young *Mastocarpus* and *Chondrus* gametophyte isolated from Helgoland are comparable to those reported by Bischof et al. (2000) on mature wild materials collected from the same locality. However, the complete acclimation of photosynthesis in both species after 3 d of repeated UVR exposure contradicts Bischof et al. (2000), as they reported a reduction in the mean $\Delta F/F_m'$ (with a lesser degree of photoinhibition in *Mastocarpus*) throughout the 5 d repeated exposure to PAB. This could be attributed to the higher experimental UV irradiance used in their study, which was twice the intensity we applied, or to the low PAR (25 µmol photons m^{-2} s^{-1} ≈ 5.4 W m^{-2}) they applied. Insufficient PAR and therefore unrealistically low PAR:UVB ratios, could exaggerate the UVB effects on plants (Caldwell et al. 1995, Rozema et al. 1997).

Chlorophyll *a* levels in both *Mastocarpus* and *Chondrus* were not affected by UVR but increased when supplemented with UV-A and UV-A+UV-B. Most studies reported significant damage and decrease in pigment contents under UVR (i.e. Aguilera et al. 2002, Bischof et al. 2002a,b), which can occur either (1) when protein-based pigments absorb UV energy directly and undergo photochemical degradation, (2) by photosensi-

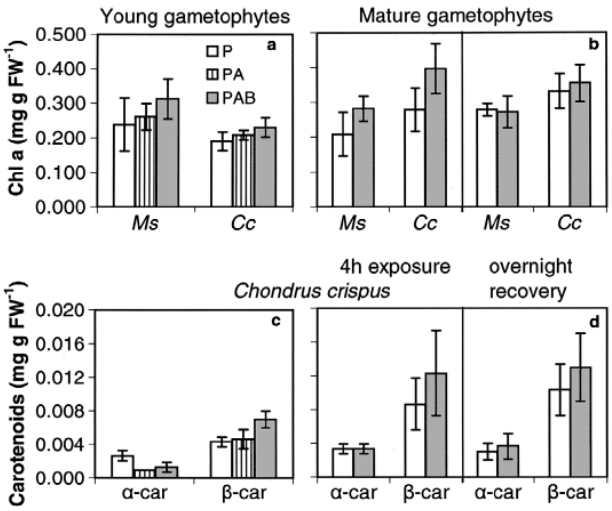

Fig. 7. *Mastocarpus stellatus* and *Chondrus crispus*. Chlorophyll *a* contents in (a) young and (b) mature gametophytes and corresponding carotenoids of *C. crispus* (c,d) exposed to P, PA, PAB. *Ms* = *M. stellatus*, *Cc* = *C. crispus*. Vertical bars are standard deviations (SD), n = 3

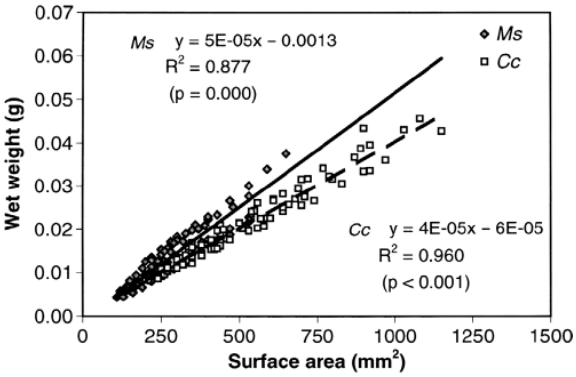

Fig. 8. *Mastocarpus stellatus* and *Chondrus crispus*. Relationship between surface area and wet weight in both algae species with corresponding regression equation. *Ms* = *M. stellatus*, *Cc* = *C. crispus*

tiser action, or (3) by oxygen radical production in addition to singlet oxygen (Vincent & Neale 2000). Although the higher chlorophyll *a* levels under PAB was statistically insignificant, the result of our study is similar to 2 other studies on *Ulva* sp., where significantly higher pigment contents were measured in the presence of UV-B (Grobe & Murphy 1998, Altamirano et al. 2000). The higher total carotenoids measured under PAB could be related to their photoprotection role (Roy 2000). Accumulation of carotenoids specifically in response to UV radiation has been previously reported in cyanobacteria and chlorophytes (Buckley & Houghton 1976, Goes et al. 1994). This mechanism could have protected the photosynthetic apparatus of *Chondrus*, which was able to acclimate its photosynthesis and growth to UVR. In *Mastocarpus*, aside from the reported higher MAA content, the thicker thalli (higher wet weight : surface area ratio) could have provided some protective mechanism against environmental stress contributing to its successful colonization in the upper eulittoral.

Exposure of mature gametophytes to natural solar radiation directly below the water surface for 4 h at noon showed no significant CPD accumulation in either *Mastocarpus* or *Chondrus*. This shows that prevention and repair mechanisms are sufficient to minimize accumulation of DNA damage. This is not surprising because both species co-exist in the eulittoral, and therefore have to be physiologically or genetically acclimated to the full spectrum of solar radiation. *Chondrus* isolates from Brittany, repeatedly exposed under comparable irradiance treatment in the laboratory for 2 wk, were also not significantly affected by UV-BR, and the number of

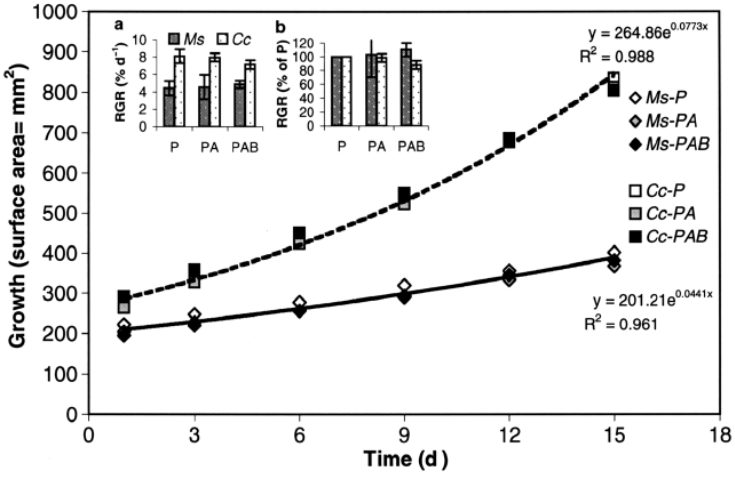

Fig. 9. *Mastocarpus stellatus* and *Chondrus crispus*. Growth, in terms of surface area increase in young gametophytes exposed to different radiation. Inlaid exponential equation is the overall growth performance of each species. Insets show relative growth rate (RGR) in % d^{-1}, per treatment (a) and growth expressed as % of PAR (b). Vertical bars are standard deviations (SD), n = 5. *Ms* = *M. stellatus*, *Cc* = *C. crispus*

CPDs per 10^6 nucleotides was found to be negligible (van de Poll et al. 2001). However, a similar outdoor experiment with several subtidal Arctic macroalgae demonstrated significant CPD accumulation after 4 h exposure to full solar radiation (van de Poll et al. 2002b).

Survival of spores is regarded as a critical life stage because once established, the vegetative plants appear not to suffer from UV-B induced DNA damage. Among kelp zoospores, the effect of UVR on spore viability was found to be correlated to the growth depth of the species (Wiencke et al. 2000) and within species, those released from adults growing in high UV environments showed higher germination and survival rates than the progeny of adults occupying lower UV environments (Swanson & Druehl 2000). In the eulittoral *Enteromorpha intestinalis*, inhibition of germination success (up to 50%) was only observed in swarmers (both spores and gametes) when exposed to elevated levels of UV-B (Cordi et al. 2001). On the other hand, in the littoral red alga *Ceramium rubrum*, 50% spore mortality was already observed after 1 h exposure to light with elevated UVR (2.2 W m^{-2} UV-B and 11 W m^{-2} UVA, Yakovleva et al. 1998). Although these experiments are not directly comparable, they suggest a high degree of variability among UV sensitivity of macroalgal spores.

There are other factors that could affect recruitment and eventual community structure. However, herbivory and wave-induced mortality were reported to have a low impact on both *Mastocarpus* and *Chondrus*. In transplant experiments to determine competitive interactions between the 2 species, coexistence was observed in the space limited intertidal flat mediated by seasonal environmental disturbances (winter storm) and physiological stress (Dudgeon et al. 1999). Aside from UV tolerance and efficient DNA repair mechanism, the apomictic reproduction (direct-type life history) of Helgolandian *Mastocarpus* may be ecologically advantageous to its successful dispersal and colonization of open spaces in the upper eulittoral. This is because the direct development female requires only 1 open space and a single establishment event; whereas a sexual female must first pass through the crustose, tetrasporophytic generation, requiring 2 open spaces and 2 establishment events (Zupan & West 1988). Moreover, spores are produced year-round, not seasonally as in sexual populations (Maggs 1988). In the field, the occurrence of foliose *Mastocarpus* on the walls and boulders around Helgoland which are always exposed during low tides (pers. obs.) could have been influenced by the hydrographic conditions (i.e. tide level and surf) during spore release (Norton 1992). Consequently, attachment and germination under favorable 'recruitment windows' (Deysher & Dean 1986, Reed et al. 1988) could have been facilitated by the presence of surface mucilage observed in both species. This blanket-like extracellular mucilaginous sheath plays a role in the ontogeny of the sporelings by influencing algal spore attachment for colonization (Fletcher & Callow 1992, Vadas et al. 1992).

In conclusion, high PAR and UV exposure could determine recruitment success. However, it remains to be determined whether there would be significant effects from exposure to UV at the magnitude and spectral composition characteristic of *in situ* environments in which PAR is already high enough to cause considerable mortality. Although mature fronds of *Mastocarpus* have been reported to be more tolerant to environmental stress (i.e. temperature, desiccation and freezing) compared with *Chondrus* (Dudgeon et al. 1989, 1990, 1995, Collén & Davison 1999), we would suggest that the foliose life stages of both species are equally resilient to UV-B stress and could persist in the rocky intertidal shore of Helgoland.

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LITERATURE CITED

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087–1095

Altamirano M, Flores-Moya A, Figueroa FL (2000) Growth, seasonality, photosynthetic pigments, and C and N content in relation to environmental factors: a field study on *Ulva olivascens* (Ulvales, Chlorophyta). Phycologia 39: 50–58.

Altamirano M, Flores-Moya A, Figueroa FL (2003a) Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophycea). Aquat Bot 75:9–20

Altamirano M, Flores-Moya A, Kuhlenkamp R, Figueroa FL (2003b) Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. Zygote 11:101–106

Bañares E, Altamirano M, Figueroa FL, Flores-Moya A (2002) Influence of UV radiation on growth of sporelings of three non-geniculate coralline red algae from southern Iberian Peninsula. Phycol Res 50:23–30

Bartsch I, Kuhlenkamp R (2000) The marine macroalgae of Helgoland (North Sea): an annotated list of records between 1845 and 1999. Helgol Mar Res 54:160–189

Bhattacharya D (1985) The demography of fronds of *Chondrus crispus* Stackhouse. J Exp Mar Biol Ecol 91:217–231

Bischof K, Hanelt D, Wiencke C (1998) UV-radiation can affect depth-zonation of Antarctic macroalgae. Mar Biol 131:597–605

Bischof K, Krābs G, Hanelt D, Wiencke C (2000) Photosynthetic characteristics and mycosporine-like amino acids

under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? Helgol Mar Res 54:47–52

Bischof K, Peralta G, Kräbs G, van de Poll WH, Pérez-Lloréns JC, Breeman AM (2002a) Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. J Exp Bot 53:2411–2421

Bischof K, Kräbs G, Wiencke C, Hanelt D (2002b) Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 215:502–509

Buckley CE, Houghton JA (1976) A study of the effects of near UV radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. Arch Microbiol 107:93–97

Buma AGJ, van Hannen EJ, Roza L, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. J Phycol 31:314–321

Buma AGJ, van Oijen T, van de Poll WH, Veldhuis MJW, Gieskes WWC (2000) The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. J Phycol 36: 296–303

Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology. Academic Press, New York, p 131–177

Caldwell MM, Teramura AH, Tevini M, Bornman JF, Björn LO, Kulandaivelu G (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio 24:166–173

Chen LCM, McLachlan J (1972) The life history of *Chondrus crispus* in culture. Can J Bot 50:1055–1060

Chen LCM, Edelstein T, McLachlan J (1974) The life history of *Gigartina stellata* (Stackh.) Batt. (Rhodophyceae, Gigartinales) in culture. Phycologia 13:287–294

Chopin T (1986) The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans: a review. Can Tech Rep Fish Aquat Sci 1514:1–69

Collén J, Davison IR (1999) Stress tolerance and reactive oxygen metabolism in the intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. Plant Cell Environ 22: 1143–1151

Cordi B, Donkin ME, Peloquin J, Price DN, Depledge MH (2001) The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis*. Aquat Toxicol 56:1–11

Deysher LE, Dean TA (1986) In situ recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L.) C.A. Agardh: effects of physical factors. J Exp Mar Biol Ecol 103:41–63

Dring MJ, Wagner A, Boeskov J, Lüning K (1996a) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. Eur J Phycol 31:293–302

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996b) Influence of ultraviolet radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126: 183–191

Dudgeon SR, Johnson AS (1992) Thick vs thin: thallus morphology and tissue mechanics influence differential drag and dislodgment of two co-dominant seaweeds. J Exp Mar Biol Ecol 165:23–43

Dudgeon SR, Davison IR, Vadas RL (1989) Effect of freezing on photosynthesis of intertidal macroalgae: relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus* (Rhodophyta). Mar Biol 101:107–114

Dudgeon SR, Davison IR, Vadas RL (1990) Freezing tolerance in the intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus*: relative importance of acclimation and adaptation. Mar Biol 106:427–436

Dudgeon SR, Kübler JE, Vadas RL, Davison IR (1995) Physiological responses to environmental variation in intertidal red algae: does thallus morphology matter? Mar Ecol Prog Ser 117:193–206

Dudgeon SR, Steneck RS, Davison IR, Vadas RL (1999) Coexistence of similar species in a space-limited intertidal zone. Ecol Monogr 69:331–352

Fletcher RL, Callow ME (1992) The settlement, attachment and establishment of marine algal spores. Br Phycol J 27: 303–329

Flores-Moya A, Posudin YI, Fernández JA, Figueroa FL, Kawai H (2002) Photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose. J Photochem Photobiol B Biol 66:134–140

Garbary DJ, DeWreede RE (1988) Life history phases in natural populations of Gigartinaceae (Rhodophyta): quantification using resorcinol. In: Lobban DS, Chapman DJ, Kremer BP (eds) Experimental Phycology. A laboratory manual. Cambridge University Press, Cambridge, p 174–178

Goes JI, Handa N, Taguchi S, Hama T (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter *Tetraselmis* sp.: relationship to cellular pigments. Mar Ecol Prog Ser 114:259–274.

Grobe CW, Murphy TM (1998) Solar ultraviolet-B radiation effects on growth and pigment composition of the intertidal alga *Ulva expansa* (Setch.) S. & G. (Chlorophyta). J Exp Mar Biol Ecol 225:39–51

Guiry MD, West JA (1983) Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. J Phycol 19:474–494

Guiry MD, West JA, Kim DH, Masuda M (1984) Reinstatement of the genus *Mastocarpus* Kützing (Rhodophyta). Taxon 33:53–63

Han T, Han YS, Kim KY, Kim JH, Shin HW, Kain JM, Callow JA, Callow ME (2003) Influences of light and UV-B on growth and sporulation of the green alga *Ulva pertusa* Kjellman. J Exp Mar Biol Ecol 290:115–131

Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361–369

Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255–266

Huovinen PS, Oikari AOJ, Soimasuo MR, Cherr GN (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. Photochem Photobiol 72:308–313

Kornmann P, Sahling PH (1977) Meeresalgen von Helgoland. Helgol Wiss Meeresunters 29:1–29

Kornmann P, Sahling PH (1994) Meeresalgen von Helgoland: zweite Ergänzung. Helgol Meeresunters 48:365–406

Lazo ML, Greenwell M, McLachlan J (1989) Population structure of *Chondrus crispus* Stackhouse (Gigartinaceae, Rhodophyta) along the coast of Prince Edward Island, Canada: distribution of gametophytic and sporophytic fronds. J Exp Mar Biol Ecol 126:45–58

Lüning K (1990) Seaweeds: their environment, biogeography, and ecophysiology. Wiley, New York

Maggs CA (1988) Intraspecific life history variability in the Florideophycidae (Rhodophyta). Bot Mar 31:465–490

Makarov MV, Voskoboinikov GM (2001) The influence of

Ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. Bot Mar 44:89–94
Norton TA (1992) Dispersal by macroalgae. Br Phycol J 27: 293–301
Pakker H, Beekman CAC, Breeman AM (2000a) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). Eur J Phycol 35:109–114
Pakker H, Martins RST, Boelen P, Buma AGJ, Nikaido O, Breeman AM (2000b) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). J Phycol 36:334–341
Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: Watanabe A, Hattori A (eds) Cultures and collection of algae. Proceedings of U.S.-Japan Conference. Japanese Society of Plant Physiologists, Hakone, p 63–75
Reed DC, Laur DR, Ebeling AW (1988) Variation in algal dispersal and recruitment: the importance of episodic events. Ecol Monogr 58:321–335
Roy S (2000) Strategies for the minimisation of UV-induced damage. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, p 177–205
Rozema J, van de Staaij J, Björn LO, Caldwell M (1997) UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol Evol 12:22–28
Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Natl Acad Sci USA 71:3363–3366
Smith RC, Prézelin BB, Baker KS, Bidigare RR and 9 others (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952–959
Swanson AK, Druehl LD (2000) Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. Mar Biol 136:657–664
Tasende MA, Fraga MI (1992) Efecto de las condiciones de cultivo en la germinación de esporas de *Chondrus crispus* Stackh, (Gigartinales, Rhodophyta). Cah Biol Mar 33: 407–415
Vadas RL Sr, Johnson S, Norton T (1992) Recruitment and mortality of early post-settlement stages of benthic algae. Br Phycol J 27:331–351
van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat related differences in UV-B tolerance. J Phycol 37: 30–37
van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2002a) Temperature dependence of UV radiation effects in Arctic and temperate isolates of 3 red macrophytes. Eur J Phycol 37:59–68
van de Poll WH, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002b) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. Photochem Photobiol 76:493–501
Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In: de Mora S Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, p 149–176
Vink AA, Bergen-Henegouwen JB, Nikaido O, Baan RP, Roza L (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. Photochem Photobiol 24: 25–31
Warner CW, Caldwell MM (1983) Influence of photon flux density in the 400–700 nm waveband on inhibition of photosynthesis by UV-B (280–320 nm) irradiation in soybean leaves: separation of indirect and direct effects. Photochem Photobiol 38:341–346
Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa FL (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implication for depth zonation. Mar Ecol Prog Ser 197:217–229
Yakovleva IM, Dring M, Titlyanov EA (1998) Tolerance of North Sea algae to UV and visible radiation. Russ J Plant Physiol 45:45–54
Zupan JR, West JA (1988) Geographic variation in the life history of *Mastocarpus papillatus* (Rhodophyta). J Phycol 24: 223–229

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Publication 2

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Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation

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Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation

MICHAEL Y. ROLEDA[1,2]*, DIETER HANELT[1], GUDRUN KRÄBS[3] AND CHRISTIAN WIENCKE[3]

[1]*Biologische Anstalt Helgoland, Alfred Wegener Institute for Polar and Marine Research, Helgoland, D-27498, Germany*
[2]*Biology Department, De La Salle University, 2401 Taft Avenue, 1004 Manila, Philippines*
[3]*Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany*

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Young sporophytes of *Laminaria ochroleuca* were exposed in the laboratory either to a full light spectrum or to light depleted of only ultraviolet-B radiation (UVB) or of the whole ultraviolet radiation (UVR) using cutoff glass filters. The plants were grown under 16:8 h light–dark cycles with 6 h additional UV exposure in the middle of the light phase. Effective quantum yield of photosystem II ($\Delta F/Fm'$) was measured daily, 1 h before UV exposure, at 2 and 5 h cumulative UV exposure and at 1 and 4 h after UV exposure. Growth was measured using two methods in separate experiments. In the first, a scanner with image analysis software was used to measure surface area every 3 days for 4 weeks. In the second, a growth chamber with online video measuring technique was used to measure growth every 10 min for 2 weeks. Pigments were measured at the end of the experiments. During the first day of UV exposure, the photosynthetic yield of plants exposed to photosynthetically active radiation (PAR) + ultraviolet-A radiation (UVA) and PAR + UVA + UVB was significantly reduced but was able to recover 1 h after the end of UV exposure. An increasing mean $\Delta F/Fm'$ during UV exposure showed partial acclimation of photosynthesis in young sporophytes in the course of several days. However, a higher growth rate was observed in plants exposed to PAR alone, whereas reduced growth and damaged tissue were observed in plants exposed to UVR. Similarly, a lower content of all pigments was measured in thalli exposed to PAR + UVR. The result shows that acclimation of photosynthesis could underestimate the negative effect of this stress factor. Growth, as an integrative process, is a better parameter to explain ecophysiological performance at organism level. It was shown that growth and morphology of young sporophytes of *L. ochroleuca* are susceptible to UV damage, which could effectively limit the upper distributional range of this species.

INTRODUCTION

Across a latitudinal gradient, *Laminaria ochroleuca* de la Pylaie is distributed along the Atlantic coast of Northern Africa to the southwestern part of the British Isles (John 1969; Price *et al.* 1978; Sheppard *et al.* 1978; Benhissoune *et al.* 2002), the Mediterranean coast (Ribera *et al.* 1992) and an isolated population in the Strait of Messina (Drew 1972, 1974). It inhabits the littoral zones between 0 and 2 m above low water (John 1969, 1971; Sheppard *et al.* 1978) and also depths in excess of 100 m (Drew 1972). Until recently, its population density was reported to vary between sites only in relation to water quality and exposure to wave action and current surge. In Spain, within the 0–2 m zone, higher density was observed in an estuarine and sheltered site (8–30 plants m^{-2}) compared to a site exposed to wave action (3 plants m^{-2}) (John 1971). At the same tide level at the French coast, much lower density (2 plants m^{-2}) was observed due to synergistic effect of wave and current surge (Sheppard *et al.* 1978). Along the Strait of Messina, a dense population was observed between 50 and 100 m depth, where water clarity is similar to Jerlov's (1976) type IB Oceanic water, and 5% of surface photosynthetically active radiation (PAR; 18 W m^{-2}) reaches the kelp community at 50 m (Drew *et al.* 1982). However, these are not the only factors that could affect population density across a vertical gradient.

* Corresponding author (mroleda@awi-bremerhaven.de).

Several physiological studies have established a correlation between stress tolerance and the vertical distribution of seaweeds. These stress factors include inhibiting PAR (e.g. Hanelt *et al.* 1997a, b; Hanelt 1998) and ultraviolet radiation (UVR; e.g. Dring *et al.* 1996a; Hanelt *et al.* 1997c; Bischof *et al.* 1998a). Eulittoral macrophytes are periodically exposed to the full solar spectrum during low tides. Consequently, chronic exposure to increasing solar UVR might present some deleterious effect. Intertidal algae may possess photoadaptive mechanisms to minimize damage by solar radiation. When exposed to irradiances exceeding the energy requirement for photosynthesis, a strong degradation of the reaction centre protein (D1) of photosystem II (PS II) can occur (Ohad *et al.* 1984). This process is called chronic photoinhibition to distinguish it from the xanthophyll cycle, in which quantum yield of photosynthesis is regulated (Demmig-Adams & Adams 1992). On the other hand, dynamic photoinhibition involves a fast reversible process where the quantum yields of PS II are diminished by increasing thermal energy dissipation controlled by carotenoids (Osmond 1994).

The increasing UVR on the earth's surface caused by stratospheric ozone depletion has been well documented in the polar and temperate regions (Smith *et al.* 1992; Pearce 1996). Aside from the extensive studies done in polar regions (e.g. Hanelt *et al.* 1997c; Bischof *et al.* 2001, 2002a), other geographical locations have received meagre attention with respect to the potential effect of UVR to the biosphere. In Spain, for example, levels of ultraviolet-B radiation (UVB) have been re-

ported to be high and persistent under long periods of open sky condition (Altamirano *et al.* 2000a, b). Despite this fact, most of the studies conducted on the ecophysiological response of the macrothalli of seaweeds to UVR in this region are limited to few species of green (e.g. Pérez-Rodríguez *et al.* 1998; Bischof *et al.* 2002b), red (e.g. Flores-Moya *et al.* 1998; Gómez *et al.* 2001) and brown (e.g. Jiménez *et al.* 1998; Häder *et al.* 2001) seaweeds but not Laminariales.

UVR sensitivity of Laminariales is known in species from polar (e.g. Bischof *et al.* 1998b, 1999; Aguilera *et al.* 1999) and cold temperate (e.g. Dring *et al.* 1996b, 2001; Makarov & Voskoboinikov 2001) waters, whereas only few data are available from lower latitudes (Yabe *et al.* 1997; Wiencke *et al.* 2000). Moreover, only few studies have been conducted on the effect of irradiance as a stress factor on *L. ochroleuca* (e.g. Wiencke *et al.* 2000; Izquierdo *et al.* 2001). On the other hand, most studies on the impact of UVR examine the vulnerability of large sporophytes. To determine the depth zonation of these species, it is also important to consider the susceptibility of other life stages to UVR (e.g. Dring *et al.* 1996b; Huovinen *et al.* 2000; Wiencke *et al.* 2000; Bañares *et al.* 2002; Altamirano *et al.* 2003). In *L. ochroleuca,* zoospores are extremely sensitive to UVR (Wiencke *et al.* 2000). However, in the field, where spores and germlings of kelps can be found to remain competent in plankton for extended periods of time (Reed *et al.* 1992), surviving spores are still capable of dispersal, settlement, attachment and initiation of new individuals across the expanse of the vertical tidal zones, especially in crevices and sheltered tide pools. We develop the hypothesis that the susceptibility of young sporophytes to UVR effectively determines the upper distribution limit of this species. Young sporophytes in the eulittoral zone are periodically exposed to air during low tides and the whole spectrum of solar radiation which may contribute to the postrecruitment mortality of this species and exclude *L. ochroleuca* from higher parts of the shore, especially at sun-exposed locations. Therefore, the present study focuses on the impact of UVR on the photosynthetic parameters, growth and morphological integrity of young *L. ochroleuca* sporophytes.

MATERIAL AND METHODS

Algal material

Cultures of *L. ochroleuca* gametophytes, originally established from fertile sporophytes collected from Puerto de San Pedro, La Coruña, NW Spain (43°22′N; 8°26′W), were used to obtain young sporophytes. They were grown aerated in glass beakers filled with Provasoli enriched seawater (Provasoli 1968) inside a temperature-controlled room at 15°C and 10 μmol m^{-2} s^{-1} white light at 12 : 12 h light–dark (LD) photoperiod.

Laboratory incubation system, experimental design and measuring procedures

In the first experiment, a large flow-through basin (600 × 400 × 120 mm) was installed inside a 15°C temperature-controlled room. Inside the basin, polyvinyl chloride–U (PVC-U) pipes (dark grey, 120 mm diameter × 70 mm height, both ends open) were placed upright and served as enclosures for the algae in each treatment. The PVC-U pipes had a 5 mm diameter hole at the bottom for water inflow through silicon tubes and water flows out into the basin through four equidistant 10 mm diameter holes around the PVC-U pipes covered with mesh. From a reservoir, 80 litres of filtered and sterile seawater was pumped into the basin through the PVC-U pipes using submersible water pump (Typ 1060, 38 litre min^{-1}; Eheim, Deizisau, Germany), which also provided water movement inside the PVC pipes. Water level in the basin was maintained at 60 mm, to simulate low tide water, by circulating water back to the reservoir. During the experiment, water temperature was maintained at 15 ± 1°C as the optimum temperature for growth (Wiencke *et al.* 1994). Water in the reservoir was changed weekly with fresh sterilized seawater to prevent depletion of nutrients.

To determine the effects of different light treatments of PAR (P), PAR + ultraviolet-A radiation (UVA) (PA) and PAR + UVA + UVB (PAB) on whole young *L. ochroleuca* thalli (average size 250–300 mm^2, $n = 5$), lamps were fixed 40 cm above the flow-through basin. Three white fluorescent lamps (L65 Watt/25S; Osram, Munich, Germany), emitting background PAR resulted in a fluence rate of about 10 W m^{-2} (50 μmol m^{-2} s^{-1}). Three UV lamps (UVA-340, 40 W; Q-Panel, Cleveland, OH, USA), emitting a spectrum similar to solar radiation in the range 295–340 nm, provided 6.0 W m^{-2} UVA and 0.5 W m^{-2} of UVB. Three kinds of glass filters – Quartz, WG320 and GG400 (Schott Glass Technologies, Duryea, PA, USA) were used to cut off different UV wavelength ranges from the spectrum. Irradiation conditions (280–700 nm) were measured using a cosine sensor connected to a UV-VIS Spectrometer (M. Kruse, Bremerhaven, Germany) below the glass filters. Acclimated whole thalli (3 days at 10 W m^{-2} white light and 15°C) were grown for 4 weeks under 16 : 8 h LD cycles (0500–2100 hours) with 6 h UV exposure in the middle of the light phase (0900–1500 hours). Photosynthetic activity was determined by measuring the variable chlorophyll (Chl) fluorescence of PS II with a Diving PAM device (Walz, Effeltrich, Germany). Measurement of the effective quantum yield ($\Delta F/Fm'$) was done daily, 1 h before UV exposure (0800 hours), at 2 and 5 h after the start of UV exposure (1000, 1400 hours) and at 1 and 4 h after the end of UV exposure (1600, 1900 hours).

Higher total light energy was measured among PAR + UV treatments (PA and PAB) in the above experiment than under PAR alone (GG 400; see Table 1). In this regard, a second experiment was conducted in the same flow-through basin culture system to determine the effects of varying photon flux density (PFD) of PAR. This was done to ensure that the negative physiological effect on the young thalli was due to light quality (presence of UVR) and not due to a different total light energy in the first experiment. Three white fluorescent lamps (L65 Watt/25S; Osram) were used and four PAR levels (30, 40, 50 and 60 μmol m^{-2} s^{-1}) were achieved by varying the distance between the basins and the light source and by using neutral grey mesh covers. Light was measured using a cosine quantum sensor (Type 1925B; LI-COR Biosciences, Bad Homburg, Germany) attached to a LI-COR data logger (LI-1000). Basal parts (± 6 mm of the phylloid, including the meristem) were cut from the whole plants (average size 30 mm^2) and acclimated for 3 days at 10 W m^{-2} white light and 15°C before being used in this experiment. They were grown

Table 1. Irradiances applied to the growth experiments in the respective laboratory incubation system.

	Irradiance (W m^{-2})			
	PAR (400–700 nm)	UVA (320–400 nm)	UVB (280–320 nm)	Total irradiance
Flow-through basin incubation system				
Quartz filter (PAR + UVA + UVB)	11.50	6.24	0.50	18.24
WG 320 filter (PAR + UVA)	11.47	6.08	0.16	17.71
GG 400 filter (PAR alone)	8.00	0.01	0.00	8.01
ISITEC growth chamber				
Quartz filter (PAR + UVA + UVB)	4.04	4.91	0.42	9.37
GG 400 filter (PAR alone)	3.59	0.01	0.00	3.60

for 3 weeks under the same LD cycles. Effective quantum yield ($\Delta F/Fm'$) was measured every 4 h from 0800–2000 hours using the Diving PAM. In both experiments, growth, in terms of surface area increase (mm^2), was measured every 3 days using a scanner connected to a personal computer (PC) and WinFolia 5.0 image analysis software (Regent Instrument, Quebec City, Canada).

Online video growth measurement technique

Three growth chambers with online video measuring technique were constructed by ISITEC (Bremerhaven, Germany). The growth chamber (attached to a water-circulating system) was equipped with a Charged Coupled Device camera coupled to a PC. A sliding metal platform with top and bottom plate of UV-transparent Plexiglas chambers at the centre was positioned 20 cm above the camera. The top Plexiglas chamber (12 × 16.5 × 3.5 cm, constructed with side frames) was designed to be laid hanging over the bottom chamber (17.5 × 17.5 × 4 cm), where the algae are fixed on tiny nails attached to the bottom chamber. The space between the top and bottom Plexiglas chambers allows the circulating seawater to pass through. The water-circulating system comprised a cooling unit (Aqua Medic, Bissendorf, Germany) and 30 litre reservoir tank filled with filtered and pasteurized seawater. Seawater was cooled to 15°C and pumped into the growth chamber by a centrifugal water pump (Eheim Typ 1060, 38 litre min^{-1}). Light sources were mounted 15 cm above the platform consisting of two white fluorescent lamps (TL 8W/965; Philips, Eindhoven, Netherlands) and two UV lamps (Q-Panel UVA-340, 40 W). Infrared diodes were mounted at the sides of the chamber to produce infrared images of the object for the video camera, also during the dark periods. The captured image was analysed by a MedeaLAB Count and Classify software (Multimedia and Software GmbH, Erlangen, Germany), which measures growth of the algae in terms of increased number of pixels. In each growth chamber, two basal pieces of the thallus (average size = 30 mm^2, ± 6 mm of the phylloid including the meristem), positioned 20 mm from each other, were fixed between the top and bottom of the UV-transparent Plexiglas chamber and acclimated for 3 days without UV. Two types of glass filters, one cutting off all UV radiation (GG400), the other UV transparent (Quartz) were laid over the top Plexiglas chamber covering the algae for the corresponding treatment. Irradiance was measured as mentioned above. All irradiances applied in each treatment are summarized in Table 1. Growth was continuously measured every 10 min for 2 weeks. Seawater was changed weekly to ensure enough nutrient supply within the medium.

Pigment extraction and characterization

At the end of each growth experiment, algal thalli were transferred to 2 ml Eppendorf tubes and frozen at −80°C for high performance liquid chromatography pigment analysis. Frozen samples were treated with 100 µl of 100% *N-N*-dimethylformamide and stored in darkness for approximately 16 h. Subsequent analyses were performed as described by Bischof *et al.* (2002c). The whole thallus of the first experiment was divided into three parts (base, mid and tip) to determine the longitudinal profile of Chl *a*. However, due to tissue sample limitation, no replicate was analysed. Samples from the online growth chamber were analysed for Chl *a*, Chl c_1, fucoxanthin and β-carotene in triplicate.

Data analysis

All data were tested for homogeneity of variances (Levene Statistics) and normality (Kolmogorov–Smirnov Test). Corresponding transformations were done to heteroskedastic (unequal variances) and nonnormal data. Time series measurements on the photosynthetic yield ($\Delta F/Fm'$) were subjected to repeated measures analysis of variance (RMANOVA) to determine the effects of light treatments across the sampling days.

Growth rate was computed by plotting all data points (entire experiment period) of each replicate per treatment. They were individually fitted to an exponential equation $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase. Slopes (r) were computed daily for the growth chamber data. Growth rate at time t, rt, is comparable to the growth equation applied by Lüning (1979): relative growth rate (% per day) = $(\ln SA_2 - \ln SA_1)/t_2 - t_1 \times 100$, where SA_1 and SA_2 are the surface areas at t_1 and t_2 in days, respectively. Subsequently, the statistical significance of differences in growth rates as affected by light treatments were tested using analysis of variance (ANOVA, $P = 0.05$). This was followed by Duncan's multiple range test (DMRT, $P = 0.05$). For the growth chamber and pigment data, where we only tested two variables, comparison between the two groups was done by *t*-test ($P < 0.05$). Statistical analyses were done using the SPSS program (SPSS, Chicago, IL, USA).

RESULTS

Chlorophyll fluorescence: effective quantum yield

During the first day of exposure of young *L. ochroleuca* sporophytes to 5 h UVR, a reduction of 53% and 61% in the

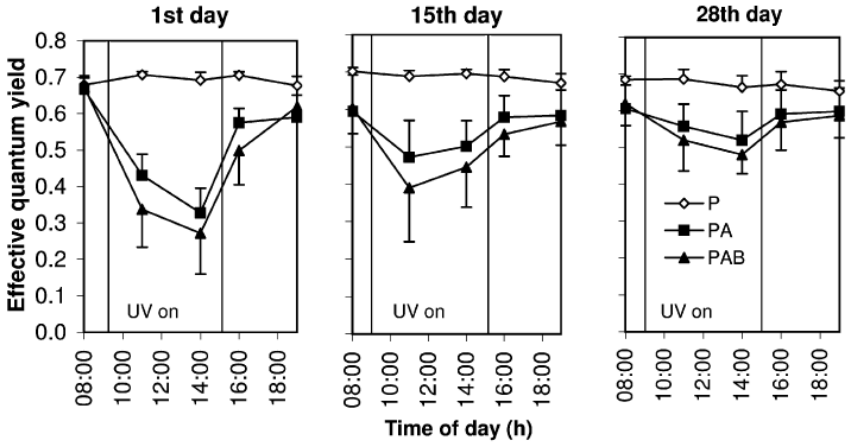

Fig. 1. Circadian pattern of the mean effective quantum yield of young *Laminaria ochroleuca* sporophytes (n = 5) exposed to different radiation (PAR = P; PAR + UVA = PA; PAR + UVA + UVB = PAB) during the light phase of the 16 : 8 h light–dark photoperiod. PFD is 40–50 μmol m^{-2} s^{-1}. Vertical bars are standard deviations (s). Corresponding statistical analysis is shown in Table 2.

mean effective quantum yield ($\Delta F/Fm'$) was observed in the PA and PAB treatments relative to P, respectively. The mean $\Delta F/Fm'$ of PA and PAB treatments were observed to recover 1 h after UV lamps were switched off, to 82% and 71% of the P treatment, respectively (Fig. 1). The reduction in the mean $\Delta F/Fm'$ of UV-exposed plants became smaller through time (15th and 28th day). After 2 and 5 h of UV exposure (PA and PAB), $\Delta F/Fm'$ at the end of the experiment (28th day) was significantly higher than during the first day (Fig. 1), indicating acclimation of the photosynthetic apparatus to UVR.

RMANOVA ($P < 0.05$) showed a significant effect of irradiance on the effective quantum yield, $\Delta F/Fm'$ (Table 2). Sporophytes exposed to PA and PAB had significantly lower $\Delta F/Fm'$ during (1100 and 1400 hours) and after (1600 and 1900 hours) UV exposures. Although photosynthetic recovery was evident when UV lamps were switched off (Fig. 1), $\Delta F/Fm'$ of P was still significantly higher compared to PA and PAB. In the morning (0800 hours), $\Delta F/Fm'$ was found to be not significantly different between treatments, indicating further recovery. Final photosynthetic recovery on the 28th day (1900 hours) was 90% of the initial value at the start of the experiment.

Plants exposed to different PFD of PAR (30, 40, 50 and 60 μmol m^{-2} s^{-1}) showed significant variation (RMANOVA, $P < 0.05$) in the time series $\Delta F/Fm'$. The DMRT ($P < 0.05$) showed significantly higher daily $\Delta F/Fm'$ in plants exposed to lower PAR (Fig. 2).

Growth

Higher growth rates were observed in plants exposed to P alone (7.2 ± 0.6% day^{-1}) compared to plants exposed to PA (4.6 ± 3.1% day^{-1}) and PAB (3.7 ± 1.1% day^{-1}). Analysis of variance showed significant effect of treatment ($P < 0.05$). However, DMRT showed that P is not significantly different with PA, and PA is not significantly different with PAB (Fig. 3). Moreover, tissue damage was evident among plants exposed to PA and PAB showing tissue deformation, necrosis, blistering, lesions, and curling and thickening of the meristematic region (Fig. 4). Different PFDs of PAR have no significant effect on growth (ANOVA, P = 0.354; Fig. 5). At the same PAR level, growth rate at 40 μmol m^{-2} s^{-1} (7.9 ± 0.6% day^{-1}) is comparable to the first experiment.

In the ISITEC growth chambers experiment, growth over the entire experimental period (fitness of the exponential curve, R^2 = 0.95 and 0.94 for P and PAB, respectively) were lower for both P (4.4 ± 0.5% day^{-1}) and PAB (1.9 ± 0.6% day^{-1}) compared to the growth experiment using the large basin flow-through incubation system. The calculated slope (r = intrinsic rate of increase) was observed to be exponential during the first day in both P (R^2 = 0.99) and PAB (R^2 =

Table 2. Repeated measures analysis of variance and significance values for the effect of light treatments (P, PA, PAB) on the photosynthetic yields ($\Delta F/Fm'$) of young *Laminaria ochroleuca* sporophytes at every time interval between sampling days (days 1, 15 and 28).

Variables					
Dependent	Independent		df	F	P value[1]
Yield (ΔF/Fm′)	Irradiance	Sampling time (h)			
		08:00 (UV off)	2	3.155	0.079 NS
		11:00 (UV on)	2	57.170	<0.001*
		14:00 (UV on)	2	90.106	<0.001*
		16:00 (UV off)	2	19.934	<0.001*
		19:00 (UV off)	2	5.589	0.019*

[1] P values represent significance level within time factor, * significant; NS, not significant.

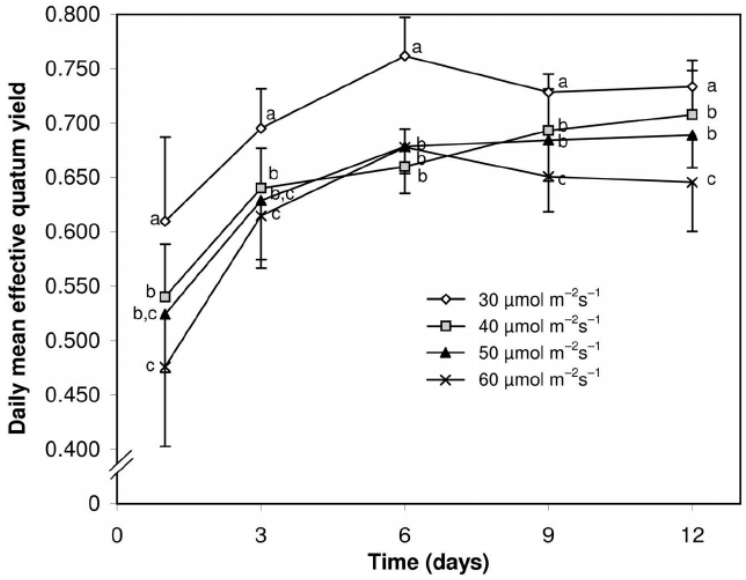

Fig. 2. Daily mean of the effective quantum yields of young *Laminaria ochroleuca* sporophytes (n = 5), exposed to different PFDs of PAR, measured every 4 h from 0800 to 2000 hours. Vertical bars are standard deviations (s). RMANOVA showed significant difference between treatments ($P < 0.001$). Letters on graph show result of DMRT ($P < 0.05$); different letters refer to significant differences between mean values.

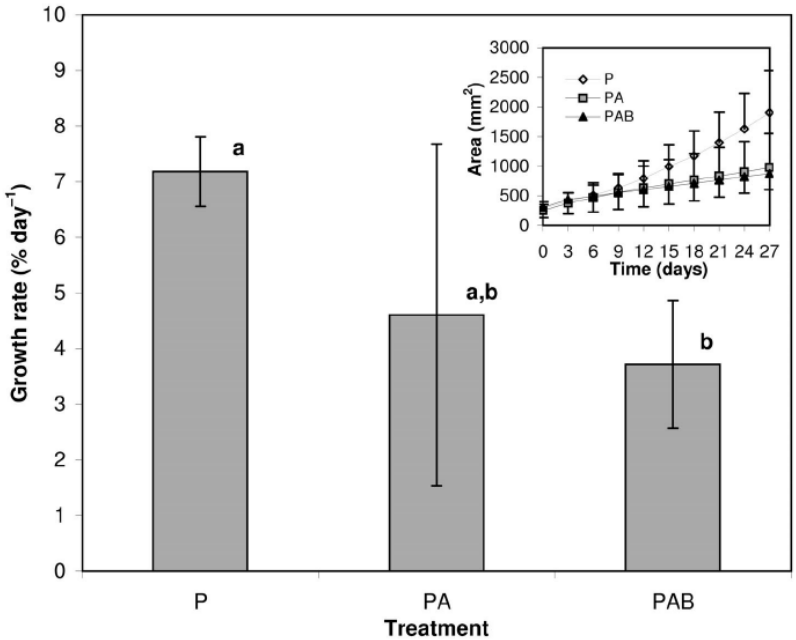

Fig. 3. Growth rates of young *Laminaria ochroleuca* sporophytes exposed to different radiation (PAR = P; PAR + UVA = PA; PAR + UVA + UVB = PAB). PFD was 40–50 µmol m^{-2} s^{-1}. Inset shows increase in surface area over time measured using a scanner and image analysis software (WinFolia). Values are $\bar{x} \pm s$ (n = 5). ANOVA showed significant difference between treatments (P = 0.038). Letters on graph show result of DMRT ($P < 0.05$); different letters refer to significant differences between mean values.

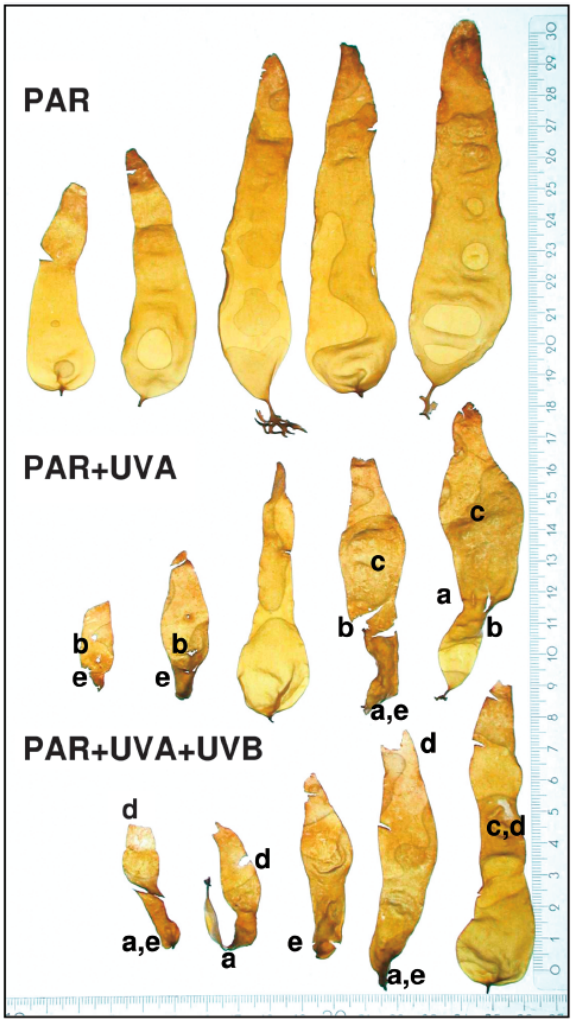

Fig. 4. Morphological responses of *Laminaria ochroleuca* after 28 days UVR exposure includes: a, tissue deformation; b, lesion; c, blistering; d, bleaching; e, curling and thickening of the meristematic lamina.

0.98) treatments. On the sixth and the 12th day, plants grown under P treatment were still growing exponentially at a lower rate (R^2 = 0.99 and 0.94, respectively) but not under PAB (Fig. 6). Growth (r) in PAB during the first day was already 36% lower relative to P treatment. It decreased further to 84% on the sixth day and was zero on the 12th day. Therefore, the velocity of increase per unit time in the young sporophytes was unable to acclimate to UVR which significantly lowered the growth rate of sporophytes exposed to PAB ($P < 0.05$, inset of Fig. 6).

Photosynthetic pigments

Although no replicates were measured, a trend was observed in the longitudinal profile of the Chl *a* content in the young sporophytes (Table 3). It was observed that regardless of the light treatment, the meristematic and young parts of the thallus contain less pigment than the rest of the thallus. Highest Chl *a* content was measured in the middle part of the thallus and close to the tip. On the other hand, total Chl *a* contents in plants exposed to PA and PAB were relatively lower compared to plants exposed to P alone. No pigment analysis was performed in the experiment using different PFD of PAR.

Pigment concentration of sporophytes incubated inside the growth chambers showed similar results. Significantly higher Chl *a,* Chl c_1, fucoxanthin and β-carotene ($P < 0.005$) levels were measured in the phylloids exposed to P alone (Fig. 7). Relative to P, different pigments showed different sensitivities to PAB. The order of sensitivity of pigment, expressed as the reduction in concentration is as follows: Chl c_1, β-carotene, fucoxanthin, and Chl *a* with 80%, 77%, 72% and 65%, respectively. The carotenoids to Chls ratio (car : chl) showed that the P treatment (car : chl = 0.349 ± 0.01) is not significantly different to PAB (car : chl = 0.305 ± 0.05) (*t*-test, $P = 0.20$).

DISCUSSION

The major result of this study is that photosynthesis is able to acclimate to UVR whereas growth cannot. Acclimation of photosynthesis to UVR in brown macroalgae has been previously reported in the Arctic Laminariales (Bischof *et al.* 1998a, 1999). This indicates that photosynthesis is a dynamic process, which can acclimate to variations in light intensity and spectral quality (reviewed by Senger & Bauer 1987; Falkowski & LaRoche 1991). Mechanisms that might have been involved in UVR acclimation include the establishment of a physical barrier that shields the photosynthetic apparatus against damaging radiation (Karentz 1994), or the induction and synthesis of phlorotannins, which have been invoked as UV-screening compounds in brown algae (reviewed by Schoenwaelder 2002). Phlorotannins of *L. ochroleuca* have been previously characterized (Koch *et al.* 1980). However, the physiological and ecological significance of these chemically complex and heterogeneous polyphenolic components isolated from *L. ochroleuca* are unknown.

In contrast to photosynthesis, growth rate of *L. ochroleuca* has been significantly affected under longer PAB treatment. This indicates that the photosynthetic capability of the algae to partially acclimate to chronic UVR exposure cannot always be equated to the ecological optimum of the plant. Although growth (size of phylloid area) is still increasing in the P treatment, the declining slope could be attributed to the increase in doubling time for the cell mass (Sorokin 1973; Brinkhuis 1985). Field experiments on the relative growth rates of three *Laminaria* J.V. Lamouroux species in Helgoland were also observed to decrease through time (Lüning 1979). Consequently, regardless at which point of the growth curve we look at, the rate of increase per unit time in the young sporophytes exposed to PAB was unable to acclimate to UVR.

Although the effect of UVA was statistically insignificant in the growth experiment, it is evident that long-term exposure to UVA and UVB resulted in tissue deformation and damage in *L. ochroleuca.* This characteristic tissue damage and morphological deformation are still undocumented and unreported in seaweeds exposed to UVR. This is probably because previous growth studies on young *Laminaria* sporophytes were too short to induce tissue injury (e.g. 2–3 weeks; Dring *et al.*

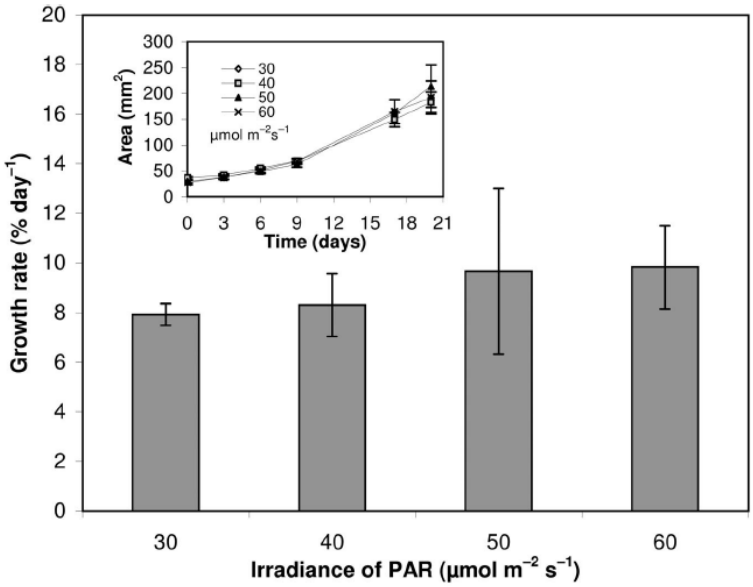

Fig. 5. Growth rates of young *Laminaria ochroleuca* sporophytes exposed to different PFDs of PAR. Inset shows increase in surface area over time measured using a scanner and image analysis software (WinFolia). Values are $\bar{x} \pm s$ ($n = 5$). ANOVA showed insignificant difference between treatments ($P = 0.354$).

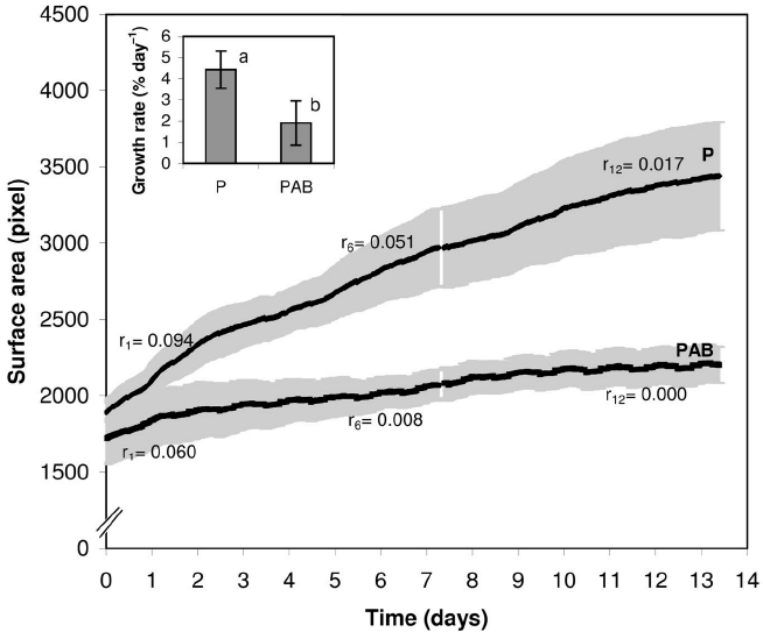

Fig. 6. Growth, in terms of surface area increase, of young *Laminaria ochroleuca* sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB) using the ISITEC growth chamber coupled to a PC with video image analysis software (MedeaLAB) determining area in pixels. PFD was ± 20 µmol m^{-2} s^{-1}. Slopes (r = rate of increase) r_1, r_6 and r_{12} on days 1, 6 and 12 for P and PAB are computed using the exponential growth $N_t = N_0 e^{rt}$, where N_t is growth at time t and N_0 is initial size. Inset is the corresponding growth rates for the entire experimental period. Values are $\bar{x} \pm s$ ($n = 3$). Letters on graph show result of *t*-test ($P < 0.05$); different letters refer to significant differences between mean values.

Table 3. Longitudinal profile of thallus Chl *a* concentration in *Laminaria ochroleuca* sporophytes exposed to different irradiances. Control is sample derived directly from bubbling culture; light is ± 10 µmol $m^{-2}s^{-1}$. Values per segment represent one replicate only.

	Chl *a* (mg g^{-1} FW)[1]			
Treatment	Base	Mid	Tip	Total
Control	0.1498	0.2968	0.2544	0.7010
P	0.1965	0.4589	0.4077	1.0631
PA	0.2485	0.3461	0.3202	0.9148
PAB	0.2486	0.3243	0.3130	0.8860

[1] FW, fresh weight.

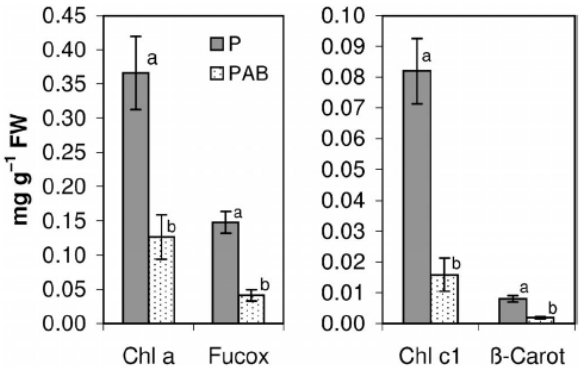

Fig. 7. Photosynthetic and accessory pigments in *Laminaria ochroleuca* sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB). Vertical bars are standard deviations (*s, n* = 3). Letters on graph show result of *t*-test ($P < 0.005$); different letters refer to significant differences between mean values.

1996b; Aguilera *et al.* 1999). However, pronounced tissue necrosis and loss of parts of the thalli was reported in the Arctic *L. solidungula* J. Agardh after 1 week of daily exposure to 18 h UVR (Michler *et al.* 2002). On the other hand, UVR-induced injuries on plant's tissue have been reported in terrestrial flora. This includes reduced leaf area, blistering and epidermal deformation, lesions, increased leaf thickness and photomorphogenesis (radiation-induced change in growth form) (Cline & Salisbury 1966; Robberecht & Caldwell 1978; Tevini *et al.* 1981; Teramura 1983; Barnes *et al.* 1990). In this regard, long-term growth measurement and observation on morphological integrity of the tissue presents a more holistic indication of the negative impact of this stress factor.

Different growth rates were obtained from the two experimental methods. Higher growth rates in both P and PAB treatments were measured in the basin incubation system compared to the growth chamber with automated online video measuring technique. There could be several reasons for this: (1) plants in the basin are subjected to water motion which could facilitate gas exchange and nutrient uptake; (2) the higher volume of circulating water in the basin incubation system (80 litres) compared to the growth chamber (30 litres) could result in a better nutrient supply; or (3) the growth rate was light-limited in the growth chamber (P = 3.6 W m^{-2}; PAB = 9.4 W m^{-2}) compared to the basin incubation system (P = 8 W m^{-2}; PAB = 18 W m^{-2}).

In the experiment with different photon fluence rates, we were not able to test the effect of the highest total amount of light energy equivalent to the PA and PAB treatments (≅ 18 W m^{-2}). Further experiment should be conducted to address this question explicitly. However, we believe that the physiological and morphological effects observed in our study are due to light quality (presence of UVR) rather than the higher amount of light energy in the UV treatments, especially because UVR cannot be used for photochemical energy conversion.

Photosynthetic and accessory pigments in *L. ochroleuca* were observed to react similarly with growth. These were significantly reduced under UVB. Pigment damage can result either (1) when protein-based pigments absorb UV energy directly and undergo photochemical degradation; (2) by photosensitizer action; or (3) by oxygen radical production in addition to singlet oxygen (Vincent & Neale 2000). Aguilera *et al.* (2002) reported that under natural solar radiation, photosynthetic pigments of six Arctic macroalgae decreased significantly upon exposure to increased PAR and UVR after sea-ice break-up. Reduced Chl concentrations were also observed in field experiments with *Ulva* Linnaeus species in Spain and Helgoland (Bischof *et al.* 2002b, c) and in the laboratory experiments of Australian seagrasses (Dawson & Dennison 1996). On the other hand, a study on *U. rigida* C. Agardh reported significantly higher pigment content (Chl *a,* Chl *b* and carotenoids) in the presence of UVB (Altamirano *et al.* 2000b). There was also an inverse relationship between growth and pigment content, which caused the authors to speculate some kind of photoprotective mechanism in the algae that deflects energetic resources to pigment biosynthesis at the expense of growth. In *L. ochroleuca,* we observed lower growth rates and pigment concentration in UVB-exposed plants. This implies that UVB reduced the synthesis of or degraded the pigments, effectively limiting the light-harvesting ability. Consequently, reduction in the photosynthetic end products also imposes constraints on the repair of cellular damage and growth. With the meagre information on the long-term effects of UVR on pigment content and its relationship to photosynthetic efficiency and growth, more studies should focus on this mechanism, which could be species-specific.

The longitudinal profile of Chl *a* concentration in young sporophytes was comparable to those of mature *L. ochroleuca* (Drew *et al.* 1982), *L. digitata* (Hudson) J.V. Lamouroux, *L. saccharina* (Linnaeus) J.V. Lamouroux and *L. hyperborea* (Gunnerus) Foslie (Küppers & Kremer 1978). The lowest Chl *a* contents were measured in the young and meristematic regions of the plant. Tissue Chl *a* concentration increases with age, but the tip contains relatively lower amounts than the middle parts. Furthermore, different pigments were observed to exhibit different sensitivities to UVR. For example, within the Chls, Chl c_1 has been found to be more sensitive than Chl *a.* The carotenoids fucoxanthin and β-carotene were more affected than the Chls (Chl *a* + Chl c_1) under UVB. These observations conflict with previous studies, where it was reported that Chl *a* was more sensitive than Chl *b* (Teramura 1983; Strid *et al.* 1990) and that carotenoids are generally less affected than Chls (Teramura 1983). Therefore, an increase in car : chl due to faster degradation of Chls can imply some photoprotection role of carotenoids (Roy 2000). However, the accumulation of carotenoids specifically in response to UVR was only observed in cyanobacteria and chlorophytes (Buck-

ley & Houghton 1976; Goes *et al.* 1994). In our study, car: chl was more or less the same in plants exposed to P alone and plants exposed to PAB.

In the field, the wide range of distribution of *L. ochroleuca* across a vertical gradient (0–100 m) suggests that competent spores and germlings can successfully recruit across the expanse of the vertical tidal zone. However, young sporophytes are probably prevented from growing successfully into adult sporophytes in the upper tide level by consistent exposure to environmental stress such as high UVR, temperature changes, desiccation and grazing. This could explain the low relative plant density reported at 0–2 m zone in the field. Although there were no previous field data on UVR in these areas, much lower plant densities were observed in the Spanish and French coasts (John 1971; Sheppard *et al.* 1978) compared to the estuarine area in Spain (John 1971). Because of the higher solar angle, lower latitude areas receive more solar radiation, and higher harmful UVB levels have been reported in Spain than in polar regions or higher latitudes (Altamirano *et al.* 2000a, b). Also, macrophytes are more affected by UVR in clear waters than in turbid waters because excessive UV can be absorbed and scattered in the water column by suspended matter, dissolved organic carbon and phytoplankton.

In conclusion, we recommend that future studies on the long-term effect of increasing UVR on aquatic macrophytes should measure growth rather than photosynthesis only, or preferably measure both physiological processes and other biochemical parameters, to understand better the mechanisms of UV damage in macroalgae. It has been demonstrated that photosynthesis is an important physiological target of UVR (Franklin & Forster 1997; Hanelt *et al.* 1997c; Bischof *et al.* 1998a). However, fluorescence data showed that photosynthesis was negatively affected only during the initial exposure to UVR and eventually acclimated to it. Conversely, long-term chronic exposure to UVR showed a significant effect on growth rate, the tissue's morphological integrity and pigment composition. Other cellular processes affected by UVR are cell division, and damage to macromolecules such as DNA, proteins and lipids (Altamirano *et al.* 2000a, b; van de Poll *et al.* 2001). UV exposure reduces the accumulation of photosynthetic products, which are diverted to the repair of cellular damage and consequently limit growth and reproduction. In this regard, growth as an integrative cellular process is better suited than photosynthesis to the study of the long-term effect of UV exposure to macroalgae.

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REFERENCES

Aguilera J., Karsten U., Lippert H., Vögele B., Philipp E., Hanelt D. & Wiencke C. 1999. Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology Progress Series* 191: 109–119.

Aguilera J., Bischof K., Karsten U., Hanelt D. & Wiencke C. 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Marine Biology* 140: 1087–1095.

Altamirano M., Flores-Moya A. & Figueroa F.-L. 2000a. Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated *in situ*. *Botanica Marina* 43: 19–26.

Altamirano M., Flores-Moya A. & Figueroa F.-L. 2000b. Growth, seasonality, photosynthetic pigments, and C and N content in relation to environmental factors: a field study on *Ulva olivascens* (Ulvales, Chlorophyta). *Phycologia* 39: 50–58.

Altamirano M., Flores-Moya A. & Figueroa F.-L. 2003. Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophycea). *Aquatic Botany* 75: 9–20.

Bañares E., Altamirano M., Figueroa F.-L. & Flores-Moya A. 2002. Influence of UV radiation on growth of sporelings of three non-geniculate coralline red algae from southern Iberian Peninsula. *Phycological Research* 50: 23–30.

Barnes P.W., Flint S.D. & Caldwell M.M. 1990. Morphological responses of crop and weed species of different growth forms to ultraviolet-B radiation. *American Journal of Botany* 77: 1354–1360.

Benhissoune S., Boudouresque C.-F. & Verlaque M. 2002. A checklist of the seaweeds of the Mediterranean and Atlantic Coasts of Morocco. II. Phaeophyceae. *Botanica Marina* 45: 217–230.

Bischof K., Hanelt D. & Wiencke C. 1998a. UV-radiation can affect depth-zonation of Antarctic macroalgae. *Marine Biology* 131: 597–605.

Bischof K., Hanelt D., Tüg H., Karsten U., Brouwer P.E.M. & Wiencke C. 1998b. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biology* 20: 388–395.

Bischof K., Hanelt D. & Wiencke C. 1999. Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biology* 1: 435–444.

Bischof K., Hanelt D. & Wiencke C. 2001. UV-radiation and Arctic marine macroalgae. In: *UV-radiation and Arctic ecosystems* (Ed. by D. Hessen), pp. 227–244. Springer, New York. [Ecological Studies Series, vol. 153.]

Bischof K., Hanelt D., Aguilera J., Karsten U., Vögele B., Sawall T. & Wiencke C. 2002a. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Marine Biology* 140: 1097–1106.

Bischof K., Peralta G., Kräbs G., van de Poll W.H., Pérez-Lloréns J.C. & Breeman A.M. 2002b. Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. *Journal of Experimental Botany* 53: 2411–2421.

Bischof K., Kräbs G., Wiencke C. & Hanelt D. 2002c. Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* 215: 502–509.

Brinkhuis B.H. 1985. Growth patterns and rates. In: *Handbook of phycological methods: ecological field methods: macrolagae* (Ed. by M.M. Littler & D.S. Littler), pp. 461–477. Cambridge University Press, Cambridge, UK.

Buckley C.E. & Houghton J.A. 1976. A study of the effects of near UV radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. *Archives of Microbiology* 107: 93–97.

Cline M.G. & Salisbury F.B. 1966. Effects of ultraviolet radiation on the leaves of higher plants. *Radiation Botany* 6: 151–163.

Dawson S.P. & Dennison W.C. 1996. Effects of ultraviolet and photosynthetically active radiation on five seagrass species. *Marine Biology* 125: 629–638.

Demmig-Adams B. & Adams W.W. III. 1992. Photoprotection and

other responses of plants to high light stress. *Annual Review of Plant Physiology & Plant Molecular Biology* 43: 599–626.

Drew E.A. 1972. Growth of a kelp forest at 60 metres in the Straits of Messina. *Memorie di Biologia Marina e di Oceanografia* 2: 135–157.

Drew E.A. 1974. An ecological study of *Laminaria ochroleuca* Pyl. growing below 50 metres in the Straits of Messina. *Journal of Experimental Marine Biology and Ecology* 15: 11–24.

Drew E.A., Ireland J.F., Muir C., Robertson W.A.A. & Robinson J.D. 1982. Photosynthesis, respiration and other factors influencing the growth of *Laminaria ochroleuca* Pyl. below 50 metres in the Straits of Messina. *Marine Ecology – Pubblicazioni Della Stazione Zoologica di Napoli I* 3: 335–355.

Dring M.J., Wagner A., Boeskov J. & Lüning K. 1996a. Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. *European Journal of Phycology* 31: 293–302.

Dring M.J., Makarov V., Schoschina E., Lorenz M. & Lüning K. 1996b. Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Marine Biology* 126: 183–191.

Dring M.J., Wagner A. & Lüning K. 2001. Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. *Plant, Cell & Environment* 24: 1153–1164.

Falkowski P.G. & LaRoche J. 1991. Acclimation to spectral irradiance in algae. *Journal of Phycology* 27: 8–14.

Flores-Moya A., Gómez I., Viñegla B., Altamirano M., Pérez-Rodríguez E., Maestre C., Caballero R.M. & Figueroa F.-L. 1998. Effects of solar radiation on the endemic Mediterranean red alga *Rissoella verruculosa*: photosynthetic performance, pigment content and the activities of enzymes related to nutrient uptake. *New Phytologist* 139: 673–683.

Franklin L.A. & Forster R.M. 1997. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology* 32: 207–232.

Goes J.I., Handa N., Taguchi S. & Hama T. 1994. Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter *Tetraselmis* sp.: relationship to cellular pigments. *Marine Ecology Progress Series* 114: 259–274.

Gómez I., Figueroa F.-L., Sousa-Pinto I., Viñegla B., Pérez-Rodríguez E., Maestre C., Coelho S., Felga A. & Pereira R. 2001. Effects of UV radiation and temperature on photosynthesis as measured by PAM flourescence in the red alga *Gelidium pulchellum* (Turner) Kützing. *Botanica Marina* 44: 9–16.

Häder D.-P., Porst M. & Lebert M. 2001. Photosynthetic performance of the Atlantic brown macroalgae, *Cystoseira abies-marina, Dictyota dichotoma* and *Sargassum vulgare,* measured in Gran Canaria on site. *Environmental and Experimental Botany* 45: 21–32.

Hanelt D. 1998. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology* 131: 361–369.

Hanelt D., Melchersmann B., Wiencke C. & Nultsch W. 1997a. Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Marine Ecology Progress Series* 149: 255–266.

Hanelt D., Wiencke C., Karsten U. & Nultsch W. 1997b. Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). *Journal of Phycology* 33: 387–395.

Hanelt D., Wiencke C. & Nultsch W. 1997c. Influence of UV-radiation on the photosynthesis of Arctic macroalage in the field. *Journal of Photochemistry and Photobiology B: Biology* 38: 40–47.

Huovinen P.S., Oikari A.O.J., Soimasuo M.R. & Cherr G.N. 2000. Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. *Photochemistry and Photobiology* 72: 308–313.

Izquierdo J.L., Pérez-Ruzafa I.M. & Gallardo T. 2001. Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. *Helgoland Marine Research* 55: 285–292.

Jerlov N.G. 1976. *Marine optics,* ed. 2. Elsevier Scientific, Amsterdam. 231 pp.

Jiménez C., Figueroa F.-L., Salles S., Aguilera J., Mercado J., Viñegla B., Flores-Moya A., Lebert M. & Häder D.-P. 1998. Effects of solar radiation on photosynthesis and photoinhibition in red macrophytes from an intertidal system of southern Spain. *Botanica Marina* 41: 329–338.

John D.M. 1969. An ecological study on *Laminaria ochroleuca*. *Journal of the Marine Biological Association of the United Kingdom* 49: 175–187.

John D.M. 1971. The distribution and net productivity of sublittoral populations of attached macrophytic algae in an estuary on the Atlantic coast of Spain. *Marine Biology* 11: 90–97.

Karentz D. 1994. Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: *Ultraviolet radiation in Antarctica: measurements and biological effects* (Ed. by C.S. Weiler & P.A. Penhale), pp. 93–110. American Geophysical Union, Washington, DC. [Antarctic Research Series no. 62.]

Koch M., Glombitza K.-W. & Eckhardt G. 1980. Phlorotannins of Phaeophycea *Laminaria ochroleuca. Phytochemistry* 19: 1821–1823.

Küppers U. & Kremer B.P. 1978. Longitudinal profiles of carbon dioxide fixation capacities in marine macroalgae. *Plant Physiology* 62: 49–53.

Lüning K. 1979. Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Marine Ecology Progress Series* 1: 195–207.

Makarov M.V. & Voskoboinikov G.M. 2001. The influence of Ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina. Botanica Marina* 44: 89–94.

Michler T., Aguilera J., Hanelt D., Bischof K. & Wiencke C. 2002. Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. *Marine Biology* 140: 1117–1127.

Ohad I., Kyle D.J. & Arntzen C.J. 1984. Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *Journal of Cell Biology* 99: 481–485.

Osmond C.B. 1994. What is photoinhibition? Some insights from comparisons of shade and sun plants. In: *Photoinhibition of photosynthesis, from the molecular mechanisms to the field* (Ed. by N.R. Baker & J.R. Bowyer), pp. 1–24. BIOS Scientific, Oxford, UK.

Pearce F. 1996. Big freeze digs a deeper hole in ozone layer. *New Scientist* 147: 7.

Pérez-Rodríguez E., Gómez I., Karsten U. & Figueroa F.-L. 1998. Effects of UV-radiation on photosynthesis and excretion of UV-absorbing compounds of *Dasycladus vermicularis* (Dasycladales, Chlorophyta) from southern Spain. *Phycologia* 37: 379–387.

Price J.H., John D.M. & Lawson G.W. 1978. Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. II. Phaeophyta. *Bulletin of the British Museum (Natural History) Botany* 6: 87–182.

Provasoli L. 1968. Media and prospects for the cultivation of marine algae. In: *Cultures and collections of algae.* Proceedings of US–Japan conference, Hakone, 1966 (Ed. by A. Watanabe & A. Hattore), pp. 63–75. Japanese Society for Plant Physiology, Tokyo.

Reed D.C., Amsler C.D. & Ebeling A.W. 1992. Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* 73: 1577–1585.

Ribera M.A., Gómez-Garreta A., Gallardo T., Cormaci M., Furnari G. & Giaccone G. 1992. Check-list of Mediterranean seaweeds. I. Fucophyceae (Warming 1884). *Botanica Marina* 35: 109–130.

Robberecht R. & Caldwell M.M. 1978. Leaf epidermal transmit-

tance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. *Oecologia* 32: 277–287.

Roy S. 2000. Strategies for the minimisation of UV-induced damage. In: *The effects of UV radiation in the marine environment* (Ed. by S. de Mora, S. Demers & M. Vernet), pp. 177–205. Cambridge University Press, Cambridge, UK.

Schoenwaelder M.E.A. 2002. The occurrence and cellular significance of physodes in brown algae. *Phycologia* 41: 125–139.

Senger H. & Bauer B. 1987. The influence of light quality on adaptation and function of the photosynthetic apparatus. *Photochemistry and Photobiology* 45: 939–946.

Sheppard C.R.C., Jupp B.P., Sheppard A.L.S. & Bellamy D.J. 1978. Studies on the growth of *Laminaria hyperborea* (Gunn.) Fosl. and *Laminaria ochroleuca* De La Pylaie on the French Channel Coast. *Botanica Marina* 21: 109–116.

Smith R.C., Prézelin B.B., Baker K.S., Bidigare R.R., Boucher N.P., Coley T., Karentz D., MacIntyre S., Matlick H.A., Menzies D., Ondrusek M., Wan Z. & Waters K.J. 1992. Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* 255: 952–959.

Sorokin C. 1973. Dry weight, packed cell volume and optical density. In: *Handbook of phycological methods: culture methods and growth measurements* (Ed. by J.R. Stein), pp. 321–343. Cambridge University Press, Cambridge, UK.

Strid Å., Chow W.S. & Anderson J.M. 1990. Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Biochimica et Biophysica Acta* 1020: 260–268.

Teramura A.H. 1983. Effects of ultraviolet-B radiation on the growth and yield of crop plants. *Physiologia Plantarum* 58: 415–427.

Tevini M., Iwanzik W. & Thoma U. 1981. Some effects of enhanced UV-B on the growth and composition of plants. *Planta* 153: 388–394.

van de Poll W.H., Eggert A., Buma A.G.J. & Breeman A.M. 2001. Effects of UV-B–induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. *Journal of Phycology* 37: 30–37.

Vincent W.F. & Neale P.J. 2000. Mechanisms of UV damage to aquatic organisms. In: *The effects of UV radiation in the marine environment* (Ed. by S. de Mora, S. Demers & M. Vernet), pp. 149–176. Cambridge University Press, Cambridge, UK.

Wiencke C., Bartsch I., Bischoff B., Peters A.F. & Breeman A.M. 1994. Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Botanica Marina* 37: 247–259.

Wiencke C., Gómez I., Pakker H., Flores-Moya A., Altamirano M., Hanelt D., Bischof K. & Figueroa F.-L. 2000. Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Marine Ecology Progress Series* 197: 217–229.

Yabe K., Makino M. & Susuki M. 1997. Growth-inhibition on gametophytes of *Laminaria religiosa* induced by UV-B radiation. *Fisheries Science* 63: 668–670.

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Publication 3

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Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation.

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ORIGINAL PAPER

Michael Y. Roleda · Dieter Hanelt · Christian Wiencke

Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation

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Abstract Young sporophytes of *Saccorhiza dermatodea* and *Alaria esculenta* cultured from Spitsbergen isolates were exposed in the laboratory to either only photosynthetically active radiation (PAR) or to a spectrum including UV-radiation (PAR + UVA + UVB) by use of cutoff glass filters. The plants were grown at 8 ± 2°C and 16:8 h light–dark cycles with 6 h additional UV exposure in the middle of the light period. Growth was measured every 10 min using growth chambers with online video measuring technique for 18–21 days. Tissue morphology and absorption spectra were measured in untreated young sporophytes while tissue chlorophyll-*a* content and DNA damage were measured from treated thalli at the end of the experiment. Under UVR, growth rates of *S. dermatodea* were significantly reduced while *A. esculenta* have a potential to acclimate. Tissue chlorophyll-*a* contents in both species were not significantly different between treatments suggesting that these algae may acclimate to moderate UVR fluence. Higher DNA damage in *S. dermatodea* effectively diverted photosynthetic products for repair constraining growth. Tissue optics (opacity and translucence) was correlated to the tissue absorbance in the UVR region characteristics of phlorotannin, an important UV-absorbing compound in brown macroalgae. Growth rates of sporophytes of both species exposed to PAR without UV was similar during day and night. The results showed that both species can recruit and inhabit a similar coastal zone when appropriate strategies are expressed to minimize damage in response to the stress factor.

M. Y. Roleda (✉)
Alfred Wegener Institute for Polar and Marine Research,
Biologische Anstalt Helgoland, 27498 Helgoland, Germany
E-mail: mroleda@awi-bremerhaven.de
Tel.: +49-4725-819230
Fax: +49-4725-819283

M. Y. Roleda
Biology Department, De La Salle University,
2401 Taft Avenue, 1004 Manila, Philippines

D. Hanelt
Biozentrum Klein Flottbek, University of Hamburg,
Ohnhorststr. 18, 22609 Hamburg, Germany

C. Wiencke
Foundation Alfred Wegener Institute for Polar and Marine
Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Introduction

Saccorhiza dermatodea (Bachelot de la Pylaie) J. Agardh has a circumboreal distribution. It is found throughout the Arctic Ocean and extends down the northeastern seaboard of North America. *Alaria esculenta* (Linnaeus) Greville, on the other hand, is found in the North Atlantic as far south as the 16°C summer isotherm on the Brittany coast and extends north towards the Arctic (Lüning 1985). In Spitsbergen, *S. dermatodea* grows in the upper sublittoral while *A. esculenta* grows in the upper-mid sublittoral (Wiencke et al. 2004). However, they can also be found co-inhabiting a depth from 3 m to 10 m (Bischof et al. 1998).

Stratospheric ozone depletion is not only reported over Antarctica (Solomon 1999; Staehelin et al. 2001) but also over the Arctic (Jokela et al. 1993; Müller et al. 1997; Rex et al. 1997, 2002), which leads to enhanced levels of Ultraviolet-B radiation (UVBR) at the earth's surface. In aquatic ecosystems, reflection and attenuation within the water body reduces irradiance levels and alters spectral composition. However, biologically significant UVBR levels are still recorded down to 8-m water depth in Kongsfjorden (Hanelt et al. 2001).

Exposure to UV radiation (UVR) can significantly affect macrophyte productivity and ecophysiology (Franklin and Forster 1997). In particular, it can induce damage directly by photochemical degradation of biomolecules and cause DNA lesions forming cyclobutane-pyrimidine dimers (CPDs) or indirectly via the production of reactive oxygen species such as hydrogen peroxide and superoxide radicals, responsible for the more widespread oxidative damage within the cell

(Vincent and Neale 2000). The resulting stress is manifested in increased energy demands for protection and repair, a decrease in growth rate resulting from the photochemical damage and disruption of cell metabolism and cell division, and under severe exposures, in mortality of the specimen.

UVR sensitivity of macrothalli of Laminariales is well documented (e.g. Clendennen et al. 1996; Aguilera et al. 1999; Karsten et al. 2001; Bischof et al. 2002a; Apprill and Lesser 2003). Most of these studies measured photosynthetic responses to UVR. In several studies (e.g. Hanelt et al. 1997a, b), sensitivity and recovery were found to be related to the depth distribution of the species while capability of acclimation is reported in upper sublittoral species (e.g. Bischof et al. 1998, 1999). However, acclimation of photosynthesis to UVR exposure cannot be equated to the ecological optimum of the plant because photosynthetic products are used for protection and repair, consequently constraining growth. For example, photosynthesis of *Laminaria ochroleuca* de la Pylaie is able to partially acclimate to chronic UVR exposure, but growth does not (Roleda et al. 2004a).

Different life stages of Laminariales are reported to be differentially susceptible to light stress (e.g. Dring et al. 1996; Hanelt et al. 1997c), whereby early developmental life stages are reported to be the most susceptible to UVR compared to the adult stages (reviewed by Coelho et al. 2000). For example, zoospore motility, germination capacity and DNA are affected under UVR (e.g. Huovinen et al. 2000; Wiencke et al. 2000, 2004; Makarov and Voskoboinikov 2001). A protective mechanism against UVR includes synthesis of the UV-absorbing compound phlorotannin (Schoenwaelder 2002; Wiencke et al. 2004).

Across a vertical gradient, growth of young sporophytes of the upper sublittoral *S. dermatodea* (Aguilera et al. 1999) and upper-mid sublittoral *A. esculenta* (Michler et al. 2002) is reported to be inhibited by UVR. Macroalgae growing in shallower depth are often more tolerant to UV and recover well after periods of high UV radiation. Morphologically, species with tougher and thicker thalli may also be less sensitive to UVR as a result of more protective tissue (Johansson and Snoeijs 2002).

In the present study, we compare growth kinetics of cultured young sporophytes under controlled laboratory condition and the potential for growth acclimation under UVR between *S. dermatodea* and *A. esculenta* using growth chambers with an automatic video growth measuring technique. Growth in terms of increase in pixel size will be measured at short time scales (e.g. every 10 min) over a 3-week period. Data obtained from these measurements will allow us to quantify growth during and after UV exposures or during light and dark phases of the daily photoperiod. DNA damage accumulation and pigment content will also be measured after repeated exposure to UVR. The effect of UVR on growth will be discussed in relation to thallus morphology, tissue absorption spectra characteristics of UV-absorbing compounds and depth distribution of the sporophytes in the field.

Materials and methods

Algal material

Cultures of *S. dermatodea* and *A. esculenta* gametophytes (hereinafter called '*Alaria*' and '*Saccorhiza*' respectively), originally established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden, Spitsbergen (78°55′N, 11°56′E), were used to obtain young sporophytes. They were grown aerated in 5 l culture bottles filled with Provasoli enriched seawater (Starr and Zeikus 1993) inside a temperature-controlled room at 8 ±2°C and 10 μmol photon m^{-2} s^{-1} white light at 12:12 h light:dark photoperiod. To compare morphological differences between cultured and wild specimen, young sporophytes were collected by SCUBA diving in May 2004 in Kongsfjorden.

Online video growth measurement technique

Three growth chambers (ISITEC GmbH, Bremerhaven, Germany) with online video measuring technique described by Roleda et al. (2004a) were operated simultaneously in the experiment (Fig. 1a). Each growth chamber contained two basal pieces of thalli (average size = 30 mm^2, ±6 mm of the phylloid including the meristem), positioned 20-mm apart from each other. They were fixed using tiny nails between the top and bottom of the UV-transparent Plexiglas chamber (Fig. 1b,c). The space between the top and bottom chambers allows circulating filtered, pasteurized and cooled seawater (8 ± 2°C) to pass through. Light sources are mounted 15 cm above the platform (Fig. 1d) consisting of two white fluorescent lamps (Philips, TL 8 W/965, Holland) and two UV lamps (Q-Panel UVA-340, 40 Watt, Cleveland, USA; modified from the figure). Infrared diodes (wavelength = 930 nm) are mounted at the sides of the chamber to produce infrared images of the object for the video camera, also during the dark periods. The captured image by the CCD camera (Fig. 1e) is analysed by a MedeaLAB Count and Classify software (Fig. 1f; Multimedia and Software GmbH, Erlangen, Germany) which measures growth, in terms of increased number of pixel, of the algae.

The algal samples were acclimated for 3 days inside the growth chambers to 16:8 light-dark photoperiod without UVR. After acclimation, UVR was supplemented in the middle of the light phase (0900–1500 H). Two types of glass filters, Schott-GG 400 (Schott, Germany) and Quartz glass, were put on the top of the Plexiglas chamber, each covering one of the two algal samples, respectively. This corresponds to two treatments per chamber with photosynthetically active

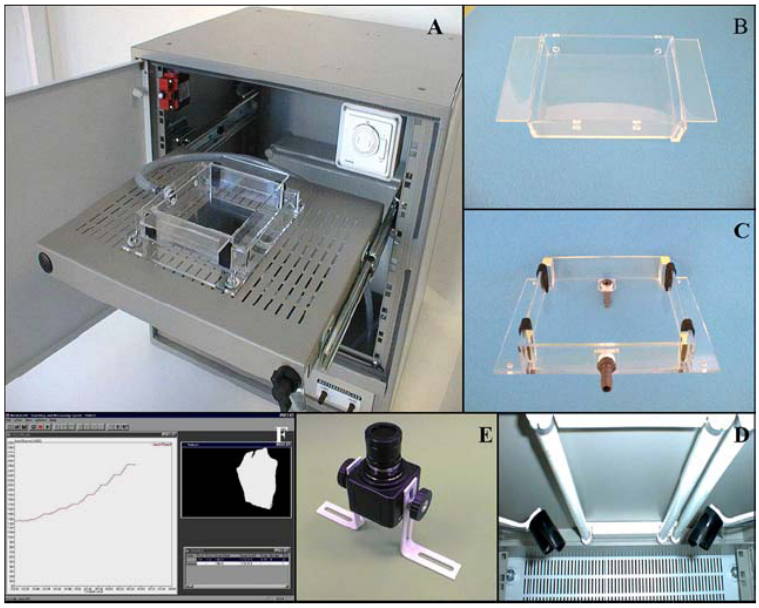

Fig. 1 a–f ISITEC growth chamber (**a**) showing UV-transparent Plexiglas chambers where the algae is fixed between the top (**b**) and bottom (**c**) of the chambers. Seawater inflow and outflow outlets are shown in the bottom part of the Plexiglas chamber. Lamps and infrared diodes (**d**) are mounted above the platform and on the sides of the growth chamber, respectively. Image captured by the CCD camera (**e**) is analysed by MedeaLAB Count and Classify software, a measurement for one thallus piece is shown in (**f**)

radiation (PAR = P) and PAR + UVA + UVB (PAB) treatment. The three growth chambers operated simultaneously per species, were used as replicates for the P and PAB treatments.

Irradiance was measured using a scanning UV-VIS spectrometer (M. Kruse, Bremerhaven, Germany) below the glass filters. Weighted irradiance was calculated using action spectra for well-known biological responses: DNA damage for *Escherichia coli* (280–320 nm, Setlow 1974) and the generalized plant damage (280–312 nm) described by Caldwell (1971). Both unweighted and weighted irradiances for each treatment are compiled in Table 1. Growth was continuously measured every 10 min for 18–21 days. Seawater was changed weekly to ensure enough nutrient supply within the medium. At the end of each growth experiment, algal thalli were transferred to 2-ml Eppendorf tubes and frozen at −80°C for further chemical analyses.

Growth rate was computed by plotting all data points (entire experimental period) of each replicate per treatment. They were individually fitted to an exponential equation $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase. Growth rate at time t (rt) is comparable to the growth equation applied by Lüning (1979): relative growth rate (% day^{-1}) = (ln SA_2 − ln SA_1)/ $t_2 - t_1 \times 100$, where SA_1 and SA_2 are the surface areas at t_1 and t_2 in days, respectively. To determine day and night growth rates, daily growth rates (% h^{-1}) during the light (16 h) and dark phase (8 h) of plants exposed to photosynthetically active radiation (P) alone were computed using the same exponential equation. Mean day and night growth rates were computed every three days. Among sporophytes exposed to PAB (PAR + UVA + UVB), circadian growth rates and three days average (% h^{-1}) were computed during the start of the light phase without UV (0500–0900 h), in the middle of the light phase with UV supplement (0900–1500 h), at the end of the light phase without UV (1500–2100 h), and during the dark phase (2100–0500 h).

Thallus morphology

The wet weight: surface area (mg:mm^2) ratio was used to determine thallus thickness (Roleda et al. 2004b).

Table 1 Unweighted and weighted irradiance applied in the growth chambers

	Irradiance (W m^{-2})			
	Unweighted		Weighted	
	GG 400 (PAR alone)	Quartz (PAR + UVA + UVB)	DNA damage (Setlow 1974)	Generalized plant damage (Caldwell 1971)
PAR (400–700 nm)	3.60	4.00	0	0
UVA (320–400 nm)	0.02	4.92	0	0
UVB (280–320 nm)	0.00	0.41	9.76×10^{-3}	3.16×10^{-2}
Total irradiance	3.62	9.33	9.76×10^{-3}	3.16×10^{-2}

Surface area of sporophytes taken directly from the unialgal cultures was measured using a scanner and WinFolia 5.0 image analysis software (Regent Instrument, Quebec City, Canada) and the corresponding wet weight of the blotted tissue was weighed using Sartorius (CP225D, Germany) weighing scale ($n = 10$).

Absorbance spectrum

To determine the presence of UV-absorbing compounds in the tissue, young sporophytes obtained from culture were inserted into seawater-filled quartz cuvettes and scanned with seawater as reference in the 250–700 nm waveband using a Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere. For comparison, young sporophytes collected in the field in Spitsbergen were also scanned.

Pigment extraction and characterisation

Frozen samples were treated with 100 µl of 100% *N*-*N*-dimethylformamide and stored in darkness for approximately 16 h. Subsequent analyses were performed using HPLC as described by Bischof et al. (2002b).

DNA extraction and quantification

Thalli were homogenized in liquid nitrogen and DNA was isolated following the CTAB extraction procedure described by Poll et al. (2001) and modified by Roleda et al. (2004b). After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 µl 10 mg ml^{-1}, 30 min, 37°C; Sigma, MO) and stored at −20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, CA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection

The immunoassay for CPDs was modified after Vink et al. (1994) and Poll et al. (2001). Heat-denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 µm, Schleicher Schuell). After a two-step antibody assay, the membrane was treated with ECL Western blotting detection reagent (Amersham Buckinghamshire, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) at different exposure time. The films were developed using X-ray film developer. Developed films were scanned using a Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 µg ml^{-1} DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis

The statistical significant differences between treatments (P versus PAB) and between species (*Saccorhiza* versus *Alaria*) were tested using *t*-test ($P < 0.05$) using SPSS software (SPSS, Chicago, IL, USA).

Results

The intrinsic rate of increase (r) in the surface area of both species exposed to P was higher compared to sporophytes exposed to PAB (Fig. 2). However, a significant effect of the treatment was only observed in the growth rate of *Saccorhiza* ($P = 0.006$) while growth rate of *Alaria* was not significantly different between treatments ($P = 0.275$; inset Fig. 2). Among sporophytes exposed to P only, average growth rates during the light and dark phase of the photoperiod was not different in *Alaria* throughout the whole experimental period while slightly higher growth rates during the light phase were observed in *Saccorhiza* from the 15th to the 18th day of measurement (Fig. 3). Among sporophytes exposed to the full light spectrum (PAB), a circadian growth pattern was observed (Fig. 4). Growth rates were mostly lowest in the morning (0500–0900 h) and at the middle of the light phase with UVR supplement (0900–1500 h). Growth rates increased in both species after UVR was switched off in the afternoon and during the dark phase in *Alaria* while variable growth pattern was observed during the dark phase in *Saccorhiza*. Comparison between species showed no significant difference in growth rates at the start of the light phase (0500–0900 h). In the middle of the light phase with UVR supplement, significant difference between species was only observed on the 18th day where growth rate of *Saccorhiza* was significantly depressed ($P < 0.05$). The same trend was observed at the end of the light phase (1500–2100 h) and at night, when *Alaria* showed significantly higher growth rates ($P < 0.05$). Time series observation on the growth rates of sporophytes during UVR exposure (0900–1500 h) showed acclimation in the growth rate of *Alaria* and continuous decrease in the growth rate of *Saccorhiza* (Fig. 5).

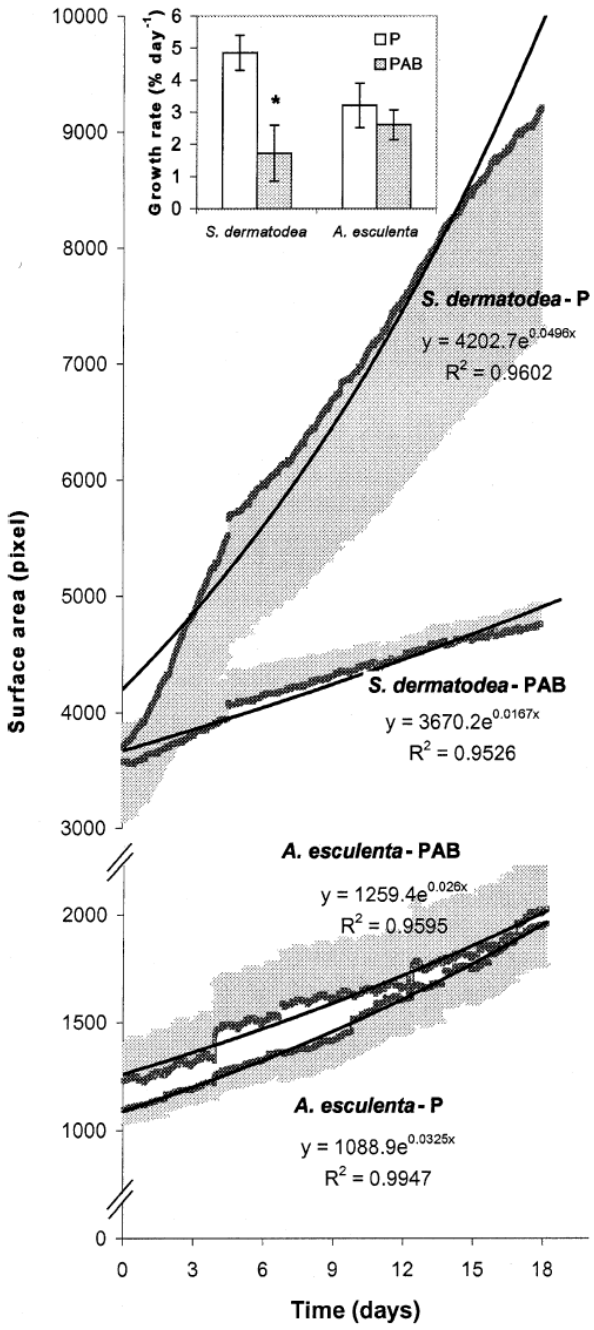

Fig. 2 Growth, in terms of surface area increase, in young sporophytes exposed to different irradiances of photosynthetically active radiation (PAR = P) and PAR + UVA + UVB(= PAB). PFD was ±20 µmol m^{-2} s^{-1}. Data points (*irregular dark gray points*) are fitted using the exponential growth equation $N_t = N_0 e^{rt}$ (*dark solid curves*), where N_t is growth at time t and N_0 is initial size. Values are means ± SD (*light gray one sided area*, $n = 3$). Inset is the corresponding growth rates for the entire experimental period. * refers to significant differences between mean values (*t*-test, $P < 0.05$)

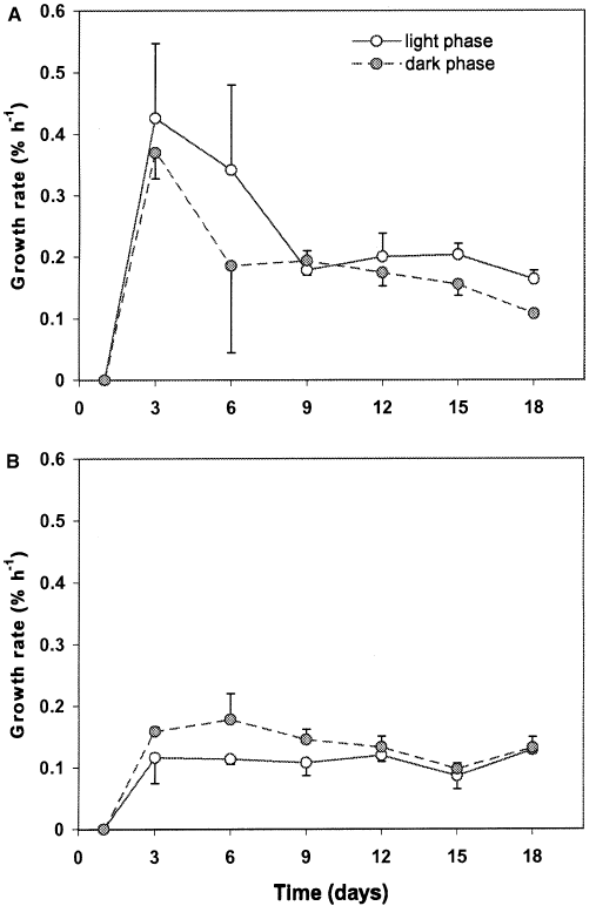

Fig. 3 a, b Growth rates of young sporophytes exposed to photosynthetically active radiation (PAR = P) during the light (16 h) and dark phase (8 h) of the photoperiod in (**a**) *Saccorhiza dermatodea* and (**b**) *Alaria esculenta*

Cultured sporophytes grown in low white light without UVR showed that *Saccorhiza* had thicker thalli compared to *Alaria* ($P < 0.0001$; Fig. 6a). Different growth forms were observed between wild and cultured sporophytes (insets Fig. 7a,b). The collected wild *Saccorhiza* specimen already exposed to naturally high solar radiation was more robust and pigmented compared to the cultured specimen. *Alaria* cultured at daylength of 12 or 16 h was darkly pigmented and opaque compared to the translucent wild specimen. When transferred from 12 h to 24 h daylength, the thalli become lightly pigmented and translucent after 2 weeks (Fig. 7b). However, cultured *Alaria* manifest broader thalli compared to the more tapered wild specimen with a very distinct midrib. Significantly higher chlorophyll-*a* concentration was measured in *Alaria* compared to *Saccorhiza* ($P < 0.05$). Comparison between treatments, however, showed only slightly and insignificantly higher chl *a* concentration in sporophytes exposed to PAB compared to P (Fig. 6b). DNA damage measured as the number of

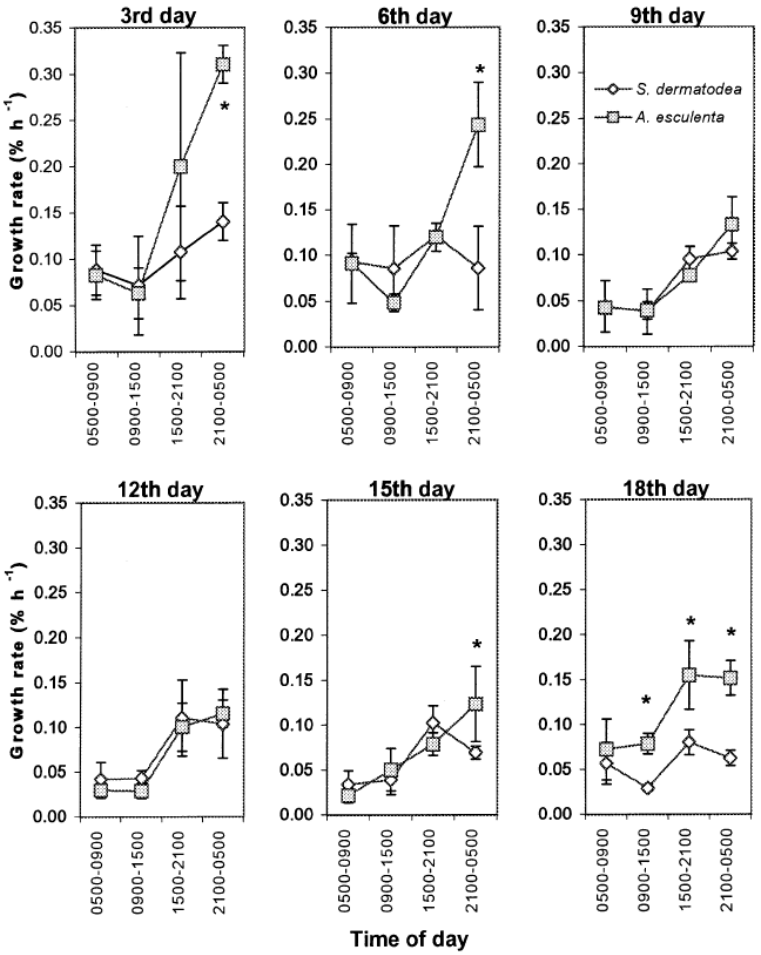

Fig. 4 Average circadian growth pattern of young sporophytes exposed at the start of the light phase to PAR without UV (0500–0900 h); in the middle of the light phase with UV supplement (0900–1500 h); at the end of the light phase again without UV (1500–2100 h); and during the dark phase (2100–0500 h). * refers to significant differences between mean values (*t*-test, $P < 0.05$)

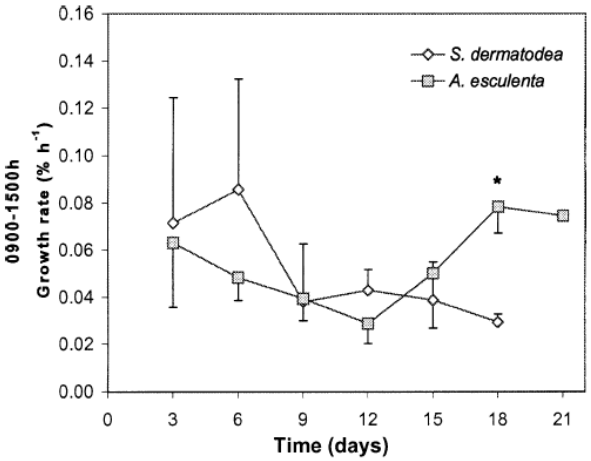

Fig. 5 Average growth rates monitored over a period of 21 days in young sporophytes of *Saccorhiza dermatodea* and *Alaria esculenta* during UVR exposure (0900–1500 h). * refers to significant differences between mean values (*t*-test, $P < 0.05$)

cyclobutane pyrimidine dimers (CPDs) showed significantly higher DNA damage induction and CPDs accumulation in *Saccorhiza* compared to *Alaria* ($P < 0.0001$; Fig. 6c). Spectral analysis of tissue showed different absorbance between the wild and cultured young gametophytes. The absorbance maxima observed in the UVR region are characteristic for the phlorotannin absorption spectrum. Strong absorption below 280 nm was measured in the wild *Saccorhiza* compared to the cultured sporophytes (Fig. 7a). Cultured *Alaria* grown at 12 h and experimentally exposed to 16 h daylength were opaque. These samples showed a very strong absorption below 360 nm while collected wild and cultured sporophytes grown at 24 h daylight showed similar but much lower absorbance spectra with maximal absorbance below 280 nm (Fig. 7b).

Discussion

Potential for growth acclimation in young sporophytes to UVR was found to be specific for one of the inves-

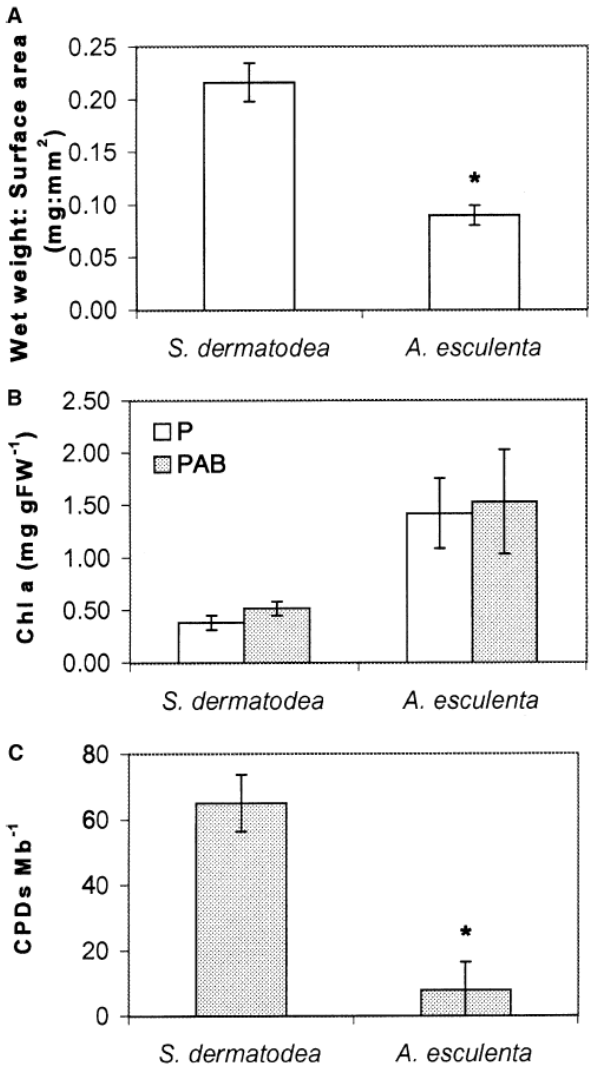

Fig. 6 a–c Morphological and biochemical characteristics in young sporophytes of *Saccorhiza dermatodea* and *Alaria esculenta* where (**a**) Surface area: wet weight ratio to determine thallus thickness of cultured sporophytes ($n = 10$); (**b**) Chlorophyll-*a* content in sporophytes exposed to photosynthetically active radiation (PAR) = P and PAR + UVA + UVB = PAB ($n = 3$); (**c**) UVB-induced DNA damage measured as cyclobutane pyrimidine dimers (CPDs) concentrations per million nucleotides ($n = 3$). *Vertical bars* are standard deviations (SD). * refers to significant differences between mean values (*t*-test, $P < 0.05$)

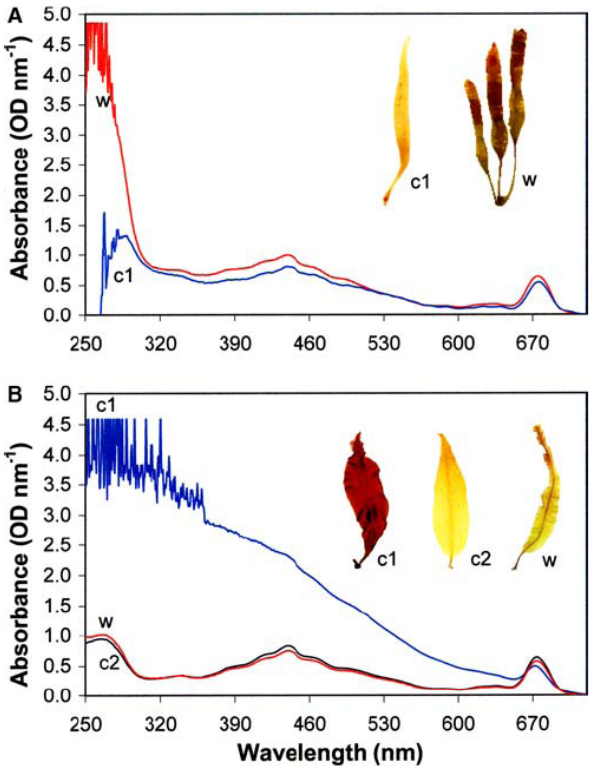

Fig. 7 a,b Tissue absorbance spectrum of cultured (c1 = 12–16 h daylength; c2 = 24 h daylength) and wild (w) young sporophytes in (**a**) *Saccorhiza dermatodea* and (**b**) *Alaria esculenta*. The insets show photographs of the typical morphology of thalli used for the experiments

tigated species and correlated to tissue DNA damage induction and CPDs accumulation while tissue chlorophyll-*a* contents in both species were able to acclimate to moderate UVR fluence. Higher DNA damage diverts photosynthetic products for repair, thus constraining growth. Moreover, we found that thallus optical property was correlated to the tissue absorbance in the UVR region, characteristic of phlorotannin, an important UV-absorbing compound in brown macroalgae. This study showed that appropriate stress protective response such as synthesis of phlorotannin, and efficient DNA damage repair mechanism can enhance survival of young recruits and may be a factor for the determination of the upper distribution range of a species.

Previous studies on the same species have shown that photosynthesis of *Alaria* acclimate to UVR (Bischof et al. 1999) while photosynthesis of *Saccorhiza* is photoinhibited under full solar spectrum (Karsten et al. 2001). In our study, we found that growth responds similarly to photosynthesis. Outdoor incubation of *Saccorhiza* to the natural solar radiation also showed the same growth inhibition under UVR (Aguilera et al. 1999). On the other hand, previous study on the growth of *Alaria* under laboratory condition showed contrasting result where significantly lower growth rate was observed in sporophytes exposed to UVR (Michler et al. 2002). This is attributed to the different parts of the sporophytes used in the experiment. In the present study, we used acclimated meristematic basal parts of the young sporophytes while Michler and co-workers used cut off pieces from the meristem of the blade, making the wounded tissue more susceptible to UVR.

UVB-R induced DNA damage can be repaired through photoreactivation or excision repair (Karentz 1994; Pakker et al. 2000a, b; Poll et al. 2002). However,

when DNA damage rate is higher, photosynthetic products will be diverted to cellular repair at the expense of growth and reproduction. Difference in thallus morphology, such as thickness, maybe regarded as protective mechanism against both high PAR and UVR in macroalgae (Dring et al. 1996; Hanelt et al. 1997c; Johansson and Snoeijs 2002). However, CPDs concentration was higher in the thicker *Saccorhiza* thallus suggesting that DNA damage rate exceeds repair rate in this species. Thallus thickness in itself is therefore not sufficient to minimize deleterious UVR effects. The optical property of the thallus is also important, which can influence reflection, attenuation, scattering, absorption or transmittance of UV radiation in plant tissues (Caldwell et al. 1983). To prevent UVR induced damage, many macroalgae produce UV screening compounds such as phlorotannins in brown macroalgae (Pavia et al. 1997; Pavia and Brock 2000; Schoenwaelder 2002; Henry and Van Alstyne 2004). In comparison to isolated phlorotannins from *Fucus gardneri* (Henry and Van Alystyne 2004), the absorbance spectrum of *Alaria* and *Saccorhiza* tissues showed a similar strong absorption in the UV-C and UV-B region. In terrestrial plants, wavelength selectivity of absorption in leaf epidermis is very pronounced and is often attributed to flavonoids and other related UV-absorbing compounds (Robberecht and Caldwell 1978). Phenolic compounds generally have substantial UV absorbance and very little absorption in the visible waveband (Caldwell et al. 1983).

Trade-offs between phlorotannin production and growth were observed in *Ascophyllum nodosum* (Pavia et al. 1999). Although phlorotannin concentration was not quantitatively analyzed in our study, the absorbance maxima in the UVR region of the tissues are characteristic of the phlorotannin absorption spectrum, showing higher concentration in cultured *Alaria* compared to *Saccorhiza*. The physiological cost of producing this UV-absorbing compound may be seen in the lower growth rate of *Alaria* compared to *Saccorhiza*. *Alaria* grown at 24 h daylength simulating polar day increased their growth rate to $11 \pm 3\%$ day^{-1} (data not shown) compared to only $3 \pm 1\%$ day^{-1} growth rate (Fig. 1) at 16:8 h light–dark photoperiod. An increase in growth rates to longer photoperiod was previously reported in *A. esculenta* from the British Isles (Han and Kain 1996).

The wild young *Saccorhiza* sporophytes, which were collected during the polar day in May in the upper sublittoral (4 m), were already exposed to higher natural solar UV radiation compared to wild young *Alaria* sporophytes, which were collected in the mid sublittoral (5–6 m) under the canopy of adult plants. At this point, wild young *Saccorhiza* sporophytes have already responded to the environmental cue by means of physiological mechanism to reduce stress such as synthesizing UV-absorbing compounds as shown in the darkly pigmented thallus of the wild isolate and the corresponding strong tissue absorption in the UVR region. On the other hand, recruitment of *Alaria* under the canopy of adult plants is thought to be an adaptive behavior which effectively protects these early life stages from stress factors, therefore minimizing ecological cost for defense, enabling the juvenile plants to allocate more photosynthate for growth (Herms and Mattson 1992). The expression of dark pigmented *Alaria* phenology in culture (12 or 16 h daylength of low white light, 10–20 μmol photon m^{-2} s^{-1}) is speculated to be some kind of physiological mechanism (Roy 2000) related to the seasonal variation of daylength. It is typical for the so called season anticipators (Kain 1989). In these species, seasonal development is finely tuned to the seasonal variation of daylength and this strategy is evident from the organism to the cellular and metabolic level (Wiencke 1990a, b; Lüder et al. 2001, 2002; Dummermuth and Wiencke 2003). When unprotected juvenile recruits (such as in our unialgal culture) start growing during the short daylength (e.g. at the end of the polar night, before the start of polar day in the field), we hypothesize that the young sporophytes are investing UV-absorbing compounds in anticipation of the polar days when they could be potentially exposed to enhanced solar radiation. When we increased the daylength to 24 h depleted of UVR, the plants responded by growing faster and stopped synthesizing UV-absorbing compounds as shown in the decreased tissue absorbance in the UVR region supporting our hypothesis.

In zoospores, phlorotannin containing physodes was observed in both species. Enlargement of these phenolic vesicles was observed after 20 h exposure to PAR + UVA + UVB, which provide protective function against UVR (Wiencke et al. 2004). More prominent physodes were observed in *Saccorhiza* compared to *Alaria* spores, which consequently enhanced germination rate in *Saccorhiza* (Wiencke et al. 2004). Allocation of phenolic compounds seems to be dependent on life stage and life history of the plant. However, among 1-μm- to <10-μm-size class cells, production of UV-absorbing compounds can afford considerable benefits but only at the expense of relatively heavy energetic investment (Garcia-Pichel 1994). Phlorotannin synthesis in juvenile recruits lowers growth rate but could enhance survival of the young sporophytes. In adult sporophytes, higher concentrations of phenolic compounds are found in the reproductive fronds (sporophylls) than in the vegetative blades of the intertidal *A. marginata* Postels & Ruprecht (Steinberg 1984) where sporogenic tissue could provide protection to zoospore containing sporangium against environmental stress factors. A conceptual model of the evolution of plant defense suggest that plant physiological trade-offs (e.g. allocation of photosynthate for repair, growth and reproduction, synthesis of UV-absorbing compounds and secondary metabolites) interact with the abiotic environment, competition and herbivory (Herms and Mattson 1992).

Seasonal variation in pigment concentration of Arctic *Saccorhiza* collected before, during and after sea ice break-up showed decreasing chlorophyll-*a* concentration coinciding with the increasing underwater radiation (Aguilera et al. 2002). In the laboratory, we observed no

significant variation in Chl-*a* concentration between sporophytes exposed to the whole spectrum and to PAR alone. This could be attributed to the higher natural solar radiation at 3–4 m depth where Aguilera and coworker collected their algal material. In *Ulva rigida* C. Agardh, however, significantly higher pigment content (Chl *a*, Chl *b* and carotenoids) was measured in the presence of UVB, which is speculated to be some kind of photoprotective mechanism (Altamirano et al. 2000). In *Palmaria decipiens* (Reinsch) Ricker, chlorophyll-*a* concentration increases after 4 h of exposure to UVR. Further exposure to 12–16 h of UVR showed no significant variation between treatments (Poppe et al. 2002). This indicates the capability of algae to acclimate to moderate fluences of UV radiation.

Circadian (daily) rhythm in gene expression, cell division timing, photosynthesis and growth has been reported in algae (e.g. Lüning 1994a, 2001; Granbom et al. 2001; Suzuki and Johnson 2001; Jacobsen et al. 2003). Seasonal growth pattern in Laminariales is shown to be environmentally as well as internally controlled (Lüning 1993, 1994b). On a shorter time scale, day and night kinetics (diurnal pattern = within 24 h) is also observed (Lüning 1992). Among Laminariales, study on growth kinetics has been performed in *Laminaria* spp. and *Pterygophora californica* Ruprecht, but not on *A. esculenta* and *S. dermatodea*. Maximal growth rate of 0.7% h^{-1} is reported in young *Laminaria saccharina* (Linnaeus) J.V. Lamouroux sporophytes (Lüning 1992), higher than the $0.42 \pm 0.1\%$ h^{-1} growth rate that we observed in *Saccorhiza*. Lüning (1994a) further reported an immediate decrease in growth rate after the onset of darkness. This contradicts our study, which showed no significant difference in the growth rate between day and night in *Alaria* while lower growth rate at night was only observed later (day 15–18) in *Saccorhiza*. Apical growth of *Ascophyllum nodosum* and *Chondrus crispus* during the night makes up 33% and 63% of their average day growth rates, respectively (Strömgren and Nielsen 1986). In UVR-exposed *Alaria* sporophytes, higher growth rates even occurred during the dark phase, compared to the early morning and late afternoon light phase without UVR. It has been hypothesized that the driving force for the early evolution of circadian clocks could be an inherent advantage in phasing cellular events that are inhibited by sunlight to occur in the night (Suzuki and Johnson 2001). If this regulation by a circadian clock is adaptive, growth during the day in the absence of UV stress may be enhanced equal to but not greater than the growth at night.

Growth kinetics is thought to be species-specific, otherwise the contradicting result could be attributed to technical limitations of the image analysis software previously used by Lüning or to the interpretation of data wherein the author interpreted thallus area as 'single projected area' (Lüning 1992) and not as the total surface area. Our advanced image analysis software is able to measure the whole surface area continuously in the course of several days without interruption. Measurements are manually stopped for a maximum of 2 h to change the seawater in the circulating system every week.

For future studies, an in situ growth kinetics measurement is recommended. Prototypes of field growth chamber with similar growth measuring technique are currently developed for future use. Synergistic effects of abiotic factors will bring new insights on how growth mechanics operate in the field. The single factor laboratory experiment performed in this study has shown that growth is inhibited by UVR. Recovery of growth was observed after the offset of the stress factor and growth acclimation under UVR was observed in *Alaria*. UVR sensitivity in the growth rate of *Saccorhiza* is attributed to the accumulation of tissue DNA damage and the lower UV-absorbing compound concentration in cultured sporophytes. The potential of some species to acclimate to increased solar radiation is indicated by the changes in the optical properties of the leaf (Caldwell et al. 1983) or in the thallus of macroalgae as exhibited by *Alaria*. In the field, both species are capable of recruiting the upper sublittoral when appropriate physiological strategies are expressed to minimize UV-induced damage, as adult sporophytes could be found to inhabit the same coastal zone in Spitsbergen.

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References

Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. Mar Ecol Prog Ser 191:109–119

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087–1095

Altamirano M, Flores-Moya A, Figueroa F-L (2000) Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated in situ. Bot Mar 43:19–26

Apprill AM, Lesser MO (2003) Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine. Mar Ecol Prog Ser 256:75–85

Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). Polar Biol 20:388–395

Bischof K, Hanelt D, Wiencke C (1999): Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol 1:435–444

Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002a) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. Mar Biol 140:1097–1106

Bischof K, Kräbs G, Wiencke C, Hanelt D (2002b) Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 215:502–509

Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology. Academic, New York, pp 131–177

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Plant 58:445–450

Clendennen SK, Zimmerman RC, Powers DA, Alberte RS (1996) Photosynthetic response of giant kelp *Macrocystis pyrifera* (Phaeophyceae) to ultraviolet radiation. J Phycol 32:614–620

Coelho SM, Rijstenbil JW, Brown MT (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. J Aquat Ecosyst Stress Recovery 7:317–333

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

Dummermuth AL, Wiencke C (2003) Experimental investigation of seasonal development in six Antarctic red macroalgae. Antarct Sci 15:449–457

Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32: 207–232

Garcia-Pichel F. (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol Oceanogr 39:1704–1717

Granbom M, Pedersén M, Kadel P, Lüning K (2001) Circadian rhythm of photosynthetic oxygen evolution in *Kappaphycus alvarezii* (Rhodophyta): dependence on light quantity and quality. J Phycol 37:1020–1025

Han T, Kain J.M. (1996). Effect of photon irradiance and photoperiod on young sporophytes of four species of the Laminariales. Eur J Phycol 31:233–240

Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997a) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255–266

Hanelt D, Wiencke C, Nultsch W (1997b) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J Photochem Photobiol B Biol 38:40–47

Hanelt D, Wiencke C, Karsten U, Nultsch W (1997c) Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). J Phycol 33:387–395

Hanelt D, Tüg GH, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. Mar Biol 138:649–658

Henry BE, Van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. J Phycol 40:527–533

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335

Huovinen PS, Oikari AOJ, Soimasuo MR, Cherr GN (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. Photochem Photobiol 72:308–313

Jacobsen S, Lüning K, Goulard F (2003) Cicadian changes in relative abundance of two photosynthetic transcripts in the marine macroalga *Kappaphycus alvarezii* (Rhodophyta). J Phycol 39:888–896

Johansson G, Snoeijs P (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar Ecol Prog Ser 244:63–72

Jokela K, Leszczynski K, Visuri R (1993) Effects of Arctic ozone depletion and snow on UV exposure in Finland. Photochem Photobiol 58:559–566

Kain JM (1989) The seasons in the subtidal. Br Phycol J 24:203–215

Karentz D (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: Measurements and Biological effects (Antarctic Research Series no. 62). American Geophysical Union, Washington DC, pp 93–110

Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters followed by exposure to natural solar radiation. Oecologia 127:11–20

Lüder UH, Knoetzel J, Wiencke C (2001) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biol 24:598–603

Lüder UH, Wiencke C, Knoetzel J (2002) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta): a study to simulate Antarctic winter sea ice cover. J Phycol 38:904–913

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Mar Ecol Prog Ser 1:195–207

Lüning K (1985) Meeresbotanik: Verbreitung, Ökophysiologie und Nutzung der marine Makroalgen. Georg Thieme Verlag, Stuttgart, pp 375

Lüning K (1992) Day and night kinetics of growth rate in green, brown, and red seaweeds. J Phycol 28:794–803

Lüning K (1993) Environmental and internal control of seasonal growth in seaweeds. Hydrobiologia 260/261:1–14

Lüning K (1994a) Circadian growth rhythm in juvenile sporophytes of Laminariales (Phaeophyta). J Phycol 30:193–199

Lüning K (1994b) When do algae grow? The third founders' lecture. Eur J Phycol 29:61–67

Lüning K (2001) Circadian growth in *Porphyra umbilicalis* (Rhodophyta): spectral sensitivity of the circadian system. J Phycol 37:52–58

Makarov MV, Voskoboinikov GM (2001) The influence of ultraviolet-B radiation on spore resease and growth of the kelp *Laminaria saccharina*. Bot Mar 44:89–94

Michler T, Aguilera J, Hanelt D, Bischof K, Wiencke C (2002) Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. Mar Biol 140:1117–1127

Müller R, Crutzen PJ, Grooß JU, Brühl C, Russel JM, Gernandt H, Mc Kenna DS, Tuck AF (1997) Severe ozone loss in the Arctic during the winter of, 1995–96. Nature 389:709–712

Pakker H, Beekman CAC, Breeman AM (2000a) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). Eur J Phycol 35:109–114

Pakker H, Martins RST, Boelen P, Buma AGJ, Nikaido O, Breeman AM (2000b) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). J Phycol 36:334–341

Pavia H, Brocks E (2000) Extrinsic factors influencing phlorotannin production in the brown alga *Ascophyllum nodosum*. Mar Ecol Prog Ser 193:285–294

Pavia H, Cervin G, Lindgren A, Åberg P (1997) Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. Mar Ecol Prog Ser 157:139–146

Pavia H, Toth G, Åberg P (1999) Trade-offs between phlorotannin production and annual growth in natural populations of the brown seaweed *Ascophyllum nodusum*. J Ecol 87:761–771

Poll WH van de, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. J Phycol 37:30–37

Poll WH van de, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. Photochem Photobiol 76:493–501

Poppe F, Hanelt D, Wiencke C (2002) Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic red alga *Palmaria decipiens* during acclimation to UV radiation. Bot Mar 45:253–261

Rex M, Harris NRP, von der Gathen P, Lehmann R, Braathen GO, Reimer E, Beck A, Chipperfield MP, Alfier R, Allaart M, O'Connor F, Dier H, Dorokhov V, Fast H, Gil M, Kyrö E, Litynska Z, Mikkelsen IS, Molyneux MG, Nakane H, Notholt J, Rummukainen M, Viatte P, Wenger J (1997) Prolonged stratospheric ozone loss in the 1995–96 Arctic winter. Nature 389:835–838

Rex M, Salawitch RJ, Harris NRP, von der Gathen P, Braathen GO, Schulz A, Deckelmann H, Chipperfield M, Sinnhuber B-M, Reimer E, Alfier R, Bevilacqua R,Hoppel K, Fromm M, Lumpe J, Küllmann H,Kleinböhl A, Bremer H, König M, Künzi K, Toohey D, Vömel H, Richard E, Aikin K, Jost H, Greenblatt JB, Loewenstein M, Podolske JR, Webster CR, Flesch GJ, Scott DC, Herman R, Margitan L, Elkins JW, Ray EA, Moore FL, Hurst DF, Romashkin P, Toon GC, Sen BJJ, Wennberg P, Neuber R, Allart M, Bojkov RB, Claude H, Davies J, Davies W, Backer H, de Dier H, Dorokhov V, Fast, H, Kondo Y, Kyrö E, Litynska Z, Mikkelsen IS, Molyneux MJ, Moran E, Murphy G, Nagai T, Nakane H, Parrondo C, Ravegnani F, Skrivankova P, Viatte P, Yushkov V (2002) Chemical loss of Arctic ozone in winter 1999/2000. J Geophys Res 107, D20, 8276. DOI 10.1029/2001JD000533

Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia (Berl.) 32:277–287

Roleda MY, Hanelt D, Kräbs G, Wiencke C (2004a) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycologia 43:603–613

Roleda MY, van de Poll WH, Hanelt D, Wiencke C (2004b) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar Ecol Prog Ser 281:37–50

Roy S (2000) Strategies for the minimisation of UV-induced damage. In: de Mora S, Demers S, Vernet, M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp 177–205

Schoenwaelder MEA (2002) The occurrence and cellular significance of physodes in brown algae. Phycologia 41:125–139

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Nat Acad Sci USA 71:3363–3366

Solomon S (1999) Statospheric ozone depletion: a review of concepts and history. Rev Geophys 37:275–316

Stähelin J, Harris NRP, Appenzeller C, Eberhard J (2001) Ozone trends: a review. Rev Geophys 39:231–290

Starr RC, Zeikus JA (1993) UTEX—the culture collection of algae at the University of Texas at Austin. J Phycol 29 (Suppl.):1–106

Steinberg PD (1984) Algal chemical defense against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata*. Science 223:405–406

Strömgren T, Nielsen MV (1986) Effect of diurnal variations in natural irradiance on the apical length growth and light saturation of growth in five species of benthic macroalgae. Mar Biol (Berl) 90:467–472

Suzuki L, Johnson CH (2001) Algae know the time of day: circadian and photoperiodic programs. J Phycol 37:933–942

Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp 149–176

Vink AA, Bergen-Henegouwen JB, Nikaido O, Baan RP, Roza L (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. Photochem Photobiol 24:25–31

Wiencke C (1990a) Seasonality of brown macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:589–600

Wiencke C (1990b) Seasonality of red and green macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601–607

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa F-L (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar Ecol Prog Ser 197:217–229

Wiencke C, Clayton MN, Schoenwaelder M (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. Mar Biol 145:31–39

Publication 4

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ORIGINAL ARTICLE

Michael Y. Roleda · Christian Wiencke · Dieter Hanelt

Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic *Laminaria* sporophytes

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Abstract Growth of young sporophytes of the brown algae *Laminaria digitata*, *L. saccharina* and *L. solidungula* from Spitsbergen were measured in the laboratory after being exposed for 21 days to either photosynthetically active radiation (PAR = P) or to full light spectrum (PAR + UV-A + UV-B = PAB) using of cutoff glass filters. The plants were grown at 8 ± 2°C and 16 h light : 8 h dark cycles with 6 h additional ultraviolet radiation (UVR) exposure in the middle of the light period. Growth was measured every 10 min using growth chambers with online video measuring technique. Tissue morphology and absorption spectra were measured in untreated young sporophytes while chlorophyll (Chl) *a* content and DNA damage were measured in treated thalli at the end of the experiment. In all species, growth rates were significantly higher in sporophytes exposed to P alone compared to sporophytes exposed to PAB. Tissue DNA damage is dependent on thallus thickness and absorption spectra characteristics of pigments and UV-absorbing compounds. In sporophytes exposed to UVR, energy demands for repair of DNA damage and synthesis of UV-absorbing compounds for protection effectively diverts photosynthate at the expense of growth. Photosynthetic pigment was not significantly different between treatments suggesting a capacity for acclimation to moderate UVR fluence. The general growth pattern in sporophytes exposed to P alone showed an increasing growth rate from the onset of light (0500–0900 hours) to a peak at the middle of the light phase (0900–1500 hours), a decline towards the end of the light phase (1500–2100 hours) and a minimum "low" growth in the dark (2100–0500 hours) relative to growth during the entire light phase. Under PAB, different growth patterns were observed such as growth compensation at night in *L. digitata*, delayed growth recovery in *L. saccharina* and minimal but continuous growth in *L. solidungula*. Growth as an integrative parameter of all physiological processes showed that the effect of UVR is correlated to the depth distribution of these species.

Keywords Cyclobutane pyrimidine dimers (CPDs) · Growth · *Laminaria digitata* · *Laminaria saccharina* · *Laminaria solidungula* · Pigments

Abbreviations PAR: Photosynthetically active radiation · UV-A: Ultraviolet-A · UV-B: Ultraviolet-B · UVR: UV radiation · P: PAR · PAB: PAR + UV-A + UV-B · PFD: Photon flux density · Chl: Chlorophyll

M. Y. Roleda (✉)
Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, 27498 Helgoland, Germany,
E-mail: mroleda@awi-bremerhaven.de
Tel.: +49-4725-819230
Fax: +49-4725-819283

M. Y. Roleda
Biology Department, De La Salle University, 2401 Taft Avenue, 1004 Manila, Philippines

C. Wiencke
Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

D. Hanelt
Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-Str. 18, 22609 Hamburg, Germany

Introduction

The kelp forest of Kongsfjorden, Spitsbergen (Svalbard) is structured by the perennial canopy species *Alaria esculenta* (Linnaeus) Greville, *Laminaria digitata* (Hudson) Lamouroux and *L. saccharina* (Linnaeus) Lamouroux, and the annual *Saccorhiza dermatodea* (Bachelot de la Pylaie) J. Agardh. The endemic Arctic species *L. solidungula* J. Agardh occurs predominantly in the inner zones of the fjord (Hop et al. 2002). The three *Laminaria* species grow in the upper sublittoral (*L. digitata*, 0.5–5 m), in the mid to lower sublittoral (*L. saccharina*, 2–15 m) and in the lower sublittoral (*L. solidungula*, 8–15 m).

Comprehensive comparative ecological studies between different *Laminaria* species from the North Sea have been performed in different life history stages (e.g. Lüning 1979; Dring et al. 1996; Roleda et al. 2005a). Studies on the three *Laminaria* species in Spitsbergen are fragmentary except for the studies on UVR effects on sporophytes photosynthesis and growth (Aguilera et al. 1999; Bischof et al. 2002a) and on zoospore germination (Wiencke et al. 2004). These studies showed that susceptibility to light stress depends on the life history stages and on the growth depth of the sporophytes.

With respect to stratospheric ozone depletion and the resulting enhanced ultraviolet-B (UV-B) radiation, several effects are reported on the physiology and productivity of marine macrophytes. The negative impact of exposure to ultraviolet radiation (UVR) includes (1) photoinhibition and eventual photodamage to the photosynthetic apparatus (Hanelt et al. 1997); (2) photochemical degradation of biomolecules inhibiting important metabolic processes (Franklin and Forster 1997); (3) formation of cyclobutane pyrimidine dimers (CPDs) in the DNA, inhibiting genome replication and expression (Wiencke et al. 2000; van de Poll et al. 2001, 2002); and (4) production of reactive oxygen species responsible for oxidative damage within the cell (Rijstenbil et al. 2000). However, UV-B induced DNA damage is repaired under photo-reactivating light (van de Poll et al. 2002; Roleda et al. 2004a, 2005a) while enlargement of phlorotannin-containing physodes is observed in zoospores exposed to UVR enhancing germination capacity (Wiencke et al. 2004). The physiological balance to counteract the negative effects can, however, increase energy demands for protection and repair at the expense of growth.

A previous in situ growth study on the three *Laminaria* species in Spitsbergen at 1 m and 3 m depth showed no significant effect of UVR in all species (Aguilera et al. 1999). This was attributed to the strong attenuation of underwater UVR during the actual field experimental campaign (June–July) when melting ice flowing into the fjord increased water turbidity and reduced underwater radiation. However, during early summer (April–May) when sea ice begins to break, biologically significant UV-B radiation levels are recorded down to 8 m water depth in Kongsfjorden (Hanelt et al. 2001).

In the present study, we compare growth kinetics of cultured young sporophytes of the three *Laminaria* species from Spitsbergen under controlled laboratory conditions. Growth rates and their potential for growth acclimation under UVR have been investigated using growth chambers with an automatic video growth measuring technique. Growth increment was measured at short time scales (e.g. every 10 min) which allowed us to quantify growth rates during and after UVR exposures or during light and dark phases of the daily photoperiod over 18–21 days period. DNA damage and pigment content was also measured at the end of the experiment after repeated UVR exposure. The effect of UVR on growth is discussed in relation to thallus morphology, tissue optics and absorption spectra characteristics of UV-absorbing compounds and depth distribution of the sporophytes in the field. Our study gives an insight into the differential negative mechanistic effects of UVR on growth and DNA damage, which could influence the survival and establishment of young recruits at different depths in the sublittoral zone.

Materials and methods

Algal material

Gametophyte cultures of *Laminaria digitata* (Hudson) Lamouroux, *L. saccharina* (Linnaeus) Lamouroux and *L. solidungula* J. Agardh, originally established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden, Svalbard, were used to obtain young sporophytes. Filaments of male and female gametophytes were fragmented together using mortar and pestle and transferred to culture dishes filled with Provasoli enriched seawater (Starr and Zeikus 1993). After this treatment gametangia are formed and in 6 weeks young sporophytes (approximately 3 mm length) were taken from the bottom of the culture dishes and transferred to aerated 5 L culture bottles until sporophyte size was ready for experiment. Cultures were maintained inside a temperature-controlled room at 8 ± 2°C and 10 μmol photons m^{-2} s^{-1} white light for 12 h light : 12 h dark photoperiod. To compare morphological differences between cultured and wild specimen, young sporophytes of *L. digitata*, *L. saccharina* and *L. solidungula* were collected by SCUBA diving in May 2004 in Kongsfjorden at 1–2, 4 and 18 m depth respectively.

Online video growth measurement technique

Three growth chambers (ISITEC GmbH, Bremerhaven, Germany) with circulating water system and online video measuring technique were operated simultaneously in the experiment as described by Roleda et al. (2004b, 2005b). Each growth chamber contained 2 basal pieces of thalli (average size = 30 mm^2, ±6 mm of the phylloid including the meristem), positioned 20 mm apart from each other. The video-captured image is analysed by MedeaLAB Count and Classify software (Multimedia and Software GmbH, Erlangen, Germany) which measures growth in terms of increased number of pixel size of the algae. After 3 days acclimation to 16 h light : 8 h dark cycle of white light, UVR was supplemented in the middle of the light phase (0900–1500 hours). Two types of glass filters: Schott-GG 400 (Schott, Germany) and Quartz glass were put on the top of the Plexiglas chamber, each covering one of the two algal samples, respectively. This corresponds to two treatments per chamber with photosynthetically active radiation (PAR = P) and PAR + UV-A + UV-B (PAB)

treatment. The three growth chambers were operated simultaneously so that three replicates for the P and PAB treatments of each species were obtained.

Light sources in each chamber consisting of two white fluorescent lamps (Philips, TL 8W/965, Holland) and two UV lamps (Q-Panel UVA-340, 40 Watt, Cleveland, OH, USA) were measured using a scanning UV-VIS spectrometer (M. Kruse, Bremerhaven, Germany) below the glass filters. Weighted irradiance was calculated using action spectra for well-known biological responses: DNA damage for *Escherichia coli* (280–320 nm, Setlow 1974) and the generalized plant damage (280–312 nm) described by Caldwell (1971). Both unweighted and weighted irradiances for each treatment are compiled in Table 1. Growth was continuously measured every 10 min for 18–21 days. Seawater was changed weekly to ensure enough nutrient supply within the medium. At the end of each growth experiment, algal thalli were transferred to 2 ml Eppendorf tubes and frozen at −80°C for further chemical analyses.

Growth rates were computed by plotting all data points over the entire experimental period of each replicate per treatment. They were individually fitted to an exponential equation $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase (Roleda et al. 2004b, 2005b). To determine circadian growth rates of the sporophytes exposed to P and PAB treatments, three days average (% h^{-1}) were computed during the start of the light phase without UVR (0500–0900 hours), in the middle of the light phase with UVR supplement (0900–1500 hours), at the end of the light phase without UVR (1500–2100 hours), and during the dark phase (2100–0500 hours).

Thallus morphology and optics

To determine thallus thickness, wet weight: surface area (mg: mm^2) ratio was measured as described by Roleda et al. (2004a, 2005b). Surface area of sporophytes taken directly from the unialgal cultures was measured using a scanner and WinFolia 5.0 image analysis software (Regent Instrument, Quebec City, Canada) and the corresponding wet weight of the blotted tissue was weighed using Sartorius (CP225D, Germany) weighing scale ($n = 10$). To determine optical properties of cultured and wild specimen, young sporophytes were inserted into seawater-filled quartz cuvettes and scanned with seawater as reference in the 250–740 nm waveband using Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere.

Pigment extraction and measurement

Frozen samples were treated with 100 µl of 100% *N-N*-dimethylformamide and stored in darkness for approximately 16 h. Subsequent chlorophyll (Chl) *a* analysis was performed using HPLC as described by Bischof et al. (2002b).

DNA extraction and quantification

Thalli were homogenized in liquid nitrogen and DNA was isolated following the CTAB extraction procedure described by van de Poll et al. (2001) and modified by Roleda et al. (2004a). After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 µL 10 mg ml^{-1}, 30 min, 37°C; Sigma, MO,USA) and stored at −20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection

The immunoassay for CPDs was modified following Vink et al. (1994) and van de Poll et al. (2001). Heat denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 µm, Schleicher & Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a two step antibody assay, the membrane was treated with ECL Western blotting

Table 1 Unweighted and weighted irradiance applied in the growth chambers

	Irradiance (W m^{-2})			
	Unweighted		Weighted	
	GG 400 (PAR alone)	Quartz(PAR + UV-A + UV-B)	DNA damage (280–320 nm, Setlow 1974)	Generalized plant damage (280–312 nm, Caldwell 1971)
PAR (400–700 nm)	3.60	4.00	0	0
UV-A(315–400 nm)[a]	0.02	5.01	4.99×10^{-4}	0
UV-B(280–315 nm)[a]	0.00	0.31	9.26×10^{-3}	3.16×10^{-2}
Total irradiance	3.62	9.32	9.76×10^{-3}	3.16×10^{-2}

[a] As defined by International Commission on Illumination (C.I.E.)

detection reagent (Amersham Buckinghamshire, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) for different exposure times. The films were developed using X-ray film developer. Developed films were scanned using a Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UVR-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 μg ml^{-1} DNA for each calibration point. The UVR-irradiated DNA (45 min exposure to 2 TL 20 W/12 lamps, Philips, Eindhoven, Netherlands) was previously calibrated against UVR-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Data Analysis

Statistically significant differences in growth rate (% day^{-1} and % P), CPD accumulation, thallus thickness (wet weight: surface area ratio; mg: mm^2) and Chl *a* contents between species were tested using ANOVA ($P < 0.05$) while differences between treatments (P vs. PAB) were tested using *T*-test ($P < 0.05$) using SPSS software (SPSS, Chicago, IL, USA). The general trends in the circadian growth pattern (% h^{-1}) were described.

Results

Significantly higher growth rates (% day^{-1}, $P < 0.001$) were observed in the lower sublittoral *L. saccharina* and *L. solidungula* compared to the upper sublittoral *L. digitata* at an irradiance of 18 μmol photons m^{-2} s^{-1} (Fig. 1a and inset). A higher intrinsic rate of surface area

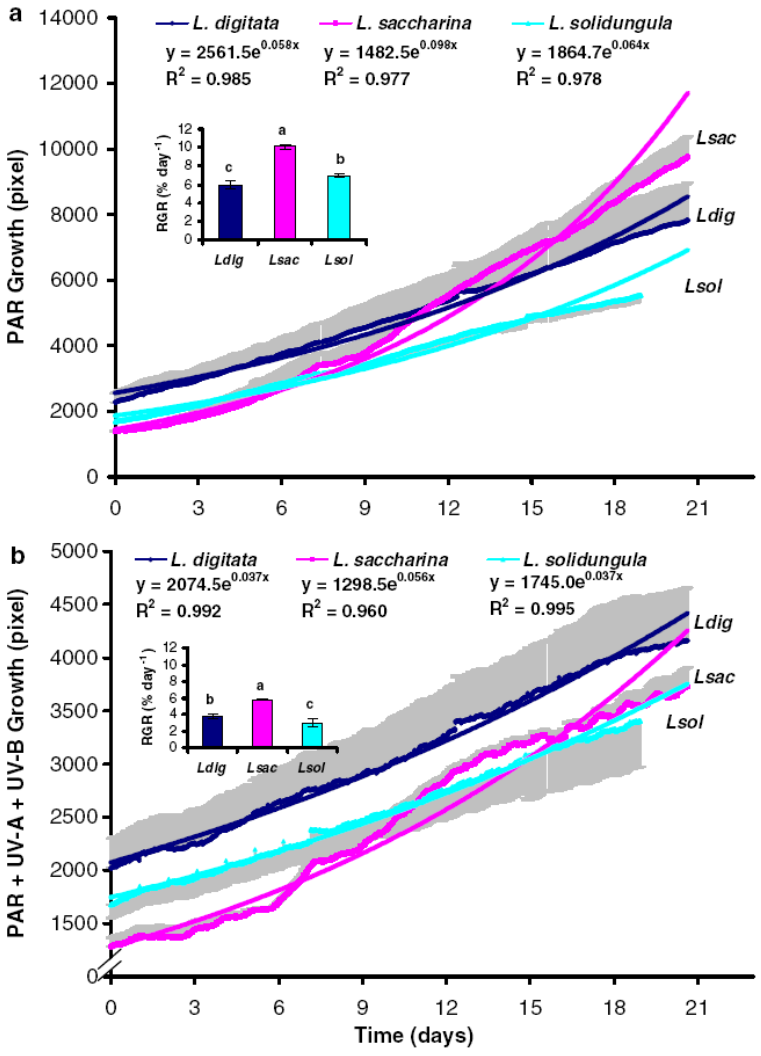

Fig. 1 Growth, in terms of surface area increase, in young sporophytes exposed to **a** photosynthetically active radiation (PAR = P) and **b** PAR + UV-A + UV-B (= PAB). Photon flux density (PFD) was ±18 μmol photons m^{-2} s^{-1}. Data points (*irregular coloured points*) are fitted using the exponential growth equation $N_t = N_0 e^{rt}$ (*solid curves*), where N_t is growth at time t and N_0 is initial size. Values are means ± SD (*light grey one-sided vertical lines*, $n = 3$). *Inset* is the corresponding relative growth rates (RGR, % day^{-1}) for the entire experimental period. ANOVA showed significant difference between species ($P < 0.05$). *Letters on graph* show result of DMRT ($P = 0.05$); *different letters* refer to significant differences between means

increase was observed in sporophytes of all three species exposed to P alone compared to sporophytes exposed to PAB (Fig. 1a, b). Supplement of daily UVR in the middle of the light phase (PAB treatment) also showed higher intrinsic rate of increase in *L. saccharina* relative to the other two species (Fig. 1b and inset). Relative growth rates of sporophytes exposed to PAB, expressed as percent of P, showed significant effect of UVR with higher growth rates in *L. digitata*, intermediate in *L. saccharina* and lowest in *L. solidungula* ($P < 0.05$, Fig. 2).

The general growth pattern in sporophytes exposed to P alone showed an increasing growth rate from the onset of light (0500–0900 hours) to a peak at the middle of the light phase (0900–1500 hours), often a decline towards the end of the light phase (1500–2100 hours) and a minimum "low" growth in the dark (2100–0500 hours) relative to growth during the entire light phase (Fig. 3a–c). Growth of sporophytes exposed to the full light spectrum (PAB) seemed to be species specific. In *L. digitata* (Fig. 3a), growth rate in the middle of the light phase with UVR supplement was lower in the first 3 days of exposure. In the course of repeated exposure, growth during this light phase was able to acclimate to UVR through time. A decrease in growth rate was observed at the end of the light phase (without UVR) but was compensated with a higher growth rate in the dark. In *L. saccharina* (Fig. 3b), growth in the middle of the light phase with UVR supplement was generally lower compared to the early morning growth without UVR. After UVR exposure, growth continuously declined to the end of the light phase and no growth compensation was observed at night. In *L. solidungula* (Fig. 3c), minimal growth was observed throughout the daily light and dark cycle but growth increased immediately after UVR exposure towards the end of the light phase and a moderate growth was also observed at night generally comparable to growth at different light phase without UVR. Growth rates (% h^{-1}) of all species under P and PAB treatments were observed to decrease through the course of the 21 days experiment (Fig. 3a–c).

Thallus morphology of sporophytes cultured in low white light without UVR showed that the thallus of young sporophytes is thickest in *L. solidungula* and thinnest in *L. digitata* (Fig. 4). After 21-days repeated exposure to 6 h daily UVR, DNA damage measured as number of cyclobutane pyrimidine dimer per million bases (CPD Mb^{-1}) was negatively correlated to thallus thickness (Fig. 4). Significantly higher remaining CPDs ($P < 0.01$) was measured in the tissue of the thinner *L. digitata* compared to the thicker *L. saccharina* and the thickest *L. solidungula*.

Different growth forms and thallus morphology were observed between wild and cultured sporophytes (insets Fig. 5a, b). Wild *L. saccharina* sporophytes collected at 4 m depth were found to be very thin and translucent. Thicker and darkly pigmented thalli were observed in *L. digitata* and *L. solidungula* collected at 1–2 and 18 m depths, respectively. Corresponding spectral analysis of the cultured tissues showed higher UVR absorbance maxima, characteristic for the phlorotannin absorption spectrum, in *L. solidungula* compared to *L. digitata* and *L. saccharina* (Fig. 5a). In wild sporophytes, strong absorbance below 280 nm was measured in wild *L. digitata* and *L. solidungula* (Fig. 5b). Lowest and comparable absorbance in the UV-B and UV-C region was observed between cultured and wild *L. saccharina*. The Chl *a* contents of experimental tissues showed no significant difference between treatments (P vs. PAB) in all species (Fig. 6). Comparison between species showed significantly higher Chl *a* content in P- and PAB-treated *L. digitata* ($P < 0.01$). No significant difference was observed in Chl *a* contents between the two lower sublittoral kelp species but was lowest in *L. saccharina*.

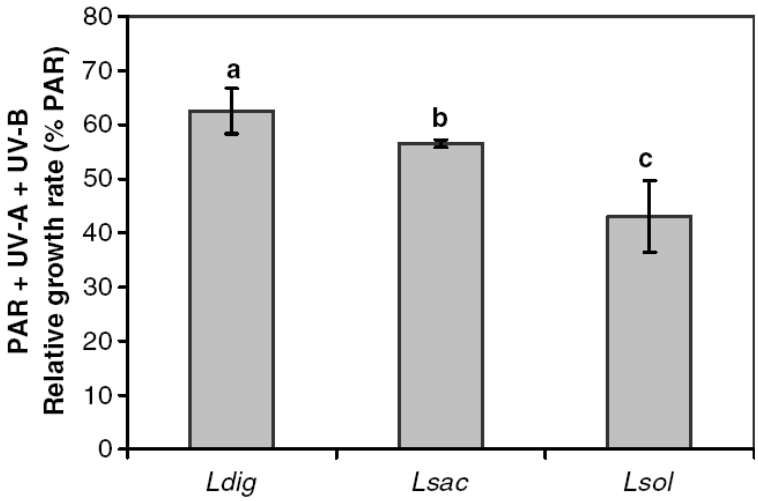

Fig. 2 Ultraviolet radiation effect on the growth rates of young *Laminaria* sporophytes. Growth rates of sporophytes exposed to PAR + UV-A + UV-B were expressed as percent of the growth rates of sporophytes exposed to PAR alone (% PAR). ANOVA showed significant difference between species ($P < 0.05$). *Letters on graph* show result of DMRT ($P = 0.05$); *different letters* refer to significant differences between means

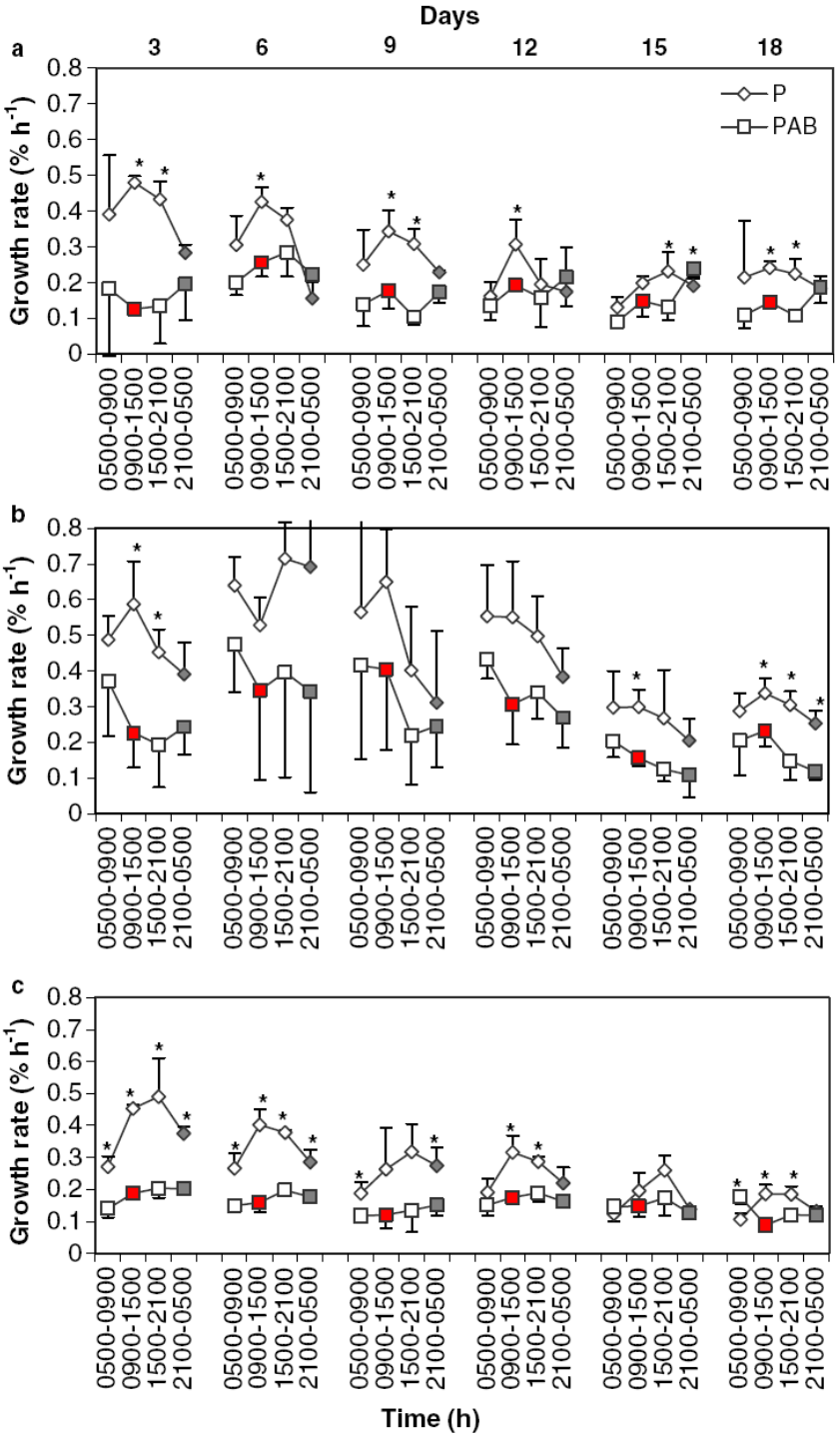

Fig. 3 Average circadian growth pattern of young **a** *Laminaria digitata*, **b** *L. saccharina*, **c** *L. solidungula* sporophytes exposed to photosynthetically active radiation (*P*) and to the full light spectrum (*PAB*) at the start of the light phase to PAR without UVR (0500–0900 hours); in the middle of the light phase with UVR supplement (0900–1500 hours; *red-colour filled symbols*); at the end of the light phase again without UVR (1500–2100 hours); and during the dark phase (2100–0500 hours; *grey-colour filled symbols*). Each point represents the mean of 3 days ± SD. * refers to significant difference between mean (*T*-test, $P < 0.05$)

Discussion

Ultraviolet radiation sensitivity of growth in young Arctic kelp sporophytes is related to the depth distribution of the species. Tissue DNA damage is, however, dependent on thallus thickness and absorption spectra characteristics of the pigments and of UV-absorbing compounds. Energy demands for repair and protection may divert photosynthates at the expense of growth.

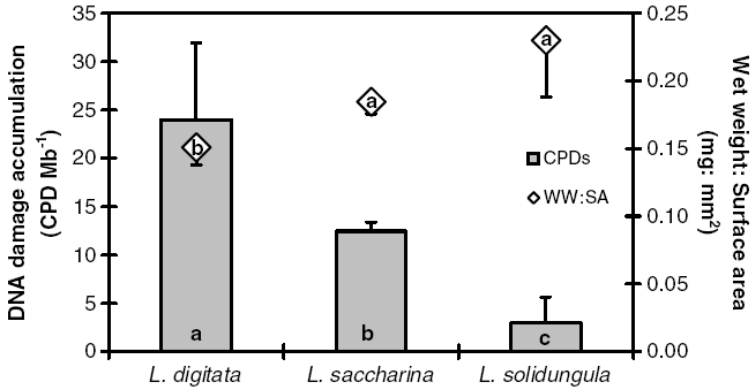

Fig. 4 Relationship between average thallus thickness and remaining tissue DNA damage after 18–21 days of repeated daily 6 h UVR exposure and 18 h recovery (6:8:4 h light:dark:light cycle) in the three Arctic *Laminaria* species. *Vertical bars* are standard deviations ($n=3$). ANOVA showed significant difference between species ($P<0.05$). *Letters on graph* show result of DMRT ($P=0.05$); *different letters* refer to significant differences between means

The higher growth rate of *L. saccharina* relative to the other two *Laminaria* species under P treatment is comparable to the in situ growth experiment of Aguilera et al. (1999). The lower growth rate of *L. digitata* ($5.98\pm0.5\%$ day^{-1}) compared to *L. saccharina* ($10.11\pm0.3\%$ day^{-1}) under 18 μmol photons m^{-2} s^{-1} of PAR suggests that this species requires more light to be able to produce sufficient photosynthate for growth (Lüning 1979). At the same photon flux density (PFD) of PAR, we observed a higher growth rate in the lower sublittoral *L. solidungula* at $6.92\pm0.2\%$ day^{-1} compared to the previous study of Michler et al. (2002) which reported growth rate of $4.25\pm0.5\%$ day^{-1} at higher PAR of 23 μmol photons m^{-2} s^{-1} . Apparently, *L. solidungula* requires minimum photon irradiance to sustain growth. In the high Arctic, in situ seasonal growth measurements showed that *L. solidungula* completes most of its growth in darkness (Chapman and Lindley 1980, Henley and Dunton 1995) while *L. saccharina*, does so when light first starts to penetrate the water column as a result of breakup of the ice canopy (Dunton 1985). Correspondingly high photosynthetic efficiency (α) and low saturation irradiance (I_k) were also reported in *L. solidungula* (Dunton and Jodwalis 1988).

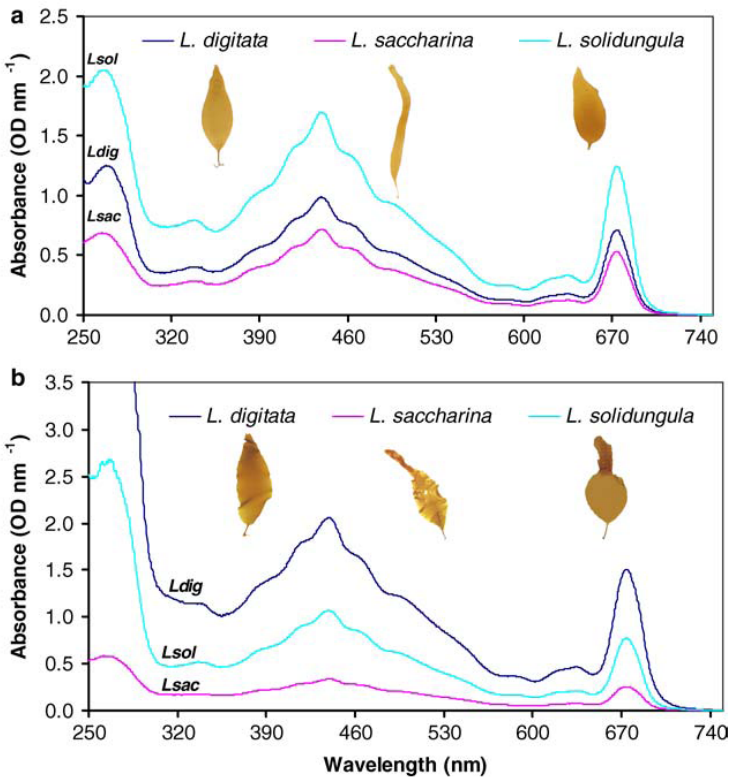

Fig. 5 Tissue absorbance spectrum of the three *Laminaria* species in **a** cultured and **b** wild young sporophytes. The *insets* show difference in morphology of cultured and wild specimen

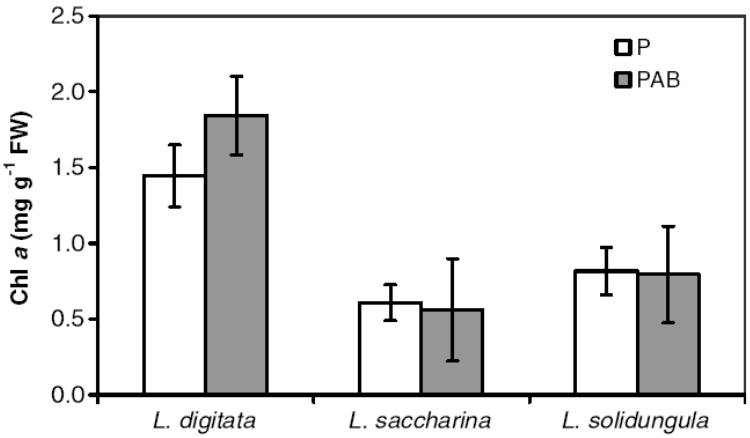

Fig. 6 Chlorophyll a concentration in young sporophytes exposed to different irradiances (PAR = P; PAR + UV-A + UV-B = PAB). *Vertical bars* are standard deviations ($n = 3$). ANOVA showed significant difference between species ($P < 0.05$) and insignificant difference between treatments

Bischof et al. (2002a) showed that photosynthetic efficiencies of the three Arctic *Laminaria* species were depressed after exposure to high UVR dose but were able to recover after 18 h. Sensitivity and recovery of photosynthetic efficiency in *L. saccharina* was also found to be related to the collection depth (Bischof et al. 1998). In *L. solidungula*, photosynthetic efficiency of sporophytes collected at 15 m and transplanted to 1 and 3 m depths were significantly affected, but no longer at 4 m depth, under full solar radiation (Karsten et al. 2001).

Growth rates of *Laminaria* species are higher compared to other Laminariales such as *Saccorhiza dermatodea* ($0.42 \pm 0.12\%\ h^{-1}$) and *Alaria esculenta* ($0.31 \pm 0.02\%\ h^{-1}$, measured during the dark phase) (Roleda et al. 2005b). The pioneering work of Lüning (1992) on online growth measurements within three days showed an increasing growth rate throughout the light phase and a subsequent continuous decrease in growth at the onset of darkness. In contrast, the present online growth measurement which lasted for 18 days recorded a general growth trend showing an initial minimum level during early morning, a mid morning or late afternoon peak and a decline during the dark phase. The decrease in growth rate through time observed in this study was also evident in the subsequent study of Lüning (1994) which recorded decreasing growth in the course of a 6-day measurement. Field experiments on the relative growth rates of three *Laminaria* species in Helgoland were also observed to decrease through time (Lüning 1979). The decrease in the intrinsic rate of increase in growth is attributed to the increase in doubling time for the cell mass (Brinkhuis 1985).

Circadian growth pattern of sporophytes exposed to UVR was first reported by Roleda et al. (2005b) where dark pigmented young *A. esculenta* sporophytes were able to acclimate to UVR while translucent young *S. dermatodea* did not. In *L. digitata*, the drop in growth rate during UVR exposure was observed only during the first 3 days. Growth was eventually able to acclimate to UVR where growth rates at this time (0900–1500 hours) were higher compared to the early morning and late afternoon growth but a corresponding growth compensation was observed at night. The presence of UV stress seems to drive this species in phasing cellular events (i.e. cell division for growth) sensitive to light to occur at night. In *L. saccharina*, growth after UVR stress took longer time to recover. Growth declined continuously after UV lamps were turned off in the afternoon (1500–2100 hours) and during the night phase (2100–0500 hours). Growth was only able to recover early in the morning (0500–0900 hours) after 14 h from previous UVR exposure. In *L. solidungula*, moderate growth rates were observed throughout the daily light cycle of PAB exposed sporophytes. However, they are able to cope with the daily UVR dose until the 15th day when growth during UVR exposure (0900–1500 hours) did not drop relative to the early morning growth (0500–0900 h) without UVR. Growth increased further after UVR exposure and at night. The first drop in growth rate during UVR exposure was only observed towards the end of the experiment (18th day).

The insignificant variation in Chl *a* concentration in sporophytes exposed to PAB and to P treatments indicates that algae are able to synthesize new pigments in replacement of degraded chlorophylls after 6 h daily UVR exposure and acclimate to moderate fluence of UVR. Acclimation of Chl *a* concentration was observed in *Palmaria decipiens* (Reinsch) Ricker exposed to 12–16 h UVR (Poppe et al. 2002). The higher (but insignificant) Chl *a* concentration in *L. digitata* sporophytes exposed to PAB is comparable to the previously reported higher pigment content in *Ulva rigida* C. Agardh exposed to UVR, which is speculated to be some kind of photoprotective mechanism (Altamirano et al. 2000). In situ seasonal variation in pigment concentration of Arctic *L. saccharina* collected before, during and after ice break-up showed decreasing Chl *a* concentration coinciding with increasing underwater radiation. However, Chl *a* concentration did not increase when melting snow contributed to water turbidity and reduction in underwater radiation (Aguilera et al. 2002).

Although *L. digitata* (24 ± 8 CPD Mb^{-1}) was the species with the highest accumulation of CPDs after 21 days of repeated 6 h daily exposure to UV-B radiation, an effective DNA damage repair mechanism is still indicated in this species. Previously, CPD induction in adult Arctic *L. saccharina* after 45 min exposure to 2.3 W m^{-2} UV-B radiation resulted in a high accumulation of 88 CPD Mb^{-1} and was effectively repaired after 5 h exposure to photo-reactivating light at the rate of 0.49 CPD Mb^{-1} h^{-1} (van de Poll et al. 2002). The other two lower sublittoral species (*L. saccharina* and *L. solidungula*) exhibited a considerably lower DNA damage. This is attributed to their thicker thalli which provided extra protective tissue against UVR (Johansson and Snoeijs 2002). However, Roleda et al. (2005b) found that thallus thickness in itself is not sufficient to minimize deleterious UVR effects. The thick but translucent thallus of *Saccorhiza dermatodea* had a higher CPDs content of 65 ± 9 CPD Mb^{-1} compared to the thin but opaque *Alaria esculenta* (8 ± 3 CPD Mb^{-1}) after exposure to the same experimental treatment (Roleda et al. 2005b). The optical property of the thallus is also important, which can influence reflection, attenuation, scattering, absorption or transmittance of UVR (Caldwell et al. 1983).

The pronounced wavelength selectivity of absorption in leaf epidermis of terrestrial plants is often attributed to flavonoids and other related UV-absorbing compounds (Robberecht and Caldwell 1978). Flavonoids absorb UVR with maximum effectiveness around 295 nm (Stapleton and Walbot 1994; Landry et al. 1995). Among Laminariales phlorotannins are accumulated within the outer cortical layer of the thalli (Lüder and Clayton 2004; Shibata et al. 2004) supporting their role as effective UV screens (Arnold 2003). The absorption spectra presented here show an increasing absorbance from 300 nm to the shorter UV-C waveband comparable to that of isolated phlorotannins from *Fucus gardneri* Silva exhibiting a peak at 265 nm (Henry and van Alstyne 2004). It is yet uncertain which UV-screening compound is involved in the current study. Profiles of the absorption spectra indicate that the presumptive UV-screening substance provides no complete protection against UV-B radiation. The absorption shoulder >265 nm can, however, decrease UV-B sensitivity of a plant. Furthermore, it is thought that absorption of tissue-bound phlorotannin might shift to different peaks compared to extracted compounds. Further studies are needed to address this question.

The observed remaining tissue DNA damage shows that the sensitivity of the young sporophytes is not related to the depth distribution of the adult sporophytes but to the thallus thickness of the species. *Laminaria solidungula*, which occurs deepest in the field, is less sensitive to UV-B radiation induced DNA damage but showed the highest reduction in growth rates. Thus, the remaining tissue DNA damage alone cannot explain the survival success in this genus. It shows that increasing thallus thickness minimizes UVR effect as a function of optical effect where outer cell layers shade inner cells, and because of longer pathlength for UVR absorption (Franklin and Forster 1997). The low amount of remaining CPDs in *L. solidungula* as well as the high amount of UV-absorbing compound present in the tissue suggests that this species has either an effective repair or protective mechanism to counteract deleterious UVR effect but at the expense of growth. The occurrence of *L. solidungula* in Kongsfjorden at great depth is attributed to its sensitivity to high PAR. At 18 m depth, a maximum of 7% PAR and no biologically significant UV-B irradiances can reach this species (Hanelt et al. 2001). Aside from its sensitivity to light, the seclusion of *L. solidungula* to the deeper and inner part of the fjord could also be attributed to its discoidal holdfast which needs secure substrate to attach making it less fit to grow on an easily disturbed environment compared to other kelp species with rhizoidal holdfast (Busdosh et al. 1985). *Laminaria solidungula* can be found at depths from 5 to >30 m on undisturbed substrate in a high-arctic fjord in NE Greenland (Borum et al. 2002) and at 5–6 m depth in Alaskan Arctic (Dunton 1990).

A simple growth model follows a growth-differentiation balance (Carr et al. 1997) 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):

$$G = P - R - L$$

Photoinhibition of photosynthesis already decreases potential carbon acquisition (P) into plant dry matter (G) (Long et al. 1994). Dark respiration (R) represents the energy used to synthesize new biomass (growth respiration) and that used to maintain metabolic activity (maintenance respiration). Cellular, enzymatic and molecular damage caused by exposure to high light and UVR could further increase loss due to respiration (R) by diverting more photosynthate for repair and defense (i.e. production of secondary metabolites). Thus growth as an integrative cellular process is a better parameter to understand the long term effect of UVR exposure to macroalgae.

This long term single factor laboratory experiment showed that UVR effect on growth is species specific. Different growth patterns were exhibited by the plants to mitigate UVR effects such as growth compensation at night in *L. digitata*, delayed (time-lagged) growth recovery in *L. saccharina* and minimal but continuous growth throughout the light cycle in *L. solidungula* coupled with effective repair or defense mechanism. Growth as an integrative parameter of all physiological processes showed that the effect of UVR may be related to the depth distribution of the species.

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References

Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. Mar Ecol Progr Ser 191:109–119

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087–1095

Altamirano M, Flores-Moya A, Figueroa F-L (2000) Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated in situ. Bot Mar 43:19–26

Arnold TM (2003) To grow and defend: lack of tradeoffs for brown algal phlorotannins. Oikos 100:406–408

Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). Polar Biol 20:388–395

Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002a) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. Mar Biol 140:1097–1106

Bischof K, Kräbs G, Wiencke C, Hanelt D (2002b) Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 215:502–509

Borum J, Pedersen MF, Krause-Jensen D, Christensen PB, Nielsen K (2002) Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. Mar Biol 141:11–19

Brinkhuis BH (1985) Growth pattern and rates. In: Littler MM, Littler DS (eds) Handbook of phycological methods: ecological field methods: macroalgae. Cambridge University Press, Cambridge, pp 461–477

Busdosh M, Beehler CL, Robilliard GA, Tarbox KR (1985) Distribution and abundance of kelp in the Alaskan Beaufort Sea near Prudhoe Bay. Arctic 38:18–22

Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (ed). Photophysiology. Academic Press, New York, pp 131–177

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Plant 58:445–450

Carr GM, Duthie HC, Taylor WD (1997) Models of aquatic plant productivity: a review of the factors that influence growth. Aquat Bot 59:195–215

Chapman ARO, Lindley JE (1980) Seasonal growth of *Laminaria solidungula* in the Canadian high Arctic in relation to irradiance and dissolved nutrient concentrations. Mar Biol 57:1–5

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

Dunton KH (1985) Growth of dark-exposed *Laminaria saccharina* (L.) Lamour, and *Laminaria solidungula* J. Ag. (Laminariales: Phaeophyta) in the Alaskan Beaufort Sea. J Exp Mar Biol Ecol 94:181–189

Dunton KH (1990) Growth and production in *Laminaria solidungula*: relation to continuous underwater light levels in the Alaskan High Arctic. Mar Biol 106:297–304

Dunton KH, Jodwalis CM (1988) Photosynthetic performance of *Laminaria solidungula* measured in situ in the Alaskan High Arctic. Mar Biol 98:277–285

Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32: 207–232

Hanelt D, Wiencke C, Nultsch W (1997) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J Photochem Photobiol B Biol 38:40–47

Hanelt D, Tüg GH, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. Mar Biol 138:649–658

Henley WJ, Dunton KH (1995) A seasonal comparison of carbon, nitrogen, and pigment content in *Laminaria solidungula* and *L. saccharina* (Phaeophyta) in the Alaskan Arctic. J Phycol 31:325–331

Henry BE, van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. J Phycol 40:527–533

Hop H, Pearson T, Hegseth EN, Kovacs KM, Wiencke C, Kwasniewski S, Eiane K, Mehlum F, Gulliksen B, Wlodarska-Kowalczuk M, Lydersen C, Weslawski JM, Cochrane S, Gabrielsen GW, Leakey RJG, Lønne OJ, Zajaczkowski M, Falk-Petersen S, Kendall M, Wängberg S-Å, Bischof K, Voronkov AY, Kovaltchouk NA, Wiktor J, Poltermann M, di Prisco G, Papucci C, Gerland S (2002) The marine ecosystem of Kongsfjorden, Svalbard. Polar Res 21:167–208

Johansson G, Snoeijs P (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar Ecol Prog Ser 244:63–72

Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters followed by exposure to natural solar radiation. Oecologia 127:11–20

Landry LG, Chapple CCS, Last RL (1995) *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol 109:1159–1166

Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Ann Rev Plant Physiol Plant Mol Biol 45:633–662

Lüder UH, Clayton MN (2004) Induction of phlorotannins in the brown algae *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory- the first microscopic study. Planta 218:928–937

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Mar Ecol Prog Ser 1:195–207

Lüning K (1992) Day and night kinetics of growth rate in green, brown, and red seaweeds. J Phycol 28:794–803

Lüning K (1994) Circadian growth rhythm in juvenile sporophytes of Laminariales (Phaeophyta) J Phycol 30:193–199

Michler T, Aguilera J, Hanelt D, Bischof K, Wiencke C (2002) Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. Mar Biol 140:1117–1127

van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. J Phycol 37:30–37

van de Poll WH, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. Photochem Photobiol 76:493–501

Poppe F, Hanelt D, Wiencke C (2002) Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic red alga *Palmaria decipiens* during acclimation to UV radiation. Bot Mar 45:253–261

Rijstenbil JW, Coelho SM, Eijsackers M (2000) A method for the assessment of light-induced oxidative stress in embryos of fucoid algae via confocal laserscan microscopy. Mar Biol 137:763–774

Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia (Berl.) 32:277–287

Roleda MY, van de Poll WH, Hanelt D, Wiencke C (2004a) PAR and UVBR effects on photosynthesis, viability, growth and

DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar Ecol Prog Ser 281:37–50

Roleda MY, Hanelt D, Kräbs G, Wiencke C (2004b) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycologia 43:603–613

Roleda MY, Wiencke C, Hanelt D, van de Poll WH, Gruber A (2005a) Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction. Plant Cell Environ 28:466–479

Roleda MY, Hanelt D, Wiencke C (2005b) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. Polar Biol 28:539–549

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Nat Acad Sci USA 71:3363–3366

Shibata T, Kawaguchi S, Hama Y, Inagaki M, Yamaguchi K, Nakamura T (2004) Local and chemical distribution of phlorotannins in brown algae. J Appl Phycol 16:291–296

Stapleton AE, Walbot V (1994) Flavonoids protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol 105:881–889

Starr RC, Zeikus JA (1993) UTEX—the culture collection of algae at the University of Texas at Austin. J Phycol 29 (Suppl):1–106

Vink AA, Bergen-Henegouwen JB, Nikaido O, Baan RP, Roza L (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. Photochem Photobiol 24:25–31

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof, K, Figueroa F-L (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar Ecol Prog Ser 197:217–229

Wiencke C, Clayton MN, Schoenwaelder M (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. Mar Biol 145:31–39

Publication 5

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RESEARCH ARTICLE

Michael Y. Roleda · Dieter Hanelt · Christian Wiencke

Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea)

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Abstract Growth as an integrative parameter of all physiological processes was measured in young sporophytes of temperate *Laminaria digitata*, *Laminaria saccharina* and *Laminaria hyperborea* exposed in the laboratory to irradiance consisting of either only photosynthetically active radiation (PAR) or to a spectrum including ultraviolet radiation (UVR) (PAR + UVA + UVB) by use of cut-off glass filters. Size increment was measured every 10 min over a period of 18–21 days using growth chambers with on-line video measuring technique. In the chamber, plants were grown at 10 ± 2°C and 16:8 h light–dark cycles with 6 h additional UVR exposure in the middle of the light period. Tissue morphology and absorption spectra were measured in untreated young sporophytes while chlorophyll a content and DNA damage were measured in treated thalli at the end of the experiment. Sensitivity of growth under UVR was found to be related to the observed upper depth distribution limit of the upper sublittoral *L. digitata*, upper to mid sublittoral *L. saccharina* and lower sublittoral *L. hyperborea*. Tissue DNA damage is, however, dependent on thallus thickness which minimizes UVR effect where outer cell layers shade inner cells and provide longer pathlength for UVR. Exposure to UVR causes cellular, enzymatic and molecular damage. Presence of UV-absorbing compounds further reduces effective UVR from reaching physiological targets. The cost of producing higher amount of UV-absorbing compounds and effective DNA repair mechanism can, however, divert photosynthate at the expense of growth. Tissue chlorophyll a content was not significantly different between treatments suggesting a capacity for acclimation to moderate UVR fluence. Growth acclimation to repeated UVR exposure was observed within a period of 12 days while growth inhibition was observed after a longer UVR exposure period of 21 days. The results give further insight into the effects of UVR on the cellular level and show how ecological parameters such as the upper depth distribution limit are dependent on cellular processes.

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M. Y. Roleda (✉)
Alfred Wegener Institute for Polar and Marine Research,
Biologische Anstalt Helgoland, 27498 Helgoland, Germany
E-mail: mroleda@awi-bremerhaven.de
Tel.: +49-4725-819230
Fax: +49-4725-819283

M. Y. Roleda
Biology Department, De La Salle University,
2401 Taft Avenue, 1004 Manila, Philippines

D. Hanelt
Biozentrum Klein Flottbek, University of Hamburg,
Ohnhorst-Str. 18, 22609 Hamburg, Germany

C. Wiencke
Foundation Alfred Wegener Institute for Polar and Marine
Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Introduction

The three kelp species from Helgoland grow in characteristic belts with *Laminaria digitata* (Hudson) Lamouroux in the upper sublittoral, *Laminaria saccharina* (Linnaeus) Lamouroux in the mid-sublittoral and *Laminaria hyperborea* (Gunnerus) Foslie in the lower sublittoral (Lüning 1979). A mixed stand of *L. digitata* and *L. saccharina* is, however, found on the upper sublittoral (0–1.5-m depth) of the western intertidal flat of the island (Lüning 1985). Moreover, timing of reproduction is different in the three species. *L. digitata* is fertile during late spring to summer (May–August), *L. saccharina* during autumn to mid winter (September–February) and *L. hyperborea* during late autumn to winter (November–February). Zoospore sensitivity in terms of recovery of photosynthetic efficiency, DNA damage repair and germination rate of Helgolandic kelp species was found to be related to the depth distribution of the adult sporophytes and to the seasonal reproductive strategy of the species (Roleda et al. 2005a). Spores

and germlings of kelps are found to remain viable in plankton for extended periods of time (Reed et al. 1992). Exposure to environmental stress factors, e.g. high light, ultraviolet radiation (UVR) and temperature, contribute to pre-settlement mortality whereas surviving spores are capable of dispersal, settlement, attachment and initiation of new recruits across the expanse of the vertical tidal zones. The sensitivity of the young recruits to the same stress factors could contribute to post-settlement mortality, which effectively determines the upper distribution limit of each species. A relation between the effect of UVR on the growth of young sporophytes and the depth distribution of kelps in Helgoland was previously sought but not found (Dring et al. 1996).

With respect to stratospheric ozone depletion and the resulting enhanced UVB radiation (UVBR), several negative effects are reported on the physiology and productivity of marine macrophytes. This includes photoinhibition and eventual photodamage to the photosynthetic apparatus (Hanelt et al. 1997a, b), photochemical degradation of biomolecules inhibiting important metabolic processes (Franklin and Forster 1997), formation of cyclobutane-pyrimidine dimers (CPDs) in the DNA inhibiting genome replication and expression (Wiencke et al. 2000; van de Poll et al. 2001, 2002) and production of reactive oxygen species responsible for oxidative damage within the cell (Aguilera et al. 2002a). Under photoreactivating light, DNA damage in macroalgal spores and young sporophytes is repaired (van de Poll et al. 2002; Roleda et al. 2004a, 2005a), whereas protection by enlargement and increase in number of phlorotannin-containing physodes is observed in zoospores exposed to UVR, enhancing germination capacity (Wiencke et al. 2004). The physiological balance to counteract the negative effects can, however, increase energy demands for protection and repair at the expense of growth.

Species morphology is an important trait in algal ecology. Littler and Littler (1980) defined functional-form groups in marine macroalgae and grouped the genus *Laminaria* in the thick leathery functional form. However, different thallus thicknesses are observed in different species of the genus, whereby *L. hyperborea* is considered the thickest leathery species (Johansson and Snoeijs 2002). With respect to photosynthetic efficiency, UVR tolerance is higher in species with increasing thallus thickness (Franklin and Forster 1997). The optical effect of the outer cell layers can influence reflection, attenuation, scattering, absorption or transmittance of UV radiation to the inner cells (Caldwell et al. 1983). Translucence and opacity of the thallus related to pigments and presence of UV-absorbing compounds was also found to affect growth and DNA sensitivity of other young Laminariales sporophytes (Roleda et al. 2005b).

In a previous study on the effect of UVR on young *Laminaria* sporophytes, unrealistically high doses of different exposure lengths ranging from 0.25 (15 min) to 144 h (6 days) of UVR were applied. Bleaching and zero growth rates were observed in all species exposed to 72 h or more of UVR (Dring et al. 1996). After development of the on-line growth measurement system by Lüning (1992, 1994) for *Laminaria* and similar species, short-term (3–6 days) day and night kinetics of growth under L:D photoperiod, continuous light and different light spectra are possible. An improved growth chamber with advance software allowed Roleda et al. (2004b, 2005b) to measure growth in intervals of 10 min over a longer period of time, which will be used in the present study.

Dispersal and potential recruitment of kelp germlings in the eulittoral zone is possible. However, they can be exposed to air and the whole spectrum of solar radiation during low tides, which may contribute to the post-recruitment mortality of the species. We will test the hypothesis that susceptibility of young sporophytes to UVR influences the upper depth distribution limit of the mature sporophytes. The present study will re-examine the effect of UVR on the growth of young *Laminaria* sporophytes exposed to moderate dose of UVR supplemented in the middle of the light phase. Growth as an integrative parameter of all physiological and biochemical processes will be measured every 10 min for 18–21 days. This study extends to investigate accumulation of CPDs in the tissue as a measure of DNA damage and repair capacity after repeated exposure to UVR. Thallus thickness and the presence of UV-absorbing compounds in young cultured and wild sporophytes were examined and discussed in relation to the species sensitivity to UVR.

Materials and methods

Algal material

Stock gametophyte cultures of *L. digitata*, *L. saccharina* and *L. hyperborea* gametophytes, originally established from spores of fertile sporophytes collected around Helgoland, North Sea, were used to obtain young sporophytes. Filaments of male and female gametophytes were fragmented together using mortar and pestle and transferred to culture dishes filled with Provasoli-enriched seawater (Starr and Zeikus 1993). Cultures were maintained inside a temperature-controlled room at 12 ± 2°C and 10 μmol m^{-2} s^{-1} white light at 12:12 h light:dark photoperiod. Gametangia were formed and at about 6 weeks, young sporophytes (approximately 3 mm length) were taken from the bottom of the culture dishes and transferred to aerated 5 l culture bottles until sporophyte size was ready for the experiment. To compare morphological differences between cultured and wild specimens, young sporophytes of *L. digitata* were collected by hand in the upper sublittoral (0.5–1 m) during low tide, while *L. saccharina* and *L. hyperborea* were collected by SCUBA diving in the mid (2–4 m) and lower (5–7 m) sublittoral, respectively, around the island of Helgoland.

On-line video growth measurement technique

Three growth chambers (ISITEC GmbH, Bremerhaven, Germany) with circulating water system and on-line video measuring technique were operated simultaneously in the experiment as described by Roleda et al. (2004b, 2005b). Each growth chamber contained two basal pieces of thalli (average size = 30 mm^2, ±6 mm of the blade including the meristem), positioned 20 mm apart from each other. The video-captured image is analysed by MedeaLAB Count and Classify software (Multimedia and Software GmbH, Erlangen, Germany), which measures growth in terms of increased number of pixel size of the algae. After 3 days of acclimation to 16:8 light–dark photoperiods of white light, UVR was supplemented in the middle of the light phase (0900–1500 h). Two types of glass filters, Schott-GG 400 (Schott, Germany) and Quartz glass slides, were put on the top of the Plexiglas chamber, each covering one of the two algal samples, respectively. This corresponds to two treatments per chamber with photosynthetically active radiation (PAR = P) and PAR + UVA + UVB (PAB) treatment. The three growth chambers operated simultaneously per species were used as replicates for the P and PAB treatments.

Light sources in each chamber consisted of two white fluorescent lamps (Philips, TL 8W/965, Holland) and two UV lamps (Q-Panel UVA-340, 40 W, Cleveland, USA). The PAR was measured using a cosine quantum sensor (Type 1925B; LI-COR Biosciences, Bad Homburg, Germany) attached to a LI-COR data logger (LI-1000) and UVR was measured using a scanning UV-VIS spectrometer (M. Kruse, Bremerhaven, Germany) below the glass filters. Weighted irradiance was calculated using action spectra for well-known biological responses: DNA damage for *Escherichia coli* (280–320 nm, Setlow 1974) and the generalized plant damage (280–312 nm) described by Caldwell (1971). Both unweighted and weighted irradiances for each treatment are compiled in Table 1. Growth was continuously measured every 10 min for 18–21 days. Seawater was changed weekly to ensure enough nutrient supply within the medium. At the end of each growth experiment, algal thalli were transferred to 2 ml Eppendorf tubes and frozen at −80°C for further chemical analyses.

Growth rate was computed by plotting all data points (of the entire experimental period) of each replicate per treatment. They were individually fitted to an exponential equation $N_t = N_0 e^{rt}$, where N_t is growth at time t, N_0 is initial size and r is the intrinsic rate of increase (Roleda et al. 2004b, 2005b). To determine the circadian growth rates of the sporophytes exposed to P and PAB treatments, 3-day averages (percent per hour) were computed during the start of the light phase without UV (0500–0900 h), in the middle of the light phase with UV supplement (0900–1500 h), at the end of the light phase without UV (1500–2100 h) and during the dark phase (2100–0500 h).

Thallus morphology and optics

To determine thallus thickness, wet weight:surface area (mg:mm^2) ratio was measured as described by Roleda et al. (2004a, 2005b). Surface area of sporophytes taken directly from the unialgal cultures was measured using a scanner and WinFolia 5.0 image analysis software (Regent Instrument, Quebec City, Canada). The corresponding wet weight of the blotted tissue was weighed using Sartorius (CP225D, Germany) weighing scale (n = 10). To determine the optical property of cultured and wild specimens, young sporophytes were inserted into seawater-filled quartz cuvettes and scanned with seawater as reference in the 250–700 nm waveband using Shimadzu photometer (UV 2401PC, Japan) equipped with an integrating sphere.

Pigment extraction and characterization

Frozen samples were treated with 100 µl of 100% N-N-dimethylformamide and stored in darkness for approximately 16 h. Subsequent analyses were performed using HPLC as described by Bischof et al. (2002).

DNA extraction and quantification

Thalli were homogenized in liquid nitrogen and DNA was isolated following the CTAB extraction procedure described by van de Poll et al. (2001) and modified by

Table 1 Unweighted and weighted irradiance applied in the growth chambers

	Irradiance (W m^{-2})			
	Unweighted		Weighted	
	GG 400 (PAR alone)	Quartz (PAR + UVA + UVB)	DNA damage (Setlow 1974)	Generalized plant damage (Caldwell 1971)
PAR (400–700 nm)	3.60	4.00	0	0
UVA (320–400 nm)	0.02	4.92	0	0
UVB (280–320 nm)	0.00	0.41	9.76×10^{-3}	3.16×10^{-2}
Total irradiance	3.62	9.33	9.76×10^{-3}	3.16×10^{-2}

Roleda et al. (2004a). After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), treated with RNAase (5 μl 10 mg ml^{-1}, 30 min, 37°C; Sigma, MO, USA) and stored at −20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection

The immunoassay for CPDs was modified after Vink et al. (1994) and van de Poll et al. (2001). Heat-denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 μm, Schleicher and Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher and Schuell). After a two-step antibody assay, the membrane was treated with ECL Western blotting detection reagent (Amersham Buckinghamshire, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham), with different exposure times. The films were developed using an X-ray film developer. Developed films were scanned using a Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, Hercules, CA, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included, giving 1 μg ml^{-1} DNA for each calibration point. The UV-irradiated DNA (45 min exposure to two TL 20 W/12 lamps, Philips, Eindhoven, Netherlands) was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). The CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis

The statistically significant differences in growth rate (percent per day), CPDs accumulation and thallus thickness (wet weight:surface area ratio; mg:mm^2) between species were tested using ANOVA ($P < 0.05$), and the difference between treatments (P versus PAB) was tested by *t*-test ($P < 0.05$) using SPSS software (SPSS, Chicago, IL, USA). The general trends in the circadian growth pattern (percent per hour) were described.

Results

Higher intrinsic rates of increase in surface area were observed in sporophytes of all three species exposed to P alone compared to sporophytes exposed to PAB (Fig. 1). Significant difference in growth rates was observed between species (percent per day; insets Fig. 1a, b) exposed to P and PAB (ANOVA, $P < 0.01$). The corresponding Duncan multiple range test (DMRT, $P = 0.05$) showed significantly lower growth rates in *L. hyperborea* sporophytes exposed to P and PAB compared to the other two kelp species. However, the difference in growth rates between *L. digitata* and *L. saccharina* is not significant in both treatments. Relative growth rates of sporophytes exposed to PAB, expressed as percent of P, showed significant effect of UVR (ANOVA, $P < 0.05$). The DMRT ($P = 0.05$), however, showed that *L. digitata* is not significantly different from *L. saccharina* and *L. saccharina* is not significantly different from *L. hyperborea* (Fig. 2).

The circadian growth pattern in sporophytes exposed to P alone generally showed an increasing growth from the onset of light (0500–0900 h) to a peak at the middle (0900–1500 h) or at the end of the light phase (1500–2100 h) and a minimum "low" growth in the dark phase (2100–0500 h) (Fig. 3a–c). Among sporophytes exposed to the full light spectrum (PAB), circadian growth pattern was observed to be species specific. After UV exposure, a relative decrease in the growth rate of *L. digitata* was observed at the end of the light phase (1500–2100), but this was compensated with a higher growth rate in the dark phase (2100–0500) (Fig. 3a). A similar pattern was observed in *L. hyperborea* (Fig. 3c) but lower growth rate was observed during the dark phase compared to *L. digitata*. In *L. saccharina*, growth rates generally increased at the end of the light phase (1500–2100) after UV exposure, while growth rates during the dark phase varied over the observation period (Fig. 3b).

Growth rates of young sporophytes were initially able to acclimate to UVR in the course of several days (4–12 days) of repeated exposure (Fig. 4). However, at longer exposure periods (13–21 days), growth rates were eventually observed to decline through time. Average growth rates during UVR exposure (0900–1500 h) over a period of 18–21 days showed significant differences between species (ANOVA, $P < 0.05$). Duncan's multiple range test showed no significant variation between *L. digitata* and *L. saccharina* except growth during the 3rd and 18th day (DMRT, $P = 0.05$), while significantly lower growth rates were observed in *L. hyperborea* during the 9th, 12th and 18th day (DMRT, $P = 0.05$).

Higher wet weight:surface area ratio corresponds to thicker thallus as observed visually and tactually among different species of cultured sporophytes. Analysis of variance (ANOVA, $P < 0.001$) and Duncan Multiple Range Test (DMRT, $P = 0.05$) showed significant differences between species with *L. saccharina* having the thinnest and *L. hyperborea* the thickest thalli (Fig. 5). DNA damage measured as number of CPDs after 18–21 days of daily 6 h exposure to UVR showed inverse relationship with thallus thickness (Fig. 5). Significantly higher remaining CPDs ($P < 0.01$) was measured in the

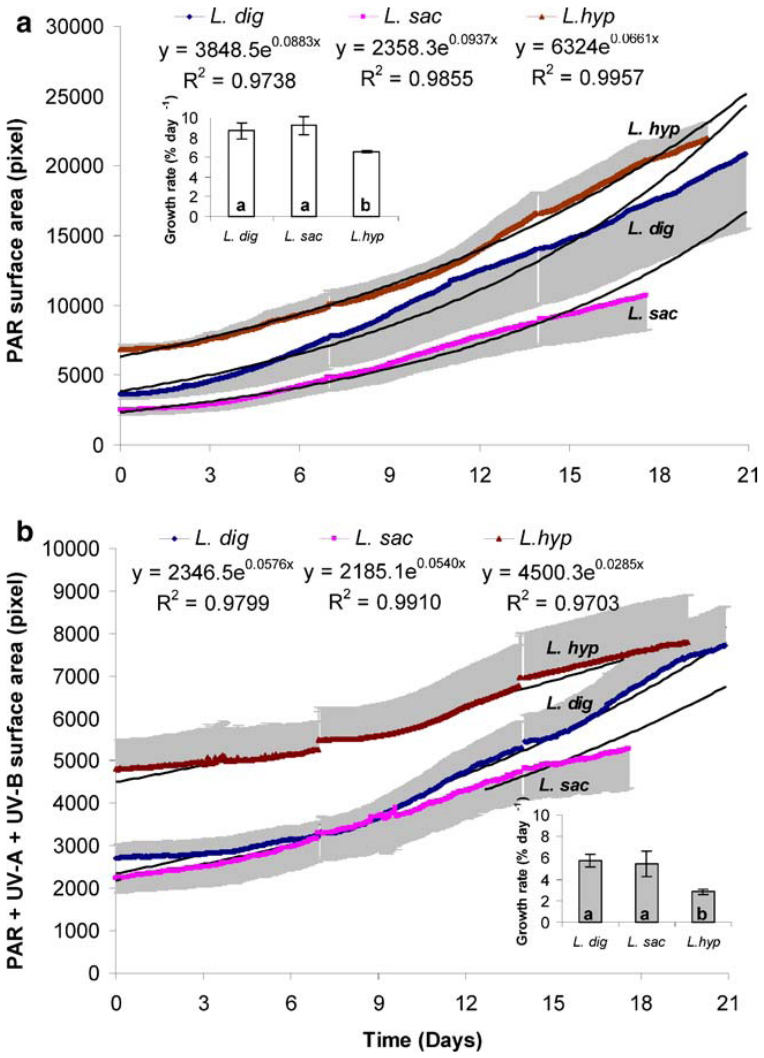

Fig. 1 Growth, in terms of surface area increase, in young sporophytes exposed to **a** photosynthetically active radiation (PAR = P) and **b** PAR + UVA + UVB (= PAB). PFD was ± 18 µmol m^{-2} s^{-1}. Data points (*irregular coloured points*) are fitted using the exponential growth equation $N_t = N_0 e^{rt}$ (*solid curves*), where N_t is growth at time t and N_0 is initial size. Values are means ± SD (*light gray* one sided *vertical lines*, $n = 3$). Inset is the corresponding daily growth rates (percent per day) for the entire experimental period. ANOVA showed significant difference between species ($P < 0.05$). Letters on graph show result of DMRT ($P = 0.05$); different letters refer to significant differences between means

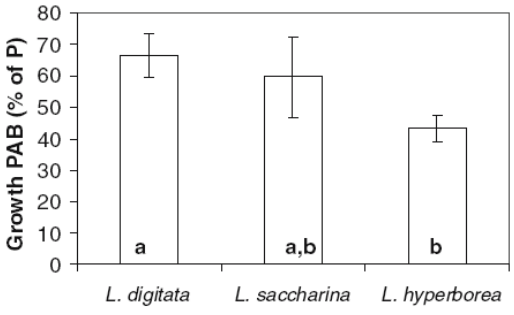

Fig. 2 Ultraviolet radiation effect on the growth rates of young *Laminaria* sporophytes. Growth rates of sporophytes exposed to PAR + UVA + UVB (PAB) were expressed as percent of the growth rates of sporophytes exposed to PAR alone (percent P). ANOVA showed significant difference between species ($P < 0.05$). *Letters* on graph show result of DMRT ($P = 0.05$); *different letters* refer to significant differences between means

tissue of the thinner *L. saccharina* compared to the two other species. No significant differences in the remaining CPDs were observed between the thicker thalli of *L. digitata* and *L. hyperborea*. No significant difference was observed in the pigment concentration between *L. digitata* and *L. hyperborea* sporophytes exposed to P and PAB (Fig. 6). Due to sample limitation, no replicates were measured in *L. saccharina*.

Different thallus morphology and growth forms were observed between young cultured and wild sporophytes (insets Fig. 7a, b). Wild sporophytes were thicker than cultured sporophytes, while both isolates are leathery in texture. *L. saccharina* was, however, optically lighter than *L. digitata* and *L. hyperborea*. Corresponding spectral analysis of wild young sporophytes showed higher absorbance peak at 678 nm representing chlorophyll a peak and peaks between 425–450 nm representing additionally higher carotenoid content in wild *L.*

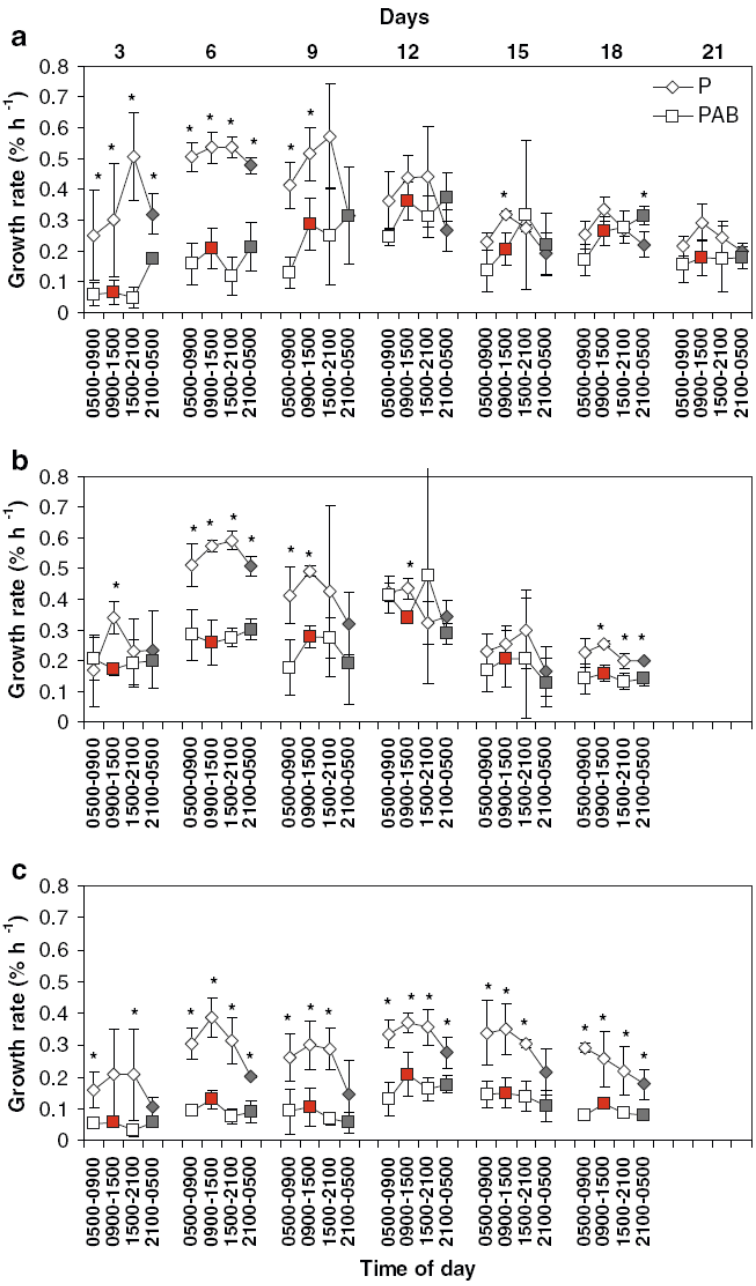

Fig. 3 Average circadian growth patterns of young **a** *L. digitata* **b** *L. saccharina* **c** *L. hyperborea* sporophytes ($n = 3$) exposed to PAR (P) and to the full light spectrum (PAB) at the start of the light phase to PAR without UV (0500–0900 h); in the middle of the light phase with UV supplement (0900–1500 h; *red-colour filled symbols*); at the end of the light phase again without UV (1500–2100 h); and during the dark phase (2100—0500 h; *grey-colour filled symbols*). Each point represents the mean of 3 days ± SD. * refers to significant difference between means (*t*-test, $P < 0.05$)

digitata and *L. hyperborea*. Strong absorbance below 280 nm was measured in the thalli of wild sporophytes, characteristic of the UV-absorbing compound phlorotannin.

Discussion

The UVR sensitivity of growth as an integrative parameter of all physiological processes is related to the upper distribution limit of the three kelp species in Helgoland. Tissue DNA damage is, however, dependent on thallus thickness, optics and absorption spectra characteristics of the pigments and UV-absorbing compounds. Energy demands for repair and protection may divert photosynthate at the expense of growth.

Growth as an integrative parameter of all cellular processes is better than photosynthetic performance to study long-term effects of UV exposure in macroalgae (Roleda et al. 2004b). A simple growth model: $G = P\text{-}R\text{-}L$,

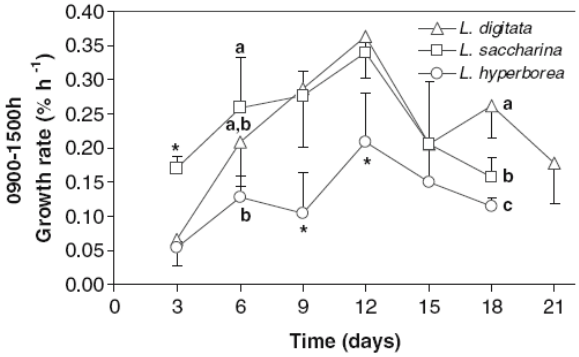

Fig. 4 Average growth rates over a period of 21 days in young sporophytes of the three *Laminaria* species ($n=3$) during UVR exposure (0900–1500 h). ANOVA showed significant difference between species ($P<0.05$). *Letters* on graph show result of DMRT ($P=0.05$); *different letters* refer to significant differences between means. * suggests that one species is significantly different from the other two species while the other two species are not significantly different from each other

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 lost or decay (L) (Carr et al. 1997). Under high-light intensity and UVR, photoinhibition of photosynthesis already decreases potential carbon acquisition (P) into plant dry matter (G) (Long et al. 1994). Dark respiration (R) represents the energy used to synthesize new biomass (growth respiration) and that used to maintain metabolic activity (maintenance respiration). Exposure to UVR causes cellular, enzymatic and molecular damage, which could further increase loss due to respiration (R) by diverting more photosynthate for repair and defense

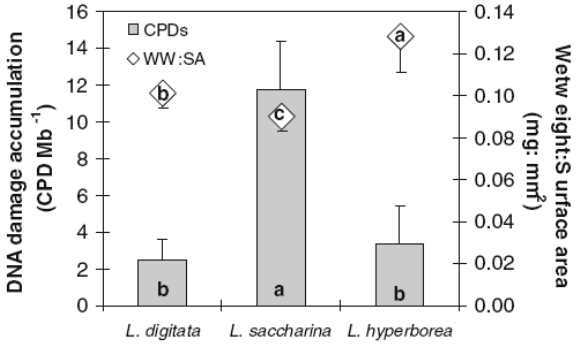

Fig. 5 Relationship between average thallus thickness and remaining tissue DNA damage after 18–21 days of repeated daily 6 h UVR exposure and 18 h recovery (6:8:4 h *light:dark:light cycle*) in the three *Laminaria* species. *Vertical bars* are standard deviations ($n=3$). ANOVA showed significant difference between species ($P<0.05$). *Letters* on graph show result of DMRT ($P=0.05$); *different letters* refer to significant differences between means

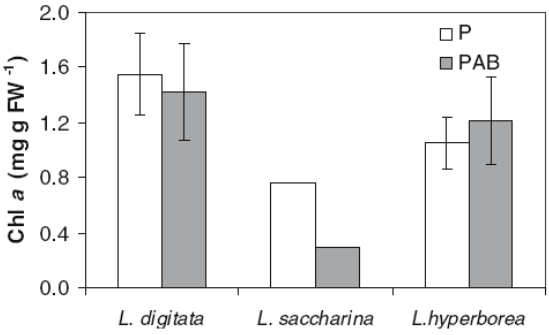

Fig. 6 Chlorophyll a content in young sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB). *Vertical bars* are standard deviations ($n=3$). Value of *L. saccharina* represents one replicate only

(i.e. production of secondary metabolites) thereby inhibiting growth.

Saturation of growth in young sporophytes of *L. digitata*, *L. saccharina* and *L. hyperborea* was previously reported at 20–30 μmol photon m^{-2} s^{-1} of continuous photon irradiance, whereas 1–2 μmol photon m^{-2} s^{-1} was able to sustain growth in culture (Han and Kain 1996). At 18 μmol photon m^{-2} s^{-1} in this study, maximum growth rate in *L. digitata* (1500–2100 h, $0.57 \pm 0.17\%$ h^{-1}) is comparable to the previous online growth measurements of Lüning (1994) on the same species from Helgoland reported at 0.5% h^{-1}. Growth rate in *L. saccharina* (1500–2100 h, $0.59 \pm 0.03\%$ h^{-1}) is, however, lower compared to the previous study reported at 0.7% h^{-1} (Lüning 1992). Comparison with other Laminariales showed higher growth rates among *Laminaria* species than in *Saccorhiza dermatodea* ($0.42 \pm 0.12\%$ h^{-1}) and *Alaria esculenta* ($0.31 \pm 0.02\%$ h^{-1}; measured during the dark phase) (Roleda et al. 2005b). The pioneering work of Lüning (1992) on on-line growth measurements within 3 days showed an increasing growth rate throughout the light phase and a subsequent continuous decrease in growth at the onset of darkness. However, the present on-line growth measurement which lasted for 18 days recorded a general growth trend showing an initial low growth during early morning, a mid morning or late afternoon peak and minimum growth during the dark phase. The circadian rhythm in growth has been reported to be associated with the circadian rhythm in gene expression, cell division and photosynthesis (Lüning 2001; Suzuki and Johnson 2001; Jacobsen et al. 2003). The decrease in growth rate through time observed in this study was also evident in the subsequent study of Lüning (1994), which recorded decreasing growth in the course of measurement over 6 days. In situ growth measurements on the three *Laminaria* species in Helgoland were also observed to decrease through time (Lüning 1979). The decrease in the intrinsic rate of increase in growth through time is attributed to the increase in doubling time for the cell mass (Brinkhuis 1985).

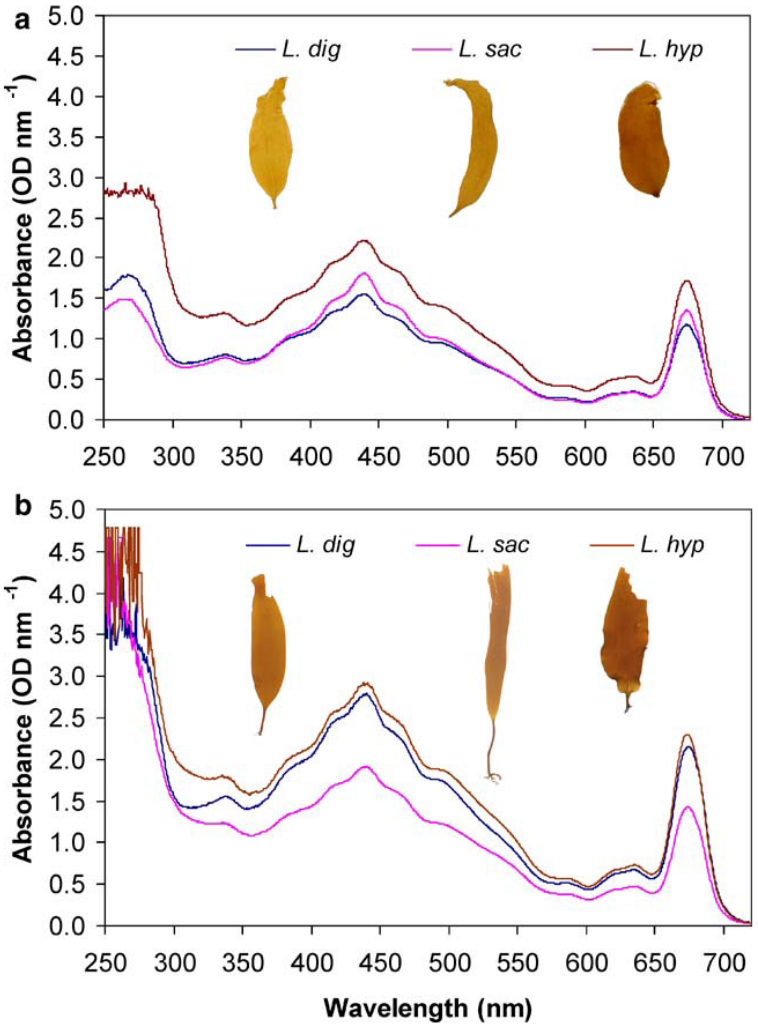

Fig. 7 Tissue absorbance spectrum of **a** cultured and **b** wild young *Laminaria* spp. sporophytes. The *insets* show photographs of the typical morphology of thalli among the three species of kelps used for the experiments

A circadian growth pattern of sporophytes exposed to UV-radiation was first reported by Roleda et al. (2005b) where dark pigmented young *A. esculenta* sporophytes were able to acclimate to UVR while translucent young *S. dermatodea* did not. The general growth pattern of *A. esculenta* and *S. dermatodea* exhibits a drop in growth rate during UVR exposure (0900–1500) relative to growth during early morning (0500–0900) and late afternoon (1500–2100) without UVR and at night (2100–0500). The circadian growth strategies of the three *Laminaria* species are different compared to that of *A. esculenta* and *S. dermatodea*. In *L. digitata*, the drop in growth during UVR exposure was observed only during the first 3 days. Growth was eventually able to acclimate to UVR, where growth rates at this time (0900–1500) were higher compared to the early morning growth. The decline in growth in the afternoon when UV lamps were turned off was compensated with a corresponding higher growth rate at night. This follows the general diurnal pattern observed among sporophytes exposed to PAR alone. However, at night, higher growth rates were observed relative to growth during the day in sporophytes exposed to UVR. In *L. saccharina*, although growth was also able to acclimate up to 12 days repeated UV exposure, the circadian growth patterns between P- and PAB-exposed sporophytes were relatively similar and follow the typical low early morning growth, midday peak, afternoon decline and low evening growth rhythm. In *L. hyperborea*, moderate growth rates were observed throughout the daily light cycle of PAB-exposed sporophytes. They were able to cope up with the daily UV dose throughout the experiment when growth during UVR exposure (0900–1500) did not drop relative to the early morning growth (0500–0900) without UVR. However, growth decreased after UVR exposure, similar to the pattern observed in *L. digitata*, but distinct growth compensation at night was observed only until the sixth day.

Based on the remaining tissue DNA damage, it is inferred that sensitivity of young sporophytes is not related to the depth distribution of the adult sporophytes but on the thallus thickness of the species. *L. saccharina*, which can occur in the upper sublittoral intermixed with *L. digitata* stand and in the mid sublittoral (Lüning 1979, 1985), is more sensitive to UVBR-induced DNA damage. This is attributed to its thinner and relatively translucent thalli. Although *L. saccharina* had the highest remaining tissue CPDs (11.7 ± 2.6 CPD mb^{-1}) after 18 days of repeated daily UVB dose of 8.6×10^3 J m^{-2}, an effective DNA damage repair mechanism is indicated in this species. After daily 6 h UVR exposure, the sporophytes were able to recover for 18 h under the daily light:dark cycle. Previously, induction of CPDs in Arctic *L. saccharina* exposed to lower UVB dose of 6.2×10^3 J m^{-2} resulted in a high accumulation of 88 CPD mb^{-1}, which was effectively repaired after 5 h exposure to photo-reactivating light at the rate of 0.49 CPD mb^{-1} h^{-1} (van de Poll et al. 2002). Efficient DNA damage repair was also observed in 8 hours UVR-exposed zoospores after 48 h recovery in low-white light whereby remaining DNA damage was undetectable in *L. digitata*, 3.8 ± 1 and 7.0 ± 4 CPD mb^{-1} in *L. saccharina* and *L. hyperborea*, respectively (Roleda et al. 2005a).

Laminaria digitata, which occurs in the upper sublittoral and *L. hyperborea*, which occurs deepest in the field reduce effective UV fluence from reaching the physiological target by means of thick thalli. It shows that thicker thalli can provide extra protective tissue against UVR (Johansson and Snoeijs 2002), where outer cell layers shade inner cells and provide longer pathlength for UVR absorption (Franklin and Forster 1997) making it less sensitive to UVB radiation-induced DNA damage. The epidermis itself already serves as a filter of UVB radiation and the optical property of the thallus can further influence reflection, attenuation, scattering, absorption or transmittance of UV radiation (Caldwell et al. 1983). The significance of thallus optics was previously reported by Roleda et al. (2005b). The thick but translucent thallus of *S. dermatodea* accumulated higher CPD content of 65 ± 9 CPD mb^{-1} compared to the thin but opaque *A. esculenta* (8 ± 3 CPD mb^{-1}) after exposure to the same experimental treatment (Roleda et al. 2005b). Thus, remaining tissue DNA damage in young sporophytes alone cannot explain the survival success between these species.

Zoospores of the same species are relatively more sensitive to DNA damage and their sensitivity is related to the vertical distribution pattern of the large sporophytes (Roleda et al. 2005a). In young sporophytes, DNA damage measured in *L. digitata* (2.5 ± 1 CPD mb^{-1}), *L. saccharina* (11.7 ± 3 CPD mb^{-1}) and *L. hyperborea* (3.4 ± 2 CPD mb^{-1}) after 18–21 days of repeated 6 h daily UVR exposure is less than the number of CPDs observed in zoospores exposed to 8 h UVR, accumulating 18.6 ± 4, 30.8 ± 8 and 49.8 ± 6 CPD mb^{-1}, respectively. Ontogenic differences in DNA damage accumulation was previously reported in *Mastocarpus stellatus* Stackhouse (Guiry) and *Chondrus crispus* Stackhouse, where carpospores of the two species were observed to be more sensitive compared to the foliose gametophytic life stages (Roleda et al. 2004a). Lower DNA damage and effective DNA damage repair mechanism in carpospores of *M. stellatus* enhanced its recruitment success to the upper eulittoral zone. In comparison across the latitudinal gradient, Arctic *Laminaria* populations were observed to be more sensitive to DNA damage compared to their temperate counterparts of the same species. Under the same light treatment, significantly higher DNA damage was observed in Arctic *L. digitata* (23.9 ± 7.9 CPD mb^{-1}), higher but insignificantly different between *L. saccharina* (Spitsbergen: 12.5 ± 0.9; Helgoland: 11.7 ± 2.6 CPD mb^{-1}) populations. Comparison between the deep and thick kelp species *L. hyperborea* (3.4 ± 2.1 CPD mb^{-1}) and *L. solidungula* (3.0 ± 2.7 CPD mb^{-1}) showed no significant difference in DNA damage accumulation (Roleda et al. 2006).

Under moderate fluence of UVR, the insignificant variation in chlorophyll a concentration in sporophytes exposed to PAB and to P treatments indicates that algae are able to synthesize new pigments in replacement of degraded chlorophylls after 6 h daily UVR exposure and acclimate to repeated moderate daily fluence of UVR. Acclimation of chlorophyll a concentration was also observed in the red macroalgae *Palmaria decipiens* (Reinsch) Ricker exposed to 12–16 h UVR (Poppe et al. 2002). In situ seasonal variation in pigment concentration of Arctic *L. saccharina* collected before, during and after ice break-up showed decreasing chlorophyll a concentration coinciding with increasing underwater radiation. Chlorophyll a concentration did not increase when melting snow contributed to water turbidity and reduction in underwater radiation (Aguilera et al. 2002b), suggesting irreparable degradation of pigments after exposure to high-light intensity.

Absorption profile and peaks in the Soret region (400–540 nm) are characteristic of carotenoids in the antenna complexes of higher plants (Pascal et al. 1999; Croce et al. 2000). Carotenoids are involved in several aspects of photosynthesis such as light absorption and energy transfer to the reaction centre complex and protection of the photosynthetic apparatus from damage by strong illumination. The higher absorption in this region among wild sporophytes of Laminariales could imply some photoprotection role of carotenoids.

Strong absorption of sporophyte thallus in the UV region of the spectrum is characteristic of phlorotannin. Isolated phlorotannins from *Fucus gardneri* Silva absorb strongly in the UV region with peak at 265 nm (Henry and van Alstyne 2004). In terrestrial plants, the pronounced wavelength selectivity of absorption in leaf epidermis is often attributed to flavonoids and other related UV-absorbing compounds (Robberecht and Caldwell 1978). Phenolic compounds generally have substantial UV absorbance and very little absorption in the visible waveband (Caldwell et al. 1983). Distribution

of phlorotannins is observed in the epidermis and cell walls of brown algae (Schoenwaelder and Clayton 1999; Lüder and Clayton 2004). UVR can therefore be attenuated by cellular UV-absorbing compounds and cell walls of the epidermal tissue, effectively reducing UV fluence from reaching physiological targets.

Several functions of phlorotannins have been reported in brown algae such as herbivore deterrents, digestion inhibitors, antibacterial agents and UV screens (Schoenwaelder 2002). Production of this compound involves a substantial cost in terms of individual growth (Pavia et al. 1999). However, synthesis of moderate levels of phlorotannins and growth could be maintained simultaneously (Steinberg 1995). Substantial UV absorbance characteristics of phlorotannin in young sporophytes grown in culture as well as in wild specimens supports the hypothesis of Arnold (2003) that synthesis and production of this compound is not strictly inducible as a secondary metabolite but also serves some primary and secondary roles in reproduction, fertilization, spore attachment and cell wall construction. Its synthesis could also be in response to specific environmental cues or stress factors and is allocated for different functions in different kelp species. The physiological cost of producing a higher amount of UV-absorbing compounds is, however, reflected in the lower growth rates of *L. hyperborea* relative to *L. digitata* and *L. saccharina*, which exhibit relatively lower absorption in the UV waveband.

Effective DNA repair mechanism in the zoospores of *L. digitata* (Roleda et al. 2005a) and in young sporophytes (this study) effectively enhanced recruitment success of this species to the upper sublittoral. The upper distribution limit of *L. saccharina* to the upper sublittoral could be attributed to the timing of its reproductive season in autumn. Zoospore release, fertilization and establishment of new recruits and growth under the canopy of *L. digitata* during low UVR environment of the season contributed to this success. Relative growth tolerance of young *L. saccharina* sporophytes to UVR extended its upper distribution limit to the upper sublittoral inter-mixed with *L. digitata* stands in some areas of the island. Sensitivity of *L. hyperborea* zoospores to UVR have been reported such that timing of its reproduction during winter is thought to be an adaptive strategy to prevent reproductive failure (Roleda et al. 2005a). In this study, a trade-off between the physiological cost of repair/defense and growth was observed in *L. hyperborea*, whilst growth inhibition of young sporophytes under UVR further established the relative sensitivity of this species where successful recruitment is confined within the lower sublittoral of the island.

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References

Aguilera J, Dummermuth A, Karsten U, Schriek R, Wiencke C (2002a) Enzymatic defenses against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. Polar Biol 25:432–441

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002b) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087–1095

Arnold TM (2003) To grow and defend: lack of tradeoffs for brown algal phlorotannins. Oikos 100:406–408

Bischof K, Kräbs G, Wiencke C, Hanelt D (2002) Solar ultraviolet radiation affects the activity of ribulose−1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 215:502–509

Brinkhuis BH (1985) Growth pattern and rates. In: Littler MM, Littler DS (eds) Handbook of phycological methods: ecological field methods: macroalgae. Cambridge University Press, Cambridge, UK, pp 461–477

Carr GM, Duthie HC, Taylor WD (1997) Models of aquatic plant productivity: a review of the factors that influence growth. Aquat Bot 59:195–215

Caldwell MM (1971) Solar ultraviolet radiation and the growth and development of higher plants. In: Giese AC (eds) Photophysiology. Academic Press, New York, pp 131–177

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Plant 58:445–450

Croce R, Cinque G, Holzwarth AR, Bassi R (2000) The Soret absorption properties of carotenoids and chlorophylls in antenna complexes of higher plants. Photosynth Res 64:221–231

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

Franklin LA, Forster RM (1997) The changing iradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32:207–232

Han T, Kain JM (1996) Effect of photon irradiance and photoperiod on young sporophytes of four species of the Laminariales. Eur J Phycol 31:233–240

Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997a) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255–266

Hanelt D, Wiencke C, Nultsch W (1997b) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J Photochem Photobiol B 38:40–47

Henry BE, van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. J Phycol 40:527–533

Jacobsen S, Lüning K, Goulard F (2003) Circadian changes in relative abundance of two photosynthetic transcripts in the marine macroalga *Kappaphycus alvarezii* (Rhodophyta). J Phycol 39:888–896

Johansson G, Snoeijs P (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar Ecol Prog Ser 244:63–72

Littler MM, Littler DS (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. Am Nat 116:25–44

Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol 45:633–662

Lüder UH, Clayton MN (2004) Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory—the first microscopic study. Planta 218:928–937

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Mar Ecol Prog Ser 1:195–207

Lüning K (1985) Meeresbotanik: Verbreitung, Ökophysiologie und Nutzung der marinen Makroalgen. Georg Thieme Verlag, Stuttgart, pp 375

Lüning K (1992) Day and night kinetics of growth rate in green, brown, and red seaweeds. J Phycol 28:794–803

Lüning K (1994) Circadian growth rhythm in juvenile sporophytes of Laminariales (Phaeophyta). J Phycol 30:193–199

Lüning K (2001) Circadian growth in *Porphyra umbilicalis* (Rhodophyta): spectral sensitivity of the circadian system. J Phycol 37:52–58

Pascal A, Gradinaru C, Wacker U, Peterman E, Calkoen F, Irrgang K-D, Horton P, Renger G, van Grondelle R, Robert B, van Amerongen H (1999) Spectroscopic characterization of the spinach Lhcb4 protein (CP29), a minor light-harvesting complex of photosystem II. Eur J Biochem 262:817–823

Pavia H, Toth G, Åberg P (1999) Trade-offs between phlorotannin production and annual growth in natural populations of the brown seaweed *Ascophyllum nodusum*. J Ecol 87:761–771

Poppe F, Hanelt D, Wiencke C (2002) Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic red alga *Palmaria decipiens* during acclimation to UV radiation. Bot Mar 45:253–261

Reed DC, Amsler CD, Ebeling AW (1992) Dispersal in kelps: factors affecting spore swimming and competency. Ecology 73:1577–1585

Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia (Berl) 32:277–287

Roleda MY, van de Poll WH, Hanelt D, Wiencke C (2004a) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar Ecol Prog Ser 281:37–50

Roleda MY, Hanelt D, Kräbs G, Wiencke C (2004b) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycologia 43:603–613

Roleda MY, Wiencke C, Hanelt D, van de Poll WH, Gruber A (2005a) Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction. Plant Cell Environ 28:466–479

Roleda MY, Hanelt D, Wiencke C (2005b) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. Polar Biol 28:539–549

Roleda MY, Wiencke C, Hanelt D (2006) Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic Laminaria sporophytes. Planta DOI 10.1007/s00425-005-0092-0

Schoenwaelder MEA (2002) The occurrence and cellular significance of physodes in brown algae. Phycologia 41:125–139

Schoenwaelder MEA, Clayton MN (1999) The presence of phenolic compounds in isolated cell walls of brown algae. Phycologia 38:161–166

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Nat Acad Sci USA 71:3363–3366

Starr RC, Zeikus JA (1993) UTEX—the culture collection of algae at the University of Texas at Austin. J Phycol 29(Suppl):1–106

Steinberg PD (1995) Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. Oecologia 102:169–173

Suzuki L, Johnson CH (2001) Algae know the time of day: circadian and photoperiodic programs. J Phycol 37:933–942

van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. J Phycol 37:30–37

van de Poll WH, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. Photochem Photobiol 76:493–501

Vink AA, Bergen-Henegouwen JB, Nikaido O, Baan RP, Roza L (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. Photochem Photobiol 24:25–31

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D Bischof K, Figueroa FL (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar Ecol Prog Ser 197:217–229

Wiencke C, Clayton MN, Schoenwaelder M (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. Mar Biol 145:31–39

Publication 6

Roleda MY, Wiencke C, Hanelt D, van de Poll WH, Gruber A

Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction

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Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction

M. Y. ROLEDA[1], C. WIENCKE[2], D. HANELT[3], W. H. VAN DE POLL[4] & A. GRUBER[1]

[1]*Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Postfach 180, D-27483 Helgoland, Germany,* [2]*Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany,* [3]*Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-Str. 18, D-22609 Hamburg, Germany and* [4]*Department of Marine Biology, University of Groningen, PO Box 14, NL-9750 AA Haren, The Netherlands*

ABSTRACT

Depth distribution of kelp species in Helgoland (North Sea) is characterized by occurrence of *Laminaria digitata* in the upper sublittoral, whereas *L. saccharina* and *L. hyperborea* dominate the mid and lower sublittoral region. *Laminaria digitata* is fertile in summer whereas both other species are fertile in autumn/winter. To determine the light sensitivity of the propagules, zoospores of *L. digitata*, *L. saccharina* and *L. hyperborea* were exposed in the laboratory to different exposure times of photosynthetically active radiation (PAR; 400–700 nm), PAR + UVA radiation (UVAR; 320–400 nm) and PAR + UVAR + UVB radiation (UVBR; 280–320 nm). Optimum quantum yield of PSII and DNA damage were measured after exposure. Subsequently, recovery of photosynthetic efficiency and DNA damage repair, as well as germination rate were measured after 2 and 3 d cultivation in dim white light. Photosynthetic efficiency of all species was photoinhibited already at 20 μmol photons m^{-2} s^{-1} PAR, whereas UV radiation (UVR) had a significant additional effect on photoinhibition. Recovery of the PSII function was observed in all species but not in spores exposed to irradiation longer than 4 h of PAR + UVA + UVB and 8 h of PAR + UVA. The amount of UVB-induced DNA damage measured as cyclobutane–pyrimidine dimers (CPDs) increased with exposure time and highest damage was detected in the spores of lower subtidal *L. hyperborea* relative to the other two species. Significant removal of CPDs indicating repair of DNA damage was observed in all species after 2 d in low white light especially in the spores of upper subtidal *L. digitata*. Therefore, efficient DNA damage repair and recovery of PSII damage contributed to the germination success but not in spores exposed to 16 h of UVBR. UV absorption of zoospore suspension in *L. digitata* is based both on the absorption by the zoospores itself as well as by exudates in the medium. In contrast, the absorption of the zoospore suspension in *L. saccharina* and *L. hyperborea* is based predominantly on the absorption by the exudates in the medium. This study indicates that UVR sensitivity of zoospores is related to the seasonal zoospore production as well as the vertical distribution pattern of the large sporophytes.

Key-words: *Laminaria digitata*; *Laminaria saccharina*; *Laminaria hyperborea*; cyclobutane-pyrimidine dimers; DNA damage and repair; F_v/F_m; germination rate; photosynthesis.

INTRODUCTION

Zoospore production and its subsequent release and recruitment on suitable substrate are important processes in maintaining kelp population in coastal marine environments (Reed, Schroeter & Raimondi 2004). Upon the release of zoospores, these propagules are confined in a viscous physical environment due of their small size (approximately 3.0–5.0 μm) and relatively slow swimming speeds (Amsler, Reed & Neushul 1992). Swimming competency of kelp zoospores has been recorded up to 72 h (Reed, Amsler & Ebeling 1992) and kelp zoospores have been identified from *in situ* plankton samples (Graham 1999). Spore swimming increases the likelihood of settlement. However, even after cessation of swimming, spores were found to germinate in the water column and retained their capacity to produce viable sporophyte recruits (Reed *et al*. 1992). This transitory planktonic phase, which is capable of photosynthesis, can, however, be exposed to variable environmental stress conditions with respect to light, ultraviolet radiation (UVR) and temperature. Spore dispersal as source of recruits has been extensively studied in Laminariales (e.g. Reed *et al*. 1992, 2004; Fredriksen *et al*. 1995), where resulting colonization has been documented over distances of at least 4000 m (Reed, Laur & Ebeling 1988). Moreover, local hydrodynamic condition can enhance dis-

Correspondence: Michael Y. Roleda. Fax: + 49 (0) 4725 819 283; e-mail: mroleda@awi-bremerhaven.de

persal; probability of gamete encounters and ensures high fertilization rates (Yund 2000) developing distinct natural population within the locality.

Surface UV wavelength to PAR ratio in Helgoland varies depending on cloud cover and season. The ratios of 320, 340 and 380 nm to PAR were 15–20% higher on the dull days than on bright days, whereas the 305 nm : PAR ratio did not vary between different cloud cover. On the other hand, seasonal variation is characterized as follows: no seasonal increase in 305 nm : PAR ratio; 50% increase in 320 nm : PAR ratio from winter to summer; constant 340 nm : PAR ratio throughout the year; and slight decrease in 380 nm : PAR ratio during summer months (Dring *et al.* 2001a). Underwater measurements estimated 1% depth for 305-nm radiation at 1 m measurable only during summer and autumn. The 1% depths recorded for the other UV wavelengths were 2.0, 2.6 and 4.6 m for 320, 340 and 380 nm, respectively, compared to 12 m for PAR (Dring *et al.* 2001a). Relative to tidal fluctuation, higher PAR transmittance is also observed during neap tides (low tide at midday) than on spring tides (high tide on midday). This association is suggested to be dependent on stronger tide flows that occur during spring tides contributing to the re-suspension of sediments effectively reducing light penetration (Dring & Lüning 1994).

Tolerance and recovery of photosynthesis of young and adult thalli to inhibiting photosynthetically active radiation (PAR) is reported to influence depth distribution of seaweeds (e.g. Han & Kain 1996; Hanelt, Wiencke & Nultsch 1997a; Hanelt *et al.* 1997b). Recent temporary lowering of stratospheric ozone concentrations enhances the UVB fluxes at the earth's surface (Smith *et al.* 1992; von der Gathen *et al.* 1995; Stähelin *et al.* 2001). Consequently, UVR has also been reported to influence the vertical distribution pattern of seaweeds (e.g. Dring *et al.* 1996a; Bischof, Hanelt & Wiencke 1998a, 2001; Hanelt 1998; Dring, Wagner & Lüning 2001b).

The effect of ultraviolet radiation (UVR) on algal metabolism and physiology is manifold. Early developmental stages of seaweeds have been reported to be more susceptible to UVR when compared to adult stages (reviewed by Coelho, Rijstenbil & Brown 2000). Exposure to increased UVR induces spore mortality and photoinhibition of photosynthesis (Wiencke *et al.* 2000; Wiencke, Clayton & Schoenwaelder 2004; Roleda *et al.* 2004a). The primary targets for the UV-suppression of photosynthetic activity are still under debate (Baker, Nogues & Allen 1997). UVBR appears to degrade the D1 protein and part of the D1/D2 heterodimer; the major structural complex within PSII (Aro *et al.* 1990; Melis, Nemson & Harrison 1992; Jansen *et al.* 1993). Other studies have demonstrated decreases in the pool size of carbon fixation enzymes such as carbonic anhydrase (Dionisio, Tsuzuki & Miyachi 1989) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Bischof, Hanelt & Wiencke 2000). Moreover, UVBR exposure causes DNA damage in spores of Laminariales and Gigartinales (Wiencke *et al.* 2000; Roleda *et al.* 2004a). Most UVB-induced lesions involve dimerization of adjacent pyrimidine bases which results in cyclobutane-pyrimidine dimers (CPDs). These lesions are cytotoxic because they disrupt cell metabolism and division (van de Poll *et al.* 2001).

To counteract the negative effects of UVBR, ultraviolet sunscreens are used as photoprotective mechanisms in planktonic organisms (Garcia-Pichel 1994). Among 1–< 10 μm size class cells, sunscreens accumulation can afford considerable benefits but only at the expense of relatively heavy energetic investment and with restricted efficiencies (Garcia-Pichel 1994). However, the protective potential of phlorotannin containing physodes in kelp zoospores (3–5 μm) has been described by Wiencke *et al.* (2004). Moreover, kelp phlorotannin exudates along coastal shores from macroalgal sources have been reported to reduce the impact of UVBR, forming UV-refugia for kelp zoospores within the water column (Swanson & Druehl 2002).

Zonation pattern of kelp species in Helgoland (North Sea) is characterized by typical occurrence of *Laminaria digitata* (Hudson) Lamouroux in the uppermost part of the sublittoral region, whereas *Laminaria saccharina* (Linnaeus) Lamouroux and *Laminaria hyperborea* (Gunnerus) Foslie dominate the middle and deeper parts of the kelp zone, respectively (Lüning 1979). Initiation of reproduction in Helgolandic Laminariales is limited to a distinct reproductive season. Reproductive tissues (sori) are observed in *L. digitata* during late spring to summer (May–August), *L. saccharina* during autumn to mid winter (September–February) and *L. hyperborea* during late autumn to winter (November–February). This reproductive strategy is speculated to be associated to the seasonal environmental pattern ensuring higher probability of success in reproduction (De Wreede & Klinger 1988). A recent study on the germination of five species of Laminariales from the Arctic showed that UVR susceptibility of zoospores is also related to the depth distribution of the adult sporophytes (Wiencke *et al.* 2004). In this regard, the present study will re-examine the susceptibility of the early developmental stages of the three Helgolandic *Laminaria* species to varying irradiance in relation to their depth distribution. This relationship was previously sought but not found by Dring *et al.* (1996b). We focus on zoospores and germinating spores, because they can be found to be planktonic for an extended period of time exposed to environmental stress such as high photon fluence rate and UVR. However, settling zoospores under algal canopies experiences different low-light microenvironment which is suitable for germination and growth. This study extends to investigate the impact of increasing exposure time to varying light spectrum on the photosynthetic efficiency and DNA damage of zoospores. The presence of UV-absorbing compounds in zoospore suspension is quantified and this is the first study on the capacity of Laminariales zoospores for DNA repair and its implication on germination capacity. We hypothesize that susceptibility of zoospores of the three *Laminaria* species to PAR and UVR influence the vertical distribution pattern of the adult sporophytes and the eventual reproductive strategies of adult sporophytes.

MATERIALS AND METHODS

Zoospore material

Fertile specimens of *L. digitata* were collected by hand in the upper sublittoral (0.5–1 m) during low tide, and *L. saccharina* and *L. hyperborea* were collected by scuba diving in the mid (2–4 m) and lower (5–7 m) sublittoral, respectively, around the island of Helgoland. Thallus parts with sori were blotted with tissue paper and kept for 2 d in a wet chamber in dim light at 5 ± 1 °C. Spores were released from five individual sporophytes per species by flooding Provasoli enriched seawater (Starr & Zeikus 1993) to the thallus in separate Petri-dishes. Spore density released from individual sporophytes was adjusted to 2.0×10^5–4.0×10^5 spore ml^{-1} using a Neubauer Chamber (Brand GmbH, Wertheim, Germany). Due to the extent of the experimental work, sori for photosynthesis, absorption spectra, DNA damage and recovery, and germination experiments were separately collected four times during the peak fertile season of *L. digitata* (May–July), *L. saccharina* (September–November) and *L. hyperborea* (December–February).

Irradiation treatments

Photosynthetically active radiation (PAR) was provided by white fluorescent tubes (L65 Watt/25S, Osram, Munich, Germany) and ultraviolet radiation (UVR) was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA), emitting a spectrum similar to solar radiation in the range 295–340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes. Experimental units were covered with the following filters: Ultraphan transparent (Digefra GmbH, Munich, Germany); Folanorm (Folex GmbH, Dreieich, Germany) or Ultraphan URUV Farblos corresponding to the PAR + UVA + UVB (PAB), PAR + UVA (PA) and PAR (P) treatments, respectively. Irradiation was measured using a cosine sensor connected to a UV-VIS Spectrometer (Marcel Kruse, Bremerhaven, Germany) below the cut-off filters. The biologically effective doses (BED) between 280 and 320 nm applied were calculated using two action spectra for well-known biological responses: the generalized plant damage (280–312 nm, Caldwell 1971) and DNA damage for *Escherichia coli* (280–320 nm, Setlow 1974). Minimal erythemal dose (MED) below the cut-off filters was also measured using an ELUV-14 UV-Data logger (El Naggar *et al.* 1995). Both unweighted and weighted irradiances for each treatment are compiled in Table 1. The 1.26 UV (total UVR) : PAR ratio in this study is within the highly variable ratio of UV (305, 320, 340, and 380) : PAR ratio measured in Helgoland which ranges between 0.002 and 1.4 depending on cloud cover and season (Dring *et al.* 2001a).

Chlorophyll fluorescence measurements

Photosynthetic efficiency measured as variable fluorescence of photosystem II (PSII), was determined using a xenon pulse amplitude modulation fluorometer (XE-PAM) connected to a PC with WINCONTROL software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of spore density (approximately 1 h after spore release), spore suspension was filled into 5 mL quartz cuvettes and the maximum quantum yield (F_v/F_m) was measured to determine initial photosynthetic efficiency at time zero (T_0, $n = 5$) as described by Hanelt (1998). Photosynthesis (in terms of relative electron transport rate, $ETR = PFR \times \Delta F/F_m'$) versus irradiance curves (P–I curve) were also measured in the T_0 samples ($n = 3$, chosen at random from the five replicates) as described by Bischof *et al.* (1998b). Saturating irradiance level (I_k) and ETR_{max} were estimated. Spore suspensions used for T_0 measurements were filled into corresponding Petri dishes. To evaluate the effect of different radiation and exposure time treatments, 5 mL of fresh spore suspension were filled into each 35 mm × 10 mm cell culture dish and exposed to the three radiation conditions in a series of time treatments (1, 2, 4, 8 and 16 h; $n = 5$ per treatment combination) at 10 ± 1 °C. After treatments, F_v/F_m was measured and spore suspension was returned to the same culture dish and cultivated under dim white light (10 μmol photons m^{-2} s^{-1}) for recovery. Spore suspension measured at T_0 was also maintained at the same condition. Measurements of photosynthetic efficiency were repeated after 2 d to determine recovery and handling effect on untreated T_0 samples (now designated as T_2 measurement), which were eventually used as control. Settled and germinating spores

Table 1. Experimental treatments applied with the corresponding weighted irradiances using the biologically effective weighing function for general plant damage (Caldwell 1971), DNA damage of *E. coli* (Setlow 1974), and minimal erythemal dose (MED, El Naggar *et al.* 1995)

Treatment	Experimental irradiance (W m^{-2})			Weighted irradiance (W m^{-2})		
	PAR (400–700 nm)	UVA (320–400 nm)	UVB (280–320 nm)	General plant damage (Caldwell 1971)	DNA damage (Setlow 1974)	MED (El Naggar *et al.* 1995)
Ultraphan transparent (PAR + UVA + UVB)	4.74	5.86	0.36	1.8×10^{-2}	4.7×10^{-3}	8.7×10^{-2}
Folanorm 320 (PAR + UVA)	4.44	4.95	0.03	0	9.5×10^{-5}	4.2×10^{-3}
Ultraphan URUV farblos (PAR)	4.59	0.04	0.00	0	0	1.2×10^{-3}

were slowly re-suspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes. F_v/F_m after exposure and after recovery was expressed as percentages of T_0 and T_2 control, respectively.

Absorbance spectrum

To determine the presence of UV-absorbing compounds in the zoospore suspension, untreated samples were filled into quartz cuvettes and scanned in the 250–700 nm waveband using Shimadzu photometer (UV 2401PC; Shimadzu, Tokyo, Japan) equipped with an integrating sphere. Absorbance spectrum of the zoospore suspension, the medium (filtrate) and zoospores were measured from: (1) zoospore suspension with seawater as reference, (2) filtrate with seawater as reference, and (3) zoospore suspension and filtrate as reference, respectively. The filtrate was obtained by filtering the zoospores out of the suspension through 44-mm-diameter, 1.0 μm pore size Nuclepore® polycarbonate membrane (Whatman, Maidstone, Kent, UK) using a vacuum pump at 400–600 millibars to minimize damage to the cells.

DNA damage and repair

DNA damage and its subsequent repair were determined after exposure to the same treatments. From the spore suspension, 40 mL was used for each experimental unit. For each treatment, six experimental units were prepared. After the irradiation treatment, three experimental units (as replicates) were processed immediately while the other three were allowed to recover for 2 d in low white light before processing. Settled and germinating spores were re-suspended from the bottom of the Petri dishes by jetting pressurized seawater from a wash bottle. The spore samples were filtered through 44-mm-diameter, 1.0 μm pore size Nuclepore® polycarbonate membrane (Whatman). Filters were individually filled into 2 mL Eppendorf tubes and frozen at –80 °C for further DNA extraction and analysis of CPDs.

Spore germination

Cover slips were put inside the 85 mm × 15 mm culture dishes and filled with 40 mL Provasoli-enriched seawater. To ensure that the density of spores per unit area is similar throughout the experiments, 2–4 drops of the working spore suspension were put into each dish. The dishes were then exposed to the same experimental treatments, in triplicates. After treatment, spores were allowed to germinate in low white light (10 μmol photons m^{-2} s^{-1}) for 3 d. Triplicate of untreated samples (control) were also allowed to grow at the same low light condition. Spores settled on the cover slip were scored as germinated or not germinated by counting 300 cells per replicate using a light microscope (Olympus CH-2; Olympus, Tokyo, Japan) equipped with a 20× seawater immersion objective. A spore was classified as germinated when at least a germ-tube was formed. Dead and living cells were not differentiated. Since non-germinated cells were also observed under control, germination rate under P, PA and PAB treatments was expressed as percentage of control.

DNA extraction

Frozen spores on polycarbonate filters were treated with extraction buffer (CTAB) and DNA was isolated as described by van de Poll *et al.* (2001) and modified by Roleda *et al.* (2004a). After DNA extraction, the pellet was dissolved in 0.2 mL TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, pH 8.0), treated with RNAase (5 μL 10 mg mL^{-1}, 30 min, 37 °C; Sigma, St Louis, MO, USA) and stored at –20 °C. The DNA concentration was quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, Palo Alto, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPDs detection

The immunoassay for CPDs was modified after Vink *et al.* (1994) and van de Poll *et al.* (2001). Heat-denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 μm; Schleicher & Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a two-step antibody assay, the membrane was treated with ECL Western blotting detection reagent (Amersham, Little Chalfont, Bucks., UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) at different exposure times. The films were developed using X-ray film developer. Developed films were scanned using Bio-Rad imaging densitometer (Model GS-700; Bio-Rad Laboratories, Hercules, CA, USA) and grey scale values were quantified using MULTI-ANALYST (Macintosh Software for Bio-Rad Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva, Heidelberg, Germany) supplemented with unexposed DNA was included giving 1 μg mL^{-1} DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the grey scales within the linear range of the film.

Statistical analysis

Data were tested for homogeneity of variances (Levene Statistics) and normality (Kolmogorov–Smirnov test). Corresponding transformations were done to heteroskedastic and non-normal data. The response of the dependent factors were tested using multiple analyses of variance (MANOVA, $P < 0.05$) with interaction effect between spe-

cies, irradiance and exposure time. When two-way and three-way interactions were observed, significantly different subgroups were determined by plotting the means of each dependent factor against the levels of each independent (main) factor (Underwood 1981). Groupings were based on *post hoc* multiple comparisons test. Statistical analyses were done using SPSS program (SPSS, Chicago, IL, USA).

RESULTS

Photosynthetic efficiency

Measurements of initial photosynthetic efficiency of the controls showed highest maximum quantum yield (F_v/F_m) in zoospores of *L. hyperborea* (0.533 ± 0.005), followed by *L. saccharina* (0.476 ± 0.003) and lowest in *L. digitata* (0.466 ± 0.010). During the short irradiation period (3 min) for the measurement of the P–I curve, ETR_{max} of the controls in *L. digitata* did not decrease until the highest actinic light level of 750 μmol photons m^{-2} s^{-1} was reached. A slight decrease in ETR_{max} was observed in *L. saccharina* at 275 μmol photons m^{-2} s^{-1} whereas the ETR_{max} of *L. hyperborea* decreases already above 200 μmol photons m^{-2} s^{-1}. Visual estimate of the P–I curve showed higher saturating irradiance (I_k) in *L. digitata* (approximately 40 μmol photons m^{-2} s^{-1}; Fig. 1a) compared to *L. saccharina* and *L. hyperborea* (approximately 30 and 20 μmol photons m^{-2} s^{-1}, respectively; Fig. 1b & c). After 1 h of higher light pretreatment at ±20 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR = P), the optimum quantum yield (F_v/F_m, expressed as a percentage of control) was already reduced to 34% in *L. digitata* (Fig. 2a) and *L. saccharina* (Fig. 2b) and to 20% in *L. hyperborea* (Fig. 2c). Increasing exposure time further decreased the F_v/F_m, indicating that zoospores are quite low light adapted. Light supplemented with UV-radiation further decreased zoospore photosynthetic efficiency. After 1 h exposure to PAR + UVA (PA) and PAR + UVA + UVB (PAB), an additional reduction in the F_v/F_m of UVR exposed samples (approximately 7–20%) was observed compared to the PAR only. Increasing exposure time further exacerbate the effect of UVR.

After 2 d in dim white light, photosynthetic efficiency of the untreated (control) germinating spores was reduced by

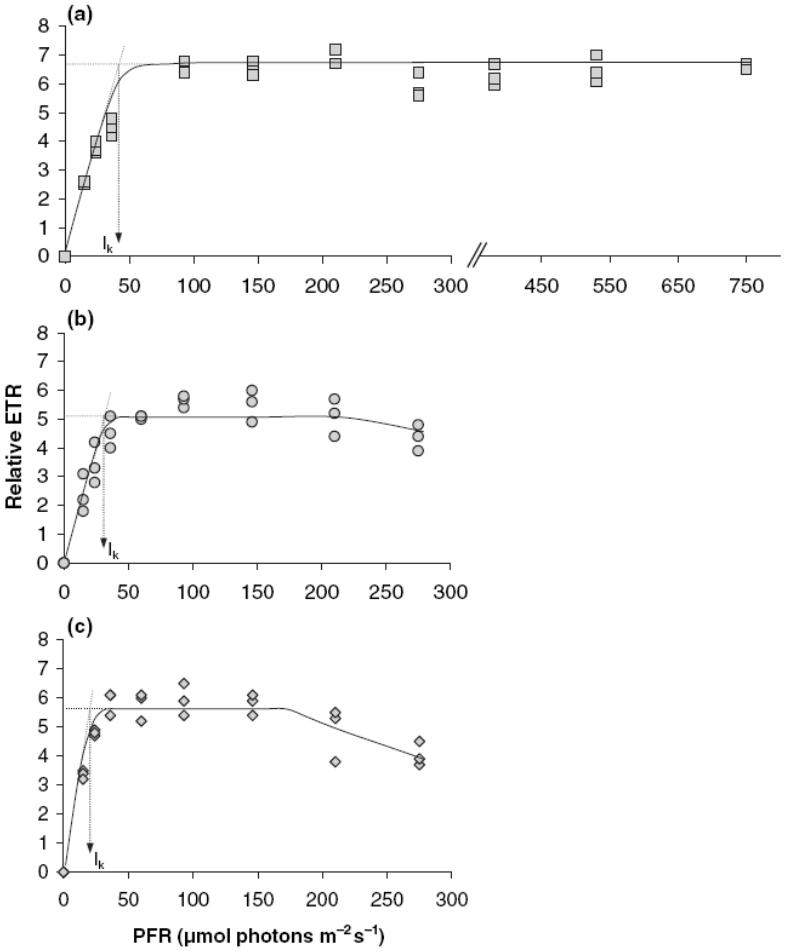

Figure 1. Photosynthetic performance (P–I curve) of zoospores from (a) *Laminaria digitata* (b) *L. saccharina* and (c) *L. hyperborea* ($n = 3$) immediately after release from the sori. PFR is the respective photon fluence rate of actinic white light and ETR is the electron transport rate. Saturating irradiance (I_k) is estimated as the point at which the extrapolated initial slope crosses maximum photosynthesis (ETR_{max}). Maximum effective quantum yields are 0.424 ± 0.01, 0.318 ± 0.09 and 0.453 ± 0.02 for *L. digitata*, *L. saccharina* and *L. hyperborea*, respectively.

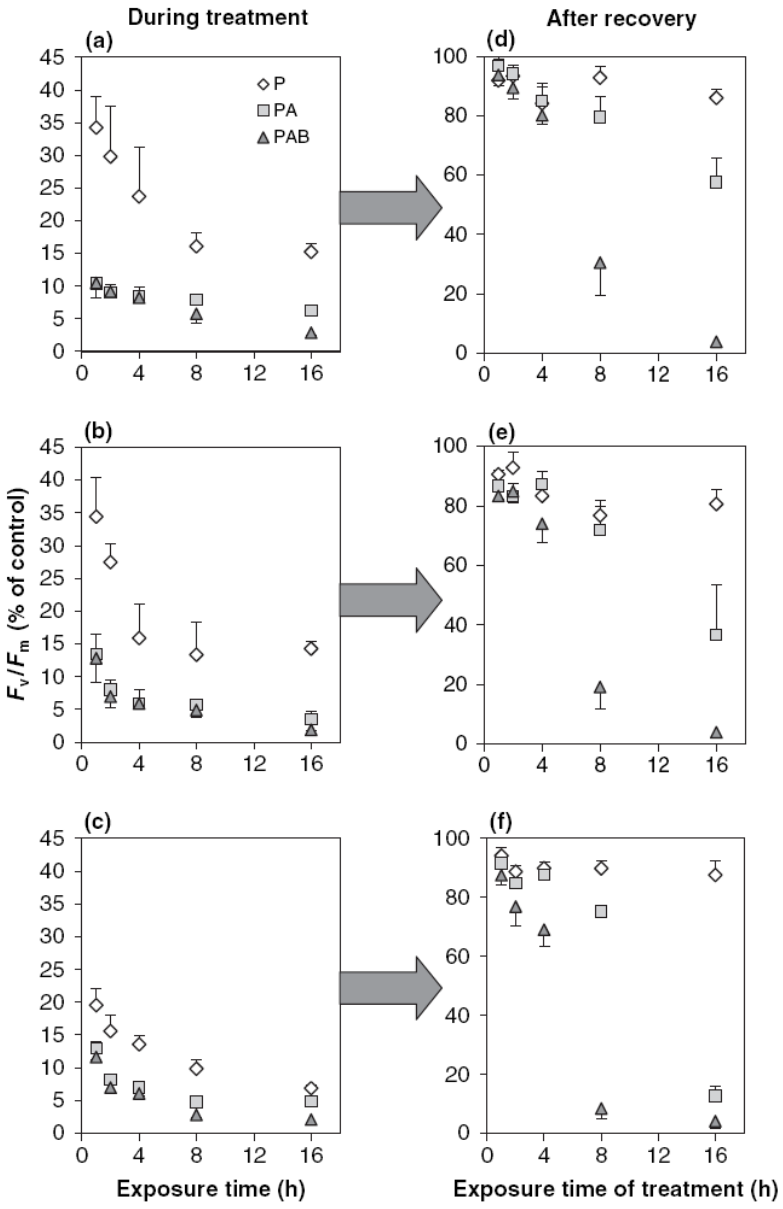

Figure 2. Mean optimum quantum yield (F_v/F_m) of zoospores during treatment to photosynthetically active radiation (PAR = P), PAR + UVA (PA) and PAR + UVA + UVB (PAB) at different exposure times in (a) *Laminaria digitata*; (b) *L. saccharina*; and (c) *L. hyperborea* expressed as percentage of control. Absolute means at T_0 are 0.466 ± 0.010, 0.476 ± 0.003 and 0.533 ± 0.005, respectively. Corresponding photosynthetic recovery (d, e and f, respectively) after 48 h post-culture in dim white light (10 μmol photons m^{-2} s^{-1}). Vertical bars are standard deviations (SD, n = 5). Absolute means at T_2 are 0.387 ± 0.005, 0.431 ± 0.008 and 0.513 ± 0.011, respectively.

4–17% in comparison with the freshly released zoospores of all controls. F_v/F_m was measured in decreasing order in *L. hyperborea* (0.513 ± 0.011); *L. saccharina* (0.431 ± 0.008); and *L. digitata* (0.387 ± 0.005). Photosynthetic efficiency of all species treated with P was able to recover in dim light to 80–95% of the control (Fig. 2d–f). An efficient recovery of photosynthetic efficiency (70–90% of control) was also observed in spores of all species treated to a maximum of 8 h PA and 4 h PAB (Fig. 2d–f). All data (absolute values) are compiled in Table 2.

Multiple analysis of variance (MANOVA, $P = 0.05$) showed a significant effect of the main factors, two-way and three-way interactions (Table 3). *Post hoc* multiple comparisons test showed several significantly different subgroups. Ranking the subgroups from the lowest to higher photosynthesis level, photosynthetic efficiency was lowest in the subgroup consisting of 16 h PAB in all species, 16 h PA in the mid and lower sublittoral species *L. saccharina* and *L. hyperborea* and 8 h PAB in *L. hyperborea*. The second from lowest subgroup showed minimal photosynthetic efficiency among 4 h PAB and 8 h PA in *L. saccharina* and *L. hyperborea*, 8 h PAB in *L. digitata* and *L. saccharina*, 16 h PA in *L. digitata* and 16 h P in *L. hyperborea*. After 2 d in dim white light, no recovery of photosynthetic efficiency was observed in the subgroup consisting of 16 h PAB treatment in all species and minimal recovery was observed in the

Table 2. Mean absolute values (± SD) of zoospore photosynthetic efficiency (optimum quantum yield, F_v/F_m) and germination after treatment to photosynthetically active radiation (PAR = P); PAR + UVA radiation (PA); PAR + UVAR + UVB radiation (PAB)

	Laminaria digitata			*Laminaria saccharina*			*Laminaria hyperborea*		
Optimum quantum yield (F_v/F_m)									
Control (T_0)	0.466 ± 0.010			0.476 ± 0.003			0.533 ± 0.005		
Control (T_2)	0.387 ± 0.005			0.431 ± 0.008			0.513 ± 0.011		
	P	PA	PAB	P	PA	PAB	P	PA	PAB
Treatment (h)									
1	0.160 ± 0.022	0.049 ± 0.004	0.049 ± 0.011	0.164 ± 0.029	0.064 ± 0.015	0.061 ± 0.018	0.104 ± 0.014	0.069 ± 0.005	0.062 ± 0.002
2	0.139 ± 0.036	0.042 ± 0.005	0.043 ± 0.004	0.131 ± 0.013	0.038 ± 0.007	0.033 ± 0.008	0.083 ± 0.013	0.043 ± 0.004	0.037 ± 0.003
4	0.110 ± 0.036	0.039 ± 0.007	0.038 ± 0.002	0.076 ± 0.025	0.028 ± 0.010	0.028 ± 0.003	0.072 ± 0.007	0.037 ± 0.003	0.032 ± 0.002
8	0.075 ± 0.010	0.037 ± 0.003	0.027 ± 0.006	0.063 ± 0.024	0.027 ± 0.003	0.023 ± 0.005	0.053 ± 0.007	0.025 ± 0.004	0.015 ± 0.003
16	0.071 ± 0.006	0.029 ± 0.004	0.013 ± 0.002	0.068 ± 0.005	0.017 ± 0.005	0.009 ± 0.001	0.037 ± 0.003	0.026 ± 0.002	0.011 ± 0.003
48 h recovery after exposure [Treatment exposure(h)]									
1	0.356 ± 0.028	0.375 ± 0.012	0.363 ± 0.015	0.391 ± 0.006	0.374 ± 0.008	0.359 ± 0.005	0.483 ± 0.013	0.470 ± 0.017	0.449 ± 0.017
2	0.362 ± 0.011	0.365 ± 0.010	0.346 ± 0.015	0.400 ± 0.022	0.359 ± 0.019	0.366 ± 0.017	0.455 ± 0.011	0.435 ± 0.010	0.395 ± 0.033
4	0.326 ± 0.022	0.330 ± 0.023	0.310 ± 0.012	0.359 ± 0.017	0.377 ± 0.018	0.319 ± 0.028	0.461 ± 0.012	0.451 ± 0.013	0.354 ± 0.030
8	0.359 ± 0.015	0.308 ± 0.027	0.118 ± 0.043	0.331 ± 0.021	0.310 ± 0.034	0.083 ± 0.032	0.461 ± 0.014	0.386 ± 0.010	0.043 ± 0.018
16	0.333 ± 0.011	0.223 ± 0.031	0.015 ± 0.006	0.348 ± 0.020	0.158 ± 0.074	0.017 ± 0.006	0.450 ± 0.023	0.065 ± 0.016	0.021 ± 0.013
Germination (%)									
Control		93 ± 2.5			90 ± 3.2			95 ± 3.2	
Treatment (h)									
1	83 ± 0.3	83 ± 2.2	69 ± 0.7	75 ± 2.2	69 ± 3.5	61 ± 0.6	80 ± 0.9	63 ± 1.2	61 ± 7.6
2	75 ± 1.8	72 ± 2.6	61 ± 3.0	63 ± 4.6	67 ± 4.4	57 ± 1.5	71 ± 1.0	55 ± 5.3	54 ± 1.6
4	69 ± 0.8	70 ± 1.3	51 ± 4.4	61 ± 4.0	61 ± 3.7	50 ± 4.5	69 ± 1.0	55 ± 0.9	50 ± 1.5
8	66 ± 5.1	69 ± 2.2	37 ± 7.9	59 ± 2.2	58 ± 1.3	26 ± 1.3	63 ± 2.8	53 ± 1.4	25 ± 3.7
16	59 ± 2.4	59 ± 7.8	26 ± 0.9	56 ± 3.6	57 ± 2.0	23 ± 0.6	62 ± 6.8	45 ± 15.5	19 ± 1.9

Photon flux density is 20–24 μmol photons m^{-2} s^{-1} (± 4–5 W m^{-2}). Photosynthetic recovery was initiated in dim white light of 10 μmol photons m^{-2} s^{-1} after treatment. Control at time zero (T_0) was measured after spore release while control at day 2 (T_2) was measured after post-cultivation at the same dim light condition with that of the treated samples. Germination controls were untreated samples simultaneously cultivated at 10 μmol photons m^{-2} s^{-1} together with the treated samples after treatment.

Table 3. Multiple analysis of variance (MANOVA) and significance values for the main effects and interactions of species, irradiance and exposure time on the photosynthesis, DNA damage and repair and germination of zoospores from three species of Laminariales in Helgoland

Experiment	Variable	Source of variation	d.f.	*F*-value	*P*-value
Photosynthesis	F_v/F_m (after treatment)	Species (A)	2	51.286	< 0.001*
		Irradiance (B)	2	533.834	< 0.001*
		Exposure time (C)	4	133.264	< 0.001*
		A × B	4	31.238	< 0.001*
		A × C	8	3.376	0.001*
		B × C	8	14.561	< 0.001*
		A × B × C	16	2.502	0.002*
	F_v/F_m (recovery)	Species (A)	2	47.502	< 0.001*
		Irradiance (B)	2	825.265	< 0.001*
		Exposure time (C)	4	729.542	< 0.001*
		A × B	4	11.663	< 0.001*
		A × C	8	5.532	< 0.001*
		B × C	8	209.925	< 0.001*
		A × B × C	16	9.352	< 0.001*
DNA damage and repair	CPDs induction	Species (A)	2	49.608	< 0.001*
		Exposure time (B)	4	40.684	< 0.001*
		A × B	8	3.769	0.004*
	Remaining CPDs	Species (A)	2	12.053	< 0.001*
		Exposure time (B)	4	5.243	0.003*
		A × B	8	1.872	0.102^{ns}
Germination		Species (A)	2	58.614	< 0.001*
		Irradiance (B)	2	390.725	< 0.001*
		Exposure time (C)	4	185.816	< 0.001*
		A × B	4	16.497	< 0.001*
		A × C	8	1.265	0.272^{ns}
		B × C	8	25.338	< 0.001*
		A × B × C	16	0.741	0.745^{ns}

*Significant; ^{ns} not significant.

subgroup of 16 h PA and 8 h PAB treatments in the lower sublittoral species *L. hyperborea*. This corresponds to the zonation pattern of the respective species at the coast.

Absorbance spectrum

Spectral analysis of the zoospore suspension (Fig. 3a), filtrate (Fig. 3b) and zoospores (Fig. 3c) showed strong absorbance below 360 nm. The absorbance maxima in the 260–280 nm range are characteristic of phlorotannins. Higher absorbance was measured in all *L. digitata* samples in comparison with the two other species. The filtrate containing exudates strongly absorbed UVBR in the medium of *L. digitata* more than the zoospores itself. UV absorbance of the filtrate was always higher than that of the zoospores. Relatively similar absorbance was measured in the zoospores of *L. saccharina* and *L. hyperborea* (Fig. 3c).

DNA damage and repair

Differential cyclobutane-pyrimidine dimers (CPDs) induction was observed in the spores of the three species examined. CPD induction significantly increases with UV exposure time and higher CPD induction was observed in *L. hyperborea* (Fig. 4a). However, significant two-way interaction was observed in CPD induction (Table 2), implying that DNA damage may either be significantly different among the three species, but not for all exposure times or significantly different among all exposure times but not for all species. After 2 d post-culture in dim white light, all species were observed to repair DNA damage. No detectable CPDs were observed in *L. digitata* spores exposed to 1–8 h of PAB (Fig. 4b). Repair of DNA damage was observed to be significantly different between species ($P < 0.001$) and exposure time ($P = 0.003$). *Post hoc* test showed that the difference between repair of *L. saccharina* and *L. hyperborea* DNA damage was not significantly different.

Germination

After 3 d in dim white light, spore germination of untreated samples were 93 ± 2.5, 90 ± 3.2 and 95 ± 3.2% in *L. digitata*, *L. saccharina* and *L. hyperborea*, respectively (all non-standardized data are presented in Table 2). Among treated samples, germination rate decreased with increasing exposure treatment in all species (Fig. 5a–c). Germination was 85–90% after 1 h PAR and 60–65% after 16 h PAR exposure. Germination capacity was effectively reduced in spores exposed to light supplemented with UVR. UVA had no effect on the germination capacity of *L. digitata* (Fig. 5a) and *L. saccharina* (Fig. 5b). In contrast, germination rate in

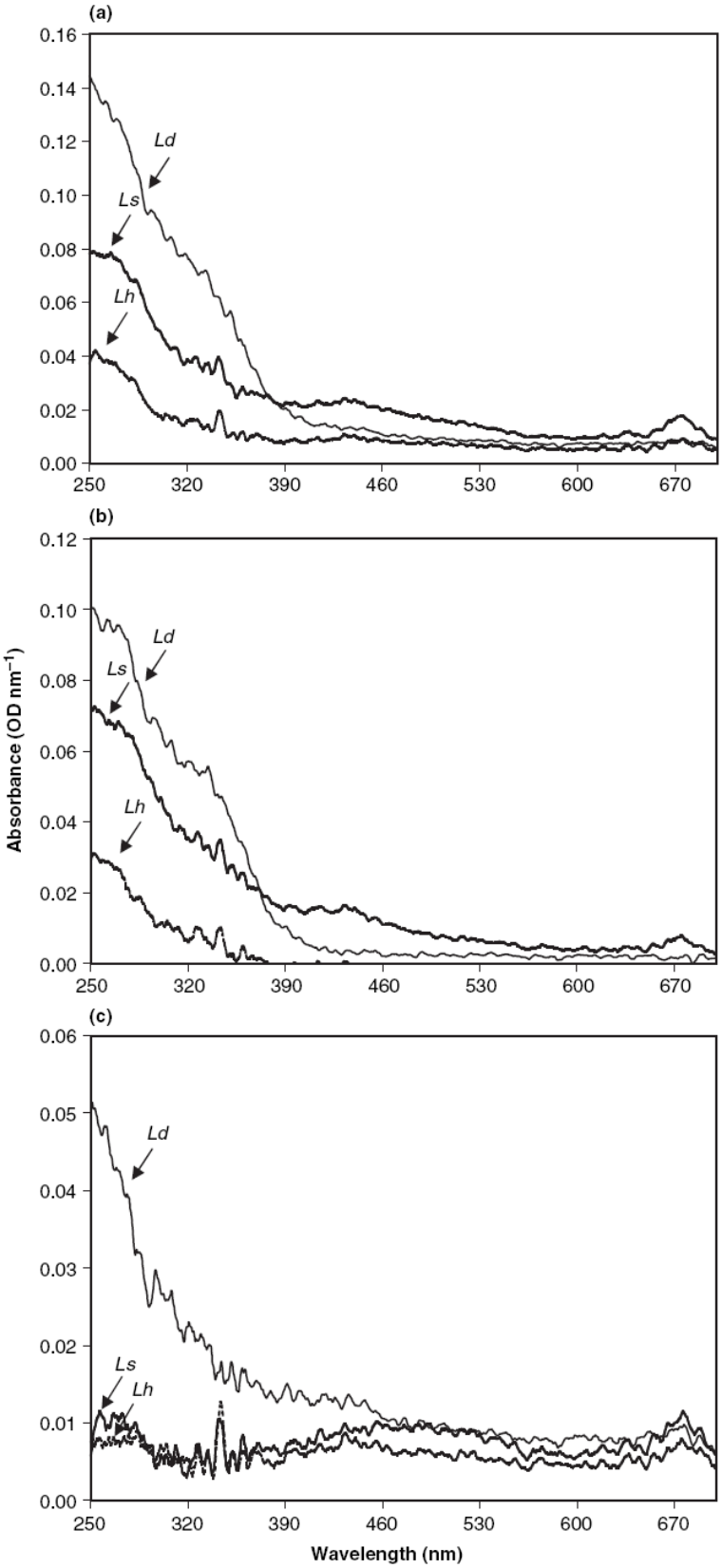

Figure 3. Absorbance spectrum of *Laminaria digitata* (*Ld*), *L. saccharina* (*Ls*) and *L. hyperborea* (*Lh*) (a) zoospore suspension (b) filtrate = seawater medium and (c) zoospores.

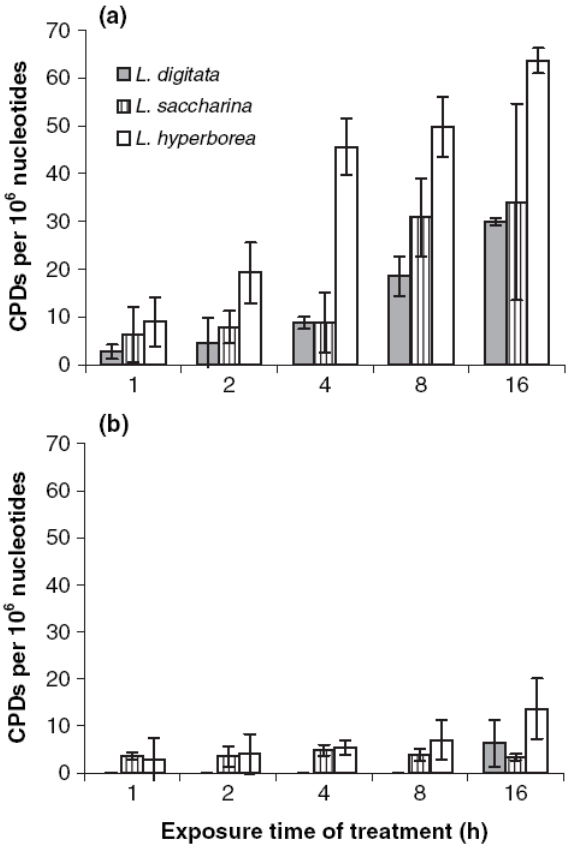

Figure 4. UVB-induced DNA damage (induced CPD concentrations per million nucleotides) in zoospores after (a) exposure to increasing time of PAR + UVA + UVB and (b) corresponding CPD repair after 2 d recovery in 10 μmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD, $n = 3$).

L. hyperborea decreased to 50% after 16 h of exposure to PA (Fig. 5c). Additional UVB reduced germination rate to less than 30% in all species exposed to 16 h PAB and to *L. saccharina* and *L. hyperborea* exposed to 8 h PAB (Fig. 5b & c). MANOVA ($P = 0.05$) showed significant effect of the main factors and two-way interaction between species and irradiance, as well as between irradiance and exposure time (Table 3).

DISCUSSION

This study indicates that zoospore photosynthesis is adapted to low light conditions and UVR causes significant photoinhibition of photosynthesis. Moreover, it is the first report of the capability of spores to repair UVB-induced DNA damage which enhanced spore viability and germination capacity of UVR treated samples. Absorbance of UVR in the zoospore suspensions was found to be based on the absorbance capacity of the spores as well as of the exudates in the medium. Tolerance of spores to PAR and UVR was found to be related to the depth distribution of the adult sporophytes.

The initial differences in I_k values of the three *Laminaria* species investigated are related to the natural depth distribution of the algae. The saturating irradiance showed a

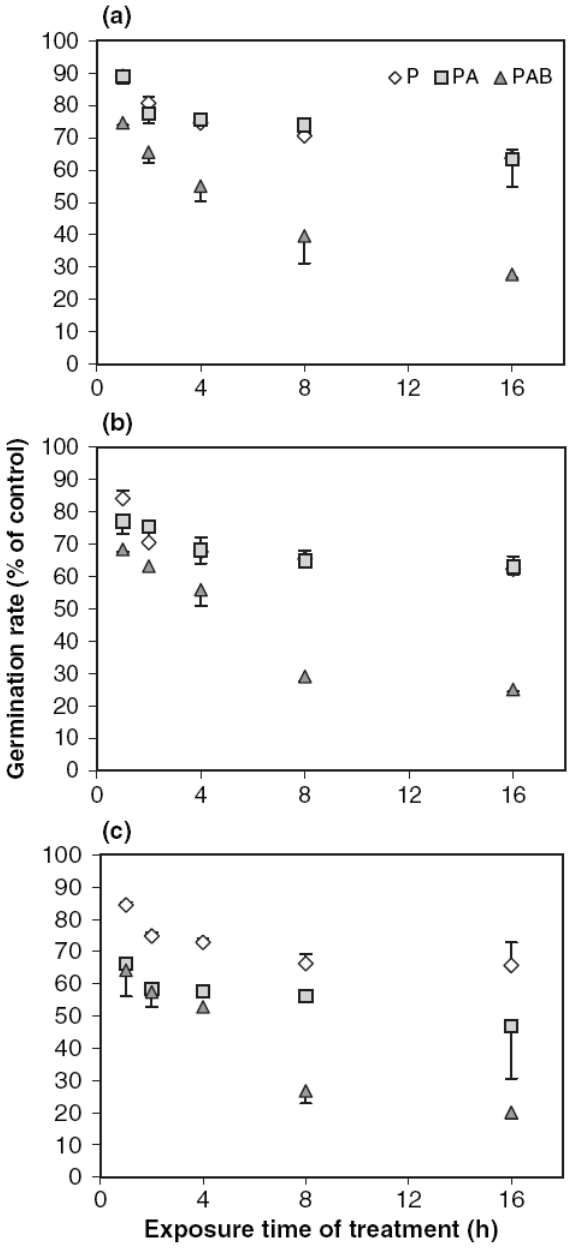

Figure 5. Spore germination, expressed as percentage of control in (a) *Laminaria digitata* (b) *L. saccharina* and (c) *L. hyperborea*, 3 d after exposure to photosynthetically active radiation (PAR = P), PAR + UVA (PA) and PAR + UVA + UVB (PAB) at different exposure time. Post-cultivation is at 10 μmol photons m^{-2} s^{-1}. Vertical bars are standard deviations (SD, $n = 3$).

correlation with habitat whereby zoospores photosynthetic performance of the upper sublittoral *L. digitata* saturated at approximately 40 μmol photons m^{-2} s^{-1} whereas the performance of the lower sublittoral *L. hyperborea* saturated at approximately 20 μmol photons m^{-2} s^{-1}. The decrease in ETR_{max} with increasing actinic light level during P–I curve measurement further established the association between light sensitivity and depth distribution of adult plants. Zoospores from *L. saccharina* and *L. hyperborea* which inhabit the deeper kelp zone are clearly more sensitive to higher photon fluence rates. This differential light adaptation is also reported in young sporophytes of *L. hyperborea*, which are able to survive in low light, requiring only 1 μmol photon m^{-2} s^{-1}, in contrast to *L. digitata* (Han & Kain 1996). Another *Laminaria* species, *Laminaria farlowii* Setchell showed an I_k (41 μmol photons m^{-2} s^{-1}; Amsler & Neushul 1991) comparable to *L. digitata* in our study. Thus, light requirement and tolerance to high light is thought to be genetically fixed. Adult *L. digitata* sporophytes can be photosynthetically characterized as 'sun plants', which are not able to produce sufficient photosynthate for growth in the 'shade' regions of the deeper kelp zones (Lüning 1979).

The sensitivity of zoospore photosynthesis is already shown in the depression of the maximum quantum yield (F_v/F_m) of PSII at the low fluence rate of 20 μmol photons m^{-2} s^{-1} of PAR. This is attributed to the occurrence of photoinhibition even below the saturating light intensity (Hanelt, Huppertz & Nultsch 1992; Osmond 1994). Ögren & Sjöström (1990) also reported that the rate of net photosynthesis can be depressed by photoinhibition over the whole natural range of the photosynthetic photon fluence rates. The primary site of photoinhibition has been suggested to be located in the PSII reaction centre. Key stages of photoinhibition are as follows: functional PSII ↔ inactivated PSII → non functional PSII → damage D1 protein removed → D1 protein resynthesized → recovery of functional PSII (Long, Humphries & Falkowski 1994). If low light adaptation is a general feature of brown algal zoospores, light may exert a significant effect on survival of all zoospores in the water column. Differences in P, PA and PAB sensitivity of maximum quantum yield are presumably caused by the degree of damage to PSII components versus the xanthophyll cycle mediated down regulation of PSII (Gevaert *et al.* 2003). Although a similar reduction in maximum quantum yield was also observed in PA and PAB treatments, recovery after prolonged exposure to PAB treatments was only minimal compared with the first, indicating that UVBR causes more damage to PSII function than UVAR. When D1 protein of the PSII is impaired, the effect is only reversible over a longer time scale (hours) because synthesis of new D1 protein is required (Mattoo *et al.* 1984; Long *et al.* 1994; Hanelt, Wiencke & Bischof 2003). This usually occurs in species growing in the lower subtidal zone exposed to high irradiances (Hanelt 1998). Due to the activity of the xanthophyll cycle, seaweeds are able to recover rapidly (within minutes) after the offset of light stress (Long *et al.* 1994; Osmond 1994; Franklin & Forster 1997). Gametophytes exposed to unnaturally high UVR (2.36×10^5 J m^{-2}) showed no full recovery in *L. digitata*, *L. saccharina* and *L. hyperborea* (Dring *et al.* 1996a). Slow photosynthetic recovery can therefore reduce the accumulation of photosynthetic products disabling cellular division and delaying initiation of germination in zoospore. Modelling studies on photoinhibition indicate a clear cost in terms of potential carbon acquisition, whereby stress-induced photoinhibition decreases the efficiency in the conversion of intercepted light into dry matter (Long *et al.* 1994).

Harmful effects of UVR on the cell is suggested to operate in a more direct way, through its absorption by aromatic and sulfhydryl-containing biomolecules causing direct molecular damage (Vass 1997) and, by proteins and DNA forming CPDs (Setlow 1974). These photoproducts inhibit transcription and replication of DNA and consequently disrupt cell metabolism and division (Buma *et al.* 1995, 2000), directly constraining cell viability and growth. Hence, UVB-induced DNA damage further compromised germination capacity where adverse effect was elicited already after 1 h of exposure, especially for zoospores of *L. saccharina* and *L. hyperborea*. Damage to microtubules causing inhibition of nuclear division in the zoospore nucleus of *Macrocystis pyrifera* (Huovinen *et al.* 2000) and cell division in *Fucus* spp. (Schoenwaelder *et al.* 2003) were also responsible for mortality and failure of germination in spores exposed to UVR. In young sporophytes, chronic exposures to UVR causes lower growth rate, tissue damage and morphological deformations in *Laminaria ochroleuca* Bachelot de la Pylaie (Roleda *et al.* 2004b).

The impact of UVR on the germination capacity of brown algal zoospores inhabiting different water depth has been demonstrated for Laminariales from Spitsbergen and from southern Spain (Wiencke *et al.* 2000, 2004). Conversely, in a previous study on UVR sensitivity of zoospore germination and gametophyte photosynthesis showed no differences among the three species of Laminariales around Helgoland (Dring *et al.* 1996b). This may be attributed to the two- to four-fold higher levels of UVA and UVB that was supplemented to about 35–50 μmol photons m^{-2} s^{-1} of PAR (UV : PAR ratio = 1.525). A higher order of magnitude in UVR : PAR ratio has been reported to intensify the UV effect on plants (Caldwell *et al.* 1995; Rozema *et al.* 1997), which may magnify UVR effect on one species and at the same time obscure its effects between species. Comparison between experimental treatments showed that the 6 h UVR applied by Dring *et al.* (1996b) is equivalent to 3.54×10^5 J m^{-2}, a dose that is higher in comparison with our 16 h PAR + UVA + UVB treatment (3.32×10^5 J m^{-2}). Consequently, the study of Dring and coworkers observed lower germination rates in *L. digitata* (approximately 20%) and *L. hyperborea* (>10%) compared with our study (UVR : PAR ratio =1.257) on the same species with germination rate of 27.6 and 20.1%, respectively. Higher UVR dose (5.46×10^5 J m^{-2}, UVR : PAR ratio =1.523) was also used by Wiencke *et al.* (2004), which resulted in >1% germination in Arctic *L. digitata* and *L. saccharina*. However, it may be possible that the Arctic population of these two

Laminariales is more susceptible to UVR in comparison with their cold temperate counterparts.

In this experiment, we supplemented lower UVA and UVB levels to the predetermined saturating light intensity (I_k) of the deep kelp species. We used a more realistic UV : PAR ratio compared to the study by Dring *et al.* (1996b). We observed that the physiological responses of *L. hyperborea* were more impaired under longer exposure time of PA and PAB treatments than those of *L. digitata* and *L saccharina*. Species-specific difference in DNA damage accumulation became obvious after 4 h and longer exposures, where the degree of DNA damage was also higher in *L. hyperborea*. This response was observed to be related to the amount of UV absorbing compounds present in the zoospore and in the medium. In brown algae, phlorotannin containing vesicles (called physodes) which strongly absorb in the UVC region of the spectrum were invoked to play a role in chemical UV defence because their synthesis is inducible by UVBR (Pavia *et al.* 1997; Schoenwaelder 2002). Exudation of this compound from macroalgae into seawater can at low concentrations reduce the impact of UVB exposure to UV-sensitive kelp meiospores (Swanson & Druehl 2002). In Arctic Laminariales zoospores, an increase in number and size of phlorotannin-containing physodes was observed after UV exposure which contributed UVR protection against cellular damage (Wiencke *et al.* 2004). Although we observed strong absorbance of UVBR in the medium where zoospores were released, the source of these phlorotannin exudates (either released from the sori or from zoospores itself) is yet to be determined. All species were able to repair DNA damage, which could either be mediated by light-dependent photolyases or light-independent nucleotide excision repair (Pakker, Beekman & Breeman 2000; van de Poll *et al.* 2002). However, higher remaining DNA damage was also observed in *L. hyperborea* previously exposed to 8 and 16 h PAB. Although no direct correlation was observed between germination and DNA repair rates among species, DNA repair mechanism certainly had contributed to the germination success in *L. digitata* relative to the other two investigated deep kelp species.

Although zoospores of all species were able to recover in dim white light, 2 d after exposure to 4 h of different UV-spectra, future study should measure time series regeneration to determine the rate of photosynthetic recovery. Field experiments are also necessary because UVB radiation applied in our experiment could be accompanied by a 10- to 20-fold higher PAR. Studies on the acclimation potential of zoospores to high PAR are also necessary because under these conditions UVB tolerance may be enhanced by increasing activity of photorepair enzymes (Warner & Caldwell 1983). To estimate the ecological impact of enhanced UVR, seasonal variation in solar radiation has to be related to the reproductive seasonality of kelps as well as the diel periodicity in zoospore release (Reed *et al.* 1988; Amsler & Neushul 1989). Zoospore production in perennial kelp is either continuous (Chapman 1984; Joska & Bolton 1987) or seasonal (tom Dieck 1991; Reed *et al.* 1996; Graham 1999). The distinct reproductive seasons of Helgolandic *Laminaria* spp. is remarkable compared to other population of the same species. *Laminaria digitata* plants from Nova Scotia are found to be fertile throughout the year (Chapman 1984). To ensure reproductive success, propagules production should be synchronized with the onset of favourable environmental conditions (e.g. light, photoperiod or temperature) (Lüning 1980; Santelices 1990; Kinlan *et al.* 2003). Inferring from the result of our study, the summer reproductive season of the upper sublittoral *L. digitata* would suggest that sporogenic tissues as well as zoospores of this species could tolerate or possess effective protective mechanism against high solar radiation. On the other hand, winter reproduction in the lower sublittoral *L. hyperborea* is thought to be a strategy to avoid reproductive failure due to the relative sensitivity of their zoospores to high PAR and UVR. Samples collected at specific sites around Helgoland belong most likely to the same population because water motion disperses spores and fertilize gametes between different individual sporophytes around the small island (approximately 35 km^2 of rocky shore area). The population of *Laminaria* around Helgoland is exposed to the same environmental factors and therefore, collection site (geographic) is unlikely to play a role in the species response to the experimental treatments. However, the difference in the abiotic factors occurring at different water depths may cause a different adaptation status of the samples. In conclusion, zoospores susceptibility to UVR could therefore determine spore viability and germination success of kelp propagules. Susceptibility of spores indicates a relation to the observed zonation pattern of kelp community around Helgoland.

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REFERENCES

Amsler C.D. & Neushul M. (1989) Diel periodicity of spore release from the kelp *Nereocystis luetkeana* (Mertens) Postels *et* Ruprecht. *Journal of Experimental Marine Biology and Ecology* **134,** 117–127.

Amsler C.D. & Neushul M. (1991) Photosynthetic physiology and chemical composition of spores of the kelps *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Laminaria farlowii*, and *Pterygophora californica* (Phaeophyceae). *Journal of Phycology* **27,** 26–34.

Amsler C.D., Reed D.C. & Neushul M. (1992) The microclimate inhabited by macroalgal propagules. *British Phycology Journal* **27,** 253–270.

Aro E.M., Hundal T., Carlberg I. & Andersson B. (1990) In vitro studies on light-induced inhibition of photosystem II and D1-protein degradation at low temperatures. *Biochimica et Biophysica Acta* **1019,** 269–275.

Baker N.R., Nogues S. & Allen D.J. (1997) Photosynthesis and photoinhibition. In *Plants and UVB: Responses to Environmen-*

tal Change (ed. P. Lumsden), pp. 95–111. Cambridge University Press, Cambridge, UK.

Bischof K., Hanelt D. & Wiencke C. (1998a) UV-radiation can affect depth-zonation of Antarctic macroalgae. *Marine Biology* **131,** 597–605.

Bischof K., Hanelt D., Tüg H., Karsten U., Brouwer P.E.M. & Wiencke C. (1998b) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biology* **20,** 388–395.

Bischof K., Hanelt D. & Wiencke C. (2000) UV-effects on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* **211,** 555–562.

Bischof K., Hanelt D. & Wiencke C. (2001) UV-radiation and Arctic marine macroalgae. In *UV-Radiation and Arctic Ecosystems. Ecological Studies Series*, Vol. 153 (ed. D. Hessen), pp. 227–244. Springer, New York, Heidelberg.

Buma A.G.J., van Hannen E.J., Roza L., Veldhuis M.J.W. & Gieskes W.W.C. (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *Journal of Phycology* **31,** 314–321.

Buma A.G.J., van Oijen T., van de Poll W.H., Veldhuis M.J.W. & Gieskes W.W.C. (2000) The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. *Journal of Phycology* **36,** 296–303.

Caldwell M.M. (1971) Solar ultraviolet radiation and the growth and development of higher plants. In *Photophysiology* (ed. A.C. Giese), pp. 131–177. Academic Press, New York.

Caldwell M.M., Teramura A.H., Tevini M., Bornman J.F., Björn L.O. & Kulandaivelu G. (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio* **24,** 166–173.

Chapman A.R.O. (1984) Reproduction, recruitment and mortality in two species of *Laminaria* in southwest Nova Scotia. *Journal of Experimental Marine Biology and Ecology* **78,** 99–109.

Coelho S.M., Rijstenbil J.W. & Brown M.T. (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. *Journal of Aquatic Ecosystem Stress and Recovery* **7,** 317–333.

De Wreede R.E. & Klinger T. (1988) Reproductive strategies in algae. In *Plant Reproductive Ecology: Patterns and Strategies* (eds J.L. Doust & L.L. Doust), pp. 267–284. Oxford University Press, New York, USA.

Dionisio M.L., Tsuzuki M. & Miyachi S. (1989) Blue light induction of carbonic anhydrase activity in *Chlamydomonas reinhardtii*. *Plant and Cell Physiology* **30,** 215–219.

Dring M.J. & Lüning K. (1994) Influence of spring-neap tidal cycles on the light available for photosynthesis by benthic marine plants. *Marine Ecology Progress Series* **104,** 131–137.

Dring M.J., Wagner A., Boeskov J. & Lüning K. (1996a) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurement: influence of collection depth and season, and length of irradiation. *European Journal of Phycology* **31,** 293–302.

Dring M.J., Makarov V., Schoschina E., Lorenz M. & Lüning K. (1996b) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Marine Biology* **126,** 183–191.

Dring M.J., Wagner A., Franklin L.A., Kuhlenkamp R. & Lüning K. (2001a) Seasonal and diurnal variations in ultraviolet-B and ultraviolet-A irradiances at and below the sea surface at Helgoland (North Sea) over a 6-year period. *Helgoland Marine Research* **55,** 3–11.

Dring M.J., Wagner A. & Lüning K. (2001b) Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. *Plant, Cell and Environment* **24,** 1153–1164.

El Naggar S., Gustat H., Magister H. & Rochlitzer R. (1995) An electronic personal UV-B-dosimeter. *Journal of Photochemistry and Photobiology B: Biology* **31,** 83–86.

Franklin L.A. & Forster R.M. (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology* **32,** 207–232.

Fredriksen S., Sjøtun K., Lein T.E. & Rueness J. (1995) Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia* **80,** 47–54.

Garcia-Pichel F. (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnology and Oceanography* **39,** 1704–1717.

von der Gathen P., Rex M., Harris N.R.P., *et al.*. (1995) Observational evidence for chemical ozone depletion over the Arctic in winter 1991–92. *Nature* **375,** 131–134.

Gevaert F., Creach A., Davoult D., Migne A., Levavasseur G., Arzel P., Holl A.-C. & Lemoine Y. (2003) *Laminaria saccharina* photosynthesis measured in situ: photoinhibition and xanthophylls cycle during a tidal cycle. *Marine Ecology Progress Series* **247,** 43–50.

Graham M.H. (1999) Identification of kelp zoospores from in situ plankton samples. *Marine Biology* **135,** 709–720.

Han T. & Kain J.M. (1996) Effect of photon irradiance and photoperiod on young sporophytes of four species of the Laminariales. *European Journal of Phycology* **31,** 233–240.

Hanelt D. (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology* **131,** 361–369.

Hanelt D., Huppertz K. & Nultsch W. (1992) Photoinhibition of photosynthesis and its recovery in red algae. *Botanica Acta* **105,** 278–284.

Hanelt D., Wiencke C. & Nultsch W. (1997a) Influence of UV-radiation on the photosynthesis of Arctic macroalage in the field. *Journal of Photochemistry and Photobiology B: Biology* **38,** 40–47.

Hanelt D., Melchersmann B., Wiencke C. & Nultsch W. (1997b) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Marine Ecology Progress Series* **149,** 255–266.

Hanelt D., Wiencke C. & Bischof K. (2003) Photosynthesis in marine macrolage. In *Photosynthesis in Algae* (eds A.W. Larkum, S.E. Douglas & J.A. Raven), pp. 413–435. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Huovinen P.S., Oikari A.O.J., Soimasuo M.R. & Cherr G.N. (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. *Photochemistry and Photobiology* **72,** 308–313.

Jansen M.A.K., Gaba V., Greenberg B., Mattoo K.A. & Edelman M. (1993) UV-B driven degradation of the D1 reaction-center protein of Photosystem II proceeds via plastosemiquinone. In *Photosynthetic Responses to the Environment* (eds H.Y. Yamamoto & C.M. Smith), pp. 142–149. American Society of Plant Physiologist, Rockville, MD, USA.

Joska M.A.P. & Bolton J.J. (1987) In situ measurement of zoospore release and seasonality of reproduction in *Ecklonia maxima* (Alariaceae, Laminariales). *British Phycology Journal* **22,** 209–214.

Kinlan B.P., Graham M.H., Sala E. & Dayton P.K. (2003) Arrested development of giant kelp (*Macrocystis pyrifera*, Phaeophyceae) embryonic sporophytes: a mechanism for delayed recruitment in perennial kelps? *Journal of Phycology* **39,** 47–57.

Long S.P., Humphries S. & Falkowski P.G. (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology* **45,** 633–662.

Lüning K. (1979) Growth strategies of three *Laminaria* species

(Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Marine Ecology Progress Series* **1,** 195–207.

Lüning K. (1980) Control of algal life-history by daylength and temperature. In *The Shore Environment*, Vol. 2*: Ecosystems* (eds J.H. Price, D.E.G. Irvine & W.F. Farnham), pp. 915–945. Academic Press, New York, USA.

Mattoo A.K., Hoffman-Falk H., Marder J.B. & Edelman M. (1984) Regulation of protein metabolism: coupling of photosynthetic electron transport in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. *Proceedings of the National Academy of Sciences of the USA* **81,** 1380–1384.

Melis A., Nemson J.A. & Harrison M.A. (1992) Damage to functional components and partial degradation of Photosystem II reaction center proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochimica et Biophysica Acta* **1100,** 312–320.

Ögren E. & Sjöström M. (1990) Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. *Planta* **181,** 560–567.

Osmond C.B. (1994) What is photoinhibition? Some insights from comparisons of shade and sun plant. In *Photoinhibition of Photosynthesis, from the Molecular Mechanisms to the Field* (eds N.R. Baker & J.R. Bowyer), pp. 1–24. BIOS Scientific Publishers, Oxford, UK.

Pakker H., Beekman C.A.C. & Breeman A.M. (2000) Efficient photoreactivation of UVBR- induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). *European Journal of Phycology* **35,** 109–114.

Pavia H., Cervin G., Lindgren A. & Åberg P. (1997) Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Marine Ecology Progress Series* **157,** 139–146.

van de Poll W.H., Eggert A., Buma A.G.J. & Breeman A.M. (2001) Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. *Journal of Phycology* **37,** 30–37.

van de Poll W.H., Hanelt D., Hoyer K., Buma A.G.J. & Breeman A.M. (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochemistry and Photobiology* **76,** 493–501.

Reed D.C., Amsler C.D. & Ebeling A.W. (1992) Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* **73,** 1577–1585.

Reed D.C., Ebeling A.W., Anderson T.W. & Anghera M. (1996) Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. *Ecology* **77,** 300–316.

Reed D.C., Laur D.R. & Ebeling A.W. (1988) Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs* **58,** 321–335.

Reed D.C., Schroeter S.C. & Raimondi P.T. (2004) Spore supply and habitat availability as sources of recruitment limitation in the giant kelp *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology* **40,** 275–284.

Roleda M.Y., Hanelt D., Kräbs G. & Wiencke C. (2004b) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. *Phycologia* **43**, 603–613.

Roleda M.Y., van de Poll W.H., Hanelt D. & Wiencke C. (2004a) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implication for recruitment and zonation pattern. *Marine Ecology Progress Series* **281,** 37–50.

Rozema J., van de Staaij J., Björn L.O. & Caldwell M. (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology and Evolution* **12,** 22–28.

Santelices B. (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology Annual Review* **28,** 177–276.

Schoenwaelder M.E.A. (2002) The occurrence and cellular significance of physodes in brown algae. *Phycologia* **41,** 125–139.

Schoenwaelder M.E.A., Wiencke C., Clayton M.N. & Glombitza K.W. (2003) The effect of elevated UV radiation on *Fucus* spp. (Fucales, Phaeophyta) zygote and embryo development. *Plant Biology* **5,** 366–377.

Setlow R.B. (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proceedings of the National Academy of Sciences of the USA* **71,** 3363–3366.

Smith R.C., Prézelin B.B., Baker K.S., *et al.* (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* **255,** 952–959.

Stähelin J., Harris N.R.P., Appenzeller C. & Eberhard J. (2001) Ozone trends: a review. *Reviews of Geophysics* **39,** 231–290.

Starr R.C. & Zeikus J.A. (1993) UTEX. – the culture collection of algae at the University of Texas at Austin. *Journal of Phycology* **29 (**Suppl.), 1–106.

Swanson A.K. & Druehl L.D. (2002) Induction, exudation and the UV protective role of kelp phlorotannins. *Aquatic Botany* **73,** 241–253.

tom Dieck I. (1991) Circannual growth rhythm and photoperiodic sorus induction in the kelp *Laminaria setchellii* (Phaeophyta). *Journal of Phycology* **27,** 341–350.

Underwood A.J. (1981) Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography and Marine Biology: an Annual Review* **19,** 513–605.

Vass I. (1997) Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. In *Handbook of Photosynthesis* (ed. M. Pessarakli), pp. 931–949. Marcel Dekker Inc. New York, USA.

Vink A.A., Bergen-Henegouwen J.B., Nikaido O., Baan R.P. & Roza L. (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. *Photochemistry and Photobiology* **24,** 25–31.

Warner C.W. & Caldwell M.M. (1983) Influence of photon flux density in the 400–700 nm waveband on inhibition of photosynthesis by UV-B (280–320 nm) irradiation in soybean leaves: separation of indirect and direct effects. *Photochemistry and Photobiology* **38,** 341–346.

Wiencke C., Clayton M.N. & Schoenwaelder M. (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. *Marine Biology* **145,** 31–39.

Wiencke C., Gómez I., Pakker H., Flores-Moya A., Altamirano M., Hanelt D., Bischof K. & Figueroa F.-L. (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Marine Ecology Progress Series* **197,** 217–229.

Yund P.O. (2000) How severe is sperm limitation in natural populations of marine free-spawners? *Trends in Ecology and Evolution* **15,** 10–13.

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Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments

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Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments

CHRISTIAN WIENCKE[1], MICHAEL Y. ROLEDA[2], ANSGAR GRUBER[3], MARGARET N. CLAYTON[4] and KAI BISCHOF[5]

[1]*Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany,* [2]*Biologische Anstalt Helgoland, Alfred Wegener Institute for Polar and Marine Research, PO Box 180, D-27483 Helgoland, Germany,* [3]*Plant Ecophysiology, University of Konstanz, Universitätsstr. 10, D-78464 Konstanz, Germany,* [4]*School of Biological Sciences, Monash University, Victoria 3800, Australia, and* [5]*Institute for Polar Ecology, University of Kiel, Wischhofstr. 1–3, D-24148 Kiel, Germany*

Summary

1 The UV susceptibility of zoospores of the brown seaweeds *Saccorhiza dermatodea*, *Alaria esculenta* and *Laminaria digitata* (Laminariales) was determined in field experiments in June 2004 on Spitsbergen (78°55′ N, 11°56′ E).
2 Freshly released zoospores were exposed for 1 or 2 days at various water depths to ambient solar radiation, ambient solar radiation depleted of UVB radiation (UVBR) and ambient solar radiation depleted of both UVBR and UVAR. Subsequently, germination rates were determined after exposure to favourable light and temperature conditions in the laboratory.
3 The radiation regime was monitored at the water surface and in the water column using data loggers attached adjacent to each experimental platform for the duration of the field exposure.
4 Under ambient solar radiation, the tolerance of zoospores to UVR was highest in the shallow water species *S. dermatodea*, intermediate in the upper to mid sublittoral *A. esculenta* and lowest in the upper to mid sublittoral *L. digitata*. There was, however, no difference in the susceptibility of the zoospores to ambient solar radiation or to solar radiation depleted of UVBR.
5 The water column was relatively UV transparent, especially in the upper water layers. The 1% UVB depth ranged between 5.35 and 6.87 m, although on one stormy day the 1% UVB depth was only 3.57 m, indicating resuspension of sediments.
6 Early developmental stages are most susceptible to environmental stress. Tolerance of zoospores to UVR is a major if not one of the most important factors determining the upper distribution limit of different Laminariales on the shore.
7 Kelps are very important primary producers in inshore coastal ecosystems, serving as food for herbivores and as habitat for many organisms. Enhanced UVBR due to stratospheric ozone depletion may lead to changes in the depth distribution of kelps and may cause significant ecological domino effects.

Key-words: *Alaria esculenta*, depth distribution, germination, *Laminaria digitata*, Laminariales, optical water characteristics, *Saccorhiza dermatodea*, stratospheric ozone depletion, UV radiation, zoospore viability

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Correspondence: Christian Wiencke (fax +49 471 48311425; e-mail cwiencke@awi-bremerhaven.de).

Introduction

The depth distribution of seaweeds is governed by a variety of biotic and abiotic factors, among which the radiation regime is very important (Lüning 1990). While the lower depth distribution is determined by the

need to maintain a positive carbon balance (Gómez *et al*. 1997), the upper limit primarily depends on the capability of seaweeds to sustain high light stress, and especially on the capacity for dynamic photoinhibition of photosynthesis (Sagert *et al*. 1997; Hanelt 1998). In addition to coping with excessive photosynthetically active radiation (PAR), tolerance of UV radiation (UVR) is regarded as a major factor determining the zonation of seaweeds in shallow waters (Maegawa *et al*. 1993; Dring *et al*. 1996; Bischof *et al.* 1998).

Most previous studies on the UV tolerance of seaweeds have focused on their macrothalli (Franklin & Forster 1997). Only a few studies have been performed using the unicellular propagules of seaweeds, the spores, despite the fact that these early developmental stages are regarded as most susceptible to UV stress (Coelho *et al*. 2000). It is known that UVR leads to an inhibition of photosynthesis in zoospores of *Laminaria digitata* and to DNA damage of zoospores of the kelps *L. digitata*, *L. saccharina* and *Alaria esculenta* (Wiencke *et al*. 2000). Both the photosynthetic apparatus and DNA can be repaired in spores of the upper sublittoral species *L. digitata*, and the red algae *Chondrus crispus* and *Mastocarpus stellatus* (Roleda *et al*. 2004), but damage is much less reversible in the mid to lower sublittoral *L. saccharina* and *L. hyperborea* (Roleda *et al*. 2005). Other negative effects of UVR include a decrease of the motility of zoospores from *L. saccharina* (Makarov & Voskoboinikov 2001) and of the phototaxis of spores of *Scytosiphon lomentaria* and *Petalonia fascia* (Flores-Moya *et al*. 2002). Finally, microtubules can be affected by UVR as shown in zoospores of *Macrocystis pyrifera* by Huovinen *et al*. (2000).

The need for repair processes is reduced if damage to biomolecules, cell structure and cell function can be prevented by the presence of UV-absorbing compounds, such as the phlorotannins in the phenolic vesicles (physodes) that are typically found in kelp zoospores. After exposure to UVR, physodes increase in number and size, particularly in the upper sublittoral *Saccorhiza dermatodea* and *A. esculenta*, and to a lesser degree in kelps from deeper waters (Wiencke, Clayton & Schoenwaelder 2004). This is consistent with the observation that zoospore suspensions of the upper sublittoral *L. digitata* absorb UVBR and UVAR below 360 nm (characteristic of phlorotannins) strongly but *L. saccharina* and *L. hyperborea* from greater water depths do not (Roleda *et al*. 2005). Zoospore suspensions of *A. esculenta*, *L. digitata* and *L. saccharina* can protect cultures of zoospores of other species from the potentially lethal effects of UVAR and UVBR with UV-protection properties varying with the species and, in particular, with the spore density (Clayton & Wiencke 2004).

If the negative effects of UVR are not fully balanced by protective and repair mechanisms, germination is impaired and germination rates decrease. This has been demonstrated clearly in *L. digitata*, *L. saccharina* and *L. hyperborea* growing in this order from the upper to the mid sublittoral on Helgoland (North Sea), with the strongest reduction in *L. hyperborea* (Roleda *et al*. 2005). Similar results were obtained in *S. dermatodea*, *A. esculenta*, *L digitata*, *L. saccharina* and *L. solidungula* growing from low tide level down to about 16 m depth in the middle zone of Kongsfjorden (Spitsbergen, 78°55′ N; Wiencke, Vögele, Kovaltchouk & Hop 2004). Again, the greatest decrease of germination rates was found in the deep water species. In all these studies the strongest effect has been obtained after exposure to both UVBR and UVAR. UVAR alone usually induces more limited effects on photosynthesis, DNA and germination rates (Wiencke *et al*. 2000, 2004; Roleda *et al*. 2005).

Apart from a very limited pilot study performed under natural solar radiation and ambient air temperature (Wiencke, Clayton & Schoenwaelder 2004), all previous studies have been conducted in the laboratory under artificial illumination. Levels of PAR are much lower in the laboratory than in the field, whereas those of UVBR are considerably higher (Wiencke, Clayton & Schoenwaelder 2004). As the effect of UVBR exposure on the performance of brown algal zoospores *in situ* has not been explored, we exposed spores of three kelp species from Spitsbergen for 1 to 2 days at different water depths in Kongsfjorden to the full ambient solar radiation, ambient solar radiation depleted of UVBR and ambient solar radiation depleted of UVBR and UVAR. Radiation regimes were monitored both at the water surface and in the water. Germination rates were subsequently determined after exposure to favourable light and temperature conditions in the laboratory. These studies may cast light on the potential impact of stratospheric ozone depletion, especially over the polar regions (von der Gathen *et al*. 1995; Rex *et al*. 2002), as this process leads to an enhancement of UVB radiation (UVBR) at the earth's surface and in the water column (Groß *et al*. 2001; Dahlback 2002). On Spitsbergen biologically significant UVBR levels are measurable down to about 8 m water depth (Hanelt *et al*. 2001; van de Poll *et al*. 2002) and a UVBR-induced loss of zoospore viability in shallow waters may therefore shift areas of successful kelp recruitment to greater water depths.

Materials and methods

Fertile specimens of *Saccorhiza dermatodea* (Pyl.) J. Ag., *Alaria esculenta* (L.) Grev. and *Laminaria digitata* (Huds.) Lamour. were collected between May and June 2004 by SCUBA divers in Kongsfjorden at Prins Heinrichøya or Blomstrandhalvøya close to Ny Ålesund (Spitsbergen, 78°55′ N, 11°56′ E). For detailed information about the physical environment and the ecosystem of Kongsfjorden in general see Svendsen *et al*. (2002), Hop *et al*. (2002) and Wiencke (2004).

Fertile sori were removed from five different individuals per species using a razor blade, blotted with tissue paper and kept in darkness in a moist chamber at 0 °C overnight or for a few days. Sori were immersed in a small amount of seawater at *c*. 15 °C and placed in the light close to a window to promote the rapid release of zoospores

(Wiencke *et al*. 2000). The initial zoospore density was counted by use of a Neubauer chamber (Brand Germany). Samples taken from this stock suspension were transferred into 5-cm diameter Petri-dishes filled with filtered (0.2 μm pore size) seawater to give spore densities between 35 000 and 60 000 spores cm^{-2}.

Sample holders for the field experiments consisted of an aluminium frame (0.25 × 0.40 m) with a black plastic bottom and a top of UV-transparent Plexiglas 'GS 2458' (Röhm, Darmstadt, Germany: mean transmission 93% of PAR, 92% of UV-A and 86% of UV-B), and accommodated 15 Petri-dishes (53 × 12 mm) arranged in a 3 × 5 grid. To determine the effect of different radiation treatments, the Plexiglas above each Petri-dish was covered either with Ultraphan URUV farblos (Digefra GmbH, Munich, Germany), Folex PR Montage (Dr Schleussner, Dreieich, Germany) and Ultraphan URT 300 (Digefra GmbH, Munich, Germany) foil to give three treatments: photosynthetically active radiation (PAR) = P, PAR + UV-A = PA, and PAR + UV-A + UV-B = PAB. The spectral properties of the foils used are published elsewhere (Bischof *et al*. 2002). Treatments were assigned randomly. Two to five drops of zoospore suspension from different sporophytes were added to each dish, before filling to the top with filtered seawater. They were then covered by UV-transparent Plexiglas, such that each dish was water-tight and free of air bubbles. Several sample holders were prepared and deployed at five depths (0.25 m, 0.5 m, 1 m, 2 m and 4 m) in the fjord between the old and the new pier in Ny Ålesund using anchors and buoys. They were exposed for about 24 hours in the field. One experiment was run for 45 hours due to very low light conditions and corresponding low UV doses.

An ELUV 14 datalogger was fixed close to each sample holder, to determine the UV-B doses (erythema weighted, UV_{ery}; El Naggar *et al*. 1995) at the different depths. The sensitivity of the datalogger was calibrated to the standard CIE-87 erythemal response after McKinlay & Diffey (1987). We used this datalogger as it is, to our knowledge, the only submersible field datalogger available. Surface PAR was measured throughout the experimental period using a cosine quantum sensor attached to a LI-COR datalogger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska, USA). Diffuse vertical attenuation coefficients of downward irradiance of UVBR were determined after Kirk (1994) using the UV_{ery} data determined at different depths.

After exposure, the sample holders were recovered from the fjord and the individual Petri-dishes were covered with lids and exposed to dim white light (10 μmol photons^{-2} s^{-1}) using daylight fluorescent tubes (Osram Daylight Lumilux De Luxe L36W/12–950) at a temperature of 10 °C for 3 days. Germination rates were determined microscopically by use of an Axioplan microscope (Zeiss, Göttingen, Germany) equipped with a 25× seawater immersion objective. A spore was classified as germinated if at least a germ-tube was formed. We did not distinguish between dead spores and those that were living, but not germinated. Approximately 300 spores were examined per sample.

Germination data were tested for homogeneity of variances (Levene Statistics) and normality (Kolmogorov-Smirnov test). Due to different environmental conditions during each field experiment, statistical tests were conducted separately on each species. The response of the dependent variable was tested using multiple analyses of variance (MANOVA, $P < 0.05$) to determine the interaction between the effects of irradiance and depth. This was followed by Duncan's multiple range test (DMRT, $P = 0.05$) to determine which groups were homogeneous or significantly different from each other. Statistical analyses were done using the SPSS program (SPSS, Chicago, IL, USA).

Biologically effective UV-B doses (UV_{ery}) resulting in a 50% inhibition in germination were determined from all germination data (expressed as percentage of the value in treatment P) using non-linear regression ($y = a + bx + cx^2$), corresponding to the best fit curves.

Results

Throughout the investigation period, the weather was relatively bright, with a mixture of sunny and cloudy periods. Even during the polar day there is a clear variation between low light conditions at midnight and high light conditions at noon (see Fig. 1a). Midnight photon fluence rates (PFR) were between about 100 and 200 μmoles photons m^{-2} s^{-1}, whereas maximum PFRs of about 1200–1400 μmoles photons m^{-2} s^{-1} were measured at noon. PAR values at the surface were

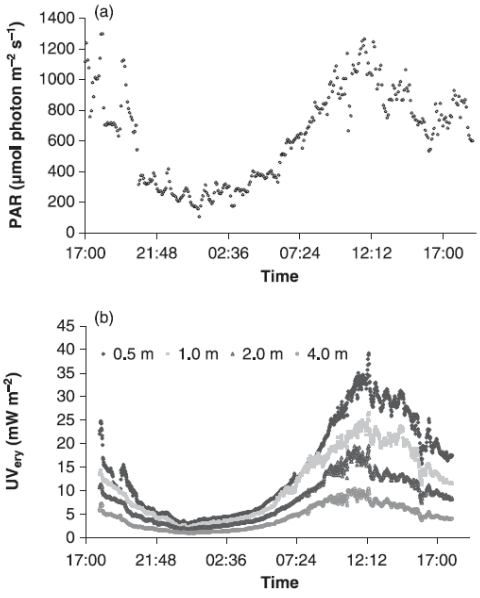

Fig. 1 Variation in radiation during a typical polar day showing (a) 24-hour surface photosynthetically active radiation and (b) corresponding Erythema-weighted UV-B radiation (UV_{ery}; El Naggar *et al*. 1995) at 0.5, 1.0, 2.0 and 4.0 m water depth.

Table 1 Surface levels of photosynthetically active radiation (PAR) and of weighted UV-B radiation (UV_{ery}; El Naggar *et al.* 1995) at different water depths during field experiments performed in Kongsfjorden (Spitsbergen). Values corresponding to different treatments of PAR = P, PAR + UV-A = PA, and PAR + UV-A + UV-B = PAB were extrapolated from the percentage transmission of UV dose (UV_{ery}) using the same cut-off filter foils in the laboratory (ND = not determined)

	Experimental duration			PAR ($J\,m^{-2}$)	UV_{ery} ($J\,m^{-2}$)			
Species	Date	Total time h:min	Treatment	Surface	0.5 m	1.0 m	2.0 m	4.0 m
Saccorhiza dermatodea	09.06.04 17.37 to 10.06.04 18.18	24:41	No filter	1.06×10^7	1.34×10^3	9.49×10^2	5.81×10^2	ND
			PAB		9.35×10^2	6.63×10^2	4.06×10^2	
			PA		3.46×10^1	2.46×10^1	1.51×10^1	
			P		0.00×10^0	0.00×10^0	0.00×10^0	
Saccorhiza dermatodea	21.06.04 16.30 to 23.06.04 13.30	45:00	No filter	1.73×10^7	8.18×10^2	3.99×10^2	1.35×10^2	ND
			PAB		5.72×10^2	2.79×10^2	9.44×10^1	
			PA		2.12×10^1	1.04×10^1	3.49×10^0	
			P		0.00×10^0	0.00×10^0	0.00×10^0	
Alaria esculenta	04.06.04 08.40 to 05.06.04 09.26	24:46	No filter	1.26×10^7	1.38×10^3	1.02×10^3	6.77×10^2	3.39×10^2
			PAB		9.67×10^2	7.16×10^2	4.73×10^2	2.37×10^2
			PA		3.58×10^1	2.65×10^1	1.75×10^1	8.77×10^0
			P		0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
Alaria esculenta	14.06.04 18.35 to 15.06.04 16.15	21:40	No filter	8.23×10^6	6.78×10^2	5.96×10^2	3.89×10^2	1.85×10^2
			PAB		4.74×10^2	4.16×10^2	2.72×10^2	1.30×10^2
			PA		1.76×10^1	1.54×10^1	1.01×10^1	4.80×10^0
			P		0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
Laminaria digitata	07.06.04 17.34 to 08.06.04 18.02	24:32	No filter	1.17×10^7	1.28×10^3	9.74×10^2	6.86×10^2	3.72×10^2
			PAB		8.95×10^2	6.81×10^2	4.79×10^2	2.60×10^2
			PA		3.32×10^1	2.52×10^1	1.78×10^1	9.62×10^0
			P		0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
Laminaria digitata	17.06.04 16.10 to 18.06.04 16.10	24:00	No filter	1.33×10^7	6.98×10^2	5.06×10^2	2.50×10^2	7.85×10^1
			PAB		4.88×10^2	3.54×10^2	1.75×10^2	5.49×10^1
			PA		1.81×10^1	1.31×10^1	6.49×10^0	2.03×10^0
			P		0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0

relatively similar in all experiments and varied between 8.23×10^6 J m^{-2} and 1.73×10^7 J m^{-2} (Table 1).

Figure 1(b) shows the UV_{ery} values obtained in a typical experiment (7–8 June). There is a clear differentiation of the underwater radiation regime at the various depths. Minimum levels of UVB radiation (UVBR, determined as UV_{ery}) at midnight were between 2 and 5 mW^{-2} at all depths, whereas maximum values between 18 and 45 mW^{-2} were recorded at noon at 0.5 m water depth (data not shown).

The UV_{ery} doses shown in Table 1 clearly reflect the spectral properties of the different cut-off filters used. No UVBR was measured under the filter used for the P treatment. The UVAR values reflect both the UVA transparency of the used filter and the relatively low sensitivity of the ELUV-14 datalogger in the UVA region of the spectrum. The filter used for the PAB treatment has a UV_{ery} transmission of about 70%.

Although the surface radiation regime was similar during the various experiments (Table 1), the underwater radiation regime exhibited clear differences, with water being UV transparent at some times and more turbid at others (7–8 June, see Fig. 1, Table 1, and 9–10 June vs. 17–18 June; 4–5 June, 21–23 June and 14–15 June represent intermediate situations). This is also reflected in Kd values ranging between 0.67 and 1.28 and 1% UVB-depths between 6.87 and 3.57 m (Fig. 2).

In the first experiment with *S. dermatodea* (9–10 June), germination rates between 66 and 78% were determined at 1 and 2 m depth under all of the three exposure conditions (Fig. 2a). At 0.5-m depth germination rates under full ambient solar radiation (PAB) and under solar radiation depleted of UVBR (PA) did not differ significantly (Table 2) from those under solar radiation depleted of both UVBR and UVAR (P). In the second experiment with this species (on 21–23 June), germination rates under all three conditions and at all depths were very similar and ranged between 17 and 30% (Fig. 2a).

UVR had no significant effect on the germination capacity of *S. dermatodea* in either field experiment. In the first, however, depth had a significant effect on germination (ANOVA, $P = 0.003$, see Table 2). Regardless of light quality, Duncan's multiple range test (DMRT, $P = 0.05$) showed significantly higher germination rate at 1.0 m depth compared with 0.5 m and 2.0 m depths.

In the first experiment with *A. esculenta* (4–5 June), germination rates of about 50% were measured under the P condition in all four tested depths (Fig. 2b). Low germination rates of 15–20% were determined under the PAB and PA condition at 0.5 and 1 m depth. These were not, however, significantly different from the rates measured under the P condition (Table 2). At 2 and 4 m depth germination rates were all very similar. In the second experiment with this species (14–15 June),

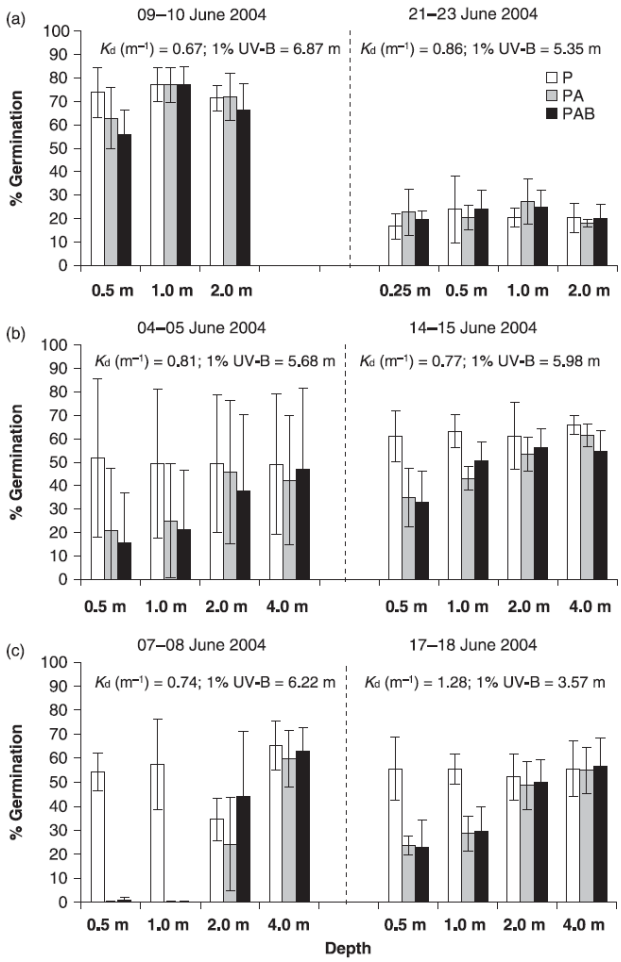

Fig. 2 Spore germination in (a) *Saccorhiza dermatodea*, (b) *Alaria esculenta* and (c) *Laminaria digitata*, 3 days after exposure to ambient solar radiation treatments consisting of photosynthetically active radiation (PAR = P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) at different depths and exposure times (see Table 1) and post-cultivated at 10 µmole photons m^{-2} s^{-1}. Inset values are corresponding average attenuation coefficients (K_d) and depth of 1% UV-B penetration. Vertical bars are standard deviations (SD, $n = 5$).

germination rates of about 60–65% were obtained under the P condition at all depths. Under the PAB and PA conditions a depression to about 35% was apparent at 0.5 m water depth.

In *A. esculenta*, significant effects of irradiance and dose (as a function of depth) (ANOVA, $P < 0.001$) were only observed in the second field experiment (Table 2). DMRT ($P = 0.05$) showed that the P condition is significantly different from PA and PAB conditions but PA and PAB conditions are not significantly different from each other. Values at 0.5 m were significantly different from the other depths. Depths 1 and 2 m are homogenous subsets, as were 2 and 4 m, although 1 m was significantly different from 4 m.

In the first experiment with *L. digitata* (7–8 June, Fig. 2c) almost no germination was observed under the PAB and PA condition at 0.5 m and 1 m depth, whereas a germination rate of about 55% was obtained under the P condition at these depths. At 4 m depth germination rates of about 60% were measured under all three conditions, whereas at 2 m very variable values between 25 and 45% were determined under all radiation conditions. During the second experiment with this species (17–18 June) germination rates exhibited little variation under the P condition at all depths studied, with values around 55% (Fig. 2c). UVBR and UVAR had no effect on the germination rate at 2 and 4 m depth. However, at 0.5 and 1 m depth there was a clear decrease of germination rates down to 22–27% under both PA and PAB.

In both field experiments with *L. digitata* there were significant effects of irradiance and depth as well as an interaction between these variables (ANOVA, $P = 0.001$). DMRT ($P = 0.05$) showed that, in both experiments, the P condition is significantly different from the PA and PAB conditions but PA and PAB conditions are not significantly different from each other. In the first experiment, depths 1 m and 2 m belong to a homogenous subset and are significantly different from depths

Table 2 Multiple analysis of variance (MANOVA) and significance values for the main effects and interactions of irradiance (PAR = P; PAR + UV-A radiation = PA; PAR + UV-A + UV-B radiation = PAB) and depth on germination of zoospores in three species of Laminariales from Spitsbergen. *Significant; NS, not significant

Date of experiment	Species	Source of variation	d.f.	*F*-value	*P*-value
9–10 June 2004	*S. dermatodea*	UV treatment (A)	2	2.412	0.104^{ns}
		Depth (B)	2	7.053	0.003*
		A × B	4	1.289	1.289^{ns}
21–23 June 2004	*S. dermatodea*	UV treatment (A)	2	0.312	0.734^{ns}
		Depth (B)	3	1.257	0.301^{ns}
		A × B	6	0.583	0.742^{ns}
4–5 June 2004	*A. esculenta*	UV treatment (A)	2	2.599	0.085^{ns}
		Depth (B)	3	1.261	0.298^{ns}
		A × B	6	0.431	0.855^{ns}
14–15 June 2004	*A. esculenta*	UV treatment (A)	2	16.195	< 0.001*
		Depth (B)	3	10.177	< 0.001*
		A × B	6	2.327	0.047*
7–8 June 2004	*L. digitata*	UV treatment (A)	2	35.096	< 0.001*
		Depth (B)	3	39.256	< 0.001*
		A × B	6	10.428	< 0.001*
17–18 June 2004	*L. digitata*	UV treatment (A)	2	16.326	< 0.001*
		Depth (B)	3	16.085	< 0.001*
		A × B	6	4.646	0.001*

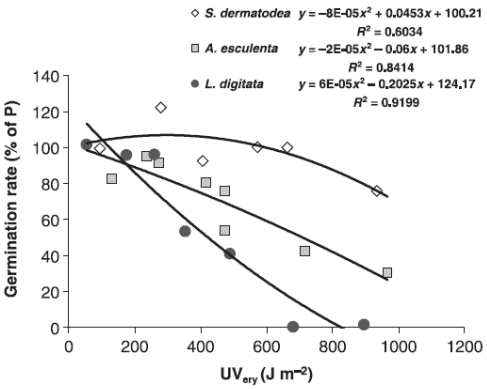

Fig. 3 Relationship between effective UV-B dose measured as UV_{ery} and germination rate expressed as percentage of PAR. Non-linear regression was used to obtain dose–response relationship. Biological effective doses needed to achieve 50% inhibition of germination BED50 are > 1000 J m^{-2}, 700 J m^{-2} and 418 J m^{-2} for *S. dermatodea*, *A. esculenta* and *L. digitata*, respectively.

2 m and 4 m, which are also significantly different from each other. In the second experiment, both 0.5 m and 1.0 m and 2.0 m and 4.0 m are homogenous subsets. The two subsets are significantly different from each other.

Overall, the most UV-sensitive species was therefore *L. digitata*, the least sensitive *S. dermatodea*, and *A. esculenta* occupied an intermediate position. The dose–response curves for the three species (Fig. 3) clearly show that the 50% biologically effective dose (BED50) for *S. dermatodea* was as high as > 1000 J m^{-2} UV_{ery}, for *A. esculenta* 700 J m^{-2} UV_{ery} and for *L. digitata* as low as 418 J m^{-2} UV_{ery}.

Discussion

The main result of this first field study of UV-effects on brown algal zoospores is that, under ambient solar radiation, the UVR tolerance is highest in the shallow water species *S. dermatodea*, intermediate in the upper to mid sublittoral *A. esculenta* and lowest in the upper to mid sublittoral *L. digitata*. Clearly, the viability of the zoospores of the species studied depends on the UV exposure and is a major if not the most important factor determining their upper distribution limit as proposed by Wiencke *et al.* (2000) and Wiencke *et al.* (2004). However, there is no additional UVBR effect on the viability of the zoospores compared with the PA condition.

Indoor and outdoor radiation conditions mainly differ in the much higher PAR levels in the field compared with the laboratory. Whereas in laboratory studies (e.g. Wiencke, Clayton & Schoenwaelder 2004) PFRs < 30 µmoles photons m^{-2} s^{-1} were applied, surface field PFRs in this study ranged between 100 and 1400 µmoles photons m^{-2} s^{-1}. A somewhat unexpected result is that high PAR values do not inhibit germination in the field, as shown by similar germination rates in the P treatment at different water depths in all species. Moreover, parallel laboratory experiments under dim light conditions with the same spore material gave similar germination rates to those in the field (data not shown). This result is in contrast to previous observations that, in addition to the inhibitory effect of UVR, high levels of PAR exert strong negative effects on photosynthesis and on the growth of seaweed macrothalli (Hanelt *et al.* 1997; Aguilera *et al.* 1999). Such patterns may, however, be confined to the sun-adapted macrothalli of seaweeds with highly active photosynthetic tissues, and may not

be appropriate for the spores of Laminariales, which are shade adapted, contain only a single chloroplast with few thylakoids (Henry & Cole 1982) and exhibit only very low photosynthetic rates (Kain 1964; Amsler & Neushul 1991).

Another important difference is the much higher UVBR levels in the laboratory compared with the field. Under ambient solar radiation, over various time intervals, about 10 times lower UVBR doses were recorded compared with those in standard laboratory experiments (data taken from Wiencke, Clayton & Schoenwaelder 2004), probably explaining lower laboratory germination under PAB filters than under PA conditions (Wiencke *et al*. 2000; Wiencke, Clayton & Schoenwaelder 2004; Roleda *et al*. 2005). Another possibility may be a better stimulation under field conditions of the blue-light dependent photolyases that remove the cyclobutane pyrimidine dimers resulting from UV-induced DNA damage (Pakker, Beekman & Breeman 2000; Pakker, Martins, Boelen, Buma, Nikaido & Breeman 2000). In higher plants, photolyase enzymes recognize UV-induced DNA lesions and reverse dimerization by absorbing light between 350 and 450 nm (Hada *et al*. 2000).

The UVR-tolerance of zoospores may be partly explained by increased activity of the repair mechanisms, which apparently operate best in the shallow water species *S. dermatodea*, to a lesser degree in the upper to mid sublittoral *A. esculenta* and least in the upper to mid sublittoral *L. digitata*, as observed in laboratory studies (Wiencke, Clayton & Schoenwaelder 2004). Repair of DNA damage has been demonstrated in a variety of seaweeds (van de Poll *et al*. 2002) and also in zoospores of *L. digitata*, *L. saccharina and L. hyperborea* (Roleda *et al*. 2005). In the latter study, efficient repair was observed in the upper sublittoral *L. digitata*, but not in species occurring in greater water depths. Although the recovery of photosynthesis after UVR exposure has been documented, repair processes have so far not been shown in macroalgae, despite the fact that such processes must be involved.

Another explanation for the differential UV tolerance is the presence of protective mechanisms. UV-absorbing compounds, in particular phlorotannins are present in kelp zoospores, with higher concentrations in upper compared with mid sublittoral species (Roleda *et al*. 2005). Moreover, an increase in the number and diameter of phlorotannin-containing physodes has been described in *S. dermatodea* and in *A. esculenta* (Wiencke, Clayton & Schoenwaelder 2004), another reason for the success of these species in shallow waters.

Beside the incident surface radiation, an important factor governing the underwater radiation regime in polar regions is the presence of sea ice, which attenuates both PAR and UVBR very strongly (Hanelt *et al*. 2001). By the time the break-up of ice occurs in spring, the solar angle is already relatively high and algae are therefore suddenly exposed for long daily periods to very high PFRs. Moreover, the water is very clear and biologically relevant UVBR penetrates the water column down to about 5–8 m depth (Fig. 2; Hanelt *et al*. 2001; van de Poll *et al*. 2002). From the end of June onwards, however, attenuation increases due to a strong inflow of turbid melt water from then until mid August (Svendsen *et al*. 2002), after which the water transparency increases again (Hanelt *et al*. 2001).

This change in the underwater radiation may be reflected also in the UVR susceptibility of algal spores. Germination is clearly inhibited in spores from *L. saccharina*, *L. digitata* and *A. esculenta* collected in spring and exposed to PA (Wiencke *et al*. 2000), but there is no equivalent UVAR effect on autumn-collected spores (Wiencke, Clayton & Schoenwaelder 2004). In our opinion, the inhibition of germination after UVAR exposure in spring is related to the fact that material is not yet acclimated to high radiation conditions. In a marine diatom, damage to carbon fixation in the cells was found to be higher under UV-A, which also induces localized loss on the acceptor side of the PSII reaction centres (Grzymski *et al*. 2001; Turcsányi & Vass 2002).

All the species studied here develop their sori in summer. *A. esculenta* and *L. digitata* are fertile between May and September, whereas young specimens of *S. dermatodea* become fertile in August and September (C. Wiencke & M. N. Clayton, unpublished data) and 18-month-old specimens in May and June. The spores of the studied species were therefore exposed to the described radiation conditions at an appropriate time, underlining the ecological relevance of our data.

In the field, *S. dermatodea* is common at depths between 0.5 and 5.5 m, *A. esculenta* at depths between 1.5 and 12.5 m and *L. digitata* grows between 1.5 and 13.5 m (Wiencke, Vögele, Kovaltchouk & Hop 2004; C. Wiencke unpublished data). The known depth distribution pattern therefore mostly reflects the data obtained here on UV tolerance of the zoospores. The only misfit is the overlap in the distribution between *A. esculenta* and *L. digitata* in shallow water. However, in such situations, *L. digitata* may grow below *A. esculenta* (C. Wiencke, unpublished observations) and settling in the shade of *A. esculenta* may allow the very UV sensitive zoospores of *L. digitata* to establish. The adult macrothalli of *L. digitata* are clearly sun adapted and can cope very well both with high PAR and with UVBR (Hanelt *et al*. 1997; Bischof *et al*. 1998). Additional biotic factors, such as competition or grazing, as exemplified by Wahl *et al*. (2004), require further investigation.

The results suggest the need for studies of the protective and repair mechanisms in the most UV-tolerant species, *S. dermatodea* and *A. esculenta*. The question remains whether they will be able to cope with the increase of harmful UVBR due to stratospheric ozone depletion. A 10% decline in ozone concentration results in a doubling of irradiance at 300 nm (Frederick *et al*. 1989). In the Ny Ålesund area a 12% increase in irradiance at 300 nm was already measured as a

consequence of a relatively minor reduction in ozone concentration of 10 Dobson units (Groß *et al*. 2001). Enhanced UVBR certainly will influence the viability of brown algal zoospores and, hence, the zonation of seaweeds around Spitsbergen.

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References

Aguilera, J., Karsten, U., Lippert, H., Vögele, B., Philipp, E., Hanelt, D. & Wiencke, C. (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology-Progress Series*, **191**, 109–119.

Amsler, C.D. & Neushul, M. (1991) Photosynthetic physiology and chemical composition of spores of the kelps *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Laminaria farlowii*, and *Pterygophora californica* (Phaeophyceae). *Journal of Phycology*, **27**, 26–34.

Bischof, K., Hanelt, D., Tüg, H., Karsten, U., Brouwer, P.E.M. & Wiencke, C. (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biology*, **20**, 388–395.

Bischof, K., Hanelt, D. & Wiencke, C. (1998) UV-radiation can affect depth-zonation of Antarctic macroalgae. *Marine Biology*, **131**, 597–605.

Bischof, K., Kräbs, G., Wiencke, C. & Hanelt, D. (2002) Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta*, **215**, 502–509.

Clayton, M.N. & Wiencke, C. (2004) UV effects on reproduction of brown algae from Kongsfjorden (Svalbard). *Berichte zur Polar- und Meeresforschung*, **492**, 215–221.

Coelho, S.M., Rijstenbil, J.W. & Brown, M.T. (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. *Journal of Aquatic Ecosystem Stress and Recovery*, **7**, 317–333.

Dahlback, A. (2002) Recent changes in surface ultraviolet solar radiation and stratospheric ozone at a high Arctic site. *UV Radiation and Arctic Ecosystems* (ed. D. Hessen), pp. 3–22. Springer Verlag, Berlin.

Dring, M.J., Wagner, A., Boeskov, J. & Lüning, K. (1996) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. *European Journal of Phycology*, **31**, 293–302.

El Naggar, S., Gustat, H., Magister, H. & Rochlitzer, R. (1995) An electronic personal UV-B dosimeter. *Journal of Photochemistry and Photobiology B: Biology*, **31**, 83–86.

Flores-Moya, A., Posudin, Y.I., Fernández, J.A., Figueroa, F.L. & Kawai, H. (2002) Photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose. *Journal of Photochemistry and Photobiology B: Biology*, **66**, 134–140.

Franklin, L.A. & Forster, R.M. (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology*, **32**, 207–232.

Frederick, J.E., Snell, H.E. & Haywood, E.K. (1989) Solar ultraviolet radiation at the earth's surface. *Photochemistry and Photobiology*, **50**, 443–450.

von der Gathen, P., Rex, M., Harris, N.R.P., Lucic, D., Knudsen, B.M., Braathen, G.O. *et al.* (1995) Observational evidence for chemical ozone depletion over the Arctic in winter 1991–92. *Nature*, **375**, 131–134.

Gómez, I., Weykam, G., Klöser, H. & Wiencke, C. (1997) Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctica). *Marine Ecology-Progress Series*, **148**, 281–293.

Groß, C., Tüg, G.H. & Schrems, O. (2001) Three years spectral resolved UV-measurements at Koldewey-Station (1997–99). *Memoirs of the National Institute of Polar Research, Special Issue*, **54**, 113–123.

Grzymski, J., Orrico, C. & Schofield, O.M. (2001) Monochromatic ultraviolet light induced damage to Photosystem II efficiency and carbon fixation in the marine diatom *Thalassiosira pseudonana* (3H). *Photosynthesis Research*, **68**, 181–192.

Hada, M., Iida, Y. & Takeuchi, Y. (2000) Action spectra of DNA photolyases for photorepair of cyclobutane pyrimidine dimers in sorghum and cucumber. *Plant and Cell Physiology*, **41**, 644–648.

Hanelt, D. (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology*, **131**, 361–369.

Hanelt, D., Tüg, G.H., Bischof, K., Groß, C., Lippert, H., Sawall, T. *et al.* (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Marine Biology*, **138**, 649–658.

Hanelt, D., Wiencke, C. & Nultsch, W. (1997) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. *Journal of Photochemistry and Photobiology B: Biology*, **38**, 40–47.

Henry, E.C. & Cole, K.M. (1982) Ultrastructure of swarmers in the Laminariales (Phaeophyceae), I. Zoospores. *Journal of Phycology*, **18**, 550–569.

Hop, H., Pearson, T., Hegseth, E.N., Kovacs, K.M., Wiencke, C., Kwasniewski, S. *et al.* (2002) The ecosystem of Kongsfjorden, Svalbard. *Polar Research*, **21**, 167–208.

Huovinen, P.S., Oikari, A.O.J., Soimasuo, M.R. & Cherr, G.N. (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. *Photochemistry and Photobiology*, **72**, 308–313.

Kain, J.M. (1964) Aspects of the biology of *Laminaria hyperborea*. III. Survival and growth of gametophytes. *Journal of the Marine Biology Association of the UK*, **44**, 415–433.

Kirk, J.T.O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press, Cambridge.

Lüning, K. (1990) *Seaweeds, Their Environment, Biogeography and Ecophysiology*. John Wiley and Sons, Chichester.

Maegawa, M., Kunieda, M. & Kida, W. (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. *Japanese Journal of Phycology*, **41**, 207–214.

Makarov, M.V. & Voskoboinikov, G.M. (2001) The influence of ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. *Botanica Marina*, **44**, 89–94.

McKinlay, A.F. & Diffey, B.L. (1987) A reference action spectrum for ultraviolet induced erythema in human skin. *CIE Research Note, CIE-Journal*, **6**, 17–22.

Pakker, H., Beekman, C.A.C. & Breeman, A.M. (2000) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). *European Journal of Phycology*, **35**, 109–114.

Pakker, H., Martins, R.S.T., Boelen, P., Buma, A.G.J., Nikaido, O. & Breeman, A.M. (2000) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). *Journal of Phycology*, **36**, 334–341.

van de Poll, W.H., Hanelt, D., Hoyer, K., Buma, A.G.J. & Breeman, A.M. (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochemistry and Photobiology*, **76**, 493–501.

Rex, M., Salawitch, R.J., Harris, N.R.P., von der Gathen, P., Braathen, G.O., Schulz, A. *et al.* (2002) Chemical loss of Arctic ozone in winter 1999/2000. *Journal of Geophysical Research* **107**, D20, 8276. DOI 10.1029/2001JD000533.

Roleda, M.Y., van de Poll, W.H., Hanelt, D. & Wiencke, C. (2004) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. *Marine Ecology-Progress Series*, **281**, 37–50.

Roleda, M.Y., Wiencke, C., Hanelt, D., van de Poll, W.H. & Gruber, A. (2005) Sensitivity of Laminariales zoospores from Helgoland (North Sea) to ultraviolet and photosynthetically active radiation: implications for depth distribution and seasonal reproduction. *Plant, Cell and Environment*, **28**, 466–479.

Sagert, S., Forster, R.M., Feuerpfeil, P. & Schubert, H. (1997) Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. *European Journal of Phycology*, **32**, 363–371.

Svendsen, H., Beszczynska-Møller, A., Hagen, J.O., Lefauconnier, B., Tverberg, V., Gerland, S. *et al.* (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Research*, **21**, 133–166.

Turcsányi, E. & Vass, I. (2002) Effect of UV-A radiation on photosynthetic electron transport. *Biologica Szegediensis*, **46**, 171–173.

Wahl, M., Molis, M., Davis, A., Dobretsov, S., Dürr, S.T., Johansson, J. *et al.* (2004) UVR effects that come and go: a global comparison of marine epibenthic community level impacts. *Global Change Biology*, **10**, 1962–1972.

Wiencke, C. (2004) The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991–2003. *Berichte zur Polar- und Meeresforschung* **492**, 1–244.

Wiencke, C., Clayton, M.N. & Schoenwaelder, M.E.A. (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. *Marine Biology*, **145**, 31–39.

Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altamirano, M., Hanelt, D. (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Marine Ecology-Progress Series*, **197**, 217–229.

Wiencke, C., Vögele, B., Kovaltchouk, N.A. & Hop, H. (2004) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. *Berichte zur Polar- und Meeresforschung*, **492**, 55–62.

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4. SUMMARY OF RESULTS

4.1 Photosynthetic performance

4.1.1 Photosynthesis of juvenile plants

Initial mean effective quantum yield ($\Delta F/F_m$') in young *Laminaria ochroleuca* sporophytes was 0.673 ± 0.02. In young Gigartinales gametophytes, higher photosynthetic efficiency was observed in *Mastocarpus stellatus* (0.575 ± 0.03) compared to *Chondrus crispus* (0.536 ± 0.03) (**Fig. 5**). During the first day of UVR exposure, higher reduction in the mean effective quantum yield was observed in *L. ochroleuca* (PA = 53%, PAB = 61% reduction) compared to *C. crispus* (PA = 23%, PAB = 43%) and *M. stellatus* (PA = 22%, PAB = 30% reduction). The mean $\Delta F/F_m$' of PA and PAB treated samples were able to recover after UV lamps were switched off. After 2 hours recovery, photosynthetic efficiencies of *Mastocarpus* and *Chondrus* were able to increase to values comparable to pre-UV treatment. Longer recovery time of 4 hours was required in *L. ochroleuca*. Time series measurements showed that the reduction in the mean $\Delta F/F_m$' of UV-exposed plants became smaller with time. *Mastocarpus* and *Chondrus* were able to completely acclimate to UVR after 3 days of repeated exposure while *L. ochroleuca* was only able to partially acclimate to UVR until the 28th exposure day.

4.1.2 Photosynthesis of spores

Photosynthetic efficiencies in dark-adapted Gigartinales carpospores and Laminariales zoospores (F_v/F_m) studied are lower compared to their respective light-adapted juvenile gametophytes and sporophytes ($\Delta F/F_m$'). Species specific differences were also observed in initial spore photosynthetic efficiencies of the untreated controls. Between Gigartinales carpospores, higher optimum quantum yield was measured in *Mastocarpus* (0.533 ± 0.01) compared to *Chondrus* (0.386 ± 0.04). Among Laminariales zoospores, F_v/F_m was 0.466 ± 0.01 for *Laminaria digitata*, 0.476 ± 0.003 for *L. saccharina* and 0.533 ± 0.005 for *L. hyperborea*. After exposure to different doses of PAR, PAR + UV-A and PAR + UV-A + UV-B (see publications 1 and 6 for different exposure times and irradiances), F_v/F_m of the investigated Gigartinales carpospores, expressed as percent of control, were observed to be

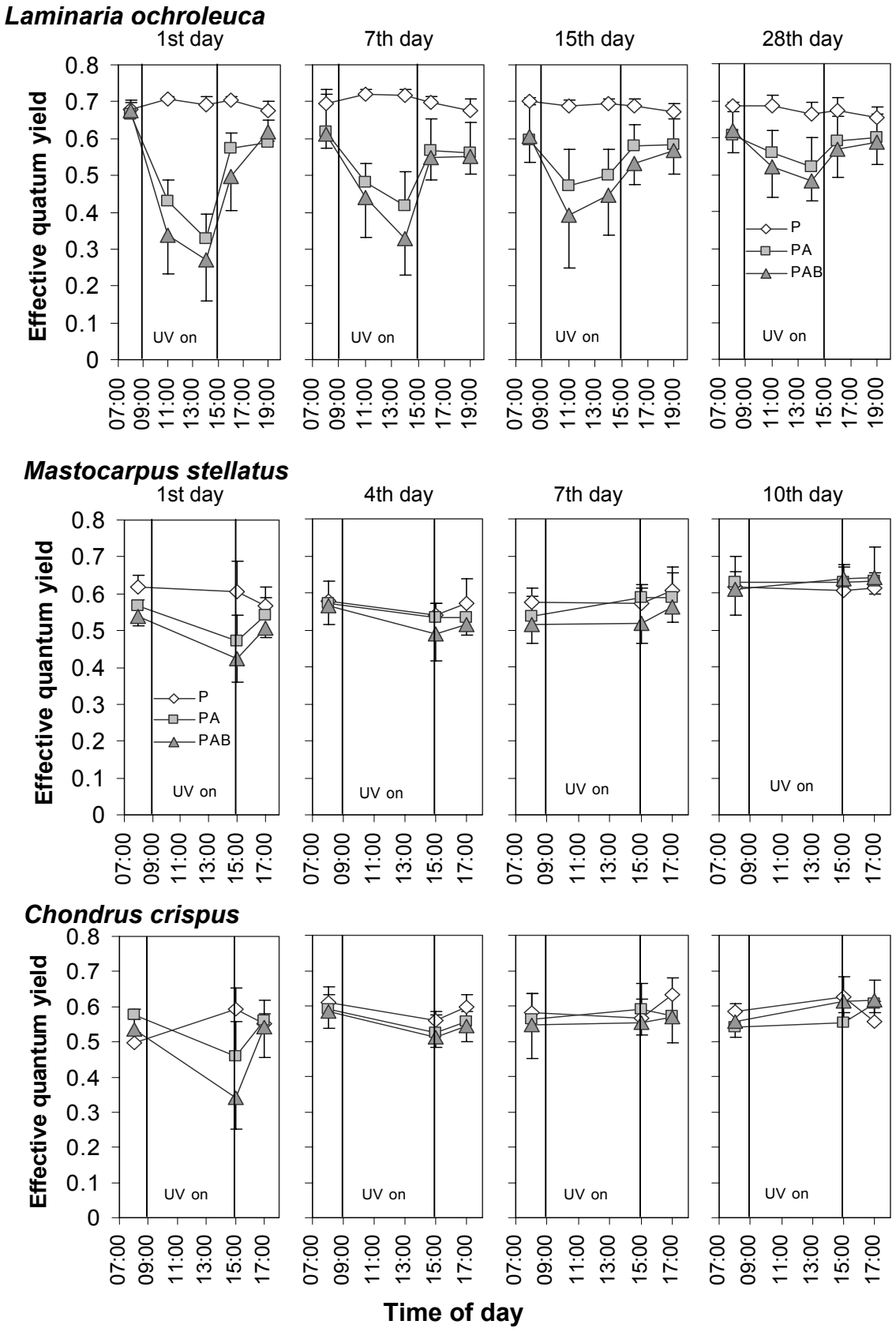

Fig. 5. Time series of the circadian pattern of the mean effective quantum yield ($\Delta F/F_m'$) of young sporophytes and gametophytes during the light phase of the 16:8 h light:dark photoperiod. PFD is 40-50 µmole photons m^{-2} s^{-1}. Between 9:00 and 15:00 h light was supplemented with 6.24 W m^{-2} UV-A and 0.50 W m^{-2} UV-B. Vertical bars are standard deviations (SD, n=5). Figure redrawn from Roleda *et al*. 2004a, 2004b.

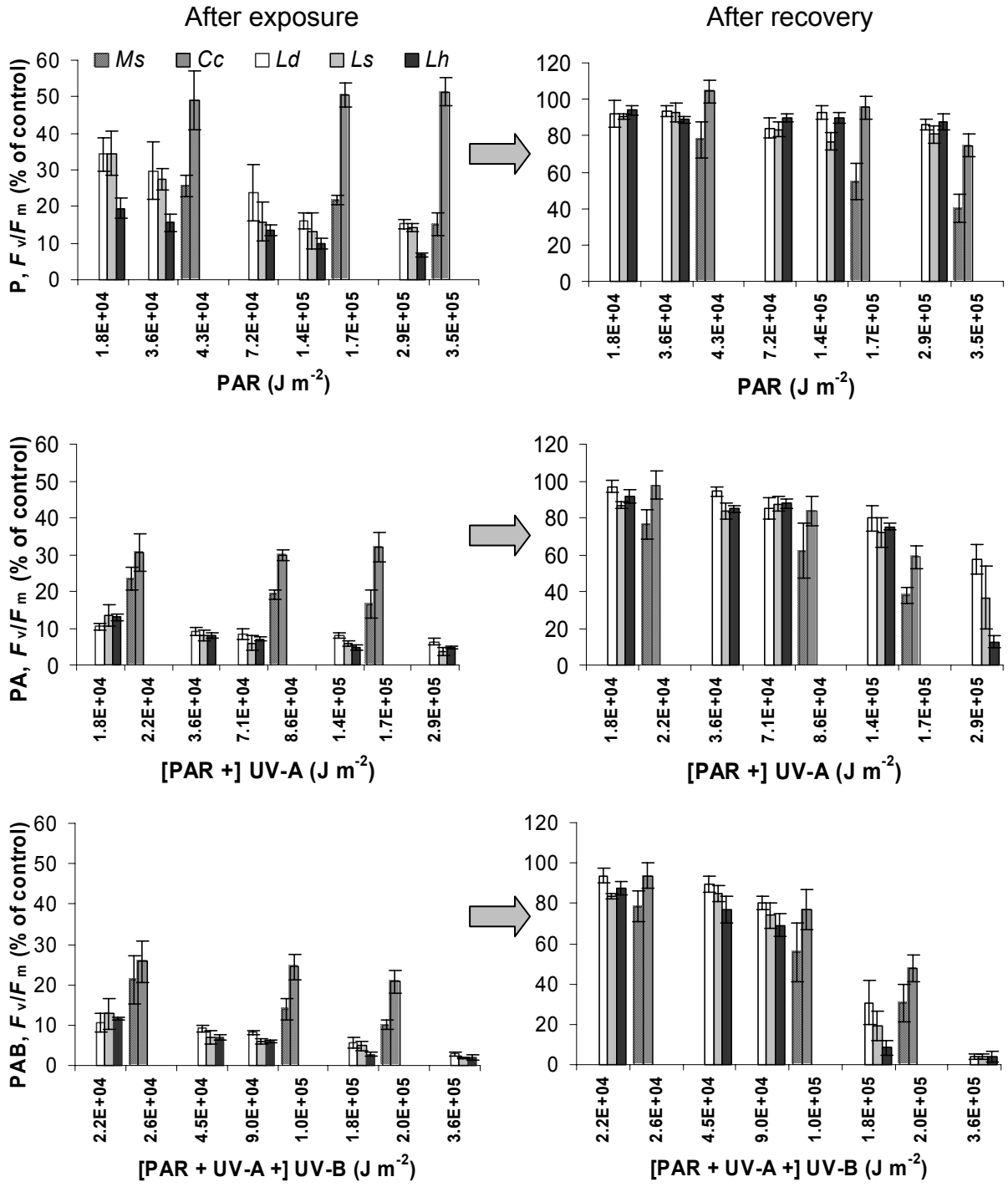

Fig. 6. Mean optimum quantum yield (F_v/F_m) of spores during treatment (left column) to photosynthetically active radiation (PAR = P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) at different doses in (*Ms) Mastocarpus stellatus*; (*Cc*) *Chondrus crispus*; (*Ld*) *Laminaria digitata*; (*Ls*) *L. saccharina*; and (*Lh*) *L. hyperborea* expressed as percent of control. Corresponding photosynthetic recovery (right column) after 48 h post-culture in dim white light (10 µmole photons m^{-2} s^{-1}). Vertical bars are standard deviations (SD, n=5). Figure redrawn from Roleda *et al*. 2004b, 2005a

less sensitive to higher doses of different radiation treatments compared to Laminariales zoospores (**Fig. 6**). Zoospores are found to be low light adapted compared to carpospores. Light supplemented with UVR further decreased photosynthetic efficiency and increasing dose exacerbates the effect of UVR.

After 2 days in low white light (10 µmol photons m^{-2} s^{-1}), photosynthetic efficiency of the untreated controls was reduced in all species except in *Chondrus* (0.394 ± 0.04). Lower F_v/F_m was measured in *Mastocarpus* (0.453 ± 0.02), *L. digitata* (0.387 ± 0.005), *L. saccharina* (0.431 ± 0.008) and *L. hyperborea* (0.513 ± 0.011). Photosynthetic efficiency of PAR-treated germinating Laminariales spores was able to recover in dim light to 80- 95% of the control (**Fig. 6**). An efficient F_v/F_m recovery (70- 90% of control) was also observed in kelp spores previously exposed to a maximum of 1.43 x 10^5 J m^{-2} UV-A and 8.96 x 10^4 J m^{-2} UV-B. Exposure to higher doses of PAR in Gigartinales carpospores affects photosynthetic recovery in *Mastocarpus*. Conversely, higher percent recovery was observed in UVR-exposed *Mastocarpus* (56- 75%) compared to *Chondrus* (45- 72%) spores.

4.2 Growth of juvenile plants

In the basin flow-through culture system, growth rates of young *Laminaria ochroleuca* sporophytes were significantly affected under different radiation treatments (ANOVA, $p < 0.05$). Duncan's multiple range test (DMRT, $p = 0.05$) showed that growth rate of sporophytes exposed to P is significantly different to PAB, while P is not significantly different to PA and PA is not significantly different to PAB. Among the investigated Gigartinales, different radiation treatments have no significant effect on the growth rates of young *Mastocarpus* and *Chondrus* gametophytes (**Fig. 7**).

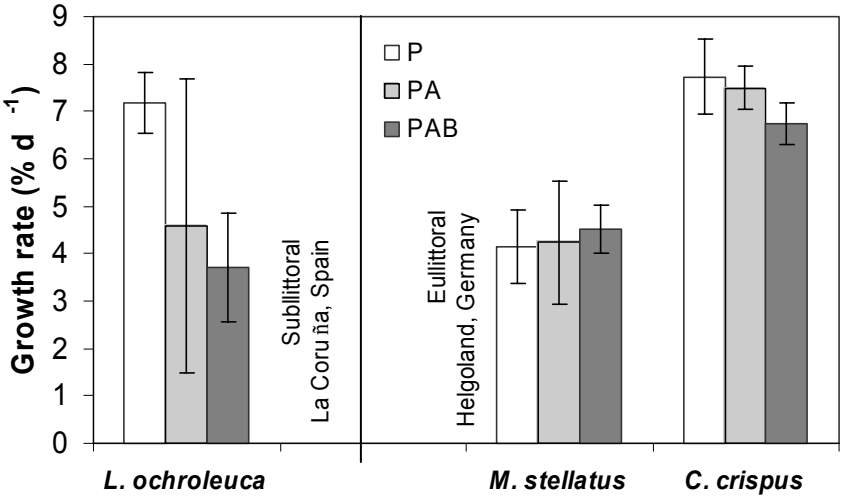

Fig. 7. Growth rates of young sporophytes and gametophytes cultured in a basin flow-through system exposed to different radiation (PAR = P; PAR + UV-A = PA; PAR + UV-A + UV-B = PAB). PFD was 40-50 µmole photons m^{-2} s^{-1}. Vertical bars are standard deviation (SD, n=5). Figure redrawn from Roleda *et al*. 2004a, 2004b

In the ISITEC automated growth chambers, growth rates of different species of Laminariales under P treatment ranges from 3.2 ± 0.6% day^{-1} (*A. esculenta*) to 10.1 ± 0.3% day^{-1} (*L. saccharina*, Spitsbergen isolate). Among sporophytes exposed to the whole light spectrum (expressed as percent of P, **Fig. 8**), growth rate was highest in *L. digitata* and lowest in *L. hyperborea* from Helgoland. In Laminariales from Spitsbergen, highest growth rates in *Alaria esculenta* were measured and lowest growth rates occurred in *Saccorhiza dermatodea* and *Laminaria solidungula*. The growth rate of the cold-temperate *L. ochroleuca* exposed to the whole light spectrum is characteristic for mid- to sublittoral kelp species.

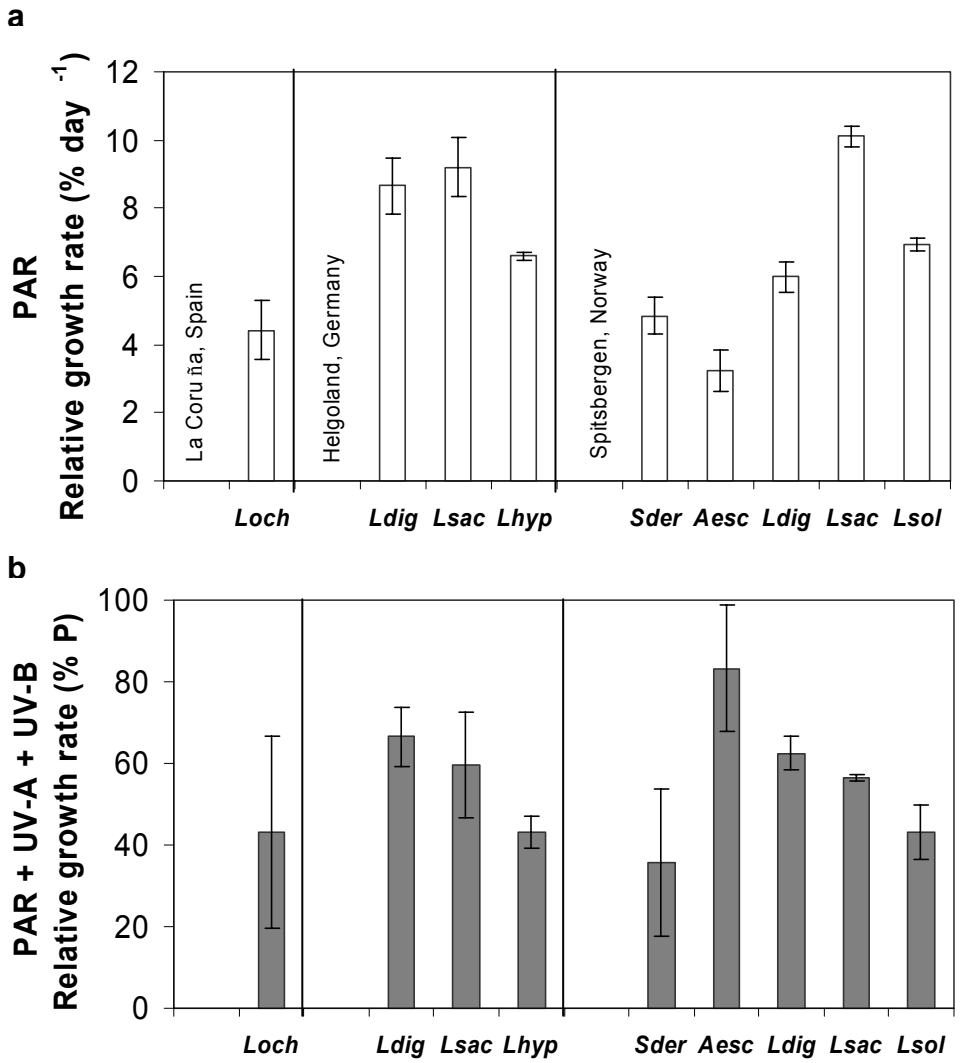

Fig. 8. Growth rates of young Laminariales sporophytes using the ISITEC growth chamber exposed to (a) PAR and (b) PAR + UV-A + UV-B expressed as percent of PAR (% P). PDF is ± 18 µmol photons m^{-2} s^{-1}. Vertical bars are standard deviations (SD, n=3). Figure redrawn from Roleda *et al*. 2004a, 2005b, 2006a, 2006b.

4.3 Thallus morphology, optical characteristics and absorption spectra

Species specific differences in growth forms and thallus morphology were observed between wild and cultured sporophytes (**Fig. 9**). With the exception of *Alaria esculenta* and *L. saccharina* (Spitsbergen isolate), all wild specimen were found to be darkly pigmented compared to their cultured counterparts. Wild sporophytes of young *A. esculenta* and arctic *L. saccharina* were found to be thin and translucent compared the cultured morphotype. Among the cultured sporophytes, *Saccorhiza dermatodea* was thick but pale and translucent. Corresponding spectral analysis showed an absorbance peak at 678 nm representing chlorophyll *a* and peaks between 425- 450 nm representing chlorophylls and carotenoids. Strong absorbance below 280 nm was also observed in all Laminariales species studied (see Figure 7 of publications 3, Figure 5 of publication 4, and Figure 7 of publication 5).

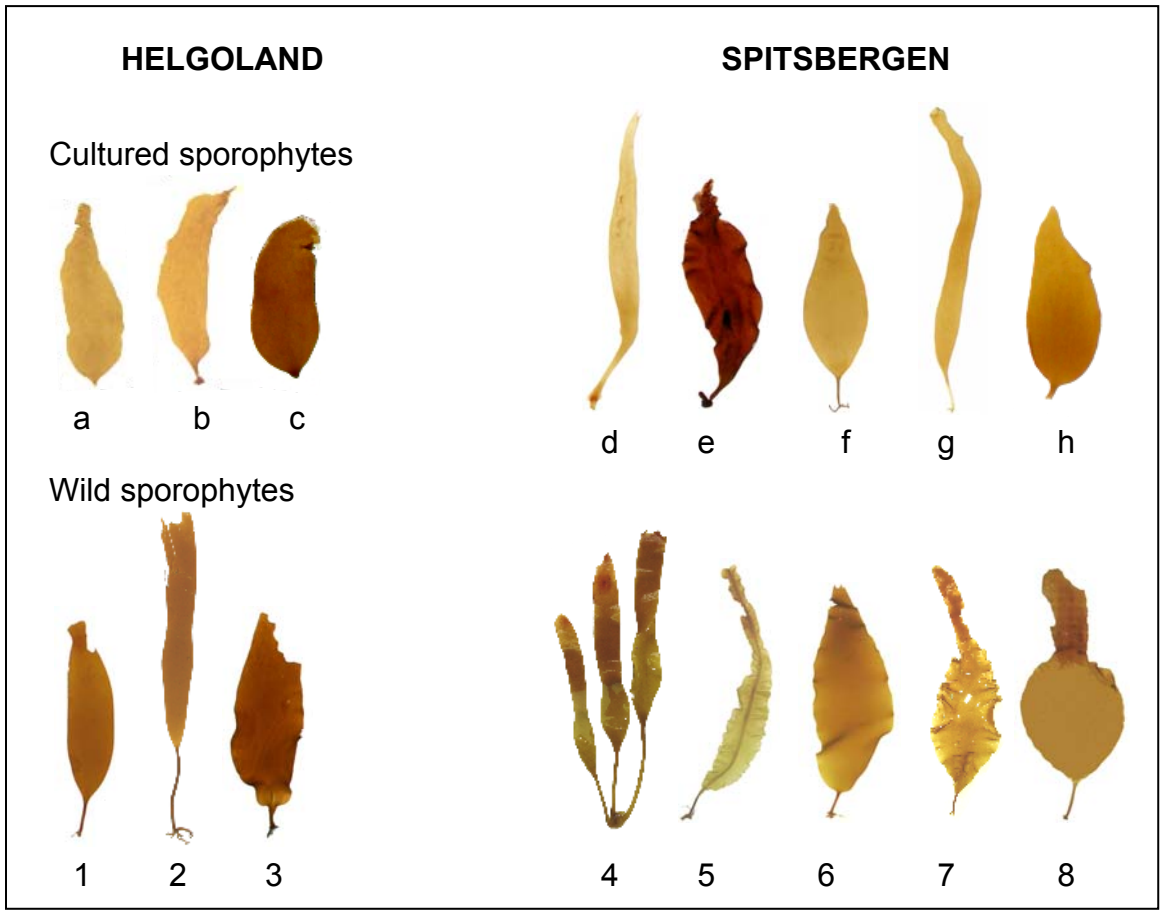

Fig. 9. Morphological differences in (a-h) cultured and (1-8) wild young Laminariales sporophytes from (a-c; 1-3) Helgoland and (d-h; 4-8) Spitsbergen. Species are (a,1; f,6) *Laminaria digitata*; (b,2; g,7) *L. saccharina*; (c,3) *L. hyperborea*; (d,4) *Saccorhiza dermatodea*; (e,5) *Alaria esculenta*; and (h,8) *L. solidungula*. Thallus for each species represents the typical morphology used for the experiments

4.4 Zoospore suspension and absorption spectra

Spectral analysis of zoospore suspensions, filtrates and zoospores also showed strong absorbance in the UV waveband. Higher UV absorbance was observed in *L. digitata* compared to *L. saccharina* and *L. hyperborea*. UV absorbance of the filtrate was also higher compared to zoospore itself in all species (see Figure 3 of publication 6).

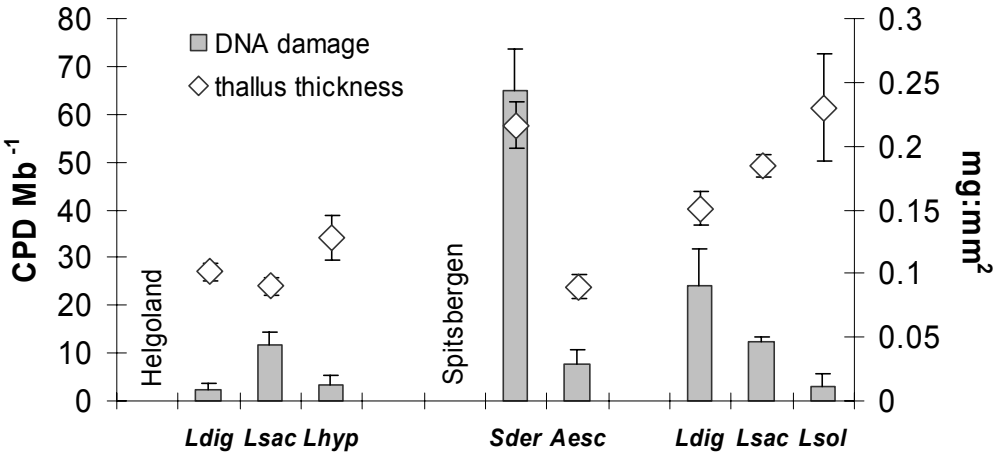

Fig. 10. Relationship between average thallus thickness and remaining tissue DNA damage in young Laminariales sporophytes from Helgoland and Spitsbergen after 18-21 days of repeated daily 6 hours ultraviolet radiation exposure and 18 hours recovery (6:8:4 h light: dark: light cycle). PFD is ± 18 µmol photons m^{-2} s^{-1}. Vertical bars are standard deviations (SD, n=3 for DNA damage; n=10 for thallus thickness). Figure redrawn from Roleda *et al*. 2005b, 2006a, 2006b.

4.5 DNA damage and repair

After repeated daily exposure of 6 hours UVR for 18- 21 days, remaining tissue DNA damage in young sporophytes was affected by thallus thickness (**Fig. 10**) and optical characteristics (publications 4, 5, and 6) but no relation to the depth distribution of the species was observed. Among *Laminaria* species from Helgoland and Spitsbergen, an inverse relationship was observed between thallus thickness and DNA damage. In *S. dermatodea* and *A. esculenta*, however, thallus translucence and opacity influenced sensitivity of young sporophytes to UV-B-induced DNA damage (see publication 4). Other than *S. dermatodea*, a relatively moderate to low cyclobutane pyrimidine dimer (CPD) content was remaining in the tissue of different Laminariales species after daily18 h recovery of 6:8:4 h light: dark: light cycle.

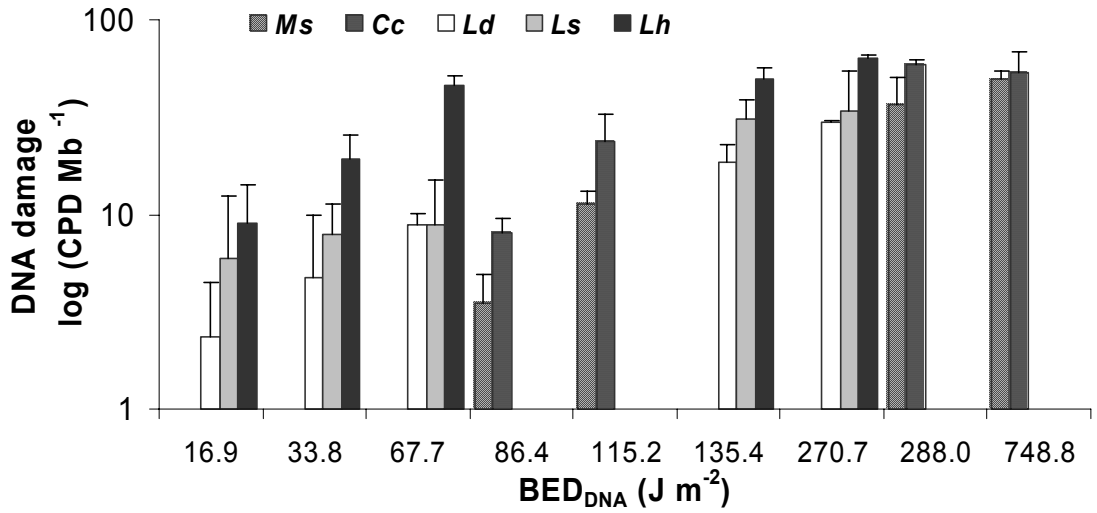

Fig. 11. UVB-induced DNA damage (induced cyclobutane pyrimidine dimers per million nucleotides) in Gigartinales carpospores and Laminariales zoospores. Species are (*Ms) Mastocarpus stellatus*; (*Cc*) *Chondrus crispus*; (*Ld*) *Laminaria digitata*; (*Ls*) *L. saccharina*; and (*Lh*) *L. hyperborea*. Vertical bars are standard deviations (SD, n=3). Figure redrawn from Roleda *et al*. 2004b and 2005a

Among reproductive cells, exposure to different UV-B doses showed that the investigated Laminariales zoospores are more sensitive to UV-B-induced DNA damage compared to the investigated Gigartinales carpospores (**Fig. 11**). At higher UV-B doses, the upper eulittoral species *Mastocarpus* sustained less DNA damage compared to the lower eulittoral *Chondrus* and the sublittoral *Laminaria* species. Post cultivation at low white light for 2 days in zoospores and 6 days in carpospores showed non linear relationship (logarithmic for Laminariales and polynomial for Gigartinales) between UV-B-induced DNA damage as a function of dose (BED_{DNA}) and DNA repair rate in spores (**Fig. 12**). This implies that at higher UV-B dose, the extent of DNA damage induced is not effectively repaired by the cells anymore. DNA repair rate was higher in Laminariales zoospores compared to Gigartinales carpospores. Among the kelp zoospores, higher repair rates were observed in the lower sublittoral *L. hyperborea* compared to the upper sublittoral *L. digitata* (**Fig. 12**).

4.6 Germination and spore mortality

Germination capacity of spores was low light adapted (20- 50 µmol photons m^{-2} s^{-1}). Spore viability after post cultivation under dim white light (10 µmol photons m^{-2} s^{-1}; 3 days in zoospores and 6 days in carpospores) showed that pre-treatment of high PAR (50- 130 µmol photons m^{-2} s^{-1}) induced higher mortality among carpospores (mortality rates ranges from 4-49% in *Mastocarpus*, and 26- 54% in *Chondrus*; see Figure 3 of publication 1) compared to

pre-treatment of lower PAR (20 µmol photons m^{-2} s^{-1}) among Laminariales (with dead and non-germinated spores of 17- 41% in *Laminaria digitata*, 25- 44% in *L. saccharina*, and 20-38% in *L. hyperborea*; data recomputed from publication 6). Relative to different doses of PAR (1- 16 hours exposure of 20- 130 µmol photons m^{-2} s^{-1}), zoospores are relatively more sensitive to white light compared to carpospores.

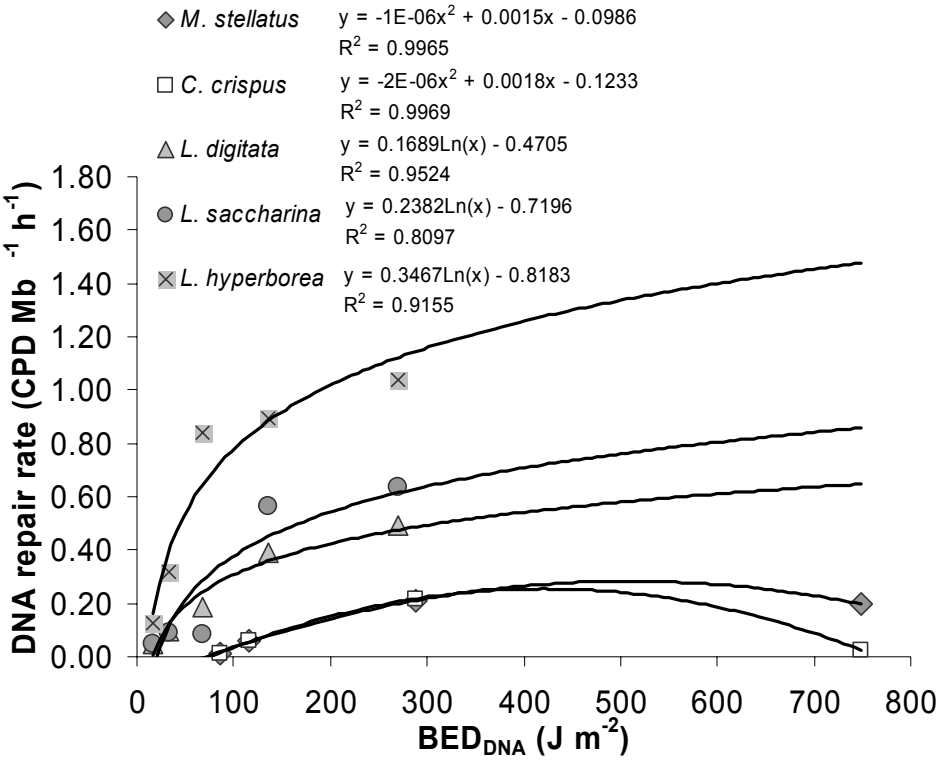

Fig. 12. Relationship between biologically effective UVB doses and DNA repair rate in carpospores of Gigartinales and zoospores of Laminariales from Helgoland. Values are means (n=3). Curves are non linear regressions corresponding to the best fit for each species

Spore mortality under UVR (PA and PAB treatments) was higher in *Chondrus* compared to *Mastocarpus* across all treatments (see Figure 3 of publication 1). A higher biological effective dose (BED_{50}) is required to achieve a 50% inhibition of germination in *Mastocarpus* (762 J m^{-2}) compared to *Chondrus* (248 J m^{-2}) (see Figure 4 of publication 1). In Laminariales zoospores, UVA had minimal effects on the germination capacity of *L. digitata* and *L. saccharina* but reduced germination rate of up to 50% in *L. hyperborea* after 16 h exposure to PA. Additional UV-B further reduced germination rates to less than 30% in all species exposed to 16 h PAB and to *L. saccharina* and *L. hyperborea* exposed to 8 h PAB (see Figure 5 of publication 6). Zoospores are more sensitive to UVR compared to carpospores where much lower biological effective doses are needed to inhibit 50%

germination in *L. digitata* (86 J m^{-2}), *L. saccharina* (67 J m^{-2}), and *L. hyperborea* (52 J m^{-2}) (**Fig. 13**).

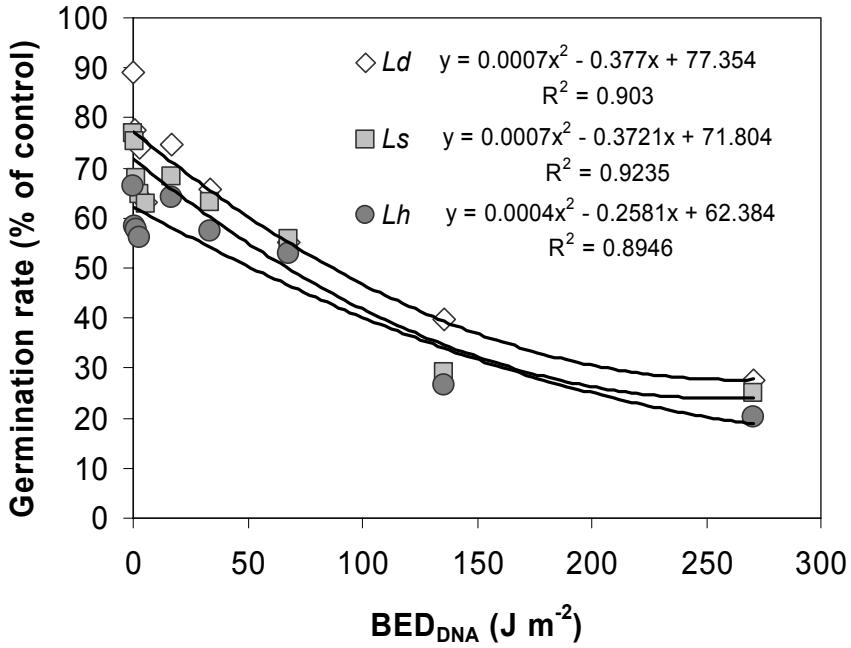

Fig. 13. Relationship between effective UV-B dose and germination rate expressed as percent of control. Non linear regression was used to obtain dose-response relationship. Biological effective dose needed to achieve 50% inhibition of germination BED_{50} are 86 J m^{-2}, 67 J m^{-2} and 52 J m^{-2} for *Laminaria digitata* (*Ld*), *L. saccharina* (*Ls*) and *L. hyperborea* (*Lh*) respectively.

4.7 Pigment contents

Chlorophyll *a* contents showed significant differences between species, but not between treatments in Gigartinales (see Figure 7a and b of publication 1) and Laminariales from Helgoland and Laminariales from Spitsbergen (**Fig. 14**). Significantly lower photosynthetic and accessory pigment contents were observed in *Laminaria ochroleuca* sporophytes exposed to UVR (see Figure 7 of publication 2). Carotenoids (α-car and β-car) contents of *Chondrus crispus* were also significantly affected by radiation treatment (see Figure 7c of publication 1). A decrease in α-car content was observed under UVR. Conversely, β-car content increased under UVR treatment.

4.8 Field experiments with spores of three Laminariales from the Arctic

Under ambient solar radiation, tolerance of zoospores to UVR exposure was highest in *Saccorhiza dermatodea*; intermediate in *Alaria esculenta* and lowest in *Laminaria digitata*

(see Figure 2 of publication 7). At exposure depth with an Erythema-weighted UV-B dose (UV_{ery}) of 9.49 x 10^2 - 1.38 x 10^3 J m^{-2}, higher germination rates were observed in *S. dermatodea* compared to the other 2 species after 3 days of cultivation in the laboratory. Moreover, no significant effect of radiation (P = PA = PAB) was observed in the germination rate of *S. dermatodea*. At the same UV-B dose, but due to high variability in spore susceptibility released from different sporophytes, insignificant variation was observed between treatments in the germination rate of *A. esculenta*. In *L. digitata*, ≤ 2% germination was observed in spores exposed to the same UV dose. At the lower UV-B exposure dose of 5.06 x 10^2 - 6.98 x 10^2 J m^{-2} (UV_{ery}), significantly lower germination rates were measured in UVR-exposed *A. esculenta* and *L. digitata* zoospores compared to PAR alone. However, zoospore susceptibility to ambient solar radiation and to radiation depleted of UV-B was in all cases not significantly different. A 10-fold higher biological effective dose (BED) (see Figure 3 of publication 7) was needed to achieve 50% inhibition of germination in the investigated Laminariales zoospores (*S. dermatodea* = 1124 J m^{-2}, *A. esculenta* = 700 J m^{-2}, and *L. digitata* = 418 J m^{-2}) compared to the laboratory condition.

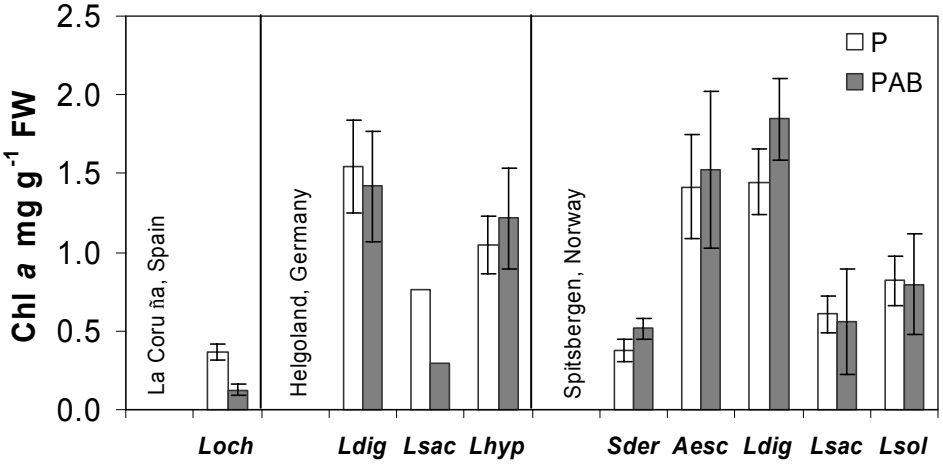

Fig. 14. Chlorophyll *a* contents in young *Laminaria* sporophytes exposed to different radiation (PAR = P; PAR + UV-A + UV-B = PAB). PFD is ± 18 µmol photons m^{-2} s^{-1}. Vertical bars are standard deviations (SD, n=3). Value for *L. saccharina* (Helgoland) represents one replicate only. Figure redrawn from Roleda *et al*. 2004a, 2005b, 2006a, 2006b.

5. SUMMARY OF DISCUSSION

5.1 Photosynthetic response to different spectral irradiance

5.1.1 Juvenile plants

Photosynthesis is a dynamic process which acclimates to variations in light intensity and spectral quality (reviewed by Senger & Bauer 1987; Falkowski & LaRoche 1991). Photosynthetic efficiencies of juvenile plants exposed to experimental irradiance of PAR alone were not photoinhibited while a decrease in photosynthetic efficiencies were observed in juvenile plants exposed to PAR supplemented with UV-A and UV-A + UV-B . Partial and complete acclimation to UVR was, however, observed in juvenile plants of *Laminaria ochroleuca* and in *C. crispus* and *M. stellatus* respectively. Acclimation of photosynthesis to UVR has been previously reported in sporophytes of Arctic Laminariales (Bischof *et al*. 1998a, 1999). Exposure to high irradiance showed that the higher susceptibility of the deep sea species *L. abyssalis* (Oliveira) to photoinhibition compared to the shallow water species *L. digitata* is due to its limited de-epoxidation capacity and reduced xanthophyll-cycle pool size (Rodrigues *et al*. 2002).

Among Gigartinales, the complete acclimation of photosynthesis in young gametophytes of *Mastocarpus* and *Chondrus* after 3 days of repeated UVR exposure contradicts the previous study of Bischof *et al*. (2000). They reported a reduction in photoinhibition but no acclimation throughout the 5 days repeated exposure to PAB. This difference may be attributed to the higher experimental UV irradiance used in their study, which was twice the intensity applied here. It might also be related to the low PAR (25 μmol photons m^{-2} s^{-1} ≈ 5.4 W m^{-2}) they applied. Insufficient PAR and therefore, unrealistically low PAR: UVB ratios could exaggerate the UV-B effects on plants (Caldwell *et al*. 1995; Rozema *et al*. 1997).

The primary targets for the UV-suppression of photosynthetic activity are still under debate (Baker *et al*. 1997). UV-B radiation appears to degrade the D1 protein and part of the D1/D2 heterodimer, the major structural complex within PSII (Aro *et al*. 1990; Melis *et al*. 1992; Jansen *et al*. 1993). Other studies have demonstrated decreases in the pool size of carbon fixation enzymes such as carbonic anhydrase (Dionisio *et al*. 1989) and ribulose-1, 5-

bisphosphate carboxylase/oxygenase (Rubisco) (Bischof *et al*. 2000). A mechanism that may be involved in UVR acclimation is the establishment of a physical barrier which shields the photosynthetic apparatus against damaging radiation (Karentz 1994). Although some algae are able to partially acclimate to chronic UVR exposure, growth reduction and tissue damage have been observed in UVR-exposed young *Laminaria ochroleuca* sporophytes (Roleda *et al*. 2004a).

5.1.2 Reproductive cells

Carpospores and zoospores are low light adapted because photosynthetic efficiencies decreased already at photon flux densities (PFD) of 56 and 22 µmol photons m^{-2} s^{-1} PAR, respectively. This can be attributed to the occurrence of photoinhibition even below the saturating light intensity (Hanelt *et al*. 1992; Osmond 1994). Ögren & Sjöström (1990) also reported that the rate of net photosynthesis can be depressed by photoinhibition over the whole natural range of the photosynthetic photon fluence rates. The primary site of photoinhibition has been suggested to be located in PSII reaction center. Key stages of the repair cycle of the D1 protein observed during photoinhibition are as follows: functional PSII ↔ inactivated PSII → non functional PSII → damage D1 protein removed → D1 protein resynthesized → recovery of functional PSII (Long *et al*. 1994).

Ultraviolet radiation contributes significantly to photoinhibition and no recovery was observed in photosynthetic efficiency of spores of the lower sublittoral species *L. hyperborea* exposed to high UV dose. Differences in P, PA and PAB sensitivity of maximum quantum yield are presumably caused by the degree of damage to PSII components versus the xanthophyll cycle mediated down regulation of PSII (Gevaert *et al*. 2003). Although a similar reduction in maximum quantum yield was also observed in PA and PAB treatments, recovery after prolonged exposure to PAB treatments was only minimal compared to PA, indicating that UV-B radiation damage the PSII function more than UV-A radiation. When the D1 protein of the PSII is impaired, the effect is only reversible over a longer time scale (hours) because synthesis of new D1 protein is required (Mattoo *et al*. 1984; Long *et al*. 1994; Hanelt *et al*. 2003). This usually occurs in species growing in the lower subtidal zone exposed to high irradiances (Hanelt 1998). Due to the activity of the xanthophyll cycle, seaweeds are able to recover rapidly (within minutes) after the offset of light stress (Long *et al*. 1994; Osmond

1994; Franklin & Forster 1997). Slow photosynthetic recovery can therefore reduce the accumulation of photosynthetic products disabling cellular division and delaying the initiation of germination in reproductive cells. Modeling studies on photoinhibition indicate a clear cost in terms of potential carbon acquisition, whereby stress-induced photoinhibition decreases the efficiency in the conversion of intercepted light into dry matter (Long *et al*. 1994).

If low light adaptation is a general feature of reproductive cells, light may exert a significant effect on survival of spores in the natural environment. The PAR waveband can, therefore, elicit a large impact on the survival of spores in the field because the UV-B radiation applied in our experiment would be accompanied by 10- to 20-fold higher PAR. Whether reproductive cells are capable of acclimating to higher PAR is unknown. In higher plants, physiological acclimation to high PAR can increase UVR tolerance. This is facilitated through photoinduction of screening compounds, increased activity of photorepair enzymes and other changes related to life under high irradiances (Warner & Caldwell 1983).

5.2 UVR effects on photosynthetic and accessory pigments

Chlorophyll *a* contents in both *Mastocarpus* and *Chondrus* were not negatively affected by UVR instead showed an increasing content when PAR was supplemented with UV-A and UV-A + UV-B. Among Laminariales, all but *L. ochroleuca* showed insignificant variation in Chl *a* contents in sporophytes exposed to P and PAB treatments. Pigment damage can result either (1) when protein-based pigments absorb UV energy directly and undergo photochemical degradation; (2) by photosensitiser action; or (3) oxygen radical production in addition to singlet oxygen (Vincent & Neale 2000). Under moderate fluence of UVR, however, Laminariales from Helgoland and Spitsbergen were able to synthesize new pigments in replacement of degraded chlorophylls after 6 hours daily UVR exposure and acclimate to repeated moderate daily fluence of UVR. Acclimation of Chl *a* concentration was also observed in the red macroalgae *Palmaria decipiens* (Reinsch) Ricker exposed to 12-16 h UVR (Poppe *et al*. 2002). However, *in situ* seasonal variation in pigment concentration of Arctic *L. saccharina* collected before, during and after ice break-up showed decreasing chlorophyll *a* concentration coinciding with increasing underwater radiation. Chlorophyll *a* concentration did not increase when melting snow contributed to water turbidity and reduction in underwater radiation (Aguilera *et al*. 2002) suggesting irreparable degradation of

pigments after exposure to high light intensity. Collection of field material during the 2004 field campaign in Spitsbergen showed eventual bleaching of young *L. saccharina* recruits at 4 m depth after prolonged exposure to ambient solar radiation when sea-ice breaks (S. Krembs, personal observation).

Most studies reported significant damage and decrease in pigment contents under UVR (i.e. Aguilera *et al*. 2002; Bischof *et al*. 2002a). A study on *Ulva rigida* C. Agardh, however, reported significantly higher pigment content (Chl *a*, Chl *b* and carotenoids) in the presence of UV-B (Altamirano *et al*. 2000). There was also an inverse relationship between growth and pigment content, which made them to speculate some kind of photoprotective mechanism in the algae, diverting energetic resources to pigment biosynthesis at the expense of growth. In *L. ochroleuca*, lower growth rates and lower pigment concentrations (Chl *a*, Chl c_1, fucoxanthin and β-carotene) in UV-B radiation exposed plant were observed. This implies that UV-B reduced the synthesis of, or degraded the pigments effectively limiting the light-harvesting ability. Consequently, reduction in the photosynthetic end products also imposes constraints on the repair of cellular damage and growth. With the meagre information on the long-term effects of UVR on pigment content and its relationship to photosynthetic efficiency and growth, more studies should focus on this mechanism which could be species specific.

Carotenoids are involved in several aspects of photosynthesis such as light absorption and energy transfer to the reaction center complex and protection of the photosynthetic apparatus from damage by strong illumination. In *Chondrus*, the higher total carotenoids measured under PAB could be related to its photoprotective role (Roy 2000). Accumulation of carotenoids specifically in response to UV radiation was already reported in cyanobacteria and chlorophytes (Buckley & Houghton 1976, Goes *et al*. 1994). This mechanism can protect the photosynthetic apparatus facilitating acclimation of photosynthesis and growth to UVR. Among Laminariales, the tissue absorption peaks in the Soret region (400- 540 nm) are characteristic of carotenoids in antenna complexes of higher plants (Pascal *et al*. 1999; Croce *et al*. 2000). Among the natural solar radiation-acclimated young wild sporophytes, the higher absorption in this region could imply some photoprotective role of carotenoids.

5.3 UV-B-induced DNA damage and its repair

In the investigated Laminariales sporophytes, the remaining tissue CPD content as a measure of the thallus sensitivity to UV-B-induced DNA damage and the activity of the repair mechanism is dependent on thallus thickness and its optical properties. Moreover, there is intra-specific difference in the sensitivity between Arctic and cold-temperate populations of *Laminaria digitata*. Arctic *L. digitata* was observed to be more sensitive to UV-B-induced DNA damage compared to the cold-temperate population. The inverse relationship between thallus thickness and remaining DNA damage was observed independently between the *Laminaria* species from Helgoland and Spitsbergen. There is no linear correlation between thallus thickness and DNA damage. In other Laminariales investigated, the thick but translucent *S. dermatodea* sustained more DNA damage compared to the thin but opaque *A. esculenta* and the darkly pigmented *Laminaria* species. The relationship between UV-sensitivity and leaf morphology was also observed in seagrasses wherein species with thicker leaves provided greater morphological protection for UV-sensitive organelles (Dawson & Dennison 1996).

Based on the remaining tissue DNA damage, sensitivity of the young sporophytes is not related to the depth distribution of the adult sporophytes. DNA damage repair entail energy loss at the expense of growth. After 18- 21 days of repeated 6 hours daily UVR exposure and 18 hours recovery, the lower sublittoral but thick species *L. hyperborea* and *L. solidungula* as well as the medium thick Helgoland *L. digitata* were found to have lower remaining DNA damage. This could be attributed either to its better repair mechanism, protection by its thallus thickness or tissue optical characteristics influencing UV absorption, but on the expense of energy which is not available for growth. Increasing thallus thickness minimizes UVR effect as a function of optical effect where outer cell layers shade inner cells and in terms of longer pathlength for UVR absorption (Franklin & Forster 1997). The combined protective mechanism due to thallus thickness and higher UV-absorbing compounds was observed to effect higher growth reduction in *L. hyperborea* and *L. solidungula*.

Conversely, the UV-sensitivity of the investigated Gigartinales carpospores and Laminariales zoospores were related to the vertical and depth distribution of the foliose gametophytes and adult sporophytes, respectively. Reproductive cells are more susceptible to

UVR compared to young thalli and to adult gametophytes and sporophytes. Differences in DNA damage between different life history stages were observed in both groups of macroalgae. DNA damage was observed in carpospores of *Mastocarpus* and *Chondrus* but not in their foliose gametophytic stages. This is not implausible because both species co-exist in the eulittoral, physiologically and genetically acclimated to the full solar radiation. The lower DNA damage and effective DNA damage repair mechanism in carpospores of *Mastocarpus* enhanced its recruitment success to the upper eulittoral zone. The number of CPDs observed in *Laminaria* zoospores exposed to 8 hours UVR are 18.6 ± 4, 30.8 ± 8 and 49.8 ± 6 CPD Mb^{-1} in *L. digitata*, *L. saccharina* and *L. hyperborea* respectively. These values are higher compared to their respective young sporophytes exposed for 18- 21 days of repeated 6 hours daily UVR at 2.5 ± 1, 11.7 ± 3 and 3.4 ± 2 CPD Mb^{-1} respectively.

Diploid carpospores were found to incur less genetic damage compared to the haploid zoospores. Haploid zoospores were, however, found to be more efficient in DNA damage repair. Long and Michod (1995) reported that haploid cells are efficient replicators, while diploid cells are resistant to damage. Mathematical models suggest that sexual organism can combine the advantage of both ploidy states: spending much of its life cycle in the haploid state, then fusing to become diploid. During the diploid state DNA damage can be repaired, since there are two copies of the gene in the cell and one copy is presumed to be undamaged (Long & Michod 1995). In the life history of Laminariales, haploid zoospores were more sensitive to DNA damage compared to diploid young sporophytes.

5.4 Absorption spectra and UV-absorbing compounds

The absorption spectra of young Laminariales sporophytes (see publications 4, 5, and 6) show an increasing absorbance from 300 nm to the shorter UV-C waveband comparable to that of isolated phlorotannins from *Fucus gardneri* Silva exhibiting a peak at 265 nm (Henry & van Alstyne 2004). It is yet uncertain which UV-screening compound is involved in the current study. Profiles of the absorption spectra indicate that the presumptive UV-screening substance provides no complete protection against UV-B radiation. The absorption shoulder >265 nm can, however, enhance UV-B tolerance of a plant. Furthermore, it is thought that absorption of tissue-bound phlorotannin might shift to different peaks compared to extracted compounds. Further studies are needed to address this question. Concentration of presumptive

phlorotannin compound in relation to the absorption intensity at the UV waveband showed no relation to the depth distribution of species. Phlorotannins are reported to have several ecological functions (see Schoenwaelder 2002). The synthesis and storage of this phenolic compound in the cultured and wild sporophytes is, therefore, not being primarily in response to ultraviolet radiation. It is known that the production of this compound (as herbivore deterrent, antibacterial agent or as UV screens) involves energy cost at the expense of growth. Its synthesis could be in response to specific environmental cues or stress factors and is allocated for different functions between different kelp species.

Secretion of phenolic compounds in reproductive cells of brown algae has been previously reported (Schoenwaelder & Clayton 1998a, 1998b, 2000). These compounds which strongly absorb in the UV-C region of the spectrum were invoked to play a role in chemical UV defence because their synthesis is inducible by UVR (Pavia *et al*. 1997; Schoenwaelder 2002). In Arctic Laminariales zoospores, an increase in number and size of phlorotannin-containing physodes was observed after UV exposure which contributed UVR protection against cellular damage and enhanced germination rate (Wiencke *et al*. 2004b). Exudation of this compound from macroalgal tissue into seawater can at low concentration reduce the impact of UV-B exposure to UV-sensitive kelp meiospores (Swanson & Druehl 2002). A recent study showed that exposure to PAR and UVR induces release of phlorotannin-containing physodes from the zoospores into the medium (Lüder, Wiencke & Roleda, unpublished data). Relative to its ecological function, the physiological trade-off of this mechanism at cellular level is yet to be elucidated.

5.5 Thallus morphology and optical characteristics

The optical property of thallus influences reflection, attenuation, scattering, absorption or transmittance of UV radiation (Caldwell *et al*. 1983). In leaf epidermis of terrestrial plants, the pronounced wavelength selectivity of absorption is often attributed to flavonoids and other related UV-absorbing compounds (Robberecht & Caldwell 1978), where phenolic compounds generally have substantial UV absorbance and very little absorption in the visible waveband (Caldwell *et al*. 1983). Optically dark pigmented wild Laminariales sporophytes showed strong absorbance in the UV waveband, characteristics of the UV-absorbing compound phlorotannin.

Among the different species studied, intra-specific morphological differences were observed between cultured and wild isolates. Moreover, morphological differences were also apparent between the Arctic and cold-temperate populations of *Laminaria digitata* and *L. saccharina*. This is attributed to the prevailing variable environmental factors in the field compared to the constant conditions in the laboratory. Light quantity can affect thallus elongation pattern while drag force as a function of water motion can affect blade width (Roberson & Coyer 2004). Thallus morphogenesis can therefore be controlled as much by the environment as by genotype.

Among the naturally solar-acclimated wild sporophytes, all but *Laminaria saccharina* and *Alaria esculenta* from Spitsbergen had stronger UV absorption compared to cultured sporophytes of the same species. *Laminaria saccharina* and *A. esculenta* were found to be thin and translucent compared the cultured morphotype. The thick but pale and translucent cultured *Saccorhiza dermatodea* also has minimal absorption in the UV waveband. In cultured *Alaria*, the development of dark pigmented thalli under 12- 16 h daylength of low white light (10- 20 µmol photons m^{-2} s^{-1}) and translucent thalli under 24 h daylength (Roleda *et al*. 2005b) is speculated to be some kind of physiological mechanism related to seasonal variation in daylength where induction and cessation in synthesis of UV-absorbing compounds respond to specific environmental cues (Roleda *et al*. 2005b). It is typical for the so called season anticipators (Kain 1989). In these species, seasonal development is finely tuned to the seasonal variation of daylength (Wiencke *et al*. 1990a, b; Lüder *et al*. 2001, 2002; Dummermuth & Wiencke 2003).

5.6 Growth response of juvenile plants to UVR

Exposure to UVR did not affect growth rates of the investigated foliose Gigartinales gametophytes. The growth rate of young Laminariales sporophytes was, however, significantly reduced under UVR. The presence of UV screening compounds such as mycosporine-like amino acids (MAAs) in *Mastocarpus* and *Chondrus* reduces the effective UVR that penetrates to UV-sensitive targets in the cell. Comparison across a latitudinal gradient showed that cold-temperate *Laminaria* species are less sensitive to UVR compared to their Arctic counterparts. However, the difference in growth rates was not significantly different.

In *Laminaria ochroleuca*, the effect of light supplemented with UV-A radiation on growth was not significantly different to PAR alone and to the whole light spectrum. However, long term exposure to UV-A and UV-B radiation resulted in tissue deformation and damage to *L. ochroleuca* (Roleda *et al*. 2004a). This characteristic tissue damage and morphological deformation were still undocumented and unreported in seaweeds exposed to UVR. This is probably because previous growth studies on young *Laminaria* sporophytes were too short to induce tissue injury (e.g. 2- 3 weeks, Dring *et al*. 1996; Aguilera *et al*. 1999). However, pronounced tissue necrosis and loss of parts of the thalli was reported in the Arctic *Laminaria solidungula* J. Agardh after 1 week of daily exposure to 18 h UVR (Michler *et al*. 2002). On the other hand, UVR-induced injuries on plant's tissue have been reported in terrestrial flora. This includes reduced leaf area, blistering and epidermal deformation, lesions, increased leaf thickness and photomorphogenesis (Cline & Salisbury 1966; Robberecht & Caldwell 1978; Tevini *et al*. 1981; Teramura 1983; Barnes *et al*. 1990). In this regard, long term growth measurements and observation on morphological integrity of the tissue present a more holistic indication of the negative impact of this stress factor.

A simple growth model: G = P - R - L, 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 lost or decay (L) (Carr *et al*. 1997). Under high light intensity and ultraviolet radiation, photoinhibition of photosynthesis already decreases potential carbon acquisition (P) into plant dry matter (G) (Long *et al*. 1994). Dark respiration (R) represents the energy used to synthesize new biomass (growth respiration) and that used to maintain metabolic activity (maintenance respiration). Exposure to UVR causes cellular, enzymatic and molecular damage which could further increase loss due to respiration (R) by diverting more photosynthate for repair and defense (i.e. production of secondary metabolites) thereby inhibiting growth. UVR sensitivity of growth, as an integrative parameter of all physiological processes, shows relations to the upper distribution limit of macroalgae. Tissue DNA damage is, however, dependent on thallus thickness, optics and absorption spectra characteristics of the pigments and UV-absorbing compounds. Energy demands for repair and protection may divert photosynthate at the expense of growth.

5.7 UVR effects on germination of spores

Low photon flux density (PFD) of PAR (10- 20 µmol photons m^{-2} s^{-1}) is optimal for germination in carpospores and zoospores. Germination capacity can also be constrained by the harmful effects of UVR. Higher BED_{50} is needed to inhibit germination rate in the eulittoral *Mastocarpus* and *Chondrus* compared to the sublittoral *Laminaria* species. Damage to microtubules causing inhibition of nuclear division in the zoospore nucleus of *Macrocystis pyrifera* (Huovinen *et al*. 2000) and cell division in *Fucus* spp. (Schoenwaelder *et al*. 2003) were responsible for mortality and failure of germination in spores exposed to UVR. To counteract the negative effects of UV-B radiation, ultraviolet sunscreens are used as photoprotective mechanisms in planktonic organisms (Garcia-Pichel 1994). Among 1- <10 µm size class cells, sunscreens accumulation can afford considerable benefits but only at the expense of relatively heavy energetic investment and with restricted efficiencies (Garcia-Pichel 1994). However, the protective potential of phlorotannin-containing physodes in kelp zoospores (3- 5 µm) has been described by Wiencke *et al*. (2004b). Traces of shinorine were observed in carpospores of *Mastocarpus* and *Chondrus* (Kräbs & Roleda, unpublished data). Whether carpospores produce sufficient MAAs to achieve the same degree of UV-protection to that of the foliose gametophytes remains to be studied. In this study, however, spores were observed to repair UV-B-induced DNA damage which enhanced spore viability and germination capacity of UVR treated samples.

The susceptibility of spores to UVR determines the upper depth distribution limit of macroalgae. This has been invoked from laboratory studies with Laminariales from Spitsbergen and from southern Spain (Wiencke *et al*. 2000, 2004b). UVR sensitivity of zoospore germination among *Laminaria* species around Helgoland was previously reported not significantly different between species (Dring *et al*. 1996). This is attributed to the 2 to 4 fold higher levels of UV-A and UV-B radiation that was supplemented to about 35-50 µmol photons m^{-2} s^{-1} of PAR (UV: PAR ratio = 1.525). Higher order of magnitude in UVR: PAR ratio has been reported to intensify the UV effect on plants (Caldwell *et al*. 1995; Rozema *et al*. 1997), which may magnify UVR effect on one species and at the same time obscure its effects between species. Comparison between experimental treatments showed that the 6 hours UVR applied by Dring *et al*. (1996) is equivalent to 3.54×10^5 J m^{-2}, a dose higher compared to the 16 hours PAR + UV-A + UV-B treatment (3.32×10^5 J m^{-2}) applied in this study. Consequently, the study of Dring and coworkers observed lower germination rate in *L.*

digitata (ca. 20%) and *L. hyperborea* (<10%) compared to our study (UVR: PAR ratio= 1.257) on the same species with germination rate of 27.6% and 20.1% respectively. Higher UVR dose (5.46 x 10^5 J m^{-2}, UVR: PAR ratio= 1.523) was also used by Wiencke *et al*. (2004b), which resulted in <1% germination in Arctic *L. digitata* and *L. saccharina*. However, it may be possible that the Arctic population of these two Laminariales is more susceptible to UVR compared to their cold temperate counterparts.

Considering the small size of zoospores (ca. 3.0- 5.0 μm), kinematic viscosity of water (Purcell 1977) contributes to their slow swimming speeds (Amsler *et al*. 1992) and confine these reproductive cells within the water column for a period of time. Field exposure of zoospores to ambient solar radiation for 24- 45 hours simulated the transitory planktonic phase of kelp zoospores exposed to environmental stress such as high photon fluence rate and UVR. Kelp zoospores have been observed to swim up to 72 hours (Reed *et al*. 1992) and have been identified from *in situ* plankton samples (Graham 1999). Spore swimming competency or positive buoyancy increases the likelihood of settlement. Settling spores in substrate at depths or under algal canopies experiences different low-light microenvironment suitable for germination and growth.

It was shown in this study that pre-exposure to low PAR in the laboratory and to different photon flux densities of high PAR in the field did not inhibit spore germination. This shows that spores can acclimate to a wide range of photon flux densities or that photosynthetic efficiency are able to recover after photoinhibition. Ecologically, the recovery process will be dependent on the settlement of spores to suitable low-light environment.

Exposure to ambient solar condition in the water column and post cultivation in the laboratory at low-light condition, simulating low-light field microenvironment upon settlement, did not affect germination of *S. dermatodea*. At approximately the same UV dose, germination of *Alaria esculenta* was susceptible to UVR while germination of *L. digitata* was totally terminated. At lower UV dose, germination of *L. digitata* was observed to be still sensitive to UVR. Persistent exposure to ambient solar radiation for extended period of time, however, might exhibit different effect on germination.

The high UVR: PAR ratio applied in the laboratory exhibit additional UV-B effect on photosynthesis and germination (Wiencke *et al.* 2000, 2004b; Roleda *et al.* 2005a). In the

field, a ten-fold increase in BED_{50} was observed under ambient solar irradiation compared to the laboratory irradiance. Higher ambient PAR in the field was observed to enhance UV tolerance of zoospores. Under high PAR, UVR tolerance may be enhanced by increasing activity of photorepair enzymes (Warner & Caldwell 1983). Moreover, there seems to be a UV-exposure threshold to effect negative impact on the germination of kelp zoospores. In all species, pre-exposure to lower dose of ambient solar radiation at 2 and 4 m depths did not affect the germination capacity of zoospores. At higher doses of ambient solar radiation at 1 and 2 m depths, no additional UV-B radiation effect on the viability of the zoospores. In all cases the viability was the same after exposure to the PA and PAB condition in the field. Damage to carbon fixation in the cells was found to be higher under UV-A waveband in marine diatoms (Grzymski *et al*. 2001). It is further indicated that localised loss on the acceptor side of the PSII reaction centres is induced by UV-A (Grzymski *et al*. 2001; Turcsányi & Vass 2002).

5.8 Concluding remarks and future perspectives

The present study has shown that young gametophytes of *Mastocarpus* and *Chondrus* are less susceptible to the detrimental effects of UVR compared to their reproductive cells. Carpospores of *Chondrus* were, however, more sensitive to UVR compared to carpospores of *Mastocarpus*. The protection strategies in *Mastocarpus* to counterbalance UV damage are responsible for its successful recruitment and colonization of the eulittoral area in Helgoland. In the sublittoral, the sensitivity of spores of the different Laminariales species studied is related to the upper depth distribution limit of the adult sporophytes in Helgoland and Spitsbergen. Among *Laminaria* species from Helgoland, efficient DNA damage repair and recovery of the PSII damage in *L. digitata* zoospores contributed to their germination success. Moreover, presence of UV-screening substance characteristics of phlorotannin, further contributed an effective protective mechanism in the zoospores of the upper sublittoral *L. digitata*. Juvenile Laminariales sporophytes were observed to possess several protective mechanisms to minimize UVR damage and, hence, are less sensitive, but at the expense of growth. Long term effects of UV exposure to macroalgae are better studied by measuring growth and reproductive success as integrative parameter of all cellular processes than photosynthetic performance.

The field spore germination experiments showed that exposure to ambient solar condition did not affect zoospores viability of the upper sublittoral species *S. dermatodea*. Settling spores under algal canopies or at depths experiences different low-light microenvironment suitable for photosynthetic recovery, germination and growth. Photosynthetic acclimation of reproductive cells to persistent high light intensity and time series of recovery of photosynthesis are recommended for future studies because under high PAR, UVR tolerance may be enhanced by increasing activity of photorepair enzymes (Warner & Caldwell 1983). To estimate the ecological impact of enhanced UVR, seasonal variation in solar radiation has to be related to the reproductive seasonality of kelps as well as the diel periodicity in zoospore release (Reed *et al*. 1988; Amsler & Neushul 1989). The distinct reproductive seasons of Helgolandic *Laminaria spp*. is remarkable compared to other populations of the same species. *L. digitata* plants from Nova Scotia are found to be fertile throughout the year (Chapman 1984). To ensure reproductive success, propagules production are synchronized with the onset of favorable environmental condition (e.g. light, photoperiod or temperature) (Lüning 1980; Santelices 1990; Kinlan *et al*. 2003). The summer reproductive season of the upper sublittoral *L. digitata* would suggest that sporogenic tissues as well as zoospores of this species could tolerate or possess effective protective mechanisms against high solar radiation. On the other hand, winter reproduction in the lower sublittoral *L. hyperborea* is thought to be a strategy to avoid reproductive failure due to the relative sensitivity of their zoospores to high PAR and UVR. Zoospores are capable of dispersal, settlement, attachment and development into new recruits across the area of the vertical tidal zones. In the field, young recruits of *Laminaria* in the eulittoral are exposed to ambient environmental stress condition. Recruitment success of individuals growing to adult sporophytes is therefore dependent on the sensitivity or tolerance of the young *Laminaria* species. Recruitment success of juvenile *Laminaria* may be, however, higher under the canopy of adult sporophytes. Susceptibility of spores indicates a relation to the observed zonation pattern of kelp community around Helgoland and in Spitsbergen.

Despite the artificial laboratory irradiance condition, it was observed that UVR affects growth and the response is related to the depth distribution in the field. Synergistic effects might exist between high PAR and UV; however, UV alone can also induce damage in the field. The irradiance of the UV wavebands used in these experiments is comparable with those occurring in nature, thus, the results are worthwhile for modelling UV-effects under natural conditions even if strong PAR is missing. This study presents a piece of the puzzle

which may lead to an explanation why zonation occurs in the observed pattern in nature. For future studies, an *in situ* growth measurement is recommended. Prototypes of field growth chambers with similar growth measuring technique are currently developed for future use. Synergistic effects of abiotic factors in the field will bring new insights on how growth mechanics operate in nature.

Results obtained from single-factor experiments impose strict limits in making ecological inferences. UVR effects on aquatic primary producers have been extensively studied in the last 20 years (see review, Franklin & Forster 1997; Franklin *et al*. 2003). The actual and potential impact of increasing temperatures has likewise received considerable amount of research (see review, Davison 1991). However, the interactive effects of multiple stressors have received little attention. Ecological effects of global environmental changes, particularly global warming and increase in UVR due to ozone depletion have recently raised considerable concern. Environmental variables such as temperature and UVR are changing simultaneously around the globe. These two factors have been found to induce interactive and independent effects on germination and growth in the early life stages of two multicellular marine algae (Hoffman *et al*. 2003). Moreover, net biological effects of UVR, which are a function of both the rate of UVR-induced damage and the rate at which that damage is repaired, is mediated by temperature and vice versa (Hoffman *et al*. 2003). For example, DNA repair rates in *Palmaria* have been shown to increase with increasing temperature (Pakker *et al*. 2000), while sensitivity of *Fucus* germlings to UV-B radiation is enhanced with increasing temperature (Altamirano *et al*. 2003).

The effect of ambient solar radiation and temperature during the transitory planktonic phase of kelp zoospores on germination capacity of different kelp species was effectively studied in the field on Spitsbergen. To investigate increasing UVR and temperature under scenario of global environmental change could, however, be achieved only using sunshine simulators (Tüg 1996; Hanelt *et al*. submitted) in which the radiation and temperature conditions can be manipulated according to the conditions forecasted. Future research using this laboratory incubation system to study the effect of spectrally enhanced artificial solar radiation on photosynthesis, DNA damage and repair, germination and growth of early life stages of macroalgae at various temperatures will considerably enhance scientific output. As the studied macroalgal species are important primary producers in inshore coastal ecosystems serving as food for herbivores and as habitats for many organisms, changes in their depth and

geographic distribution under a scenario of climate change due to stratospheric ozone depletion and global warming can entail significant ecological domino effects.

6. REFERENCES

Adamska I. (1997) ELIPs – light-induced stress proteins. *Physiologia Plantarum* **100**, 794-805.

Aguilera J., Karsten U., Lippert H., Vögele B., Philipp E., Hanelt D. & Wiencke C. (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology Progress Series* **191**, 109-119.

Aguilera J., Bischof K., Karsten U., Hanelt D. & Wiencke C. (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Marine Biology* **140**, 1087-1095.

Altamirano M., Flores-Moya A. & Figueroa F-L. (2000) Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated *in situ*. *Botanica Marina* **43**, 19-26.

Altamirano M., Flores-Moya A. & Figueroa F-L. (2003) Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophycea). *Aquatic Botany* **75**, 9-20.

Amsler C.D. & Neushul M. (1989) Diel periodicity of spore release from the kelp *Nereocystis luetkeana* (Mertens) Postels *et* Ruprecht. *Journal of Experimental Marine Biology and Ecology* **134**, 117-127.

Amsler C.D., Reed D.C. & Neushul M. (1992) The microclimate inhabited by macroalgal propagules. *British Phycological Journal* **27**, 253-270.

Arnold T.M. (2003) To grow and defend: lack of tradeoffs for brown algal phlorotannins. *Oikos* **100**, 406-408.

Aro E.M., Hundal T., Carlberg I. & Andersson B. (1990) In vitro studies on light-induced inhibition of photosystem II and D1-protein degradation at low temperatures. *Biochimica et Biophysica Acta* **1019**, 269-275.

Baker N.R., Nogues S. & Allen DJ (1997) Photosynthesis and photoinhibition. In *Plants and UVB: Responses to Environmental Change* (ed P. Lumsden), pp 95-111. Cambridge University Press, Cambridge, UK.

Bañares E., Altamirano M., Figueroa F-L. & Flores-Moya A. (2002) Influence of UV radiation on growth of sporelings of three non-geniculate coralline red algae from southern Iberian Peninsula. *Phycological Research* **50**, 23-30.

Barnes P.W., Flint S.D. & Caldwell MM (1990) Morphological responses of crop and weed species of different growth forms to ultraviolet-B radiation. *American Journal of Botany* **77**, 1354-1360.

Bartsch I. & Kuhlenkamp R. (2000) The marine macroalgae of Helgoland (North Sea): an annotated list of records between 1845 and 1999. *Helgoland Marine Research* **54**, 160-189.

Bartsch I. & Tittley I. (2004) The rocky intertidal biotopes of Helgoland: present and past. *Helgoland Marine Research* **58**, 298-302.

Benhissoune S., Boudouresque C.-F. & Verlaque M. (2002) A checklist of the seaweeds of the Mediterranean and Atlantic Coasts of Morocco. II. Phaeophyceae. *Botanica Marina* **45**, 217-230.

Bhattacharya D. (1985) The demography of fronds of *Chondrus crispus* Stackhouse. *Journal of Experimental Marine Biology and Ecology* **91**, 217-231.

Bird C.J., Sosa P.A. & MacKay R.M. (1994) Molecular evidence confirms the relationship of *Petrocelis* in the western Atlantic to *Mastocarpus stellatus* (Rhodophyta, Petrocelidaceae). *Phycologia* **33**, 134-137.

Bischof K., Hanelt D., Tüg H., Karsten U., Brouwer P.E.M. & Wiencke C. (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biology* **20**, 388-395.

Bischof K., Hanelt D. & Wiencke C. (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biology* **1**, 435-444.

Bischof K., Kräbs G., Hanelt D. & Wiencke C. (2000a) Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? *Helgoland Marine Research* **54**, 47-52.

Bischof K., Hanelt D. & Wiencke C. (2000b) UV-effects on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* **211**, 555-562.

Bischof K., Kräbs G., Wiencke C. & Hanelt D. (2002) Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* **215**, 502-509.

Bold H.C. (1973) Morphology of plants. 3rd edition. Harper & Row Publishers, New York.

Buckley C.E. & Houghton J.A. (1976) A study of the effects of near UV radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. *Archieves of Microbiology* **107**, 93-97.

Buma A.G.J., Hannen E.J. van, Roza L., Veldhuis M.J.W. & Gieskes W.W.C. (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *Journal of Phycology* **31**, 314-321.

Buma A.G.J., Oijen T. van, Poll W.H. van de, Veldhuis M.J.W. & Gieskes W.W.C. (2000) The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. *Journal of Phycology* **36**, 296-303.

Caldwell M.M. (1971) Solar ultraviolet radiation and the growth and development of higher plants. In *Photophysiology* (ed A.C. Giese), pp 131-177. Academic Press, New York.

Caldwell M.M., Robberecht R. & Flint S.D. (1983) Internal filters: prospects for UV-acclimation in higher plants. *Physiologia Plantarum* **58**, 445-450

Caldwell M.M., Teramura A.H., Tevini M., Bornman J.F., Björn L.O. & Kulandaivelu G. (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio* **24**, 166-173.

Carr G.M., Duthie H.C. & Taylor W.D. (1997) Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany* **59**, 195-215.

Carrington E., Grace S.P. & Chopin T. (2001) Life history phases and the biochemical properties of the red alga *Chondrus crispus* (Rhodophyta). *Journal of Phycology* **37**, 699-704.

Chapman A.R.O. (1984) Reproduction, recruitment and mortality in two species of *Laminaria* in southwest Nova Scotia. *Journal of Experimental Marine Biology and Ecology* **78**, 99-109.

Chen L.C-M. & McLachlan J. (1972) The life history of *Chondrus crispus* in culture. *Canadian Journal of Botany* **50**, 1055-1060.

Chen L.C-M., Edelstein T. & McLachlan J. (1974) The life history of *Gigartina stellata* (Stackh.) Batt. (Rhodophyceae, Gigartinales) in culture. *Phycologia* **13**, 287-294.

Chopin T. (1986) The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans: a review. *Canadian Technical Report of Fisheries and Aquatic Sciences* **1514**, 1-69.

Cline M.G. & Salisbury F.B. (1966) Effects of ultraviolet radiation on the leaves of higher plants. *Radiation Botany* **6**, 151-163.

Cordi B., Donkin M.E., Peloquin J., Price D.N. & Depledge M.H. (2001) The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis*. *Aquatic Toxicology* **56**, 1-11.

Croce R., Cinque G., Holzwarth A.R. & Bassi R. (2000) The Soret absorption properties of carotenoids and chlorophylls in antenna complexes of higher plants. *Photosynthesis Research* **64**, 221-231.

Crutzen P.J. (1992) Ultraviolet on the increase. *Nature* **356**, 104-105.

Dahlback A. (2002) Recent changes in surface ultraviolet solar radiation and stratospheric ozone at a high Arctic site. *UV radiation and Arctic Ecosystem* (ed D. Hessen), pp. 3-22. Springer Verlag, Berlin, Heidelberg.

Davison I.R. (1991) Environmental effects on algal photosynthesis: temperature. *Journal of Phycology* **27**, 2-8.

Davison I.R. & Pearson G.A. (1996) Stress tolerance in intertidal seaweeds. *Journal of Phycology* **32**, 197-211.

Dawson S.P. & Dennison W.C. (1996) Effects of ultraviolet and photosynthetically active radiation on five seagrass species. *Marine Biology* **125**, 629-638.

Dean T.A., Thies K. & Lagos S.L. (1989) Survival of juvenile giant kelp: the effects of demographic factors, competitors, and grazers. *Ecology* **70**, 483-495.

Demmig-Adams B. & Adams III W.W. (1992) Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology & Plant Molecular Biology* **43**, 599-626.

Dionisio M.L., Tsuzuki M. & Miyachi S. (1989) Blue light induction of carbonic anhydrase activity in *Chlamydomonas reinhardtii*. *Plant and Cell Physiology* **30**, 215-219.

Drew E.A. (1972) Growth of a kelp forest at 60 metres in the Straits of Messina. *Memorie di Biologia Marina e di Oceanografia* **2**, 135-157.

Drew E.A., Ireland J.F., Muir C., Robertson W.A.A. & Robinson J.D. (1982) Photosynthesis, respiration and other factors influencing the growth of *Laminaria ochroleuca* Pyl. below 50 metres in the Straits of Messina. *Marine Ecology- Pubblicazioni Della Stazione Zoologica di Napoli I*. **3**, 335-355.

Dring M.J., Makarov V., Schoschina E., Lorenz M. & Lüning K. (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Marine Biology* **126**, 183-191.

Dring M.J., Wagner A., Franklin L.A., Kuhlenkamp R. & Lüning K. (2001) Seasonal and diurnal variations in ultraviolet-B and ultraviolet-A irradiances at and below the sea

surface at Helgoland (North Sea) over a 6-year period. *Helgoland Marine Research* **55**, 3-11.

Dummermuth A.L. & Wiencke C. (2003) Experimental investigation of seasonal development in six Antarctic red macroalgae. *Antarctic Science* **15**, 449-457.

Dudgeon S.R. & Johnson A.S. (1992) Thick vs thin: thallus morphology and tissue mechanics influence differential drag and dislodgement of two co-dominant seaweeds. *Journal of Experimental Marine Biology and Ecology* **165**, 23-43.

Dunlap W.C. & Yamamoto Y. (1995) Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. *Comparative Biochemistry and Physiology* **112B**, 105-114.

El Naggar S., Gustat H., Magister H. & Rochlitzer R. (1995) An electronic personal UV-B-dosimeter. *Journal of Photochemistry and Photobiology B: Biology* **31**, 83-86.

Falkowski P.G. & LaRoche J. (1991) Acclimation to spectral irradiance in algae. *Journal of Phycology* **27**, 8-14.

Farman J.C., Gardiner B.G. & Shanklin J.D. (1985) Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. *Nature* **314**, 207-210.

Flores-Moya A., Posudin Y.I., Fernández J.A., Figueroa F-L. & Kawai H. (2002) Photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose. *Journal of Photochemistry and Photobiology B: Biology* **66**, 134-140.

Franklin L.A. & Forster R.M. (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology* **32**, 207-232.

Franklin L.A., Osmond C.B. & Larkum A.W.D. (2003) Photoinhibition, UV-B and algal photosynthesis. In *Photosynthesis in Algae* (eds A.W. Larkum, S.E. Douglas & J.A. Raven JA), pp 351-384. Kluwer Academic Publishers, Netherlands.

Garbary D.J. & DeWreede R.E. (1988) Life history phases in natural populations of Gigartinaceae (Rhodophyta): quantification using resorcinol. In *Experimental Phycology. A laboratory manual* (eds D.S. Lobban, D.J. Chapman & B.P. Kremer), pp 174-178. Cambridge University Press, Cambridge.

Garcia-Pichel F. (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnology and Oceanography* **39**, 1704-1717.

Gevaert F., Creach A., Davoult D., Migne A., Levavasseur G., Arzel P., Holl A-C. & Lemoine Y. (2003) *Laminaria saccharina* photosynthesis measured in situ: photoinhibition and xanthophylls cycle during a tidal cycle. *Marine Ecology Progress Series* **247**, 43-50.

Goes J.I., Handa N., Taguchi S. & Hama T. (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter *Tetraselmis* sp.: relationship to cellular pigments. *Marine Ecology Progress Series* **114**, 259-274.

Graham M.H. (1997) Factors determining the upper limit of giant kelp, *Macrocystis pyrifera* Agardh, along the Monterey Peninsula, central California, USA. *Journal of Experimental Marine Biology and Ecology* **218**, 127-149.

Graham M.H. (1999) Identification of kelp zoospores from in situ plankton samples. *Marine Biology* **135**, 709-720.

Guiry M.D. & West J.A. (1983) Life history and hybridization studies on *Gigartina stellata* and *Petrocelis cruenta* (Rhodophyta) in the North Atlantic. *Journal of Phycology* **19**, 474-494.

Guiry M.D., West J.A., Kim D-H. & Masuda M. (1984) Reinstatement of the genus *Mastocarpus* Kützing (Rhodophyta). *Taxon* **33**, 53-63.

Grzymski J., Orrico C. & Schofield O.M. (2001) Monochromatic ultraviolet light induced damage to Photosystem II efficiency and carbon fixation in the marine diatom *Thalassiosira pseudonana* (3H). *Photosynthesis Research* **68**, 181-192.

Hall D.O. & Rao K.K. (1994) *Photosynthesis*. Cambridge University press, Cambridge

Han T., Han Y-S., Kim K-Y., Kim J-H., Shin H-W., Kain J.M., Callow J.A. & Callow M.E. (2003) Influences of light and UV-B on growth and sporulation of the green alga *Ulva pertusa* Kjellman. *Journal of Experimental Marine Biology and Ecology* **290**, 115-131.

Hanelt D. (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology* **131,** 361-369.

Hanelt D., Huppertz K. & Nultsch W. (1992) Photoinhibition of photosynthesis and its recovery in red algae. *Botanica Acta* **105**, 278-284.

Hanelt D, Wiencke C, Nultsch W (1997a) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. *Journal of Photochemistry and Photobiology B: Biology* **38**, 40-47.

Hanelt D., Melchersmann B., Wiencke C., Nultsch W. (1997b) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Marine Ecology Progress Series* **149**, 255-266.

Hanelt D., Wiencke C. & Bischof K. (2003) Photosynthesis in marine macrolage. In *Photosynthesis in Algae* (eds A.W. Larkum, S.E. Douglas & J.A. Raven JA), pp. 413-435. Kluwer Academic Publishers, Netherlands.

Hanelt D., Hawes I. & Rae R. (submitted) Reduction of UV-B radiation causes an enhancement of photoinhibition in high light stressed aquatic plants from New Zealand lakes. *Journal of Photochemistry and Photobiology B: Biology*.

Henry B.E. & van Alstyne K.L. (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *Journal of Phycology* **40**, 527-533.

Herman J.R., Bhartia P.K., Ziemke J., Ahmad Z. & Larko D. (1996) UV-B increases (1979-1992) from decreases in total ozone. *Geophysical Research Letters* **23**, 2117-2120.

Hoffman J.R., Hansen L.J. & Klinger T. (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *Journal of Phycology* **39**, 268-272.

Hoffman A.J. & Ugarte R. (1985) The arrival of propagules of marine macroalgae in the intertidal zone. *Journal of Experimental Marine Biology and Ecology* **92**, 83-95.

Hop H., Pearson T., Hegseth E.N., Kovacs K.M., Wiencke C., Kwasniewski S., Eiane K., Mehlum F., Gulliksen B., Wlodarska-Kowalczuk M., Lydersen C., Weslawski J.M., Cochrane S., Gabrielsen G.W., Leakey R., Lønne O.J., Zajaczkowski M., Falk-Petersen S., Kendall M., Wängberg S.Å., Bischof K., Voronkov A.Y., Kovaltchouk N.A., Wiktor J., Poltermann M., di Prisco G., Papucci C. & Gerland S. (2002) The ecosystem of Kongsfjorden, Svalbard. *Polar Research* **21**, 167-208.

http://www.mbari.org/staff/conn/botany/reds/mastocar/lifehist.htm

Huovinen P.S., Oikari A.O.J., Soimasuo M.R. & Cherr G.N. (2000) Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. *Photochemistry and Photobiology* **72**, 308-313.

Izquierdo J.L., Pérez-Ruzafa I.M. & Gallardo T. (2002) Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. *Helgoland Marine Research* **55**, 285-292.

Jansen M.A.K., Gaba V., Greenberg B., Mattoo K.A. & Edelman M. (1993) UV-B driven degradation of the D1 reaction-center protein of Photosystem II proceeds via plastosemiquinone. In *Photosynthetic Responses to the Environment* (eds H.Y. Yamamoto & C.M. Smith), pp 142-149. American Society of Plant Physiologist, Rockville, Maryland.

Jerlov N.G. (1976) *Marine Optics* (2nd edition). Elsevier Scientific Publ. Co., Amsterdam, N.Y. & London.

Johansson G. & Snoeijs P. (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. *Marine Ecology Progress Series* **244**, 63-72.

John D.M. (1971) The distribution and net productivity of sublittoral populations of attached macrophytic algae in an estuary on the Atlantic coast of Spain. *Marine Biology* **11**, 90-97.

Kain J.M. (1989) The seasons in the subtidal. *British Phycological Journal* **24**, 203-215.

Karentz D. (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In *Ultraviolet radiation in Antarctica: Measurements and Biological effects*. (eds C.S. Weiler & P.A. Penhale), pp.93-110. American Geophysical Union, Washington DC. [Antarctic Research Series no. 62].

Kinlan B.P., Graham M.H., Sala E. & Dayton P.K. (2003) Arrested development of giant kelp (*Macrocystis pyrifera*, Phaeophyceae) embryonic sporophytes: a mechanism for delayed recruitment in perennial kelps? *Journal of Phycology* **39**, 47-57.

Kirk J.T.O. (1994) *Light and photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge.

Kornmann P. & Sahling P-H. (1994) Meeresalgen von Helgoland: zweite Ergänzung. *Helgoländer Merresuntersungen* **48**, 365-406.

Landry L.G., Chapple C.C.S. & Last R.L. (1995) *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. *Plant Physiology* **109**, 1159-1166.

Lao K. & Glazer A.N. (1996) Ultraviolet-B photodestruction of a light-harvesting complex. *Proceedings of the National Academy of Sciences, USA* **93**, 5258-5263.

Lazo M.L., Greenwell M. & McLachlan J. (1989) Population structure of *Chondrus crispus* Stackhouse (Gigartinaceae, Rhodophyta) along the coast of Prince Edward Island, Canada: distribution of gametophytic and sporophytic fronds. *Journal of Experimental Marine Biology and Ecology* **126**, 45-58.

Lindgren A. & Åberg P. (1996) Proportion of life cycle stages of *Chondrus crispus* and its population structure: a comparison between a marine and estuarine environment. *Botanica Marina* **39**, 263-268.

Littler M.M. & Littler D.S. (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *American Naturalist* **116**, 25-44.

Lobban C.S. & Harrison P.J. (1994) *Seaweed ecology and physiology*. Cambridge University Press, Cambridge.

Long A. & Michod R.E. (1995) Origin of sex for error repair I. sex, diploidy, and haploidy. *Theoretical Population Biology* **47**, 18-55.

Long S.P., Humphries S. & Falkowski P.G. (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**, 633-662.

Lotze H.K. & Worm B. (2002) Complex interaction of climatic and ecological controls on macroalgal recruitment. *Limnology and Oceanography* **47**, 1734-1741.

Lubchenco J. (1980) Algal zonation in the New England rocky intertidal community: an experimental analysis. *Ecology* **61**, 333-344.

Lubchenco J. & Menge B.A. (1978) Community development and persistence in a low rocky intertidal zone. *Ecological Monograph* **59**, 67-94.

Lüder U.H., Knoetzel J. & Wiencke C. (2001) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. *Polar Biology* **24**, 598-603.

Lüder U.H., Wiencke C. & Knoetzel J. (2002) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta): a study to simulate Antarctic winter sea ice cover. *Journal of Phycology* **38**, 904-913.

Lüder U.H. & Clayton M.N. (2004) Induction of phlorotannins in the brown algae *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory- the first microscopic study. *Planta* **218**, 928-937.

Lüning K. (1970) Tauchuntersuchungen zur Vertikalverbreitung der sublitoralen Helgoländer Algenvegetation. *Helgoländer Wissenschaftlicher Meeresuntersuchungen* **21**, 271-291.

Lüning K. (1979) Growth strategies of three Laminaria species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Marine Ecology Progress Series* **1**, 195-207.

Lüning K. (1980) Control of algal life-history by daylength and temperature. In *The Shore Environment, Vol. 2: Ecosystems* (eds J.H. Price, D.E.G. Irvine & W.F. Farnham), pp. 915-945. Academic Press, New York.

Lüning K. (1985) Meeresbotanik: Verbreitung, Ökophysiologie und Nutzung der marine Makroalgen. Georg Thieme Verlag, Stuttgart.

Maggs C.A. (1988) Intraspecific life history variability in the Florideophycidae (Rhodophyta). *Botanica Marina* **31**, 465-490.

Makarov M.V. & Voskoboinikov G.M. (2001) The influence of Ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. *Botanica Marina* **44**, 89-94.

Mann K.H. (1973) Seaweeds: their productivity and strategy for growth. *Science* **182**, 975-981.

Markager S. & Sand-Jensen K. (1992) Light requirements and depth zonation of marine macroalgae. *Marine Ecology Progress Series* **88**, 83-92.

Masuda M. & Hashimoto Y. (1993) Autecological studies on *Chondrus nipponicus* Yendo (Gigartinales, Rhodophyta). *Japanese Journal of Phycology* **41**, 99-111.

Mattoo A.K., Hoffman-Falk H., Marder J.B. & Edelman M. (1984) Regulation of protein metabolism: Coupling of photosynthetic electron transport in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. *Proceedings of the National Academy of Sciences USA* **81**, 1380-1384.

Melis A., Nemson J.A. & Harrison M.A. (1992) Damage to functional components and partial degradation of Photosystem II reaction center proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochimica et Biophysica Acta* **1100**, 312-320.

Michler T., Aguilera J., Hanelt D., Bischof K. & Wiencke C. (2002) Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. *Marine Biology* **140**, 1117-1127.

Müller D.G. (1989) The role of pheromones in sexual reproduction of brown algae. In *Algae as Experimental Systems* (eds A.W. Coleman, L.J. Goff & J.R. Stein-Taylor), pp 201-213. Alan R. Liss., New York.

Müller R., Crutzen P.J., Grooß J.-U., Brühl C., Russel J.M. III, Gernandt H., McKenna D.S. & Tuck A.F. (1997) Severe ozone loss in the Arctic during the winter of 1995-96. *Nature* **389**, 709-712.

Ohad I., Kyle D.J. & Arntzen C.J. (1984) Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *Journal of Cell Biology* **99**, 481-485.

Ögren E. & Sjöström M. (1990) Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. *Planta* **181**, 560-567.

Osmond C.B. (1994) What is photoinhibition? Some insights from comparisons of shade and sun plant. In *Photoinhibition of photosynthesis, from the molecular mechanisms to the field* (eds N.R. Baker & J.R. Bowyer), pp 1-24. BIOS Scientific Publ., Oxford.

Pakker H., Martins R.S.T., Boelen P., Buma A.G.J., Nikaido O. & Breeman A.M. (2000) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). *Journal of Phycology* **36**, 334-341.

Pascal A., Gradinaru C., Wacker U., Peterman E., Calkoen F., Irrgang K-D., Horton P., Renger G., van Grondelle R., Robert B. & van Amerongen H. (1999) Spectroscopic characterization of the spinach Lhcb4 protein (CP29), a minor light-harvesting complex of photosystem II. *European Journal of Biochemistry* **262**, 817-823.

Pavia H., Cervin G., Lindgren A. & Åberg P. (1997) Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Marine Ecology Progress Series* **157**, 139-146.

Pavia H., Toth G. & Åberg P. (1999) Trade-offs between phlorotannin production and annual growth in natural populations of the brown seaweed *Ascophyllum nodosum*. *Journal of Ecology* **87**, 761-771.

Pearce F. (1996) Big freeze digs a deeper hole in ozone layer. *New Scientist* 147: 7.

van de Poll W.H., Eggert A., Buma A.G.J. & Breeman A.M. (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. *Journal of Phycology* **37**, 30-37.

van de Poll W.H., Hanelt D., Hoyer K., Buma A.G.J. & Breeman A.M. (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochemistry and Photobiology* **76**, 493-501.

Poppe F., Hanelt D. & Wiencke C. (2002) Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic red alga *Palmaria decipiens* during acclimation to UV radiation. *Botanica Marina* **45**, 253-261.

Purcell E.M. (1977) Life at low Reynolds number. *American Journal of Physics* **45**, 3-11.

Reed D.C. (1990) The effects of variable settlement and early competition on patterns of kelp recruitment. *Ecology* **71**, 776-787.

Reed D.C., Laur D.R. & Ebeling A.W. (1988) Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs* **58**, 321-335.

Reed D.C., Amsler C.D. & Ebeling A.W. (1992) Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* **73**, 1577-1585.

Rijstenbil J.W, Coelho S.M. & Eijsackers M. (2000) A method for the assessment of light-induced oxidative stress in embryos of fucoid algae via confocal laserscan microscopy. *Marine Biology* **137**, 763-774.

Ribera M.A., Gómez-Garreta A., Gallardo T., Cormaci M., Furnari G. & Giaccone, G. (1992) Check-list of Mediterranean Seaweeds. I. Fucophyceae (Warming 1884). *Botanica Marina* **35**, 109-130.

Roberson L.M. & Coyer J.A. (2004) Variation in blade morphology of the kelp *Eisenia arborea*: incipient speciation due to local water motion? *Marine Ecology Progress Series* **282**, 115-128.

Robberecht R. & Caldwell M.M. (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. *Oecologia (Berl.)* **32**, 277-287.

Rodrigues M.A., dos Santos C.P., Young A.J., Strbac D. & Hall D.O. (2002) A smaller and impaired xanthophyll cycle makes the deep sea macroalgae *Laminaria abyssalis* (Phaeophyceae) highly sensitive to daylight when compared with shallow water *Laminaria digitata*. *Journal of Phycology* **38**, 939-947.

Roleda M.Y., Hanelt D., Kräbs G. & Wiencke C. (2004a) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. *Phycologia* **43**, 603-613.

Roleda M.Y., van de Poll W.H., Hanelt D. & Wiencke C. (2004b) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. *Marine Ecology Progress Series* **281**, 37-50.

Roleda M.Y., Wiencke C., Hanelt D., van de Poll W.H. & Gruber A. (2005a) Sensitivity of Laminariales zoospores from Helgoland to ultraviolet and photosynthetically active radiation: implication for depth distribution and seasonal reproduction. *Plant, Cell and Environment* **28**, 466-479.

Roleda M.Y., Hanelt D. & Wiencke C. (2005b) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. Polar Biology **28**, 539-549.

Roleda M.Y., Wiencke C. & Hanelt D. (2006a) Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic *Laminaria* sporophytes. *Planta* **223**, 407-417.

Roleda M.Y., Hanelt D. & Wiencke C. (2006b) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). *Marine Biology* **148**, 1201-1211.

Roy, S. (2000) Strategies for the minimisation of UV-induced damage. In *The effects of UV radiation in the marine environment* (eds S. de Mora, S. Demers & M. Vernet), pp 177-205. Cambridge University Press, U.K.

Rozema J., van de Staaij J., Björn L.O. & Caldwell M. (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology and Evolution* **12**, 22-28.

Rueness I. (1978) A note on development and reproduction in *Gigartina stellata* (Rhodophyceae, Gigartinales) from Norway. *British Phycological Journal* **13**, 87-90.

Santelices B. (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology Annual Review* **28**, 177-276.

Schoenwaelder M.E.A. (2002) The occurrence and cellular significance of physodes in brown algae. *Phycologia* **41**, 125-139.

Schoenwaelder M.E.A. & Clayton M.N. (1998a) Secretion of phenolic substances into the zygote wall and cell plate in embryos of *Hormosira* and *Acrocarpia* (Fucales, Phaeophyceae). *Journal of Phycology* **34**, 969-980.

Schoenwaelder M.E.A. & Clayton M.N. (1998b) The secretion of phenolic compounds following fertilization in *Acrocarpia paniculata* (Fucales, Phaeophyta). *Phycologia* **37**, 40-46.

Schoenwaelder M.E.A. & Clayton M.N. (2000) Physode formation in embryos of *Phyllospora comosa* and *Hormosira banksii* (Phaeophyceae). *Phycologia* **39**, 1-9.

Schoenwaelder M.E.A., Wiencke C., Clayton M.N. & Glombitza K.W. (2003) The effect of elevated UV radiation on *Fucus* spp. (Fucales, Phaeophyta) zygote and embryo development. *Plant Biology* **5**, 366-377.

Senger H. & Bauer B. (1987) The influence of light quality on adaptation and function of the photosynthetic apparatus. *Photochemistry and Photobiology* **45**, 939-946.

Setlow R.B. (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proceedings of the National Academy of Sciences USA* **71**, 3363-3366.

Sheppard C.R.C., Jupp B.P., Sheppard A.L.S. & Bellamy D.J. (1978) Studies on the growth of *Laminaria hyperborea* (Gunn.) Fosl. and *Laminaria ochroleuca* De La Pylaie on the French Channel Coast. *Botanica Marina* **21**, 109-116.

Shibata T., Kawaguchi S., Hama Y., Inagaki M., Yamaguchi K. & Nakamura T. (2004) Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology* **16**, 291-296.

Smith R.C., Prézelin B.B., Baker K.S., Bidigare R.R., Boucher N.P., Coley T., Karentz D., MacIntyre S., Matlick H.A., Menzies D., Ondrusek M., Wan Z. & Waters K.J. (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* **255**, 952-959.

Solomon S. (1990) Progress towards a quantitative understanding of Antarctic ozone depletion. *Nature* 347:347-354.

Solomon S. (1999) Stratospheric ozone depletion: a review of concepts and history. *Reviews of Geophysics* **37**, 275-316.

Staehelin J., Harris N.R.P., Appenzeller C. & Eberhard J. (2001) Ozone trends: a review. *Reviews of Geophysics* **39**, 231-290.

Stapleton A.E. & Walbot V. (1994) Flavonoids protect maize DNA from the induction of ultraviolet radiation damage. *Plant Physiology* **105**, 881-889.

Starr R.C. & Zeikus J.A. (1993) UTEX. – the culture collection of algae at the University of Texas at Austin. *Journal of Phycology* **29 (Suppl.)**, 1-106.

Steinberg P.D. (1995) Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. *Oecologia* **102**, 169-173.

Suzuki L. & Johnson C.H. (2001) Algae know the time of day: circadian and photoperiodic programs. *Journal of Phycology* **37,** 933-942.

Swanson A.K. & Druehl L.D. (2000) Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. *Marine Biology* **136**, 657-664.

Swanson A.K. & Druehl L.D. (2002) Induction, exudation and the UV protective role of kelp phlorotannins. *Aquatic Botany* **73**, 241-253.

Teramura A.H. (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. *Physiologia Plantarum* **58**, 415-427.

Tevini M., Iwanzik W. & Thoma U. (1981) Some effects of enhanced UV-B on the growth and composition of plants. *Planta* **153**, 388-394.

Tüg H. (1996) Sunshine simulation. Annual Report of Alfred Wegener Institute for Polar and Marine Research 1994/1995, 43-46.

Turcsányi E. & Vass I. (2002) Effect of UV-A radiation on photosynthetic electron transport. *Biologica Szegediensis* **46**, 171-173.

Vass I. (1997) Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. In *Handbook of Photosynthesis* (ed M. Pessarakli), pp 931-949. Marcel Dekker Inc. New York.

Vincent W.F. & Neale P.J. (2000) Mechanisms of UV damage to aquatic organisms. In *The effects of UV radiation in the marine environment* (eds S. de Mora, S. Demers & M. Vernet), pp 149-176. Cambridge University Press, U.K.

Vink A.A., Bergen-Henegouwen J.B., Nikaido O., Baan R.P. & Roza L. (1994) Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. *Photochemistry and Photobiology* **24**, 25-31.

de Vooys C.G.N. (1979) Primary production in aquatic environments. In *The Global Carbon Cycle* (eds B. Bolin, E.T. Degen, S. Kempe & P. Ketner), pp 1-21. International Council of Scientific Unions, Paris, France.

Wängberg S.A., Selmer J.S., Ekelund N.G.A. & Gustavson K. (1996) UV-B effects on nordic marine ecosystem. Tema Nord 1996, Nordic Council of Ministers, Copenhagen.

Warner C.W. & Caldwell M.M. (1983) Influence of photon flux density in the 400-700 nm waveband on inhibition of photosynthesis by UV-B (280-320 nm) irradiation in soybean leaves: separation of indirect and direct effects. *Photochemistry and Photobiology* **38**, 341-346.

West J.A., Polanshek A.R. & Guiry M.D. (1977) The life history in culture of *Petrocelis cruenta* J. Agardh (Rhodophyta) from Ireland. *British Phycological Journal* **12**, 45-53.

Wiencke C. (1990a) Seasonality of brown macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. *Polar Biology* **10**, 589-600.

Wiencke C. (1990b) Seasonality of red and green macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. *Polar Biology* **10**, 601-607.

Wiencke C., Gómez I., Pakker H., Flores-Moya A., Altamirano M., Hanelt D., Bischof K. & Figueroa F-L. (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Marine Ecology Progress Series* **197**, 217-229.

Wiencke C., Vögele B., Kovaltchouk N.A. & Hop H. (2004a) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. *Berichte zur Polar- und Meeresforschung* **492**, 55-62.

Wiencke C., Clayton M.N. & Schoenwaelder M. (2004b) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. *Marine Biology* **145**, 31-39.

Wiencke C., Roleda M.Y., Gruber A., Clayton M.N. & Bischof K. (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments. *Journal of Ecology* **94**, 455-463.

WMO Report No. 44 (1998) Scientific assessment of ozone depletion: 1998 www.WMO.ch/indexflash.html (updated 2002).

Worm B. & Chapman A.R.O. (1996) Interference competition among two intertidal seaweeds: *Chondrus crispus* strongly affects survival of *Fucus evanescens* recruits. *Marine Ecology Progress Series* **145**, 297-301.

Worm B. & Chapman A.R.O. (1998) Relative effects of elevated grazing pressure and competition from a red algal turf on two post-settlement stages of *Fucus evanescens* C. Ag. *Journal of Experimental Marine Biology and Ecology* **220**, 247-268.

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