

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)

Received: 11 May 2005 / Accepted: 22 September 2005 / Published online: 24 November 2005
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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 $10 \pm 2^\circ\text{C}$ 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.

Communicated by O. Kinne, Oldendorf/Luhe

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 $12 \pm 2^\circ\text{C}$ and $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 mm², ± 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:mm²) 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 μ l 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 μ m, 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 μ g 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:mm²) 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 $\pm 18 \mu\text{mol m}^{-2} \text{s}^{-1}$. 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 \pm 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

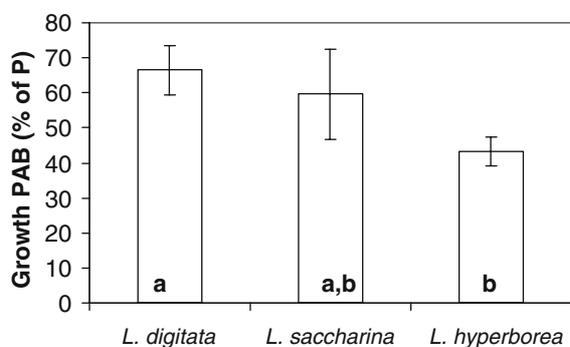
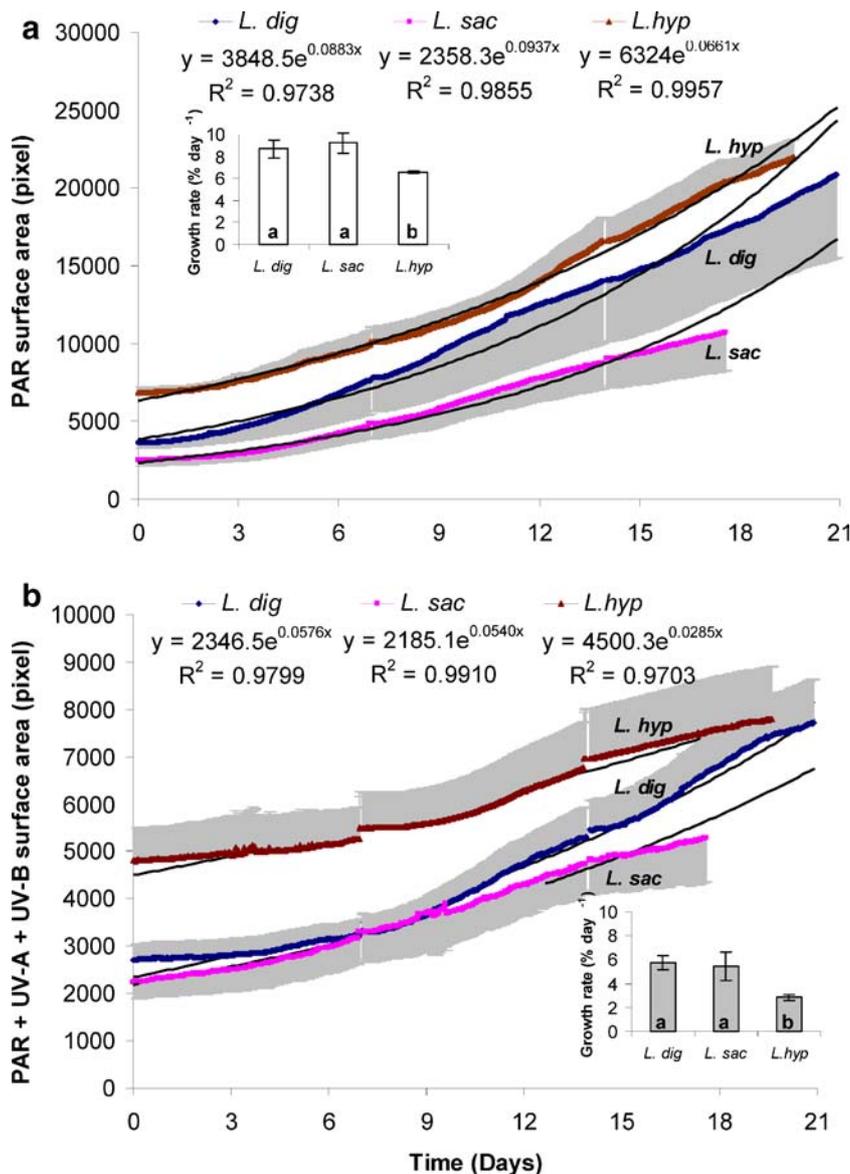
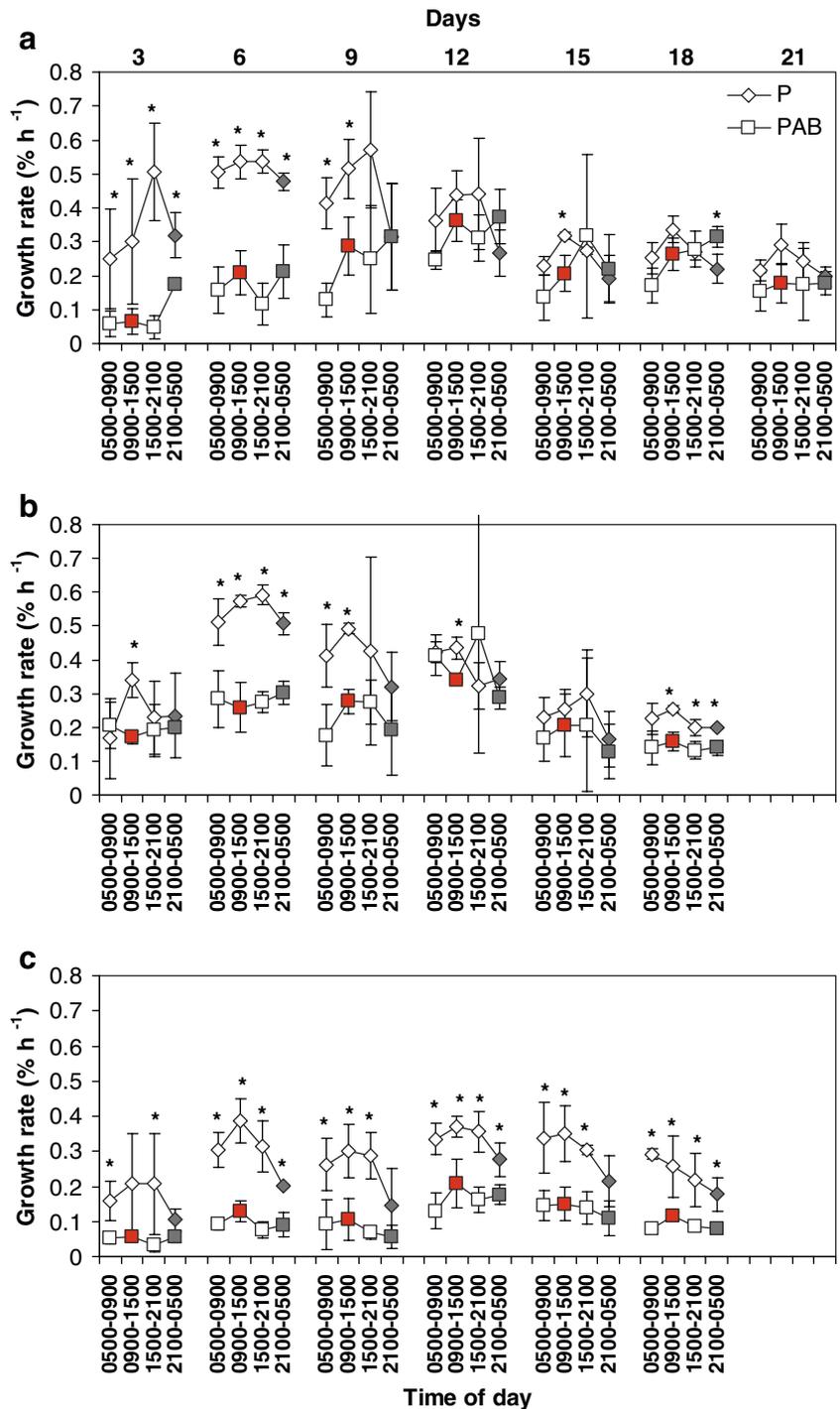


Fig. 2 Ultraviolet radiation effect on the growth rates of young *Laminaria* sporophytes. Growth rates of sporophytes exposed to PAR + UVA + UVB (PAB) were expressed as percent of the growth rates of sporophytes exposed to PAR alone (percent P). ANOVA showed significant difference between species ($P < 0.05$). 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 \pm 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: $G = P - R - L$,

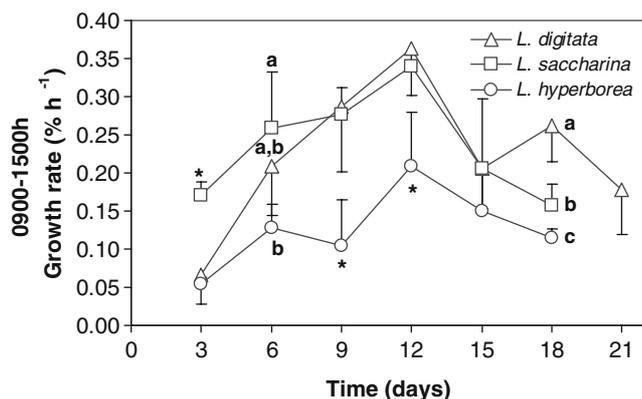


Fig. 4 Average growth rates over a period of 21 days in young sporophytes of the three *Laminaria* species ($n=3$) during UVR exposure (0900–1500 h). ANOVA showed significant difference between species ($P < 0.05$). Letters on graph show result of DMRT ($P=0.05$); different letters refer to significant 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

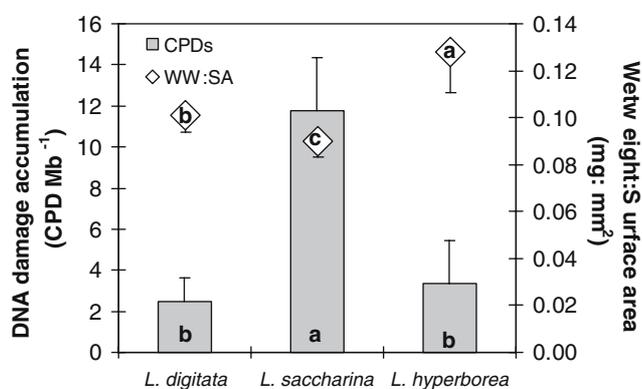


Fig. 5 Relationship between average thallus thickness and remaining tissue DNA damage after 18–21 days of repeated daily 6 h UVR exposure and 18 h recovery (6:8:4 h light:dark:light cycle) in the three *Laminaria* species. Vertical bars are standard deviations ($n=3$). ANOVA showed significant difference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

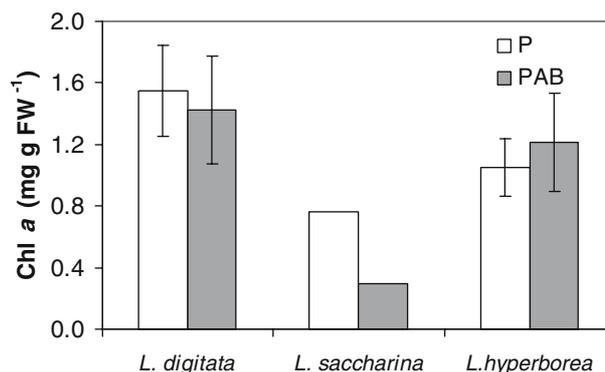
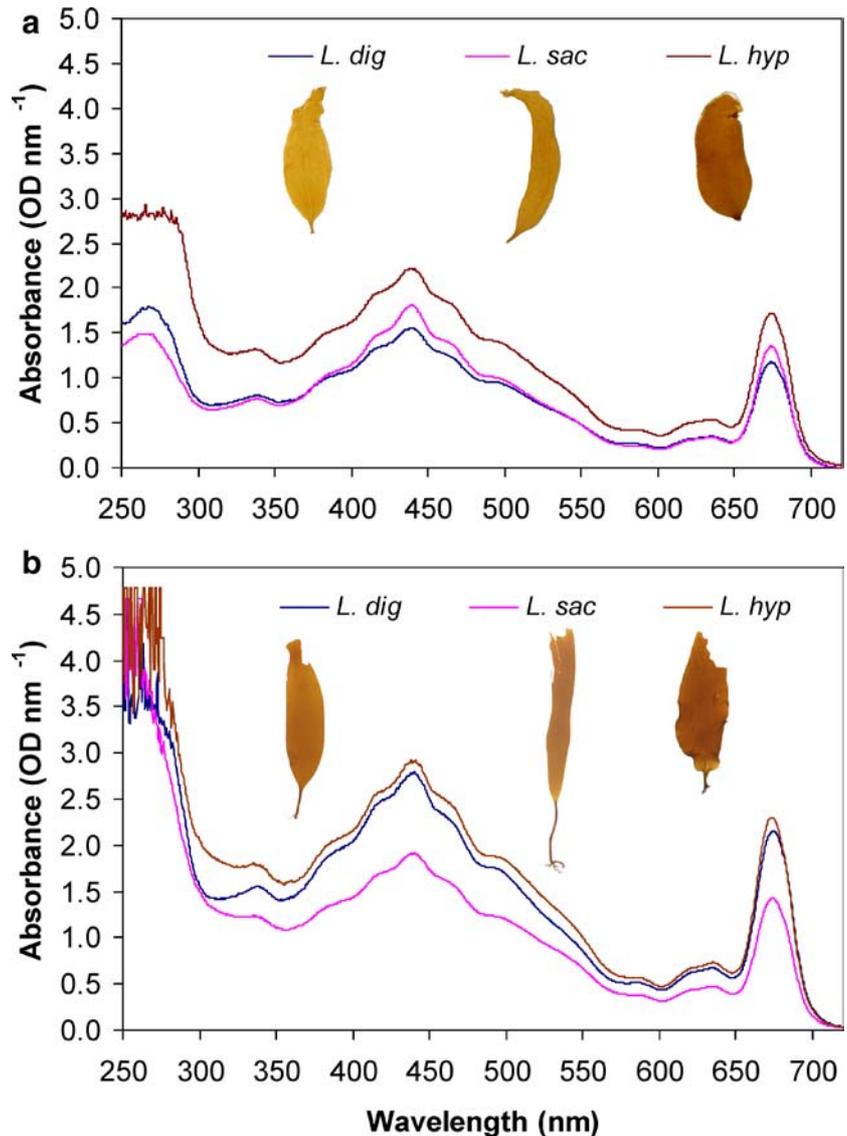


Fig. 6 Chlorophyll a content in young sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB). Vertical bars are standard deviations ($n=3$). Value of *L. saccharina* represents one replicate only

(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 $20\text{--}30 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ of continuous photon irradiance, whereas $1\text{--}2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ was able to sustain growth in culture (Han and Kain 1996). At $18 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ in this study, maximum growth rate in *L. digitata* (1500–2100 h, $0.57 \pm 0.17\% \text{ h}^{-1}$) is comparable to the previous online growth measurements of Lüning (1994) on the same species from Helgoland reported at $0.5\% \text{ h}^{-1}$. Growth rate in *L. saccharina* (1500–2100 h, $0.59 \pm 0.03\% \text{ h}^{-1}$) is, however, lower compared to the previous study reported at $0.7\% \text{ h}^{-1}$ (Lüning 1992). Comparison with other Laminariales showed higher growth rates among *Laminaria* species than in *Saccorhiza dermatodea* ($0.42 \pm 0.12\% \text{ h}^{-1}$) and *Alaria esculenta* ($0.31 \pm 0.02\% \text{ h}^{-1}$; 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 thallus can provide extra protective tissue against UVR (Johansson and Snoeijs 2002), where outer cell layers shade inner cells and provide longer path-length 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.

Acknowledgements MY Roleda is supported by a scholarship from the German Academic Exchange Service (DAAD). We thank the divers of Biologische Anstalt Helgoland for collecting field materials and C. Daniel for pigment analysis. This is publication awi-n 15416 of the Alfred Wegener Institute for Polar and Marine Research. The experiments performed comply with the current laws of Germany.

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