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ORIGINAL ARTICLE

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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

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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

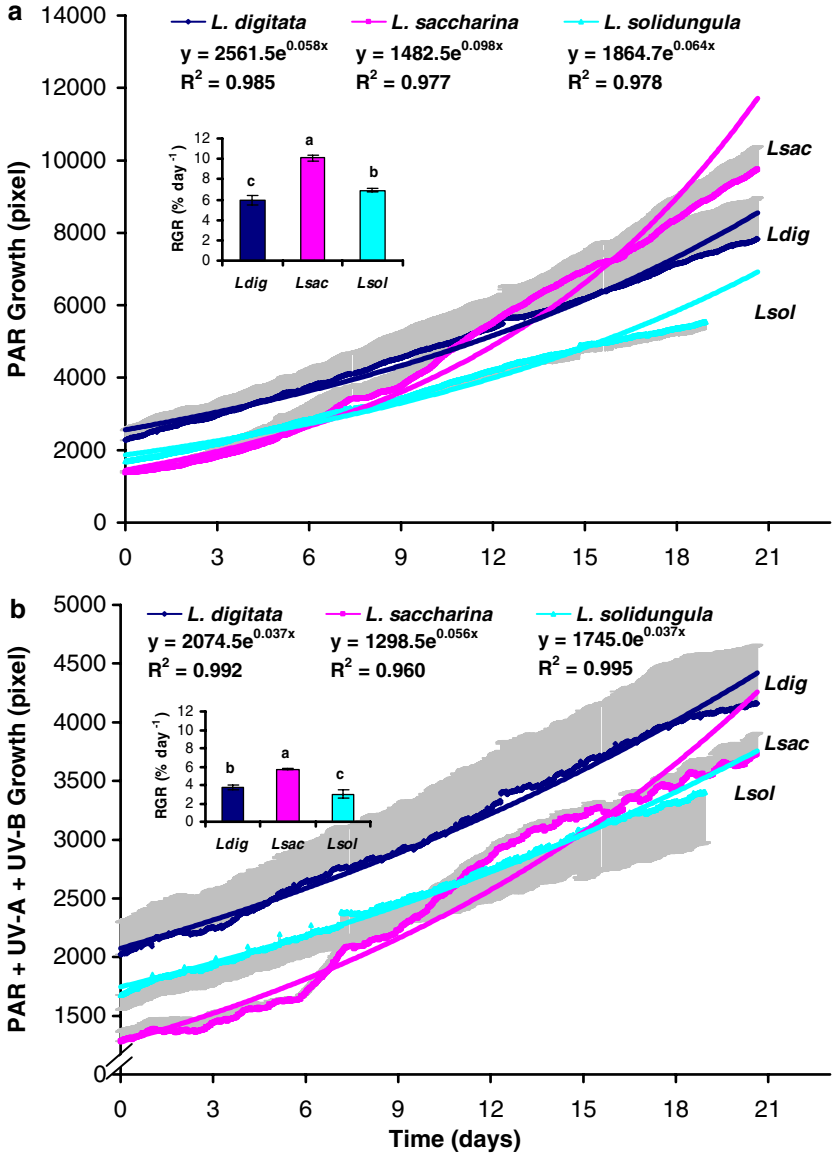

Fig. 1 Growth, in terms of surface area increase, in young sporophytes exposed to **a** photosynthetically active radiation (PAR = P) and **b** PAR + UV-A + UV-B (= PAB). Photon flux density (PFD) was ±18 μmol photons m^{-2} s^{-1}. Data points (*irregular coloured points*) are fitted using the exponential 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*.

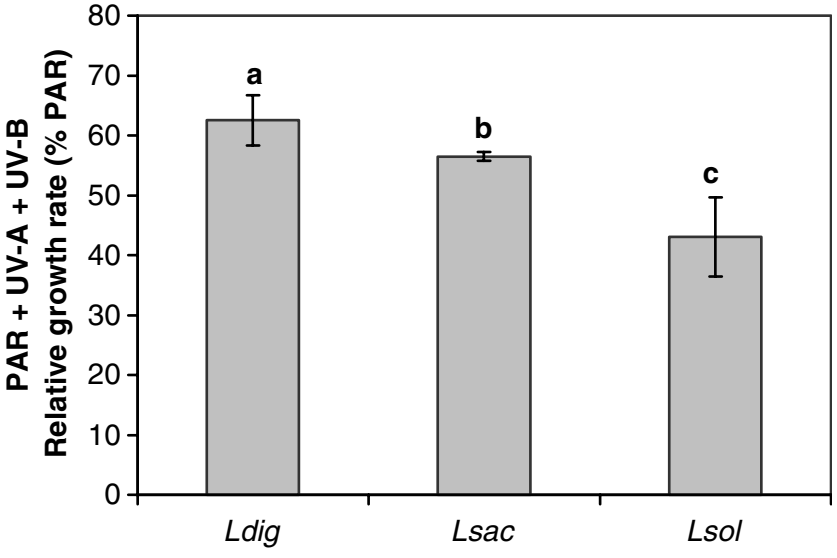

Fig. 2 Ultraviolet radiation effect on the growth rates of young *Laminaria* sporophytes. Growth rates of sporophytes exposed to PAR + UV-A + UV-B were expressed as percent of the growth rates of sporophytes exposed to PAR alone (% PAR). ANOVA showed significant difference between species ($P < 0.05$).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

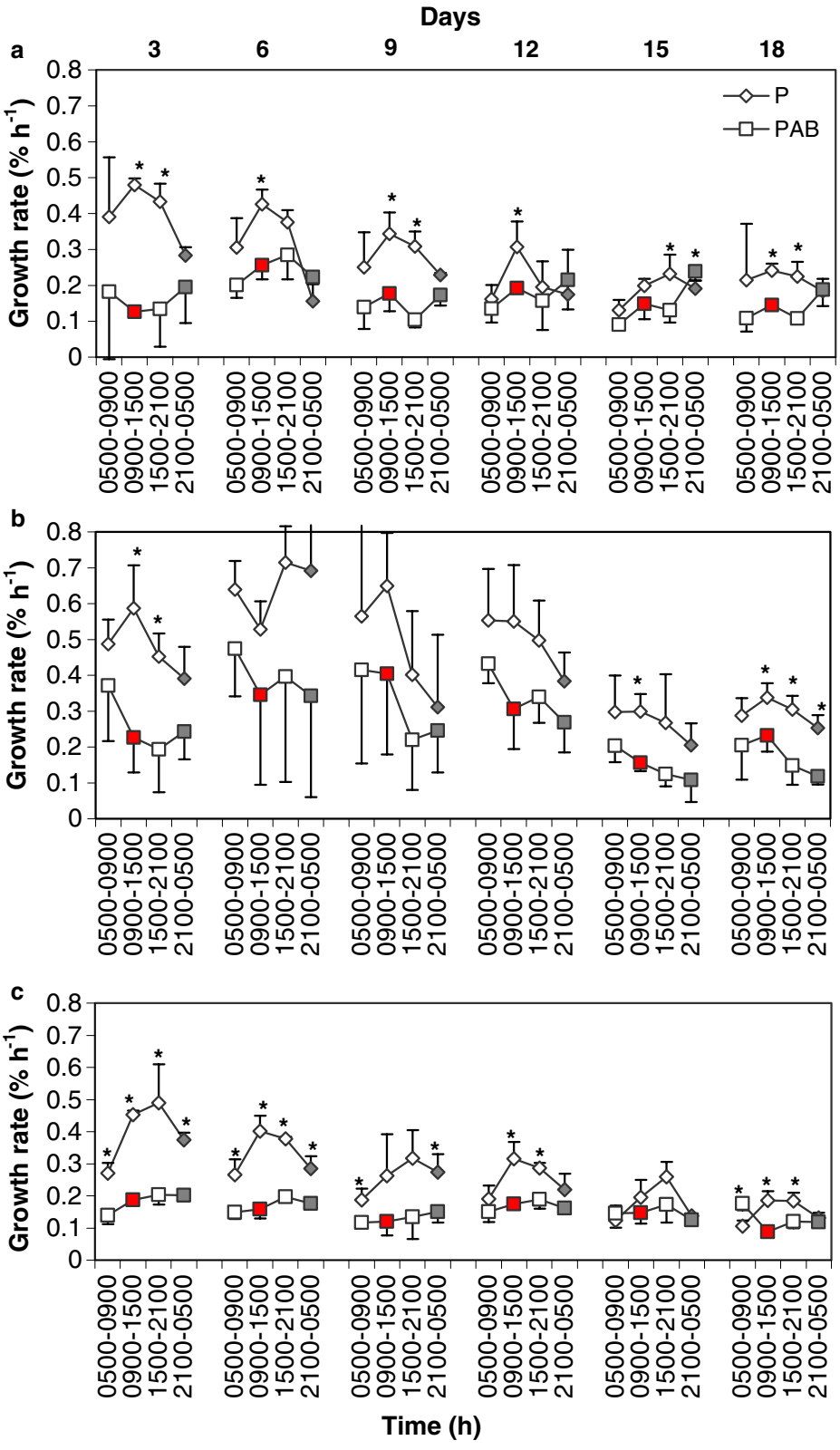

Fig. 3 Average circadian growth pattern of young **a** *Laminaria digitata*, **b** *L. saccharina*, **c** *L. solidungula* sporophytes exposed to photosynthetically active radiation (*P*) and to the full light spectrum (*PAB*) at the start of the light phase to PAR without UVR (0500–0900 hours); in the 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.

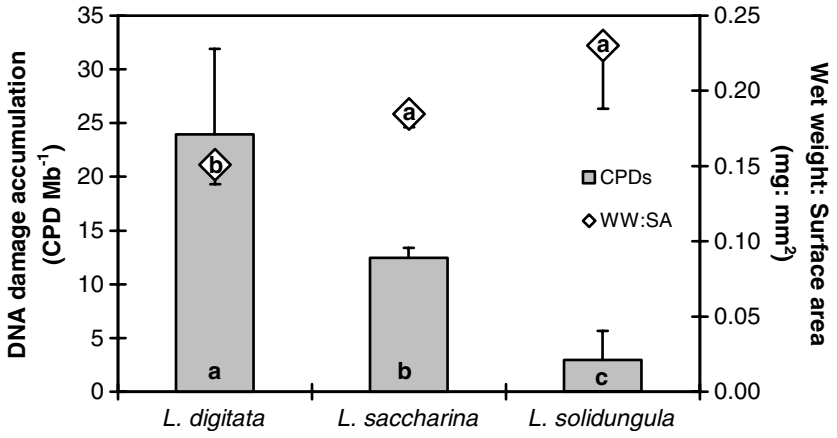

Fig. 4 Relationship between average thallus thickness and remaining tissue DNA damage after 18–21 days of repeated daily 6 h UVR exposure and 18 h recovery (6:8:4 h light:dark:light cycle) in the three Arctic *Laminaria* species. *Vertical bars* are standard deviations ($n=3$). ANOVA showed significant 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).

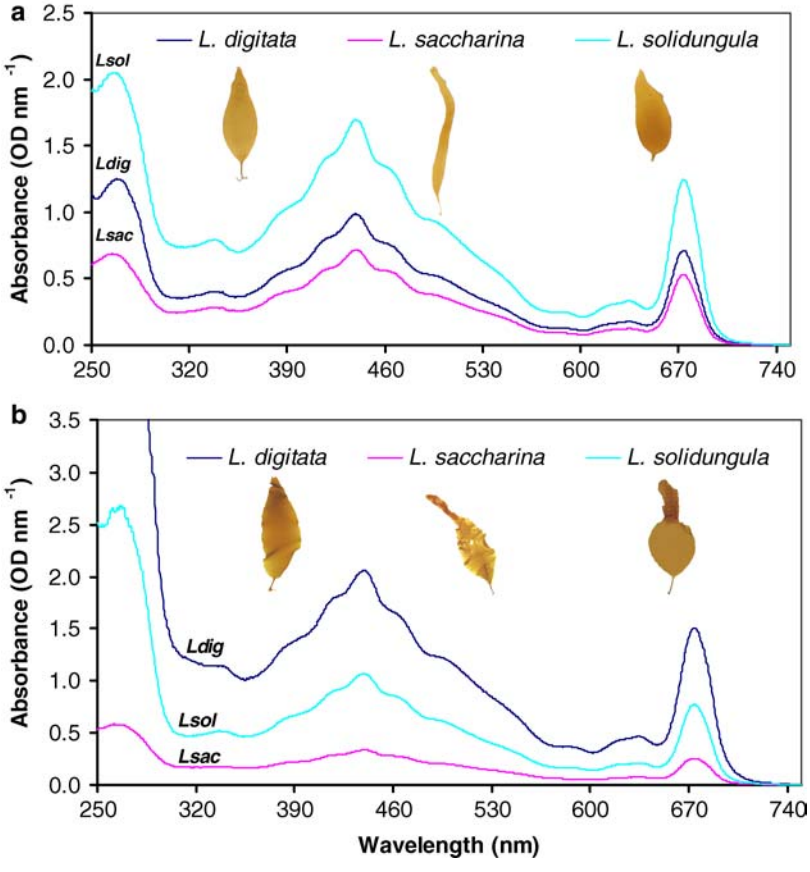

Fig. 5 Tissue absorbance spectrum of the three *Laminaria* species in **a** cultured and **b** wild young sporophytes. The *insets* show difference in morphology of cultured and wild specimen

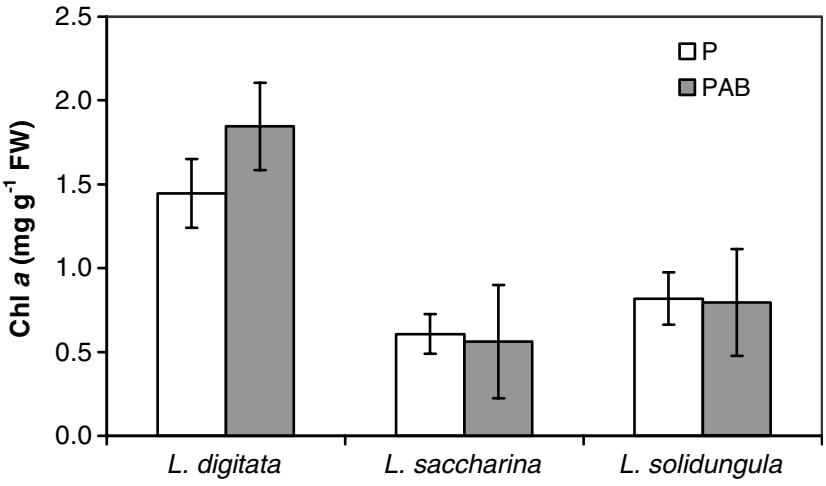

Fig. 6 Chlorophyll a concentration in young sporophytes exposed to different irradiances (PAR = P; PAR + UV-A + UV-B = PAB). *Vertical bars* are standard deviations ($n = 3$). ANOVA showed significant difference between species ($P < 0.05$) and insignificant difference between treatments

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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