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

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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.

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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 ($\Delta F/F\mathrm{m}'$) 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 ($12 \times 16.5 \times 3.5$ cm, constructed with side frames) was designed to be laid hanging over the bottom chamber ($17.5 \times 17.5 \times 4$ 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 ($\Delta F/F\mathrm{m}'$) 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

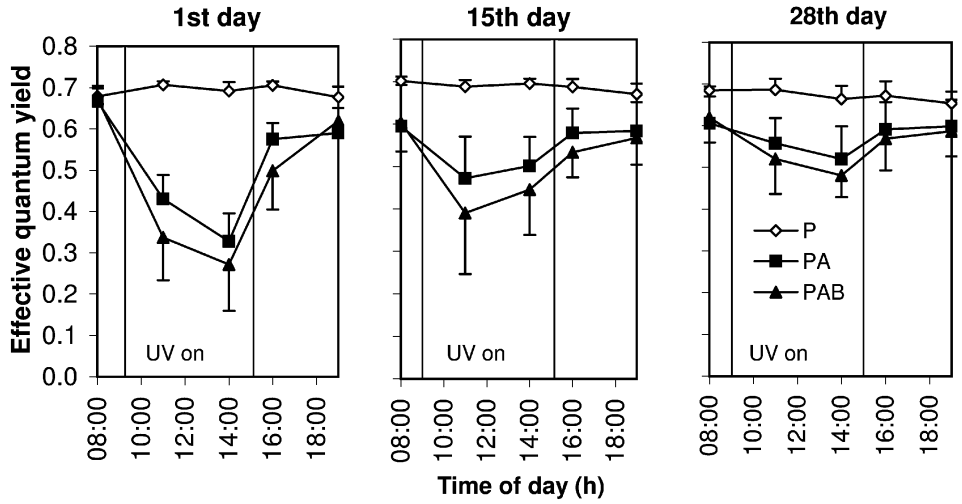

Fig. 1. Circadian pattern of the mean effective quantum yield of young *Laminaria ochroleuca* sporophytes (n = 5) exposed to different radiation (PAR = P; PAR + UVA = PA; PAR + UVA + UVB = PAB) during the light phase of the 16 : 8 h light–dark photoperiod. PFD is 40–50 μmol m^{-2} s^{-1}. Vertical bars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

mean effective quantum yield ($\Delta F/F\mathrm{m}'$) was observed in the PA and PAB treatments relative to P, respectively. The mean $\Delta F/F\mathrm{m}'$ 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 $\Delta F/F\mathrm{m}'$ of UV-exposed plants became smaller through time (15th and 28th day). After 2 and 5 h of UV exposure (PA and PAB), $\Delta F/F\mathrm{m}'$ 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, $\Delta F/F\mathrm{m}'$ (Table 2). Sporophytes exposed to PA and PAB had significantly lower $\Delta F/F\mathrm{m}'$ 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), $\Delta F/F\mathrm{m}'$ of P was still significantly higher compared to PA and PAB. In the morning (0800 hours), $\Delta F/F\mathrm{m}'$ 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 $\Delta F/F\mathrm{m}'$. The DMRT ($P < 0.05$) showed significantly higher daily $\Delta F/F\mathrm{m}'$ 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 ($\Delta F/F\mathrm{m}'$) 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.

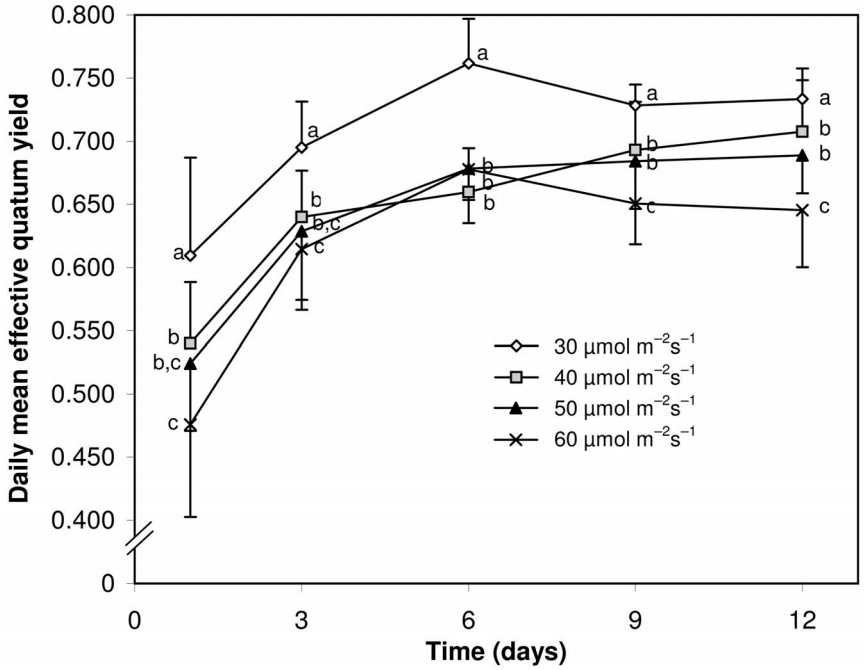

Fig. 2. Daily mean of the effective quantum yields of young *Laminaria ochroleuca* sporophytes ($n = 5$), exposed to different PFDs of PAR, measured every 4 h from 0800 to 2000 hours. Vertical bars are standard deviations (s). RMANOVA showed significant difference between treatments ($P < 0.001$). Letters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

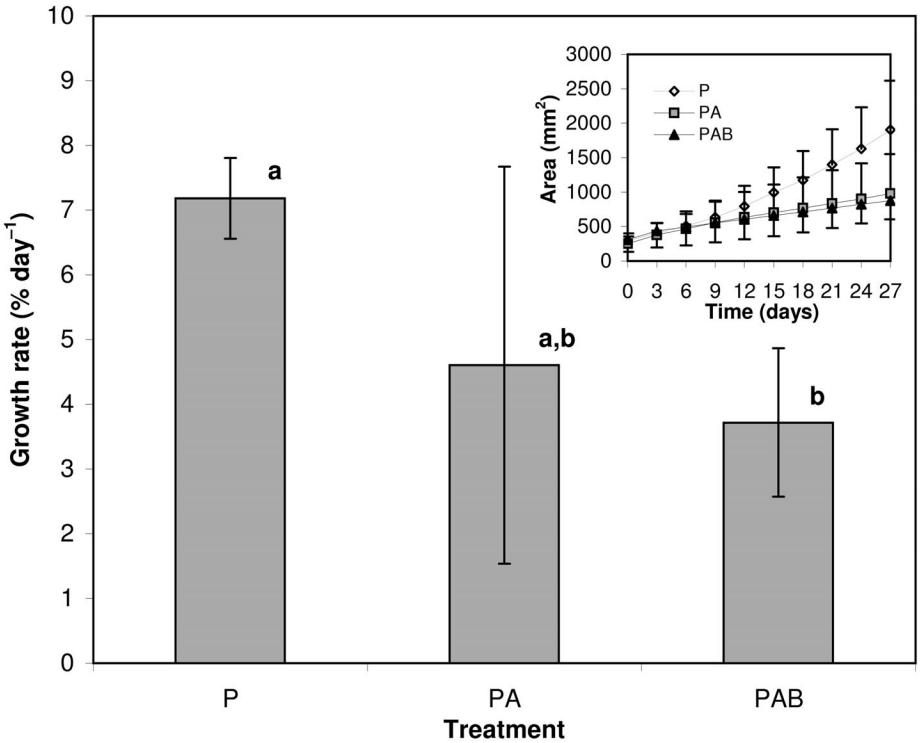

Fig. 3. Growth rates of young *Laminaria ochroleuca* sporophytes exposed to different radiation (PAR = P; PAR + UVA = PA; PAR + UVA + UVB = PAB). PFD was 40–50 µmol m^{-2} s^{-1}. Inset shows increase in surface area over time measured using a scanner and image analysis software (WinFolia). Values are $\bar{x} \pm s$ ($n = 5$). 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.

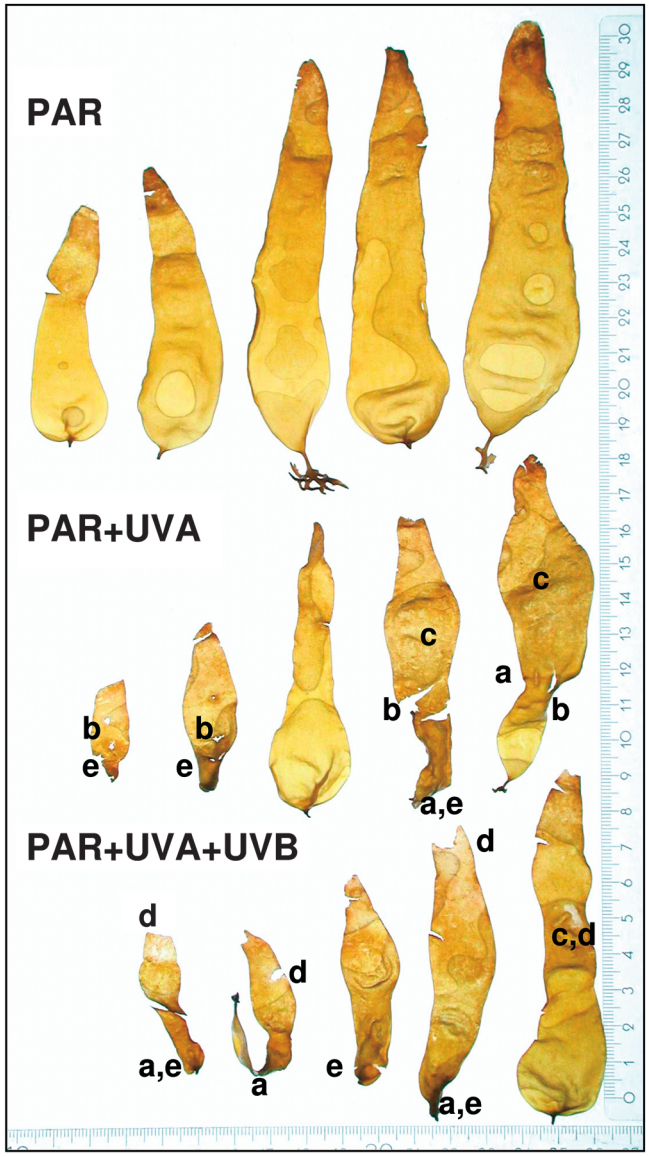

Fig. 4. Morphological responses of *Laminaria ochroleuca* after 28 days UVR exposure includes: a, tissue deformation; b, lesion; c, blistering; d, bleaching; e, curling and thickening of the meristematic lamina.

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.*

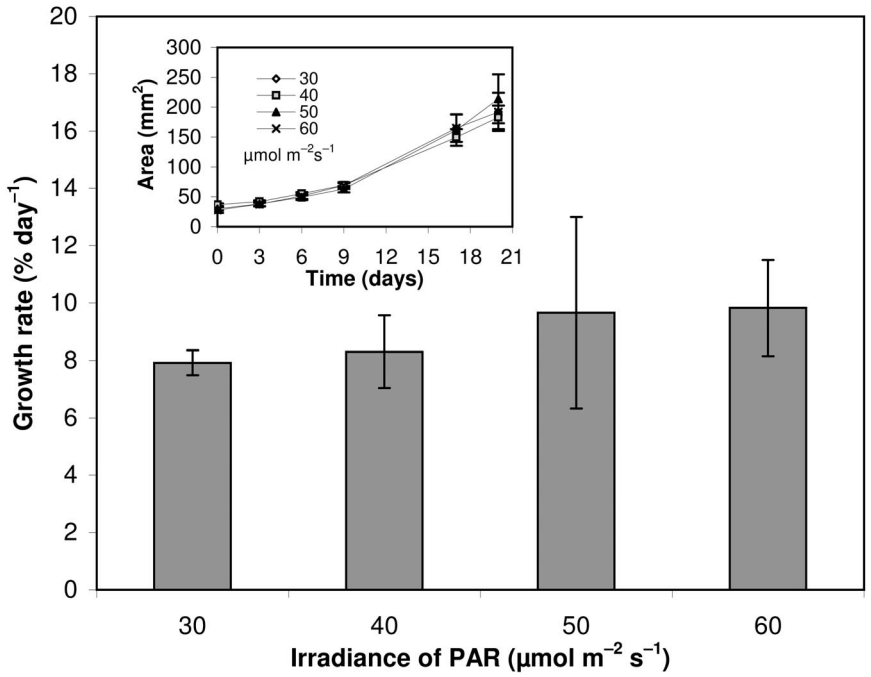

Fig. 5. Growth rates of young *Laminaria ochroleuca* sporophytes exposed to different PFDs of PAR. Inset shows increase in surface area over time measured using a scanner and image analysis software (WinFolia). Values are $\bar{x} \pm s$ ($n = 5$). ANOVA showed insignificant difference between treatments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

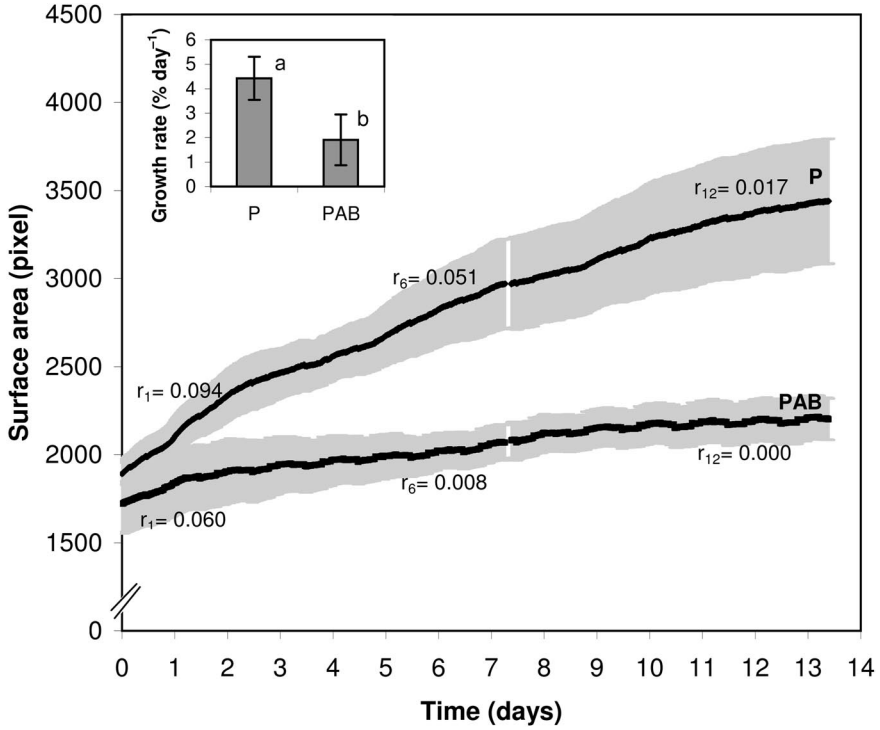

Fig. 6. Growth, in terms of surface area increase, of young *Laminaria ochroleuca* sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB) using the ISITEC growth chamber coupled to a PC with video image analysis software (MedeaLAB) determining area in pixels. PFD was ± 20 µmol 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.

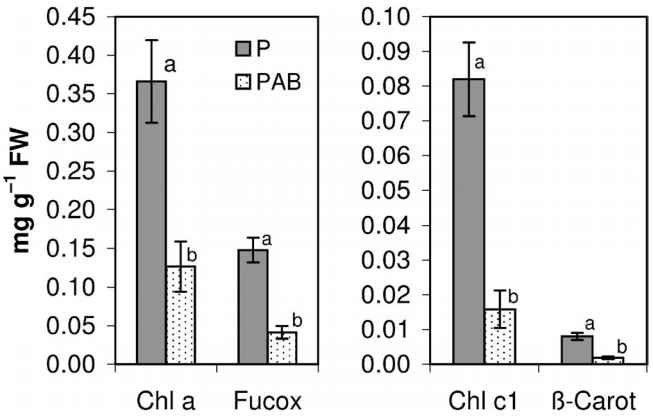

Fig. 7. Photosynthetic and accessory pigments in *Laminaria ochroleuca* sporophytes exposed to different irradiances (PAR = P; PAR + UVA + UVB = PAB). Vertical bars are standard deviations (*s, n* = 3). Letters on graph show result of *t*-test ($P < 0.005$); different letters refer to significant differences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