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Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine

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ABSTRACT: The effects of solar radiation, both visible and ultraviolet (UV-R, 290 to 400 nm), on the kelp *Laminaria saccharina* (L.) Lamour were examined in relation to depth distribution and tidal height in the Gulf of Maine. Despite the high attenuation of visible and UV-R, shallow subtidal *L. saccharina* exhibited significant decreases in midday measurements of steady-state quantum yields of Photosystem II (PSII) fluorescence compared to deeper conspecifics. Decreases in PSII quantum yields under low-tide conditions at midday were more pronounced and associated with higher coefficients of non-photochemical quenching than yields of the same algae measured at midday under high-tide conditions. Steady-state quantum yields in shallow algal populations had not recovered by early morning of the following day. Laboratory experiments were conducted to partition out the effects of high visible radiation versus UV-R effects on steady-state quantum yields observed in the field. Algae exposed to solar visible PAR (photosynthetically active radiation; 400 to 700 nm), PAR + UV-A (ultraviolet-A radiation; 320 to 400 nm), and PAR+UV-A+UV-B (ultraviolet-B radiation; 290 to 320 nm) treatments exhibited daily midday depressions in steady-state quantum yields, similar to those observed in field samples at low tide. The PAR+UV-A+UV-B treatment showed a significantly greater depression in PSII quantum yields than field samples at low tide, and took longer to recover at night. Algae exposed to the UV-R component of the spectrum also showed a significant decrease in gross primary production and contained less areal chlorophyll *a* than field samples, while non-photochemical quenching (a measurement of the dissipation of excess excitation energy) was significantly higher during midday exposures to UV-B. Significantly greater concentrations of the UV-B-absorbing compound mycosporine-glycine, a mycosporine-like amino acid (MAA), were present in the shallow field and experimental PAR+UV-A+UV-B–treated algae. Our results suggest that exposure to midday visible irradiances resulted in dynamic photoinhibition, while exposure to visible and UV-R irradiance in the Gulf of Maine results in both dynamic and chronic photoinhibition that causes a decrease in gross primary production of *L. saccharina* in shallow waters or under low-tide conditions.

KEY WORDS: Macroalgae · Chlorophyll fluorescence · *Laminaria saccharina* · UV-B radiation · Photoinhibition · Mycosporine-like amino acids · Gulf of Maine

INTRODUCTION

Ultraviolet (UV-R, 290 to 400 nm), and (in particular) ultraviolet-B (UV-B, 290 to 320 nm) radiation are known to be harmful to many photoautotrophic marine organisms (Karentz et al. 1994, Shick et al. 1996, Franklin & Forster 1997), especially the effects of UV-B on phytoplankton (Cullen & Lesser 1991, Cullen et al. 1992, Smith et al. 1992, Helbling et al. 1994, Neale et al. 1994, Lesser et al. 1996, Neale et al. 1998) resulting

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from the decrease in stratospheric ozone in polar regions (Gleason et al. 1992, Stolarski et al. 1992, Madronich et al. 1998). Exposure of marine and freshwater photoautotrophs to the biologically damaging wavelengths of UV-B has been shown to have adverse effects on primary productivity, DNA, proteins, and biological membranes (Vincent & Neale 2000). Ultimately, damage to marine primary producers could cause decreases in production and changes to the structure of marine ecosystems (e.g. Bothwell et al. 1994).

Recently there have been a number of studies examining the effects of UV-R on macrophytes from the Arctic and Antarctic (Hanelt et al. 1997, Bischof et al. 1998a,b, Brouwer et al. 2000, Karsten et al. 2001). These studies have largely been short-term studies on UV-R effects on photosynthesis, specifically photoinhibition, based on active fluorescence or oxygen-flux measurements (Brouwer et al. 2000, Karsten et al. 2001). Other studies have differentiated between chronic and dynamic photoinhibition in macrophytes (Hanelt et al. 1993, Franklin & Forster 1997, Hanelt 1998), while long-term studies on the effects of UV-R on macrophyte growth have also been documented (Michler et al. 2002). Fewer studies on the effects of UV-R have been conducted on temperate populations of macrophytes (Wood 1987, Dring et al. 1996b, 2001, Gevaert et al. 2002).

Studies examining the effects of UV-R exposure must distinguish between the effects of UV-R and high irradiances of PAR. Excessive exposure to PAR irradiance has been demonstrated to depress the maximum rate of photosynthesis, a process known as photoinhibition. Two types of photoinhibition are now commonly recognized: (1) chronic photoinhibition, whereby a decrease in photosynthetic efficiency is the result of damage to the reaction centers of PSII and degradation of the D1 protein requires *de novo* synthesis of new D1 protein; and (2) dynamic photoinhibition, a protective regulatory mechanism that dissipates excess excitation energy as heat and involves the xanthophyll cycle (Falkowski & Raven 1997). The thermally dissipated energy can be characterized, using active fluorescence techniques, as non-photochemical quenching (NPQ). Dynamic photoinhibition has recently been shown to be an important mechanism of photoprotection for the symbiotic dinoflagellates of corals (Brown et al. 1999, Gorbunov et al. 2001), whereby midday irradiances of solar radiation in optically clear waters cause a depression in steady-state quantum yields of PSII fluorescence, but net photosynthesis shows no accompanying decrease (Lesser & Gorbunov 2001). Other photoprotective strategies such as changes in the abundance and composition of photosynthetic pigments, or production of UV-R-absorbing compounds known as mycosporine-like amino acids (MAAs) may also play a role in preventing chronic photoinhibition.

The brown algal kelps, which include species of *Macrocystis* and *Laminaria*, are one of the most productive groups of marine organisms in temperate marine environments (Mann 1973). Temperate coastal waters are characterized by the high attenuation of UV-R due to sediments, phytoplankton, and dissolved organic carbon that serves as a natural blocker of UV-R for temperate subtidal organisms (Kirk 1994). In the first 4 m of many coastal temperate waters, up to 90% of the available UV-R is attenuated (Franklin & Forster 1997), although biologically damaging UV-B can penetrate as deep as 7 to 8 m in temperate latitudes such as the Gulf of Maine (Banaszak et al. 1998, Lesser et al. 2001). In addition to the seasonal variability in the optical properties of the water column, the Gulf of Maine also exhibits a dynamic tidal range that averages approximately 3 to 4 m and can be as high as 10 m. The combination of changes in the optical properties of the water and tidal range variability will expose shallow subtidal macrophytes to an increase in UV-R and possibly result in decreased primary production. This study presents data describing the effects of solar radiation on shallow subtidal field populations of the brown alga *L. saccharina* (L.) Lamour from different depths and tidal heights. Additionally, using natural solar radiation, we conducted laboratory experiments to partition out the effects of PAR versus UV-R wavelengths on the photobiology of this important member of the subtidal community in the Gulf of Maine.

MATERIALS AND METHODS

Characterization of underwater light field. The spectral irradiance of PAR and UV-R (300 to 700 nm) were measured daily at 13:00 h at 6.3 m mean low water (MLW) over many days during the summer of 2000 at Appledore Island, Isles of Shoals, off the coast of Maine and New Hampshire (42° 59.29′ N, 70° 37.01′ W), as were simultaneous measurements of surface irradiance using a LiCor LI-1800UW scanning spectroradiometer (LiCor) calibrated with National Institute of Standards and Technology (NIST) traceable standards. We took 3 scans, and the mean is reported in units of W m^{-2} nm^{-1}. The cosine-corrected collector and sensors were programmed to scan from 300 to 700 nm at 2 nm intervals. The sensor has a full bandwith at half maximum of 8 nm and a wavelength accuracy of ±1.5 nm with minimum excitation energies on the order of 10^{-8} W cm^{-2} nm^{-1}. A comparison of this instrument showed very good agreement with other commercial instruments (e.g. Biospherical PUV) and radiative transfer models based on comparisons

of spectral scans, calculated attenuation coefficients, and stray light-rejection underwater (when PAR:UV-R ratios would be very high; Kirk et al. 1994). Vertical diffusive attenuation coefficients (k_d m^{-1}) for both visible and UV-R were calculated as described by Kirk (1994).

Field experiments. Subtidal samples of *Laminaria saccharina* were studied using SCUBA. The study site contained sunken pier pilings that provided substrate for the algae to attach and grow at various depths. Sporophytes of *L. saccharina* from 0.5, 2.5 and 5.0 m (N = 8 for each depth, length 1 to 3 m), relative to MLW, were tagged at the base of the stipe to represent shallow, mid, and deep algal populations at this site. *In situ* fluorescence yields on these populations were examined as described below.

Using a pulse amplitude-modulated fluorometer (PAM; Diving-PAM) fluorescence measurements were made on all field samples daily at ~10:00 h for 10 d with the exception of the diel study, which was conducted during opposing tidal cycles with 5 fluorescence measurements per day: a low-tide of 0.2 m below MLW occurred at 11:59 h on 25 July 2000 and a high tide of 3.3 m above MLW on 31 July 2000 at 11:56 h, and on these dates fluorescence measurements were conducted at 08:00, 12:00, 16:00, 20:00, and 00:00 h and ambient irradiance was measured hourly not daily. A magnetic clip held the end of the fiber optics cable 10 mm from the lower meristematic tissue region of the alga near the base of the thallus and with no visible epiphytes. Each measurement was made with the blade of the alga parallel to the bottom and therefore directly exposed to downwelling irradiance with minimal shadowing, and the fiberoptic probe was held at an angle of 35 to 45° relative to the blade. For consistency, and to minimize shading artifacts, all measurements were conducted in this manner for algae sampled in the field or laboratory experiments. The PAM was used to measure steady-state quantum yields with an initial fluorescence (F) measurement at a low intensity of red light (~0.244 µmol quanta m^{-2} s^{-1} at 650 nm). Maximal fluorescence (F_m') was achieved by a saturating actinic light pulse (3700 µmol quanta m^{-2} s^{-1}) of 700 ms. Steady-state quantum yields of PS II fluorescence, $\Delta F/F_m'$ (a coefficient of photosynthetic efficiency), were calculated as the ratio of change in fluorescence before and after saturation to F_m', without a dark-adaptation period:

$$\Delta F/F_m' = (F_m' - F)/F_m' \quad (1)$$

$\Delta F/F_m'$ ranges from 0 to 1, with a higher value indicating a greater proportion of functional PSII units. In reality, values of $\Delta F/F_m'$ cannot exceed dark-acclimated quantum yields (F_v/F_m), which are considerably less than 1. Additionally, a coefficient of non-photochemical quenching (NPQ) was determined as described by Maxwell & Johnson (2000) using the 00:00 h measurements (dark-adapted) of F_m for the calculations:

$$NPQ = (F_m - F_m')/(F_m') \quad (2)$$

Laboratory experiments. *Laminaria saccharina* sporophytes (length 0.5 to 1.0 m without epiphytes) were collected from the 5 m field population described above, where the algae were attached to shells of the mussel *Mytilus edulis*. Before manipulative experiments began, the samples (N = 30) were placed in an outdoor sea-table (1.0 m deep) with flowing seawater for 1 wk to acclimatize them to the new natural solar irradiance regime. Randomly chosen samples of algae were then exposed to different solar UV-R treatments during July and August 2000. Treatments (N = 5 algae per treatment) were established using UV-R transparent Plexiglas (50% cutoff at 285 nm) for all treatment groups and Mylar plastic of different compositions to create the UV-A (50% cutoff at ~320 nm) and ultraviolet opaque (UV-O 50% cutoff at ~395 nm) treatment groups as described below. One group was exposed to full solar radiation (PAR and UV-R) and designated the ultraviolet-transparent (UV-T) treatment (290 to 700 nm), a second group was designated the UV-A treatment + PAR (320 to 700 nm), and a third group that allowed only PAR wavelengths (400 to 700 nm) to transmit was designated the UV-O treatment. All experimental samples were acclimatized to the different treatments for 5 d with flowing seawater at 15 to 17°C.

Fluorescence and photosynthesis measurements. *In vivo* chlorophyll fluorescence was measured as described above (subsection 'Field experiments') using the PAM fluorometer for 5 consecutive d at ~09:00 h. On the fifth day of the experiment, a diel study of photosynthetic parameters and oxygen production was conducted. Fluorescence was measured from 07:00 to 23:00 h in 2 h increments. Steady-state quantum yields and NPQ were calculated for the laboratory experiment as described above.

Photosynthesis, assessed by oxygen production, was measured at 08:00, 12:00, 16:00, and 21:30 h in light and dark biological oxygen demand (BOD) bottles (UV-R transmission >295 nm, 50% transmission at 320 nm) in the experimental sea-table during the diel study. We cut 2 pieces of thalli per alga per treatment group (N = 3). The thalli and light (N = 3) and dark (N = 3) blanks were incubated for 1.5 h in foil-covered and uncovered 0.3 l glass bottles of unfiltered seawater. After the incubation period, the bottles were immediately capped and the dissolved oxygen measured with a polarographic oxygen meter (YSI Model 51B) and the blotted wet mass measured. The thalli were placed back in their respective treatment groups until the next

incubation period. After the final measurement, the thalli were dried to determine dry weight. Oxygen production was defined as:

$$O_2 = (\Delta O_2 \times v)/(m_d \times t) \quad (3)$$

where ΔO_2 represents the change in oxygen production in mg O_2 l^{-1}, v = bottle volume (l), m_d = dry mass (g) of alga, and t = incubation time (h). Gross primary production (GPP) was determined using both the oxygen production in the light bottles and respiration in the dark bottles, and is defined as:

$$GPP = O_2 \text{ light bottle} - O_2 \text{ dark bottle} \quad (4)$$

All oxygen measurements were corrected using the light and dark blanks described above to remove the influence of any photo- and heterotrophs.

Photosynthetic pigments and UV-R-absorbing compounds. Samples for the analysis of photosynthetic pigments from all treatments were collected at midday between 11:00 and 13:00 h. Samples from the meristomatic region, without obvious epiphytes, were then extracted for pigment analysis by cutting 44.2 mm^2 discs using a cork borer. The disks were placed in 10 ml of 100% HPLC grade methanol and stored at –20°C in the dark until analysis. Photosynthetic pigment concentrations were determined by high-performance liquid chromotrography (HPLC), and 50 to 150 µl of methanol-extracted photosynthetic pigments were injected and analyzed as described by Wright et al. (1991). Pigments were separated using a gradient system of 80:20 methanol:ammonium acetate, 90:10 acetonitrile:water, and ethyl acetate with a Spheri-ODS, 25 cm × 4.6 mm i.d., 5 µm particle-size column at a flow rate of 1.0 ml min^{-1} with detection at 436 nm. Authentic standards were obtained from Sigma (chlorophyll *a*, β-carotene) and VKI Water Quality Institute (Hørsholm, Denmark) (zeaxanthin, fucoxanthin, violaxanthin, chlorophyll c_2) as described by the SCOR Carotenoid Workshop (Wright et al. 1991). Peaks were integrated and quantification of individual pigments was accomplished using HPLC peak areas and calibration factors determined by analysis of the standards listed above. Concentrations are expressed in µg cm^{-2}.

From the same samples, the concentration of mycosporine-like amino acids (MAAs) was also measured by HPLC according to the procedures of Shick et al. (1992). Individual MAAs were separated by reverse-phase, isocratic HPLC on a Brownlee RP-8 column (Spheri-5, 4.6 mm i.d. × 250 mm) protected with an RP-8 guard column (Spheri-5, 4.6 mm i.d. × 30 mm). The mobile phase consisted of 40 to 55% methanol (v/v) and 0.1% glacial acetic acid (v/v) in water, run at a flow rate of 0.6 ml min^{-1}. MAA peaks were detected by UV absorbance at 313 and 340 nm. Standards were available for 7 MAAs (mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene). Identities of peaks were confirmed by co-chromatography with standards. Peaks were integrated and quantification of individual MAAs was accomplished using HPLC peak areas and calibration factors determined by analysis of the standards listed above. All MAAs were normalized to soluble protein from an aliquot of the methanol-extracted sample, and concentrations are expressed in µg MAA mg $protein^{-1}$. Protein content were determined using a BioRad protein kit that utilizes the procedure of Bradford (1976) and bovine serum albumin as a standard.

Statistical analysis. Significant differences between treatment groups were assessed using an analysis of variance (ANOVA) at significance level of 0.05. Significant treatment effects were followed by multiple-comparison testing using the Student-Newman-Keuls (SNK) test at a significance level of 0.05 (StatView software, SAS Institute). All data were tested for homogeneity of variances and normal distribution. Data were transformed where necessary for analysis and back-transformed for presentation.

RESULTS

Summer measurements of ambient PAR and UV-R around Appledore Island on a cloudless day at midday (~13:00 h) showed maximum surface PAR irradiance (integrated from 400 to 700 nm) ranging between 2100 and 2400 µmol quanta m^{-2} s^{-1} or 450 to 520 W m^{-2} (Fig. 1a,b), with a typical PAR irradiance of 264 µmol quanta m^{-2} s^{-1} or 57 W m^{-2} at low-tide (Fig. 1a, 6.3 m) and 50 µmol quanta m^{-2} s^{-1} or 11 W m^{-2} at high tide (Fig. 1b, 9.2 m). At 6.3 m (low tide), UV-R (290 to 400 nm) was 0.6 W m^{-2}, and UV-B (290 to 320 nm) was 0.0 W m^{-2}; at 9.2 m (high tide), UV-R was 0.04 W m^{-2} and UV-B was 0.0 W m^{-2}. Algae in the field deeper than 5 m were never exposed to UV-B radiation during this study. Spectral changes occur with increasing depth in temperate coastal waters, with the highest attenuation coefficients in the UV-R and red portions of the spectrum (Fig. 1a,b). During the experimental period, on days with similar ambient irradiances and cloud cover, the irradiance data and spectral attenuation coefficients at midday varied by ±10%, suggesting that the optical properties of the water column did not change dramatically during our study period. The high attenuation coefficients for the PAR portion of the spectrum averaged between 0.39 and 0.47 m^{-1} and are typical of Case II waters as defined by Kirk (1994).

Throughout the experimental period, algae were exposed to different solar irradiances, depending on the weather conditions, with the most consistent changes resulting from cloud cover: irradiance was lower in all

portions of the spectrum on a cloudy day (>50% sky cover) than a sunny day (Fig. 1c) with differences of 2100 µmol quanta m^{-2} s^{-1} (Fig. 1c) for sunny versus 600 µmol quanta m^{-2} s^{-1} (Fig. 1c) for cloudy periods, with changes on a timescale of minutes. In addition to the changes in PAR irradiance, ambient UV-R varied from 48 W m^{-2} UV-R and 2.21 W m^{-2} UV-B (sunny) to 17 W m^{-2} UV-R and 0.7 W m^{-2} UV-B (cloudy).

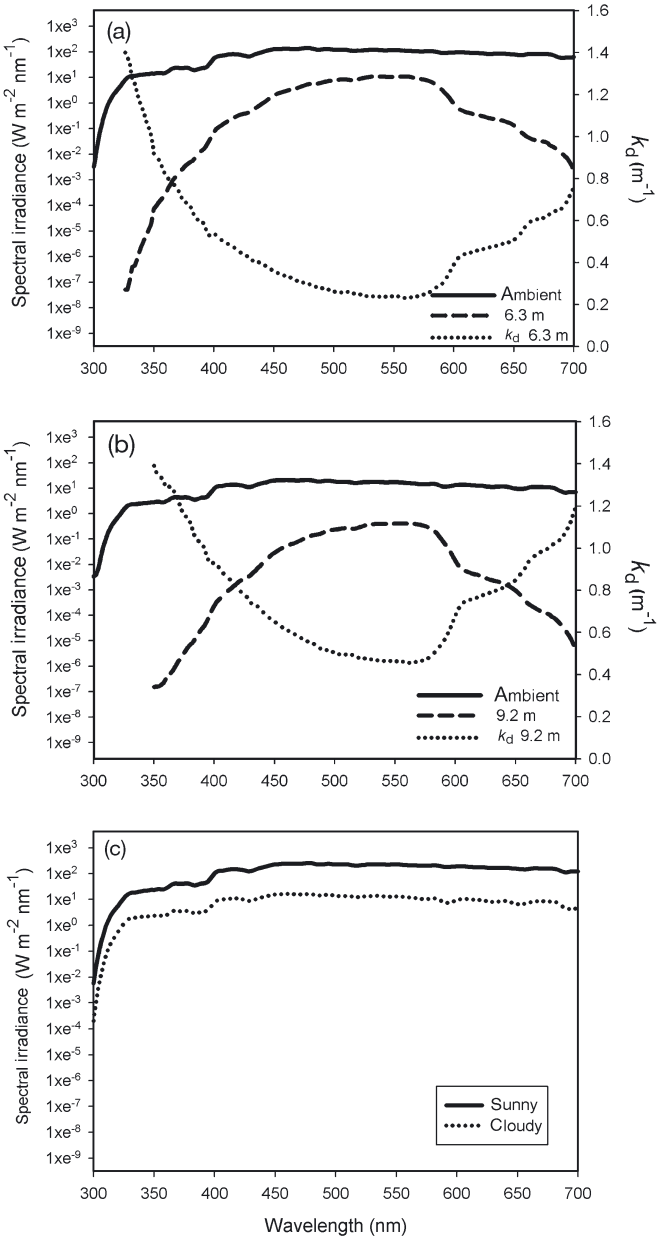

Fig. 1. (a,b) Ambient and underwater spectral irradiance (300 to 700 nm) at (a) low tide (6.3 m) and (b) high tide (9.2 m) at Appledore Island, Isles of Shoals, July 2000 (13:00 h). k_d: calculated spectral attenuation coefficients. (c) Ambient spectral irradiance on a typical sunny and for a typical cloudy day at 13:00 h

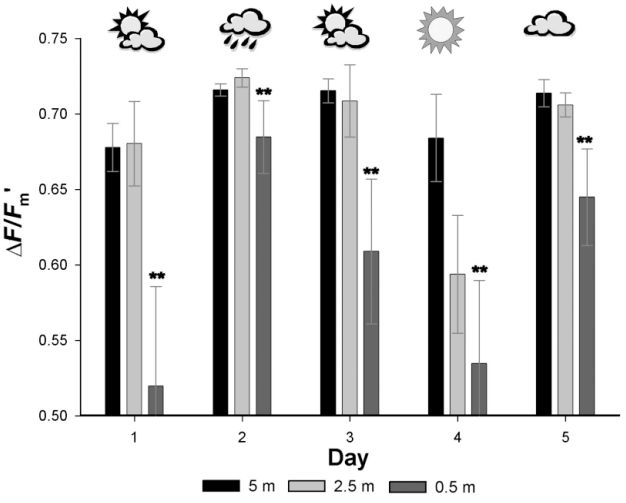

Fig. 2. *Laminaria saccharina.* Comparison of daily (~10:00 h) steady-state quantum yield (mean ±SE; N = 8 per depth) of PS II fluorescence ($\Delta F/F_m'$) at depths of 5.0, 2.5, and 0.5 m at mean low water. Average weather conditions prior to and during 10:00 h measurements are shwon above bars. **Statistically significant differences between shallow depth and all other treatments (SNK: $p < 0.05$). Magnitude of daily differences depended on differences in ambient irradiance which in turn were affected by daily weather changes

Field experiments

Both depth (ANOVA: $p < 0.0001$) and day (ANOVA: $p = 0.0004$) had a statistically significant effect on the steady-state quantum yield of PSII $\Delta F/F_m'$ for *Laminaria saccharina*. $\Delta F/F_m'$ was significantly (SNK: $p < 0.05$) depressed in shallow algae compared to deep and mid-depth algae on all days sampled (Fig. 2a). The magnitude of the differences in $\Delta F/F_m'$ between depths was dependent upon weather condition, with clear sunny days (Fig. 2, Day 4) exhibiting the largest depression in midday $\Delta F/F_m'$ and significant differences (SNK: $p < 0.05$) between all depths (Fig. 2: Day 4).

A diel study conducted during a low tide at midday revealed a statistically significant effect of depth (ANOVA: $p = 0.0006$) and time (ANOVA: $p = 0.0001$) on $\Delta F/F_m'$ measurements. At all depths, significantly (SNK: $p < 0.05$) lower $\Delta F/F_m'$ measurements were observed at 12:00 h, when both low tide and high solar irradiances occur, compared to 08:00, 16:00, 20:00, and 00:00 h measurements (Fig. 3a). For all sampling times during the low-tide study, there were significant differences (SNK: $p < 0.05$) in $\Delta F/F_m'$ between the shallow depths and the mid and deep depths (Fig. 3a). A similar study conducted at high tide at midday showed no effect of time (ANOVA: $p = 0.39$). A significant effect of depth (ANOVA: $p < 0.0001$) on $\Delta F/F_m'$ was observed for shallow algae despite the lower PAR and UV-B irradiances and the longer pathlength for damaging solar radiation at high tide, but the mid and deep depths dis-

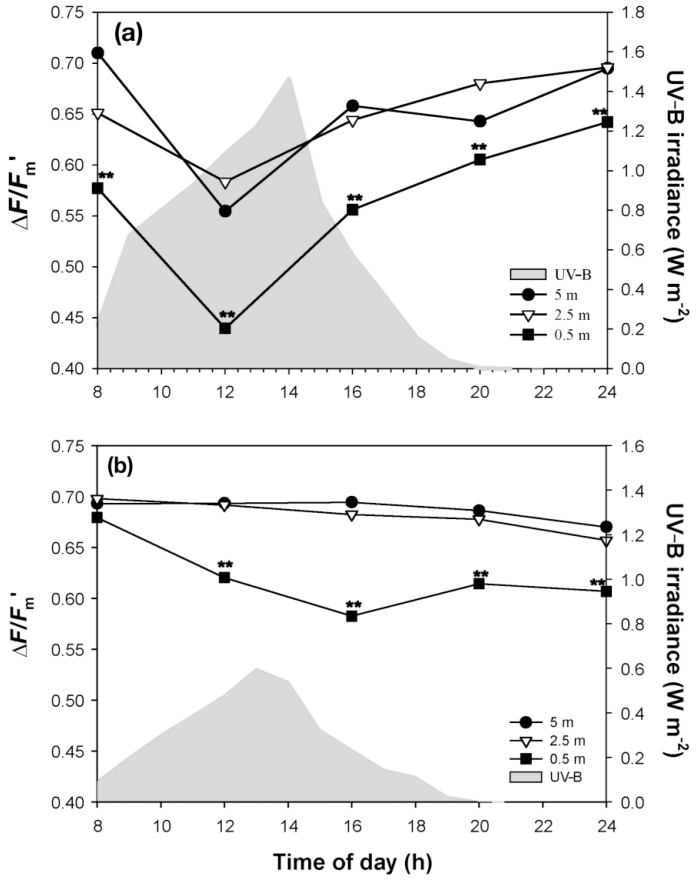

Fig. 3. *Laminaria saccharina.* Diel patterns of steady-state quantum yields of PS II fluorescence ($\Delta F/F_m'$) at depths of 5, 2.5, and 0.5 m (N = 8 per treatment) under different tidal conditions. (a) Low tide of –0.2 m at ~12:00 h; (b) high tide of 3.3 m at ~12:00 h. Shaded area: integrated ambient UV-B irradiance (300 to 320 nm); **: treatments statistically significantly different from all other treatments (SNK: $p < 0.05$)

played nearly constant $\Delta F/F_m'$ throughout the study (Fig. 3b). For the low-tide diel study, there was a strong inverse correlation between $\Delta F/F_m'$ and NPQ in shallow depths at 12:00 h (Fig. 4a), with significantly (ANOVA: $p = 0.01$, SNK: $p < 0.05$) greater NPQ coefficients than either mid or deep populations of algae (data not shown). During the high-tide study, NPQ occurred only at shallower depths (Fig. 4b) during midday, but was not significantly different than at other sampling times. There were no significant changes in $\Delta F/F_m'$ (Fig. 4b).

Pigment concentrations of chlorophyll *a* and fucoxanthin varied significantly with depth (ANOVA: $p < 0.05$, Fig. 5). Concentrations were significantly greater in deep than in shallow algae (SNK: $p < 0.05$), and both chlorophyll *a* and fucoxanthin showed a typical inverse relationship with irradiance. The carotenoid violaxanthin showed no effect of depth (ANOVA: $p = 0.562$), while zeaxanthin showed a non-significant (ANOVA: $p = 0.058$) trend of increasing concentrations with decreasing depth (Fig. 5). Violaxanthin/zeaxanthin ratios, however, did show significant effects of depth (ANOVA: $p = 0.007$), with significantly (SNK: $p < 0.05$) lower ratios for shallow (0.07 ± 0.01 SE) *Laminaria saccharina* than mid (0.14 ± 0.02 SE) and deep (0.18 ± 0.03 SE) populations, which were not significantly (SNK: $p > 0.05$) different from each other.

A single UV-absorbing MAA, mycosporine-glycine, was found in samples of *Laminaria saccharina*, and significantly (ANOVA: $p = 0.0001$) higher concentrations were observed in shallow (25.4 ± 1.9 SE nmol mg^{-1} protein) than in mid (14.1 ± 1.7 SE nmol mg^{-1} protein) and deep (12.7 ± 0.9 SE nmol mg^{-1} protein) algae, which were not significantly different from each other.

Laboratory experiments

Fig. 6 shows the transmission properties of natural solar radiation in the 3 treatments (UV-T, UV-A, UV-O)

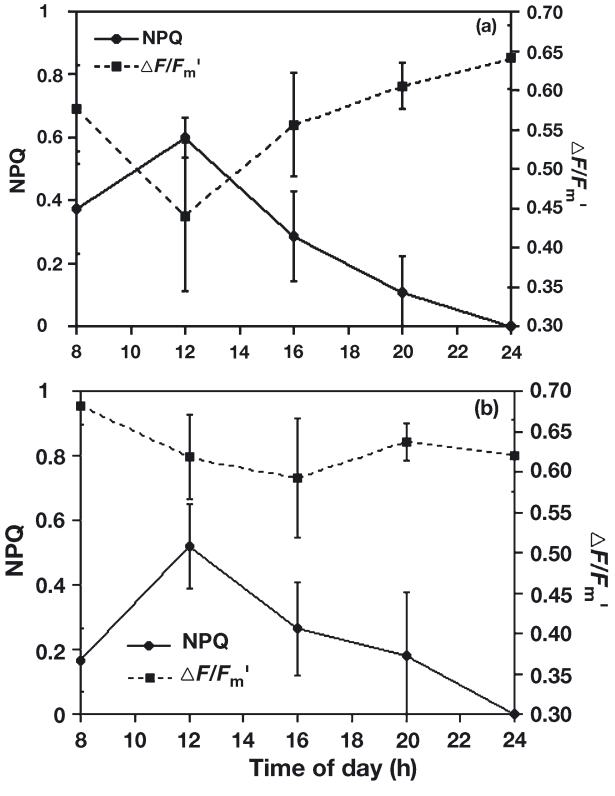

Fig. 4. *Laminaria saccharina.* Mean (± SE; N = 8) diel patterns of steady-state quantum yields of PS II fluorescence ($\Delta F/F_m'$) and non-photochemical quenching (NPQ) for shallow (0.5 m) algae under different tidal conditions. (a) Low tide of ~0.2 m at ~12:00 h; (b) high tide of ~3.3 m occurring at ~12:00 h (N = 8)

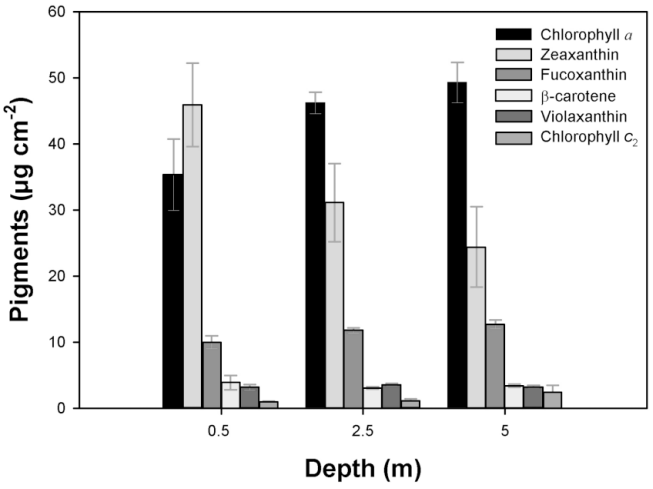

Fig. 5. *Laminaria saccharina.* Photosynthetic pigment concentrations (mean ± SE; N = 8 per depth) in field samples collected from 0.5, 2.5, and 5.0 m

compared to ambient irradiance without any filters. The integrated PAR irradiances under the filters varied by only 2 to 4%. For all treatment groups, $\Delta F/F_m'$ did not significantly change from day to day, but changes in $\Delta F/F_m'$ were observed on a diel basis (Fig. 7a), as in the field populations of *Laminaria saccharina*. All treatments groups displayed a decrease in $\Delta F/F_m'$ at midday between 10:00 and 15:00 h, when solar irradiance was highest, which was followed by a late afternoon recovery between 15:00 and 17:00, as solar irradiance decreased (ANOVA: $p < 0.05$). $\Delta F/F_m'$ values of ~0.20 were observed from 11:00 to 15:00 h in the UV-T treatment (Fig. 7a) that were significantly (SNK: $p < 0.05$) lower at 11:00 and 13:00 h than either the UV-A and UV-O treatments, which ranged from 0.26 to 0.32. Additionally, while UV-A and UV-O treatments nearly recovered to their early morning reading of 0.56 to 0.61 by 19:00 h, the UV-T treatment remained significantly lower than 0.50 until all treatment groups fully recovered at 23:00 h.

During the midday $\Delta F/F_m'$ depression, both the UV-T and UV-A treatments exhibited statistically significant decreases in GPP at 12:00 h (ANOVA: $p = 0.029$; SNK: $p < 0.05$) and 16:00 h (ANOVA: $p = 0.008$; SNK:

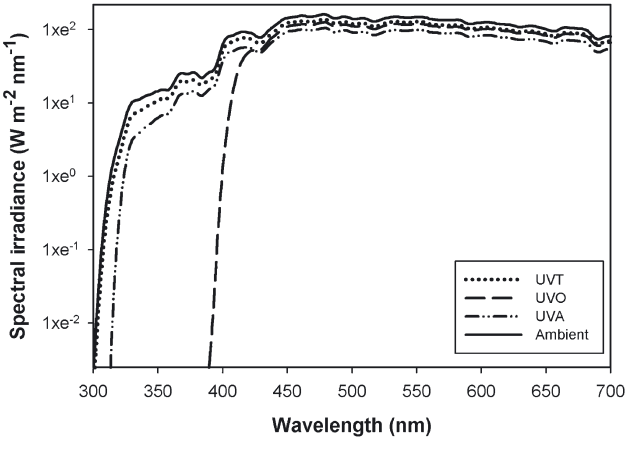

Fig. 6. Spectral irradiance of treatment filters for laboratory experiments. Treatment groups are UV-T (ultraviolet transparent, 50% cutoff at ~285 nm), UV-A (ultraviolet-A, 50% cutoff at ~320 nm), and UV-O (ultraviolet opaque, 50% cutoff at ~395 nm)

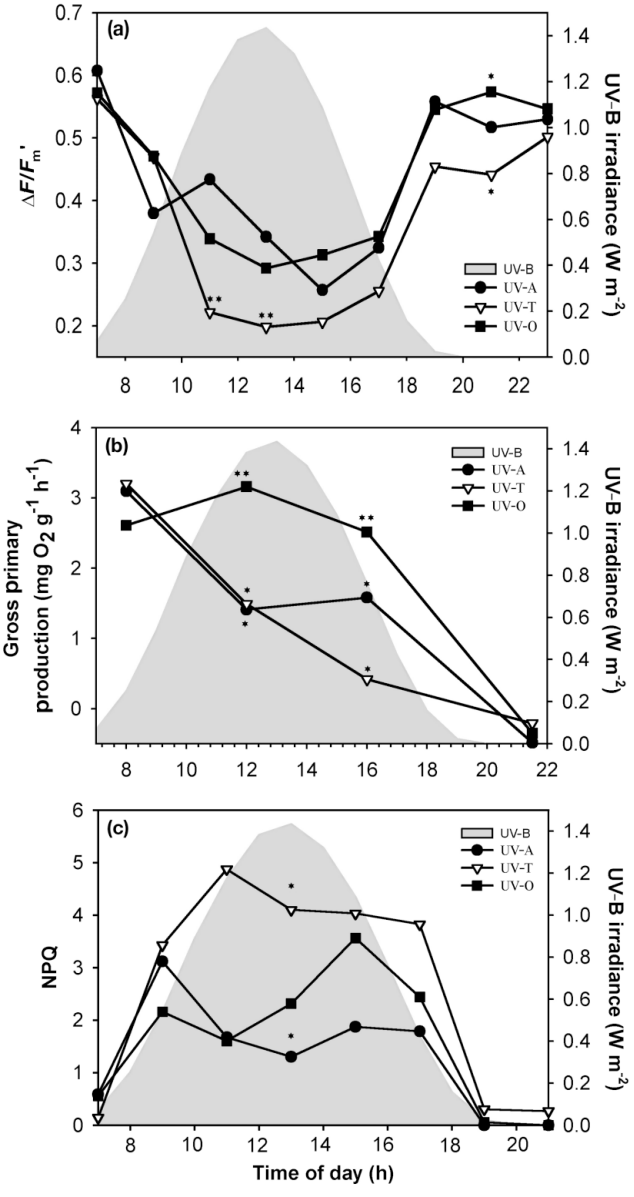

Fig. 7. *Laminaria saccharina.* Diel changes (laboratory experiments) in (a) steady-state quantum yields of PS II fluorescence ($\Delta F/F_m'$); (b) gross primary production; (c) non-photochemical quenching (NPQ) of treatment groups (N = 5 per treatment). *,**: statistically significant intra- and intertreatment differences (SNK: $p < 0.05$). Further details as in Fig. 6

$p < 0.05$) compared to the UV-O treatment (Fig. 7b). Comparison of the $\Delta F/F_m'$ and GPP measurements shows that the UV-O treatment group did not sustain any decrease in midday photosynthetic rates, despite a midday depression in $\Delta F/F_m'$ that is characteristic of dynamic photoinhibition. The UV-T and UV-A treatments, however, show a significant decrease in GPP, indicating some damage to PSII reaction centers (also, an increase in F′; data not presented), and also exhibited dynamic photoinhibition. While the UV-T treatment exhibited the lowest $\Delta F/F_m'$ and decreased GPP at midday, it also displayed significantly higher (ANOVA: $p = 0.002$; SNK: $p < 0.05$) NPQ coefficients compared to the UV-A and UV-O treatments (Fig. 7c).

Significant differences in pigment concentrations were observed between treatments (Fig. 8). Chlorophyll *a* (ANOVA: $p = 0.009$) concentration was significantly lower in the UV-T treatment than the UV-A and UV-O treatments, which were indistinguishable from each other (SNK: $p < 0.05$), as were the concentrations of chlorophyll c_2 (ANOVA: $p < 0.0001$, SNK: $p < 0.05$). The β-carotene (ANOVA: $p = 0.005$) concentration was significantly higher in the UV-T treatment than the UV-A and UV-O treatments, which again were indistinguishable from each other (SNK: $p < 0.05$). No differences were observed in fucoxanthin or violaxanthin concentrations, but zeaxanthin was significantly (ANOVA: $p < 0.0001$) greater in the UV-T treatment (SNK: $p < 0.05$) than the UV-A and UV-O treatments (SNK: $p < 0.05$). Violaxanthin/zeaxanthin ratios showed significant treatment effects (ANOVA: $p = 0.001$) with all treatment groups (UV-T: 0.04 ± 0.01 SE, UV-A: 0.08 ± 0.01 SE, UV-O: 0.12 ± 0.01 SE) significantly (SNK: $p < 0.05$) different from each other.

As in the field experiments, only mycosporine-glycine was found in samples of *Laminaria saccharina*, and significantly (ANOVA: $p = 0.0001$) higher concentrations were observed in the UV-T (81.3 ± 16.2 SE nmol mg^{-1} protein) than the UV-O (16.5 ± 3.4 SE nmol mg^{-1} protein) and UV-A (21.4 ± 5.9 SE nmol mg^{-1} protein) treatments.

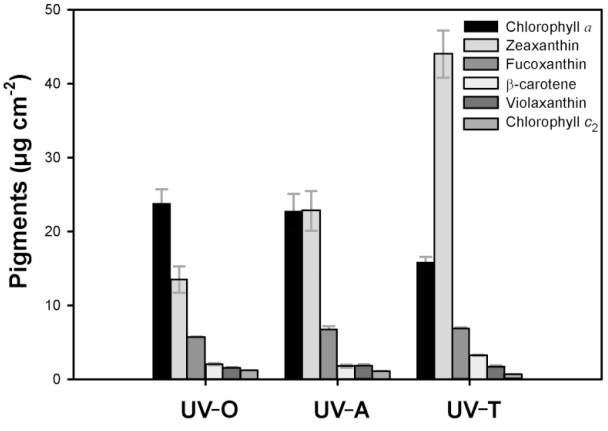

Fig. 8. *Laminaria saccharina.* Photosynthetic pigment concentrations (mean ± SE; N = 5) in laboratory samples (N = 5) from UV-T. Further details as in Fig. 6

DISCUSSION

To determine the effects of exposure to solar radiation on the photobiology of *Laminaria saccharina* in the Gulf of Maine we investigated those variables affecting the amount of PAR and UV-R reaching populations of algae at different depths. These variables included the spectral diffuse attenuation coefficient (k_d), changes in daily cloud cover, depth of algae, pigment content, and tidal influences. The high attenuation coefficients observed in these coastal waters are typical of Case II waters (Kirk 1994) but still allow UV-R, and in particular UV-A, to penetrate to depths ≥5 m. Similar spectral attenuation coefficients and UV-R irradiances have been reported for an Arctic fjord (Hanelt et al. 2001), where populations of *L. saccharina* also occur. Shallow *L. saccharina* are consistently more susceptible to overexcitation of the photosynthetic apparatus, and exhibit significant midday decreases in steady-state quantum yields consistent with dynamic photoinhibition regardless of tidal state. This is further supported by the significantly lower violaxanthin/zeaxanthin ratios in shallow algal populations that suggests the interconversion of violaxanthin to zeaxanthin and a functioning xanthophyll cycle (Demming-Adams & Adams 1992), as previously reported to occur in *L. saccharina* (Gevaert et al. 2002). Algae at deeper depths require either low tides and/or sunny days to exhibit similar decreases in steady-state PSII quantum yields. Decreases in midday dark-adapted quantum yields and co-occurring increases in NPQ have also been observed in experiments by Gevaert et al. (2002) during a simulated tidal cycle with European populations of *L. saccharina*. Changes in cloud cover also have a significant effect on shallow populations of *L. saccharina*, as shown by the daily morning measurements of $\Delta F/F_m'$, which improve significantly for shallow algae on cloudy days. This shows that cloud cover relaxes the excitation pressure on these shallow algae but still provides enough photons to saturate photosynthesis. These short-term responses to changes in irradiances of solar radiation are similar to the longer-term seasonal changes observed for populations of *L. saccharina* in an Arctic fjord that is affected by ice cover and changes in the optical properties of the water column caused by glacial melting (Bischof et al. 2002).

Laminaria saccharina populations in this study showed an inverse relationship between the midday depression in $\Delta F/F_m'$ and NPQ; this strongly supports

an important photoprotective role for dynamic photoinhibition. The diel studies from the laboratory, however, also suggest that shallow field populations of *L. saccharina* experience chronic photoinhibition, despite having significantly higher concentrations of the MAA mycosporine-glycine that has a maximum absorption in the UV-B portion of the spectrum (310 nm). The observed changes in steady-state quantum yields in the field are consistent with recent work on scleractinian corals and changes in the underwater light field as a function of depth (Lesser & Gorbunov 2001). Similar results on *L. saccharina* from the Arctic have also been reported (Hanelt 1998, Bischof et al. 2002).

The $\Delta F/F_m'$ of shallow field populations rarely recovers fully at night, even when UV-B irradiance, and also PAR, are reduced by 50%. Using the dark-adapted quantum yields of PSII fluorescence (F_v/F_m) from the diel studies in the field, one can calculate the fraction of inactive PSII reactions centers using the relationship $1 - (F_v/F_m)/0.65$, as described by Falkowski & Kolber (1995) for phytoplankton but utilizing a maximum quantum yield of 0.75 as described for macrophytes (Dring et al. 1996b). Such calculations for the low-tide and high-tide diel studies reveal that the fraction of inactive PSII reaction centers for shallow populations of *Laminaria saccharina* ranges from 43 to 49%, with higher numbers during the shallower tides. We also calculated that 37 to 40% of the reaction centers are inactive in mid and deep algal populations. One explanation for this is that during the late spring and summer, when these studies were conducted, these macrophytes are nitrogen-limited; this would affect protein turnover and the number of functional PSII reaction centers (Korb & Gerard 2000).

To partition out the effects of PAR versus UV-R on the photobiology of *Laminaria saccharina* and to determine whether UV-B is responsible for the chronic photoinhibition observed in shallow field populations, we conducted laboratory experiments using natural solar radiation. The results of the diel laboratory study indicate that dynamic photoinhibition from high midday PAR irradiances occurred in all treatments. This is supported by the changes in the violaxanthin/zeaxanthin ratios at midday in both the field and laboratory and by changes due to xanthophyll cycling previously observed in this alga (Gevaert et al. 2002). This type of photoinhibition is characterized by a quick recovery, and is in agreement with other studies on brown macrophytes (Hanelt et al. 1993, Hanelt 1998). The *L. saccharina* in our laboratory study exposed to UV-B irradiances in addition to PAR and UV-A displayed components of both dynamic and chronic photoinhibition. During the midday depression in $\Delta F/F_m'$, a large increase in non-photochemical quenching occurred, suggesting dynamic photoinhibition. Additionally, during the period of peak UV-R exposure, there was a significantly greater depression in $\Delta F/F_m'$ for UV-T treatments, and the $\Delta F/F_m'$ of UV-T-exposed algae took longer to recover at night than either the UV-O or UV-A treatments — symptomatic of chronic photoinhibition. Lastly, the UV-T-treated algae showed higher minimum fluorescence yields in the dark (F_o) and during exposure to ambient irradiance (data not shown), indicative of damage to PSII reaction centers (Osmond 1994). The UV-T-treated algae also exhibited a decrease in gross primary production, as did the UV-A treatment, which is also symptomatic of damage to PSII. These results are in contrast to experiments using artificial UV-R lamps (Q-Panel), where 6 species of brown and red macrophytes showed very few instances of UV-R-induced photoinhibtion when assessed using dark-adapted chlorophyll fluorescence yields (Dring et al. 2001). The results reported here are, however, in agreement with those of many studies on macrophytes, in particular on *L. saccharina* (Hanelt et al. 1997, Bischof et al. 1998a,b, Brouwer et al. 2000, Karsten et al. 2001). Damage to PSII reaction centers can be caused directly by UV-R (Greenberg et al. 1989) or indirectly by the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$) (Richter et al. 1990). The induction of enzymatic antioxidant defenses (e.g. superoxide dismutase) to prevent the formation of ROS has been demonstrated in phytoplankton (Lesser 1996a), the dinoflagellate symbionts of corals (Lesser 1996b) and recently for macrophytes, including *L. saccharina* (Aguilera et al. 2002). This important photoprotective response can still be overwhelmed by the direct and indirect damage caused by UV-R. Field and laboratory samples of *L. saccharina* contain the MAA mycosporine-glycine at high concentrations; this MAA has been shown to have an antioxidant activity (Dunlap & Yamamoto 1995), but the presence of this UV-B-absorbing compound does not appear to fully protect these algae from damage to PSII.

Our field and laboratory studies indicated that photoinhibition under PAR exposure elicits a dynamic photoinhibition response that does not affect primary production. However, there is also a UV-R effect that results in chronic photoinhibition and a decrease in primary production. These processes would be exacerbated in shallow algal populations during changes in local weather conditions, tides, or optical properties of the water column that favor increased UV-R irradiances.

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