Physiological acclimation to gradients of solar irradiance within mats of the filamentous green macroalga *Chaetomorpha linum* from southern Spain

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ABSTRACT: *Chaetomorpha linum* (Müller) Kützing is a dominant mat-forming green macroalgal species within eutrophic systems. Its structure, comprising thick mats of interwoven filaments, requires acclimation to either high or low irradiance along a steep gradient of light arising from self-shading. Occurring in high-light environments, such as that along the southern coast of Spain, *C. linum* mats represent an excellent model system for studying spatial differences in photoacclimation and also the interactive effects of photosynthetically active and ultraviolet radiation on photoinhibition. This study was conducted in eutrophic brine-pond systems along the Bay of Cádiz (southern Spain). For periods of up to 1 wk, *C. linum* mats were exposed to natural solar radiation under different cut-off filters to study the effects of different wavelength ranges on photosynthetic activity, pigment and protein composition, and the occurrence of oxidative stress and changes in enzymatic activity of superoxide dismutase (SOD). The results revealed a particularly high tolerance of *C. linum* photosynthesis to high light and UV exposure. Addition of the UV-B range (280 to 315 nm) to photosynthetically active radiation, resulted in inhibition of non-photochemical quenching, a delay in recovery from photoinhibition, and an increase in both lipid peroxidation and SOD activity. In contrast, pigment and protein data revealed acclimation to the high irradiances of photosynthetically active radiation, but no additional effect of UV radiation. The data suggest that even in a high-radiation environment such as southern Spain, UV-B radiation plays a minor role in photoacclimation of *C. linum*, but is effective in increasing both the generation and scavenging of oxidative stress. The UV-induced increase in SOD activity represents an important physiological acclimation mechanism of *C. linum*, enabling it to thrive under high UV radiation and high-light conditions.

KEY WORDS: *Chaetomorpha linum* · Photosynthesis · Superoxide dismutase · Ultraviolet radiation

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INTRODUCTION

Eutrophication of estuaries and coastal soft-bottom ecosystems favour the development of mats of ephemeral green macroalgae (Hernández et al. 1997, Taylor et al. 2001, Cohen & Fong 2004), thus increasing competition for essential resources, such as solar radiation, for other photosynthesising organisms. As a result of shading, macroalgal canopies strongly contribute to the decline of eelgrass *Zostera marina* in temperate estuarine ecosystems (Hauxwell et al. 2001, McGlath-

ery 2001). Because of their increasing ecological impact, green macroalgae blooms have been intensively studied, primarily with respect to their nutrient-uptake dynamics (e.g. Vergara et al. 1997, 1998, Krause-Jensen et al. 1999, Taylor et al. 2001, Malta et al. 2003, Cohen & Fong 2004). However, the enormous ecological success of green mat-forming macroalgae is due to their physiological responses to a combination of various abiotic factors, characterising their purely opportunistic life strategy (Cohen & Fong 2004). Typically, during mass development, bloom-forming green algae

form canopies of mat-like structures (e.g. Krause-Jensen et al. 1996, Vergara et al. 1997, Bischof et al. 2002b). Within these structures, steep gradients of light persist due to self-shading effects. Thus, the individuals comprising the mat must acclimate to either high or low light conditions (Vergara et al. 1998). In particular, the top layers of these structures are exposed to surface irradiance that may include high levels of photosynthetically active radiation (PAR) and also UV radiation. The potential effects of UV radiation on photosynthesis of algae have already been studied on specimens from polar, temperate and tropical regions (see Franklin & Forster [1997] and Häder & Figueroa [1997] for reviews). Laboratory studies tend to overestimate UV effects due to the unnatural spectrum of experimental radiation conditions used, and thus are of limited use in ecological interpretations. Furthermore, most studies rarely discriminate between the inhibitory effects of UV radiation and of high irradiances of PAR. Thus, field experiments examining the effects of different wavelength ranges on algal photosynthesis are most valuable. In a study on the differential effects of PAR and UV radiation on the green alga *Ulva rotundata* from southern Spain, it was shown that both wavelength ranges act synergistically to induce pronounced photodamage, while exposure of samples to either PAR or UV radiation alone does not impair photosynthesis in this species (Bischof et al. 2002b, 2003). Individual thalli of *U. rotunda* are very sensitive to solar radiation, but the arrangement of its thallus layers in mat-like structures effectively shields subcanopy individuals from harmful radiation.

The filamentous green alga *Chaetomorpha linum* (Müller) Kützing is another abundant macrophyte inhabiting eutrophic soft-bottom brine-pond systems along the shallow coastal zone of southern Spain. At these sites, algae have to cope with high nutrient loads, high temperature and high solar irradiance (PAR *and* UV radiation). At our study site, *C. linum* also forms cushion-like mats of interwoven filaments with a thickness often extending to 50 cm. Previous studies on *C. linum* mats mostly focussed on the effect of nutrient loads on algal production (Krause-Jensen et al. 1996, 1999, Menendez et al. 2002). The information available on photoacclimation of *C. linum* (Krause-Jensen et al. 1996, McGlathery & Pedersen 1999) mainly derives from laboratory experiments. Only few data are available from field experiments or on the effect of UV radiation on photoacclimation on *C. linum* mats (see Gómez et al. 2004).

High levels of PAR and UV radiation can impair the photosynthetic apparatus in various ways. While excessive PAR irradiance is harmful due the potential increase in oxidative stress (Asada & Takahashi 1987, Andersson et al. 1992), the harmful effects of solar UV-B exposure include direct damage to proteins, pigments and nucleic acids, thus significantly impairing photosynthesis (see Bornman [1989] and Vass [1997] for reviews) and growth. Knowledge on how protective mechanisms enable algae from intertidal and shallow waters to resist high levels of PAR and UV-B is still limited. One of the primary consequences of impaired photosynthesis is elevated production of reactive oxygen species (ROS), and it is likely that appropriate protection and scavenging mechanisms against ROS play an important role in algae in high-light environments (Aguilera et al. 2002, Dummermuth et al. 2003). In previous laboratory experiments, an arctic isolate of *Chaetomorpha linum* was characterised as particularly tolerant to oxidative stress (Dummermuth et al. 2004), but data on its performance under field conditions are still lacking.

In contrast to the single thalli of *Ulva rotundata*, at our study site bleached filaments of *Chaetomorpha linum* are rarely observed under conditions of high irradiation in the field, indicating the higher tolerance of the latter species to high solar irradiance. Thus, different acclimation and/or adaptation mechanisms allowing them to thrive under identical abiotic conditions may be present in these 2 species; such mechanisms have not previously been explored.

Herein we report the results of the first field study on the effects of high PAR and UV radiation on this dominant green alga species from a site in southern Spain. Filaments of *C. linum* were arranged into artificial mats covered by screening filter foils, and a wide range of physiological parameters was studied to elucidate the impact of UV and high PAR on algal photosynthesis. Photosynthetic activity, the composition of photosynthetic and xanthophyll cycle pigments, the amount of D1-protein, the activity of ROS-scavenging superoxide dismutase (SOD), and the impact of oxidative stress, measured as lipid peroxidation, were assessed along the depth gradient within the artificial mats.

MATERIALS AND METHODS

Study site. The experiment was conducted between 28 August and 7 September 2003 in the creeks of the 'Salinas de la Calavera' (36° 25' N, 6° 13' W; property of Acuinova, San Fernando, Spain) within the 'Parque Natural Bahia de Cádiz', Spain. A detailed description of the study site has been given by Bischof et al. (2002b). Within the creek chosen, salinity varied between 35 and 42, depending on tidal conditions. Water temperature changed according to tidal conditions and solar insolation in the range of 24 to 31°C. Vertical attenuation coefficients (k_d) of downward PAR varied between 0.7 and 1.2 m^{-1}, as determined by par-

allel irradiance measurements conducted in different water depths using 2 cosine-corrected flathead underwater sensors (Li 192 SA, Licor Quantum) connected to a Licor Li-1000 data logger. During the experimental period, sunny and cloudless conditions prevailed. Maximal solar irradiance of up to 2370 µmol photons m^{-2} s^{-1} PAR (approx. 515 W m^{-2}, 400 to 700 nm), 79 W m^{-2} UV-A (315 to 400 nm) and 1.6 W m^{-2} UV-B (280 to 315 nm) was measured. Solar UV radiation was determined by a broadband radiometer (RM-21, Gröbel).

Algal material. At the study site, *Chaetomorpha linum* forms extensive mats of interwoven filaments floating on the water surface. For the experiment, artificial mats were designed as follows (see Fig. 1): well-pigmented filaments were chosen from the understory and arranged between 2 layers of nylon mesh (cut to an appropriate size to keep the thalli in the desired position). The size of each mat was approx. 30 × 30 cm with an average thickness of 3 cm. The artificial mats had the same algal density as natural mats (see Fig. 2). We tested 4 different radiation treatments using filter foils: Ultraphan 395 (Digefra) for the PAR treatment (excluding UV-A and UV-B), and Folex 320 (Folex) for the PAR + UV-A exposure (thus excluding UV-B). Additionally, Ultraphan 295 was used to correct for the decrease in irradiance produced by the plastic filter foils. The effects of the respective foils on the spectral distribution of solar radiation have been previously published (Pérez-Rodríguez et al. 1998). Upon solar exposure, the temperature underneath the filter foils increased moderately by 2 to 3°C compared to uncovered algal stands. However, artefacts resulting from increased temperature can be excluded, since for none of the parameters tested (effective as well as maximal quantum yield, photosynthetic pigments) significant differences were found between samples receiving unfiltered radiation and those under the Ultraphan 295 filter. Therefore, data from the 'unfiltered' treatment are not presented in the 'Results' section; the data for the PAR + UV-A + UV-B treatment derive from samples under the Ultraphan 295 filter. We used 6 artificial canopies per treatment, and exposed them on 2 experimental floats designed as follows: the individual mats were fixed into a floating system of aluminium frames attached to buoys, enabling uniform exposure at the water surface and ensuring exact positioning of the canopies throughout the exposure period (Fig. 1). After 5 d (2 September) and 8 d (5 September) exposure, samples from the surface, middle and lower parts of each mat were collected for measurement of photosynthetic activity (maximal quantum yield, F_v/F_m; effective quantum yield, $\Delta F/F_m'$, photosynthesis-irradiance-curves; oxygen production), and after 8 d the mats were sampled again for physiological analyses

(pigments, proteins, activity of superoxide dismutase [SOD] and malondialdehyde [MDA] formation). Collection of material during the course of the experiment resulted in substantial thinning of the mats and thus increased solar exposure of the remaining material. To minimise this artefact, we did not collect material from all replicate treatments simultaneously, but harvested the different mats sequentially at different sampling times.

Photosynthetic activity. F_v/F_m was measured by pulse amplitude-modulated (PAM) flourometry using a PAM 2000 chlorophyll fluorometer (Walz). Samples were dark-acclimated in a black plastic box with a hole in the lid through which the fibre optic of the fluorometer was inserted. The general protocol for F_v/F_m determinations followed that of Hanelt (1998). Each F_v/F_m determination used 5 replicates. Subsequently, rapid-light curves were recorded according to Schreiber et al. (1994) and White & Critchley (1999). The halogen lamp integrated in the fluorometer was used as actinic light source: actinic irradiance ranged from 65 to 3000 µmol m^{-2} s^{-1} and was gradually increased at intervals of 30 s. From the recorded curves, we extracted the parameter NPQ (non-photochemical quenching), and compared NPQ values for all samples at an actinic irradiance of 2150 µmol photons m^{-2} s^{-1} (corresponding to Illumination Step 9 during the rapid-
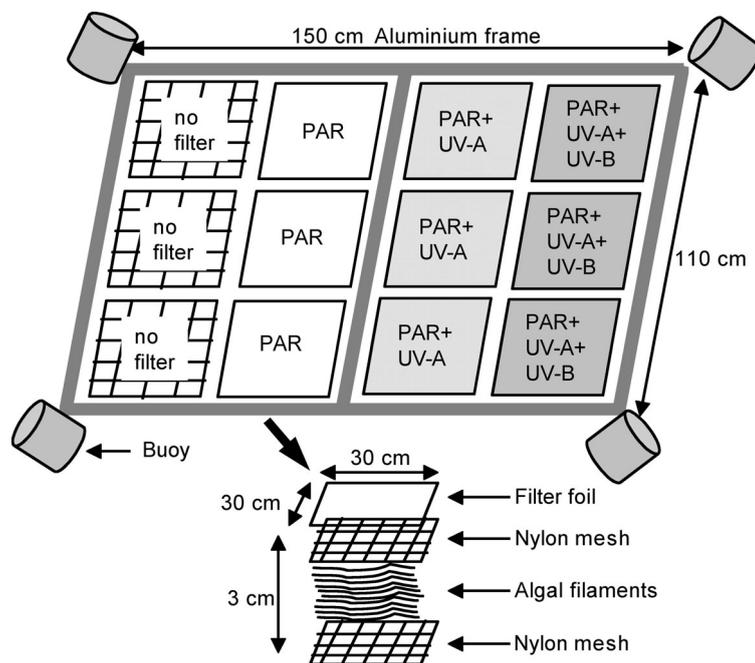

Fig. 1. Floating experimental set-up consisting of aluminium frames attached to buoys and holding 12 artificial mats of *Chaetomorpha linum*, either covered with nylon mesh only ('no filter'), or with different filter foils. Filter foils were held in desired position by tie-wraps

light curve). Because of the length of time needed to conduct each measurement, only 1 P-I curve could be recorded under each sampling condition. However, repeated records on different days and at different times of the day revealed the uniformity of NPQ patterns from the various treatments. *In situ* measurements of effective quantum yield of photosynthesis ($\Delta F/F_\mathrm{m}'$) were performed using an underwater chlorophyll fluorometer ('Diving-PAM', Walz, Effeltrich; see Beer et al. 2000 for details). At each sampling time, the fibre optics measured the top (0 cm), intermediate (~1.5 cm) and bottom (~3 cm) regions of the mats; approximately 40 replicate measurements were made. During data processing, all measurements performed at fluorescence (Ft) signals below 100 relative units were excluded from calculations, as low fluorescence signals may result in low accuracy of measurements. Consequently, the number of replicates used for analysis varied between 15 and 40. As the micro light sensor of the Diving-PAM was used to record light attenuation within natural and artificial algal populations, it was previously intercalibrated with the Licor sensor system. Gradients of dissolved oxygen within the mats were measured using a needle-type microelectrode system (Microscale Measurements), mounted on top of a rubber boat manoeuvred into the desired position. Oxygen electrodes were attached to a tripod rammed into the mud surface of the creek and lowered in steps of 0.5 cm. Calibration of the oxygen electrodes was conducted according to the manufacturer's instructions.

Biochemical analysis. For biochemical analysis, algal material was collected from the different radiation treatments, immediately frozen in liquid nitrogen and stored at −80°C until analysis. Changes in photosynthetic pigment composition (chlorophyll *a + b*, β-carotene, lutein) and pool sizes of the xanthophyll cycle were analysed by HPLC as described by Bischof et al. (2002a). Pigment data were obtained from independent triplicate samples. Crude extracts of samples for protein analysis were prepared, and protein separation by sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) and subsequent detection of the D1 reaction centre protein of photosystem II was conducted by Western blotting. For detection of D1-protein, global primary antibodies (Agrisera) raised in chicken eggs were used. Secondary immunodecoration was performed using a polyclonal alkaline phosphatase-conjugated rabbit-to-chicken antibody (Abcam). For quantification, blots were scanned by an EASY-Win 32 gel documentation system (Herolab) and relative differences in the abundance of D1 were analysed with image-analysis software (EASY Win). The activity of SOD was measured applying a photometric enzyme assay, following the xanthine-oxidase-cytochrome c

reduction method (McCord & Fridovich 1969). Frozen samples were ground to a fine powder and extracted in a 50 mM Kpi buffer containing 50 mM KH_2PO_4 with 0.1 mM EDTA at pH 7. Activity of SOD was measured in a spectrophotometer at 550 nm after the addition of 0.06 mM xanthine (Sigma X-0626), 4.5 U xanthine oxidase (Sigma X-4875) ml^{-1}, and 10 µM cytochrome *c* (Merck 124804). Enzyme activity was expressed as units per mg of total soluble protein (TSP). The protein content of the extracts was determined using a commercial protein assay (Bio Rad) and calculated according to a calibration curve prepared with known concentrations of bovine serum albumin. Finally, as an indication of oxidative stress, lipid peroxidation was studied as MDA formation. The original procedure described by Heath & Packer (1968) was modified according to Bischof et al. (2003). Results are expressed as µmol MDA g^{-1} fresh wt. Measurements of SOD activity and MDA formation were performed on triplicate samples.

Data treatment. Means and standard deviations were calculated from the respective replicates. To compare the effects of the correlated independent variables (radiation treatment, vertical position within the mat and time of day), data sets were analysed by MANOVA. When only 2 of the independent variables were compared, 2-way ANOVA was applied. In addition, post-hoc comparisons of means were applied. Significance level was set at $p < 0.05$ (Sokal & Rohlf 1995). Calculations were performed using Statistica 6.0 (Stat Soft).

RESULTS

Photosynthetic performance

Within the natural *Chaetomorpha linum* mats, a steep gradient in irradiance persists (Fig. 2). A rapid decline in PAR resulted in a 1% depth of surface irradiance at 9.27 cm, indicating high algal density of the natural mat. Due to the differences in light availability, the resulting variation in photosynthetic activity and the vertical diffusion of dissolved oxygen, well pronounced gradients of dissolved oxygen concentration were also measured within natural and artificial mats (see e.g. Fig. 3). The dissolved oxygen concentration strongly depends on the salinity and temperature of the water. We recorded values of 10 mg O_2 l^{-1} in the upper layer down to 3.8 mg O_2 l^{-1} at approx. 2.5 cm depth inside the mats. Measured values did not differ significantly between the different radiation treatments. In the example in Fig. 3, the exponential decrease in oxygen concentration can be described by a logarithmic function. However, the measured oxygen
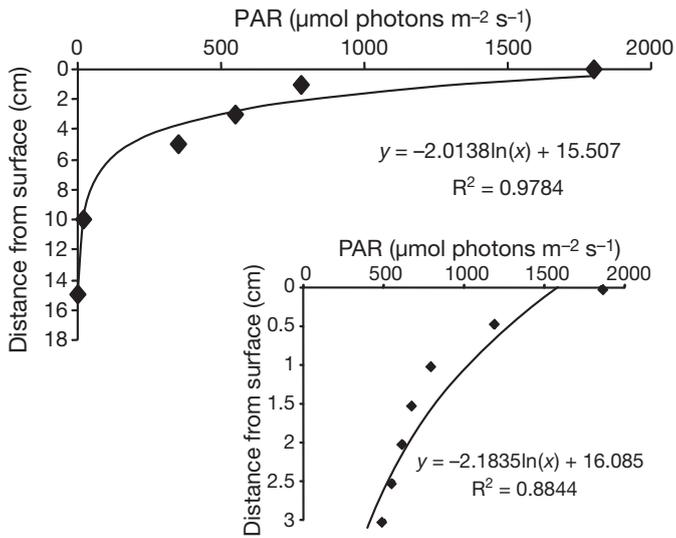
Fig. 2. Gradient of photosynthetically active radiation (PAR) within natural mats of *Chaetomorpha linum* measured by the light sensor of an underwater chlorophyll fuorometer (Diving-PAM) previously intercalibrated with Licor sensors. Inset: light gradient in artificial algal mats
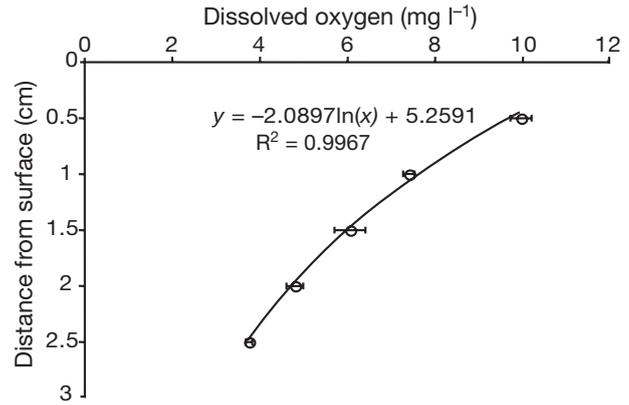

Fig. 3. *Chaetomorpha linum*. Typical gradient of dissolved oxygen in artificial mats measured by microelectrodes. Example is for mats under PAR + UV-A treatment on 5 September at 14:00 h. Note: oxygen content shown is product of photosynthesis and respiration within thallus fragments, but is also affected by water motion and diffusion

does not directly reflect photosynthetic activity, as only the oxygen content of the surrounding seawater was measured and not the photosynthetic oxygen evolution of the algal filaments. Therefore, we regarded chlorophyll fluorescence measurements more suitable for assessing photosynthetic activity within the algal mat.

Measurements of $\Delta F/F_m'$ conducted by the Diving-PAM reflected the steep gradient in irradiance within the mats (Table 1). Significant effects of radiation treatment, position inside the mat and time of day were found on both sampling days (Table 2). Yield differed as a function of the position of the algal filaments inside the mat, with the upper layers generally exhibiting lower values than the shaded specimens at the bottom of the mat. In accordance with the diurnal changes in irradiance, the slope of the yield gradient altered during the course of the day. At 14:00 h, yield of specimens in the upper layers was much lower than in the morning, while that of specimens at the bottom of the mat remained fairly unchanged. For morning (09:00 h) measurements, statistical analysis revealed a significant UV effect on the reduction of quantum yield in the upper layers; values for samples receiving no UV (i.e. receiving PAR alone) were significantly (p <

0.05) higher than values for samples exposed to PAR + UV-A either with or without UV-B. The difference diminished during the course of the day. In the early afternoon, effective quantum yield in top layers was even further reduced in all treatments, but there were no significant differences among the radiation treatments, indicating a relative increase in PAR-induced reduction of quantum yield. A significant interaction of radiation conditions and time of day was found on 5 September, but not on 2 September (Table 2). This result was supported by another MANOVA, in which

Table 1. *Chaetomorpha linum*. Effective quantum yield ($\Delta F/F_m'$) along the depth gradient of artificial mats under various radiation conditions *in situ* on 2 and 5 September 2003 at 09:00 and 14:00 h (n = 15 to 40). Values are mean ± SD; different superscripts indicate significantly different values (p < 0.05) determined by post-hoc comparison. Average thickness of artificial mats was 3 cm; samples were collected from top (~0 cm), middle (~1.5 cm) and bottom (~3 cm) depths. PAR: photosynthetically active radiation

Sample		PAR + UV-A + UV-B	PAR + UV-A	PAR
2 September				
09:00	top	0.470[b] ± 0.214	0.504[b,c]± 0.135	0.619[c] ± 0.073
	middle	0.590[c] ± 0.209	0.669[d] ± 0.146	0.700[d,e]± 0.068
	bottom	0.680[d] ± 0.138	0.634[c,d]± 0.213	0.762[e] ± 0.036
14:00	top	0.279[a] ± 0.088	0.264[a] ± 0.077	0.402[a,b]± 0.129
	middle	0.411[a,b]± 0.106	0.475[b] ± 0.158	0.539[b,c]± 0.141
	bottom	0.590[c] ± 0.180	0.637[c,d]± 0.090	0.699[d] ± 0.035
5 September				
09:00	top	0.461[b] ± 0.206	0.475[b] ± 0.154	0.618[c] ± 0.070
	middle	0.672[d] ± 0.133	0.657[c,d]± 0.143	0.696[d] ± 0.068
	bottom	0.647[c,d]± 0.16)	0.641[c,d]± 0.220	0.759[e] ± 0.035
14:00	top	0.278[a] ± 0.084	0.268[a] ± 0.087	0.381[a,b]± 0.101
	middle	0.425[a,b]± 0.116	0.454[b] ± 0.141	0.531[b,c]± 0.130
	bottom	0.628[c,d]± 0.163	0.619[c] ± 0.129	0.705[d,e]± 0.041

Table 2. Analysis of variance (MANOVA) on effect of radiation treatment, vertical position within mat and time of day on effective quantum yield ($\Delta F/F_m{}'$) of photosynthesis within artificial mats of *Chaetomorpha linum* for 2 and 5 September 2003; corresponding yield measurements and post-hoc comparisons are shown in Table 1. In a second MANOVA, date of sampling was included as additional independent variable and revealed a significant interaction of date and radiation treatment (df: 2; *F*: 19.79; p: <0.001)

Variable	df	*F*	p
2 September			
Radiation treatment	2	12.47	<0.001
Vertical position	2	48.95	<0.001
Time of day	1	58.95	<0.001
Radiation × Time	2	0.023	0.978
Radiation × Position	4	0.013	0.609
Time × Position	2	10.28	<0.001
Radiation × Time × Position	4	0.734	0.570
Error	191		
5 September			
Radiation treatment	2	8.20	<0.001
Vertical position	2	67.98	<0.001
Time of day	1	57.37	<0.001
Radiation × Time	2	3.48	0.032
Radiation × Position	4	4.17	0.003
Time × Position	2	8.29	<0.001
Radiation × Time × Position	4	9.15	<0.001
Error	447		

Fig. 4. *Chaetomorpha linum*. Mean (+SD) differences in maximal quantum yield of photosynthesis (F_v/F_m) in surface layers of artificial mats exposed to solar PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) or PAR (P) alone, measured in morning (09:00 h) and afternoon (14:00 h) of 2 and 5 September (n = 5). Different letters above bars indicate significantly different values (p < 0.05) determined by post-hoc comparison

the date of sampling was included as an additional independent variable: a significant interaction of sampling date and radiation conditions was found (see Table 2 legend).

In contrast to the effective quantum yield, F_v/F_m was maintained at remarkably high levels throughout the study period, even in the surface layers of the mats (Fig. 4). High F_v/F_m values (approx. 0.7) measured in the morning on both sampling days under all radiation treatments indicated that the algae had completely recovered from any photoinhibitory event during the previous day. Because of effective self-shading, F_v/F_m in specimens at the bottom of the mats was not inhibited at any time (data not shown). Differences in the maximal quantum yield of photosynthesis were found in the upper layers as a function of the different radiation conditions applied. In contrast to the morning (09:00 h) values, measurements in the early afternoon (14:00 h) revealed inhibitory effects of solar radiation on F_v/F_m. In particularly, F_v/F_m measured in the early afternoon of 5 September revealed a significant UV-B effect on the inhibition of maximal quantum yield (p < 0.05), which was 36% less than values measured in the morning and 27% below values measured at the same time for samples receiving PAR + UV-A but not UV-B. In contrast, F_v/F_m values in samples kept either under PAR alone or under PAR + UV-A did not differ
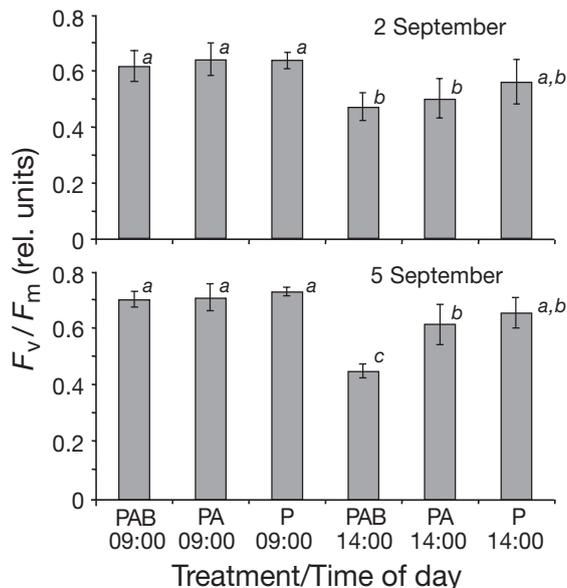
Table 3. Analysis of variance (MANOVA) on effect of radiation treatment, vertical position within mat and time of day on maximal quantum yield (F_v/F_m) of photosynthesis within artificial mats of *Chaetomorpha linum* for 2 and 5 September 2003; corresponding F_v/F_m measurements and post-hoc comparisons are shown in Fig. 4. In a second MANOVA, date of sampling was included as an additional independent variable and revealed no significant interaction of date and radiation treatment (df: 2; *F*: 0.600; p: 0.555)

Variable	df	*F*	p
2 September			
Radiation treatment	2	2.39	0.113
Vertical position	1	121.9	<0.001
Time of day	1	25.31	<0.001
Radiation × Time	2	0.470	0.631
Radiation × Position	2	1.09	0.350
Time × Position	1	10.37	0.004
Radiation × Time × Position	2	1.58	0.226
Error	24		
5 September			
Radiation treatment	2	10.77	<0.001
Vertical position	1	111.1	<0.001
Time of day	1	48.42	<0.001
Radiation × Time	2	6.67	0.005
Radiation × Position	2	9.00	0.001
Time × Position	1	30.95	<0.001
Radiation × Time × Position	2	5.90	0.008
Error	16		

Table 4. *Chaetomorpha linum*. Effect of radiation conditions on extent of non-photochemical quenching (NPQ). NPQ data extracted from rapid-light curves; readings from all sampling times pooled to provide more solid database. Mean ± SD values are shown at irradiance of 2150 µmol photons $m^{-2} s^{-1}$, corresponding to Illumination Step 9 of rapid-light curves. NPQ under exposure to PAR + UV-A and to PAR + UV-A + UV-B calculated as percentage NPQ in PAR-exposed samples; results of 1-way ANOVA are also given

	NPQ	% NPQ$_{PAR}$
PAR + UV-A + UV-B	0.953 ± 0.519	75.78
PAR + UV-A	1.209 ± 0.675	96.15
PAR	1.257 ± 0.617	100
	df	*F*	p
ANOVA	1	26.13	0.024

significantly from each other and showed only a minor decrease (down to 12%) compared to morning values. Overall, analysis of variance revealed no significant effect of the respective radiation conditions on 2 September, but a strong effect on 5 September (Table 3). Also, significant interactions of radiation and position and time of day were recorded on 5 September but not on 2 September. In contrast, the pooled data showed no significant interaction between radiation conditions and date of sampling (see Table 3 legend).

Non-photochemical quenching (NPQ) extracted from recorded P-I curves indicated variations with respect to quantitative and qualitative changes in irradiance (Table 4). Data pooled over the whole experimental period indicate a significant effect of radiation conditions on NPQ, namely a 25% reduction of NPQ values in samples receiving the whole spectral range compared to those receiving no UV. Generally, samples

exposed to UV-B exhibited lower NPQ values than those in the PAR and PAR + UV-A treatments collected at the same time.

Biochemical parameters

Pigment composition was mostly affected by the position of filaments inside the mat, and to a lesser degree by radiation conditions (Table 5). Under all radiation treatments similar patterns were found, i.e. a 2- to 3-fold higher pigment concentration in samples from the bottom layers than in those from the upper layers. This pattern was found for chlorophyll *a + b*, β-carotene and xanthophylls, but not for lutein, for which the opposite was true: under all treatments, higher concentrations were always found in the upper layers. For all pigments but lutein, a significant interaction of radiation and vertical position inside the mat on pigment concentration was found (Table 6). With the exception of chlorophyll *b*, pigment concentrations in samples from the bottom of the mat were not significantly different from the concentration in the material initially sampled from the subcanopy (Table 5). Changes in the ratio of chlorophyll *a*:*b* were analysed but did not reveal specific responses to the experimental treatments.

No significant differences in D1-protein content were detected linked to radiation treatments or to the respective position of filaments within the mat (data not shown). In contrast, algae apparently responded to the different radiation conditions by an alteration of SOD activity (Fig. 5A). Significant differences were found in the activity of SOD in UV-treated samples compared to samples exposed to PAR only. Algal samples from the mat surface and receiving the whole

Table 5. *Chaetomorpha linum*. Mean ±SD concentration of photosynthetic and accessory pigments in artificial mats in initial samples taken from subcanopy and after 8 d exposure. Samples taken at 14:00 h on 5 September 2003. For each irradiance treatment, material was sampled from the upper and bottom layers. Data are mg g^{-1} fresh wt (n = 3) for each pigment, different superscripts indicate significantly different values (p < 0.05) determined by post-hoc comparisons

Treatment Position	Chlorophyll *a*	Chlorophyll *b*	β-carotene	Lutein	Xanthophylls
Initial sample					
sub-canopy	6.135[c] ± 0.047	3.032[c] ± 0.033	0.409[c] ± 0.068	0.351[a] ± 0.064	0.442[c] ± 0.076
PAR + UV-A + UV-B					
top	2.727[a] ± 0.010	1.199[a] ± 0.045	0.183[a] ± 0.011	0.410[a] ± 0.023	0.158[a] ± 0.007
bottom	4.578[b] ± 0.364	2.409[b] ± 0.191	0.239[b] ± 0.020	0.234[b] ± 0.013	0.362[c] ± 0.021
PAR + UV-A					
top	2.723[a] ± 0.168	1.220[a] ± 0.097	0.188[a,b] ± 0.048	0.431[a] ± 0.113	0.112[b] ± 0.007
bottom	6.509[c] ± 0.754	3.391[c] ± 0.464	0.387[c] ± 0.034	0.392[a] ± 0.020	0.458[c] ± 0.054
PAR					
top	2.653[a] ± 0.301	1.063[a] ± 0.097	0.256[b] ± 0.037	0.449[a] ± 0.060	0.144[a,b] ± 0.056
bottom	5.654[b,c] ± 0.403	2.607[b] ± 0.195	0.381[c] ± 0.016	0.349[a] ± 0.026	0.420[c] ± 0.029

Table 6. Analysis of variance (2-way ANOVA) on effect of radiation treatment and vertical position within mat on concentration of photosynthetic pigments within artificial mats of *Chaetomorpha linum*. Corresponding pigment concentrations and post-hoc comparisons in Table 5

Variable	df	F	p
Chlorophyll *a*			
Radiation treatment	2	8.14	0.004
Vertical position	1	231.4	<0.001
Radiation × Position	2	8.13	0.004
Error	16		
Chlorophyll *b*			
Radiation treatment	2	10.09	<0.001
Vertical position	1	239.1	0.002
Radiation × Position	2	7.02	0.007
Error	16		
β-carotene			
Radiation treatment	2	18.26	<0.001
Vertical position	1	79.54	<0.001
Radiation × Position	2	8.16	0.004
Error	16		
Lutein			
Radiation treatment	2	4.24	0.033
Vertical position	1	17.07	<0.001
Radiation × Position	2	2.25	0.138
Error	16		
Xanthophylls			
Radiation treatment	2	0.789	0.474
Vertical position	1	279.3	<0.001
Radiation × Position	2	5.92	0.012
Error	16		

Table 7. Analysis of variance (2-way ANOVA) on effect of radiation treatment and vertical position within mat on activity of superoxide dismutase (SOD) and formation of malondialdehyde (MDA) within artificial mats of *Chaetomorpha linum*. Measured values and post-hoc comparisons in Fig. 5

Variable	df	F	p
SOD			
Radiation treatment	2	5.10	0.024
Vertical position	1	10.94	0.006
Radiation × Position	2	5.27	0.023
Error	12		
MDA			
Radiation treatment	2	3.22	0.076
Vertical position	1	3.06	0.106
Radiation × Position	2	4.82	0.029
Error	12		

Fig. 5. *Chaetomorpha linum*. (A) Activity of superoxide dismutase (SOD), expressed as units mg^{-1} total soluble protein (TSP), and (B) degree of lipid peroxidation, measured as formation of malondialdehyde (MDA), in top and bottom layers of artificial mats exposed to solar PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) or PAR (P) sampled at 14:00 h on 5 September (n = 3). Values are means (+SD). Black horizontal line shows mean of initial samples taken from subcanopy (grey horizontal shading = SD). Different letters above bars indicate significantly different values (p < 0.05) determined by post-hoc comparison

MDA formation arising from UV-B exposure in samples from the mat surface, with all other samples exhibiting similar and significantly lower values. A significant interaction of the effects of radiation conditions and vertical position inside the mat was found for SOD activity as well as for MDA formation (Table 7).

DISCUSSION

Our data provide new information on the ability of *Chaetomorpha linum* to thrive in high-light environments, such as the shallow-water ecosystems along the southern coast of Spain. To avoid the harmful effects of exposure to excessive radiation, either ecological (Bischof et al. 2002b) or physiological acclimation strategies or (more probably) a combination of the two may be employed by this alga. In a previous study on the structuring effects of solar radiation on canopies of *Ulva* spp. from the same location, the sensitivity of individual thallus layers to solar exposure was demon-

solar spectrum exhibited the highest SOD activity. Samples from the same position but receiving no UV-B still showed a trend of elevated SOD activity, but this was not significantly different from that of samples exposed to PAR alone. No difference was found in SOD activity in samples from the understory. A similar pattern was shown in the extent of lipid peroxidation (Fig. 5B), whereby there was a marked increase in
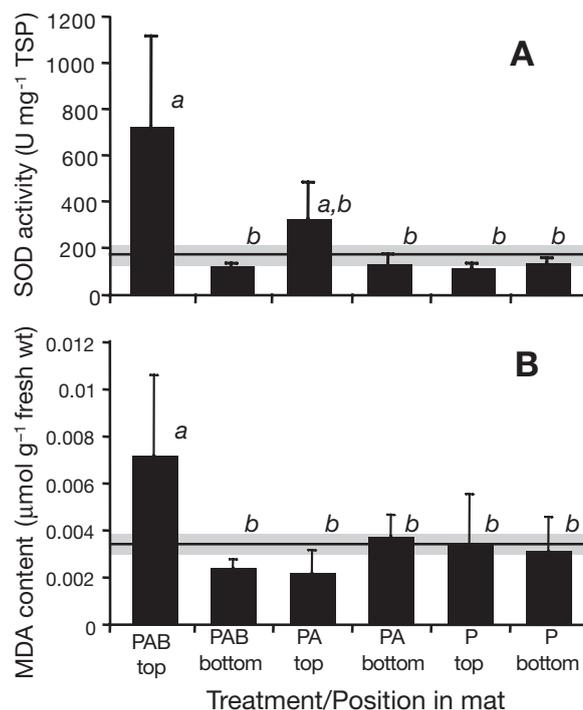
strated (Bischof et al. 2002b). However, mass development of *Ulva* spp. does occur during the summer, as the typical mat-like structure of their canopies provides effective shielding of photosynthetically active thallus layers by the topmost layer, which are subject to disintegration. Measurements of thallus absorption revealed that screening is particularly effective within the UV range of the spectrum, and may be part of an ecological strategy to protect the physiologically active subcanopy material. In the experiment conducted with *Ulva* spp. (Bischof et al. 2002b), it was also shown that the upper thallus layers rapidly exhibit bleaching by losing photosynthetic pigments, and disintegrate within 3 d. In contrast, in the present study on *C. linum*, visible bleaching of thalli did not occur even after >8 d exposure. This is an indication of a generally higher tolerance of *C. linum* to high-light exposure compared to *Ulva* spp. Although our data demonstrate a significant effect of the vertical positions of algal fragments inside the canopy for most parameters tested (Tables 2, 3, 6 & 7), top-canopy material showed no sign of disintegration. Thus, the presence of additional physiological strategies to withstand high-light stress is very likely.

The mat-like structure of interwoven filaments results in a steep gradient of light within algal stands (Fig. 2). Light absorption within the algal mats was in the same range as that previously reported by Krause-Jensen et al. (1996) for artificial *Chaetomorpha linum* mats. Thus, subcanopy filaments are effectively shielded from high radiation, but may be light-limited (Fig. 2); consequently algal productivity usually declines with increasing depth inside the mat (Krause-Jensen et al. 1996). Accordingly, a decline in oxygen concentration with increasing depth was also measured in our field study (Fig. 3). The ability of *C. linum* to thrive under low- and high-light condition was characterised in a laboratory study by Taylor et al. (2001), who recorded growth rates of field-collected *C. linum* under different irradiance levels from 0 to 175 µmol photons m^{-2} s^{-1}. Among the 8 bloom-forming green algal species they tested, *C. linum* initially exhibited the most rapid increase in growth rate under light-limiting conditions, a growth-saturating irradiance as low as 20 µmol photons m^{-2} s^{-1}, and the highest growth rate of all species tested when irradiance increased to 175 µmol photons m^{-2} s^{-1}. From their experiment, the ability of *C. linum* to acclimate to both low- and high-light environments is obvious. In a field study, Gómez et al. (2004) measured photosynthesis in *C. linum* from the coast of Chile over diurnal cycles: despite large natural fluctuations in light, *C. linum* exhibited only minor changes in effective quantum yield, illustrating its capacity for highly flexible adjustment of its photosynthetic performance.

The present study focussed on the response of *Chaetomorpha linum* to exposure to high irradiance, including PAR and UV radiation. While *Ulva* spp. receiving high levels of natural solar radiation during the summer in southern Spain showed marked inhibition of photosynthesis, and even damage to their photosynthetic apparatus (i.e. pigments and proteins; Bischof et al. 2002b), no such response was found in *C. linum*. The absence of marked damage induced by UV-B is demonstrated by the fact that none of the pigments tested showed pronounced losses in concentration other than those arising from the effect of PAR. Changes in pigment composition were mostly related to the positions of the individual filaments inside the mat, and only to a minor degree to radiation quality (Table 5), indicating a general response to exposure to high light. However, an unusual effect was the variation in lutein concentration in the various layers of the mat (Tables 5 & 6). In contrast to all other pigments analysed, lutein was always present in highest concentrations in the surface layers in each treatment, perhaps indicating its synthesis as a protective pigment under high-light stress. This was also observed in a field study on *Ulva lactuca* from Helgoland (Bischof et al. 2002a). For certain species of higher plants, the occurrence of a lutein epoxide cycle has been proposed as an additional mechanism for energy dissipation under excessive irradiance (Bungard et al. 1999, Garcia-Plazaola et al. 2003). To our knowledge this function has not yet been described for an alga; however, various protective functions are generally attributed to the presence of lutein in plants, e.g. radical quenching and heat dissipation (Niyogi et al. 1997). Thus, induction of lutein synthesis may be involved in physiological acclimation to high-light exposure.

Flexible acclimation to changing irradiances may involve changes in the scavenging activity against oxidative stress. In a survey on isolates of arctic macroalgae, both *Chaetomorpha linum* and C. *melagonium* were shown to be fairly resistant to oxidative stress and to display high antioxidative activities (Aguilera et al. 2002, Dummermuth et al. 2003, 2004). Our study showed a significant increase in SOD activity in the upper layers under UV exposure (Fig. 5A). As previously found for *Ulva* spp. (Bischof et al. 2003), the alga clearly responded to additional radiation stress in the UV range. Alternatively, as UV is a natural compound in the solar spectrum, SOD activity may be reduced when UV radiation, and particularly UV-B, is lacking. As also found for *Ulva* spp. (Bischof et al. 2003), the degree of lipid peroxidation was highest under the full solar spectrum (Fig. 5B), despite the higher activity of SOD. This could indicate insufficient antioxidative activity or an increase in SOD activity as a result of emerging oxidative stress. For higher plants

it is generally accepted that ROS themselves play an important role in signal transduction to induce protective mechanisms against UV-induced oxidative stress (Mackerness et al. 1999).

Comparison of results of measurements of effective quantum yield (Tables 1 & 2) and maximal quantum yield (Fig. 4, Table 3) revealed the additional effect of UV radiation in decreasing photosynthetic performance. The reduction in effective quantum yield indicates that the instantaneous decrease is predominately caused by PAR, with the UV range merely comprising a minor additional suppression. However, measurements of F_v/F_m revealed stronger inhibition arising from UV-B exposure. This difference may be due to the highly dynamic character of photoinhibition in this species (Osmond 1994, Gómez et al. 2004). Yield values at 14:00 h in PAR-exposed samples from the top of the mat were greatly reduced to 0.381 (Table 1), whereas F_v/F_m in samples under the same conditions displayed no inhibition at all (Fig. 4). This indicates that the yield values responded extremely quickly, namely during the 5 min of dark acclimation prior to the F_v/F_m measurement. Comparison of the F_v/F_m values shows that differences in inhibition of photosynthesis were much more pronounced between PAR- and UV-exposed samples, indicating that UV may induce inhibition of quantum yield or delay recovery from such inhibition (Fig. 4), as also suggested for various polar and temperate macroalgal species (Hanelt et al. 1997, Hanelt 1998). An important mechanism involved in dynamic photoinhibition is the activity of the xanthophyll cycle (Demmig-Adams 1990, Frank et al. 1994), which enables the harmless dissipation of excessively absorbed light energy as heat. Previous studies on higher plants and algae have suggested that the activity of this cycle is particularly affected by UV-B exposure via inhibition of violaxanthin de-epoxidation, thus hampering a plant's ability for dynamic photoinhibition (Pfündel et al. 1992, Bischof et al. 2002a, b). Consequently, the high energy of PAR may not be dissipated as heat as efficiently in the antenna. At the level of the photosynthetic electron transport chain this could result in increased cyclic electron transport and a higher number of electrons participating in the Mehler reaction. The present study cannot present direct experimental evidence for these phenomena; however lower values of NPQ (Table 4), the inhibition of maximal quantum yield induced by UV-B (Fig. 4), and elevated lipid peroxidation as a marker of oxidative stress under UV-B exposure (Fig. 5B) do at least indicate that similar interactive effects of PAR and UV on inhibition of photosynthesis are present in *Chaetomorpha linum* as well.

Comparison of F_v/F_m values measured on 2 and 5 September does not indicate acclimation of the quantum yield to the respective radiation conditions, but rather a slow increase in photoinhibition (Fig. 4), which unfortunately could not be followed any further due to time limitations. Also, an extended experimental treatment reveal differences in pigment composition arising from different radiation conditions. Thus, UV-B effects may become increasingly prominent during the further course of exposure, e.g. through increased production of ROS (Fig. 5B). Nevertheless, taking into account that in a similar set of experiments *Ulva* spp. thalli completely disintegrated within only 3 d, *Chaetomorpha linum* must be regarded as a species that is very tolerant of solar UV exposure. The results of the present study do not point to direct photoacclimation in terms of pigment composition and photosynthetic activity, but indicate the important contribution of an UV-induced increase in SOD activity as part of a physiological acclimation strategy to resist prolonged exposure under UV-B irradiation.

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