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Effects of ultraviolet and visible radiation on the cellular concentrations of dimethylsulfoniopropionate (DMSP) in *Emiliania huxleyi* (strain L)

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ABSTRACT: *Emiliania huxleyi* is an important component of the global carbon and sulfur cycles and is known to be sensitive to ultraviolet (UV) radiation. We investigated the influence of radiation intensity and of short-term exposure to UV radiation on the per-cell amount and intracellular concentration of dimethylsulfoniopropionate (DMSP). *E. huxleyi* (strain L) was exposed to artificial radiation intensities similar to those at 15 m (700 µmol PAR [photosynthetically active radiation] m^{-2} s^{-1}) and 25 m depth (400 µmol PAR m^{-2} s^{-1}) in the subtropical Atlantic Ocean. Exposure to UV radiation led to a 10 to 25% increase in the per-cell amount of DMSP as compared to *E. huxleyi* exposed to only PAR, whereas photosynthetic activity (measured via oxygen production) of UV-exposed *E. huxleyi* was reduced by 18 to 22%. Furthermore, the intracellular DMSP concentration was always higher in PAR+UV-exposed *E. huxleyi* than in PAR-exposed *E. huxleyi*, despite the small but significant increase in cell volume of *E. huxleyi* after exposure to PAR+UV as compared to PAR exposure only. A shift of the radiation conditions to higher levels resulted in a short-term increase in the per-cell amount and intracellular concentration of DMSP. *E. huxleyi* cultured in turbidostats under different radiation intensities (ranging from 5.6 to 400 µmol PAR m^{-2} s^{-1}) revealed a significant monotonical increase in the per-cell amount and the intracellular concentration of DMSP with increasing radiation intensity.

KEY WORDS: Coccolithophorida · *Emiliania huxleyi* · Ultraviolet radiation (UV) · DMSP · DMS · Marine sulfur cycle

INTRODUCTION

Emiliania huxleyi is the dominant species within the coccolithophorids (Prymnesiophyceae) and is widespread in the oceans, with the only exception being polar waters (Holligan et al. 1993, Brown & Yoder 1994). It can form extensive blooms in both open oceans and coastal waters, leading to a characteristic coloring of the waters (Holligan et al. 1983, Balch et al. 1991, Van der Wal et al. 1995). Its significance in the cycling of carbon and sulfur is frequently emphasized due to its incorporation of inorganic carbon into coccoliths (calcite plates) (Honjo 1976, Sekino et al. 1996), because of the export of carbon from the euphotic zone due to high sinking rates (Bramlette 1958, Van der Wal et al. 1995, Buitenhuis et al. 1996), and because of the production of substantial amounts of dimethylsulfoniopropionate (DMSP) (Keller et al. 1989a).

Since *Emiliania huxleyi* is one of the most prominent producers of the osmolyte DMSP (Vairavamurthy et al. 1985), DMSP production and release have been extensively investigated during bloom events (Malin et al. 1993, Matrai & Keller 1993, Levasseur et al. 1996). The release of dissolved DMSP occurs mostly either in the

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senescent phase of blooms (Ngyuen et al. 1988) due to viral lysis (Bratbak et al. 1995, Hill et al. 1998, Malin et al. 1998) or due to grazing by zooplankton (Dacey & Wakeham 1986, Wolfe & Steinke 1996). The dissolved fraction of DMSP is enzymatically cleaved into acrylic acid and dimethylsulfide (DMS) (Cantoni & Anderson 1956, Ngyuen et al. 1988, Belviso et al. 1990, Stefels et al. 1995) or demethylated (Taylor & Gilchrist 1991, Kiene 1992, Kiene et al. 2000). DMS is a volatile sulfur compound and thought to be involved in regulating the Earth's climate (Charlson et al. 1987, Andreae 1990). In the atmosphere, DMS-derived sulfate aerosols can act as cloud condensation nuclei and contribute to the back-scattering of incoming solar radiation (Charlson et al. 1992, Ayers et al. 2000). Recently, DMS has been suggested to play another important role in the atmosphere in the form of a hypothetical 'halogen activation' autocatalytic cycle. Sulfuric acid derived from DMS oxidation in the atmosphere might catalyze the release of highly reactive and ozone-destroying halogens (such as bromide and chloride) from sea-salt particles into the air (Vogt et al. 1996, Ayers et al. 2000).

In addition to global warming, stratospheric ozone depletion and the associated increase in ultraviolet-B (UV-B) radiation (280 to 320 nm) have attracted considerable attention. UV radiation can penetrate at biologically significant radiation levels down to about half of the euphotic zone (Gieskes & Kraay 1990, Smith et al. 1992, Obernosterer et al. 2001). Generally, UV radiation exerts stress on organisms and alters the chemical environment of aquatic organisms (Palenik et al. 1991, Franklin & Forster 1997, Greenberg et al. 1997). The impact of UV radiation on phytoplankton, as the base of the aquatic food chain, has been intensively studied. UV radiation retards phytoplankton growth (Ekelund 1990, Arrigo 1994, Davidson et al. 1994) and activity (Cullen et al. 1992, Helbling et al. 1992, Smith et al. 1992), and it can damage intracellular macromolecules or alter their composition (Buma et al. 1995, 1996, Goes et al. 1995, Boelen et al. 2000). At the community level, UV radiation can cause changes in the species composition and the food-web dynamics (Bothwell et al. 1994, Davidson et al. 1996, Wängberg et al. 1996).

Only a few studies, so far, have focused on the effects of UV radiation on the production and release of DMSP and DMS by phytoplankton. In a mesocosm study running over 48 h, Sakka et al. (1997) identified 2 major effects: (1) on a short-term basis (<24 h), natural UV radiation reduced the DMS production rate, and (2) on a long-term basis (>24 h), the per-cell amount of algal DMSP decreased. In another study, *Phaeocystis antarctica* cultures were incubated under artificial light with low PAR (photosynthetically active radiation, 400 to 700 nm) intensities compared to the intensities of UV-A and UV-B radiation (Hefu & Kirst 1997). The authors found that the DMSP production was strongly inhibited by UV radiation, whereas the conversion rate of DMSP to DMS was enhanced. In a very recent paper, Sunda et al. (2002) found that intracellular DMSP and DMS levels in *Emiliania huxleyi* (CCMP374) were elevated upon long-term exposure (3 to 5 d) to total solar radiation as compared to *E. huxleyi* exposed to solar radiation without UV radiation.

In this study, we determined the short-term effect of UV radiation on the intracellular DMSP levels and possible release of DMSP of *Emiliania huxleyi* strain L. *E. huxleyi* is known to be severely impaired by UV radiation, and its growth rate is substantially reduced (Buma et al. 2000). Furthermore, we investigated the effect of visible radiation intensity (PAR) on the per-cell amount of DMSP and the intracellular concentration in the presence and absence of UV radiation. The rationale behind this study is, therefore, to determine the consequences of UV radiation for an important organism involved in the global biogenic sulfur cycle.

MATERIALS AND METHODS

Culture conditions. *Emiliania huxleyi* (calcifying strain L; originally isolated from the Oslo Fjord by O. K. Andersen) was cultivated axenically (for details see Ietswaart et al. 1994, Stolte et al. 2000) in turbidostat cultures (1 l volume) at 15 ± 0.2°C. PAR ranging from 5.6 to 400 µmol m^{-2} s^{-1} was provided continuously by circular fluorescent tubes (Splendor, color number 45). Scalar irradiance was measured with a radiometer (QSL-101, Biospherical Instruments) in the center of the culture vessel through a glass tube mounted in the vessel lid. Cells were kept in suspension using a spherically shaped magnetic stirring bar at 30 rpm. The medium was prepared according to Riegman et al. (2000), with 300 µM of $NaNO_3$ and 25 µM NaH_2PO_4. Under these culture conditions cells produced coccoliths. Subsamples were taken after the abundance of cells remained stable (variation less than 5%) for at least 3 d. Cell counts and cell volume were measured by flow cytometer using a Coulter Epics XL MCL with triggering set on red fluorescence. Possible bacterial contamination was checked regularly by epifluorescence microscopy (Hobbie et al. 1977).

Experimental set-up. Subsamples of 200 ml from the turbidostat culture were withdrawn and diluted in 2 l medium. The diluted culture was left at 15°C for adaptation to the new medium for 1 h. Then 120 ml quartz BOD (biological oxygen demand) bottles were filled with the diluted culture and incubated in a water bath at 15°C under artificial radiation. Artificial PAR was

provided by a HQI-T Powerstar lamp (250 W, Osram), UV-A radiation by 2 Philips TL100W/10R fluorescent tubes, and UV-B radiation by 2 UVA-340 fluorescent lamps (Q-Panel). Two different radiation regimes (400 and 700 µmol m^{-2} s^{-1}) were chosen as listed in Table 1. These radiation regimes were chosen according to solar-radiation measurements in the subtropical Atlantic Ocean representing the radiation intensity at ~25 m depth (PAR intensity: 400 µmol m^{-2} s^{-1}) and 15 m depth (PAR intensity: 700 µmol m^{-2} s^{-1}) (for details on the site and hydrography see Obernosterer et al. 2001). The short UV-B wavelength range (measured at 305 nm) was higher than natural UV-B intensity at the corresponding depth layer, whereas the longer UV-A wavelength range (measured at 380 nm) was lower (Table 1). Samples were incubated at 400 and 700 µmol PAR m^{-2} s^{-1} for 6.5 h (referred to subsequently as the 400-6 and 700-6 treatments, respectively) and 24 h (the 400-24 treatment) under the same radiation intensity as the parent culture (400-6 and 400-24) or at a higher intensity than the parent culture (700-6). Radiation intensity was measured with a radiometer (QSL-101) below the water surface of the water bath at the depth of the incubated samples. Half of the samples were incubated under PAR plus UV radiation (PAR+UV), the other half under PAR only (PAR), for which UV radiation was cut off by acrylic plastic (XT 20013, 3 mm, Röhm). All treatments were performed in quadruplicate bottles (2 for oxygen and 2 for DMSP determination, see below). Due to the limited availability of quartz incubation bottles, experiments with only UV-B excluded were not performed.

Table 1. Artificial solar radiation conditions in the different experimental treatments (400-6, 400-24, 700-6) established according to depth profiles measured in the Atlantic Ocean (data from Obernosterer et al. 2001). Radiation regimes of 400-6 and 400-24 are equivalent to noontime radiation at 25 m depth, 700-6 is equivalent to 15 m depth. *Emiliania huxleyi* (originally grown at 400 µmol m^{-2} s^{-1}) was exposed either for 6.5 h (400-6 and 700-6) or for 24 h (400-24). Radiation in the Atlantic Ocean was measured at 305, 320, 340, 380 nm wavelength (given in µW cm^{-2} nm^{-1}) and in the PAR (400–700 nm, in µmol m^{-2} s^{-1}) with a biospherical PUV-500 radiometer, using a correction factor for the 305 nm channel as suggested by Kirk (1994). Radiation for the experimental set-up was measured in the UV range with a PUV-510, whereas the PAR was measured with a QSL-101 radiometer. PAR: photosynthetically active radiation

Radiation range/ wavelength	400-6 and 400-24	700-6	Atlantic Ocean 25 m	Atlantic Ocean 15 m
PAR	400	700	430	714
380 nm	12.428	28.470	29.9	45.3
340 nm	7.631	16.314	7.8	16.48
320 nm	3.471	7.950	3.0	8.32
305 nm	0.512	1.094	0.14	0.48

In total, 11 experiments were made, 5 with 400-6, 2 with 400-24 and 4 with 700-6.

Measurements of oxygen production. To examine the effect of UV radiation on the photosynthetic activity of *Emiliania huxleyi* during the incubation period, 2 of the replicate bottles were used to determine the oxygen production due to photosynthetic activity. Dissolved oxygen concentrations were measured by determining the concentration of total iodine spectrophotometrically (Pai et al. 1993), following in principle the standard protocol for Winkler titration (Parsons et al. 1984). One ml of $MnCl_2 \cdot 4H_2O$ and 2 ml of NaOH-NaI were added; the bottles were closed, thoroughly shaken and allowed to react for at least 1 h. Then, 0.8 ml of concentrated sulfuric acid was added and stirred by a magnetic stirrer. After complete dissolution of the precipitate, the amount of iodine was measured spectrophotometrically (using a sipper) at a wavelength of 456 nm with a Hitachi U-1000 spectrophotometer and a 1 cm flow-through cuvette. The instrument was zeroed with Milli-Q water. Calibration was performed with iodate in distilled water resulting in an empirical factor of 0.54455 nM cm^{-1} at 456 nm wavelength (G. Kraay pers. comm.). Analytical standard deviation was $<0.5\%$ ($n = 43$). Whatman GF/F-filtered samples served as the control for photochemical oxygen consumption mediated by UV radiation in the absence of *E. huxleyi*.

Determination of particulate and dissolved DMSP and DMS. After the incubations were terminated, 5 ml subsamples were gently gravity-filtered through glass fiber filters (Whatman GF/F). The filters were placed into 25 ml glass vials filled with 20 ml 5 N NaOH and left for alkaline DMSP cleavage at 4°C for 24 h. For the determination of dissolved DMSP and DMS, 25 ml of the incubated sample was filtered. An aliquot of 10 ml of the filtrate was degassed for 20 min to determine dissolved DMSP concentrations. Thereafter, 2 ml of the degassed and non-degassed (for determination of dissolved DMSP and DMS) samples were transferred into 2.2 ml vials and 200 µl of 5 N NaOH was added. Samples were allowed to complete DMSP cleavage at 4°C for 24 h. All vials (25 and 2.2 ml) were closed by means of a Teflon-lined silicon septum and a screw cap. Dissolved DMS concentrations were calculated by subtracting the concentration in the degassed sample from that in the non-degassed sample. The precision of the determination of DMS was better than 5% for GF/F-filtered samples.

DMS analysis. DMS analysis was performed using a modified purge-and-trap system as described elsewhere (Andreae & Barnard 1983, Kiene & Service 1991). Briefly, the volatile sulfur compounds were stripped from water samples, cryo-trapped in liquid nitrogen and analyzed by gas chromatography (Hewlett Packard Model 5890 Series II, equipped with a flame

photometric detector). Ethylmethylsulfide (EMS) served as an internal standard and was injected in appropriate amounts directly into the bubbling chamber and sparged together with the samples (Brugger et al. 1998, Slezak et al. 2001). Separation was carried out on a Chromosil 330 column (Supelco Corp.) operated isothermally at 70°C and a carrier gas flow rate of 50 ml min^{-1}. Under these conditions retention times of DMS and EMS were 1.2 and 2.1 min, respectively. The analytical precision was generally better than 5% for replicate determinations.

RESULTS

Inhibition of photosynthetic activity due to UV radiation

The photosynthetic activity of *Emiliania huxleyi* incubated under PAR+UV radiation was significantly lower than that under PAR (Wilcoxon, $p < 0.001$, $n = 20$; Fig. 1). At a constant PAR radiation of 400 µmol m^{-2} s^{-1} in short-term (400-6) and long-term (400-24) experiments, photosynthetic activity in the presence of UV was reduced by 18 to 22% of the PAR treatment (Fig. 1). Increasing the PAR intensity from 400 to 700 µmol m^{-2} s^{-1} resulted in net respiration of *E. huxleyi* in the PAR+UV treatment (700-6; Fig. 1). Abiotic photochemical oxygen consumption was not detectable under PAR and PAR+UV radiation (Wilcoxon, $p = 0.18$, $n = 6$).

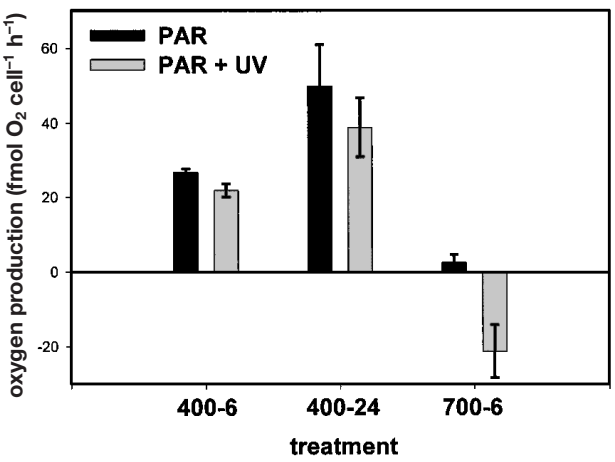

Fig. 1. Oxygen production of *Emiliania huxleyi* (grown at 400 µmol PAR m^{-2} s^{-1}) after exposure to photosynthetically active radiation only (PAR) and to PAR plus UV radiation (PAR+UV) at 3 different radiation regimes. Samples were exposed to 400 µmol PAR m^{-2} s^{-1} for 6 h (400-6) or 24 h (400-24) or to 700 µmol PAR m^{-2} s^{-1} for 6 h (700-6). Half of the samples were additionally exposed to UV radiation according to the radiation regimes listed in Table 1. Error bars indicate standard error (SE) of the mean of all experiments. SE is substantially higher than the standard deviation (SD) of the replicate samples of each experiment due to variations between experiments

Effects of UV-radiation on the per-cell amount and intracellular concentration of DMSP

Aliquots of the parent culture of *Emiliania huxleyi* grown at a PAR intensity of 400 µmol m^{-2} s^{-1} prior to incubation were exposed either to PAR or to PAR+UV radiation for 6.5 h (400-6) and 24 h (400-24; Fig. 2). *E. huxleyi* incubated under PAR+UV conditions exhibited a significantly higher per-cell amount of DMSP by, on average, 25 and 10% in the 400-6 and 400-24 treatments, respectively, as compared to the per-cell amount of DMSP in the PAR treatments (Wilcoxon, $p < 0.001$, $n = 13$; Table 2).

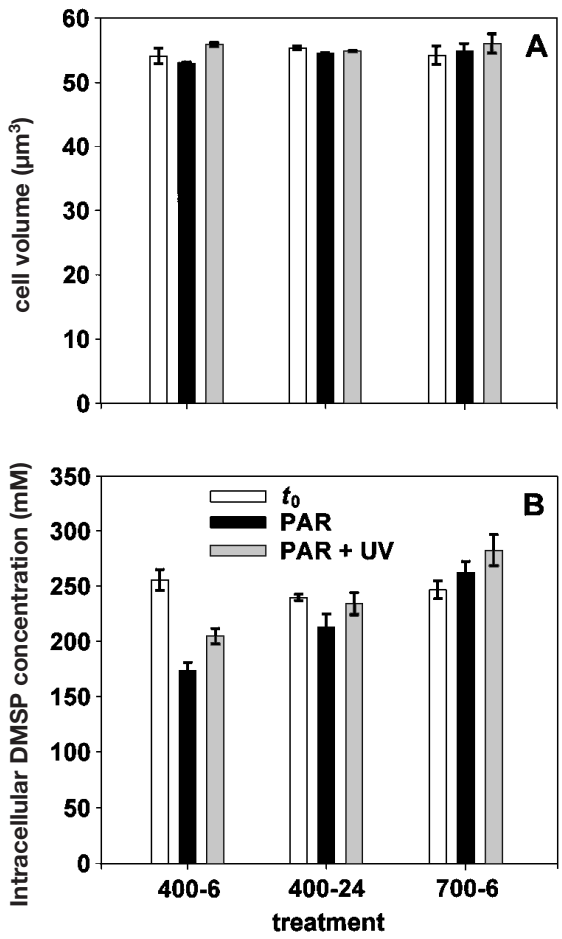

Fig. 2. *Emiliania huxleyi*. (A) Cell size and (B) intracellular DMSP concentration before (t_0) and after exposure to PAR and PAR+UV radiation at the 3 different light regimes as described in Table 1. For other information see Fig. 1. Error bars indicate SE of the mean of all experiments

Table 2. Per-cell amount of DMSP (in fmol cell^{-1} ± SE) of *Emiliania huxleyi* (originally grown at 400 µmol m^{-2} s^{-1}) at t_0 and after exposure to PAR and PAR+UV radiation. Experiments were performed as in Figs. 1 & 2. Treatments are described in Table 1

Treatment	t_0	PAR	PAR+UV
400-6	13.7 ± 0.3	9.2 ± 0.4	11.5 ± 0.4
400-24	13.3 ± 0.1	11.6 ± 0.7	12.8 ± 0.6
700-6	13.3 ± 0.4	14.2 ± 0.4	15.6 ± 0.5

Experiments were also carried out by exposing *Emiliania huxleyi* to a higher PAR intensity (700 µmol m^{-2} s^{-1}) than the parent culture (for radiation regimes see Table 1) with and without UV radiation. Again, a significantly higher per-cell amount of DMSP in the PAR+UV treatment was obtained than that in the PAR treatment (Wilcoxon, $p < 0.05$, $n = 5$; Table 2). The per-cell amount of DMSP of *E. huxleyi* was 14.2 ± 0.4 fmol cell^{-1} for the PAR treatment and 15.6 ± 0.5 fmol cell^{-1} for the PAR+UV treatment, leading to an overall increase in per-cell amount of DMSP in the presence of UV radiation of 10% as compared to that for the PAR treatment (Table 2).

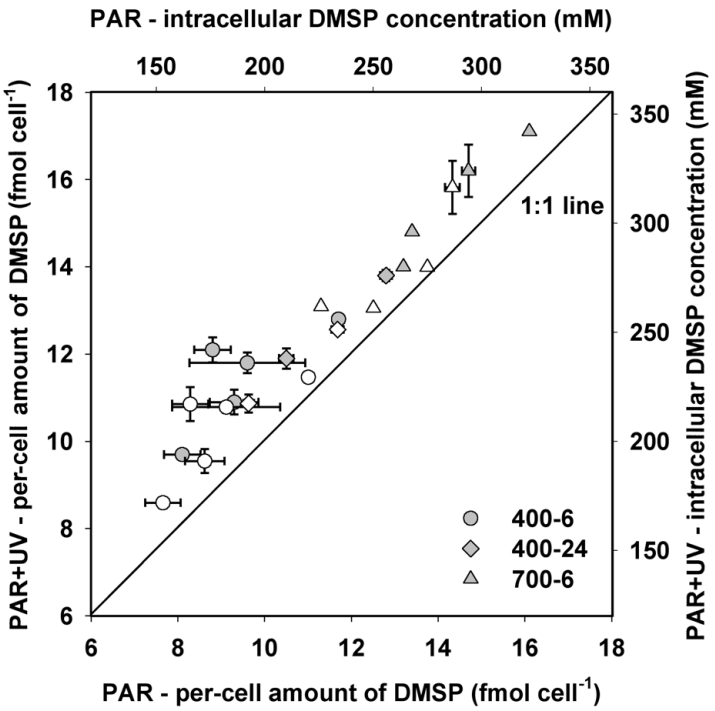

Fig. 3. *Emiliania huxleyi*. Per-cell amount (solid symbols) and intracellular concentration (open symbols) of DMSP in PAR vs PAR+UV treatments. Symbols represent the mean and error bars the SD of each experiment. Different symbols represent different treatments as listed in Table 1

A similar pattern was observed when calculating the intracellular DMSP concentration. The cell volume was slightly higher after exposure to UV radiation (53.9 ± 0.2 µm^3 for the PAR treatment, 55.6 ± 0.2 µm^3 for the PAR+UV treatments; Wilcoxon, $p < 0.05$, $n = 22$; Fig. 2A). Because the increase in cell volume following UV radiation exposure was relatively small (Fig. 2A), the observed higher per-cell amount of DMSP in the PAR+UV treatment was also reflected in the significantly higher intracellular DMSP concentration (7 to 18%; Wilcoxon, $p < 0.01$, $n = 13$ for 400-6 and 400-24; $p < 0.05$, $n = 5$ for 700-6) as compared to the intracellular DMSP concentration in PAR treatments (Fig. 2B). The higher per-cell amount and intracellular concentration of DMSP in *Emiliania huxleyi* exposed to PAR+UV radiation as compared to cells exposed to PAR was observed in all experiments. Plotting the per-cell amount and intracellular concentration of DMSP obtained in the PAR treatments versus the PAR+UV treatments clearly demonstrates that the mean and SD of every experiment is above the 1:1 line (Fig. 3). For oxygen production all data points are below the 1:1 line, as O_2 production was always higher in the PAR treatments (data not shown).

Per-cell amount and intracellular concentration of DMSP under variable radiation intensity

The per-cell amount and intracellular concentration of DMSP of *Emiliania huxleyi* was also investigated as a function of PAR intensity with and without UV radiation. *E. huxleyi* exposed to the same PAR conditions as the parent culture showed a significant decrease in the per-cell amount and intracellular concentration of DMSP as compared to the initial intracellular concentration (Wilcoxon, $p < 0.001$, $n = 27$; Fig. 2B). Exposure of *E. huxleyi* to higher radiation levels resulted in a significantly increased per-cell amount and intracellular concentration of DMSP (Wilcoxon, $p < 0.01$, $n = 11$; Fig. 2B, Table 2). A shift from 400 to 700 µmol PAR m^{-2} s^{-1} increased the intracellular DMSP concentration, on average, by 6.5 and 13.1% in the PAR and PAR+UV treatments, respectively, as compared to the initial intracellular DMSP concentration (700-6; Fig. 2B).

To assess the dependence of the per-cell amount and intracellular concentration of DMSP on radiation intensity, *Emiliania huxleyi* was grown in turbidostats at different PAR intensities ranging from 5.6 to 400 µmol m^{-2} s^{-1}. The per-cell amount of DMSP exhibited a significant correlation with PAR intensity, ranging, on average, from 4.4 fmol cell^{-1} at a PAR intensity of 5.6 µmol m^{-2} s^{-1}

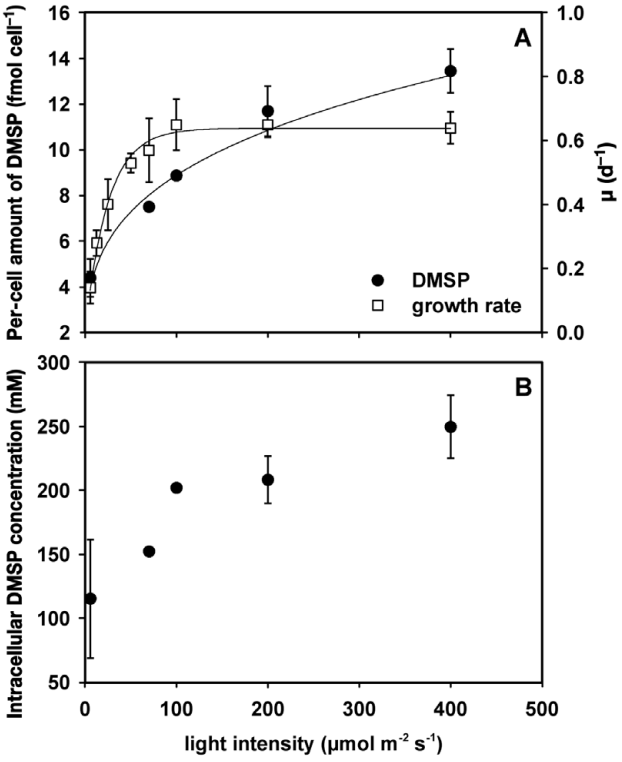

Fig. 4. *Emiliania huxleyi*. Effects of PAR intensity on the per-cell amount and intracellular concentration of DMSP. *E. huxleyi* was cultured in turbidostats at a PAR intensity ranging from 5.6 to 400 µmol m^{-2} s^{-1} at 15°C. (A) Per-cell amount of DMSP and steady-state growth rates at various PAR intensities. Solid lines represent a power fit for the per-cell amount of DMSP and an exponential fit for the growth rates. Steady-state growth rates are partly from Stolte et al. (2000) and completed by values obtained from additional turbidostat cultures (200 and 400 µmol m^{-2} s^{-1}). (B) Intracellular DMSP concentration. Values are means of replicate measurements: n = 1 for the 70 and 100 µmol m^{-2} s^{-1}, n = 3 for the 5.6 µmol m^{-2} s^{-1}, and n = 10 for the 400 µmol m^{-2} s^{-1} treatment for the DMSP determinations and n = 8 to 10 for the growth-rate determinations. Bars indicate SD

to 13.5 fmol cell^{-1} at a PAR intensity of 400 µmol m^{-2} s^{-1} (Kendall's robust line fit, $p < 0.05$, n = 5; Fig. 4A). In terms of intracellular DMSP concentration, increasing PAR intensity caused a significantly increasing intracellular DMSP concentration from 115 mM DMSP at 5.6 µmol PAR m^{-2} s^{-1} to 250 mM DMSP at 400 µmol PAR m^{-2} s^{-1} ($p < 0.05$, n = 5; Fig. 4B). Steady-state growth rates of *E. huxleyi* for the turbidostat cultures are shown in Fig. 4A. Maximum growth rates were achieved at around 100 µmol PAR m^{-2} s^{-1}.

Effects of UV radiation on the concentration of dissolved DMSP and DMS

The pattern of dissolved DMSP and DMS from *Emiliania huxleyi* exposed to the 700-6 treatment was investigated in a time-course experiment to determine the possible UV-radiation-mediated release of DMSP and its subsequent cleavage due to DMSP lyase activity by *E. huxleyi*. No significant difference was observed in the dissolved DMSP concentrations between the PAR+UV and PAR treatments over the course of the incubation (slope comparison with Student's *t*-test, p = 0.059, n = 9; Fig. 5A). The DMS concentration in the PAR treatment increased at a rate of 0.16 nmol l^{-1} h^{-1}, probably due to DMSP lyase activity. In the PAR+UV treatment, DMS concentrations remained stable (Fig. 5B). The significant difference in the time course of the DMS concentration between the PAR and PAR+UV treatments is probably due to additional photochemical removal of DMS in the PAR+UV treatment (slope comparison with Student's *t*-test, $p < 0.001$, n = 8).

DISCUSSION

In this paper we addressed the questions of whether UV radiation and PAR affect the per-cell amount and

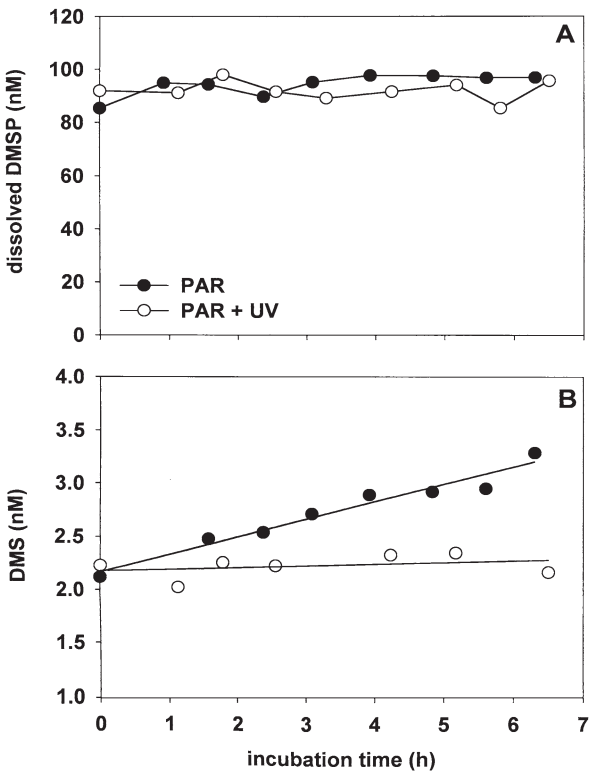

Fig. 5. Time course of (A) dissolved DMSP and (B) DMS in an *Emiliania huxleyi* culture (originally grown at 400 µmol PAR m^{-2} s^{-1} and then shifted to 700 µmol PAR m^{-2} s^{-1} for the experiment) exposed to PAR and PAR+UV radiation for approximately 6 h. Each data point represents a single incubation, as for every time point a bottle from parallel incubations was sacrificed for the analysis

intracellular concentration of DMSP in *Emiliania huxleyi* on a short-term basis and whether there is a relation between the intracellular DMSP levels and the radiation intensity in steady-state cultures. *E. huxleyi* is among the best-studied phytoplankton species that produces significant amounts of DMSP. It is a cosmopolitan species that plays an important role in the global carbon and sulfur cycles (Honjo 1976, Keller et al. 1989a,b, Sekino et al. 1996), and it forms nearly monospecific blooms (Holligan et al. 1993, Malin et al. 1993, Matrai & Keller 1993). In the present study *E. huxleyi* was cultured axenically to investigate the production and release of *E. huxleyi* without the possible interference of bacterioplankton involved in the DMSP/DMS dynamics.

Artificial solar radiation was provided at intensities similar to the radiation regimes at 25 and 15 m depth in the open subtropical Atlantic Ocean (see Obernosterer et al. 2001). However, short-wavelength UV-B radiation was higher as compared with natural conditions, and longer UV-A wavelength intensities were lower, due to the characteristics of the radiation sources used. This might have resulted in reduced photorepair, since the DNA repair mechanism is induced by UV-A (Friedberg 1985). Variable radiation conditions were used to investigate the effect of radiation intensities on the per-cell amount and intracellular concentration of DMSP.

UV radiation caused a reduction in photosynthetic activity of *Emiliania huxleyi* by ~20% when exposed to constant radiation conditions (400-6; Fig. 1), whereas elevated radiation conditions (700 µmol m^{-2} s^{-1}) compared with those of the parent culture enhanced the inhibitory effect of UV radiation (700-6; Fig. 1). The observed higher photosynthetic rates in the 24 h incubations as compared to the 6 h incubations might be due to the presence of a lag phase after dilution when cells are adapting to the new culture conditions (see also below). The combined effect of the dilution procedure and shifts to a higher radiation intensity might be the reasons for the more pronounced inhibition of oxygen production in the 700-6 treatment as compared with the other treatments.

Helbling et al. (1994) found a decrease in carbon fixation of phytoplankton exposed to changing irradiance levels (including UV radiation) as compared with phytoplankton exposed to a single radiation intensity at high radiation levels. The authors attributed this to the fact that reciprocity does not hold under high radiation conditions, but that irradiance intensity is of greater importance than the total dose (Cullen & Lesser 1991, Helbling et al. 1994). Our data support those findings as the amount of inhibition of photosynthetic activity due to UV exposure was greater when shifted to higher radiation intensities as compared with *Emiliania huxleyi* experiencing stable radiation conditions.

After an incubation period of ~6 h, which is about the period of intense UV radiation on a sunny day in the upper layers of the ocean, the per-cell amount of DMSP was, on average, 25% higher than in *Emiliania huxleyi* exposed to PAR only. Similarly, intracellular DMSP concentration increased, on average, by 18% when *E. huxleyi* was exposed to UV radiation. The differences in intracellular DMSP levels between PAR+UV-exposed *E. huxleyi* and those exposed to only PAR were, on average, smaller when the samples were exposed to UV radiation for 24 h (about 10% higher per-cell amount and intracellular concentration) than for only 6 h (Fig. 2, Table 2); however, these differences were statistically not significant (Mann-Whitney, $p = 0.246$).

We also determined the intracellular DMSP concentration, as there is evidence that the cell volume of *Emiliania huxleyi* depends on radiation intensity (W. Stolte pers. comm., M. van Rijssel pers. comm.). Furthermore, an increased cell volume upon UV exposure has been found also for other prymnesiophytes (Mostajir et al. 1999). We detected a small but significant increase in the cell volume between PAR and PAR+UV treatments when all data are pooled. Computation of statistical tests for each treatment resulted in significant differences only for the 400-6 treatment, while differences in the 400-24 and 700-6 treatments were not significant. Buma et al. (2000) found an increased cell volume after exposure to UV radiation, as the cells were arrested in the cell cycle (G1 phase). Furthermore, they found that cells remain in the G1 phase until the UV-radiation-induced DNA damage is repaired, which points to a more pronounced difference in cell volume at longer exposure times. In our experiments, *E. huxleyi* exhibited only a small increase in cell volume upon UV-radiation exposure. At longer exposure periods, the differences in cell volume became smaller (Fig. 2A) and insignificant (Wilcoxon, $p = 0.102$, $n = 4$). We therefore conclude that arrest in the cell cycle was only a minor effect of UV radiation in our experiments, but DMSP production continued despite a severe reduction of the carbon assimilation in UV-radiation-exposed *E. huxleyi* (Figs. 1 & 2B, Table 2).

Due to the high variability of the initial intracellular DMSP levels of *Emiliania huxleyi* (20 and 37% for per-cell amount and intracellular concentration, respectively), the SE of the mean of all experiments of 1 treatment are sometimes overlapping (e.g. 700-6; Fig. 2B). Fig. 3 demonstrates that the variability in the total per-cell amount and concentration is substantially higher than the differences between PAR+UV and PAR treatments. Nevertheless, the mean values ± SD of all experiments are clearly above the 1:1 line.

Our findings on the short-term effects of UV radiation on the intracellular DMSP levels seem to be in contrast to the long-term effects as observed in the seawater mesocosm study of Sakka et al. (1997). They found a decrease in DMSP per cell (total phytoplankton community) after exposure to UV radiation for more than 24 h in a day/night cycle. The decrease in per-cell amount of DMSP coincided with a decrease in the flagellate community, while the total phytoplankton abundance increased. Hence, in their study the decrease in per-cell amount of DMSP was probably due to a shift in the phytoplankton-community composition.

In contrast, our findings agree well with the long-term effects of solar radiation on intracellular DMSP levels described by Sunda et al. (2002). After 3 to 5 d exposure of *Emiliania huxleyi* to 30% of surface solar radiation levels, the DMSP and DMS concentrations per cell volume increased as compared to those for *E. huxleyi* exposed to solar radiation with UV radiation excluded. However, they found the most pronounced effect in *E. huxleyi* experiencing solar radiation including UV-A but no UV-B, which was not determined in our study.

Hefu & Kirst (1997) concluded from their experiments with *Phaeocystis antarctica* that particulate DMSP decreased under UV exposure, but they did not determine cell abundance. Thus, the observed decrease in particulate DMSP could be also due to a decrease in abundance. Furthermore, their ratios between PAR and UV radiation intensities were substantially lower than natural ratios in surface layers. In addition, the wavelength range involved in photorepair mechanisms of potential DNA damage (360 to 430 nm; Friedberg 1985) was probably underrepresented in the study of Hefu & Kirst (1997).

The decrease in the intracellular DMSP levels between the parent culture and the newly established ones in the exposure experiments at equal radiation intensities (PAR) might be due to the dilution procedure. *Emiliania huxleyi* exposed to radiation for 24 h exhibited higher oxygen production as compared with *E. huxleyi* exposed for only 6.5 h, with intracellular DMSP levels close to the initial value (Figs. 1 & 2B, Table 2). We attribute these differences in oxygen production to the presence of a lag phase when cells recover from the handling procedure or adapt to the new conditions. Furthermore, the radiation quality of the PAR between the fluorescence lamp to which the parent culture was exposed and the metal halide lamp used for the subsequent experiments could have also caused the lag phase.

Our data indicate a strong dependency of the intracellular DMSP levels of *Emiliania huxleyi* to PAR intensity. When shifting *E. huxleyi* from 400 to 700 µmol PAR m^{-2} s^{-1}, a short-term increase in intracellular DMSP levels for both treatments (PAR and PAR+UV) was observed. This suggests that the effect of radiation intensity outweighs the possible disturbance of the cells by diluting the culture.

There are a few investigations on the effect of radiation intensity on the intracellular DMSP levels in macro- and microalgae. In some cases, e.g. in the macroalgae *Enteromorpha bulbosa*, the microalgae *Tetraselmis subcordiformis* and *Emiliania huxleyi*, an increase in DMSP content was observed when the algae were grown under higher radiation intensities (Karsten et al. 1991, Levasseur et al. 1994, Keller & Korjeff-Bellows 1996, Meyerdiercks 1997). Wilhelm et al. (1997), however, found for *Prymnesium parvum* a positive correlation of the chlorophyll-*a*-normalized DMSP concentration with radiation intensity, but not on a per-cell basis. The data from our turbidostat cultures demonstrate clearly that under steady-state conditions a significant non-linear relation exists between per-cell amount and intracellular concentration of DMSP, on the one hand, and a PAR intensity of up to 400 µmol m^{-2} s^{-1}, on the other hand (Fig. 4). This is consistent with the findings of Meyerdiercks (1997). Furthermore, our data show a significant short-term increase in the per-cell amount and intracellular concentration of DMSP (within 6 h; Fig. 2B, Table 2) after *E. huxleyi* has been exposed to higher radiation intensities. This indicates a fast response in DMSP synthesis, which is in contrast to the findings of Kirst (1996) and Stefels (2000). Whether the short-term response to higher radiation intensities is caused by the same intracellular mechanisms as the adjustment of intracellular DMSP levels to radiation intensities in turbidostats remains to be investigated.

The increase of intracellular DMSP in UV-radiation-exposed *Emiliania huxleyi* might represent an overflow mechanism under certain physiological conditions (Stefels 2000). DMSP is synthesized from methionine and cysteine, which are kept at very low levels within algal cells (Giovanelli 1990). Under unbalanced growth, as, for instance, under nitrogen limitation, DMSP production keeps methionine and cysteine concentrations low. Continuous sulfate assimilation can take place, while the nitrogen, after a transamination reaction to form DMSP from methionine, will be redistributed to other amino acids (Gage et al. 1997). Furthermore, the use of the DMSP pathway could also allocate the nitrogen of methionine to other amino acids, if methionine is present in excess due to protein degradation (Gröne & Kirst 1992). Investigations of the amino-acid composition and assimilation of phytoplankton species indicate that UV-radiation exposure leads to nitrogen limitation, due to a decline in the amount of reducing power (Döhler 1989, 1992,

Goes et al. 1995). Furthermore, Goes et al. (1995) observed a decrease in combined amino acids and an increase in dissolved free amino acids after exposure to UV radiation, which supports the idea of Döhler (1992) that UV radiation could lead to a breakdown of proteins. Therefore, elevated intracellular DMSP levels could counteract possible nitrogen limitation and redistribute nitrogen originating from UV-radiation-mediated protein degradation in *E. huxleyi*.

Sunda et al. (2002) proposed that DMSP, DMS, and acrylate and the oxidation products of DMS might serve as an anti-oxidation system in phytoplankton cells. DMSP, but especially its breakdown products DMS, DMSO and acrylate, are readily oxidized by reactive oxygen species and would remove toxic oxidants from the cell. They found several indications that DMSP and DMS cell contents are increased in the presence of oxidative stressors, such as iron and CO_2 limitation or UV radiation (Lesser 1996). The increase in intracellular DMSP levels of *Emiliania huxleyi* upon short-term UV-exposure and elevated radiation levels (700-6) and the strong positive correlation with radiation intensity in turbidostat cultures observed in this study support the proposed hypothesis of an anti-oxidant function of DMSP and DMS.

The fact that no decrease in the dissolved DMSP pool due to lyase activity was detected despite an accompanying increase in DMS concentration might result from the high DMSP concentrations, where small variations among samples might have masked the slight decrease in dissolved DMSP. Steinke et al. (1998) investigated 6 strains of *Emiliania huxleyi* for their DMSP lyase activity and also found only low DMSP lyase activity for strain L, consistent with our findings. The DMSP lyase activity might be underestimated due to photochemical DMS removal (Kieber et al. 1996, Brugger et al. 1998). The low DMS concentration in the experiments, despite the high dissolved DMSP pool and the lyase activity, is probably the result of the dilution procedure, and the handling of the flasks could have allowed DMS present in the parent culture to escape into the air. In comparison to the PAR-exposed treatment, DMS concentration in the PAR+UV treatment remained stable, probably as a consequence of additional photochemical DMS removal in the UV-radiation range (Brimblecombe & Shooter 1986, Kieber et al. 1996). A similar observation was made by Sunda et al. (2002), who found the DMS per cell volume to be lower in *E. huxleyi* exposed to total solar radiation as compared with that in *E. huxleyi* exposed to PAR only.

Overall, our data indicate that the intracellular DMSP levels of *Emiliania huxleyi* are likely to be elevated in the presence of UV radiation as compared with those of *E. huxleyi* exposed to radiation without UV radiation on a short-term basis. On a long-term basis, intracellular DMSP levels increase with radiation intensity.

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