

University of Groningen

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

Slezak, D; Herndl, G.J.

Published in:
Marine Ecology Progress Series

DOI:
[10.3354/meps246061](https://doi.org/10.3354/meps246061)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2003

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Slezak, D., & Herndl, G. J. (2003). Effects of ultraviolet and visible radiation on the cellular concentrations of dimethylsulfoniopropionate (DMSP) in *Emiliana huxleyi* (strain L). *Marine Ecology Progress Series*, 246, 61 - 71. <https://doi.org/10.3354/meps246061>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Effects of ultraviolet and visible radiation on the cellular concentrations of dimethylsulfonio-propionate (DMSP) in *Emiliana huxleyi* (strain L)

Doris Slezak^{1,3,*}, Gerhard J. Herndl²

¹Department of Marine Biology, Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

²Department of Biological Oceanography, Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

³Present address: Department of Marine Sciences, University of South Alabama, Dauphin Island Sea Lab, 101 Bienville Blvd, Alabama 36528, USA

ABSTRACT: *Emiliana 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 $\mu\text{mol PAR}$ [photosynthetically active radiation] $\text{m}^{-2} \text{s}^{-1}$) and 25 m depth (400 $\mu\text{mol PAR}$ $\text{m}^{-2} \text{s}^{-1}$) 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 $\mu\text{mol PAR}$ $\text{m}^{-2} \text{s}^{-1}$) revealed a significant monotonical increase in the per-cell amount and the intracellular concentration of DMSP with increasing radiation intensity.

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

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Emiliana 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 cocco-

liths (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 dimethylsulfonio-propionate (DMSP) (Keller et al. 1989a).

Since *Emiliana 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

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 *Emiliana 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 *Emiliana 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. *Emiliana 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 $15 \pm 0.2^\circ\text{C}$. PAR ranging from 5.6 to $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 15 m depth (PAR intensity: 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (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 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ 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. *Emiliana huxleyi* (originally grown at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $\mu\text{W cm}^{-2} \text{nm}^{-1}$) and in the PAR (400–700 nm, in $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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	
	400	700	700	25 m	15 m
PAR	400	700	700	430	714
380 nm	12.428	28.470	28.470	29.9	45.3
340 nm	7.631	16.314	16.314	7.8	16.48
320 nm	3.471	7.950	7.950	3.0	8.32
305 nm	0.512	1.094	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 *Emiliana 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 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 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 *Emiliana 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ resulted in net respiration of *E. huxleyi* in the PAR+UV treatment (700-6; Fig. 1). Abiotic

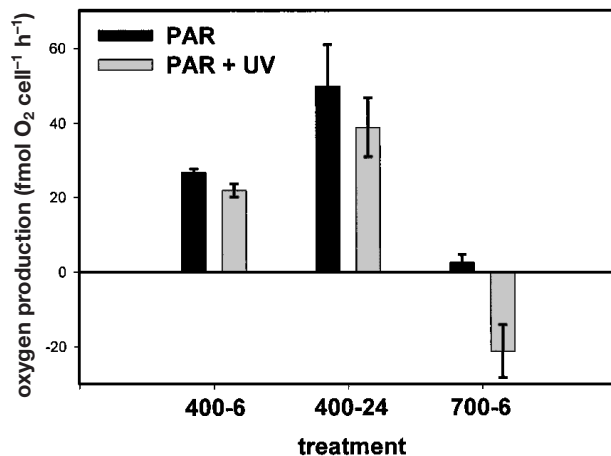


Fig. 1. Oxygen production of *Emiliana huxleyi* (grown at 400 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$) after exposure to photosynthetically active radiation only (PAR) and to PAR plus UV radiation (PAR+UV) at 3 different radiation regimes. Samples were exposed to 400 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ for 6 h (400-6) or 24 h (400-24) or to 700 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ 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

photochemical oxygen consumption was not detectable under PAR and PAR+UV radiation (Wilcoxon, $p = 0.18$, $n = 6$).

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

Aliquots of the parent culture of *Emiliana huxleyi* grown at a PAR intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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).

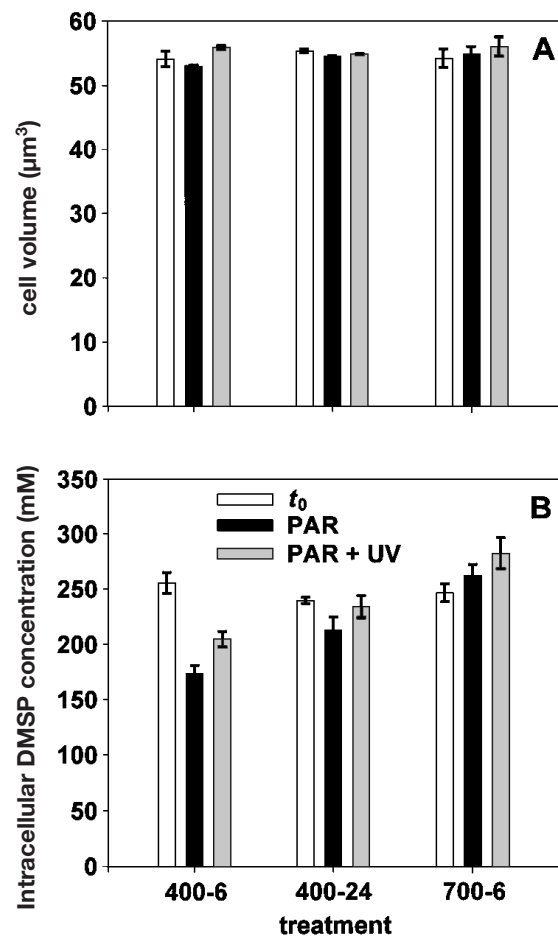


Fig. 2. *Emiliana huxleyi*. (A) Cell size and (B) intracellular DMSP concentration before (t_0) and after exposure to PAR and PAR+UV radiation at the 3 different light regimes as described in Table 1. For other information see Fig. 1. Error bars indicate SE of the mean of all experiments

Table 2. Per-cell amount of DMSP (in $\text{fmol cell}^{-1} \pm \text{SE}$) of *Emiliana huxleyi* (originally grown at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$) 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 *Emiliana huxleyi* to a higher PAR intensity ($700 \mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $14.2 \pm 0.4 \text{ fmol cell}^{-1}$ for the PAR treatment and $15.6 \pm 0.5 \text{ fmol cell}^{-1}$ 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).

A similar pattern was observed when calculating the intracellular DMSP concentration. The cell volume was slightly higher after exposure to UV radiation ($53.9 \pm 0.2 \mu\text{m}^3$ for the PAR treatment, $55.6 \pm 0.2 \mu\text{m}^3$ 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 *Emiliana 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

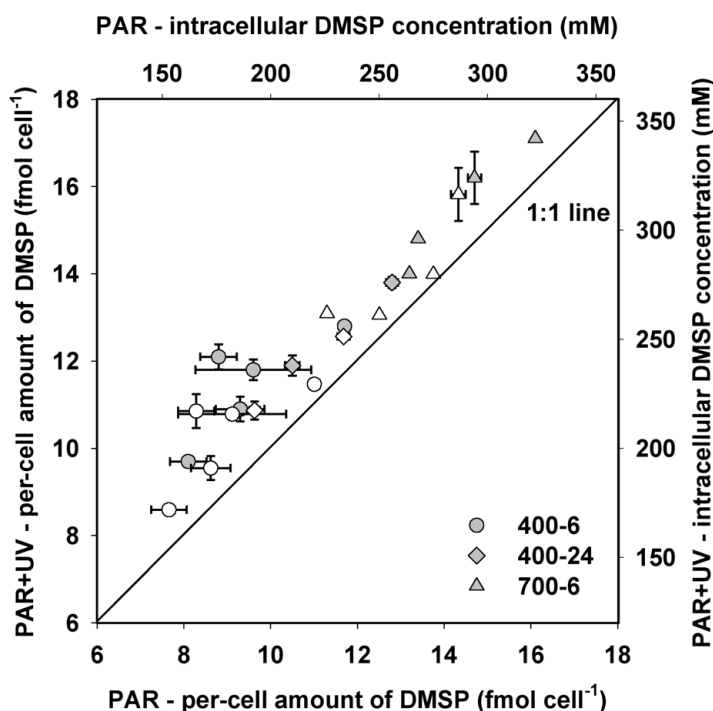


Fig. 3. *Emiliana huxleyi*. Per-cell amount (solid symbols) and intracellular concentration (open symbols) of DMSP in PAR vs PAR+UV treatments. Symbols represent the mean and error bars the SD of each experiment. Different symbols represent different treatments as listed in Table 1

The per-cell amount and intracellular concentration of DMSP of *Emiliana 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 $700 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ 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, *Emiliana huxleyi* was grown in turbidostats at different PAR intensities ranging from 5.6 to $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The per-cell amount of DMSP exhibited a significant correlation with PAR intensity, ranging, on average, from $4.4 \text{ fmol cell}^{-1}$ at a PAR intensity of $5.6 \mu\text{mol m}^{-2} \text{s}^{-1}$

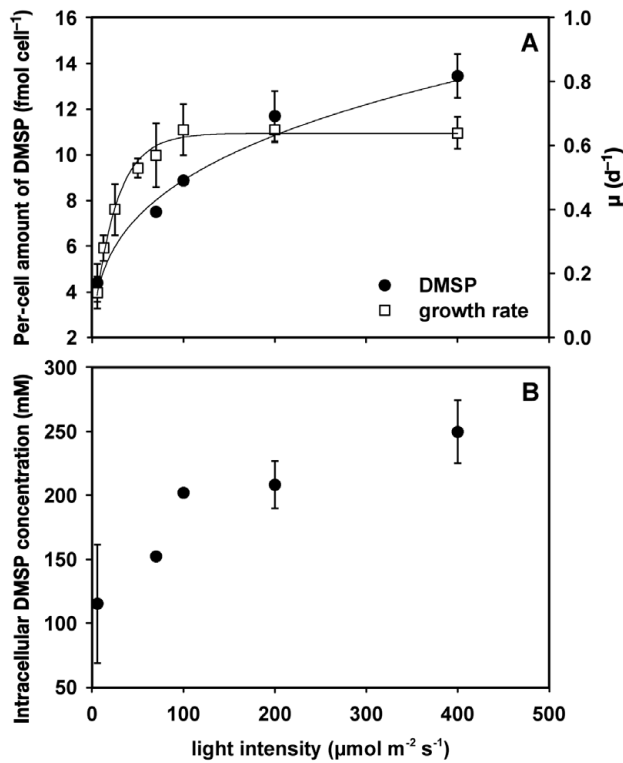


Fig. 4. *Emiliana huxleyi*. Effects of PAR intensity on the per-cell amount and intracellular concentration of DMSP. *E. huxleyi* was cultured in turbidostats at a PAR intensity ranging from 5.6 to 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 15°C. (A) Per-cell amount of DMSP and steady-state growth rates 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$). (B) Intracellular DMSP concentration. Values are means of replicate measurements: $n = 1$ for the 70 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $n = 3$ for the 5.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and $n = 10$ for the 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (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 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ to 250 mM DMSP at 400 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ($p < 0.05$, $n = 5$; 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 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$.

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

The pattern of dissolved DMSP and DMS from *Emiliana huxleyi* exposed to the 700-6 treatment was in-

vestigated 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 $^{-1} \text{ h}^{-1}$, 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

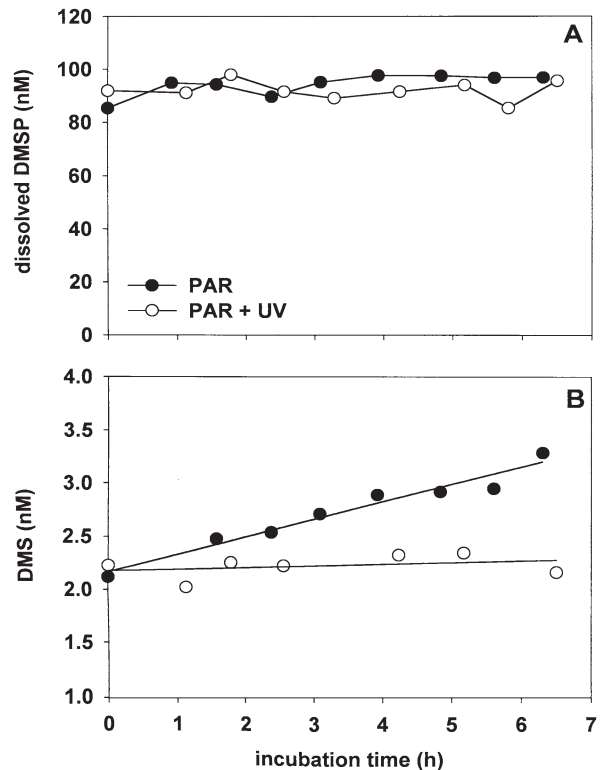


Fig. 5. Time course of (A) dissolved DMSP and (B) DMS in an *Emiliana huxleyi* culture (originally grown at 400 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ and then shifted to 700 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ for the experiment) exposed to PAR and PAR+UV radiation for approximately 6 h. Each 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 *Emiliana 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 *Emiliana huxleyi* by ~20% when exposed to constant radiation conditions (400-6; Fig. 1), whereas elevated radiation conditions (700 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 com-

pared with *Emiliana 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 *Emiliana 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 *Emiliana 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 (Moustajir 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 *Emiliana 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 \pm 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 *Emiliana 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. *Emiliana 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 *Emiliana huxleyi* to PAR intensity. When shifting *E. huxleyi* from 400 to 700 μmol

$\text{PAR m}^{-2} \text{ s}^{-1}$, 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 *Emiliana huxleyi*, an increase in DMSP content was observed when the algae were grown under higher radiation intensities (Karsten et al. 1991, Lévassieur et al. 1994, Keller & Korjef-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 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, 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 *Emiliana 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 (Giovaneli 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 CO₂ limitation or UV radiation (Lesser 1996). The increase in intracellular DMSP levels of *Emiliana 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 *Emiliana 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 *Emiliana 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.

Acknowledgements. The authors thank Anna Noordeloos, Willem Stolte and Roel Riegman for their assistance in setting up the turbidostat cultures of *Emiliana huxleyi*. Furthermore, we would like to thank our colleagues at the NIOZ, Texel, for all their help. E. Lorbeer is acknowledged for his invaluable help in establishing the analytical method. R. P. Kiene and 3 anonymous reviewers are acknowledged for their constructive comments on an earlier version of the manuscript. Financial support was provided by the Environment & Climate Program of the European Community (Microbial community response to UV-B stress in European Waters, project # EV5V-CT94-0512). This work is in part of the fulfillment of the requirements towards a PhD degree at the University of Vienna for D.S. This is contribution no. 3732 of the NIOZ.

LITERATURE CITED

- Andreae MO (1990) Ocean-atmosphere interactions in the global biochemical cycle. *Mar Chem* 30:1–29
- Andreae MO, Barnard WR (1983) Determinations of trace quantities of dimethyl sulfide in aqueous solutions. *Anal Chem* 55:608–612
- Arrigo KR (1994) Impact of ozone depletion on phytoplankton growth in the Southern Ocean: large-scale spatial and temporal variability. *Mar Ecol Prog Ser* 114:1–12
- Ayers GP, Gillet RW, Caaney JM, Dick AL (2000) DMS and its oxidation products in the remote marine atmosphere: implications for climate and atmospheric chemistry. *J Sea Res* 43:275–286
- Balch WM, Holligan PM, Ackleson SG, Voss KJ (1991) Biological and optical properties of mesoscale coccolithophore blooms in the Gulf of Maine. *Limnol Oceanogr* 36:629–643
- Belviso S, Kim SK, Rassoulzadegan F, Krajka B, Nguyen BC, Mihalopoulos N, Buat-Menard P (1990) Production of dimethylsulfonium propionate (DMSP) and dimethylsulfide (DMS) by a microbial food web. *Limnol Oceanogr* 35(8):1810–1821
- Boelen P, Boer MKD, Kraay GW, Veldhuis MJW, Buma AGJ (2000) UVBR induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean. *Mar Ecol Prog Ser* 193:1–9
- Bothwell ML, Sherbot DMJ, Pollock CM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. *Science* 265:97–100
- Bramlette MN (1958) Significance of coccolithophorids in calcium carbonate deposition. *Bull Geol Soc Am* 69:121–126
- Bratbak G, Levasseur M, Michaud S, Cantin G, Fernández E, Heimdal BR, Heldal M (1995) Viral activity in relation to *Emiliana huxleyi* blooms: a mechanism of DMSP release? *Mar Ecol Prog Ser* 128:133–142
- Brimblecombe P, Shooter D (1986) Photo-oxidation of dimethylsulfide in aqueous solution. *Mar Chem* 19:343–353
- Brown CW, Yoder JA (1994) Coccolithophorid blooms in the global ocean. *J Geophys Res* 99:7467–7482
- Brugger A, Slezak D, Obernosterer I, Herndl GJ (1998) Photolysis of dimethyl sulfide in coastal waters: dependence on substrate concentration, irradiance and DOC concentration. *Mar Chem* 59:321–331
- Buitenhuis E, Bleijswijk JV, Bakker D, Veldhuis M (1996) Trends in inorganic and organic carbon in a bloom of

- Emiliana huxleyi* in the North Sea. Mar Ecol Prog Ser 143: 271–282
- Buma AGJ, Hannen EJV, Roza L, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. J Phycol 31:314–321
- Buma AGJ, Zemmeling HJ, Sjollem K, Gieskes WWC (1996) UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms. Mar Ecol Prog Ser 142:47–54
- Buma AGJ, van Oijen T, van de Poll W, Veldhuis MJW, Gieskes WWC (2000) The sensitivity of the marine prymnesiophyte *Emiliana huxleyi* to ultraviolet-B radiation. J Phycol 36:296–303
- Cantoni GL, Anderson DG (1956) Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. J Biol Chem 222:171–177
- Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655–661
- Charlson RJ, Schwartz SE, Hales JM, Cess RD, Coackley JA Jr, Hansen JE, Hofmann DJ (1992) Climate forcing by anthropogenic aerosols. Science 255:423–430
- Cullen JC, Neale PJ, Lesser MP (1992) Biological weighting functions for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. Science 258:646–650
- Cullen JJ, Lesser MP (1991) Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar Biol 111:183–190
- Dacey JWH, Wakeham SG (1986) Oceanic dimethyl sulfide: production during zooplankton grazing on phytoplankton. Science 233:1314–1316
- Davidson AT, Bramich D, Marchant HJ, McMinn A (1994) Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms. Mar Biol 119:507–515
- Davidson AT, Marchant HJ, de la Mare WK (1996) Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culture. Aquat Microb Ecol 10:299–305
- Döhler G (1989) Impact of UV-B radiation on ¹⁵N incorporation into free amino acids *Ditylum brightwellii*. Oceanis 15:571–586
- Döhler G (1992) Impact of UV-B radiation on uptake of ¹⁵N-ammonia and ¹⁵N-nitrate by phytoplankton in the Wadden Sea. Mar Biol 112:485–489
- Ekelund NGA (1990) Effects of UV-B radiation on growth and motility of four phytoplankton species. Physiol Plant 78: 590–594
- Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32:207–232
- Friedberg EC (1985) DNA repair. WH Freeman, New York
- Gage DA, Rhodes D, Nolte KD, Hicks WA, Leustek T, Cooper AJL, Hanson AD (1997) A new route for synthesis of dimethylsulfoniopropionate in marine algae. Nature 387: 891–894
- Gieskes WWC, Kraay GW (1990) Transmission of ultraviolet light in the Weddell Sea: report of the first measurements made in the Antarctic. Biomass Newslett 12:12–14
- Giovanelli J (1990) Regulatory aspects of cysteine and methionine biosynthesis. In: Rennberg H, Brunold C, Kok LJD, Stulen I (eds) Sulfur nutrition and sulfur assimilation in higher plants: fundamental environmental and agricultural aspects. SPB Academic Publishing, The Hague, p 33–48
- Goes JI, Handa N, Taguchi S, Hama T, Saito H (1995) Impact of UV radiation on the production patterns and composition of dissolved free and combined amino acids in marine phytoplankton. J Plankton Res 17:1337–1362
- Greenberg BM, Wilson MI, Huang XD, Duxbury CL, Gerhardt KE, Gensemer RW (1997) The effects of ultraviolet-B radiation on higher plants. In: Wang W, Gorsuch JW, Hughes JS (eds) Plants for environmental studies. Lewis Publishers, Boca Raton, FL, p 2–35
- Gröne T, Kirst GO (1992) The effect of nitrogen deficiency, methionine and inhibitors of methionine metabolism on the DMSP contents of *Tetraselmis subcordiformis*. Mar Biol 112:497–503
- Hefu Y, Kirst GO (1997) Effect of UV-radiation on DMSP content and DMS formation of *Phaeocystis antarctica*. Polar Biol 18:402–409
- Helbling EW, Villafañe V, Ferrario M, Holm-Hansen O (1992) Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. Mar Ecol Prog Ser 80:89–100
- Helbling EW, Villafañe V, Holm-Hansen O (1994) Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. American Geophysical Union, Washington, DC, p 207–227
- Hill RW, White BA, Cottrell MT, Dacey JWH (1998) Virus-mediated total release of dimethylsulfoniopropionate from marine phytoplankton: a potential climate process. Aquat Microb Ecol 14:1–6
- Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228
- Holligan PM, Viollier M, Harbour DS, Camus P, Champagne-Philippe M (1983) Satellite and ship studies of coccolithophore production along a continental shelf edge. Nature 304:339–342
- Holligan PM, Fernández E, Aiken J, Balch WM and 11 others (1993) A biogeochemical study of the coccolithophore, *Emiliana huxleyi*, in the north Atlantic. Global Biogeochem Cycles 7:879–900
- Honjo S (1976) Coccoliths: production, transportation and sedimentation. Mar Micropaleontol 1:65–79
- Ietswaart T, Schneider PJ, Prins RA (1994) Utilization of organic nitrogen sources by two phytoplankton species and a bacterial isolate in pure and mixed cultures. Appl Environ Microbiol 60:1554–1560
- Karsten U, Wiencke C, Kirst GO (1991) Growth pattern and β-dimethylsulfoniopropionate (DMSP) content of green macroalgae at different irradiances. Mar Biol 108:151–155
- Keller MD, Korjef-Bellows W (1996) Physiological aspects of the production of dimethylsulfoniopropionate (DMSP) by marine phytoplankton. In: Kiene RP, Visscher P, Keller M, Kirst G (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, p 131–142
- Keller MD, Bellows WK, Guillard RRL (1989a) Dimethyl sulfide production in marine phytoplankton. In: Saltzman ES, Cooper WJ (eds) Biogenic sulfur in the environment. American Chemical Society, Washington, DC, p 167–182
- Keller MD, Bellows WK, Guillard RRL (1989b) Dimethylsulfide production and marine phytoplankton: an additional impact of unusual blooms. In: Cosper EM, Bricelj VM, Carpenter EJ (eds) Novel phytoplankton blooms. Springer-Verlag, Heidelberg, p 101–115
- Kieber DJ, Jiao J, Kiene RP, Bates TS (1996) Impact of dimethylsulfide photochemistry on methyl sulfur cycling in the equatorial Pacific Ocean. J Geophys Res 101(C2): 3715–3722

- Kiene RP (1992) Dynamics of dimethyl sulfide and dimethylsulfoniopropionate in oceanic water samples. *Mar Chem* 37:29–52
- Kiene RP, Service SK (1991) Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration. *Mar Ecol Prog Ser* 76:1–11
- Kiene RP, Linn LJ, Bruton JA (2000) New and important roles for DMSP in marine microbial communities. *J Sea Res* 43:209–224
- Kirk JTO (1994) Optics of UV-B radiation in natural waters. *Arch Hydrobiol Beiheft* 43:1–16
- Kirst GO (1996) Osmotic adjustment in phytoplankton and macroalgae. In: Kiene RP, Visscher P, Keller M, Kirst G (eds) *Biological and environmental chemistry of DMSP and related sulfonium compounds*. Plenum Press, New York, p 121–129
- Lesser MP (1996) Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorocentrum micans*. *Mar Ecol Prog Ser* 132:287–297
- Levasseur M, Gosselin M, Michaud S (1994) A new source of dimethylsulfide (DMS) for the arctic atmosphere: ice diatoms. *Mar Biol* 121:381–387
- Levasseur M, Michaud S, Egge J, Cantin G and 6 others (1996) Production of DMSP and DMS during a mesocosm study of *Emiliana huxleyi* bloom: influence of bacteria and *Calanus finmarchicus* grazing. *Mar Biol* 126:609–618
- Malin G, Turner S, Liss P, Holligan P, Harbour D (1993) Dimethylsulphide and dimethylsulphoniopropionate in the northeast Atlantic during the summer coccolithophore bloom. *Deep-Sea Res* 40:1487–1508
- Malin G, Wilson WH, Bratbak G, Liss PS, Mann NH (1998) Elevated production of dimethylsulfide resulting from viral infection of cultures of *Phaeocystis pouchetti*. *Limnol Oceanogr* 43:1389–1393
- Matrai PA, Keller MD (1993) Dimethylsulfide in a large-scale coccolithophore bloom in the Gulf of Maine. *Cont Shelf Res* 13:831–843
- Meyerdiercks D (1997) Ecophysiology of the dimethylsulfoniopropionate (DMSP) content of temperate and polar phytoplankton communities in comparison with cultures of the coccolithophore *Emiliana huxleyi* and the antarctic diatom *Nitzschia lecontei*. Doctoral thesis, University of Bremen
- Mostajir B, Sime-Negando T, Demers S, Belzile C and 7 others (1999) Ecological implications of changes in cell size and photosynthetic capacity of marine Prymnesiophyceae induced by ultraviolet-B radiation. *Mar Ecol Prog Ser* 187:89–100
- Ngyuen BC, Belviso S, Mihalopoulos N (1988) Dimethyl sulfide production during natural phytoplankton blooms. *Mar Chem* 24:133–141
- Obernosterer I, Ruardij P, Herndl GJ (2001) Spatial and diurnal dynamics of DOM fluorescence and H₂O₂ and the photochemical oxygen demand of surface water DOM across the subtropical Atlantic Ocean. *Limnol Oceanogr* 46:632–643
- Pai SC, Gong GC, Liu KK (1993) Determination of dissolved oxygen in seawater by direct spectrophotometry of total iodine. *Mar Chem* 41:343–351
- Palenik B, Price NM, Morel FMM (1991) Potential effects of UV-B on the chemical environment of marine organisms: a review. *Environ Pollut* 70:117–130
- Parsons T, Maita Y, Lalli C (1984) *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Oxford
- Riegman R, Stolte W, Noordeloos AAM, Slezak D (2000) Nutrient uptake and alkaline phosphatase (EC 3:1:3:1) activity of *Emiliana huxleyi* (strain L) during growth under N- and P-limitation in continuous cultures. *J Phycol* 36:87–96
- Sakka A, Gosselin M, Levasseur M, Michaud S, Monfort P, Demers S (1997) Effects of reduced ultraviolet radiation on aqueous concentrations of dimethylsulfoniopropionate and dimethylsulfide during a mesocosm study in the Lower St. Lawrence estuary. *Mar Ecol Prog Ser* 149:227–238
- Seokino K, Kobayashi H, Shiraiwa Y (1996) Role of coccoliths in the utilization of inorganic carbon by a marine unicellular coccolithophorid, *Emiliana huxleyi*: a survey using intact cells and protoplasts. *Plant Cell Physiol* 37:123–127
- Slezak D, Brugger A, Herndl GJ (2001) Impact of solar radiation on the biological removal of dimethylsulfoniopropionate and dimethylsulfide in marine surface waters. *Aquat Microb Ecol* 25:87–97
- Smith RC, Prézelin BB, Baker KS, Bidigare RR and 9 others (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* 255:952–959
- Stefels J (2000) Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *J Sea Res* 43:183–197
- Stefels J, Dijkhuizen L, Gieskes WWC (1995) DMSP-lyase activity in a spring phytoplankton bloom off the Dutch coast, related to *Phaeocystis* sp. abundance. *Mar Ecol Prog Ser* 123:235–243
- Steinke M, Wolfe GV, Kirst GO (1998) Partial characterization of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliana huxleyi*. *Mar Ecol Prog Ser* 175:215–225
- Stolte W, Kraay GW, Noordeloos AAM, Riegman R (2000) Genetic and physiological variation in pigment composition of *Emiliana huxleyi* (Prymnesiophyceae) and the potential use of its pigment ratios as a quantitative physiological marker. *J Phycol* 36:529–539
- Sunda W, Kieber DJ, Kiene RP, Huntsmann S (2002) An antioxidant function for DMSP and DMS in marine algae. *Nature* 418:317–320
- Taylor BF, Gilchrist DC (1991) New routes for aerobic biodegradation of dimethylsulfoniopropionate. *Appl Environ Microbiol* 57(12):3581–3584
- Vairavamurthy A, Andreae MO, Iverson RL (1985) Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source. *Limnol Oceanogr* 30(1):59–70
- Van der Wal P, Kempers RS, Veldhuis MJW (1995) Production and downward fluxes of organic matter and calcite in a North Sea bloom of the coccolithophore *Emiliana huxleyi*. *Mar Ecol Prog Ser* 126:247–265
- Vogt R, Crutzen PJ, Sander S (1996) A mechanism for halogen release from sea-salt in the remote marine boundary layer. *Nature* 383:327–330
- Wängberg SÅ, Selmer JS, Gustavson K (1996) Effects of UV-B radiation on biomass and composition in marine phytoplankton communities. *Sci Mar* 60(Suppl 1):81–88
- Wilhelm C, Bida J, Domin A, Hilse C, Kaiser B, Kesselmeier J, Lohr M, Mueller AM (1997) Interaction between global climate change and physiological responses of algae. *Photosynthetica* 33:491–503
- Wolfe GV, Steinke M (1996) Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliana huxleyi*. *Limnol Oceanogr* 41:1151–1160