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UV radiation induced stress does not affect DMSP synthesis in the marine prymnesiophyte *Emiliania huxleyi*

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ABSTRACT: A possible coupling between UV radiation (UVR; 280 to 400 nm) induced stress and the production of dimethylsulfoniopropionate (DMSP), the precursor of the climate-regulating gas dimethylsulfide (DMS), was investigated in the marine prymnesiophyte *Emiliania huxleyi*. To this end, axenic cultures of *E. huxleyi* were exposed to a range of UVR doses for 2 consecutive days. During and after these treatments, growth, photosynthetic activity, cell size, DNA damage, sugar accumulation and DMSP concentrations were followed. The vulnerability of *E. huxleyi* for relatively low UVR doses was demonstrated by the inhibition of growth and the simultaneous occurrence of DNA damage. Also, mean cell size increased and sugars accumulated as a result of the UVR treatments. In contrast, no effect was observed on the optimal quantum yield of Photosystem II (PSII), a measure of the efficiency of photosynthesis. With increasing UVR dose, cellular DMSP content increased. However, the intracellular DMSP concentrations remained constant at the level typical for the applied temperature and salinity conditions, due to accompanying increase in cell size. The increased cellular DMSP content did not compensate, therefore, for the decreased growth rates, resulting in an overall decrease in the total amount of DMSP produced in the cultures. The UVR effects as induced in this study are assumed to be severe as compared with natural solar conditions, especially because high *in situ* UVAR (315 to 400 nm) may ameliorate UVBR damage by activation of photorepair. Yet the presented results imply that when (increased) UV(B)R causes growth rate reduction of *E. huxleyi in situ*, DMSP fluxes are likely to be reduced too.

KEY WORDS: UV radiation · Phytoplankton · *Emiliania huxleyi* · Cyclobutane pyrimidine dimers · DNA · Thymine dimers · Dimethylsulfide · Dimethylsulfoniopropionate · Salinity · F_v/F_m

INTRODUCTION

Prymnesiophytes are the main producers of dimethylsulfoniopropionate (DMSP), the precursor of the volatile sulfur compound dimethylsulfide (DMS). DMS is involved in climate regulation because its emission gives rise to cloud condensation nuclei that affect cloud reflectiveness (Charlson et al. 1987). *Emiliania huxleyi*, a cosmopolitan member of the prymnesiophytes, is known for its massive blooms mainly in temperate and subpolar regions (Holligan et al. 1993) and for its high DMSP content (Keller & Korjeff-Bellows 1996). DMSP is a compatible solute that is produced as an osmolite, especially at low temperatures (Karsten et al. 1996, Van Rijssel & Gieskes 2002). Furthermore, it may serve as an overflow metabolite during stress conditions when cells are not capable of balanced growth (Stefels 2000). Under stress the production of stress-adapted enzymes is mediated by certain proteases, which facilitate the reuse of amino acids present in enzymes that are not essential in adverse conditions. DMSP production then serves as a means to reallocate nitrogen from methionine to the synthesis of new

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amino acids. In addition, Stefels (2000) postulated that DMSP could be used as an overflow of carbon when carbohydrate production exceeds cellular carbon requirements. Additions of methionine to *Tetraselmis subcordiformis* and *Wollastonia biflora* resulted in an increased DMSP production (Gröne & Kirst 1992, Hanson et al. 1994).

It is indisputable that solar UV radiation (UVR; 280 to 400 nm) negatively affects marine microalgae, judging from the many field experiments that have demonstrated UVBR-related decreases in primary production (Smith et al. 1992, Helbling et al. 1994, Neale et al. 1994, Prezelin et al. 1994, 1998, Boucher & Prezelin 1996, McMinn et al. 1999). Inside the cell, UVBR can affect Photosystem II (PSII) efficiency (Kroon et al. 1994, Schofield et al. 1995) or the ribulose 1,5-bisphosphate carboxylase/oxygenase (RUBISCO) pool (Lesser et al. 1996). In addition, UVBR induces DNA damage, notably cyclobutane pyrimidine dimers (CPDs) (Karentz et al. 1991, Karentz 1994, Buma et al. 1996) that may arrest the cell cycle in the DNA synthesis phase. Such damage obstructs de novo synthesis of cellular components and substances required for growth and cell maintenance by replication inhibition. This may result in unbalanced growth if the damage is not readily repaired. There are several ways in which DNA damage can be repaired, one of which is photoreactivation, known to be controlled by light in the UVAR-photosynthetically active radiation (PAR) region (330 to 450 nm, Sancar & Sancar 1988). CPDs have been detected *in situ* in marine tropical picophytoplankton (Jeffrey et al. 1996a,b, Boelen et al. 2000, 2001), in temperate phytoplankton assemblages (Buma et al. 2001a, Helbling et al. 2001), and in Antarctic ice algae and phytoplankton (Prezelin et al. 1998, Buma et al. 2001b).

On top of naturally occurring UVBR stress, enhanced UVBR as a result of springtime ozone reduction further inhibits water column productivity (Smith et al. 1992, Helbling et al. 1994). Ozone depletion extends from polar into subpolar and temperate regions including those where the prymnesiophytes *Phaeocystis* sp. and *Emiliania huxleyi* form massive blooms. *E. huxleyi* is rather sensitive to UVBR, as concluded from laboratory studies (Buma et al. 2000, Garde & Cailliau 2000). DNA damage and complete growth inhibition already occurred at a daily weighted UVBR dose of 400 J m^{-2} (biological effective dose $[BED]_{DNA_{300\,nm}}$). It is therefore imaginable that increased levels of UVBR as a result of ozone depletion cause damage and unbalanced growth in *E. huxleyi*. If so, DMSP production could be affected as well. Hefu & Kirst (1997) already showed that high levels of UVBR inhibited DMSP production in *P. antarctica* cultures. Also, Sakka et al. (1997) observed elevated DMSP levels in microcosms under reduced UVR.

Based on current knowledge it is likely that a coupling exists between ozone depletion-related increases in UVBR and DMSP production by microalgae. In the present study, the effect of UVR on DMSP synthesis in *Emiliania huxleyi* was studied with the aim to investigate whether *E. huxleyi* uses DMSP as an overflow metabolite under UVR-mediated disturbance of balanced growth. UVR stress was induced in axenic cultures of *E. huxleyi*, after which the effects on growth, photosynthetic activity, cell size, DNA damage, sugar accumulation and DMSP concentration were followed.

MATERIALS AND METHODS

Axenic *Emiliania huxleyi* strain L was cultured in cotton-plugged Erlenmeyer flasks filled with artificial seawater medium described by Veldhuis & Admiraal (1987), except for the vitamins and concentrations of NO_3^- and PO_4^{3-} (88.3 and 3.6 µM, respectively) that were taken from Guillard (1975). The concentration of bicarbonate was doubled to 4.5 mM to avoid carbon limitation of this coccolithophorid. Stock cultures were regularly checked for bacterial contamination by staining with Hoechst dye 33258 (Paul 1982).

At the start of a UVR experiment (both pilot and final experiment), a stock culture (adapted to 15°C and 200 µmol m^{-2} s^{-1} PAR for 3 wk) was inoculated (1% v/v) into 1 l medium and incubated until a cell density of 9.10^8 l^{-1} was reached. Seven quartz tubes (200 ml) were filled with 3-fold diluted culture, closed with silicone stoppers and submerged in a glass aquarium that was placed inside a culture cabinet to ensure identical temperatures during the experiments (15°C). The aquarium was continuously illuminated from below with 6 tubes providing PAR (200 µmol m^{-2} s^{-1}). Above the aquarium, 2 UVR lamps (Philips TL 20W/12) were placed. Each quartz tube was covered with a UVR-opaque cut-off filter sleeve (Farblos 125 µm, Digefra) until the beginning of the experiment to allow PAR only to the cultures. During UVR exposures this filter was replaced by a 294 nm cut-off filter (Ultraphan UBT 500 µm), to allow UVAR and UVBR and to exclude UVCR emitted by the TL 20W/12 lamps. UVR exposure times for the individual tubes were 0, 0.5, 1, 1.5, 2, 4 and 7 h d^{-1} on 2 consecutive days. To minimize the risk of location artifacts, the tubes were repositioned 8 times a day. Lamp spectra were measured with a spectroradiometer (MACAM SR9910 double monochromator scanning spectroradiometer, Macam Photometrics, equipped with a 4.2 m quartz cable connected to a 4π collector) (Fig. 1), weighted with the DNA action spectrum of Setlow (normalized at 300 nm, Setlow 1974) and multiplied by the exposure period to give the daily $BED_{DNA_{300\,nm}}$. For all tubes the 2 UVR periods ended

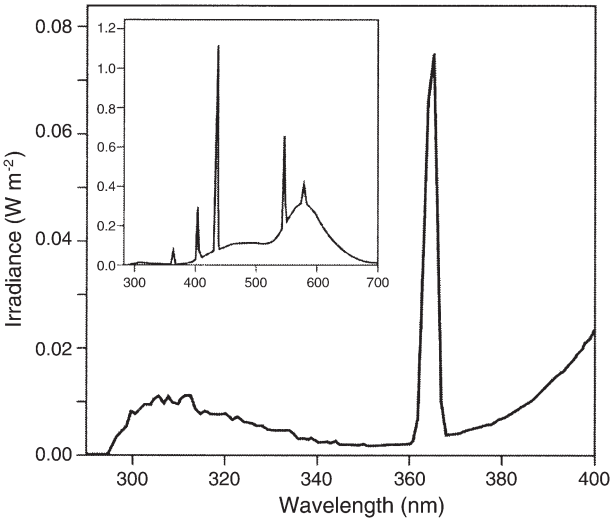

Fig. 1. Combined spectrum of the photosynthetically active radiation (PAR) and UV radiation (UVR) lamps to which the quartz tubes filled with *Emiliania huxleyi* culture were exposed

simultaneously (24 and 48 h after the beginning of the experiment). The pilot and final experiment were essentially the same except for the additional parameters measured in the final experiment (see below). After the first UVR treatment (Day 1), samples (0.5 ml) were taken from the tubes for analysis of biomass (pilot and final experiment), after which the tubes were placed back in the water bath. After the second UVR treatment (Day 2), the tubes were harvested, during which samples were taken for biomass, as well as for analyses of DMSP (pilot and final experiment), DNA damage, PSII efficiency and sugar content (final experiment).

An electronic particle analyzer (Coulter Counter ZM equipped with Channelyser 256 and a counting tube with an 30 µM inlet, Coulter Electronics) was used for analysis of cell density, average diameter and total biovolume. Particle size was calibrated with latex particles 8.7 µm in diameter (Coulter Electronics). Measurement of the diameter of cells in 1 sample using a microscope revealed that the Coulter counter measurements did not include the coccolithosphere. Samples were diluted (20×) in 0.2 µm filtered medium and placed in the dark before analysis within 1 h.

Total sugar was measured (3 samples per quartz tube) as described by Liu et al. (1973). Glucose was used as a standard.

Photosynthetic activity was assessed by measuring *in vivo* chlorophyll *a* fluorescence with a pulse-amplitude modulated fluorometer (PAM 2000, Walz). Samples were kept in the dark (to prevent photorepair) before filtering 10 ml on a GF/F filter (1 cm diameter). Filters were mounted on the end of the fiberoptic probe and inserted into a temperature-controlled cuvette (15°C) filled with seawater. The maximal quantum efficiency of 'dark-adapted' algae was determined as the ratio of variable to maximum fluorescence (F_v/F_m) (Van de Poll et al. 2001). After application of a 5 s far red pulse (~30 µmol m^{-2} s^{-1}) used to oxidize the electron transport chain, initial fluorescence (F_0) was measured with a red measuring light pulse (~0.3 µmol m^{-2} s^{-1}, 650 nm), and F_m was determined with a 800 ms completely saturating white light pulse (~9200 µmol m^{-2} s^{-1}). The samples that received the highest UVR doses were measured first. All samples were measured within 2.5 h.

DNA damage was measured following the immunochemical method described earlier (Buma et al. 2001b), slightly modified after Boelen et al. (1999). CPD was quantified by comparing sample DNA with a dilution series of damaged standard DNA. The amount of CPDs in the standard DNA was determined by calibrating against DNA isolated from irradiated HeLa cells, with a known amount of CPDs (kindly provided by Dr. A. Vink, TNO Rijswijk, The Netherlands). The amount of CPDs in this DNA was determined by Roza et al. (1988) by means of HPLC. All measurements were done in duplicate.

For DMS and DMSP analyses, 5 ml samples were transferred in 20 ml crimp top vials (Chrompack) containing 50 µl phosphoric acid (85%). The acid brings the pH below 1, preventing conversion of DMSP to DMS. Vials were immediately sealed with a Teflon-lined, butyl rubber septum (Chrompack) and stored at 4°C in the dark until analysis of the headspace. Vials were incubated in a 30°C water bath and shaken firmly before injecting a 0.5 ml headspace sample into a Packard 437 gas chromatograph with flame ionization detector (Visscher & van Gemerden 1991); a Supelpak S column was used instead of a Porapak column. This headspace value, after calibration, gives DMSconc. Then 0.5 ml of 10 M NaOH was injected into the vial to convert all DMSP into DMS and acrylate overnight. Again, DMS was measured in the headspace (DMStotal). For calibration, DMSP (prepared according to Chambers et al. 1987) standard (0 to 10 µM) was converted to DMS in the same way as the samples and measured. The detection limit was 0.1 µM and the duplicate measurements differed less than 5%. The concentration of DMSP is usually calculated according to the formula DMSP = DMStotal – DMSconc, but DMS concentrations were never above the detection limit. Hefu & Kirst (1997) described a UVR-related conversion of DMSP to DMS of 1 to 2% h^{-1}. We did not correct for this conversion because the UVR used in their study was 15 times higher than that applied here.

For the calculations of DMSP per cell and per cell volume, we did not discriminate between particulate and dissolved DMSP. An earlier experiment with

cultures adapted to different salinities (same amount of PAR, same temperature) revealed a linear relationship between salinity and the particulate DMSP per biovolume (DMSP concentration [mM] = 13.3 salinity [‰] – 301, $r^2 = 0.994$, $p < 0.0005$, n = 5 at 15°C) and a small percentage (increasing from 3.9 to 12.7%) of DMSP in the medium (GF/F filtration, gravity only). The expected percentage of dissolved DMSP at the salinity used in the UVR experiments is 5.6%.

Statistical analysis of the data involved linear regression on the average values obtained for each of the 7 independent tubes. In case of a significant linear relationship (at $p < 0.05$ level) regression lines were drawn in the figures; if not, data were connected by lines.

RESULTS

Tubes received both UVBR and UVAR (Fig. 1). The UVB/UVA ratio was 0.28, more than 10 times higher than under natural solar radiation conditions (Sakka et al. 1997). Cultures receiving biologically effective UVR doses up to 366 J m^{-2} d^{-1} were growing at 0.3 d^{-1} (data not shown). Higher UVR doses increasingly affected growth, as was observed by the difference in cell production after the 2 UVR treatments (Fig. 2C). DNA damage, however, as measured by CPD concentration, could already be observed at a dose of 244 J m^{-2} d^{-1} and higher (Fig. 2A). Above this threshold a linear dose-response relationship was found between the UVR dose and the induced CPDs (CPD Mega $base^{-1}$ = 0.228 $BED_{DNA300\,nm}$ J m^{-2} d^{-1} – 39.53, $r^2 = 0.98$, $p < 0.005$). As a result of UVR exposure, the mean cell size of *Emiliania huxleyi* increased especially after the second UVR treatment, as is illustrated for the highest UVR dose (Fig. 3). An overall increase in mean cell size was observed with increasing UVR doses after the second UVR treatment: cell volume (μm^3) = 0.0071 $BED_{DNA300\,nm}$ J m^{-2} d^{-1} + 42.07, $r^2 = 0.92$, $p < 0.001$, data not shown).

There was no effect ($r^2 = 0.42$, $p = 0.116$) of UVR on the optimal quantum yields of PSII (F_v/F_m = 0.646 ±

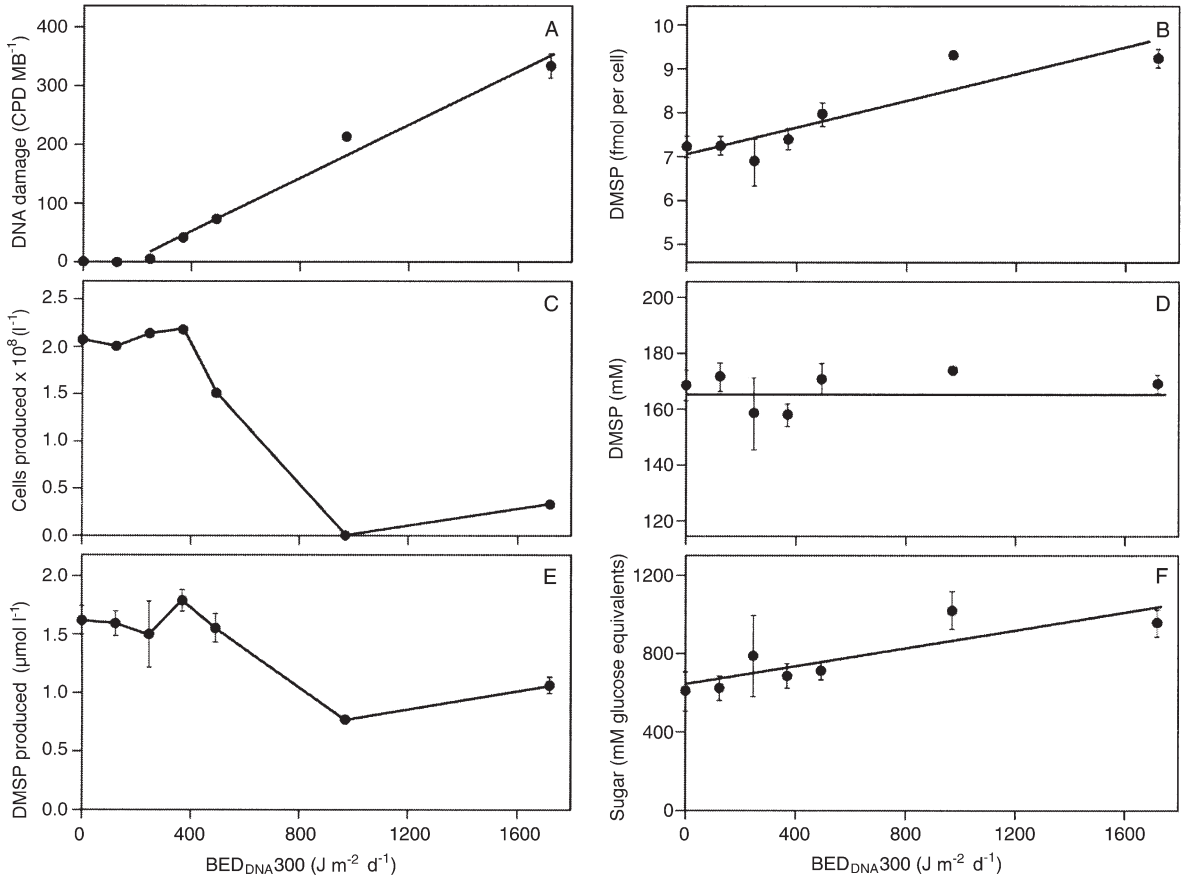

Fig. 2. Effects of UVR on *Emiliania huxleyi* after 2 d of exposure. (A) Amount of cyclobutane thymine dimers (CPDs) present in extracted DNA as a measure of DNA damage; (B) amount of dimethylsulfoniopropionate (DMSP) per cell; (C) growth, expressed as the increase in cell density; (D) concentration of DMSP in the cells (DMSP in culture divided by total biovolume); (E) DMSP (µmol l^{-1}) produced in the cultures; (F) concentration of sugars in the cells

0.03, average [AVG] ± standard error [SE], data not shown). Sugars accumulated in the cells when exposed to UVR. Although the cells became bigger as well, the concentration of total sugars in the cell increased with higher doses (sugar [mM] = 0.242 $BED_{DNA300\,nm}$ J m^{-2} d^{-1} + 636.74, r^2 = 0.73, $p < 0.0001$; Fig. 2F).

UVR exposure resulted in an increase in total DMSP per cell (DMSP [fmol $cell^{-1}$] = 0.00153 $BED_{DNA300\,nm}$ J m^{-2} d^{-1} + 7.03, r^2 = 0.81, $p < 0.001$; Fig. 2B). However, the amount of total DMSP per biovolume did not change (r^2 = 0.10, p = 0.498; Fig. 2D) and was 167.7 ± 2.4 mM (AVG ± SE) in the final experiment. This value, which includes both particulate and soluble DMSP, corresponds well with the 169 mM expected for the salinity that was used (34.7‰), based on the relation between salinity, particulate DMSP and dissolved DMSP measured in an earlier experiment without UVR (sum of 160.5 mM particulate DMSP and an additional 5.6% of soluble DMSP).

The pilot experiment, which comprised only the biomass and DMSP data, gave similar results: cells became bigger with increasing UVR doses (volume [μm^3] = 0.013 $BED_{DNA300\,nm}$ J m^{-2} d^{-1} + 45.5, r^2 = 0.77, $p < 0.01$, data not shown), and the DMSP per biovolume was 173.2 ± 8.8 mM (r^2 = 0.002, p = 0.933, data not shown). The difference with the final experiment was the slightly bigger cells and a slightly higher DMSP per biovolume at the start of the experiment. Therefore, these results were not combined with those of the final experiment.

The total amount of DMSP produced in the cultures during the experiment at the 2 highest doses was less than that produced at low doses (Fig. 2E). The apparent slight increase in cell production in the tube that received the highest dose seems to be consistent with the slight increase in total DMSP in the cultures (both independent measurements). However, it should be realized that both values are the result of subtracting the starting value from the value at the end of the experiment. The difference between the actual amount of cells and DMSP in the cultures receiving 970 and 1700 $BED_{DNA300\,nm}$ (J m^{-2} d^{-1}) was 10 and 13%, respectively. Although significant effects were found between increasing UVR doses and some measured parameters using linear regression analysis, the combined effects (for growth and total DMSP production) do not necessarily have to be linear as well. Perhaps these respond with an abrupt change at a certain threshold and virtually the same values for cultures above that threshold.

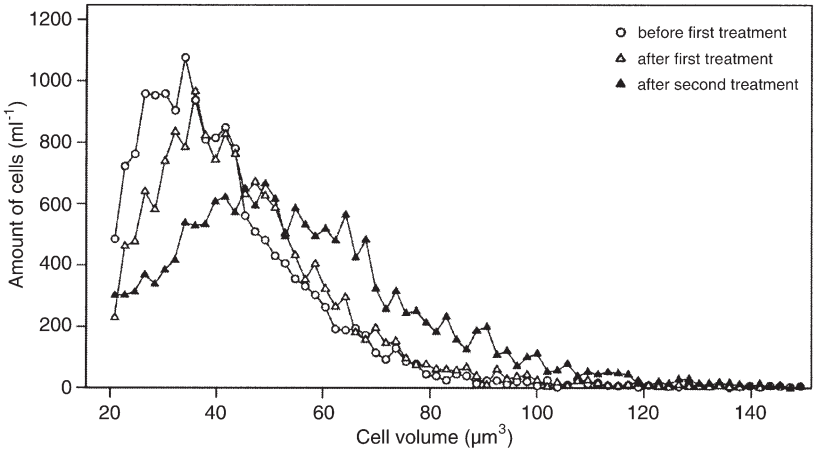

Fig. 3. Effects of UVR (daily weighted dose of 1700 J m^{-2}) on the cell size distribution of *Emiliania huxleyi* before and after the 2 UVR treatments

DISCUSSION

The present study shows that UVR stress in *Emiliania huxleyi* does not alter the intracellular DMSP concentration. The observed UVR stress-related increase in cellular DMSP content, presumably coupled to cell size increase, could not compensate for decreased growth at doses higher than a $BED_{DNA300\,nm}$ of 488 J m^{-2} d^{-1}. The total amount of DMSP produced in the cultures, therefore, decreased at high UVR doses.

The ratio of UVB to UVA applied here was such that the adverse effects of the UVR can be attributed mostly to UVBR. Clearly, low levels of biologically effective UVR caused growth rate reduction and DNA damage, whereas between 200 and 250 CPDs Mega $base^{-1}$ brought about complete cell cycle arrest in *Emiliania huxleyi*. Obviously, repair pathways were not sufficient to prevent the accumulation of damage during UVR treatments. The weighted UVR doses used here can be considered representative for daily incident UVR doses in summer in temperate regions (Madronich 1992). However, the observed characteristics of UVR stress cannot be extrapolated to the situation in the field due to the low levels of UVAR in our experimental design. On the one hand, natural levels of UVAR addition could have favored DNA repair, thereby ameliorating the effect of UVBR on DNA (Sancar & Sancar 1988). Indeed, the effect of UVR on CPD formation, growth rate or cell size was virtually absent in another experimental series when low levels of UVAR (approximately 4 W m^{-2}) were given in addition (results not shown). On the other hand, only 40 CPDs Mega $base^{-1}$ were tolerated before cell production was affected (Fig. 2A,C), whereas between 200 to 250 CPDs Mega

base^{-1} caused complete cell cycle arrest. These CPD levels as recorded in our study fall within or are slightly above *in situ* ranges found in temperate or Antarctic phytoplankton assemblages (Buma et al. 2001a,b, Helbling et al. 2001). Obviously, high *in situ* UVAR and PAR conditions do not always guarantee an optimal balance between damage and repair. This may, for instance, lead to the accumulation of CPDs during UVR exposure hours, as found in a number of regions and phytoplankton assemblages (Jeffrey et al. 1996b, Boelen et al. 2001, Buma et al. 2001a,b, Helbling et al. 2001). In addition, this imbalance in damage and repair is likely to escalate under conditions of ozone depletion, because the selective increase in UVBR would cause increased ratios of UVBR to UVAR and UVBR to PAR.

UVR-mediated increases in cell size and DNA damage (Fig. 3) imply that the cell cycle was arrested in the S or G2 phase, as found in earlier studies (Buma et al. 2000, Garde & Cailliau 2000). At the same time, no effect was observed on the optimal quantum yield of PSII, an indication of unchanged photosynthetic efficiency (Hofstraat et al. 1994), whereas sugars accumulated as a result of UVR treatment. These results indicate that, as a result of continued photosynthetic performance, the carbon that otherwise would have been incorporated into a balanced set of substances (among others, DNA) was temporarily stored in the sugar pool. Such overflow metabolism is often observed when cells are not able to produce biomass in an optimal way, for example in the case of nutrient limitation (Myklestad 1989). More than 10-fold higher UVR levels than in this experiment (14×) did affect ^{14}C incorporation by *Emiliania huxleyi* in the study of Garde & Cailliau (2000).

The stress induced by UVR did not result in altered DMSP physiology, as could be judged from the unchanged DMSP concentration in the cells. Values corresponded with earlier observations for this temperature and salinity. Apparently, DMSP synthesis was not hampered by UVR, nor was it used as a way to release superfluous carbon. This observation corresponds well with the DO^{14}C excretion rates estimated by Garde & Cailliau (2000) that followed PO^{14}C with and without UVR treatment. Although we cannot exclude that the percentage of DMSP in the medium at stress conditions was higher than the 6% measured in earlier experiments, increased production appears unlikely since it would have resulted in higher values for DMSP volume^{-1}. The observed increase in the amount of DMSP cell^{-1} upon increased UVR shows how misleading this expression of DMSP content is in studies related to DMSP physiology (Keller 1991, Stefels 2000).

Despite the fact that DMSP synthesis was in balance with the increase in cell volume, the total amount of biomass formed was affected by UVR, and as a consequence the total production of DMSP in the cultures was depressed by UVR (Fig. 2E). Similarly, the inhibition of DMSP production observed in cultures of *Phaeocystis antarctica* under high levels of UVR (Hefu & Kirst 1997) is most likely due to growth inhibition rather than to specific effects on DMSP metabolism.

In conclusion, this study has shown that in a worst case scenario, where UVR stress causes unbalanced growth in *Emiliania huxleyi*, the intracellular DMSP concentration remains unchanged. DMSP, in this case of stress, was not used as an overflow metabolite. Yet, when UVBR stress occurs in *E. huxleyi* in the field, DMSP production might be affected simply due to reduced production of biomass. Whether *E. huxleyi* experiences DNA damage under natural solar conditions or ozone depletion events should be revealed by field experiments. Only then can we estimate to what extent DMSP production is negatively affected by solar UVBR. Finally, how this affects DMS formation depends on other factors as well, such as UVR-mediated changes in bacterial composition and activity.

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