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Effect of short-term light- and UV-stress on DMSP, DMS, and DMSP lyase activity in *Emiliania huxleyi*

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ABSTRACT: The ecological conditions and cellular mechanisms which affect the production of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) in marine ecosystems are still enigmatic. This information is crucial for deriving accurate oceanic ecosystem models for the dynamics of these major players in the Earth's sulfur cycle and climate. In the present study, we examined the effect of short-term increases in photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) on the production of DMSP and DMS and on DMSP lyase potential activity (DLPA) in an axenic culture of the coccolithophore *Emiliania huxleyi* (CCMP 1742). Algal cells were subjected to a rapid shift from a low intensity of PAR (50 µE m^{-2} s^{-1}; low light, LL) to a high intensity of PAR (198 µE m^{-2} s^{-1}) and elevated UVR conditions (high light, HL), simulating what may occur during upward mixing in the surface mixed layer or during changes in cloud cover. During the 4.5 h exposure to HL, the intracellular DMSP normalized to cell volume increased by ca. 30%, and dissolved DMSP doubled relative to control values. However, the DLPA per unit of cell volume decreased by ~45% compared to the control value. The up-regulation of cellular DMSP concentration is consistent with an antioxidant and/or energy dissipation role for DMSP. The decrease in DLPA may indicate that the DMSP lyase enzyme plays no role in antioxidant protection in this algal species, but rather serves some other cellular function, such as grazing protection.

KEY WORDS: DMSP · DMS · DMSP lyase · Light stress · Ultraviolet radiation · Phytoplankton

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INTRODUCTION

Dimethylsulfoniopropionate (DMSP) is a zwitterionic sulfonium compound present in the sea. It occurs at high intracellular concentrations in certain taxonomic groups of marine microalgae and macroalgae (Stefels et al. 2007) and is a major source of organic sulfur to microorganisms in the ocean (Kiene et al. 2000). It is also the precursor of dimethylsulfide (DMS), a trace gas which plays a major role in the global sulfur cycle by transferring sulfur from the oceans to the atmosphere and ultimately to land (Lovelock et al. 1972). In addition, both DMSP and DMS serve as major signaling molecules in planktonic food webs (Steinke et al. 2006, Seymour et al. 2010, Lewis et al. 2013, Garren et al. 2014). Following its emission from the ocean's surface, DMS is rapidly oxidized to acidic sulfur species in the atmosphere,

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which form aerosols that serve as cloud condensation nuclei and that contribute to the natural acidity of rain (Nguyen et al. 1992). The resulting aerosols and clouds reflect solar radiation and thereby contribute to climate cooling, particularly in aerosol-poor atmospheric regions such as those near the poles (Charlson et al. 1987, Chang et al. 2011, Quinn & Bates 2011).

The conditions and mechanisms that affect the production (or transformation) of DMSP in marine ecosystems are still enigmatic. More knowledge is needed to develop better predictions of concentrations of DMSP and DMS in surface ocean waters from ecosystem models (Vézina 2004, Le Clainche et al. 2010). Evidence suggests that DMSP serves multiple functions in marine phytoplankton. It functions as a compatible solute (Welsh 2000), provides cryo-protection (Karsten et al. 1996), and deters zooplankton grazing (Wolfe et al. 1997, Strom et al. 2003, Lewis et al. 2013, Savoca & Nevitt 2014). Three other hypothetical roles are also frequently invoked: DMSP could act as a methyl donor in metabolic reactions (Ishida 1968), as an overflow metabolite for photosynthetically fixed carbon and reducing equivalents (e.g. NADPH) during times of unbalanced growth (Stefels 2000), and as an antioxidant in marine algae, the so-called 'antioxidant hypothesis' (Sunda et al. 2002).

Laboratory experiments have suggested that DMSP and DMS and their stepwise oxidation products (DMSO and methane sulfinic acid) constitute an antioxidant system in marine phytoplankton, which scavenges harmful hydroxyl radicals (•OH) and other reactive oxygen species (ROS) produced under oxidative stress (Sunda et al. 2002). The DMSP antioxidant system is hypothesized to be partly regulated through the activity of DMSP lyase (DL), as the enzyme cleavage products, DMS and acrylic acid, are ~60 and ~20 times more efficient in removing •OH radicals than DMSP. DMSP, DMS, DMSO, and methane sulfinic acid are all effective •OH radical scavengers, with a reactivity order of DMS > methane sulfinic acid > DMSO > DMSP (Sunda et al. 2002). As with other antioxidants, cellular DMSP has been observed to be up-regulated in marine algae under increased oxidative stress (Sunda et al. 2002). This increase in cellular DMSP would raise the antioxidant capacity of the cells to more efficiently scavenge intracellular ROS, which are produced at higher rates under increased levels of oxidative stress.

There is circumstantial evidence for the DMSP antioxidant hypothesis in nature. DMS concentrations and production rates in surface ocean waters are broadly correlated with the daily dose of solar radiation and solar UV radiation (Toole & Siegel 2004, Vallina & Simó 2007). Furthermore, cellular concentrations of DMSP, DMS, and DMSO as well as algal DMSP lyase potential activities (DLPA) are often significantly correlated with algal photoprotective or antioxidant carotenoids in seawater (Belviso et al. 1993, Steinke et al. 2002, Harada et al. 2004, Riseman & DiTullio 2004).

Apart from their direct antioxidant role, DMSP and its degradation products have been proposed to constitute a photosynthetic overflow mechanism, which helps balance carbon fixation and utilization during periods of unbalanced growth. Under this scenario, algal cells may induce net cellular DMSP biosynthesis as well as its enzymatic lysis to DMS to discard unneeded fixed carbon and/or reduced sulfur when the rates of carbon fixation and/or sulfur reduction are too high relative to the assimilation of other cellular nutrients such as nitrogen (Stefels 2000). For example, in response to different abiotic stressors such as cold temperature (Karsten et al. 1992, van Rijssel & Gieskes 2002), nitrogen limitation (Stefels 2000), or exposure to ultraviolet radiation (UVR) (Stefels & Van Leeuwe 1998), a putative DMSP overflow mechanism could provide a sink for unneeded photosynthetic products (NADPH and ATP) during periods of decreasing cell biosynthesis and growth. By serving as a sink for unneeded electrons, increases in DMSP and DMS production could help prevent over-reduction of the photosynthetic apparatus, which helps protect algal cells from oxidative stress (Niyogi 1999).

To refine our limited knowledge of the role and regulation of methylated organic sulfur compounds in phytoplankton, we cultured the coccolithophore *Emiliania huxleyi* (CCMP 1742) in axenic laboratory cultures and rapidly exposed low-light acclimated cells to an increase in PAR and UVR as they might experience between cloudy and clear skies or when transported from the bottom of the photic zone toward the surface by wind mixing. Both the antioxidant hypothesis (Sunda et al. 2002) and the photosynthetic overflow hypothesis (Stefels 2000) predict that such high light and UV stress should increase cellular DMSP, DL activity, and cellular release of DMS. To test these predictions, we measured intracellular and extracellular DMSP, cellular DLPA, dissolved DMS, maximum quantum yield of Photosystem II (F_v/F_m), and the epoxidation state of photoprotective pigment diadinoxanthin in *E. huxleyi* cultures before and after cellular expo-

sure to oxidative stress linked to high light and solar UVR.

MATERIALS AND METHODS

Microbial strains and culture conditions

Experiments were performed with axenic cultures of *Emiliania huxleyi* strain CCMP 1742 (Provasoli-Guillard National Center for Marine Algae and Microbiota, East Boothbay, Maine, USA), a non-calcifying oceanic strain isolated from the northeast Pacific. Cells were grown in f/2-Si medium (Guillard 1975) made with 0.2 µm sterile-filtered artificial seawater (salinity 35) and incubated at 15°C. Stock cultures were maintained aseptically under a 14:10 h light:dark cycle. Light was provided at an intensity of 50 µE m^{-2} s^{-1} by GE FS40 40 W fluorescent bulbs. The stock cultures were kept in mid-exponential growth phase by dilution with fresh medium. They were maintained in this low light environment for at least 6 cell divisions before the beginning of the experiments. All glassware and plastic bags were soaked for 24 h in 5% v/v HCl and rinsed with ultrapure water before use.

Light experiments

Due to logistic considerations, 2 separate experiments were conducted. In the first, 5 sterile 1 l (0.05 mm thick) Tedlar® gas sampling bags (SKC) were filled with 1 l of f/2-Si medium and were subsequently inoculated with exponentially growing cells acclimated to a light intensity of 50 µE m^{-2} s^{-1}. The Tedlar® gas sampling bags were ≥89% transparent to PAR and ultraviolet A and B radiation (UVAR and UVBR) (Twardowski & Donaghay 2002) and were impermeable to DMS. After inoculation, 5 experimental cultures were placed on a clear acrylic shelf above 2 PAR fluorescent light bulbs and were grown for 5 d at a light intensity of 50 µE m^{-2} s^{-1} (low light; LL). Continuous light was used to eliminate the effects of the photoperiod. Immediately prior to the experiment, all experimental cultures were gently mixed to re-suspend cells and were repositioned along the acrylic shelf. At the start of the experiment, the algal cells were in the mid-to-late exponential phase of growth. Subsequently, 2 bags were transferred to a high light environment (HL; PAR intensity of 198 µE m^{-2} s^{-1}), while 3 remained under low light exposure (LL; PAR intensity of 50 µE m^{-2} s^{-1}). For the HL environment, the cultures were placed on a second clear acrylic shelf and irradiated from above by 2 UVR fluorescent bulbs and from below by 6 PAR fluorescent bulbs (see description below). Samples for cell counts, pigments, photosynthetic physiology, and particulate and dissolved DMSP were taken from each culture bag immediately and thereafter at intervals of ~1.5 h over a period of 4.5 h in the HL treatment and 4.6 h in the LL treatment.

The second experiment was designed to determine the impact of the same light treatment as described above on DMSP lyase potential activity (DLPA) and DMS production in *E. huxleyi*. In this case, 6 sterile 1 l Tedlar® gas sampling bags were filled with 0.58 l of f/2-Si medium and were subsequently inoculated with exponentially growing cells (acclimated to LL) as in the first experiment. Two bags were initially harvested at the beginning of the experiment (LL, 0 h). Two bags were immediately transferred to the HL environment while 2 remained at LL. These 4 culture bags were then harvested 4 h later (LL, 4 h and HL, 4 h) and measured for DMS, DLPA, cell concentration, and total cell volume.

The irradiances for the HL and LL treatments are detailed in Table 1 and in the Supplement at www.int-res.com/articles/suppl/a074p173_supp.pdf. PAR was provided by 32 W cool white fluorescent bulbs (Sylvania). UVR (UVA and UVB) was provided by 40 W UVA-340 fluorescent bulbs (Q-Panel Lab Products) which had been pre-burned for >300 h to stabilize lamp emissions. The UV cutoff point of these lamps was 295 nm (Q-Panel Lab Products). Irradiances were measured from inside an empty Tedlar® gas sampling bag using an IL1700 Radiometer (International Light) fitted with 2 π sensors for PAR (400–700 nm), UVAR (315–400 nm), and UVBR (280–315 nm). Our ratio of PAR to UVBR energy intensity (in W m^{-2}) in the HL treatment was 483:1 W:W, a ratio equivalent to that prevailing at ca. 3.8 m depth during the height of an *E. huxleyi* bloom in the northeast

Table 1. Values of photosynthetically active radiation (PAR; 400–700 nm), ultraviolet A radiation (UVAR; 315–400 nm), and UV B radiation (UVBR; 280–315 nm) under low light (LL) and high light (HL) treatments. All measurements were made inside a 0.05 mm thick Tedlar® gas sampling bag (SKC). UVAR and UVBR were not measured for the LL treatment

Radiation	Treatment	
	LL	HL
PAR (µE m^{-2} s^{-1})	50.0	198
UVAR (W m^{-2})	–	2.10
UVBR (W m^{-2})	–	0.0885

Pacific Ocean (C. Fichot unpubl. data). However, the ratio of UVAR to UVBR was ca. 2.7 times lower than found in this environment due to the spectral output of the UVR lamps.

Sampling and measurements of DMS and DMSP

For DMS analysis, 20 ml samples were filtered by gravity through 47 mm GF/F filters (Whatman). Intracellular (particulate) DMSP (DMSPp) and dissolved DMSP (DMSPd) samples were prepared for analysis as described by Kiene & Slezak (2006) followed by base hydrolysis to DMS. For DMSPd analysis, 3.5 ml of culture were filtered by gravity through 47 mm GF/F filters into polypropylene tubes containing 50 µl 50% (v/v) sulfuric acid. The tubes were incubated in the dark for a minimum of 12 h to destroy DMS. Subsequently, a 3 ml sub-sample was transferred to a 25 ml glass septum vial containing 21 ml deionized water. One ml of 5 M NaOH was added to the vial and the vial was quickly crimp-sealed. Total DMSP samples were treated the same as those for DMSPd except that 3 ml of unfiltered culture were added to tubes containing sulfuric acid. The sealed vials containing base for measurement of total and dissolved DMSP were stored in the dark for at least 12 h before analysis to allow the complete hydrolysis of DMSP to DMS.

The DMS originally present in the samples and that liberated by base hydrolysis of DMSP was measured by gas chromatography following preconcentration of DMS by a purge and cryotrap system (Scarratt et al. 2002). DMSPp was determined by subtracting the concentration of DMSPd from that of total DMSP. The gas chromatograph (Varian CP-3800) used for DMS analysis was fitted with a pulsed-flame photometric detector. A Chromopak fused-silica capillary chromatographic column (CP-Sil 5 CB for sulfur) was used and was maintained isothermally at 70°C. The output of the detector was analyzed using a Star 6.2 chromatography workstation integration program (Varian). The detection limit was approximately 3 pmol DMS per injection. For sulfur analyses, the system was calibrated (7.9–195 pmol DMS) using inert loops of different sizes filled with DMS diffusing at 497 ng min^{-1} at 40°C from a permeation tube (KIN-TEK).

DLPA assay

In the DLPA assay, cells were isolated from 475–500 ml culture samples via centrifugation at 4°C. The resulting cell pellet was placed in 1.8 ml of 163 mM citric acid/phosphate buffer and sonicated on ice at 5 W with 5 pulses of 5 to 10 s duration using a Misonex 2000 homogenizer. The lysate was immediately frozen at −20°C and stored until analysis less than 4 d later. Freezing was found not to affect DLPA values (data not shown). DLPA was assayed by following the cleavage of DMSP to DMS in thawed cell lysate samples using the headspace technique of Steinke et al. (2000). The DLPA measurements were conducted at 30°C in a 0.5 M NaCl solution containing a 182 mM citric acid/phosphate buffer at pH 7 (see Fig. S1 in the Supplement). The evolved DMS from DMSP cleavage by DL was analyzed using the gas chromatographic technique described above. The experimental DLPA assays were run in duplicate. DLPA was normalized to the cell number or total cell volume of the centrifuged cells (Evans et al. 2007).

Cell abundances and volumes

In the first experiment, *E. huxleyi* cell abundance and mean volume per cell were measured using a FlowCAM (Fluid Imaging Technologies). The FlowCAM was fitted with a 20× microscopic objective and 2 × 0.1 mm flow chamber. Samples were diluted 11-fold in f/2-Si medium that was freshly filtered through 0.2 µm cellulose acetate filters. Cell abundances and mean total volumes (in liter of algal cells or l_{cell}) were subsequently determined by continuous imaging at 3 frames s^{-1} at a flow rate of 0.2 ml min^{-1} for 5 min. Cell concentrations and mean volume per cell were calibrated with a range of concentrations of 5 µm latex beads (1.76×10^5 to 14.1×10^5 beads ml^{-1}; Beckman Coulter), which were of similar size to our experimental algal species. Total cell volumes (µl cells $l^{-1}_{culture}$) were determined by multiplying the cell concentration by the mean volume per cell.

The second experiment assessed the effect of HL on culture DLPA and dissolved DMS concentrations. In that experiment, cell abundances were measured using a light microscope and a hemocytometer (n = 6). Tests on exponentially growing *E. huxleyi* cultures showed that counts between the hemocytometer (y) and FlowCAM (x) were comparable ($y = 1.09x$, $r^2 = 0.98$, $p < 0.001$, n = 24, range = 0.9–3.1×10^6 cells ml^{-1}). The cellular biovolume was estimated using the mean biovolume per cell measured in the first experiment.

Bacterial contamination was checked at the end of both experiments. Bacterial count samples were preserved in 2.2% (w/v) formaldehyde and stored at 4°C

in the dark. Sub-samples were subsequently stained with 0.01 µg ml^{-1} 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) for 5 min and analyzed by epifluorescence microscopy following the method described by Porter & Feig (1980). No bacterial contamination was found (n = 5, filtration of 5 ml of culture medium and visualization of the whole filter at 1000×).

Pigment analysis

Cells were collected onto GF/F filters under dim green light within 2 to 6 min after removal from the experimental chamber to minimize re-conversion of the xanthophyll diatoxanthin into diadinoxanthin. The filters were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Pigments were extracted in 3 ml of a 95/5% methanol/water mixture (v/v) using a tip sonicator. The extracts were centrifuged, filtered through 0.22 µm Gelman Acrodisc filters, and stored under argon gas at 4°C in the dark. They were then analyzed by high pressure liquid chromatography (HPLC) within 1 to 12 h of extraction. One hundred microliters of the extracts were automatically injected into a ProStar HPLC (Varian) equipped with a Symmetry C8 column (3.5 mm pore size, 4.6 × 150 mm, Waters). Chlorophylls were detected by fluorescence (excitation at 440 nm, emission at 650 nm) and carotenoids by on-line diode-array spectroscopy (slit width of 1 nm; absorbance at 450 nm used for quantification). Calibration was done with external standards obtained commercially from Sigma and DHI Water & Environment. The standard concentrations were determined from their extinction coefficients. The HPLC solvent protocol followed the procedure of Zapata et al. (2000) based on the gradient dilution with 2 solvent mixtures: a methanol, acetonitrile, and aqueous pyridine (50:25:25 v/v/v) solution and a methanol, acetonitrile, and acetone (20:60:20 v/v/v) solution. The flow rate was 1 ml min^{-1} and the equilibration time was 5 min. The xanthophyll molar ratio, i.e. the concentration of diatoxanthin (DT) divided by that of the xanthophyll pool (diatoxanthin [DT] + diadinoxanthin [DD]), was calculated as an index of xanthophyll cycle activity.

Photosynthetic physiology

Cells were dark-adapted at room temperature (~20°C) for 30 to 70 min to allow relaxation of non-photochemical quenching. A 3 ml aliquot was then transferred to a quartz glass cuvette, and induced chlorophyll fluorescence was measured using a pulse-amplitude modulated (PAM) fluorometer (Phyto-PAM field version, Walz). The maximum quantum yield of PS II photochemistry, i.e. the ratio of variable to maximum fluorescence (F_v/F_m) of the photosynthetic apparatus, was determined to establish the physiological state of the cells. Preliminary tests showed that a 200 ms pulse at a light intensity of ca. 4000 µE m^{-2} s^{-1} was sufficient to achieve maximum fluorescence. Thus, 200 ms was used in all experimental measurements.

Statistical analyses

The differences from the mean of each of the dependent variables (i.e. cell abundance, cell volume, xanthophyll ratio (DT/[DT+DD]), F_v/F_m ratio, DMSPp, and DMSPd) at low light intensity as a function of time were evaluated using 1-way ANOVAs. The ANOVAs were done on the raw data when the assumptions of the ANOVA, i.e. the normality and homogeneity of variance of residuals, proved to be valid with Kolmogorov-Smirnov and Levene tests, respectively. Otherwise, the Box-Cox transformation was used and the ANOVAs were run on transformed data. Due to the low sample size (n = 2) at HL intensities, we could not use ANOVAs since the assumptions of homogeneity of variance of residuals cannot be tested for this low sample size. Thus, at HL intensity, we used Pearson simple correlation analysis (on raw or Box-Cox transformed data that passed the Kolmogorov-Smirnov test for normality) to test whether each of the measured dependent variables increased or decreased significantly over time. Differences in mean DMSP cellular quotas or concentrations, F_v/F_m ratios, mean DLPA, and DMS concentrations between LL or HL treatments were evaluated using unpaired Student's *t*-tests. The *t*-tests were run, after having tested on each variable the assumptions of normality and homogeneity of variances of data with the Kolmogorov-Smirnov and the 2-sample *F*-test, respectively. All errors in the present study are presented as ± 1 SD. The SDs of DMSPp and DMSPd normalized per cell volume or cell number were computed taking into account error propagation from all measured parameters.

RESULTS

The first experiment examined the effect of HL treatment on cell physiology and xanthophyll ratios

and on intracellular and extracellular DMSP concentrations. In both light regimes, algal growth (on a cell number or total cell volume basis) over the 4.5 to 4.6 h exposure was not statistically significant ($p > 0.05$). Exposure to HL caused an immediate small increase in the mean cell volume from 26.5 to 29.5 μm^3 and cell size remained ca. 8 to 22% larger in the HL treatment than in the LL treatment for the remaining 4.5 h, but these differences were not statistically significant (Fig. 1B). The mean maximum quantum yield of PS II photochemistry (F_v/F_m) remained very stable ($p = 0.96$) at ca. 0.45 in the LL treatment, whereas it declined significantly ($p < 0.01$) from 0.52 to 0.27 by 1.5 h in the HL treatment and remained at this low level for the rest of the experiment (Fig. 1C). The mean xanthophyll ratio (DT/[DT+DD]) increased from 0.77 to 0.86 over the first 1.5 h in the HL treatment and remained 9 to 17% higher than in the LL treatment for the rest of the experiment. However, the differences between both light regimes at each time point were not significant ($p > 0.10$, Student's *t*-tests). By contrast, the ratio remained unchanged under the LL treatment over the 4.6 h exposure period ($p = 0.28$; Fig. 1D).

In the LL treatment, mean DMSP cell quotas did not change by more than 9% at t = 3 and 4.6 h with respect to initial DMSP quotas at t = 0 h (5.04 ± 0.31 fmol $cell^{-1}$), but they reached a transient peak of 6.2 ± 1.2 fmol $cell^{-1}$ at t = 1.5 h (Fig. 1E). Intracellular DMSP concentrations exhibited a similar pattern to DMSP cell quotas with mean values differing by less than 9% at t = 0, 3, and 4.6 h (199 ± 18 mmol l_{cell}^{-1}). However, there was a significantly higher mean DMSP value of 273 ± 29 mmol l_{cell}^{-1} at t = 1.5 h ($p < 0.05$, Fig. 1F). In addition, there were no significant changes in ambient DMSPd concentration ($p = 0.10$) and DMSPd normalized per cell ($p = 0.24$; Fig. 1G) in the LL treatment. The mean values in this treatment were 57 nM and 0.15 fmol $cell^{-1}$, respectively.

Exposure to HL resulted in an increase in the mean DMSP cell quotas from 5.3 to 7.1 fmol $cell^{-1}$ between the start of the experiment and 1.5 h, and they remained at this level for the remaining 3 h (Fig. 1E). Intracellular concentrations of DMSP also increased. They rose from 182 to 269 mmol l_{cell}^{-1} (a 48% increase) between t = 0 and 1.5 h and stayed at this level until the experiment ended (Fig. 1F). DMSP cell quotas were 55 and 45% higher, respectively, at 3 h ($p < 0.05$) and 4.5 h ($p < 0.05$) in the HL treatment than in the LL treatment (Fig. 1E). Intracellular DMSP concentrations were 34 and 30% higher, respectively, at 3 h and 4.5 h in the HL treatment than in the LL treat-

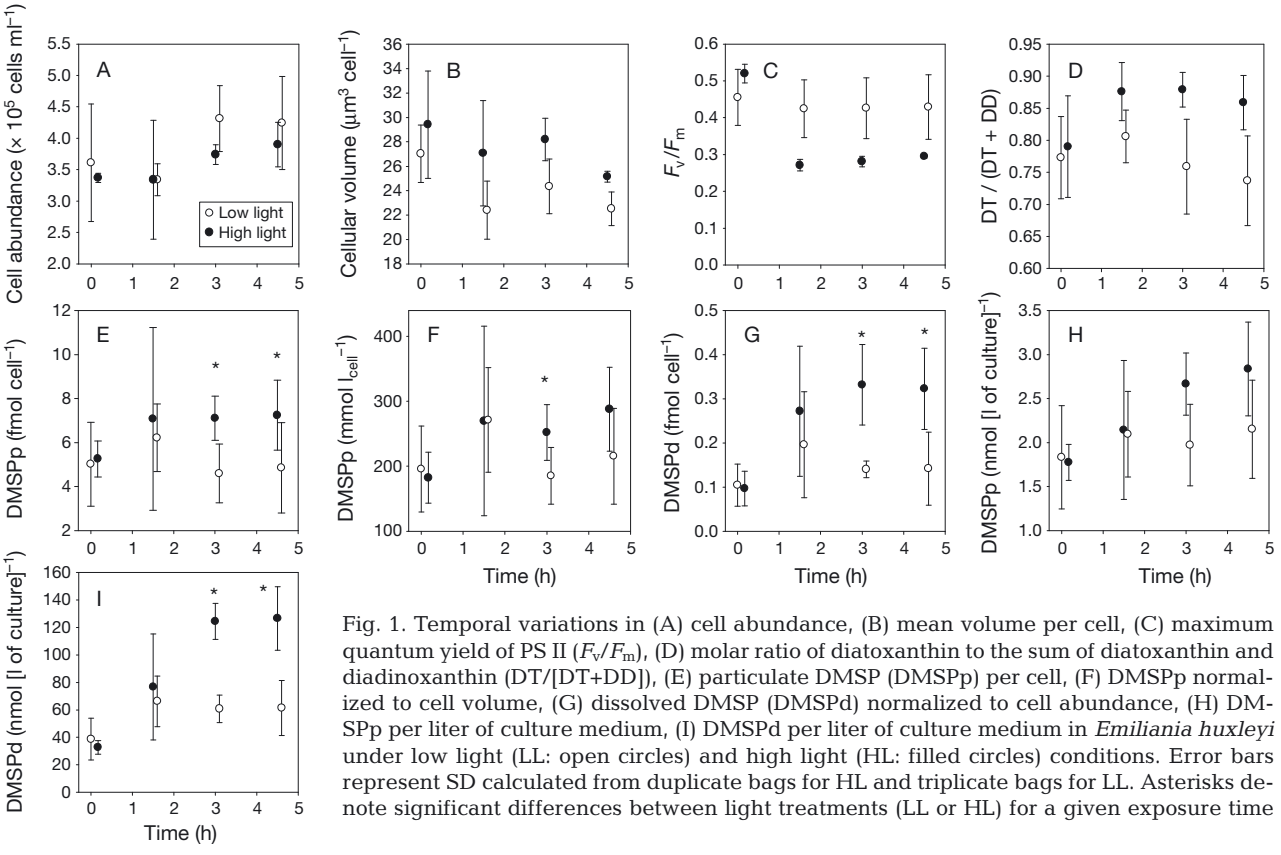

Fig. 1. Temporal variations in (A) cell abundance, (B) mean volume per cell, (C) maximum quantum yield of PS II (F_v/F_m), (D) molar ratio of diatoxanthin to the sum of diatoxanthin and diadinoxanthin (DT/[DT+DD]), (E) particulate DMSP (DMSPp) per cell, (F) DMSPp normalized to cell volume, (G) dissolved DMSP (DMSPd) normalized to cell abundance, (H) DMSPp per liter of culture medium, (I) DMSPd per liter of culture medium in *Emiliania huxleyi* under low light (LL: open circles) and high light (HL: filled circles) conditions. Error bars represent SD calculated from duplicate bags for HL and triplicate bags for LL. Asterisks denote significant differences between light treatments (LL or HL) for a given exposure time

ment (Fig. 1F). However, the difference was only statistically significant at 3 h ($p < 0.05$). The absence of a significant difference in the intracellular DMSP concentration at 4.5 h ($p = 0.18$) could have been due to the larger volume per cell in the HL than in the LL treatment and to the relatively large variance in intracellular DMSP concentrations in the LL treatment (Fig. 1B). DMSPp and DMSPd normalized per liter of culture medium also significantly increased over the 4.5 h exposure to HL ($p = 0.033$ for DMSPp and $p = 0.035$ for DMSPd), whereas no significant increases in both variables were observed in the LL treatment ($p > 0.05$; Fig. 1H,I). Thus, there was a higher net synthesis of DMSP in the HL than in the LL treatment.

Mean values of ambient DMSPd and of DMSPd normalized per cell increased from 33 to 97 nM and from 0.11 to 0.27 fmol cell^{-1} after 1.5 h of exposure to HL treatment and then remained at ca. 126 nM and ca. 0.31 fmol cell^{-1}, respectively, for the rest of the experiment (Fig. 1G,I). Ambient DMSPd and DMSPd per cell abundance were 2.0 to 2.4 times higher, respectively, at 3 h and 4.5 h in the HL treatment than in the LL treatment ($p < 0.05$; Fig. 1G,I).

The effect of enhanced PAR and UVR on DLPA and DMS concentrations was determined in a second experiment, using the same light conditions as in the first experiment. The mean DLPA was 47.6 ± 16.1 fmol DMS cell^{-1} h^{-1} (or 1.76 ± 0.60 mol DMS l_{cell}^{-1} h^{-1}) at t = 0 and remained close to this level in the LL treatment after 4 h (Table 2). In the HL treatment, the mean DLPA normalized to cell volume significantly declined to 1.05 ± 0.21 mol DMS l_{cell}^{-1} h^{-1} over 4 h ($p < 0.05$), a value ~45% lower than that observed in the LL cultures after 4 h. When normalized to cell number, the mean DLPA decreased by 38% during the 4 h of exposure at HL, but the decrease was not statistically significant ($p = 0.066$). In spite of the decrease in DLPA, exposure to HL did not affect the DMS concentration in the medium after 4 h; this remained at ca. 0.32 fmol cell^{-1} or 13 mmol l_{cell}^{-1} (Table 2).

Table 2. Mean (SD) DMSP lyase potential activity (DLPA) and DMS concentration in *Emiliania huxleyi* (strain CCMP 1742) cultures acclimated to low light prior to the experiment (LL 0 h) and after a 4 h exposure to low light (LL 4 h) and high light (HL 4 h) conditions. The values were normalized to cell abundance and cell volume

	Irradiance treatment		
	(LL 0 h)	(LL 4 h)	(HL 4 h)
DLPA (fmol DMS cell^{-1} h^{-1})	47.6 (16.1)	43.0 (3.35)	26.5 (5.35)
DMS (fmol cell^{-1})	0.34 (0.14)	0.33 (0.03)	0.32 (0.08)
DLPA (mol DMS l_{cell}^{-1} h^{-1})	1.76 (0.60)	1.91 (0.15)	1.05 (0.21)
DMS (mmol l_{cell}^{-1})	13 (5)	15 (1)	13 (3)

DISCUSSION

Impact of short-term light stress on cell physiology and photosynthesis

Our data suggest that harvested cells were stressed at the beginning of the first experiment. The initial value of F_v/F_m (0.46 ± 0.08) is low compared to other photosynthetic efficiencies measured in exponentially growing *Emiliania huxleyi* cells using PAM fluorometry (~0.6–0.7; van Rijssel & Buma 2002, Feng et al. 2008, Barcelos e Ramos et al. 2010). Moreover, the initial de-epoxidation state of xanthophyll cycle pigments (DT/[DT+DD] ~0.78 mol:mol) was very high compared to the range of values (0.1–0.3 mol:mol) previously observed in exponentially growing *E. huxleyi* cultures (Archer et al. 2010). Both the low F_v/F_m and high xanthophyll ratio suggest that the cells used in the present experiment were physiologically stressed as might occur with the onset of nutrient or CO_2 limitation (Bucciarelli & Sunda 2003). Based on the measured cellular carbon concentrations in 2 other strains of *E. huxleyi* (22 mol l_{cell}^{-1}; Sunda & Huntsman 1992) and the total cell volume in our experiments (mean of all pooled data: 107 µm^3 ml^{-1}), we estimated that the total cellular carbon content per liter of culture medium represented 29% of the total dissolved inorganic carbon concentration in the medium, enough to decrease CO_2 concentrations by 14-fold based on equilibrium calculations of the CO_2 system (Sunda & Cai 2012). This calculation thus indicates that the experimental cells might have been carbon-limited, a known oxidative stress factor (Sunda et al. 2002).

The sharp decrease in F_v/F_m between t = 0 and t = 1.5 h in the HL treatment (Fig. 1C) indicates an increase in chlorophyll fluorescence. This increase is caused by an over-excitation of photosynthetic pigments due to the increase in light intensity and perhaps also to damage to PS II reaction centers (Bouchard et al. 2008). There was also a slightly higher xanthophyll ratio in the HL than in the LL treatment during the period from 1.5 to 4.5–4.6 h (Fig. 1D), which also suggests a physiological stress on the photosynthetic apparatus in response to higher PAR or the damaging effects of UVR on photosynthetic electron transport. The systematic increase in this ratio indicates an increased enzymatic conversion of DD to DT, which functions to dissipate excess excitation energy as heat (Porra

et al. 1997). The over-excitation and over-reduction of the photosynthetic apparatus resulting from increased light absorption or damage to the photosynthetic apparatus promotes the production of a variety of ROS, including singlet oxygen, superoxide radicals, hydrogen peroxide, and •OH radicals (Niyogi 1999). The possible link between light stress (and likely increases in ROS production), and the metabolism of methylated sulfur compounds will be addressed in the following sections.

Impact of PAR and UVR on DMSP synthesis and exudation

In the HL cultures in the first experiment, the DMSP per cell increased by ≈40% during the initial 1.5 h and then remained at that elevated value for the duration of the experiment. By contrast, the LL cultures showed no increase in DMSP per cell relative to the initial value at 3 and 4.5 h, but they also exhibited a transient increase of ≈23% after 1.5 h. We have no ready explanation for this transient increase in the LL culture. A similar transient increase and subsequent decrease in the xanthophyll ratio (DT/[DT +DD]) suggests a short-term increase in oxidative stress within the chloroplast. However, a similar transient decrease was not observed in photosynthetic efficiency (F_v/F_m; Fig. 1C). One possible explanation for the short-term DMSP increase in the LL cultures is that it resulted from a change in the growth conditions and DMSP synthesis caused by mixing and re-suspension of cells during the experimental set up.

The intracellular concentration of DMSP is determined by the balance between the net DMSP synthesis rate and the biodilution rate (the decrease in the intracellular DMSP concentration due to cell growth). Under HL, the net DMSP biosynthesis rate increased relative to the LL control cultures, since DMSPp and DMSPd normalized per cell and cell volume increased by more than 1.3 times and more than 2-fold, respectively, relative to the LL treatments in the presence of high PAR and UVR, but biodilution from growth was not significantly affected (Fig. 1A,B). We note that 3.9 ± 0.1% of the total net DMSP increase in the HL cultures was released into the dissolved phase. This increase in DMSPd in the HL treatment may have been caused by UV damage to the cells, including damage to cell membranes (Mody et al. 1989, Dainiak & Tan 1995).

Several studies have been conducted on the effect of light and UV stress on cellular DMSP in microalgae. Sunda et al. (2002) reported a 38 to 98% increase in intracellular DMSP concentration in *E. huxleyi* (CCMP 374) grown in axenic cultures under fluorescent light without UV (800 µE m^{-2} s^{-1}) and then exposed for up to 3 d to a similar intensity of attenuated natural sunlight with and without UVA or UVB filters. Since the algal growth rate was not affected by the presence of UVR, their data indicate that net DMSP biosynthesis is stimulated by UVR. The experimental protocol used in our study was quite different but led to similar results. In our experiment, the exposure of LL-adapted cells to high PAR+UVR resulted in a 30 to 34% increase in intracellular DMSP concentration within 3 to 4.5 h (198–221 mmol l_{cell}^{-1} to 252–288 mmol l_{cell}^{-1}; Fig. 1E,F). Slezak & Herndl (2003) also showed increased cellular DMSP concentrations in *E. huxleyi* (clone L) exposed to increased intensities of PAR and UVR. They acclimated *E. huxleyi* to a PAR intensity of 400 µE m^{-2} s^{-1} with no UVR and subsequently exposed the cells to a combination of higher PAR (700 µE m^{-2} s^{-1}) and UVA and UVB radiation. They observed a 17% increase in DMSP $cell^{-1}$ and a 13% increase in intracellular DMSP concentration after 6.5 h of HL exposure, which is less than the response we observed. Another strain of *E. huxleyi* (B92/11) acclimated to low PAR (30 µE m^{-2} s^{-1}) without UVR showed a 20% increase in DMSP per cell and a 2-fold higher DMSP concentration in the medium after 1 h of exposure to elevated UVR (UVA ≈24 W m^{-2}; UVB ≈0.4 W m^{-2}) and PAR (500 µE m^{-2} s^{-1}) (Archer et al. 2010).

In summary, our data along with other reports in the literature indicate that short-term stress from high PAR and UVR consistently increases DMSP net production and intracellular concentrations in different *E. huxleyi* strains. The variable (20 to 60%) increase in intracellular DMSP obtained in different studies might be related to different algal strains or different experimental conditions, such as exposure time or PAR and UVR intensities and spectral composition. The consistent increase in net DMSP synthesis under short-term high PAR and UVR exposure in various clones of *E. huxleyi* supports both the antioxidant and the overflow hypotheses.

In the proposed antioxidant hypothesis, DMSP and its breakdown products (DMS, DMSO, and methane sulfinic acid) act as cellular scavengers of ROS species produced in PAR- and UVR-stressed algal cells. Indeed, UVR is a potent producer of ROS in plant cells (Asada 1999, Hideg et al. 2002). It is also known to induce significant ROS production in algal cells (van de Poll et al. 2006, Archer et al. 2010) and to trigger increases in antioxidant molecules and enzymes in algae (Janknegt et al. 2009). The decrease in F_v/F_m

(Fig. 1C) and the increasing trend in the xanthophyll ratio (Fig. 1D) suggest that an increase in ROS production occurred in the HL treatment in our experiment. Thus, the up-regulation of net DMSP synthesis measured in response to high PAR and UVR supports the antioxidant hypotheses. It could also be linked, at least partly, to energy dissipation processes, as these would also tend to alleviate oxidative stress within the chloroplast.

DLPA

The regulation of DLPA in response to abiotic factors in phytoplankton remains enigmatic since few experiments have addressed this topic. Nitrogen limitation (Sunda et al. 2007) as well as increases in temperature and [CO_2] (Lee et al. 2009) have been shown to increase DLPA, but, to our knowledge, no experiments until now have looked at the effect of light and UVR stress on DLPA in algae. We observed 39 and 45% decreases in DLPA normalized per cell or per unit of cell volume, respectively. We also observed no change in dissolved culture DMS concentration in response to short-term exposure to elevated PAR and UVR (Table 2), although there could have been some increase in the cellular release of dissolved DMS if some portion of it was photooxidized by the increased exposure to UVR (Kieber et al. 1996). These results are not consistent with the involvement of this enzyme in oxidative stress protection (Sunda et al. 2002) or energy dissipation (Stefels 2000), which both predict an increase in DL activity at high PAR and UVR. In the antioxidant hypothesis, DL activity is postulated to increase under oxidative stress because the enzymatic products, DMS and acrylic acid, react 60 and 20 times, respectively, more rapidly with •OH radicals than does DMSP (Sunda et al. 2002). If DMSP functions as a photosynthetic overflow metabolite, the intracellular excess reduced carbon or sulfur diverted into cellular DMSP production under conditions of increased light or decreasing growth rate could be further converted into DMS by the DL enzyme (Stefels 2000). Reduced sulfur and carbon incorporated in DMS could then diffuse out of the cells, which could further help alleviate oxidative stress. Thus, our results suggest that DL is not involved in antioxidant protection or energy dissipation in *E. huxleyi* strain CCMP 1742. UVR and ROS can damage enzymes and inhibit enzyme synthesis and repair (Vincent & Neale 2000, Lesser 2006), and such harmful effects may have been responsible for the observed decline in the DLPA in the HL treatment.

Although we observed no increase in DMS concentrations and a decrease in DLPA in the HL treatment in our experiments, other experiments with different isolates of *E. huxleyi* have shown quite different results for cells exposed to increased oxidative stress linked to increasing solar UVR or to growth rate limitation by CO_2 or nitrogen. Our experiments were conducted with an oceanic strain of *E. huxleyi* (CCMP 1742) isolated from the northeast Pacific. In contrast to our results, experiments with a coastal strain of this same species (possessing a much lower DLPA than strain CCMP 1742) isolated from the Gulf of Maine (CCMP 374) showed a 36-fold increase in DMS:cell volume ratios in cells exposed to solar UVA radiation compared to ratios in cells grown under a similar high intensity of fluorescent light (800 µE m^{-2} s^{-1}) containing no UVR (Sunda et al. 2002). Interestingly, culture aliquots exposed to the full solar UV spectrum containing both UVA and UVB showed a much smaller 2.4-fold increase in DMS:cell volume ratios relative to values in the fluorescent light controls (Sunda et al. 2002). Since solar UVR can both promote increased DMS production via increased oxidative stress, and remove DMS by reaction with ROS species (e.g. •OH radicals) and by UV-photolysis (Kieber et al. 1996), higher exposure to UVR or to more energetic UVB will not necessarily increase the net accumulation of DMS in algal cultures, even when gross DMS synthesis rates increase. The differing responses of DMS:cell volume ratios to UV stress in different *E. huxleyi* strains may be due to biological differences among strains, but may also be affected by different experimental conditions (UV doses, UV intensity, or exposure time) used in the experiments of Sunda et al. (2002) and in the present study.

The exposure of *E. huxleyi* (CCMP 374) to solar UVA radiation and to UVA and UVB together increased intracellular DMSP concentrations by 98% and 59%, respectively (Sunda et al. 2002). In addition to solar UV effects, exposure of *E. huxleyi* (CCMP 374) to another oxidative stress factor, CO_2-limitation, caused 2-fold increases in intracellular DMSP concentrations and order of magnitude increases in DMS:cell volume ratios (Sunda et al. 2002). The exposure of this same *E. huxleyi* strain to another oxidative stress factor (limitation of the growth rate by nitrogen) caused both DLPA and DMS:cell volume ratios to increase by 20-fold (Sunda et al. 2007). Thus, in this coastal *E. huxleyi* isolate, cellular DMSP, DLPA, and DMS:cell volume ratios all increase with increased solar UVR and with CO_2 and nitrogen limitation of growth rate, consistent with predictions of the antioxidant hypothesis and the metabolic 'spill over' hypothesis.

Why are the DLPA and DMS responses to UVR and/or growth rate limitation by nutrients and CO_2 so different in the 2 *E. huxleyi* strains? The answer may be found in the large differences in DLPA in different strains, and differences in the regulation of DL enzyme, and perhaps in the structure and cellular location of the enzyme in different *E. huxleyi* strains. In exponentially growing cultures of 6 diverse *E. huxleyi* strains isolated from different habitats, the coastal strains CCMP 374 (discussed above) and CCMP 370 isolated from the Oslo Fjord had DLPA values that were around 6000- and 1000-fold lower, respectively, than the value for an oceanic strain from the Sargasso Sea (CCMP 373) (Steinke et al. 1998; Table S1 in the Supplement). Furthermore, in a comparison between the low-DLPA coastal strain (CCMP 370) and the high-DLPA oceanic strain (CCMP 373), cellular DMS cell^{-1} values were up to an order of magnitude lower in the high-DLPA strain. In addition, DMS cell^{-1} values increased under nutrient/CO_2 limitation during the stationary phase of growth in the low-DLPA strain (CCMP 370), but not in the high-DLPA strain (CCMP 373). The low DMS cell^{-1} values in strain CCMP 373 could be explained at least partly by cellular uptake and reassimilation of DMS, which has been shown to occur in strain CCMP 373, but not in strain CCMP 370 (Wolfe et al. 2002). However, even if both strains had similar DMS cell^{-1} values, that would still suggest that a greater proportion of DL enzyme remains inactive in the high-DLPA strain than in the low-DLPA strain. Thus, while the DL potential activity in cell homogenates is around 1000-fold higher in strain CCMP 373 than in strain CCMP 370, a higher fraction of the total cellular enzyme appears to be inactive in intact cells of the high lyase strain compared to the low lyase strain, and the culture DMS levels in the high lyase strain remain relatively low, even for nutrient- or CO_2-stressed cells.

Other experiments showed that the DL enzyme is activated in the high-DLPA strains only in response to chemical or physical disruption of the cells, either from sonication, the addition of chemical reagents that disrupt cell membranes (e.g. 2-(N-morpholino) ethanesulfonic acid or 2-Amino-2(hydroxymethyl)-1,3-propanediol pH buffers; Sunda et al. 2007), or during grazing by zooplankton (Wolfe & Steinke 1996). Cellular ingestion during zooplankton grazing is particularly effective in activating the enzyme, and thereby promoting the production of the enzyme products DMS and acrylate. Acrylate is a microbial poison (Sieburth 1960), while DMS is a signaling molecule (Garcés et al. 2013) that has been recently shown to function in grazing protection (Savoca & Nevitt 2014). Wolfe & Steinke (1996) proposed that the combination of high intracellular concentrations of DMSP and DL enzyme in the high DLPA strains of *E. huxleyi* constituted a grazing-activated, grazing defense system in the high-lyase strains. They further speculated that the DL enzyme was physically separated from DMSP in intact cells and that the enzyme and substrate were only able to contact one another following disruption of cellular membranes, such as occurs during grazing and subsequent cell digestion in the guts and feeding vacuoles of zooplankton. Follow-up experiments showed that microzooplankton actively avoided consumption of high-DL strains (CCMP 373 and 379) in mixed algal populations, and preferentially grazed on the low-DL strain (CCMP 370) or a green alga containing no DMSP or DL, supporting the grazing deterrence hypothesis (Wolfe et al. 1997).

The precise mechanism whereby high-DLPA algal strains elicit grazing deterrence is currently unknown. However, a recent study strongly suggests that DMS produced in the Southern Ocean mediates a tritrophic mutualistic interaction between phytoplankton and seabirds, acting as a grazer-defense system (Savoca & Nevitt 2014). In this defense mechanism, DMS produced by phytoplankton attracts seabirds, which in turn feed on phytoplankton grazers and thereby decrease grazing rates on phytoplankton. Our experimental *E. huxleyi* strain also has a high DLPA (Table S1) and like other high DLPA strains of this species may also primarily function in grazing defense, and be activated only by disruption of cells or cellular membranes. If this is the case, that could explain why our experimental DLPA values and dissolved DMS concentrations did not increase with increased exposure to PAR and UVR. However, this function for DL may not be universal, and in many cells, including the low-DLPA strains of *E. huxleyi*, the primary function of DL may be for antioxidant protection (Sunda et al. 2002, 2007).

CONCLUSIONS

Our results clearly show that net DMSP biosynthesis in *Emiliania huxleyi* (CCMP 1742) increased in response to short-term light and UV stress, which supports predictions of the antioxidant hypothesis as well as the energy dissipation hypothesis. In contrast, our observed decrease in DLPA was not consistent with the predictions of either hypothesis.

Cellular DMSP and DL enzyme(s) may have other important cellular functions, such as grazing defense,

which are not affected by increasing light or UVR. Indeed, DMSP and its enzymatic and oxidative breakdown products appear to have multiple functions within the cell — cellular osmolyte, antioxidant protection, and grazing defense to name a few — which can operate simultaneously. However, the regulation of DL activity in cells is likely dependent on only 1 or 2 main cellular function(s) (e.g. grazing defense or antioxidant protection), which vary among algal species and individual strains.

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