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DMS production in a coccolithophorid bloom: evidence for the importance of dinoflagellate DMSP lyases

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ABSTRACT: During an experiment in the North Atlantic in June 1998, water samples were collected approximately 400 km south of Iceland inside and outside of a bloom of the coccolithophorid *Emiliania huxleyi*. *In vitro* dimethylsulphoniopropionate (DMSP) lyase activity (DLA) was quantified using gas chromatography and found to vary from 0.1 to 142.3 nM dimethyl sulphide (DMS) h^{-1}. Inside the bloom area the majority of DLA (>74%) occurred in particles >10 µm, indicating that *E. huxleyi* (5 to 7 µm diameter) made only a minor contribution to total DLA. In surface waters, phototrophic dinoflagellates (>10 µm) made up a high proportion of the total phytoplankton biomass (~27%) towards the end of the coccolithophorid bloom and may have been the source of most of the DLA. This was also indicated by a significant correlation ($p < 0.02$) between DLA and the concentration of peridinin, a pigment used as a chemotaxonomic marker for dinophytes. The data presented here are the first field measurements of DLA in a coccolithophorid bloom and suggest that even a relatively low concentration of photosynthetic dinoflagellates larger than 10 µm (during our study 18 to 105 cells ml^{-1}) may contribute significantly to DMS production. Although dinoflagellates are recognised as an important source of particulate DMSP, recognition of their significance for DMS production in the field has previously been limited to a few observations in highly concentrated coastal and shelf blooms. Very little information exists on DLA in dinophytes and further investigations are warranted in order to improve our understanding of the biogeochemical and ecophysiological significance of DMSP lyases in this group of phytoplankton.

KEY WORDS: DMSP lyase · Dimethyl sulphide · DMS · Dinoflagellates · Coccolithophorids · *Emiliania huxleyi* · North Atlantic

INTRODUCTION

Biological activity is the main source of oceanic dimethyl sulphide (DMS), a volatile compound that is of considerable importance in the global sulphur cycle (Lovelock et al. 1972). DMS influences atmospheric acidity and is thought to play a significant role in climate regulation (Charlson et al. 1987). The major precursor of this biogenic gas is dimethylsulphoniopropionate (DMSP), a compatible solute found in various groups of marine algae, particularly the dinophytes and haptophytes (Keller 1989). The abiotic conversion of DMSP to DMS, acrylate and a proton is slow at seawater pH, and alternative precursors are considered to be of minor importance for DMS production. Therefore, enzymatic cleavage by DMSP lyase (dimethylpropiothetin dethiomethylase; enzyme classification [EC] number 4.4.1.3.) is thought to be the major process for DMS production in marine environments.

DMSP lyase isozymes have been found in various marine organisms including bacteria (de Souza & Yoch 1995, van der Maarel et al. 1996), a fungus (Bacic & Yoch 1998), and micro- and macroalgae (Cantoni & Anderson 1956, Kadota & Ishida 1968, Stefels & van

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Boekel 1993, Steinke et al. 1996, 1998, Niki et al. 2000, Wolfe 2000). The role of algal DMSP lyases is being investigated and it has been suggested that this isozyme is important for the osmotic acclimation of algal cells via the degradation of DMSP (Stefels 2000). Additionally, the build-up of acrylate within the microzone or mucus layer surrounding a cell (Noordkamp et al. 2000) may result in concentrations harmful to microbial organisms (Sieburth 1960, Slezak et al. 1994). Intracellular DMSP lyases are probably segregated from their substrate so that DMS production is only initiated following cell damage (Wolfe & Steinke 1996). Furthermore, the grazing-induced production of DMS and acrylate has been suggested as the basis of a chemical defence mechanism in phytoplankton (Wolfe et al. 1997).

It is assumed that DMS is produced via a complex network of processes within the marine microbial foodweb, but the relative contributions of prokaryotes and eukaryotes to DMSP turnover to DMS is still unclear. Recently, pathways for non-bacterial production of DMS have been more widely acknowledged. The findings of a modelling study showed that omission of algal DMS production led to an underestimate of the sea-to-air flux by up to 25% (van den Berg et al. 1996). Additionally, the relationship of DMS production with mixing layer depth and its similarities to photosynthetic activity suggest that the role of phytoplankton in DMSP-to-DMS conversion is greater than recognised (Simó & Pedrós-Alió 1999a).

The haptophyte coccolithophorid *Emiliania huxleyi* accumulates millimolar concentrations of intracellular DMSP, and *in vitro* enzyme assays showed high DMSP lyase activity (DLA) (12 to 25 fmol DMS cell^{-1} min^{-1}; Steinke et al. 1998, values corrected as described in Steinke et al. 2000) in isolates from the English Channel and the Sargasso Sea. Additionally, blooms of this phytoplankton species can cover large areas and change the optical properties of surface waters, resulting in the characteristic reflectance signals detected by satellites (Brown & Yoder 1994). Several research cruises that focussed on regions with *E. huxleyi* blooms have demonstrated the importance of these areas for the production of DMSP and DMS (Holligan et al. 1993, Malin et al. 1993, Matrai & Keller 1993, Simó & Pedrós-Alió 1999b) and contributed to our understanding of DMSP/DMS production, transformation and cycling.

The present study formed part of the Atmospheric Chemistry Studies in the Oceanic Environment (ACSOE) North Atlantic Experiment 1998 (Liss et al. unpubl.), which investigated the biogeochemical controls and cycling of a variety of marine trace gases. Despite the knowledge that the enzymatic cleavage of DMSP is the major pathway for DMS production, only very few measurements of enzyme activity have been carried out in the field (Stefels et al. 1995) and the ecophysiology of this reaction is not well understood. In this paper we present the first data for particulate DLA within a bloom of the coccolithophorid *Emiliania huxleyi*. We hypothesised that this species is the most important producer of DMS in a typical North Atlantic coccolithophorid bloom and, hence, would contribute most of the DLA. Measurements of DLA were conducted in tandem with analysis of a suite of biological parameters in order to identify the source of the DMSP lyase. Our results suggest that dinoflagellates, although low in numerical abundance, can be an important source of DLA in the field.

MATERIALS AND METHODS

Sampling. Water samples were taken inside and outside a bloom of the coccolithophorid *Emiliania huxleyi* during cruise D234 of RRS 'Discovery' between 14 June and 3 July 1998 (Table 1). The bloom was located in the centre of an anticyclonic eddy (40 to 50 km diameter) at approximately 59.5° N and 21.1° W (~400 km south of Iceland; Fig. 1). In common with other coccolithophore bloom areas (e.g., Balch et al. 1991) and in contrast with the surrounding waters, the eddy core was characterised by high water-leaving radiance at 555 nm as seen in SeaWiFS satellite images (Fig. 1) and relatively low chlorophyll (chl) *a* concentrations. The inert tracer sulphur hexafluoride (SF_6) was deployed within a few kilometres of the eddy centre on 13 June (7 to 9 m depth, 7 by 7 km area). The initial patch moved towards the northeast during 14 to 27 June and was re-injected with tracer on 22 June. Daily surveys were conducted with a semi-automated analytical system connected to the ship's seawater supply (intake at ~4 m depth) in order to locate the centre of the SF_6 patch (Nightingale et al. 2000). Sampling stations were positioned where the highest concentrations of SF_6 were encountered. Further general information about the study will be presented by Liss et al. (in preparation).

A conductivity, temperature, depth rosette sampler (CTD) was used to collect discrete water samples in Niskin bottles from various depths. Samples were immediately gravity filtered through a 200 µm Nitex screen while transferring the water into 2 l polycarbonate bottles. Water samples were kept in low light at 12°C for a maximum of 1 h before proceeding with further filtrations onto polycarbonate filters.

Filter fractionations and storage of DLA samples. An experimental set-up similar to the gravity filtrations detailed in Stefels et al. (1995) was used to gently

Table 1. Stations sampled for dimethylsulphoniopropionate (DMSP) lyase activity inside and outside of a bloom of the coccolithophorid *Emiliania huxleyi* during the North Atlantic Experiment 1998. The date in 1998, position and station number, location of the station relative to the bloom area, sampling depth, data for dissolved nutrients and DMSP lyase activity are shown

Date	Position	Stn	Location relative to *E. huxleyi* bloom	Sampling depth (m)	Dissolved nutrients (µM) NO_3^-	PO_4^{3-}	SiO_2	DMSP lyase activity in 2 to 200 µm (nM h^{-1})[d]
Size-fractionation experiments								
14 June	59° 30′ N, 21° 08′ W	CTD 7	Inside	40	9.4	0.56	2.01	6.1
16 June	59° 34′ N, 21° 07′ W	CTD 16	Inside	4	7.0[a]	0.39[a]	0.54[a]	9.9
21 June	59° 33′ N, 21° 02′ W	CTD 25	Inside	Surface	7.8	0.46	0.83	40.6
03 July	59° 53′ N, 20° 22′ W	CTD 43	Outside	Surface	4.0[b]	0.2	<0.5[c]	142.3
Depth profile								
27 June	59° 46′ N, 21° 00′ W	CTD 37	Inside	2	5.4	0.32	0.08	42.2
				10	5.2	0.31	0.08	22.9
				20	5.8	0.34	0.08	13.7
				40	7.7	0.51	0.97	1.7
				70	11.3	0.74	3.34	0.1
				100	12.7	0.78	4.93	0.2

[a]No data available for 4 m depth, sampling depth for nutrients was 21 m
[b]No data available for the surface, sampling depth for NO_3^- was 16 m
[c]Instrumentation problem on 3 July increased detection limit to 0.5 µM. Other detection limits for SiO_2 are 0.05 µM (T. Jickells pers. comm.)
[d]Data not corrected for ~20% loss of activity due to storage of samples at Stns 37 and 43

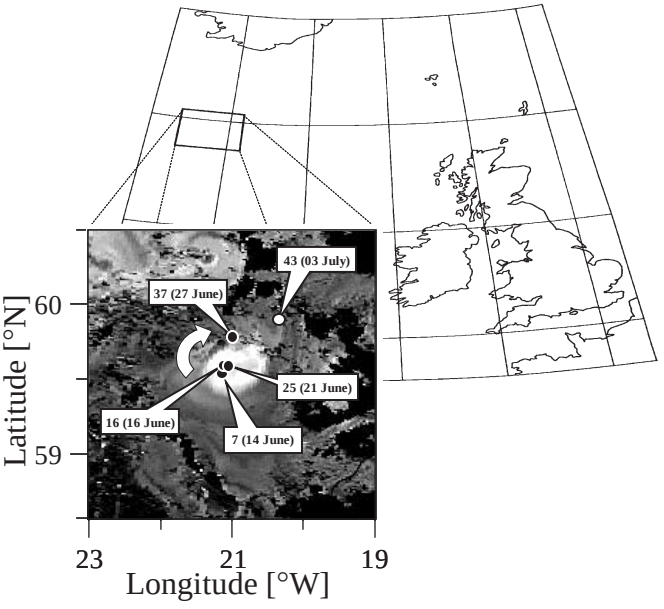

Fig. 1. Outline map of the Northeast Atlantic Ocean indicating the study area (box south of Iceland). The inset shows a composite SeaWiFS satellite picture (14 and 16 June 1998) depicting water-leaving radiance at 555 nm, which indicates high light scattering from coccoliths in the centre of an *Emiliania huxleyi* bloom at 59.5° N and 21.1° W. Black areas represent locations with complete cloud coverage. Sampling stations are pointed out in black and white circles and labels give station numbers and date in 1998. Note that CTDs 7 to 37 were in the same water mass that was moved to the northeast by clockwise turning of the eddy (indicated by the white arrow) during 14 to 27 June

concentrate particles from 1.3 to 4 l of 200 µm prefiltered seawater. Three 100 ml size fractions were collected using 47 mm diameter polycarbonate filters as follows: for CTDs 7 and 16 the size fractions used were 2 to 5, 5 to 10 and 10 to 200 µm, and for CTDs 25 and 43 the size classes were modified to 2 to 5, 5 to 18 and 18 to 200 µm. The remaining <2 µm filtrate was concentrated to 100 ml over a 0.2 µm filter under low vacuum (<100 mmHg). Particles attached to the 10, 5, 2 and 0.2 µm or 18, 5, 2 and 0.2 µm filters were gently resuspended in the concentrate before subsamples for measurement were taken of chl *a* and particulate DMSP ($DMSP_p$) concentrations, and to confirm successful fractionation using analytical flow cytometry (AFC). For DLA analysis, the remaining concentrates were filtered onto their respective polycarbonate filters under low vacuum. Further 1.3 to 2.1 l aliquots of seawater were filtered with low vacuum to investigate particulate DLA in 2 to 200 µm particles at all stations. All filtrations were carried out under dim light at 12°C. Filtration time was dependent on biomass and sample volume, and required 15 to 30 min (vacuum filtrations) or 2 to 4 h (gravity filtrations). DLA was measured

either immediately or after snap-freezing of filters in liquid nitrogen and storage at –80°C for 3 d (CTD 37; vertical profile) or 4 mo (CTD 43; size fractionation). Methodology tests showed that storage time did not affect DLA but freezing resulted in ~20% loss of activity compared to DLA measurements carried out immediately after sampling (Steinke et al. 2000).

DLA measurements. The particles collected on polycarbonate filters were analysed for DLA with a method used to test the activity of DMSP lyase isozymes in different strains of *Emiliania huxleyi* (Steinke et al. 2000). In brief, filters were extracted by sonication in 1.5 to 1.7 ml of ice-cold 344 mM citric acid/phosphate buffer with 500 mM sodium chloride at pH 6. The resulting crude extract was directly used in the enzyme tests. Filters prepared at 8 stations north of the study area discussed here were stored frozen and analysed for DLA in 2 to 200 µm particles after the cruise in order to investigate methodological and analytical variability. DLA ranged from 5 to 32 nM h^{-1}, and the average relative variation between duplicates gave a methodological uncertainty of ±12% (range of relative variation was ±2 to 24%, n = 16). The analytical precision was calculated from pseudo-duplicates (2 DLA measurements from the same filter, n = 32 individual analyses) and showed an average relative variation of ±2.7% with a range of ±0.3 to 6.3%.

After addition of 20 mM DMSP (final concentration), the DMS production was monitored for 45 to 258 min, depending on the activity of DMSP lyase, at room temperature (22.5 to 26.6°C). Controls with buffer alone were used to quantify abiotic DMS production. The time-course of DMS concentration in the headspace of the vials was investigated using on-column injections (50 µl of headspace sample) into a GC-14 gas chromatograph (Shimadzu) fitted with a Chromosil 330 packed column (Supelco) and flame-photometric detector. A series of known concentrations of commercial DMSP (Centre for Analysis, Spectroscopy and Synthesis [CASS], University of Groningen Chemical Laboratories, The Netherlands) in 10 M sodium hydroxide were used for calibration (Steinke et al. 2000).

Seawater DMS and DMSP measurements. The same gas chromatography system as above was used to quantify DMS and $DMSP_p$ in 3 ml water samples using a purge-and-trap system as described by Turner et al. (1990). Calibrations were carried out with dilutions of a primary standard prepared gravimetrically from commercial DMSP (CASS), which were prepared in distilled water, filter sterilised (0.2 µm) and stored frozen.

Pigment analyses. A fluorometric method (Parsons et al. 1984) was used to determine chl *a* concentrations in size-fractionated samples. Applying a low vacuum, 20 ml of the concentrates were filtered through glass fibre filters (25 mm diameter, Whatman GF/F). Filters were snap-frozen in liquid nitrogen and stored at –80°C. Pigments were extracted for 24 h at –20°C in the dark, using 20 ml 90% (v/v) acetone. To maximise the chl *a* extraction yield, filters were vigorously shaken on a vortex mixer immediately and 12 h after immersion in acetone.

For a vertical profile at station CTD 37, phytoplankton pigments were analysed using a reverse-phase HPLC technique as described in Gibb et al. (2000). Briefly, 2.15 l of sample was filtered through a glass fibre filter (25 mm diameter, Whatman GF/F). Filters were stored in liquid nitrogen until pigments were extracted in 100% methanol with the aid of sonication. Pigments were separated on a Hypersil MOS2 C-8 column (Shandon, Runcorn, UK), using a binary mobile phase/linear gradient and isocratic hold (70:30% methanol:1 M ammonium acetate/100% methanol at 1 ml min^{-1}). Peak detection was by absorbance at 440 nm and quantification with respect to a canthaxanthin internal standard via relative response factors.

Flow cytometry. For the filter-fractionation experiments, flow-cytometric counts were carried out on size-fractionated concentrates and whole water samples with a FACSort flow cytometer (Becton Dickinson) equipped with a 15 W laser exciting at 488 nm and a standard filter set-up. Parameters were collected as logarithmic signals, and graphs were drawn and analysed using the WinMDI freeware (Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA). Phytoplankton were identified by a combination of red fluorescence (>650 nm), orange fluorescence (585 ± 21 nm) and 90° light scatter. A laboratory-grown culture (strain 92E, Plymouth Culture Collection) was used as a reference to identify lithed *Emiliania huxleyi* cells in natural waters.

Microscopic cell counts at CTDs 25, 37 and 43. After the cruise, phytoplankton were identified and cells were counted on settled 10 to 100 ml samples preserved in either 4% formaldehyde or iodine Lugol's solution with an inverted microscope using ×200 and ×400 magnification. Carbon estimates for each species were derived from volume determinations (Kovala & Larrance 1966) and the cell volume/carbon relationships given by Eppley et al. (1970).

Bacterial abundance, biomass and production at CTD 37. Samples for bacterial abundance determination were fixed with 1% paraformaldehyde and 0.5% glutaraldehyde, incubated for 10 min, snap-frozen in liquid nitrogen and stored at –70°C. After thawing, samples were stained for 10 min with 2.5 µM Syto 13 (Molecular Probes) and analysed with a FACScalibur flow cytometer (488 nm laser; Beckton Dickinson)

using yellow-green 0.92 µm latex beads (Polysciences, Eppelheim, Germany) as internal standards. Bacterial size was calculated after the average fluorescence of the bacterial population was normalised to that of the beads (calibration by comparison to image analysis of epifluorescence preparations following Massana et al. 1997) and converted to biomass. Bacterial production was determined through the incorporation of ^{3}H-thymidine (Fuhrman & Azam 1982) as described in Pedrós-Alió et al. (1999). Samples were incubated with 20.4 nM ^{3}H-thymidine, a final concentration that was found to be saturating in a wide range of open marine environments (Pedrós-Alió et al. 1999).

RESULTS

Initial satellite images, AFC cell counts and concentrations of the major nutrients NO_3^- and PO_4^{3-} (Table 1) suggested that the coccolithophorid bloom could develop to higher cell densities over the period proposed for our observations. Furthermore, the hydrographic conditions in the eddy centre allowed us to deploy the tracer SF_6 in order to track the same water mass until the end of our study. Hydrographic data and SF_6 tracer information indicate that CTDs 7, 16, 25 and 37 were inside the same water mass, whereas CTD 43 was in a different water mass outside of the investigated bloom area (Fig. 1; Liss et al. unpubl.). Phytoplankton counts for the period of 14 to 19 June (stations not sampled for DLA but within the investigated eddy) confirm the dominance of coccolithophorids, which contributed 35 to 65% of total phytoplankton carbon in surface waters. High winds on 19 June induced mixing and homogenisation of the water column to 80 m, well below the depth of the previous nutricline. After the storm, stations inside the tracked water mass (21 and 27 June) showed lower coccolithophorid biomass with 18 and 10 mg C m^{-3} (Table 2) or 28 and 12% of total phytoplankton carbon, respectively, and the ratio of detached coccoliths to intact coccospheres (Table 3; Balch et al. 1991, Matrai & Keller 1993) suggested that the coccolithophore bloom was probably in a late stage by mid-June. In contrast to this, dinoflagellate biomass increased from 9 to 26% of phytoplankton carbon at these stations, and the shift from a coccolithophorid to a dinoflagellate-dominated phytoplankton assemblage corresponded with an increase in DLA at the surface. Inside the bloom area DLA increased from 9.9 nM h^{-1} (16 June) to 42.2 nM h^{-1} (27 June) in surface waters. Samples from a station on 3 July (CTD 43; different water mass) showed higher DLA of 142.3 nM h^{-1} (Table 1).

Table 2. Comparison of DMSP lyase activity (DLA) with carbon biomass of phytoplankton (Phyto. C), selected and total coccolithophorids, and phototrophic dinoflagellates at CTDs 25 and 43 (size fractionations) and CTD 37 (depth profile). Please note that carbon biomass data are not available for CTDs 7 and 16

Stn	Depth (m)	DLA 2 to 200 µm (nM h^{-1})[a]	Phyto. C (mg C m^{-3})	Carbon biomass (mg C m^{-3})						
				Coccolithophorids				Phototrophic dinoflagellates		
				Emiliania huxleyi	*Calcidiscus leptoporus*	*Syracosphaera rotula*	Total	*Gymnodinium* sp.	*Glenodinium* sp.	Total
Stations where size-fractionation experiments were carried out										
CTD 25	Surface	40.6	66	13.4	0.8	1.9	18.3	1.7	2.4	5.9
CTD 43	Surface	142.3	110	1.9	4.4	2.1	12.3	9.7	14.2	30.0
Depth profile station										
CTD 37	2	42.2	87	3.5	4.4	1.7	10.4	7.0	13.7	22.9
	10	22.9	78	2.0	2.7	0.6	5.7	8.1	13.7	23.8
	20	13.7	83	3.9	4.8	2.9	14.0	5.6	10.1	18.6
	40	1.7	34	7.3	0.5	0.7	8.8	2.0	1.0	3.4
	70	0.1	5	0.5	0.1	0.0	0.7	0.3	0.3	0.7
	100	0.2	4	0.6	0.0	0.1	0.8	0.3	0.1	0.8

[a]Data not corrected for ~20% loss of activity due to storage of samples at CTDs 37 and 43

Table 3. Comparison of DMSP lyase activity (DLA) with coccolith and cell density of selected and total coccolithophorids and phototrophic dinoflagellates at CTDs 25 and 43 (size fractionations) and CTD 37 (depth profile). Please note that coccolith and cell density data are not available for CTDs 7 and 16

Stn	Depth (m)	DLA 2 to 200 µm (nM h^{-1})[a]	Coccoliths (liths ml^{-1})	Ratio of detached coccoliths to coccolithophorids	Cell density (cells ml^{-1})					
					Coccolithophorids				Phototrophic dinoflagellates	
					Emiliania huxleyi	*Calcidiscus leptoporus*	*Syracosphaera rotula*	Total	*Gymnodinium* sp.	*Glenodinium* sp. Total
Stations where size fractionation experiments were carried out										
CTD 25	Surface	40.6	25583	23	1045	9	21	1102	13	4 18
CTD 43	Surface	142.3	8917	31	149	49	23	287	73	22 105
Depth profile station										
CTD 37	2	42.2	19917	54	274	49	19	366	53	21 77
	10	22.9	7208	34	153	30	6	213	61	22 85
	20	13.7	26542	61	309	53	32	432	42	16 61
	40	1.7	16813	29	570	5	7	585	15	2 17
	70	0.1	788	18	42	1	0	43	2	0 3
	100	0.2	1496	29	49	0	1	51	2	0 2

[a]Data not corrected for ~20% loss of activity due to storage of samples at CTDs 37 and 43

Size-fractionation experiments

In water samples used for size fractionations, DLA ranged from 6.1 to 142.3 nM h^{-1} in the 2 to 200 µm fraction (Table 1). The analyses for CTDs 37 and 43 probably underestimate DLA by about 20% due to loss of activity during freezing and storage of filters (Steinke et al. 2000). Relative distributions of DLA, $DMSP_p$, *Emiliania huxleyi* cells and chl *a* between the size fractions was similar for CTDs 7, 16 and 25, despite sampling from different depths and changing filter pore sizes (Fig. 2). The highest DLA was always associated with the biggest size class. At CTDs 7 and 16, particles from 10 to 200 µm accounted for 75% and 80% of the total DLA, respectively (Fig. 2A,B). DLA decreased in the next 2 smaller size classes (5 to 10 and 2 to 5 µm) and showed values of 4% and 2% in the smallest size class (0.2 to 2 µm). Flow cytometric counts of *E. huxleyi* cells were highest in the fraction corresponding to the 5 to 10 µm particle sizes (83% and 60%, respectively, of the total) and indicate good separation of particles into the respective size classes. Although 8% and 39% of *E. huxleyi* cells were found in the largest size class (10 to 200 µm), cell numbers were low in the 2 smaller size classes (2 to 5 and 0.2 to 2 µm). Most of the $DMSP_p$ (>84%) and chl *a* (>57%) was found in the 2 biggest size classes (5 to 10 and 10 to 200 µm).

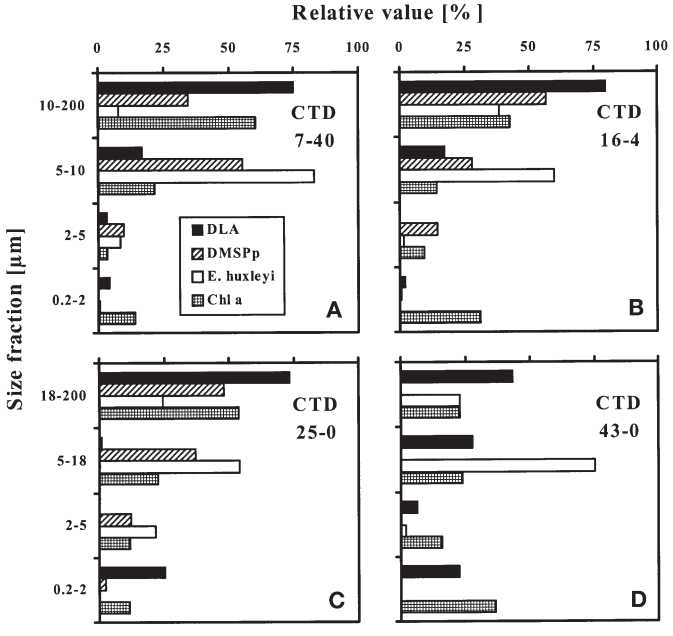

Fig. 2. Relative values for dimethylsulphoniopropionate (DMSP) lyase activity (DLA), particulate DMSP ($DMSP_p$) concentration, *Emiliania huxleyi* density and chlorophyll *a* (chl *a*) concentration in size-fractionated samples. (A) CTD 7 (40 m depth), (B) CTD 16 (4 m depth), (C) CTD 25 (surface) and (D) CTD 43 (surface)

Since *Emiliania huxleyi* cell counts were high in the largest size class at CTD 16, we used a filter with 18 µm pore size instead of a 10 µm filter at CTDs 25 and 43 (Fig. 2C,D). Despite this methodological change, the general patterns of investigated parameters were similar at CTD 25 to those reported for CTD 7 and CTD 16. DLA accounted for 74% in the biggest size class, although it was relatively higher for the 0.2 to 2 µm particles (25%). Maximum *E. huxleyi* cell numbers were found in the 5 to 18 µm size class (54%). At CTD 43 (outside the bloom), DLA in the largest size class was less (43%) but higher in the 5 to 18 µm fraction (28%) than at the other stations.

For CTDs 25 and 43, microscopic cell counts were conducted on preserved samples and phytoplankton carbon biomass was calculated (Tables 2 & 3). Coccolithophorids showed high densities at both stations and were dominated by *Emiliania huxleyi* (5 to 7 µm diameter), *Calcidiscus leptoporus* (20 µm diameter) and *Syracosphaera rotula* (5 to 7 µm diameter). The phototrophic dinoflagellates were dominated by *Gymnodinium* sp. (30 µm length) and *Glenodinium* sp. (20 µm length) but their cell densities were low relative to the density of *E. huxleyi* cells. However, the carbon biomass of dinoflagellates was similar to the coccolithophorid biomass at CTD 25 (5.9 mg C m^{-3}) but 2.4-fold higher (30 mg C m^{-3}) than the biomass of coccolithophorids at CTD 43. Flow-cytometric counts of bacterio- and picoplankton indicated low cell numbers for *Synechococcus* sp. and picoeukaryotes at 3 of the 4 CTD stations (CTDs 7, 16 and 25: range <500 to 4668 *Synechococcus* cells ml^{-1} and <500 to 4800 picoeukaryote cells ml^{-1}). However, at CTD 43 (surface) cell densities increased to 38 922 and 7540 cells ml^{-1}, respectively.

Depth profile at CTD 37

The temperature and salinity distributions at CTD 37 revealed a mixed layer from the surface to about 25 m depth, where rapid temperature and salinity changes indicated a transition into the thermo- and halocline zones (Fig. 3A). Most of the DLA was confined to the mixed layer and activity increased towards the surface (maximum value 42.2 nM h^{-1}; Table 1). $DMSP_p$ concentration was highest at 10 m depth (93 nM) and DMS showed a maximum at 20 m depth (4 nM; Fig. 3B). The carbon biomass of phytoplankton and bacteria indicated a uniform distribution within the mixed layer (surface to 20 m depth: averages of 83 and 13 mg C m^{-3}, respectively) but bacterial heterotrophic production was highest at 10 and 20 m depth (33.2 and 33.6 µg C m^{-3} h^{-1}) and 34% lower at the surface (21.9 µg C m^{-3} h^{-1}; Fig. 3C).

Measurements of phytoplankton pigment concentrations showed variable distribution patterns (Fig. 3D). Values for chl *a* were similar throughout the mixed layer (surface to 20 m depth: average 717 µg m^{-3}) and comparable to measurements in a coccolithophorid bloom in 1991 (300 to 1000 µg m^{-3}; Holligan et al. 1993). Concentrations of 19'-hexanoyloxyfucoxanthin (hex-fuco), a pigment characteristic for *Emiliania huxleyi* and other haptophytes (Jeffrey et al. 1997), were highest at 20 m depth (353 µg m^{-3}) and lower at the surface (306 µg m^{-3} at 2 m depth). The characteristic pigment for dinoflagellates, peridinin, showed the opposite trend: high at the surface (37 µg m^{-3}) and lower at 20 m depth. Linear regressions (n = 6) between DLA and the pigments chl c_1, chl c_2, chl c_3, fucoxanthin and hex-fuco were characterised by non-

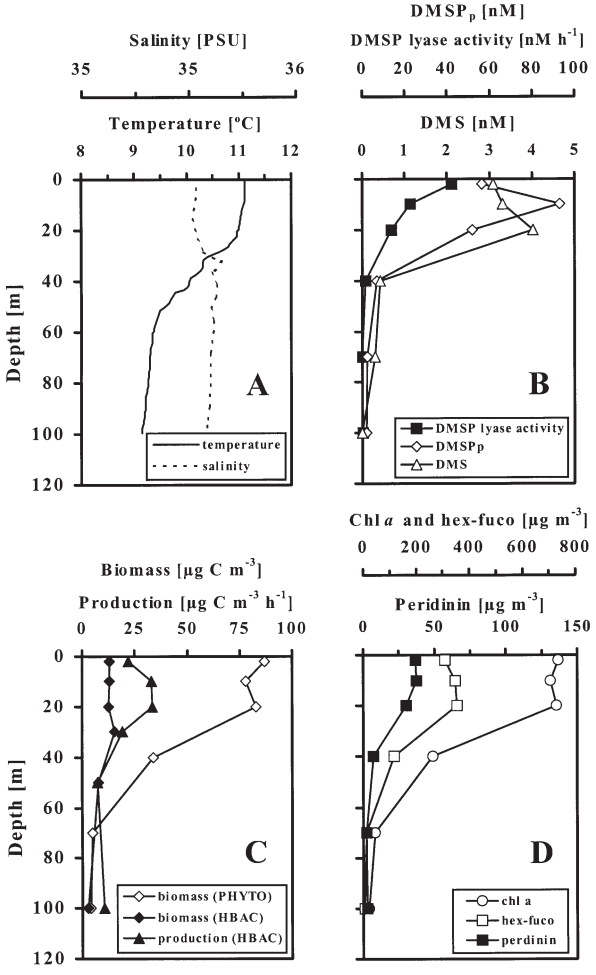

Fig. 3. Hydrographic and biological parameters at depth profile station CTD 37. (A) Salinity and temperature, (B) DMSP lyase activity (DLA), DMS and DMSP concentration, (C) carbon biomass of phytoplankton (PHYTO) and heterotrophic bacteria (HBAC), and production of HBAC and (D) concentration of selected phytoplankton pigments (chl *a*, 19′-hexanoyloxyfucoxanthin [hex-fuco], peridinin)

Table 4. Phytoplankton pigments, their linear regressions with DLA at station CTD 37, taxonomical distribution and chemotaxonomic value. NS: not significant at 0.05 level

Phytoplankton pigment Name	Abbreviation	Correlation coefficient (r) using linear regression with DLA[a]	Main distribution of phytoplankton pigments in algal divisions/classes[b]	Chemotaxonomic value
Fucoxanthin	fuco	0.61 (NS)	Bacillariophyta, Haptophyceae, Chrysophyceae, Raphidophyceae	Low
Chlorophyll c_3	chl c_3	0.73 (NS)	Chrysophyceae, some Haptophyceae, some Bacillariophyta	Low
19'-hexanoyloxyfucoxanthin	hex-fuco	0.76 (NS	Haptophyceae	High
Chlorophylls c_1 and c_2	chl c_1 and c_2	0.79 (NS)	Bacillariophyta, Haptophyceae, Raphidophyceae	Low
Chlorophyll *a*	chl *a*	0.82 (<0.05)	All phytoplankton except most Prochlorophyta	None
Chlorophyll *b*	chl *b*	0.82 (<0.05)	Chlorophyceae. Prasinophyceae, Euglenophyta	Low
Alloxanthin	allo	0.83 (<0.05)	Cryptophyta	High
Zeaxanthin	zea	0.83 (<0.05)	Prochlorophyta, Cyanophyta, Rhodophyta, Chlorophyceae	Low
19'-butanoyloxyfucoxanthin	but-fuco	0.86 (<0.05)	Haptophyceae (mainly *Phaeocystis* sp.), Chrysophyceae, some Dinophyta	Low
Peridinin	perid	0.88 (<0.02)	Dinophyta	High
Diadinoxanthin	diadino	0.96 (<0.01)	Dinophyta, Bacillariophyta, Haptophyceae, Chrysophyceae, Raphidophyceae, Euglenophyta	Low

[a]Model II regression analysis after Sokal & Rohlf (1995). Level of significance (using a 2-tailed *t*-test and [n – 2] degrees of freedom, n = 6) is presented in parentheses
[b]After Jeffrey et al. (1997)

significant relationships ($p \geq 0.05$) and low correlation coefficients ($r \leq 0.79$) (Table 4). The relationships between DLA and chl *a*, chl *b*, alloxanthin, zeaxanthin and 19'-butanoyloxyfucoxanthin (but-fuco) indicated better coefficients (r = 0.82 to 0.86; $p < 0.05$) but correlations of higher significance were only found with diadinoxanthin (diadino; r = 0.96; $p < 0.01$) and the dinoflagellate-specific peridinin (r = 0.88; $p < 0.02$). Regression analyses of phytoplankton biomass with either DLA or $DMSP_p$ were not significant when coccolithophorid biomass was used ($r < 0.1$; Fig. 4A), but significant for phototrophic dinoflagellates (DLA: r = 0.88, $p < 0.05$; $DMSP_p$: r = 0.96, $p < 0.01$; Fig. 4B).

Emiliania huxleyi was numerically the most abundant coccolithophorid at all sampling depths with highest densities below the mixed layer at 40 m depth (570 cells ml^{-1}; Table 3). The other significant coccolithophorid species, *Calcidiscus leptoporus* and *Syracosphaera rotula*, were relatively low in numbers but contributed substantially to the carbon biomass at depths of 2 m (6.1 mg C m^{-3}; 57% of total cocco-

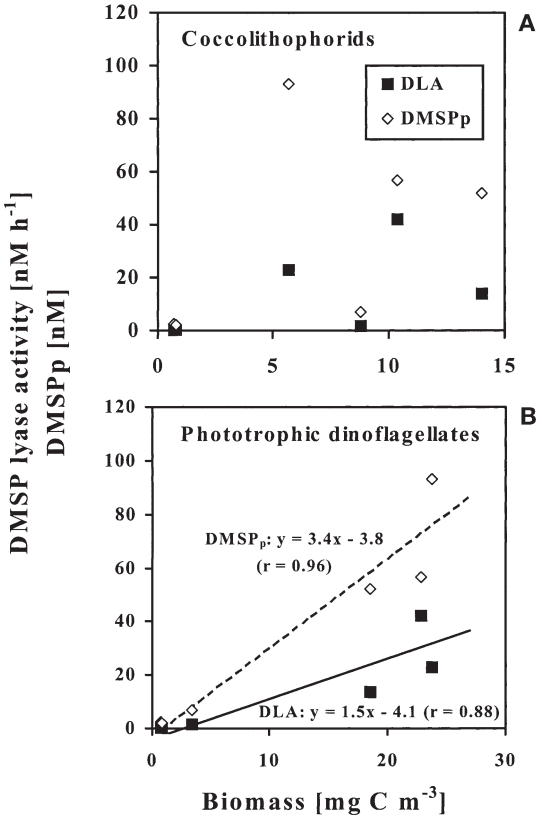

Fig. 4. Relationships between (A) coccolithophorid and (B) phototrophic dinoflagellate biomass with DMSP lyase activity (DLA) and $DMSP_p$ at CTD 37. Linear regressions are indicated by the dashed line ($DMSP_p$: $p < 0.01$) and solid line (DLA: $p < 0.05$) using a Model II analysis after Sokal & Rohlf (1995)

lithophorid carbon), 10 m (3.3 mg C m^{-3}; 58 %) and 20 m (7.7 mg C m^{-3}; 55 %). Although the density of phototrophic dinoflagellates was low (2 to 20 m depth, average 74 cells ml^{-1}), they represented a high proportion of the phytoplankton carbon biomass with 22.9 (26 % of total phytoplankton biomass), 23.8 (30 %) and 18.6 (22 %) mg C m^{-3} at 2, 10 and 20 m depth, respectively, and were dominated by *Gymnodinium* sp. and *Glenodinium* sp. Picoplankton carbon biomass was similar to the biomass of the dinoflagellates (2 to 20 m average of 25.9 mg C m^{-3}) but the diatom biomass was relatively low (2 to 20 m average of 11.9 mg C m^{-3}), which is in agreement with the low silicate concentrations found at this station.

DISCUSSION

At 5 stations in the Northeast Atlantic Ocean, DLA was measured in particles of various size classes. Maximum activity was associated with particles >10 µm and, surprisingly, not in the size fraction corresponding to the coccolithophorid *Emiliania huxleyi*. This phytoplankton was numerically the most abundant species, has been shown to be a major producer of DMSP (Keller 1989, Malin et al. 1994) and has strain-specific DLA ranging from 0.004 to 25 fmol DMS $cell^{-1}$ min^{-1} (Steinke et al. 1998, values corrected as described in Steinke et al. 2000). Preliminary data on cell-specific DLA in 3 clonal isolates of *E. huxleyi* from CTD 44 (20 m depth, 4 July 1998, 21 km southwest of CTD 43) indicate relatively low average activities of 0.04 fmol DMS $cell^{-1}$ min^{-1}.

Are dinoflagellates contributing to the DMS production in coccolithophorid blooms?

It is often assumed that coccolithophorid blooms are almost mono-specific and that *Emiliania huxleyi* is of great importance for the production of DMS, especially towards the end of a bloom (Matrai & Keller 1993). However, in samples collected towards the later stages of the bloom (CTDs 25, 37 [2, 10 and 20 m] and 43), dinoflagellate biomass was generally greater than that of *E. huxleyi* (Table 2). There is evidence that some dinoflagellates not only may be important producers of DMSP (Keller 1989) but also can enzymatically convert DMSP to DMS (Kadota & Ishida 1968, Steinke et al. 1996, Niki et al. 2000, Wolfe 2000). Furthermore, our measurements indicate that phototrophic dinoflagellates could have been the main source of $DMSP_p$ and DMSP lyase at CTD 37 (Fig. 4B), whereas coccolithophorid biomass was not significantly correlated with either of these parameters (Fig. 4A). Using our data for total DLA at CTDs 25, 37 (2, 10 and 20 m) and 43, we can calculate cell-specific DLA in the phototrophic dinoflagellates after making the following assumptions: (1) *E. huxleyi* and phototrophic dinoflagellates are the only sources of DLA and (2) cell-specific DLA in *E. huxleyi* is 0.04 fmol $cell^{-1}$ min^{-1} (as indicated for clonal isolates at station CTD 44). With these presumptions the dinoflagellates contributed a cell-specific DLA of 3.5 to 35.3 fmol $cell^{-1}$ min^{-1}, which is in good agreement with the activities reported for phototrophic dinoflagellates such as *Alexandrium* sp. (~40 µm diameter; Wolfe 2000).

Size-fractionation data also provide evidence that larger phytoplankton, such as the dominating dinoflagellates *Gymnodinium* sp. (30 µm length) and *Glenodinium* sp. (20 µm length), produced DMSP lyase. The flow-cytometric counts showed that most of the *Emiliania huxleyi* cells were successfully fractionated into the 5 to 10 and 5 to 18 µm size classes. Despite this, the highest percentage of DLA was found in the largest size classes (10 to 200 and 18 to 200 µm), which most likely had a high amount of dinoflagellate carbon biomass. The use of size-fractionated assays coupled with taxonomic analysis provides a simple means of estimating the relative importance of major taxa or functional groups in DMSP lyase production in natural assemblages. Although manipulation of samples can result in artefacts, particularly when long filtration times are necessary for gentle separation of particles, we found that vacuum size fractionations carried out in parallel with gravity filtrations at CTD 7 resulted in poor size separation with an artificially high number of small particles and 90 % of DLA collected on the filter with the largest pore size (10 µm). Therefore, we decided to use gravity filtrations in the size-fractionation experiments and believe that this is the best possible approach available.

The pigment data for CTD 37 confirm the outcome of our size-fractionation experiments and provide further evidence for the importance of dinoflagellates for potential DMS production in the investigated area at the time of this study. Peridinin, characteristic of dinoflagellates, was significantly correlated ($p < 0.02$) to DLA in samples from the depth profile (Table 4). Interestingly, diadinoxanthin was also closely correlated ($p < 0.01$) to DLA activity. Diadinoxanthin is a photoprotective pigment in the 2-component xanthophyll cycle of some phytoplankton, which is necessary for the thermal dissipation of light energy under high irradiance levels.

Influence of bacterial DMSP lyases

The interpretation of our field results is complicated by the fact that heterotrophic bacteria can also be a

source of DLA (de Souza & Yoch 1995, van der Maarel et al. 1996). DMSP lyases from unattached bacteria were probably concentrated in the smaller size fractions. Indeed, in samples from CTDs 25 and 43, about 24% of total DLA activity was found in the 0.2 to 2 µm size class. Additionally, we cannot eliminate the possibility that particle-attached bacteria may have resulted in relatively high bacterial DLA in the larger size fractions. However, in the depth profile at CTD 37, productivity of heterotrophic bacteria was high at 10 and 20 m depths but relatively low close to the surface. In contrast to this, DLA was 1.8 and 3 times higher at 2 m than at 10 and 20 m depths, respectively. This suggests that bacterial production and DLA were not closely linked and, hence, bacteria were probably not the primary source of DMSP lyase at this station.

Little is known about the role of cyanobacteria in DMS production. Corn et al. (1996) reported that the contribution of prokaryotes to total $DMSP_p$ in a time-series experiment in the Mediterranean Sea and 3 areas of the subtropical Atlantic Ocean was negligible. In contrast to this, a mesocosm study suggested that the cyanobacterium *Synechococcus* may produce high $DMSP_p$ concentrations (Wilson et al. 1998). It is unknown whether phototrophic bacteria are also a source of DMSP lyase.

Vertical distribution of DLA

The biological parameters measured at the depth profile station (CTD 37) indicate that most of the biological activity was restricted to the mixed layer (above 25 m depth; Fig. 3C,D). However, the phytoplankton counts suggest that the density of *Emiliania huxleyi* was high at about 40 m depth (Table 3). This contrasts with the relatively low concentrations of hex-fuco and chl *a* at this depth. Additionally, flow-cytometric cell counts were lower than microscopic cell counts at CTD 38 (5 h after CTD 37 and in the same water mass inside the *E. huxleyi* bloom area). Therefore, it is possible that some of the particles that were seen in the microscope counts were empty coccospheres sinking through the thermocline below the euphotic zone (1% light level at 30 m depth; G. Moncoiffé pers. comm.).

It is intriguing that highest DMS concentrations did not coincide with the depth of maximum DLA. Therefore, how our measurements of *in vitro* DLA (i.e., the potential for DMS production) relate to DMS production *in situ* remains unclear (see discussion in Steinke et al. 2000). Parallel measurements of DMS production rates in short-term (24 h) incubation experiments were much lower than the potential for DMS production and ranged from 0.05 to 0.6 nM DMS h^{-1} (Simó & Pedrós-Alió 1999b). Several production and loss processes, such as photochemical oxidation, microbial consumption and sea-to-air transfer, influence the concentration of DMS. Particularly microzooplankton grazing (Wolfe & Steinke 1996) and viral lysis (Bratbak et al. 1995, Malin et al. 1998) can be important factors for the conversion of DMSP to DMS in coccolithophorids. The vertical distribution of DMSP lyases could also be affected by species-specific production of this enzyme. Similar specificity has been shown for the accumulation of DMSP in various phytoplankton taxa (Keller 1989), and species associated with the ocean surface may provide proportionally more DLA than the phytoplankton below. Furthermore, high light intensity at the surface not only may influence phytoplankton pigment composition (e.g., accumulation of xanthophyll cycle pigments such as diadinoxanthin) but also could affect the physiology of DMSP lyases. In a coccolithophorid bloom in the northern North Sea in 1999, the ratio of DLA to chl *a* showed a similar pattern to the ratio of diadinoxanthin to chl *a*, and the highest values were always associated with the surface (Steinke et al. 2002). However, detailed studies on the effect of environmental factors (temperature, light, nutrients, etc.) on DMSP lyase physiology are unavailable. This lack of information will be addressed in our future investigations.

In summary, our results provide evidence that dinoflagellates were an important source of DLA and by extension they could also have been significant for the conversion of DMSP to DMS during the latter stages of the coccolithophorid bloom. These findings reinforce indirect evidence from other studies that related dinoflagellate abundance with DMS production (Turner et al. 1988, Leck et al. 1989). Cell-specific DLA in *Emiliania huxleyi* appeared to be relatively low in this particular bloom (0.04 fmol DMS $cell^{-1}$ min^{-1}) and dinoflagellates with high DLA per cell may contribute to the increased concentrations of DMS associated with the demise of such coccolithophorid blooms.

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