Vol. 369: 1–11, 2008
doi: 10.3354/meps07664

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 13

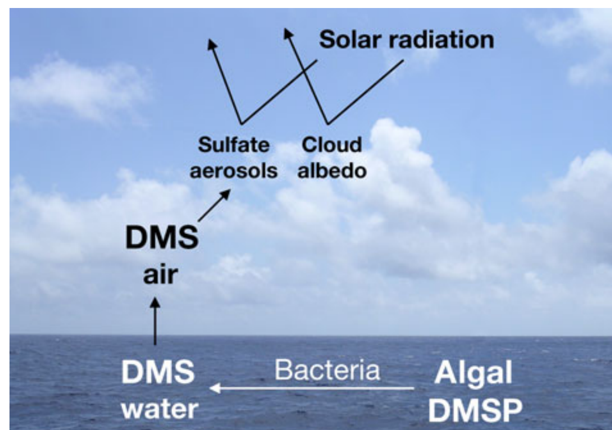
FEATURE ARTICLE

Bacterial DMSP metabolism during the senescence of the spring diatom bloom in the Northwest Atlantic

Anissa Merzouk[1,*], Maurice Levasseur[2], Michael Scarratt[3], Sonia Michaud[3], Martine Lizotte[2], Richard B. Rivkin[4], Ronald P. Kiene[5]

[1]University of British Columbia, Earth and Ocean Sciences/Oceanography, 1461-6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada
[2]Université Laval, Département de biologie (Québec-Océan), Pavillon Alexandre-Vachon, Quebec, Quebec G1K 7P4, Canada
[3]Fisheries and Oceans Canada, Maurice Lamontagne Institute, 850 Route de la mer, Mont-Joli, Quebec G5H 3Z4, Canada
[4]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland A1C 5S7, Canada
[5]University of South Alabama, Department of Marine Sciences, Mobile, Alabama 36688, USA

ABSTRACT: The impact of the decline of the vernal bloom on the bacterial metabolism of dimethylsulfoniopropionate (DMSP), the precursor of dimethylsulfide (DMS), was investigated during a 7 d Lagrangian study conducted in the Northwest Atlantic in spring 2003. Daily variations in bacterial leucine incorporation, dissolved DMSP ($DMSP_d$) uptake and DMS production rates were measured in the surface mixed layer (SML) and in the deep chlorophyll *a* maximum (DCM) that formed as the bloom collapsed. Seawater samples were amended with ^{35}S-$DMSP_d$, and the products of bacterial $DMSP_d$ degradation were measured during 3 h on-board incubations. The gradual decrease in phytoplankton biomass and diatom abundance measured in the SML was accompanied by a sharp doubling of the bacterial abundance and a peak in leucine incorporation rate on Day 2, suggesting that bacteria responded to a transient pulse in dissolved organic matter. Bacterial $DMSP_d$ uptake and DMS production were highest on Days 1 and 2 (1.2 and 0.10 nmol l^{-1} h^{-1}, respectively), but rapidly decreased by Day 3, suggesting that $DMSP_d$ was becoming a less important substrate for the growing bacterial assemblage as other substrates became available. Bacterial $DMSP_d$ uptake and DMS production rates were also low in the DCM despite very high DMS yields (40 to 50%), showing that neither the decline of the diatom spring bloom in the SML nor the accumulation of cells in the DCM resulted in a stimulation of bacterial DMSP metabolism or accumulation of DMS. The present study provides new field evidence for the potential uncoupling between bacterial production and DMS dynamics likely due to variations in the availability of other S-containing organic compounds released during the decay of phytoplankton blooms.



Bacterioplankton has a key role in dimethylsulfide production, thereby helping to regulate the Earth's climate.

Photo: Julien Pommier

KEY WORDS: DMSP cycling · Bacterial metabolism · Bloom senescence · Northwest Atlantic Ocean

Resale or republication not permitted without written consent of the publisher

*Email: amerzouk@eos.ubc.ca

© Inter-Research 2008 · www.int-res.com

INTRODUCTION

Dimethylsulfide (DMS) produced by marine plankton is the main natural source of reduced sulfur to the atmosphere (Andreae & Crutzen 1997), where it is oxidized into sulfate aerosols that scatter solar radiation and increase the albedo of clouds. Oceanic DMS emissions thus alter the radiative balance of the earth and can potentially exert a cooling effect on climate (Charlson et al. 1987).

DMS is produced in the ocean from the degradation of dimethylsulfoniopropionate (DMSP), an osmolyte synthesized by phytoplankton. High DMSP-producing taxa are mainly found among the prymnesiophytes and dinoflagellates, whereas diatoms generally have low intracellular DMSP concentrations. DMSP is released as part of the dissolved organic matter (DOM) during algal senescence, grazing by micro- and mesozooplankton and viral lysis (Yoch 2002, Simó 2004). Dissolved DMSP ($DMSP_d$) is an important organic substrate for marine bacteria that can satisfy 1 to 15% and 50 to 100% of their carbon and sulfur demands, respectively (Kiene & Linn 2000a, Simó et al. 2002, Zubkov et al. 2002). Bacteria use $DMSP_d$ via 2 main metabolic pathways. The dominant pathway is demethylation/demethiolation, which allows bacteria to use both C and reduced S contained in $DMSP_d$ and prevents the production of DMS (Kiene et al. 2000, Yoch 2002). The alternative pathway of bacterial $DMSP_d$ degradation is cleavage to DMS and acrylate, which provides bacteria with C, while the S moiety of the molecule is released in the form of DMS. Kiene et al. (2000) proposed that the demethylation/demethiolation pathway is preferred over cleavage to DMS when the S and C demands of the bacterial community are high. The proportion of $DMSP_d$ cleaved (i.e. the DMS yield) would then be determined by the availability of DMSP relative to the physiological status of the bacterial community (i.e. its C and S demand).

DMS production is intimately linked to bacterial metabolism, but also to the composition and evolution of phytoplankton blooms, since the $DMSP_d$ available for bacterial degradation depends on the phytoplankton community structure and the developmental stage of a bloom (van Duyl et al. 1998, Levasseur et al. 2004, Pinhassi et al. 2005). Bloom decline and phytoplankton senescence are associated with the release of large amounts of DOM (and DMSP), which become available for bacterial degradation and are accompanied by an increase in bacterial heterotrophic production (Ducklow et al. 1993). The decline of phytoplankton blooms also often coincides with an increase in DMS concentrations (Nguyen et al. 1988, Matrai & Keller 1993, Zubkov et al. 2004). Reported exceptions to this general trend suggest the existence of a more complex relationship between decaying blooms, DOM/DMSP release and bacterial DMSP metabolism that can preclude the accumulation of DMS. For example, DMS concentrations decreased or remained unchanged during the decline of diatom-dominated spring blooms in the Barents Sea (Matrai & Vernet 1997) and in the Northwest Atlantic (Levasseur et al. 2004). These DMS responses may be related to the combined effect of DMSP-poor dominant species (diatoms) and bacterial DMSP metabolism preventing the conversion of DMSP into DMS. Bacterial DMSP metabolism and DMS production can vary widely during the development and decline of phytoplankton blooms (Merzouk et al. 2006), but the mechanisms responsible for these variations are still poorly understood. The objective of the present study was to determine the impact of the decline of a diatom bloom on bacterial processes involved in $DMSP_d$ metabolism and DMS production.

MATERIALS AND METHODS

Study area, tracking and sampling of the water mass. The Lagrangian study of the vernal diatom bloom was conducted aboard the CCGS 'Hudson' in the region of the continental slope off the Nova Scotia coast in the Northwest Atlantic (Stn L, 43° 20′ N, 57° 42′ W; Fig. 1) from April 25 (Day 1) to May 1 (Day 7) 2003. A water mass where the phytoplankton bloom was declining was followed using a drifting buoy equipped with an Argos transponder.

Seawater samples were collected daily between 07:45 and 10:45 h local time near the drifting buoy using a Rosette equipped with a CTD (SeaBird SBE9) and 10 l SCRIPPS bottles. Seawater samples were prescreened through a 202 µm Nitex mesh to remove large zooplankton grazers and transferred into 10 l insulated containers. Bacterial $DMSP_d$ cycling experiments were conducted daily with seawater collected at 10 m within the surface mixed layer (SML) and at ca. 45 m within the deep chlorophyll *a* maximum (DCM) on Days 5 to 7.

Nitrate concentration, chlorophyll *a* concentration and diatom abundance. Samples for dissolved inorganic nutrients were filtered through pre-combusted (450°C for 5 h) Whatman GF/F glass fibre filters, and the filtrate was collected in 5 ml acid-washed polycarbonate cryovials. Samples were stored in liquid nitrogen and analyzed in the laboratory with an Alpkem FS III autoanalyzer (Knap et al. 1996).

Samples for chlorophyll *a* (chl *a*) determination were filtered onto 25 mm Whatman GF/F filters, and concentrations were determined aboard the ship with a Turner Designs 10-005R fluorometer after 18 h extraction in 90% acetone in the dark (Parsons et al. 1984).

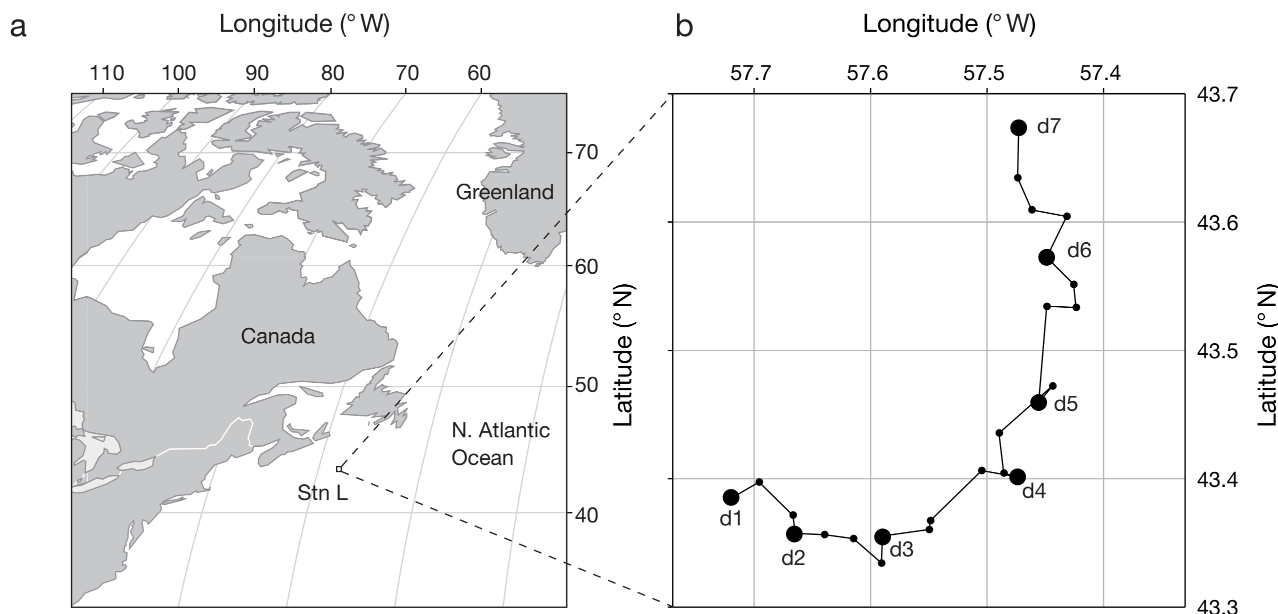


Fig. 1. (a) Map of the North Atlantic Ocean and location of Stn L where the Lagrangian study was carried out. (b) Trajectory of the drifting buoy with positions every 6 h (from Lizotte et al. 2008)

Phytoplankton identification and enumeration were performed on acid Lugol-preserved samples (0.4% final concentration) using a settling column and inverted microscope (Utermöhl 1931).

Bacterial abundance and leucine incorporation. Seawater samples for the determination of bacterioplankton abundance were preserved with 1% final concentration glutaraldehyde and kept in sterile 5 ml Falcon tubes in the dark at –75°C. Preserved samples were then stained with SYBR Green I, and cells were counted by flow cytometry (Becton-Dickinson, FACS Calibur) according to the methods described in Marie et al. (1999) and Brussaard et al. (2000).

Bacterial [^{14}C]-leucine incorporation rates were measured according to Simon & Azam (1989). Triplicate seawater samples were amended with [^{14}C]-leucine and incubated in the dark for 4 to 6 h in sterile test tubes, at ambient surface seawater temperatures, and processed using standard protocols. The coefficient of variation of leucine incorporation rates for triplicate samples was approximately 10% of the mean.

DMSP and DMS concentrations. Seawater samples (71 ml) were filtered by gravity through GF/F filters (Whatman, 47 mm, 0.7 µm retention). The filtrate was used to determine $DMSP_d$ and DMS, and the filter was used to determine particulate DMSP ($DMSP_p$). DMSP samples were hydrolyzed into DMS with KOH (10 M) and allowed to react overnight in the dark. Samples were analyzed using a purge and trap system coupled to a Varian 3400 or 3800 gas chromatograph (GC), each GC was equipped with a pulsed flame photometric detector (PFPD) (Scarratt et al. 2000). DMS analyses were calibrated with microlitre injections of DMS diluted with ultra high purity (UHP) helium supplied by a permeation tube (permeation rate certified by KinTek Laboratories Inc.). DMSP analyses were calibrated with millilitre injections of a 5 µg l^{-1} solution of hydrolyzed DMSP (Research Plus Inc.).

Bacterial $DMSP_d$ cycling experiments. Samples from the SML (Days 1 to 7) and from the DCM (Days 5 to 7) were incubated in parallel. Seawater was transferred gently into 71 ml brown polyethylene bottles previously washed with HCl and rinsed with deionised water. Samples were amended with ^{35}S-$DMSP_d$ (synthesized as described in Kiene & Linn [2000b]; specific activity 2.7 Ci $mmol^{-1}$) at tracer level concentration (final concentration <0.1 nmol l^{-1} ^{35}S-$DMSP_d$), gently mixed, and processed according to the method presented in Fig. 2 (Kiene & Linn 2000b). Then, 1 ml was immediately pipetted into a 20 ml scintillation vial containing 10 ml CytoScint (MP Biomedicals) to determine the total amount of isotope added. The samples were subsequently incubated in the dark for 3 h at *in situ* water temperature and sub-sampled after approximately 5 min (considered Time 0), and after 30, 60 and 180 min. At each time point, incubation bottles were gently mixed, and 5 ml was pipetted into 120 ml serum vials containing 0.5 ml sodium dodecyl sulfate (SDS, 0.2% final concentration) and 1 ml of $DMSP_d$ (100 µM final concentration). The addition of SDS and $DMSP_d$

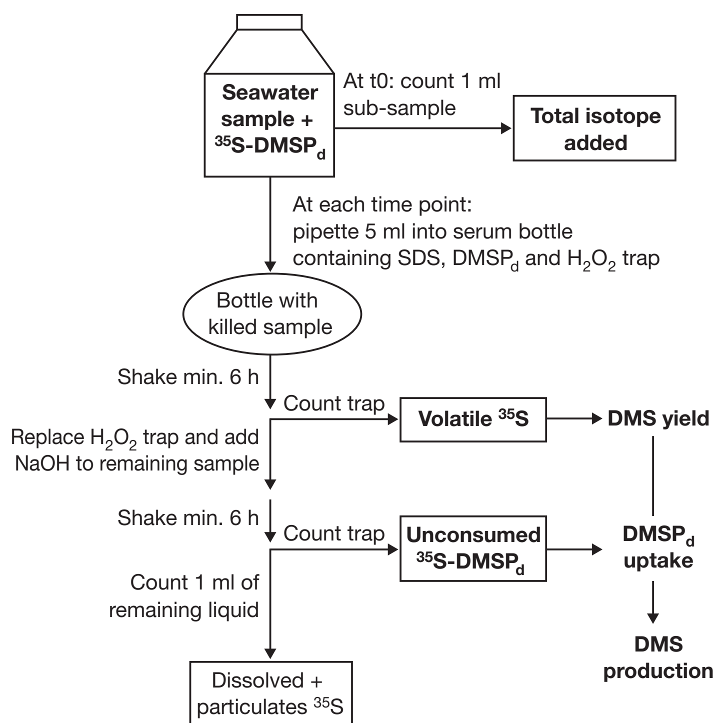


Fig. 2. Methods used to determine bacterial dissolved dimethylsulfoniopropionate (DMSP$_d$) uptake and dimethylsulfide (DMS) production after incubation of seawater samples with ^{35}S-DMSP$_d$

stopped further reaction or bacterial utilization of ^{35}S-DMSP$_d$ and its degradation products (Kiene & Linn 2000b). The serum bottles were sealed with a rubber stopper fitted with a plastic cup holding a H_2O_2-soaked filter (Gelman AE glass fibre) in the headspace. Samples were shaken for at least 6 h, during which ^{35}S volatiles (i.e. ^{35}S-DMS and ^{35}S-methanethiol) degassed into the headspace and were oxidized on the H_2O_2-soaked filters. Since methanethiol (MeSH) is consumed and degraded by bacteria 10 to 100 times faster than DMS, the amount of ^{35}S recovered on the filters at the end of the 3 h incubations was considered to be solely ^{35}S-DMS (Kiene & Linn 2000b). However, since this assumption was not verified, the DMS yields presented in this paper should be considered as upper limits. The filters were placed in 10 ml CytoScint, and the radioactivity on the filters represented the amount of DMS produced from bacterial DMSP$_d$ degradation at each time point. A new H_2O_2-soaked filter was placed in the plastic cup, and the serum bottles were resealed. A 0.2 ml aliquot of NaOH (5 M) was added to the remaining sample through the rubber septum to hydrolyze unreacted ^{35}S-DMSP$_d$ into ^{35}S-DMS. Samples were again shaken for at least 6 h, after which the filters were placed in 10 ml of CytoScint and used to determine the amount of unconsumed ^{35}S-DMSP$_d$ at each time point. One millilitre of the remaining liquid was pipetted into a 20 ml scintillation vial containing 10 ml CytoScint to determine the dissolved + particulate ^{35}S. The amount of isotope in each sample was analyzed with a RackBeta scintillation counter (LKB Wallac). These experimental and analytical procedures were conducted in duplicate on 2 occasions during the study, and the coefficient of variation for estimates of DMSP$_d$ loss rate constants and DMS yields was <12 % of the mean.

The DMSP$_d$ loss rate constant was calculated by taking the slope of the natural logarithm-transformed activity of unconsumed ^{35}S-DMSP$_d$ during the 3 h time course (Kiene & Linn 2000b). Bacterial DMSP$_d$ uptake rate was calculated by multiplying the loss rate constant by the initial DMSP$_d$ concentration. The bacterial DMS yield, expressed as a percentage, is the proportion of ^{35}S-DMSP$_d$ consumed that was recovered as ^{35}S-DMS at the end of the incubations. The bacterial DMS production rate was estimated by multiplying the DMS yield by the bacterial DMSP$_d$ uptake rate.

RESULTS

The water mass followed an eastward trajectory from Days 1 to 3, then moved north until Day 7 (Fig. 1). Temperature and salinity at the sampling depths of 10 and 45 m remained relatively stable (Table 1), and temperature-salinity diagrams not shown show similar features in the water column throughout the study (Pommier 2007), indicating that the drifting buoy successfully marked and followed the water mass. The water column was well stratified with a SML ranging from 10 to 26 m (Table 1) and a defined DCM located at around 45 m, at the base of the euphotic zone near the nitracline.

NO_3^- + NO_2^- concentrations were already low in the SML (0.9 µmol l^{-1}) at the beginning of the study and decreased below the detection limit after Day 3. They showed no distinct pattern at the DCM, with concentrations varying from 0.3 to 4.3 µmol l^{-1} (Fig. 3a). Chl *a* concentrations steadily decreased from 6.1 to 1.1 mg m^{-3} in the SML, whereas they were consistently higher at the DCM, ranging from 8 to 16 mg m^{-3}, with the highest values measured on Days 3 and 4 (Fig. 3b). At the onset of the experiment, large centric diatoms dominated the phytoplankton community in surface waters both in terms of abundance and biomass. As with chl *a* concentration, diatom abundance steadily declined in the SML over the course of the experiment (Fig. 3c). Although only a few data points are available at the DCM, diatom abundances were consistently higher than in the SML (Fig. 3c).

Table 1. Characteristics of seawater from the surface mixed layer (SML; 10 m) and the deep chl *a* maximum (DCM; 43 to 50 m) where DMSP cycling experiments were conducted

	Day	Date in 2003	Sampling depth (m)	Mixed layer depth (m)	Salinity	Temperature (°C)
SML	1	25 Apr	10	10	32.63	2.60
	2	26 Apr	10	12	32.69	2.80
	3	27 Apr	10	11	32.69	3.08
	4	28 Apr	10	23	32.72	3.08
	5	29 Apr	10	19	32.64	3.06
	6	30 Apr	10	26	32.69	3.25
	7	1 May	10	18	32.67	3.39
DCM	1	25 Apr	45		33.00	2.02
	2	26 Apr	43		32.91	1.97
	3	27 Apr	45		32.91	2.23
	4	28 Apr	50		32.93	1.87
	5	29 Apr	50		32.92	1.85
	6	30 Apr	50		32.98	2.14
	7	1 May	45		32.92	1.65

Bacterial abundance doubled from 0.8×10^9 to 1.5×10^9 cells l^{-1} in the SML at the beginning of the study, remained relatively stable between Days 2 and 5, then increased again to reach 1.7×10^9 cells l^{-1} on Days 6 and 7 (Fig. 3d). Bacterial abundance was generally lower in the DCM, ranging from 0.8×10^9 to 1.1×10^9 cells l^{-1}, with maximum values measured on Days 3 to 5 (Fig. 3d). As observed with bacterial abundance, leucine incorporation rates by heterotrophic bacteria increased sharply from Days 1 to 2 (65 to 121 pmol l^{-1} h^{-1}) in the SML, but decreased to the initial rates between Days 3 and 6 to finally increase again to 100 pmol l^{-1} h^{-1} on Day 7. Leucine incorporation was generally lower in

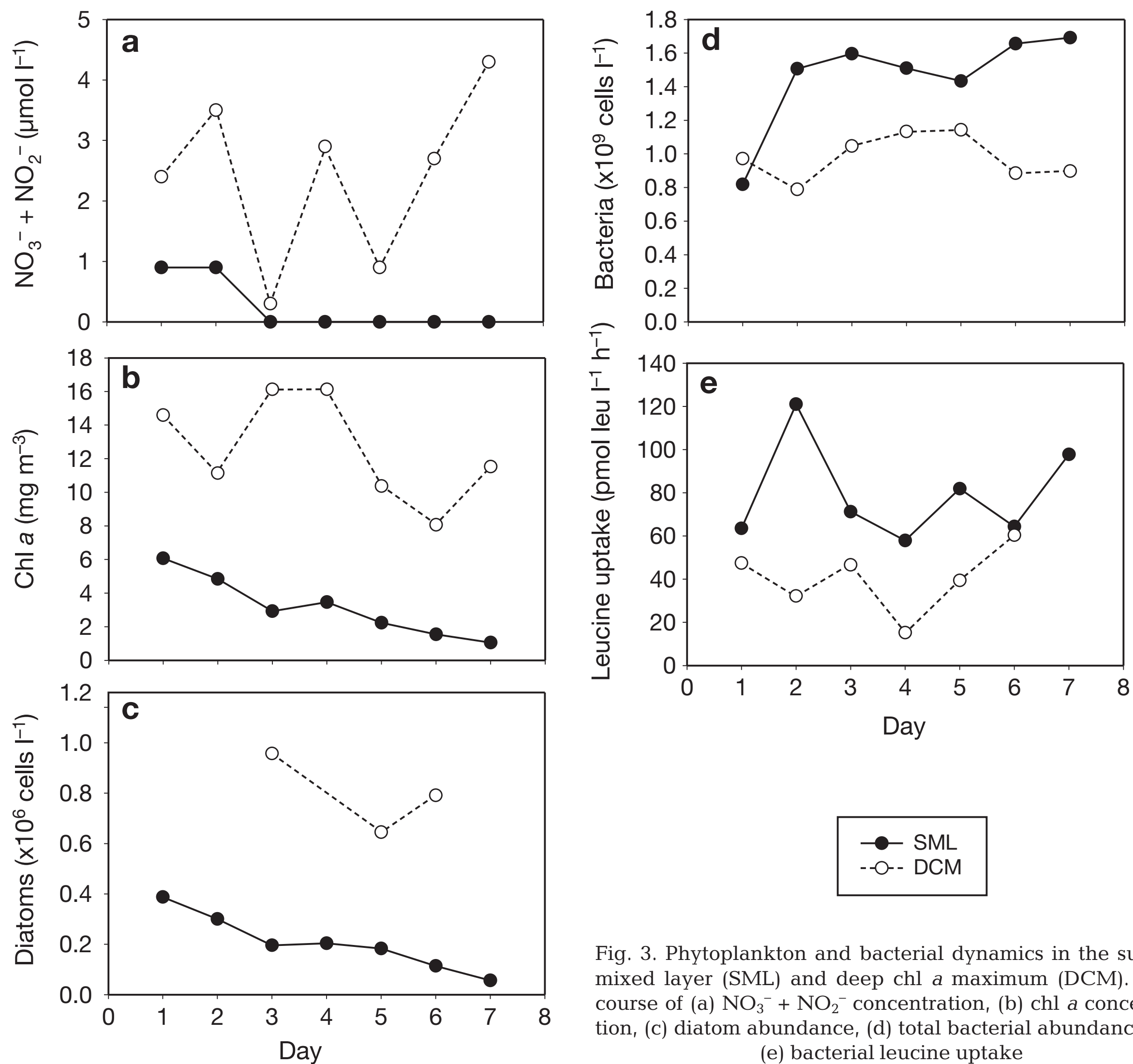


Fig. 3. Phytoplankton and bacterial dynamics in the surface mixed layer (SML) and deep chl *a* maximum (DCM). Time course of (a) NO_3^- + NO_2^- concentration, (b) chl *a* concentration, (c) diatom abundance, (d) total bacterial abundance and (e) bacterial leucine uptake

the DCM, with a minimum on Day 4 (15 pmol l^{-1} h^{-1}) and a maximum on Day 6 (60 pmol l^{-1} h^{-1}) (Fig. 3e).

In the SML, $DMSP_p$ and $DMSP_d$ concentrations also peaked on Day 2 (29.3 and 3.9 nmol l^{-1}, respectively) then steadily decreased to 12.4 and 0.7 nmol l^{-1}, respectively, by the end of the sampling period (Fig. 4a,b). A sharp increase in $DMSP_p$ from 16.7 to 36.0 nmol l^{-1} was observed in the DCM between Days 1 and 2 (Fig. 4a), then the concentration remained stable except for a peak of 46.5 nmol l^{-1} on Day 6. $DMSP_d$ concentrations at the DCM decreased sharply from 3.5 to 0.2 nmol l^{-1} between Days 1 and 3 and remained low afterward (Fig. 4b). DMS concentrations varied between 0.4 and 1.0 nmol l^{-1} both at the SML and the DCM throughout the study. In the SML, DMS slowly increased to 1.1 nmol l^{-1} by Days 4 to 5, then dropped back to the initial level (0.7 to 0.8 nmol l^{-1}) on the last 2 d (Fig. 4c). In the DCM, maximum DMS concentrations were measured on Day 3 (1.0 nmol l^{-1}), and minimum values, on Day 6 (0.4 nmol l^{-1}), with no consistent pattern over the course of the study.

In the SML, the $DMSP_d$ loss rate constant was highest on Day 1 (0.54 h^{-1}) and rapidly decreased to 0.07 h^{-1} by Day 3, and remained low for the remainder of the sampling period (Fig. 4d). Similarly to the $DMSP_d$ pool, $DMSP_d$ uptake was maximum on Days 1

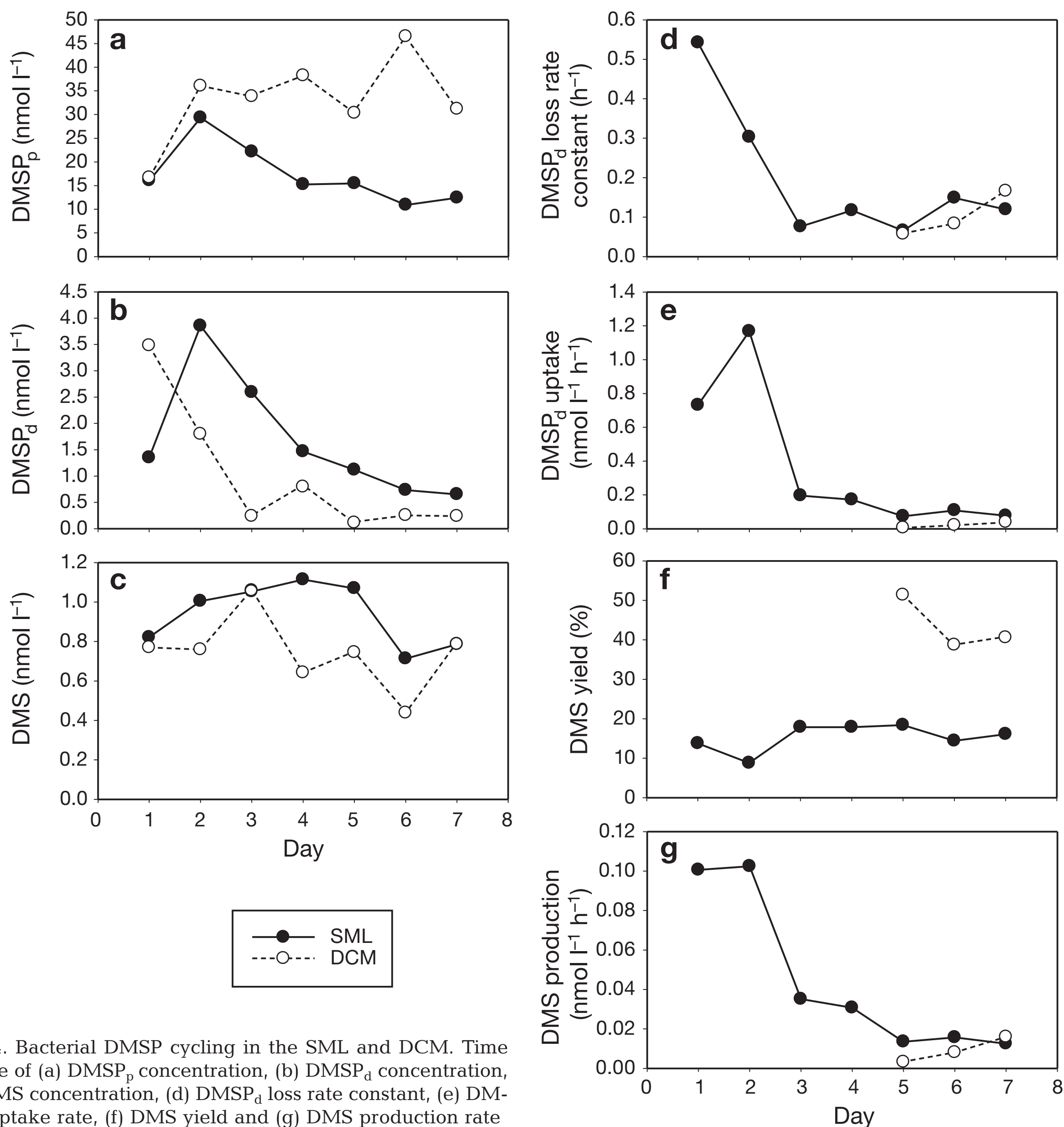


Fig. 4. Bacterial DMSP cycling in the SML and DCM. Time course of (a) $DMSP_p$ concentration, (b) $DMSP_d$ concentration, (c) DMS concentration, (d) $DMSP_d$ loss rate constant, (e) $DMSP_d$ uptake rate, (f) DMS yield and (g) DMS production rate

and 2 (0.7 and 1.17 nmol l^{-1} h^{-1}, respectively) and decreased to very low rates for Day 3 onward (<0.20 nmol l^{-1} h^{-1}; Fig. 4e). The $DMSP_d$ loss rate constant and $DMSP_d$ uptake were also low in the DCM on Days 5 to 7 (Fig. 4d,e) and fell within the range of values observed in the SML over the same period (0.06 to 0.17 h^{-1} and 0.12 to 0.26 nmol l^{-1} h^{-1}, respectively). In the SML, the DMS yield remained relatively unchanged at 13.8 to 18.4 %, with a minimum of 8.8 % measured on Day 2 (Fig. 4f). Given the relative stability of the DMS yield, the variations in DMS bacterial production rates were mostly governed by changes in bacterial $DMSP_d$ uptake rate. They exhibited high values on Days 1 and 2 (ca. 0.10 nmol l^{-1} h^{-1}), a sharp decline to 0.04 nmol l^{-1} h^{-1} between Days 2 and 3, and low rates thereafter (Fig. 4g). The DMS yield was much higher in the DCM than in the SML, with values ranging from 38.7 to 51.4 % (Fig. 4f). In the DCM, the DMS production rates were low and increased from 0.003 nmol l^{-1} h^{-1} on Day 5 to 0.02 nmol l^{-1} h^{-1} on Day 7 (Fig. 4g).

DISCUSSION

General characteristics of the Lagrangian study

The small variations in salinity and temperature measured in the SML and DCM during the 7 d experiment show that the drifting buoy tracked a coherent water mass relatively well. Nitrate concentrations were low at the onset of the sampling period (0.9 µmol l^{-1}) and fell below detection levels after Day 3, suggesting N-limiting conditions for the remainder of the phytoplankton spring bloom (Pommier 2007). Accordingly, the sampling period was characterised by a gradual decrease in chl *a* concentrations and diatom abundance in the SML (Fig. 3b,c). During the initial 4 d, diatoms sinking from the SML accumulated in the DCM, resulting in the high diatom abundances and chl *a* concentrations observed at that depth (Fig. 3b,c). The loss of $DMSP_p$ from the SML and its accumulation at the DCM suggest that the diatoms were responsible for most of the synthesis and release of DMSP despite their low intracellular DMSP quotas (Keller 1989). This is further supported by fractionated $DMSP_p$ results showing that most of the $DMSP_p$ was found in the large size fraction (>20 µM) dominated by diatoms at both depths (Lizotte et al. 2008). The depletion of $DMSP_d$ in surface waters after Day 2 shows that $DMSP_d$ losses exceeded the production rates during the decay of the bloom. The $DMSP_d$ concentrations presented here should, however, be considered with some caution in view of the recent demonstration that the filtration procedure used here may have induced cell breakage and increased the release of DMSP (Kiene & Slezak 2006). Although diatoms are less prone to cell breakage, DMSP leakage may still be important.

The low DMS concentrations (0.4 to 1.0 nmol l^{-1}) measured throughout the water column (Fig. 4c) during the senescence and sinking of the diatom bloom suggest that bacterial DMSP metabolism was oriented toward the demethylation/demethiolation pathway rather than cleavage to DMS.

Bacterial DMSP cycling in the SML

The bacterial community exhibited marked variations in abundance and activity during the decay of the diatom spring bloom and its sinking from the SML. The sharp increase in bacterial abundance between Days 1 and 2 suggests that the bacterial community responded to the declining diatom bloom, probably due to the release of DOM (Ducklow et al. 1993). This is supported by the peaks in leucine incorporation and in $DMSP_d$ uptake measured on Day 2 (Figs. 3e & 4e). Leucine is a substrate readily assimilated by the natural microbial community, and its uptake is an indicator of activity and protein synthesis by the bulk of the bacterial community (Kirchman 1993).

The increase in bacterial abundance and the peak in activity at the beginning of the study translated into a transient stimulation of $DMSP_d$ uptake. The $DMSP_d$ loss rate constants measured on Days 1 and 2 were high (Fig. 4d), and, in the absence of simultaneous DMSP release by algal cells, bacteria could consume the entire $DMSP_d$ pool in <4 h. The turnover time of the $DMSP_d$ pool may have been even faster if our filtration method artificially increased our estimate of the $DMSP_d$ pool. Similarly high loss rate constants have been measured in the productive coastal waters of the Gulf of Mexico (0.58 h^{-1}) and the Gulf of Maine (0.43 h^{-1}) (Malmstrom et al. 2004) and during the peak of an iron-induced diatom bloom in the northeast Pacific (0.32 h^{-1}; Merzouk et al. 2006). The high bacterial $DMSP_d$ uptake rates measured on Days 1 and 2 (Fig. 4e) are, however, slightly lower that the rates reported for the productive systems mentioned above (1.9 and 3.2 nmol l^{-1} h^{-1} [Malmstrom et al. 2004]; 2.2 nmol l^{-1} h^{-1} [Merzouk et al. 2006]). During our study, the highest bacterial $DMSP_d$ uptake rates were measured when the diatom abundance was still high in the SML, a period also corresponding to high rates of primary production and DOC release (Pommier 2007). Surprisingly, the rates of $DMSP_d$ uptake dropped to very low levels during most of the following bloom decay period. This shows that as the bloom declined, $DMSP_d$ became a less important substrate for

the thriving bacteria. It is interesting to note that in spite of the substantial reduction in microbial $DMSP_d$ uptake, the levels of $DMSP_d$ in the SML decreased progressively, indicating a parallel decrease in the supply rate of $DMSP_d$ (assuming steady-state concentrations). A recent microcosm study also reported that the bacterial community became less efficient at consuming $DMSP_d$ during the decay of a phytoplankton bloom due to a shift of the bacterial metabolism toward more readily available organic S compounds (Pinhassi et al. 2005).

Vila-Costa et al. (2006) recently showed that some eukaryotic phytoplankton can also take up DMSP sulfur. Although details about this new metabolic pathway for DMSP are still to be explored, we cannot preclude direct algal DMSP uptake as a partial cause for the high $DMSP_d$ uptake rates measured on Days 1 and 2 when diatom abundance was high. On the other hand, the parallel decrease in DMS production (a process attributed to bacteria) measured between Days 1 and 3 (Fig. 4g) suggests that heterotrophic bacteria were responsible for most of the DMSP uptake and degradation.

The shift in sulfur substrate used by bacteria that seems to have taken place during the decay of the diatom bloom may reflect the elemental composition of the algal community. DMSP constitutes 1 to 10 % of the intracellular carbon and 50 to 60 % of the intracellular sulfur in most phytoplankton species (Matrai & Keller 1994, Kiene et al. 2000, Simó et al. 2002). The contribution of C-$DMSP_p$ to total particulate organic carbon was <1 % during the present study (M. Lizotte pers. obs.), consistent with the low DMSP quota of diatoms. Particulate organic sulfur was not measured during this study, but diatoms are known for their high intracellular sulfur concentrations, resulting in lower C:S molar ratios (38 to 67) than reported for strong DMSP producers such as the prymnesiophyte *Emiliania huxleyi* (C:S molar ratio of 100) (Ho et al. 2003). During senescence, diatoms would thus release labile DOM rich in sulfur but poor in DMSP and bacteria would be exposed to readily available S-rich substrates, chiefly amino acids and glutathione (Ho et al. 2003). We hypothesize that during the decline of the diatom bloom, $DMSP_d$ became less available and the bacterial community used other, more accessible C and S substrates, causing the rapid decline in bacterial $DMSP_d$ uptake.

The contribution of a shift in bacterial species dominance to explain the change in bacterial DMSP metabolism is unlikely but cannot be excluded. Bacterial taxonomic composition determined by fluorescence *in situ* hybridization (FISH) showed that the proportion of the major taxa within the bacterial assemblage remained largely unchanged except for the β-proteobacteria, which almost disappeared from the community between Days 1 and 4 (K. Keats pers. comm.). However, since β-proteobacteria usually account for only a small fraction of the DMSP-assimilating bacteria (Malmstrom et al. 2004), we cannot firmly link their disappearance with the observed variations in the bacterial $DMSP_d$ uptake rate.

The DMS yield in the SML remained surprisingly stable at ca. 15 to 18 % during most of the decay of the diatom bloom (Fig. 4f). It is only on Day 2 when leucine incorporation and $DMSP_d$ uptake reached their peaks that the yield dropped below 10 %. This low yield is consistent with those reported in a wide range of marine environments, where cleavage to DMS generally represents <10 % of bacterial $DMSP_d$ consumption (Kiene & Linn 2000a, Kiene et al. 2000, Yoch 2002, Zubkov et al. 2002). On the other hand, rates >15 %, as measured for most of the sampling period (and in the DCM; as discussed below), would indicate a relatively low sulfur demand of the bacterial community. Kiene et al. (2000) first hypothesized that the DMS yield depends on $DMSP_d$ availability and the S demand of the bacterial community, which, in turn, is controlled by bacterial biomass and growth rate. This hypothesis was further refined by Pinhassi et al. (2005) and Levasseur et al. (2006), who proposed that the DMS yield varies with the bacterial S demand and the relative contribution of $DMSP_d$ to the total pool of labile DOS. The S demand (as in Merzouk et al. 2006) calculated using leucine incorporation rates (Fig. 3e) and a C:S molar ratio of 248 as reported for marine bacteria (Cuhel et al. 1983), ranged between 1.4 nmol S l^{-1} d^{-1} on Day 4 and 2.9 nmol S l^{-1} d^{-1} on Day 2 (data not shown). According to this hypothesis, the relatively high DMS yields measured during most of the study in spite of high leucine incorporation rates would indicate that other organic S substrates satisfied the high bacterial S demand, keeping DMSP as a secondary S substrate. Assuming that 5 % of $DMSP_d$ taken up was incorporated into bacterial biomass (see Merzouk et al. 2006 for details), the proportion of the bacterial S demand satisfied by DMSP-S was 57 and 48 % on Days 1 and 2, respectively, declined sharply between Day 2 and 3, and remained between 4 and 15 % for the rest of the sampling period. These results suggest that even when DMSP uptake was maximal on Days 1 and 2, DMSP-S accounted for only half of the high bacterial S demand and was never consumed in excess of the S demand, a situation that has been shown to favour DMS production over demethylation (Kiene et al. 2000, Merzouk et al. 2006). These calculations add support to the hypothesis that DMSP was a minor S substrate during the decline of the spring diatom bloom and that the high S demand was satisfied by other available S compounds.

During the decay of the diatom bloom, bacterial DMS production was mostly controlled by $DMSP_d$ uptake. Bacterial DMS production decreased in parallel with bacterial $DMSP_d$ uptake, while the DMS yield remained more or less constant (Fig. 4g). The high bacterial DMS production rates measured on Days 1 and 2 are slightly lower than biological gross DMS production rates measured during the decline of a diatom bloom in the Northwest Atlantic (0.3 nmol l^{-1} h^{-1}; Levasseur et al. 2004) and during an iron-induced diatom bloom in the Northeast Pacific (0.24 nmol l^{-1} h^{-1}; Merzouk et al. 2006). Except for the higher rates measured on Days 1 and 2, bacterial DMS production was relatively low in the SML, resulting in a modest increase in DMS concentrations from 0.6 to 1.1 nmol l^{-1} between Days 1 and 5. Biological DMS consumption was low throughout the study period (M. Lizotte pers. obs.), while DMS sea–air fluxes were roughly equivalent to DMS production rates in the SML, resulting in low and generally positive net change in surface DMS concentrations (0.05 to 0.18 nmol l^{-1} d^{-1}; Lizotte et al. 2008), except on Day 5 when increased wind speeds resulted in a DMS net change of –0.36 nmol l^{-1} d^{-1} (Lizotte et al. 2008). This is consistent with another study conducted in the Northwest Atlantic in spring, which reported low net DMS production rates and no changes in DMS concentrations during the decline of a diatom bloom (Levasseur et al. 2004). The low DMS production rates and concentrations measured during the decline of the spring diatom bloom in spite of the high bacterial activity are consistent with the release of labile DOM rich in S but poor in DMSP by the diatom community.

Bacterial DMSP cycling in the DCM

The Lagrangian study was conducted during low sea state conditions with wind speeds generally <10 m s^{-1}. These conditions provided the opportunity to follow the gradual sinking of the nutrient-limited diatom cells without major mixing and re-suspension events and the formation of the DCM at ca. 45 m (Pommier 2007). The accumulation of phytoplankton biomass and $DMSP_p$ in the DCM may favour DMS production and the formation of a DMS pool that could be eventually ventilated upon wind mixing events.

In spite of the prevailing high phytoplankton biomass, DMS production was not stimulated in the DCM. On Days 1 and 2, the relatively high $DMSP_d$ concentrations measured in the DCM, concomitant with the increase in $DMSP_p$, probably reflected the new arrival of nitrogen-limited diatom cells. The high $DMSP_d$ concentrations were, however, short-lived, and $DMSP_d$ concentrations became very low after Day 3 (Fig. 4b). The bacterial abundance and leucine incorporation remained consistently low in the DCM (Fig. 3d,e), probably reflecting a healthy algal community with low DOM release rates.

Rate measurements were only performed toward the end of the sampling period. During that time (Days 5 to 7), the bacterial $DMSP_d$ loss rate constant and $DMSP_d$ uptake were comparable to the low rates measured in the SML, showing that the bacterial utilization of $DMSP_d$ was not stimulated by the accumulation of biomass and $DMSP_p$ at the DCM. On the other hand, the bacterial DMS yields were exceptionally high (40 to 50%), more than twice the yield measured in the SML (9 to 18%) (Fig. 4f). The lower bacterial leucine incorporation rates and very high DMS yields thus suggest that the bacterial community at the DCM had a low S demand and used $DMSP_d$ mostly as a carbon source. In spite of the high DMS yields, the very low $DMSP_d$ concentrations (Fig. 4b) and low $DMSP_d$ uptake rates (Fig. 4e) resulted in low bacterial DMS production (Fig. 4g). These low DMS production rates had no consistent effect on DMS concentrations, which remained <1.0 nmol l^{-1}, indicating that the DCM was not an important source of DMS for the upper mixed layer during that post-diatom bloom period.

CONCLUSIONS

The decline of the spring diatom bloom was characterised by low and stable DMS concentrations in the SML and the DCM. The overall impact of the declining bloom on bacterial DMSP metabolism was to decrease $DMSP_d$ uptake (which decreased with $DMSP_d$ availability) and DMS production despite an increase in bacterial abundance and high bacterial activity (as indicated by leucine incorporation). At the beginning of the Lagrangian experiment, when diatom abundance and productivity were still high, direct release of DMSP by the still-growing diatom assemblage was apparently the major $DMSP_d$ source for the bacteria, and $DMSP_d$ uptake and DMS production were high. The near-exhaustion of nitrate on Day 3 resulted in a rapid loss of the diatoms from the SML and coincided with decreases in both DMSP pools, as well as in bacterial DMSP uptake and DMS production rates. This low bacterial DMSP metabolism when abundance and activity of the bacterial community were otherwise high suggests that the bacteria shifted their preference toward non-DMSP organic compounds released by the decaying bloom. Overall, our results highlight a potential uncoupling between bacterial production and DMS dynamics that could result from the uptake of other S-containing organic compounds released during the decay of low-DMSP but high particulate organic S diatom blooms.

Acknowledgements. This project was funded by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canadian Foundation for Climate and Atmospheric Sciences (CFCAS) and the Department of Fisheries and Oceans Canada. A.M. received postgraduate scholarships from the Fonds québécois de la recherche sur la nature et les technologies (FQRNT), from Université Laval and from Québec-Océan. The authors thank the captain and crew of the CCGS 'Hudson' for their work and help during the cruise. They also thank the cruise coordinator Sylvie Roy for her excellent work before, during and after the cruise. This research was part of the Canadian Surface Ocean Lower Atmosphere Study (C-SOLAS) network.

LITERATURE CITED

Andreae MO, Crutzen PJ (1997) Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. Science 276:1052–1058

Brussaard CPD, Marie D, Bratbak G (2000) Flow cytometric detection of viruses. J Virol Methods 85:175–182

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655–661

Cuhel RL, Jannasch HW, Taylor CD (1983) Microbial growth and macromolecular synthesis in the northwestern Atlantic Ocean. Limnol Oceanogr 28:1–18

Ducklow HW, Kirchman DL, Quinby HL, Carlson CA, Dam HG (1993) Stocks and dynamics of bacterioplankton carbon during the spring bloom in the eastern North Atlantic Ocean. Deep-Sea Res II 40:245–263

Ho TY, Quigg A, Finkel ZV, Milligan AJ, Wyman K, Falkowski PG, Morel FMM (2003) The elemental composition of some marine phytoplankton. J Phycol 39:1145–1159

Keller MD (1989) Dimethyl sulfide production and marine phytoplankton: the importance of species composition and cell size. Biol Oceanogr 6:375–382

Kiene RP, Linn LJ (2000a) Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. Limnol Oceanogr 45:849–861

Kiene RP, Linn LJ (2000b) The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: tracer studies using ^{35}S-DMSP. Geochim Cosmochim Acta 64:2797–2810

Kiene RP, Slezak D (2006) Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnol Oceanogr Methods 4: 80–95

Kiene RP, Linn LJ, Bruton JA (2000) New and important roles for DMSP in marine microbial communities. J Sea Res 43: 209–224

Kirchman DL (1993) Leucine incorporation as a measure of biomass production by marine bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 509–512

Knap A, Michaels A, Close A, Ducklow H, Dickson A (1996) Protocols for the Joint Global Ocean Flux Study (JGOFS) core measurements. In: JGOFS Report No. 19, reprint of the IOC manuals and guides, No. 29 (1994). UNESCO, Paris

Levasseur M, Scarratt M, Roy S, Laroche D and others (2004) Vertically resolved cycling of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) in the Northwest Atlantic in spring. Can J Fish Aquat Sci 61:744–757

Levasseur M, Scarratt MG, Michaud S, Merzouk A and others (2006) DMSP and DMS dynamics during a mesoscale iron fertilization experiment in the Northeast Pacific — Part I: temporal and vertical distributions. Deep-Sea Res II 53: 2353–2369

Lizotte M, Levasseur M, Scarratt MG, Michaud S, Merzouk A, Gosselin M, Pommier J (2008) Fate of dimethylsulfoniopropionate (DMSP) during the decline of the northwest Atlantic Ocean spring diatom bloom. Aquat Microb Ecol 52:159–173

Malmstrom RR, Kiene RP, Kirchman DL (2004) Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. Limnol Oceanogr 49:597–606

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1999) Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl Environ Microbiol 65:45–52

Matrai PA, Keller MD (1993) Dimethylsulfide in a large-scale coccolithophore bloom in the Gulf of Maine. Cont Shelf Res 13:831–843

Matrai PA, Keller MD (1994) Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: intracellular variations. Mar Biol 119:61–68

Matrai PA, Vernet M (1997) Dynamics of the vernal bloom in the marginal ice zone of the Barents Sea: dimethylsulfide and dimethylsulfoniopropionate budgets. J Geophys Res Oceans 102:22965–22979

Merzouk A, Levasseur M, Scarratt MG, Michaud S and others (2006) DMSP and DMS dynamics during a mesoscale iron fertilization experiment in the Northeast Pacific — Part II: biological cycling. Deep-Sea Res II 53:2370–2383

Nguyen BC, Belviso S, Mihalopoulos N, Gostan J, Nival P (1988) Dimethyl sulfide production during natural phytoplanktonic blooms. Mar Chem 24:133–141

Parsons TR, Maita Y, Lallil CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Toronto

Pinhassi J, Simó R, González JM, Vila M and others (2005) Dimethylsulfoniopropionate turnover is linked to the composition and dynamics of the bacterioplankton assemblage during a microcosm phytoplankton bloom. Appl Environ Microbiol 71:7650–7660

Pommier J (2007) Variabilité de la production primaire et de l'exportation du carbone organique particulaire dans la zone mésopélagique de l'océan Atlantique nord-ouest lors du déclin d'un bloom printanier de diatomées. PhD dissertation, Université du Québec à Rimouski, Rimouski, QC

Scarratt MG, Levasseur M, Schultes S, Michaud S and others (2000) Production and consumption of dimethylsulfide (DMS) in North Atlantic waters. Mar Ecol Prog Ser 204: 13–26

Simó R (2004) From cells to globe: approaching the dynamics of DMS(P) in the ocean at multiple scales. Can J Fish Aquat Sci 61:673–684

Simó R, Archer SD, Pedrós-Alió C, Gilpin L, Stelfox-Widdicombe CE (2002) Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. Limnol Oceanogr 47:53–61

Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213

Utermöhl von H (1931) Neue Wege in der quantitativen Erfassung des Planktons (mit besonderer Berücksichtigung des Ultraplanktons). Verh Int Ver Theor Angew Limnol 5: 567–595

van Duyl FC, Gieskes WWC, Kop AJ, Lewis WE (1998) Bio-

logical control of short-term variations in the concentration of DMSP and DMS during a *Phaeocystis* spring bloom. J Sea Res 40:221–231
Vila-Costa M, Simó R, Harada H, Gasol JM, Slezak D, Kiene RP (2006) Dimethylsulfoniopropionate uptake by marine phytoplankton. Science 314:652–654
Yoch DC (2002) Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide. Appl Environ Microbiol 68: 5804–5815
Zubkov MV, Fuchs BM, Archer SD, Kiene RP, Amann R, Burkill PH (2002) Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. Deep-Sea Res II 49:3017–3038
Zubkov M, Linn LJ, Amann R, Kiene RP (2004) Temporal patterns of biological dimethylsulfide (DMS) consumption during laboratory-induced phytoplankton bloom cycles. Mar Ecol Prog Ser 271:77–86

Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

Submitted: March 6, 2008; Accepted: July 7, 2008
Proofs received from author(s): September 19, 2008