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Turnover of dimethylsulfoniopropionate and dimethylsulfide in the marine environment: a mesocosm experiment

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ABSTRACT: The production of dimethylsulfoniopropionate (DMSP) by marine phytoplankton and the fate of the produced DMSP and dimethylsulfide (DMS) were studied in 4 pelagic mesocosms during an algal bloom over a period of 1 mo. Bacterial numbers, concentrations of particulate and dissolved DMSP, DMS, and chlorophyll *a* were monitored, as well as the turnover rates of DMS and DMSP. Of the total amount of DMSP produced, only a fraction could be detected as DMS in the water column. DMS production in the water column did not necessarily correlate with algal senescence, but also occurred during the maximum of the algal bloom. The flux of DMS to the atmosphere played a minor role as a sink for DMS. Evidence is presented that shows bacterial consumption to be a major sink for DMS, under conditions of both high and low DMS water concentrations. DMSP was degraded either via cleavage or via demethylation; the results indicate a predominant role for the latter route.

KEY WORDS: Dimethylsulfoniopropionate · Dimethylsulfide · Bacteria Phytoplankton Flux

INTRODUCTION

On the basis of its concentration and turnover, dimethylsulphide (DMS) is one of the most important biogenic sulphur compounds in the marine environment. It accounts for over 50% of the total biogenic sulphur (10^{11} kg S) entering the atmosphere annually, and about 90% of the DMS originates from marine sources (Andreae 1990). In the marine environment, DMS is mainly formed by the cleavage of β-dimethylsulphoniopropionate (DMSP), an osmoregulator in marine algae (Vairavamurthy et al. 1985, Dickson & Kirst 1987a, b).

The efflux of DMS to the atmosphere and the factors controlling this deserve special attention because of their (3-fold) effects. Firstly, since the work of Lovelock et al. (1972), it has been accepted that DMS is the main vehicle by which the global sulphur cycle is balanced. Secondly, photochemical oxidation of atmospheric DMS into methanesulphonic acid and sulphate leads to acid precipitation. Thirdly, the efflux of DMS to the atmosphere results in an increase in the so-called 'cloud-condensation-nuclei', or CCN, which have a backscattering effect on solar radiation and influence cloud formation. A regulatory effect of CCN on global warming has been postulated by several authors (Bates et al. 1987, Charlson et al. 1987, 1991, Ayers & Gras 1991).

Factors controlling the concentration of DMS in the water column (the parameter driving the release to the atmosphere) are not yet clearly understood, but include zooplankton grazing, algal senescence, algal and bacterial DMSP-lyase activity, and chemical breakdown of DMS (Dacey & Wakeham 1986, Nguyen et al. 1988, Belviso et al. 1990, Belviso et al. 1993, Stefels & Van Boekel 1993, De Souza & Yoch 1995, Stefels et al. 1995).

The most important loss factors for DMS from surface waters are bacterial oxidation, (photo)oxidation, and efflux to the atmosphere (Brimblecombe & Shooter

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1986, Kiene 1992). Bacterial DMSP demethylation may also occur, providing an alternative route for DMSP metabolism that circumvents the formation of DMS (Fig. 1; Taylor & Gilchrist 1991). Products thus formed include methylmercaptopropionate (MMPA), and mercaptopropionate (MPA). Stefels & Van Boekel (1993) showed that bacteria-free cultures of the algae *Phaeocystis* sp. are able to convert extracellular DMSP into DMS using a DMSP-lyase enzyme; the algal contribution under field conditions is still unknown, however.

The particulate DMSP concentration in seawater shows seasonal fluctuations which correlate well with algal densities of certain species (Nguyen et al. 1988, Keller et al. 1989, Leck et al. 1990, Matrai & Keller 1994). During algal blooms, high concentrations of DMSP and DMS are reached (Leck et al. 1990, Malin et al. 1993), especially when algae such as *Phaeocystis* sp. or *Emiliania huxleyi* are the dominant species (Matrai & Keller 1993, Liss et al. 1994). For this reason, algal blooms may have pronounced effects on the annual global sea-air exchange of DMS. It is therefore of great importance to know what mechanisms control the DMS concentration in the watercolumn (DMS_{water} concentration) under various conditions.

Kwint & Kramer (1995) could not balance the production and consumption of DMS in an earlier mesocosm study in Den Helder, The Netherlands, unless bacterial degradation was used as 'a missing sink'. In the present study, quasi-field experiments were performed in mesocosms in which an algal bloom was induced to study the fate of DMSP and the relative importance of bacterial degradation and other sinks for DMS. Mesocosm experiments have the advantages that the same water mass and planktonic community can be followed over a relatively long period under quasi-field conditions, and that the system is easily accessible for sampling. Previous studies have shown that in such pelagic mesocosms replicable phytoplankton successions occurred and that the results could be extrapolated to the field situation (Kuiper 1977, 1981). Because of the high DMSP content of *Phaeocystis* sp., a bloom in which this species dominated was monitored.

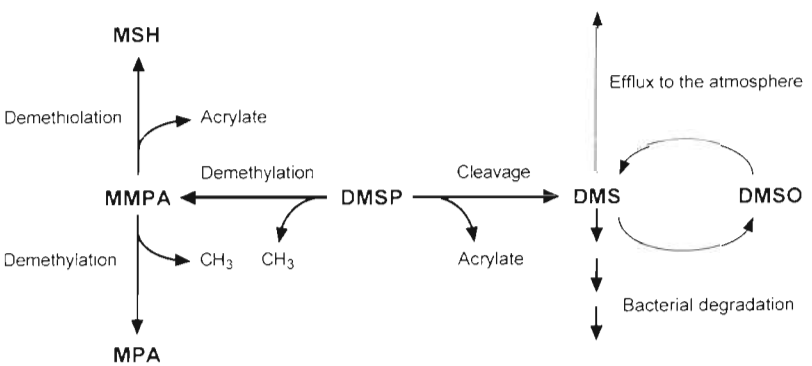

Fig. 1 Bacterial conversions of dimethylsulfoniopropionate (DMSP). Cleavage of DMSP yields dimethylsulfide (DMS) and acrylate. An alternative route for the degradation of DMSP is the demethylation via methylmercaptopropionate (MMPA) to mercaptopropionate (MPA). Demethiolation of MMPA results in methanethiol (MSH) and acrylate

MATERIALS AND METHODS

Mesocosms. The quasi-field experiments were carried out in 4 similar, free-floating, pelagial mesocosms as used by Kwint & Kramer (1995). The same experimental setup was used for all 4 systems except for the presence of sediment traps in mesocosms 1 and 2. The sediment traps (perspex cylinders (diameter 7.4 cm, height 20 cm) were mounted in a rigid PVC frame, at water depths of 0.5 and 2 m and were emptied daily. Results obtained from the sediment traps in the experiments are described in detail by Osinga et al. (1996). Two days before commencement, the mesocosms were installed in the Den Helder harbour, The Netherlands. The mesocosms consisted of large plastic bags (polythene/polyamide 2-layered foil), each with a diameter of 72 cm and an approximate depth of 3.2 m, thus having a volume of about 1300 l. The mesocosms were simultaneously filled with water from the Marsdiep (The Netherlands) tidal inlet on April 14, 1993, according to Kwint & Kramer (1995). On Day 0, 40 µM nitrate and 6 µM phosphate were added to restore the levels of these nutrients to the winter levels in the nearby Marsdiep, thereby ensuring high planktonic growth. For 35 d almost daily representative samples were taken for the analysis of chlorophyll *a* (chl *a*), DMSP DMS, and nutrient concentrations.

Analysis. Water samples were collected in a depth-transect through the bags using a 3 l glass bottle and a peristaltic pump with teflon tubing The oxygen concentration, pH, Secchi depth, particle data (Coulter Counter) and water temperature were measured daily. Subsamples for the determination of chl *a*, nutrient concentrations, DMS, phytoplankton, bacteria densities and DMSP and for the short-time incubation experiments were immediately taken from this large sample. The remainder was returned to the mesocosm.

Chl a: Subsamples were stored in 1 l polyethylene bottles in the dark until analysis within 2 h of sampling Samples were filtered on glass fibre filters (Whatmann GF/C), extracted with 90% acetone and analyzed by spectropho-

tometry according to standard procedures (Parsons et al. 1984).

Nutrient concentrations: Subsamples were stored deep-frozen (−25°C) until analysis for orthophosphate, ammonia, nitrate, nitrite and reactive silicate, using a 'Technicon' auto-analyzer (Parsons et al. 1984).

DMS: Subsamples were taken in amber 100 ml glass-stoppered bottles and stored on ice. To check for losses during storage, occasionally duplicate samples were taken for DMS and DMSP and analyzed with an interval of about 1 h. No obvious losses of particulate DMSP ($DMSP_p$), dissolved DMSP ($DMSP_d$) or DMS were detected. The samples were processed immediately after returning to the laboratory, usually within 4 h of sampling.

A gas-stripping cryotrapping method was used to concentrate the DMS samples (Kwint & Kramer 1995). DMS was analyzed according to Lindqvist (1989) on a Varian 3700 gas chromatograph equipped with a capillary linear plot column and a photoionization detector using hydrogen as the carrier gas. Calibration was performed using DMS permeation tubes in a dynamic dilution system. Quantitative analysis of DMSP was possible after its alkaline conversion to acrylate and DMS (Dacey & Blough 1987). Gravity filtration (Whatmann GF/C filters) was used to separate $DMSP_p$ from $DMSP_d$ (Stefels & Van Boekel 1993).

Phytoplankton: Samples preserved with Lugols Iodine were counted and identified by inverted microscopy. At least 20 fields were counted per sample (magnification 400×).

Zooplankton: Samples were taken twice weekly with a 3 m long PVC pipe, equipped with a ball valve at the end, according to Kuiper (1981). In total, 35 l per sample were filtered over a 55 µm mesh-sized nylon filter and immediately fixed with neutral buffered formalin. The filtered water was returned to the mesocosm. Zooplankton were counted and identified microscopically in a 1 ml chamber Copepods were subdivided into adult copepods, copepodites and nauplii.

Bacteria: Samples for the estimation of total bacterial numbers and DMS consuming bacteria were taken 3 times per week from 2 mesocosms (1 and 3). Total bacterial cells were counted by epifluorescence microscopy (Hobbie et al. 1977). The filter-fixed cells were incubated for 10 min in the dark using bisbenzimide (10 µM) (T A. Hansen pers. comm.) instead of acridine orange. For the quantification of the DMS oxidizing population a most probable number (MPN) method was used (De Man 1975). Bacteria were incubated on a mineral medium with DMS as the only carbon source. The medium used for the triplicate 10-fold dilution series (10^{-3} to 10^{-9}) consisted of 5 µm-filtered autoclaved seawater supplemented with 37.4 mM NH_4Cl, 16.5 mM 2-bis[2-hydroxyethyl]amino-2-[hydroxymethyl]-1,3-propanediol, 1 mM KH_2PO_4 and 0.2 mM $FeNH_4$-citrate (Janvier et al. 1985). The dilution series was incubated in an atmosphere of DMS in the dark at 25°C for 4 wk. The added amount of DMS resulted in a concentration of DMS in the medium (DMS_{medium}) of 200 µM. The atmosphere was refreshed weekly to assure the availability of sufficient oxygen and DMS. Positive tubes were scored on the basis of both acidification and increased turbidity. Positive tubes were double checked by transferring 5 ml from the tubes to 15 ml crimp seal vials closed with teflon-lined septa. After overnight incubation, DMS consumption was determined by headspace analysis. The computer program of Klee (1993) was used for data analysis.

Isolation of DMSP-utilizing bacteria: To determine the bacterial population diversity, subsamples from the mesocosms were directly spread on solid agarose plates [15 g agarose MP l^{-1} (Boehringer Mannheim, Germany), washed 3 times with a 100-fold volume of demineralised water] consisting of the MPN medium (Janvier et al. 1985) supplemented with 10 mM DMSP as substrate. After 3 wk distinct colonies were transferred to fresh plates and control media without substrate to check for DMSP utilization. Subsamples inoculated in mineral medium supplemented with 10 mM DMSP were used to screen the isolates for DMSP utilization and DMS, MMPA, or MPA production (Fig. 1).

DMSP and DMS conversion rate estimation: Samples for the duplicate estimation of bacterial DMSP and DMS conversion rates were taken twice weekly from mesocosm 1. Incubation took place in the dark at the *in situ* temperature. The samples (50 ml volume) were incubated in amber 250 ml screw-cap bottles with and without the addition of 500 µM chloroform. At $t = 0$ and after 2, 4 and 6 h, duplicate bottles were used to determine the $DMSP_p$, $DMSP_d$ and DMS_{water} concentrations, meaning that for each time t, 2 new bottles with and 2 new bottles without chloroform were used. The production and consumption rates for these compounds were calculated from the initial linear parts of the curves describing their changes in time. The results of an incubation were only used when the linear part of the curve covered at least 3 (duplicate) points. Consumption of DMS was blocked with 500 µM of chloroform (Kiene & Bates 1990), allowing an estimation of the net production rate of DMS. In control experiments the chloroform added had no effect on the DMSP-lyase activity of an axenic *Phaeocystis* sp. culture, while it completely blocked DMS oxidation by a DMS-utilizing bacterial culture (Matrai et al. 1995, J. Stefels pers. comm.). The concentration of $DMSP_d$ did not increase in the presence of chloroform, which is in contrast with the findings of Wolfe & Kiene (1993), but this is probably due to the high biomass of *Phaeocystis* sp. In this experiment the assumption was made that no

$DMSP_p$ was produced by phytoplankton during the 6 h incubation in the dark.

Flux: The DMS flux to the atmosphere was calculated on the basis of the model of Liss & Merlivat (1986), taking actual water temperature and windspeed, obtained from a nearby meteorological station (within 1 km distance), into account. The concentration of DMS in the air (DMS_{air}) was insignificant compared to the concentration in the water column (A. Baart pers. comm.) and therefore could be ignored in the calculation.

Reagents and chemicals. DMS and acrylate were obtained from Merck (PA quality). The HCl salt of DMSP was synthesized according to Chambers et al. (1987). Its identity and purity were checked by melting point determination, proton NMR, and after alkaline conversion to DMS, by comparison to a DMS standard. The bisbenzimide used was Hoechst dye 3258.

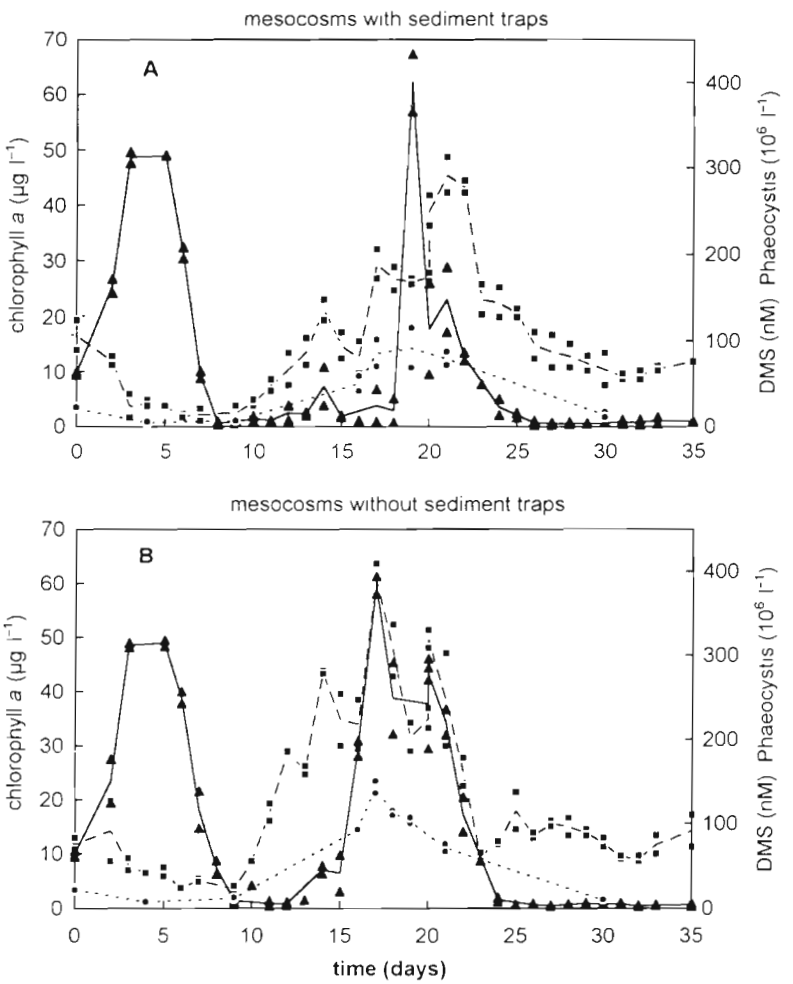

Fig. 2. Development of the DMS_{water} concentration (▲, —), chl *a* concentration (■, – – –) and *Phaeocystis* cell density (●, - - - -) during the algal bloom: (A) mesocosms 1 and 2 with sediment traps; (B) mesocosms 3 and 4 without sediment traps. Points show measurements and lines show averages between duplicate mesocosms

RESULTS AND DISCUSSION

Plankton development

After sampling started at Day 0, it took 5 d for the algae already present in the water column of the mesocosms to settle. These algae appeared unable to stay buoyant in the less turbulent environment of the mesocosms and as a result sedimentation took place. Fig 2 shows the chl *a* and the DMS_{water} concentrations together with *Phaeocystis* sp. cell density with time. The settling of algae during the initial days gave rise to elevated levels of $DMSP_d$ and DMS concentrations (Figs. 2 & 3).

A bloom dominated by *Phaeocystis* sp., a DMSP producing species, started to develop from Day 10 onwards. After a peak at around Day 18 (up to 150×10^6 cells l^{-1}) the bloom collapsed at around Day 22. This was indicated by a decrease in the chl *a* concentration. The collapse was accompanied by nitrogen depletion; a decrease from 100 to about 1 µM NO_3 was observed, suggesting that nutrient limitation rather than zooplankton grazing had caused the collapse. Diatoms, mainly *Nitszchia* sp., were present in only moderate numbers (1 to 3×10^6 cells l^{-1}) due to the low silicate concentrations, which were just above the detection limit (1 µM)

The sedimentation of algae in the first week coincided with a DMS peak that was very similar in all systems (Fig. 2). The DMS_{water} concentration peak value was about 300 nM at Days 4 and 5. The DMS_{water} concentration was up to 50% of the amount of DMSP that could be detected during the first 7 d of the experiment. Whether DMS during this period originated from algal or bacterial DMSP-lyase activity remains unclear. When both the efflux of DMS to the atmosphere and the bacterial consumption are taken into account, it seems likely that during this first DMSP peak most of the DMSP present in the water column was converted to DMS. The high DMS concentrations may be an effect of the density of the bloom induced, which practically consisted of a monoculture of the algae *Phaeocystis* sp. These high concentrations may also occur occasionally in the field during dense *Phaeocystis* sp. blooms in the Southern Ocean and North Sea (Crocker et al. 1995, S. Turner

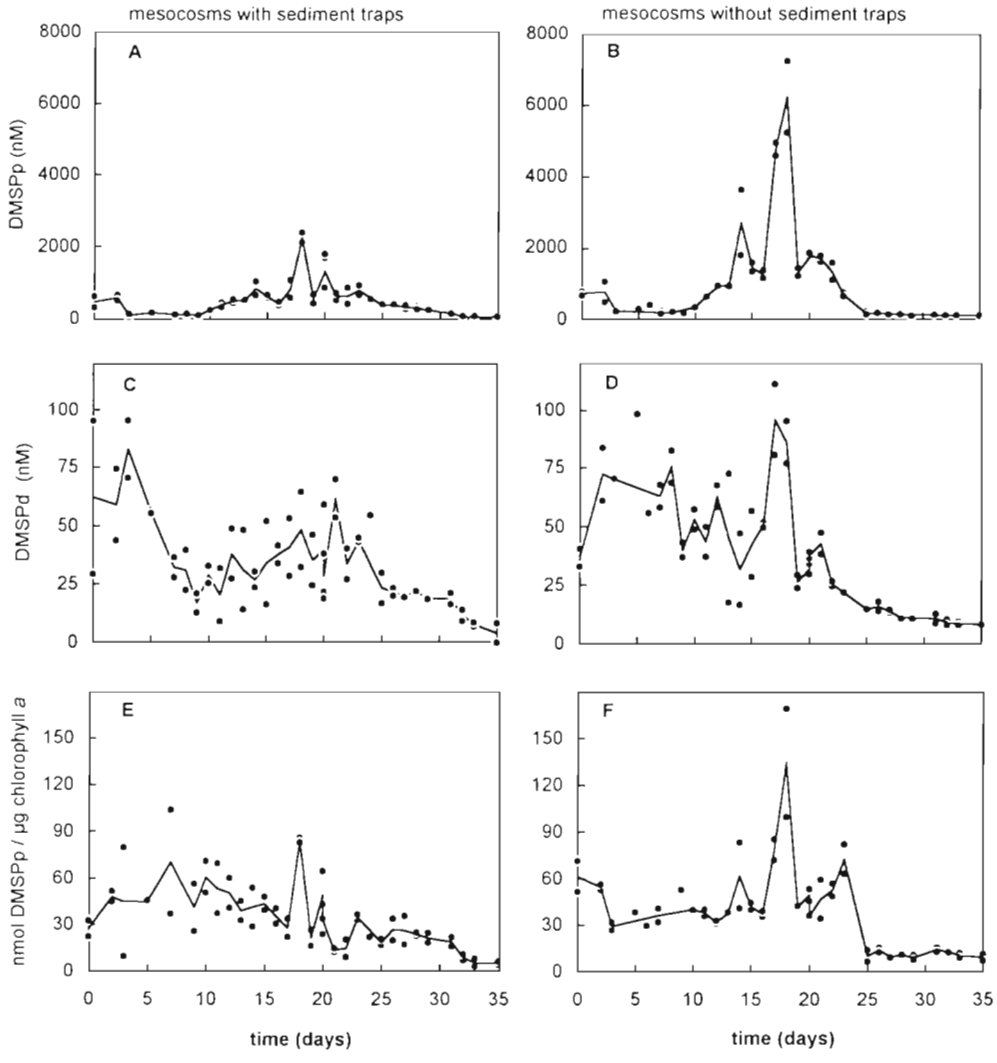

Fig. 3. DMSP concentrations and the $DMSP_p$/chl *a* ratio for the 4 mesocosms. (A), (C) and (E): mesocosms 1 and 2 with sediment traps; (B), (D) and (F): mesocosms 3 and 4 without sediment traps. (A) and (B): particulate DMSP concentration; (C) and (D): dissolved DMSP concentration; (E) and (F): $DMSP_p$/chl *a* ratio. Points show measurements and lines show averages between duplicate mesocosms

pers. comm.). From about Day 8 the DMS_{water} concentration reached its base level again (about 4 nM). In mesocosms 3 and 4 (without sediment traps), the DMS_{water} concentration started to rise at Day 14, reaching a value of approximately 400 nM at the same day of the maximal chl *a* concentration (Day 17), after which the DMS_{water} concentration decreased to a value of about 4 nM DMS at Day 24. The mesocosms with sediment traps (1 and 2) showed a much more rapid rise (Day 18 and 19) and fall (Days 20 to 23) in the concentration of DMS_{water}. The chl *a* peak was reached at Day 22. This substantial difference between mesocosms with and without traps remains to be explained. It is discussed in more detail in Osinga et al. 1996. Towards the end of the experiment the DMS_{water} concentration remained at a value of about 4 nM. From Fig. 2 it is evident that the second release of DMS started well before the *Phaeocystis* sp. bloom reached its maximum. This is consistent with our results obtained from the natural environment (Kwint & Kramer 1996) and the results of Stefels & Van Boekel (1993) who found the highest DMSP-lyase activity for exponentially growing cells of *Phaeocystis* sp. It is, however, in contrast to our findings reported before, for earlier mesocosm experiments carried out in spring and in autumn (Kwint & Kramer 1995). This apparent discrepancy will be discussed below.

Towards the climax of the bloom, the $DMSP_p$ concentration rose sharply to a value of about 2500 nM in mesocosms 1 and 2 (with sediment traps) and even up to 6500 nM in mesocosms 3 and 4 (without sediment traps) (Fig. 3A, B). This concentration was 20-fold higher than the DMS_{water} concentration. The DMSP concentrations found in this experiment were extremely high compared to what is usually found in the field. Kwint & Kramer (1996) reported maximum concentrations of $DMSP_p$ of about 1500 nM. Chl *a* concentrations were not unusually high, however (a maxi-

mum of about 60 µg l^{-1} in the mesocosms compared to 65 µg l^{-1} during the field monitoring). The plankton bloom in this experiment consisted of an almost monoculture of *Phaeocystis* sp., which was not the case in the field.

The $DMSP_p$/chl *a* ratio varied around a value of 50 to 60 nmol DMSP µg chl a^{-1} (Fig. 3E, F), with its highest values coinciding with the DMS_{water} maxima. In general the amount of $DMSP_p$ reflected the number of *Phaeocystis* sp. cells (Fig. 2). A maximum $DMSP_p$/chl *a* occurred at around Day 18, just before the end of the phytoplankton bloom, which agrees well with the results of Matrai & Keller (1993) who described a maximum DMSP content and release of DMS at the maximum of a bloom of *Emiliania huxleyi* in the Gulf of Maine.

Towards the end of our experiment where *Phaeocystis* sp. became less important, the $DMSP_p$/chl *a* ratio decreased to about 10 nmol μg^{-1}. These ratios are comparable to the DMSP/chl *a* ratios found under natural conditions in this area. During a *Phaeocystis* sp. bloom in the Marsdiep (The Netherlands) tidal inlet, Kwint & Kramer (1996) found a ratio of approximately 20 nmol DMSP µg chl a^{-1} that decreased to 12 nmol DMSP µg chl a^{-1} after the bloom had declined. We observed the general trend that a decrease in the $DMSP_p$ concentration coincides with an increase in the DMS_{water} concentration, although this is true only in a qualitative sense. The $DMSP_d$ concentration appeared to be highly variable during the entire experiment, although there was a trend of a decrease from 100 nM $DMSP_d$ at the start of the experiment to about 10 nM $DMSP_d$ at the end, with some elevation during the DMS_{water} peaks (Fig. 3C, D).

Interestingly, the increase in the DMS_{water} concentration following the decrease in $DMSP_p$ over the first 5 d represented about 50% of the $DMSP_p$ lost, while during the decline of the major bloom (Days 18 to 25) DMS accumulation accounted for only 5% of the $DMSP_p$ lost. The differences in the 2 periods cannot be accounted for by accumulation of $DMSP_d$ (Fig. 3C, D). This indicates either that in the first period a lesser turnover of DMS occurred, or that during the second bloom demethylation had a larger contribution to the degradation of DMSP than cleavage.

Maximum numbers of the copepod *Temora longicornis* occurred before the maximum of chl *a*, and corresponded with the onset of maximum DMS release into the water column (Fig. 4). Differences between mesocosms with and without sediment traps did occur. Mesocosms without sediment traps (Fig. 4B) had lower copepod numbers (ca 100 vs 150 μl^{-1}) and high numbers of rotifers and rotifer eggs were found in the first 2 wk of the experiment. The zooplankton may have stimulated $DMSP_d$ formation by feeding on the algal

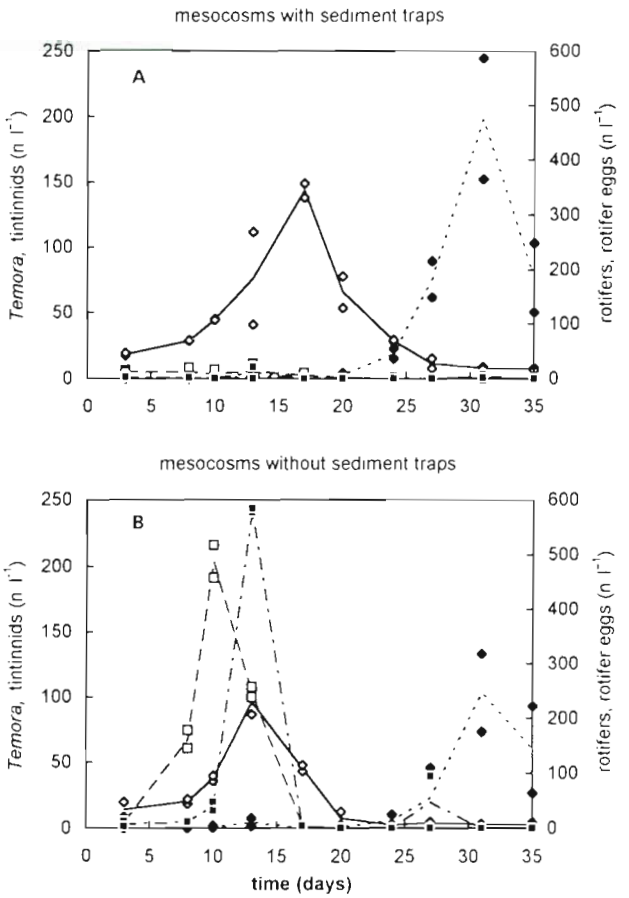

Fig. 4. Zooplankton numbers during the algal bloom: (◇) *Temora longicornis*, (◆) tintinnids, (□) rotifer eggs and (■) rotifers. (A) mesocosms 1 and 2 with sediment traps; (B) mesocosms 3 and 4 without sediment traps. Points show measurements and lines show averages between duplicate mesocosms. Note different scales

cells (Dacey & Wakeham 1986, Belviso et al. 1990, 1993, Hansen & Van Boekel 1991, Hansen et al. 1994). However, the maxima of the zooplankton densities appear to have no direct relation to the accumulation of $DMSP_d$ and DMS_{water}.

Our experiment shows that in the mesocosms used, replicable results can be obtained concerning the course of the algal bloom, the production of DMSP and the release of DMS into the water column. The differences in the results between mesocosms with and without sediment traps may be explained by different resuspension of sedimented material. Due to the rigid sediment traps, the water column in those mesocosms with traps may have been less well mixed than in those without. The result was that less material was mineralized in the water column, explaining a less pronounced phytoplankton bloom, as well as the difference in zooplankton composition and sequence.

Mesocosms are thus useful for studying the mechanisms controlling the turnover of DMSP and DMS. However, in mesocosm experiments the timescale of a phytoplankton bloom is compressed in time 2 to 3 times (Kuiper 1977). As the zooplankton bloom is restricted by the development of their different larval stages, the interrelations of phyto- and zooplankton can be seriously affected.

The total bacterial population density peaked twice during the mesocosm experiment, possibly due to the increase of particulate and dissolved organic matter originating from senescent algae (Fig. 5). The bacterial population had a base level of 2×10^9 cells l^{-1} and peaks of 8×10^9 and 6×10^9 cells l^{-1} at Days 16 and 26, respectively. The relatively moderate increase in bacterial numbers between base level and peaks (only 4-fold) was already pointed out by several authors for a broad range of pelagic environments (as reviewed by Thingstad 1987). They proposed a mechanism where a strict regulation of bacterial densities would occur caused by viruses or predators such as nanoflagellates in pelagic systems.

The DMS-utilizing bacteria, as determined by MPN method, constituted only a small fraction of the total bacterial population density (note the difference in scales in Fig. 5). The DMS-utilizing bacterial population started to grow shortly after the DMS_{water} maximum. It peaked only after the second DMS peak with a maximal density of 5×10^6 cells l^{-1} at Day 26. From laboratory studies (data not shown) we know that there is a long lag-phase for bacteria to switch to DMS utilisation. The delay in response can also be explained by the low growth rate of most marine heterotrophic strains (Thingstad 1987). The observation that there are only few DMS-utilizing bacteria during the first 5 d of the experiment may account for the relatively high DMS_{water} concentration in relation to the available $DMSP_p$.

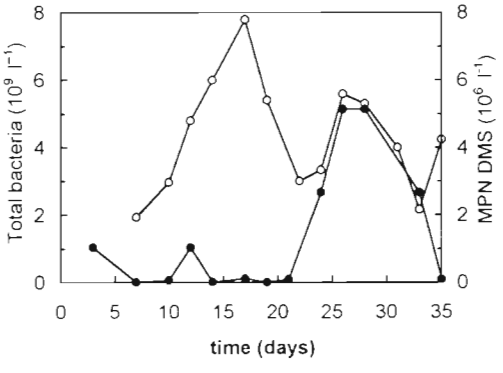

Fig. 5. Average bacterial numbers measured during the experiment in mesocosms 1 and 3. Total bacteria (o) and most probable number of DMS-utilizing bacteria (MPN DMS; ●). Note different scales

DMSP cleavage versus demethylation

In order to elucidate the role of DMSP-utilizing bacteria, subsamples collected from the mesocosms on 4 different days were incubated in the laboratory. The variables are summarized in Table 1. In addition to the $DMSP_p$ and $DMSP_d$ concentrations, the estimated DMSP conversion rates are presented. The turnover rates (V) are subdivided into turnover by cleavage and demethylation. Finally, the turnover time (τ, in d) was given by the ratio of concentration to turnover rate. A high number thus indicates a slow turnover.

It appears that at Day 7, where a rather low concentration of DMSP is available (and possibly also a low number of DMSP-utilizing bacteria), the process of demethylation was the most important.

It was assumed that no increase in $DMSP_p$ would occur in the dark during the incubation experiments. This assumption was found to be not completely true, however. In some experiments an increase was detected. Quantification was not possible as the increase was the net result of production and consumption. The increase in $DMSP_p$ may be due to intracellular accumulation of $DMSP_d$ by bacteria (Ledyard & Dacey 1994), or to the actual production of $DMSP_p$ by algae in the dark (J Stefels pers. comm.). As a result, the net decrease in $DMSP_d$ can be underestimated. The turnover of $DMSP_d$ by demethylation was calculated by subtracting the net increase in DMS from the net $DMSP_d$ decrease, leading to an underestimation of the importance of demethylation, also because some DMS may have been consumed during the incubation. Due to the large headspace in the incubations, up to 28% of the DMS formed during the incubation may have been lost on sampling a bottle. As a different bottle was used for each point on the incubation curves, the results were not influenced in a relative sense.

Table 1. DMSP concentrations ([$DMSP_p$] and [$DMSP_d$]), DMSP conversion rates (Δ $DMSP_p$ and Δ $DMSP_d$), turnover rates by cleavage ($V_{cleavage}$) and demethylation ($V_{demethylation}$), turnover time by cleavage (τ_{Cl}) and demethylation (τ_{Dm}) and the ratio τ_{Cl}/τ_{Dm}

Parameter	Unit	Day 7	Day 24	Day 31
[$DMSP_p$]	nM	5.2	501.8	167.1
[$DMSP_d$]	nM	25.9	54.9	21.4
Δ $DMSP_p$	nM d^{-1}	39.0	−795.0	682.0
Δ $DMSP_d$	nM d^{-1}	−35.0	−1529.0	29.0
$V_{cleavage}$	nM d^{-1}	0.0	21.0	47.0
$V_{demethylation}$	nM d^{-1}	35.0	1508.0	0
τ_{Cl}	d	∞	2.6	0.5
τ_{Dm}	d	0.8	0	∞
τ_{Cl}/τ_{Dm}	–	∞	70.0	0

The DMSP-degrading population consisted of a variety of bacteria, using either of the 2 routes shown in Fig. 1. The inoculation of DMSP agarose plates with subsampled seawater from the mesocosms resulted in a broad spectrum of morphologically different, colony types. Some of them (less than 5%) were agarolytic, but most of them were found to be positive for DMSP utilization. Fourteen different strains were non-randomly selected (based on colony types) and characterized. Of these strains, 6 were capable of DMSP cleavage (P. Quist unpubl.).

Turnover of DMS

A comparison was made between the (calculated) flux of DMS to the atmosphere and the degradation of DMS by bacteria (Table 2). The flux was calculated from temperature, windspeed and the DMS_{water} concentration. The bacterial production and consumption followed from the incubation experiments for the 4 d of measurements. As before, τ represents the turnover time, τ_{bio} for biological consumption and τ_{atm} for atmospheric flux. When the ratio τ_{atm}/τ_{bio} is larger than 1, it reflects the dominance of bacterial degradation over flux to the atmosphere as a sink for DMS. It appears that for all 4 d for which there was data, the bacterial breakdown is by far the most important process, usually by a factor of 10 or more. Only at the end of the mesocosm experiment (Day 31), did both processes become comparable. The bacterial breakdown was still, by a factor of 3, more important than the flux to the atmosphere. In field experiments, Kiene & Bates (1990) found a similar 10% atmospheric flux for a system with a low DMS_{water} concentration (1 to 10 nM). Our results may be influenced by the fact that chloroform did not always totally inhibit the biological DMS consumption. In samples with a high algal biomass content, the chloroform appeared to be less effective than in samples with low algal biomass, so these data were not used. The exact cause for this is not yet clear. After the completion of these mesocosm experiments, Visscher & Taylor (1993) reported a new mechanism for the bacterial breakdown of DMS which was not sensitive to inhibition by chloroform. Wolfe & Kiene (1993) showed that in some cases chloroform may cause a release of $DMSP_d$ and stimulate DMS production leading to an overestimation of the DMS consumption by a factor of about 2. In our experiments, the estimated bacterial DMS consumption was a factor 10 or more important than the flux to the atmosphere, so we can still conclude that bacterial degradation, rather than atmospheric flux, is a main sink for DMS.

Because of the direct relation between the flux and the DMS_{water} concentration in our experiment, the sea-air exchange of DMS may be heavily affected by the short periods of high DMS_{water} concentration. To expand our findings to periods of high DMS concentration, the daily fluxes to the atmosphere were calculated for the entire period of the experiment. In Fig 6 the flux of DMS is compared to the total amount of DMS present in the mesocosm. A decrease in the total amount of DMS is the result of flux to the atmosphere and bacterial degradation and/or photooxidation. It is not possible to comment on the importance of the

Table 2. Temperature, windspeed, DMS concentrations, rates of bacterial production and consumption, biological turnover time (τ_{bio}), turnover time by efflux of DMS (τ_{atm}) and the ratio τ_{atm}/τ_{bio}

Parameter	Unit	Day			
		7	10	24	31
Temperature	°C	10.2	12.2	13.6	13.6
Windspeed	$m\ s^{-1}$	5.0	4.5	4.5	7.0
[DMS]	nM	64.7	6.0	13.4	8.0
Bacterial production	$nM\ d^{-1}$	0	26.0	21.0	47.0
Bacterial consumption	$nM\ d^{-1}$	178.0	26.0	28.0	14.0
τ_{bio}	d	0.4	0.2	0.5	0.6
Flux	$\mu mol\ m^{-2}\ d^{-1}$	61.1	4.3	9.4	15.5
τ_{atm}	d	3.6	4.8	4.9	1.8
τ_{atm}/τ_{bio}	-	10.1	21.0	10.0	3.1

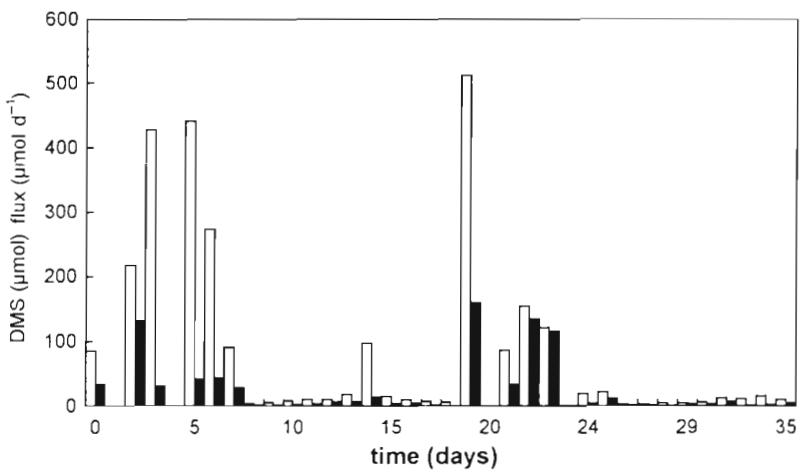

Fig. 6. Calculated total flux of DMS (black bars) from mesocosm 1 and the total amount of DMS (open bars) present in mesocosm 1

atmospheric flux in periods with a high production of DMS, as the DMS_{water} concentration is the net result of production and degradation/efflux. However, in situations where a strong decrease in the DMS concentration is observed, the calculated flux can only be responsible to a minor extent for the decrease in DMS_{water}. During Days 5 to 8 the flux to the atmosphere was very small compared to the amount of DMS that disappeared from the mesocosm. For Days 19 to 24 the flux to the atmosphere appeared to be more important, but the bacterial consumption still remains the dominant sink for DMS (compare Day 24 in Table 2 and Fig. 6).

In order to investigate whether the bacterial conversion rates are realistic, a comparison was made with data obtained from cultures of bacterial strains as documented in the literature. In continuous culture experiments, a specific conversion rate of 100 µmol DMS mg protein^{-1} d^{-1} was found for *Thiobacillus thioparus* (Visscher et al. 1991).

From a bacterial strain isolated from the epidermal surface of *Ulva lactuca*, conversion rates in the order of 36 and 27 µmol DMS mg protein^{-1} d^{-1} were measured in continuous culture growth experiments and by monitoring the oxygen consumption in batch cultures, respectively (P. Quist unpubl.). In our experiments described here, a specific conversion rate on the order of 10 to 1000 µmol DMS mg protein^{-1} d^{-1} was found by combining the population density of 5×10^6 cells l^{-1} with the bacterial turnover of DMS (assuming 20 fg protein per cell). As MPNs are most likely an underestimation of the true DMS-utilizing population (due to the selectivity of the medium), the specific uptake rates may be lower under field conditions.

Conclusion

Our data show that DMS release or production is not necessarily associated with the senescent phase of a phytoplankton bloom as found by Kwint & Kramer (1995). The first DMS_{water} peak coincided with the senescent phase of the algae, while the second DMS peak corresponded to a maximum in *Phaeocystis* sp. cells per litre. Most probably the first DMS peak was an artefact of the experiment caused by the settlement and degradation of algae. During this period $DMSP_d$ became quickly available and its degradation led to a build up of DMS.

Data on the flux of DMS to the atmosphere, bacterial DMS consumption rates and DMSP turnover show that bacterial consumption is the main sink for DMS even under conditions of high DMS_{water} concentrations present during an algal bloom. We found that during a phytoplankton bloom an increasing part of the DMSP produced is not metabolized to DMS at all, but that the alternative pathway involving DMSP demethylation (Fig. 1) is more important. During the initial phase of a phytoplankton bloom, DMSP may be converted to DMS with high efficiency.

Our results suggest that DMS and DMSP are rapidly cycled in the water column due to bacterial activity and that the flux of DMS to the atmosphere is highly dependent on factors other than the production of cellular DMSP by phytoplankton alone.

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