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## Production of dimethylsulfonium propionate (DMSP) and dimethylsulfide (DMS) by a microbial food web

**Abstract**—Production and decomposition of DMSP and DMS in a microbial food web were investigated by means of a 5-d incubation of pre-screened seawater samples (<1, <10, <100  $\mu\text{m}$ ) from northwestern Mediterranean coastal waters. The major goal of size fractionation was to create predator-free compartments. Aplastidic flagellates, plastidic nanoflagellates (<10  $\mu\text{m}$ ), or both contributed to the particulate DMSP pool, but DMSP was found to be predominantly associated with the populations of small dinoflagellates (~20  $\mu\text{m}$ , *Prorocentrum* sp., *Gymnodinium* sp.). Particulate-DMSP values and free-cell counts in the <100- $\mu\text{m}$  fraction, however, were not significantly correlated. This result reflects the grazing pressure of ciliates on the DMSP carriers since significant amounts of DMSP can be stored temporarily by the microzooplanktonic predators. The initial level of soluble DMSP was 12% of the total DMSP pool of seawater and less than 2% at the end of the experiment. This result suggests that free DMSP is not produced in significant amounts by the predator-prey interactions in the microbial food web. Conversely, DMS was shown to be excreted following ciliate grazing on dinoflagellates and DMSP decomposition. Mass balance between DMSP and DMS was achieved after a time lag of ~1.5 d. We suggest that DMSP decomposition into DMS in the marine environment can occur through microplanktonic catabolism by two pathways: enzymatic cleavage and formation of an intermediate compound after demethylation. Overall, our work indicates that microzooplankton grazing is significant in DMS release in open-ocean, surface waters.

There is considerable evidence that dimethylsulfide, DMS, is present ubiquitously in surface oceanic and nearshore wa-

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ters (Andreae 1986; Bates et al. 1987 and references herein; Turner et al. 1988) and has a major biogenic precursor in the form of dimethylsulfonium propionate (DMSP; Ishida 1968; Vairavamurthy et al. 1985; Turner et al. 1988; Keller et al. 1989). In the marine atmosphere the photochemical oxidation of DMS released from the ocean surface leads to the production of submicrometer sulfate aerosol particles which, acting as a source of cloud condensation nuclei, can affect the atmospheric radiation balance (Nguyen et al. 1983; Charlson et al. 1987). Because the oceanic DMS source could be involved in long-term climate control (Legrand et al. 1988), the parameterization of the production and decomposition of DMSP and DMS in the marine environment is of major concern. The factors controlling the production of these compounds, however, are still not well understood.

Up to now, three biological processes have been identified and found to be able to contribute significantly to DMS release into marine waters from planktonic DMSP standing stocks, i.e. metabolic excretion following phytoplankton growth (Vairavamurthy et al. 1985), phytoplankton grazing by zooplankton (Dacey and Wakeham 1986), and senescence of the microalgae (Nguyen et al. 1988). Nevertheless, the significance of these mechanisms on the marine DMS cycle is questionable. Indeed, they have been inferred separately either from laboratory experiments or with the help of excessively simplified planktonic food chains: batch cultures of monospecific planktonic populations (phyto and zooplankters), plankton densities several times higher than those measured at sea, or enhanced amounts of nutrients.

Because revised conceptual models of pelagic food webs are emerging (Laval-Peuto et al. 1986; Sherr and Sherr 1988), it becomes necessary to consider that mi-

crobes play a major role in primary production and in supplying food resources for metazoans in oligotrophic ocean waters and coastal waters. This work is a first attempt to investigate the production mechanisms of DMSP and DMS with the help of field observations of the major trophic interactions, in conjunction with experimental approaches designed to study the interactions between microbial food webs and sulfur compounds. Here we present data on the particle size distribution of DMSP and the production rates of DMSP and DMS in the microbial food web up to 100  $\mu\text{m}$ . We used a differential filtration method and subsequent dialysis-cage incubation techniques.

The experiments were carried out at the Station Zoologique at Villefranche-sur-mer in mid-June 1988. The experimental approach was that of Rassoulzadegan and Sheldon (1986), modified to maintain microbial in situ interactive feedback as much as possible. Population interactions between microplankton, nanoplankton, and bacteria were investigated by means of long (5 d) incubations of prescreened seawater samples. Northwestern Mediterranean nearshore surface seawater (Bay of Villefranche-sur-mer) was collected with buckets. In order to create predator-free compartments, we used a size fractionation method based on gravity filtration through large Nuclepore membranes (Sheldon and Rassoulzadegan 1987). The particulate material was fractionated into three size classes:  $<1$ ,  $<10$ , and  $<100$   $\mu\text{m}$ . Fourteen subsamples of the respective filtrates were stored in 1-liter Plexiglas cylinders equipped with dialysis membranes on the top. The cylinders were put into a 1- $\text{m}^3$  capacity translucent seawater tank placed in a shaded part of the garden of the Station Zoologique. The content of the tank was continuously recycled with fresh seawater pumped from 25 cm below the surface of the Bay of Villefranche-sur-mer. The flow rate of 80 liters  $\text{h}^{-1}$  assures thermal stability.

The dialysis membranes were used to provide a continuous supply of seawater nutrients to the microplanktonic food web during incubation. This procedure avoids

problems associated with the use of batch cultures over several days. Dialysis membranes were autoclaved and stored with deionized water before their use. We checked that the dialysis membranes did not release DMS and thus did not contaminate the samples. A sulfur compound that could not be identified or quantified was indeed released. Chromatograms suggest that it is a light sulfur compound, possibly  $\text{CS}_2$ .

Enumeration of pico- and microplanktonic organisms was performed following Rassoulzadegan and Sheldon (1986). After enumeration of pico-microplankton, cell numbers were converted to ppm by volume,  $\text{No. ml}^{-1} \times \text{cell volume} (\mu\text{m}^3)$ , with the measured respective cell volumes (heterotrophic bacteria, 0.04  $\mu\text{m}^3$ ; cyanobacteria, 0.2  $\mu\text{m}^3$ ; picoflagellates, 2.1  $\mu\text{m}^3$ ; nanoflagellates 65.4  $\mu\text{m}^3$ ; oligotrichous ciliates  $<30$   $\mu\text{m}$ ,  $1.5 \times 10^3$   $\mu\text{m}^3$ ; oligotrichous ciliates  $30$   $\mu\text{m} < \text{size} < 50$   $\mu\text{m}$ ,  $4.7 \times 10^3$   $\mu\text{m}^3$ ). The C equivalences were made on geometrically calculated wet weight of organisms, using conversion coefficients of 0.2 for bacteria and cyanobacteria, 0.15 for nanoflagellates, and 0.08 for ciliates and microplankton (Sheldon and Rassoulzadegan 1987).

Analysis of DMS in seawater was performed with the technique of Belviso et al. (1987) for carbonyl sulfide in rainwater. This method has already been applied to the measurement of DMS production during natural phytoplankton blooms (Nguyen et al. 1988). About 60 ml of seawater were collected from a cylinder, transferred into Pyrex-stoppered glassware, and immediately purged with high-grade He. DMS was trapped cryogenically with Tenax GC (Alltech) and subsequently analyzed by gas chromatography and a flame photometric detector. The DMS calibration was modified to improve accuracy. The permeation device was replaced by liquid DMS standards prepared by dissolving liquid DMS in degassed ethylene glycol, following the method of Andreae and Barnard (1983). Because of the relatively high amounts of DMS in the Villefranche-sur-mer area, we did not improve GC performances to measure DMS levels below the detection limit

of 0.2 nM (nM = nmol liter<sup>-1</sup>). Total errors ( $\pm 1$  SD) in the analysis of DMS are estimated to be  $\pm 7\%$ .

Samples were analyzed for DMS without prior filtration. Our experience has been that filtration does not significantly alter DMS levels. We collected 10 water samples from the bay, filtered them on GF/F filters, and compared the results of the DMS levels of unfiltered samples. The unfiltered DMS concentration has been calculated as a percentage of the concentration measured after filtration. The values fall in the range of 96–110%. Recent results obtained on larger data sets (North Sea water samples,  $n = 174$ ) have led to a similar conclusion (S. Turner pers. comm.).

Samples were analyzed for DMSP after cold alkali treatment following a method modified from Turner et al. (1988). Kinetics of hydroxide decomposition forming DMS have been investigated in detail by Dacey and Blough (1987); 1 ml of 15 M NaOH was added to 60 ml of unfiltered and filtered (Whatman GF/F glass-fiber; effective retention, 0.7  $\mu\text{m}$ ) seawater. The samples were then stored in the dark at +4°C for 12 h to allow 100% recovery of DMSP into DMS. Standard amounts of DMSPBr<sup>-</sup> (Ircha) and replicated water samples were used. The DMSP concentration was calculated by subtracting the initial DMS content of the sample from the total DMS amount after cold alkali treatment. Total errors in the analysis of DMSP are estimated to range from  $\pm 10$  to  $\pm 15\%$ . Fractionation of total DMSP into particulate and dissolved DMSP was carried out in the 10- and 100- $\mu\text{m}$  fraction at the end of the incubation experiment only. The DMSP content of the <1- $\mu\text{m}$  filtrate was used to estimate the soluble DMSP pool (free DMSP) of the seawater.

Dissolved dimethylsulfide, total DMSP, and cell numbers were measured every 4 h during the first day, then twice daily for 4 d at 0800 and 2000 hours, respectively.

At time  $t_0$ , the DMSP amount of the <10- $\mu\text{m}$  fraction (22.8 nM) was roughly equal to a third of the DMSP level of the <100- $\mu\text{m}$  fraction (65.5 nM, Fig. 1a). Although the DMSP content of the 10- $\mu\text{m}$  filtrate markedly increased from 18.7 to 48.7 nM

during the period from  $t = 36$  to  $t = 84$  h, no corresponding positive trend was observed for the 100- $\mu\text{m}$  fraction. A significant departure from the DMSP background in the 100- $\mu\text{m}$  fraction ( $67 \pm 14$  nM;  $\pm 14$  corresponds to 2 SD) is quite noticeable between  $t = 14$  h and  $t = 16$  h when DMSP reaches values of 82 and 90 nM.

A significant decreasing DMS trend was observed for the 10- $\mu\text{m}$  filtrate during the first 72 h, parallel to that of the 1- $\mu\text{m}$  fraction (Fig. 1b). Then, DMS increased slightly from 11.2 to 14.1 nM at the end of the incubation, whereas the DMS content of the 100- $\mu\text{m}$  fraction markedly increased from 16.2 nM 12 h after the beginning of the experiment to 30.7 nM 48 h later.

In 10- $\mu\text{m}$  screened water (Fig. 2), populations of cyanobacteria remained stable for the first day, then declined dramatically. Populations of heterotrophic bacteria grew gradually during the first 2 d. During this period, the biomass and growth patterns of bacteria were similar to that in 1- $\mu\text{m}$  screened water. In this fraction bacterial populations still grew gradually during the last 2.5 d; in the <10- $\mu\text{m}$  fraction, conversely, bacterial biomasses declined markedly until the end of the experiment (Fig. 2a). Aplastidic flagellates of unidentified species grew exponentially during the first 3.5 d, then declined markedly for the last 24 h. The data suggest that the populations of cyanobacteria and heterotrophic bacteria were mainly controlled by the aplastidic flagellates. The two populations of plastidic flagellates reached maximal biomasses 24 h (picoflagellates of unidentified species) and 108 h (nanoflagellates, unidentified prymnesiophytes) after the onset of the experiment (Fig. 2b).

In the <100- $\mu\text{m}$  fraction (Fig. 3), we have not reported the data on bacterial and cyanobacterial populations because as will be shown later they do not significantly influence DMS and DMSP levels either in the <10- $\mu\text{m}$  or in the <100- $\mu\text{m}$  fraction. Furthermore, we found that bacterial biomass (2.63–6.97  $\mu\text{g C liter}^{-1}$ ) in the <100- $\mu\text{m}$  fraction was lower than in the <10- $\mu\text{m}$  fraction (2.65–12.96  $\mu\text{g C liter}^{-1}$ ).

In the 100- $\mu\text{m}$  screened water, the dinoflagellate populations ( $\sim 20$   $\mu\text{m}$ , *Proro-*

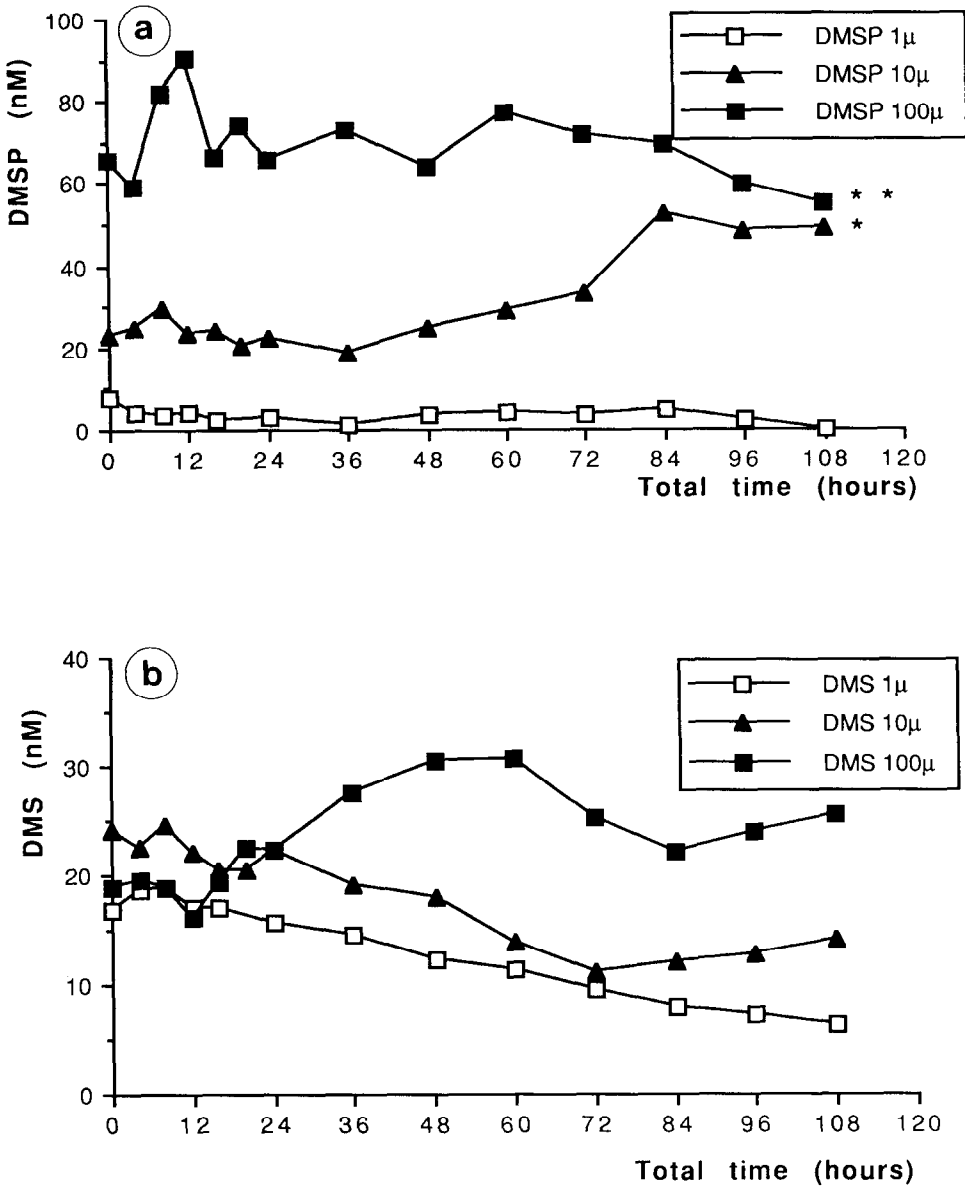


Fig. 1. DMSP and DMS trends of the <1-, <10-, and <100- $\mu$ m fractions. Units are nM. Asterisk—no free DMSP; double asterisk—free DMSP = 0.7 nM.

*centrum* sp., *Gymnodinium* sp. predominantly) declined dramatically for the first 24 h, remained stable the 2 d following, then grew slowly, and finally declined at the end of the experiment. The plastidic picoflagellates reached a density comparable to that of the first population in 10- $\mu$ m screened water, but the plastidic nanoflagellates apparently did not grow or were

efficiently grazed. Aplastidic flagellates followed a comparable trend for the first 2 d, then their biomass fluctuated up to a level of 50% of that of the <10- $\mu$ m filtrate. Populations of diatoms remained stable for the first 1.5 d, grew steeply for 2 d, and then declined on the last day. The ciliates were composed of oligotrichs, predominantly in the size range 30–50  $\mu$ m, tintinnids, and

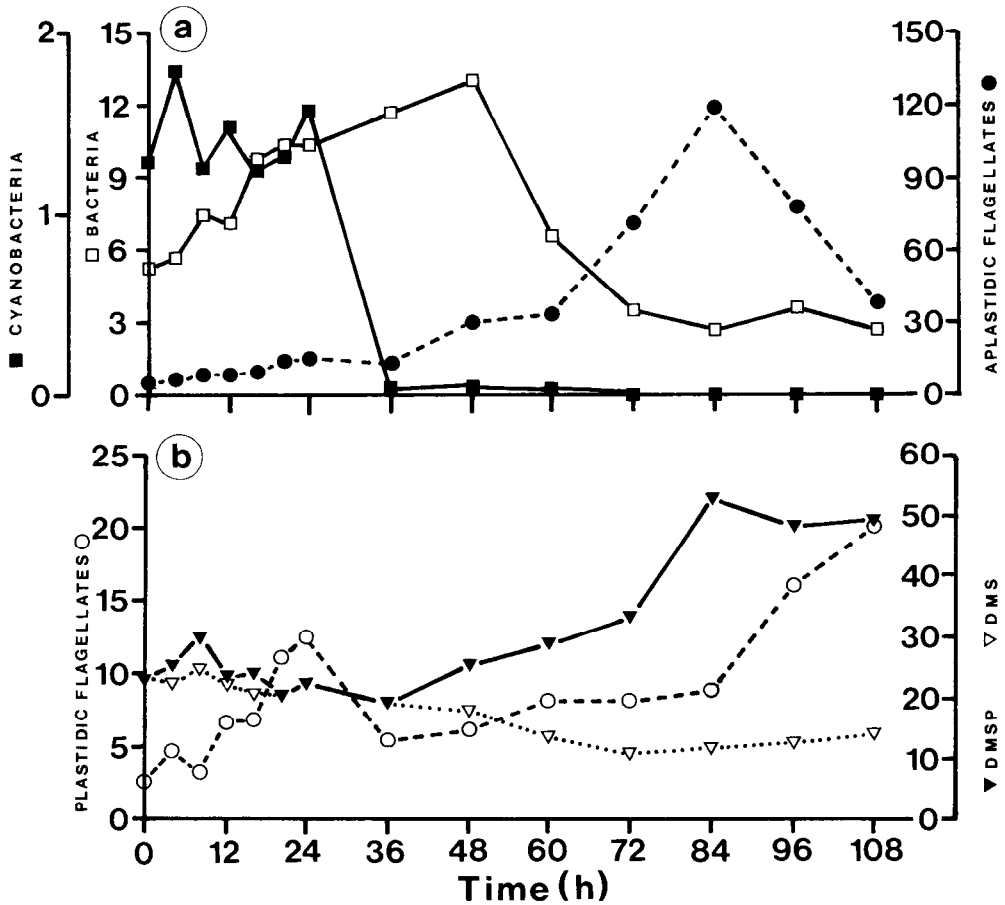


Fig. 2. Time series of the standing stocks of the microbial planktonic populations (bacteria, cyanobacteria, aplastidic and plastidic flagellates) and of the sulfur compounds (DMSP, DMS) in the  $<10\text{-}\mu\text{m}$  filtrate. Units are nM and  $\mu\text{g C liter}^{-1}$ .

some rare oligotrichs of  $<30\ \mu\text{m}$ . The ciliate population grew rather steadily until the end of the experiment. The observed temporal evolution suggests that ciliates were controlling the development of dinoflagellates and aplastidic and plastidic flagellates by predation.

It is well known that dialysis depends on the differential transport of solutes of different sizes across a porous barrier separating two liquids, with the driving force a concentration gradient. Free DMSP and DMS are low molecular weight compounds and, consequently, are dialyzable. Therefore, the free DMSP and DMS levels in the cages can change as a function of the temporal evolution of the external levels. Before interpreting our data base in terms of

DMSP and DMS production and decomposition, we needed to know how the exchanges through the membranes could have contributed to the temporal evolution of DMSP and DMS in the cages. The results must be interpreted cautiously because the experiment was not replicated.

At time  $t_0$ , the free DMSP ( $<1\text{-}\mu\text{m}$  fraction: 7.8 nM) contribution to the total DMSP pool ( $<100\text{-}\mu\text{m}$  fraction: 65.5 nM) was 12% (Fig. 1a). In this size fraction, free DMSP varied from 7.8 nM to a value below the detection limit of 0.2 nM at the end of the experiment. On the assumption that this range of values reflects the maximal external levels of free DMSP, the free DMSP content of seawater cannot account for the much higher DMSP concentrations

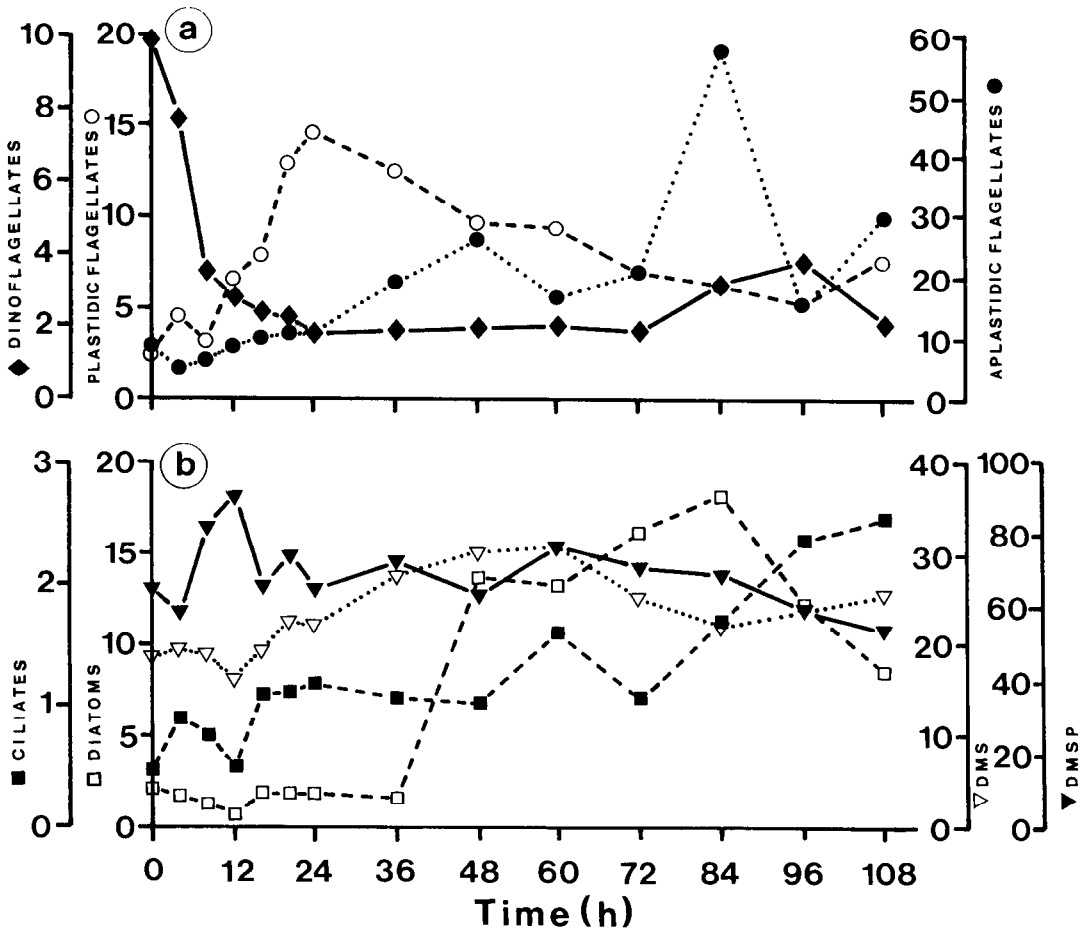


Fig. 3. Time series of the standing stocks of the microbial planktonic populations (aplastidic and plastidic flagellates, dinoflagellates, diatoms, and ciliates) and of the sulfur compounds (DMSP, DMS) in the  $<100\text{-}\mu\text{m}$  filtrate. Units are nM and  $\mu\text{g C liter}^{-1}$ .

found in the 10- and 100- $\mu\text{m}$  fractions. At the end of the experiment ( $t_0 + 108$  h), we checked that the levels of free DMSP in the 10- and 100- $\mu\text{m}$  fractions were very low (below detection limit and 0.7 nM respectively) while DMSP levels were 48.7 and 54.7 nM, respectively. Consequently, we believe that the DMSP present in the 10- and 100- $\mu\text{m}$  fractions is mainly particulate DMSP that cannot exit the cages. Some minor amounts of free DMSP produced in the cages during the incubation, however, could have been lost through the dialysis membranes.

At present, there are still few determinations of DMSP levels in marine waters. We measured 65 nM ( $2,080$  ng S  $\text{liter}^{-1}$ ) in

the Bay of Villefrance-sur-mer, a value comparable with those reported by Turner et al. (1988) in the English Channel (up to 5,500 ng S  $\text{liter}^{-1}$ ), by Wakeham et al. (1987) in a coastal pond (up to 80 nM), by Iverson et al. (1989) in Delaware Bay (up to 150 nM), and by Gibson et al. (1990) in Antarctic coastal waters (10–100 nM during austral spring). These levels are certainly higher than in the open marine environment. Lee and Wakeham (1987) found 5 nM of DMSP at the sea surface in the Cariaco Trench region and Iverson et al. (1989) found DMSP surface concentrations ranging from 15 to 40 nM in the North Atlantic Ocean.

Obviously, the cyanobacteria and the

heterotrophic bacteria populations do not contribute significantly to the 10  $\mu\text{m}$  DMSP pool since the DMSP levels were maximal ( $\sim 50$  nM) when their biomasses were minimal (Fig. 2a, b). Thus, in this size fraction the populations of plastidic nanoflagellates and aplastidic flagellates should contribute to the enhanced particulate DMSP pool observed toward the end of the experiment. Indeed, in the  $<10\text{-}\mu\text{m}$  fraction, the growth trends of the plastidic nanoflagellates, aplastidic flagellates, and DMSP were clearly positive. The level of particulate DMSP found at the end of the experiment was equal to 48.7 nM. Since the total biomass of flagellates was equal to 58.1  $\mu\text{g C liter}^{-1}$  at that time, we estimate that the contribution of flagellates to the DMSP pool is at least equal to 0.8 nmol DMSP ( $\mu\text{g C}^{-1}$ ) (i.e. 125 mmol liter $^{-1}$  of cell volume). There is a moderately strong correlation between DMSP and the individual populations of flagellates (aplastidic flagellates:  $r^2 = 0.69$ ,  $n = 14$ ,  $P < 0.001$ ; plastidic nanoflagellates:  $r^2 = 0.55$ ,  $n = 7$ ,  $P = 0.06$ ). Because of these weak correlations and the small sample sizes, the calculation of a precise representative ratio between DMSP and cell-specific C is unrealistic. The best fit, however, has been found with the aplastidic flagellates. These strictly heterotrophic organisms appear to be able to produce DMSP like other heterotrophic marine algae do. For example, Ishida (1968) reported that large amounts of DMSP evolved from the heterotrophic growth of the dinoflagellate *Gyrodinium cohnii*.

At time  $t_0$ , we have shown that the amount of DMSP in the  $<10\text{-}\mu\text{m}$  fraction (22.8 nM) was roughly equal to a third of the DMSP level of the  $<100\text{-}\mu\text{m}$  fraction (65.5 nM, Fig. 1a). Thus, at the onset of the experiment, DMSP was predominantly in particles of the 10–100- $\mu\text{m}$  size range. The DMSP values found in the  $<100\text{-}\mu\text{m}$  fraction did not covary with any specific planktonic biomass (Fig. 3a,b). It seems clear that the diatoms, the aplastidic flagellates, the plastidic flagellates, and the ciliates (oligotrichous ciliates and tintinnids) were minor contributors because their biomass increased by factors of 5 (ciliates and plastidic flagellates) to 20 (diatoms) with-

out a corresponding DMSP increase. Consequently, dinoflagellates would be initially the major contributors to the particulate DMSP pool present in this size fraction. We have assumed that the amount of DMSP in dinoflagellates at time  $t_0$  can be calculated as the difference between the DMSP found in the  $<100\text{-}\mu\text{m}$  fraction (65.5 nM) and the DMSP found in the  $<10\text{-}\mu\text{m}$  fraction (22.8 nM). Consequently, the total biomass of dinoflagellates (9.88  $\mu\text{g C liter}^{-1}$ , at time  $t_0$ ) has a DMSP:C ratio of about 4.3 nmol ( $\mu\text{g C}^{-1}$ ) (i.e. 640 mM cell volume).

The biomass of dinoflagellates decreased with time, however, whereas DMSP remained stable or surprisingly increased between 4 and 12 h. Grazing by ciliates during the first 24 h lowered the biomass of dinoflagellates by 8.08  $\mu\text{g C}$  (Fig. 3b). If we use the appropriate DMSP:C ratio, this decrease would correspond to an ingested DMSP amount of  $\sim 35$  nmol. Since we observed, during this period, a significant production of about 20 nmol of DMSP, concomitant with the decrease of the dinoflagellate biomass, we conclude that production of DMSP can take place during the digestion of organic matter. This amount of DMSP,  $\sim 60\%$  of the ingested amount, could originate from the metabolic decomposition of some precursors of DMSP (methionine-derived compounds possibly).

There is, however, an alternative interpretation of the result. During grazing of the dinoflagellates there could exist free cells still able to produce DMSP. Because our microscopic observations did not reveal that a dinoflagellate population increased relative to other dinoflagellates during the first 12 h of incubation, it is most probable that the amount of 20 nM of DMSP produced through this alternative process has been ingested by the ciliates. Overall, we estimate that about 55 nmol of DMSP could have been transferred through dinoflagellate grazing and temporarily stored in the food vacuoles of the ciliates.

Aplastidic flagellates, plastidic nanoflagellates, and dinoflagellates are the planktonic organisms identified here as significant producers of DMSP. Our results, as from other laboratory and field studies



(Turner et al. 1988; Keller et al. 1989), suggest that DMSP is an important metabolite for dinoflagellates and flagellates because levels of DMSP (cell volume)<sup>-1</sup> reach hundreds of mM. Our observations also reveal that when microplanktonic grazers are associated with phytoplankton, as in many marine environments, a significant fraction of the particulate DMSP content of seawater can be temporarily associated with microbial communities that are not the original producers of DMSP.

Before interpreting DMS data it is necessary to examine the possible artifacts deriving from the experimental protocols. Did the unidentified sulfur compound (*X*) deriving from the dialysis membranes chemically interact with DMS? If chemical interactions of major importance had occurred between *X* and DMS, i.e. *X* being able to control the temporal evolution of DMS by removing it from seawater, we should have observed similar temporal trends for the three types of filtrates because the same type of membranes of the same sizes have been used in all cases and the concentration of *X* always appeared to be in excess to that of DMS. The temporal trends of DMS were distinctly different (Fig. 1b). Thus, our data do not support the hypothesis of significant chemical interactions between compounds derived from the membranes and DMS.

During the first 12 h, the means of the DMS concentrations in the 1- and 100- $\mu$ m filtrates were not significantly different,  $17.7 \pm 2.1$  ( $\pm 2$  SD) and  $18.3 \pm 3.0$  nM, respectively. The DMS trend in the <1- $\mu$ m fraction is significantly negative. The values decreased linearly from 16.7 (at  $t_0$ ) to 6.5 nM after 108 h, at a rate of  $0.12$  nM  $h^{-1}$  ( $r^2 = 0.97$ ,  $n = 13$ ,  $P < 0.001$ ). Assuming that this range of values reflects the DMS content of external seawater, it cannot then account for the higher DMS concentrations found in the <100- $\mu$ m filtrate. Consequently, the significant DMS increase occurring in the 100- $\mu$ m fraction (Fig. 1b) is certainly the result of production taking place in the cages and cannot originate from a prefiltration artifact.

On the other hand, the mean DMS concentration in the 10- $\mu$ m fraction was

$23.3 \pm 2.4$  nM at the beginning of the experiment—significantly higher than we measured in the 1- and 100- $\mu$ m filtrates. This difference was most probably the result of DMS release via dinoflagellate cell lysis on filtration. Thus, the interpretation of the DMS data from the <10- $\mu$ m filtrate is ambiguous. This DMS release was found to be reproducible, however. Consequently, we believe that this artifact does not significantly influence the temporal evolution of DMS in the 10- $\mu$ m filtrate.

Our results clearly suggest that significant DMS production takes place only in the 100- $\mu$ m filtrate. The total amount of DMS produced in this size fraction has been calculated over every 12-h interval as follows:

$$\text{DMS production} = \text{DMS}_{t+\Delta t} - (\text{DMS}_t - 0.12 \Delta t) \quad (1)$$

where  $\Delta t = 12$  h and  $0.12$  nM  $h^{-1}$  is the slope of the DMS loss through the dialysis membranes as obtained from the results of the <1- $\mu$ m fraction. This corrective term minimizes the DMS production because the DMS loss through the membranes is proportional to the DMS concentration in the cage.

Our experiment showed that the major increase of DMS concentration occurred in the 100- $\mu$ m fraction between 12 and 60 h (Fig. 1b). Dinoflagellates are the planktonic organisms identified in this fraction as the most important contributors to the DMSP pool. Since those organisms were efficiently grazed by ciliates, the more likely explanation for DMS production is DMSP decomposition to DMS during food intake or digestion of organic matter. During the first 12-h period, while ciliates grazed 3/4 of the biomass of dinoflagellates, DMS production was minimal [ $1.3$  nM ( $12$  h)<sup>-1</sup>, Fig. 4]. The production rate of DMS was highest [ $7.4$  nM ( $12$  h)<sup>-1</sup>] during the second 12-h period. This pattern suggests that DMS is not produced during food intake but during digestion of organic matter. During that period,  $24 \pm 4$  nM of DMSP were decomposed. Thus, only a third of this amount was converted into DMS. Further on in the experiment, during the last 2 d, the DMSP concentrations decreased by  $24 \pm 4$  nM (Fig.

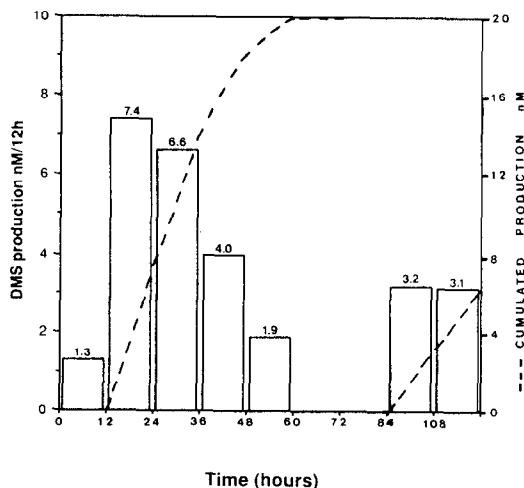


Fig. 4. Time scales of DMS production ( $12 \text{ h}^{-1}$ ) and cumulated production in the  $100\text{-}\mu\text{m}$  screened water.

3b), whereas about 7 nM of DMS were produced during the last day (Fig. 4). These results are similar to those obtained between  $t = 12$  and  $t = 24$  h and show that DMS production is not linearly related to DMSP decomposition.

The integrated DMS production during the first 60 h is about 20 nM (Fig. 4). As discussed above, this DMS production is probably a lower limit because we calculated it with a constant DMS loss through the membranes ( $0.12 \text{ nM h}^{-1}$ ). Indeed, if we take into account the ratio of the DMS levels between the  $100\text{-}\mu\text{m}$  and the  $1\text{-}\mu\text{m}$  fractions, we obtain a more realistic production of 25 nM. Over this 60-h period, mass balance between DMS production (25 nM) and DMSP decomposition ( $24 \pm 4 \text{ nM}$ ) was roughly achieved. There was a time lag of about 1.5 d, however, until the DMSP that was decomposed was found as DMS in the dissolved phase. This time lag should reflect the rates of DMSP decomposition occurring during the digestion of organic matter or ciliate catabolism.

We suggest that the decomposition of DMSP into DMS follows two pathways. A well-known pathway is enzymatic cleavage by which DMSP is converted to DMS and acrylate (Cantoni and Anderson 1956). As the second pathway, we suggest the existence of an intermediate compound that

does not yield DMS after cold alkali treatment. We hypothesize that 3-methiolpropionate ( $\text{CH}_3\text{SCH}_2\text{CH}_2\text{COO}^-$ ) is a possible intermediate during anaerobic digestion of DMSP in ciliate food vacuoles. Indeed, the work of Kiene and Taylor (1988) has shown that demethylation of DMSP in anoxic marine sediments yields 3-methiolpropionate.

As discussed above, about 55 nmol of DMSP could have been transferred through dinoflagellate grazing and stored in ciliate food vacuoles. Therefore, we estimate that a fraction of 40–50% of this DMSP pool was decomposed into DMS during the first 60 h. The remaining DMSP fraction would exist in the form of detritus or would be retained temporarily in ciliate cells.

Our results suggest that decomposition of DMSP into DMS in the microbial food web involves several steps, including DMSP storage and perhaps production in microzooplanktonic organisms, enzymatic cleavage of DMSP, demethylation, and formation of intermediate compounds. It is interesting to see that grazing at higher levels of the marine trophic web also leads to DMSP decomposition into DMS. Dacey and Wakeham (1986) reported that DMS accumulated in the water when fed zooplankton (copepods grazing on dinoflagellates) were placed in algal-free water, presumably as the result of the continuous digestion of DMSP or microbial degradation, or both. Iida and Tokunaga (1986) have shown that DMS was stored seasonally in various parts (viscera, gill, mantle, adductor) of live shellfish and cultured oysters. Thus, these observations together with ours show that, for a large set of marine organisms, DMS production follows food intake and digestion of organic matter.

In our experiment, dinoflagellate grazing by oligotrichous ciliates ( $30\text{--}50 \mu\text{m}$ ) and tintinnids produced dimethylsulfide at a mean rate of  $0.5 \text{ nM h}^{-1}$ . The mean biomass of ciliates between  $t = 12$  and  $t = 60$  h (major DMS production event) was  $1.1 \mu\text{g C liter}^{-1}$ . This single value is lower than the long-term (1973–1978) mean ( $\pm 2 \text{ SD}$ ) of the biomass of oligotrichous ciliates ( $30\text{--}50 \mu\text{m}$  size) and tintinnids in the coastal NW Mediterranean Sea ( $2.2 \pm 0.5 \mu\text{g C liter}^{-1}$ ).

ter<sup>-1</sup> in May,  $3.39 \pm 0.71$  in June; Rassoulzadegan et al. 1988). An average value of  $1 \mu\text{g C liter}^{-1}$  is observed typically in April. Since our laboratory densities and the field biomasses of ciliates in April are comparable, we assume that  $0.5 \text{ nM h}^{-1}$  corresponds to the early spring DMS production rate by oligotrichous ciliates ( $30\text{--}50 \mu\text{m}$ ) and tintinnids in the coastal NW Mediterranean. However,  $30\text{--}50\text{-}\mu\text{m}$  size oligotrichous ciliates and tintinnids are not the only populations of ciliates in the area in spring. Rassoulzadegan et al. (1988) have shown that, in April, May, and June, the biomass of  $30\text{--}50\text{-}\mu\text{m}$  oligotrichous ciliates and tintinnids represents 34, 65, and 60% of the total biomass of ciliates  $>30 \mu\text{m}$ . The data from their study suggest that although ciliates are potential grazers of bacteria their main role is that of consumers of the  $2\text{--}20\text{-}\mu\text{m}$  plankton (Fenchel 1980). Consequently, we assume that all populations of ciliates are able to graze small dinoflagellates and thus to produce DMS. Therefore we suggest that a value of  $1 \text{ nM h}^{-1}$  would be a realistic production rate of DMS in this region during spring. In July and August, the biomass of oligotrichous ciliates ( $30\text{--}50\text{-}\mu\text{m}$  size) and tintinnids is similar to that found in spring, but represents only 20% of the total biomass of ciliates  $>30 \mu\text{m}$ . Therefore it is possible that during that period the DMS production rate would reach levels up to  $4 \text{ nM h}^{-1}$  in this region.

In this discussion, we have not yet taken into account the production of DMS by the population of dinoflagellates. The estimation of the corresponding DMS production rate in the Ligurian Sea is based on the results of Dacey and Wakeham (1986). They measured a DMS production rate (mean  $\pm 2$  SD) for the dinoflagellate *Gymnodinium nelsoni* ( $0.35 \pm 0.21 \text{ nM h}^{-1}$ ). The initial biomass of *G. nelsoni* had been set to  $960 \mu\text{g C liter}^{-1}$  [calculated from the number of cells  $\text{ml}^{-1}$  (500), the cell volume ( $24,000 \mu\text{m}^3$ ), and a C:vol ratio equal to 0.08], about an order of magnitude higher than ours. Consequently, the estimated DMS production rate by free dinoflagellates would be equal to  $0.03 \text{ nM h}^{-1}$ , assuming that it is linearly related to the bio-

mass of microorganisms. Thus, we conclude that in our experiment this process can be neglected. This conclusion is sustained by the measurement of similar levels of DMS in the  $<1\text{-}\mu\text{m}$  and  $<100\text{-}\mu\text{m}$  fractions for the first 12 h of the experiment (Fig. 1b). At sea, the release of DMS by phytoplankton is certainly dependent on physiological state and most probably is a function of growth rate, but its relative importance has yet to be documented.

Another known process of DMS production is dinoflagellate grazing by copepods as reported by Dacey and Wakeham (1986). The estimate of DMS production rate by copepods in the Ligurian Sea is based on their results. Dacey and Wakeham found that copepod grazing produced  $8.5 \pm 0.6 \text{ nM h}^{-1}$  of DMS. During their experiment the selected densities of grazers ( $30\text{--}40$  copepods  $\text{liter}^{-1}$ ), however, were much higher than the usual oceanic ones. Five copepods  $\text{liter}^{-1}$  are reasonable in situ copepod densities in the Ligurian Sea. Thus, we assume that the production rate of DMS from copepods in the Ligurian Sea would range between 1 and  $1.5 \text{ nM h}^{-1}$ . Consequently, the DMS flux from copepod and ciliate grazing pressure on dinoflagellates would be of the same order of magnitude in the coastal waters of the NW Mediterranean. This calculation suggests that ciliates produce DMS as efficiently as do copepods. These considerations, however, are still speculative for many reasons related to, for example, the existence of competition between ciliates and copepods for the consumption of dinoflagellates. The impact of such competition on DMS production is unknown. It will be interesting to determine whether the grazing of ciliates by copepods increases or decreases the production rate of DMS in the marine environment.

Nevertheless, we suggest that the role of ciliates as DMS producers could be dominant in oligotrophic waters. Indeed, copepods cannot efficiently take the nanoplanktonic organisms that predominate there (Nival and Nival 1976; Paffenhöfer 1984). These organisms, which are the major DMSP carriers, must be consumed by

zooplankton smaller than copepods, i.e. ciliates.

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## Heterotrophic, autotrophic, and mixotrophic nanoflagellates: Seasonal abundances and bacterivory in a eutrophic lake

**Abstract**—Abundances of heterotrophic (HF), autotrophic (AF), and mixotrophic (MF) nanoflagellates in Lake Oglethorpe, Georgia, were in the range  $10^2$ – $10^4$  cells  $\text{ml}^{-1}$ . Pigmented and nonpigmented flagellate abundances were positively correlated with each other in samples spanning a year, but were not significantly correlated to the same physical parameters (temperature, oxygen, light). The highest density of nonpigmented flagellates ( $>1 \times 10^4 \text{ ml}^{-1}$ ) was found in spring 1986 at the surface, although most abundance peaks ranged from 4 to  $7 \times 10^3$  cells  $\text{ml}^{-1}$  and occurred in the metalimnion during summer stratification. Abundances of pigmented flagellates (AF + MF) were greatest ( $9 \times 10^3$  cells  $\text{ml}^{-1}$ ) in surface waters in early spring and late fall when the lake was not stratified. Up to 38% (avg 10%) of all pigmented flagellates at any depth also ingested particles, while the percentage of HF that were identified as bacterivores ranged from 30 to 100%. In situ grazing rate measurements indicated strong seasonal differences in the relative grazing impact of pigmented and nonpigmented flagellates, with HF dominating grazing on picoplankton except in winter.

Small heterotrophic flagellates are major bacterivores in planktonic food webs and may also regenerate significant amounts of nutrients and serve as prey for micro- and macrozooplankton (Azam et al. 1983; Sherr

and Sherr 1984; Sanders and Porter 1990). Some photosynthetic flagellates are also bacterivorous, but their relative abundance in nature is not well known (Bird and Kalff 1987; Sanders and Porter 1988). Flagellates in the nanoplankton size range (2–20  $\mu\text{m}$ ) are often fragile, so they may be difficult to detect with light microscopy. Inadequate sampling, preservation, or staining can lead to underestimates or even exclusion of these flagellates from plankton counts. Fluorescent staining and examination at high magnification (1,000 $\times$ ) by epifluorescence microscopy are required to include the smallest ( $<4 \mu\text{m}$ ) flagellates and to allow rapid differentiation between flagellates with and without chloroplasts (Caron 1983).

Potential for error exists when defining trophic status by the presence or absence of photosynthetic pigments. Pigmented flagellates are usually considered autotrophic, and nonpigmented flagellates are often assumed to be bacterivorous. Pigmented flagellates that ingest particles (mixotrophs or phagotrophic phytoflagellates) have been noted in the algal literature for decades (see Sanders and Porter 1988). Recent evidence indicates that mixotrophs in natural systems may impose a major grazing impact on bacterioplankton (Bird and Kalff 1987; Sanders et al. 1989). Heterotrophic flagellates also have several modes of carbon and nutrient acquisition including bacterivory, herbivory, and uptake of dissolved organics (Sherr and Sherr 1984; Parslow et al. 1986; Sherr 1988). Methods of determining ingestion rates that

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