

# Assessment of the role of copepods and ciliates in the release to solution of particulate DMSP

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**ABSTRACT:** The exchanges between pools of particulate DMSP (pDMSP) and the total dissolved pool of DMSP and DMS [dDMS(P)], as well as dDMS(P) removal rates were investigated in 4 shipboard incubations of amended and size-fractionated natural planktonic assemblages of the Mediterranean Sea in spring 1995 and in a laboratory experiment with cultured populations. In the shipboard experiments, the effects of different concentrations of copepods and the presence/absence of micrograzers were assessed. Removal rates of dDMS(P), obtained from seawater samples spiked with dDMSP (dissolved DMSP), were linearly correlated with dDMS(P) levels in the range 10 to 50 nM and were unrelated to size-fractionation treatments. The biological turnover rate constant of dDMS(P) was 0.5 d<sup>-1</sup>. In most of the experiments, production of dDMS(P) was independent of copepod concentration and lowest in waters from which both copepods and micrograzers >10 µm had been removed. Overall, the results of the shipboard experiments suggested that (1) dDMS(P) production occurred in the microplanktonic food web, probably because pDMSP occurred predominately in the size fraction <10 µm and was unavailable for direct copepod consumption, and (2) dDMS(P) removal rates were likely due to organisms <10 µm in size, probably bacteria. The laboratory experiment involved a common Mediterranean microplanktonic ciliate species (*Strombidium sulcatum*) grazing on a DMSP-containing Prymnesiophyceae (*Isochrysis galbana*). The rate of dDMS(P) release from algal DMSP was greatly increased when the phytoplankton was subjected to grazing by ciliates. The majority of prey pDMSP (>66%) was released to solution.

**KEY WORDS:** DMSP · Ciliates · Phytoplankton · Grazing · Copepods · Mediterranean Sea

## INTRODUCTION

The marine biogeochemical cycle of dimethylsulfide (DMS) involves a complex network of biological and chemical production and utilisation processes which may ultimately influence the flux of DMS to the atmosphere (Groene 1995). DMS is produced from the enzymatic cleavage of dimethylsulfoniumpropionate (DMSP), a compound found in marine particulate matter, notably among certain phytoplankton taxa (Belviso et al. 1993). Several pathways through which DMS might be formed in seawater have been identified.

A grazer pathway was first addressed in the pioneering work of Dacey & Wakeham (1986) in which DMS

release rates were reported to be several-fold higher when cultured dinoflagellates (*Gymnodinium nelsoni* and *Prorocentrum micans*) were grazed by marine copepods (*Labidocera aestiva* and *Centropages hamatus*), relative to the ungrazed DMSP-containing phytoplankton. It was concluded that DMS production associated with copepod grazing may be the major mechanism in many marine settings. Some field evidence of the role played by zooplankton in DMS production was provided by Leck et al. (1990). In contrast, it was recently reported (Kwint & Kramer 1995, Kwint et al. 1996) that copepod grazing on natural phytoplanktonic populations resulted in low DMS production possibly because DMSP was stored in various parts of the body of copepods and was compacted into faecal pellets.

Investigations into the role of the microbial food web in DMS production have also been conducted and yielded conflicting results. For example, Belviso et al.

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(1990) showed that during a long-term (5 d) incubation experiment with size-fractionated plankton populations, the potential for DMS production was confined to the samples containing microplanktonic organisms including micrograzers (ciliates). It was concluded that ciliates grazing on small phytoplankton may be of greater importance than phytoplankton themselves in releasing DMS in the water column. In the more recent study by Wolfe et al. (1994) in which *Emiliana huxleyi* was offered as a prey to a benthic heterotrophic dinoflagellate (*Oxyrrhis marina*), it was shown that grazing by herbivorous protists only slightly increased DMS production because not all ingested DMSP was degraded to DMS; some DMSP was stored in the digestive vacuoles and most was metabolised by the grazer.

The importance of zooplankton in DMS production is thus questionable as are the respective roles of protists and copepods. Studies thus far have relied on laboratory cultures (e.g. Dacey & Wakeham 1986) or long-term incubations of populations in mesocosms or microcosms (e.g. Belviso et al. 1990, Kwint & Kramer 1995). To our knowledge, there have been very few studies of grazer effects on DMSP using natural communities in short-term incubations ( $\leq 24$  h) and none focusing on plankton from oligotrophic systems. We investigated the role of algae, copepods and protists in releasing dissolved compounds from pDMSP (particulate DMSP) in surface waters of the NW Mediterranean Sea during spring, when levels of pDMSP are particularly high. Results are presented of 4 experiments involving shipboard incubations which were designed to examine the effects of copepod grazing on natural plankton populations. We followed communities containing natural concentrations of copepods, communities to which copepods were added, assemblages without copepods (64  $\mu\text{m}$  screening) and populations without micrograzers (10  $\mu\text{m}$  screening). The results suggested a major effect of micrograzers. In a laboratory experiment, the role of micrograzers was specifically addressed in an attempt to estimate transformation rates. We used a pDMSP algal source, a Prymnesiophyceae (*Isochrysis galbana*) as prey for a common planktonic ciliate of the Mediterranean Sea, *Strombidium sulcatum*. While Wolfe et al. (1994) have reported on an in-depth study of a herbivorous protist (*Oxyrrhis marina*), it is not a species typical of the microzooplankton. Here we report, for the first time, on the efficiency with which a common microzooplankton grazing on a phytoplankton can transform particulate DMSP (referred to as pDMSP in the text) to the dissolved pool of DMSP and DMS (referred to as dDMS(P) in the text). Note that dissolved DMSP (referred to as dDMSP in the text) constitutes a fraction of the total dissolved pool.

## MATERIALS AND METHODS

**Copepods and dDMS(P) production in the Mediterranean Sea.** Four shipboard incubations were performed using seawater samples collected at the DYFAMED station (43° 25' N, 7° 51' E) during the JGOFS-France cruise 'DYNAPROC' from 1 May to 1 June 1995 on board RV 'Le Suroît'. A size-fractionation approach was used to evaluate the role of the different components of the food web, with a focus on copepods, in the production and consumption of the dissolved compounds dDMSP and DMS. We measured total dissolved DMS(P) pool—referred to as dDMS(P) in the text—without distinguishing between dDMSP and DMS in our analyses.

The incubation experiments were conducted on 11–12 May (Expt A), 14–15 May (Expt B), 27–28 May (Expt C), and 30–31 May (Expt D). Each experiment consisted of incubating seawater subjected to 3 different treatments and a control of untreated seawater. All seawater was collected with Go-Flo bottles from 20 m depth where the maximum concentration of DMS(P) was found during May 1995 (the control). Water was incubated in 2 l polycarbonate (PC) bottles for 24 h at 30% of surface irradiance in a flow-through on-deck incubator flushed with seawater from 3 m depth. Gas exchange and photochemical decomposition of DMS was likely reduced this way. All materials used were acid-cleaned and rinsed with Milli-Q water. Three bottles were filled with untreated seawater to serve as controls; treatments were as follows.

**Treatment 1: copepods added.** *Centropages typicus* were collected from surface waters (0 to 50 m depth) with a 200  $\mu\text{m}$  mesh size zooplankton net and starved for several hours before the start of the grazing experiment. Actively swimming females were added to untreated seawater to yield 10 individuals  $\text{l}^{-1}$ , roughly comparable to 5 $\times$  natural concentrations. Duplicate bottles were prepared.

**Treatment 2: copepods removed.** Seawater was poured gently through a 64  $\mu\text{m}$  Nitex mesh to remove adult and immature stages of copepods. The <64  $\mu\text{m}$  size fraction represents micrograzers, largely ciliate microzooplankton, and phytoplankton without copepod grazers. Triplicates were prepared.

**Treatment 3: micrograzers removed.** Seawater was screened through 90 mm diameter 10  $\mu\text{m}$  pore-size Nuclepore PC filters. The method of fractionation was adapted from Verity et al. (1993). The filter was mounted on an acid-washed teflon cylinder that was allowed to sink slowly through seawater contained in a clean 2 l PC beaker, thus producing a <10  $\mu\text{m}$  size fraction. The 10  $\mu\text{m}$  cutoff was designed to remove micrograzers, primarily ciliate microzooplankton, while allowing nanograzers and most of the phytoplankton to pass. Triplicates were prepared.

In parallel incubations, the removal rate of dDMS(P) in each of the 3 different treatments and the control was evaluated in Expts A, B and D. Four 2 l bottles, each filled with treated or untreated seawater, were spiked with  $\mu$ l quantities of an acidified DMSP aqueous solution freshly prepared from DMSP.HCl (Research Plus, Inc.), to yield final concentrations of dDMSP 2- to 3-fold higher than ambient. Microvolumes ( $\mu$ l) of the acid stock solution were added to minimise the effect on the pH of seawater.

#### Laboratory experiment with a ciliate micrograzer.

Incubations were carried out in six 2 l PC bottles (Nalgene) which had been acid washed, Milli-Q water rinsed, and autoclaved. The bottles were filled with DMS(P)-free GF/F filtered seawater (collected at 1000 m depth, 52 km off Villefranche-sur-Mer, France) previously kept in the dark with continuous oxygenation with bubbled air.

*Strombidium sulcatum*, originally isolated from the bay of Villefranche-sur-Mer, was maintained on a bacterised wheat-grain media at 15°C as described in Rivier et al. (1985). Ciliates for the experiment were transferred into a bacterised yeast extract medium (0.03 g l<sup>-1</sup>) and grown at the experimental temperature of 22°C in a temperature controlled incubator. Late log-phase cultures of ciliates (300 to 400 cells ml<sup>-1</sup>) were employed for the experiment. *Isochrysis galbana* was grown on Guillard's f/2 medium (Guillard 1975) without silicate at 22°C in continuous light. Algae used for the experiment came from a log growth culture (10<sup>5</sup> cells ml<sup>-1</sup>). Final concentrations of ciliates and algae added in experimental bottles were calculated to be around 30 ciliates ml<sup>-1</sup> and 1 × 10<sup>4</sup> algal cells ml<sup>-1</sup>.

Three solutions were prepared in duplicate: (1) a ciliate control (ciliates alone), (2) a phytoplankton control (phytoplankton alone) and (3) phytoplankton and ciliates together (treatments C, P and PC respectively).

To evaluate the potential of the cultures to remove dDMS(P), 1 bottle of each solution was spiked at the onset of the incubation with dDMSP giving a 10-fold higher initial concentration of dDMSP. These bottles are referred as Ca, Pa and PCa.

Each of the 6 bottles was sampled at 0, 1, 2, 3, 6, 9, 12, 24, 36 and 48 h. The tight sampling (every hour) during the first 3 h was to check if the dDMSP spike might be very rapidly removed in the spiked bottles (Ca, Pa and PCa).

Phytoplankton cells were enumerated in samples taken at 0, 12, 24, 36 and 48 h, by epifluorescence microscopy after preservation with sodium tetraborate buffered formalin (4% final concentration) and staining with DAPI (4,6-diamidino-2-phenylindole, final concentration 500  $\mu$ g l<sup>-1</sup>). Ciliates were enumerated at 0, 24 and 48 h on an inverted microscope by Utermöhl's (1958) counting technique. A volume of 10 ml of the samples containing ciliates was fixed with alkaline

Lugol's solution and cleared with 3% sodium thiosulfate, allowing observation of ingested algae using an inverted microscope equipped with epifluorescence (Zeiss Axiovert, with a 50 W mercury lamp).

**Sulfur analyses.** Samples were analysed using a Varian 3300 gas chromatograph equipped with a Pulsed Flame Photometric Detector (PFPD). The PFPD was optimised using repeated injections of a precise amount of gaseous DMS standard and adjusting the hydrogen/air split valve to maximise the DMS peak area. A precision of 1% was obtained by filling a 1/16" (0.158 cm) outside diameter FEP-Teflon loop, mounted on a Valco Nitronic-60 6-port gas sampling valve, with a calibration mixture made from a permeation tube (Metronics) isothermally operating at 40°C in a glass chamber flushed with high-grade helium. The analytical column was a 10 m × 0.32 mm PoraPLOT Q from Chrompack operating isothermally at 80°C. Helium was the gas carrier flowing at a rate of 2 ml min<sup>-1</sup>.

Water samples were size-fractionated by gravity filtration (<GF/F, <10  $\mu$ m) as in Belviso et al. (1993) but only an 8 ml aliquot of the filtrates (20 to 50 ml) was transferred to glass tubes, treated with cold alkali, sealed with a Teflon faced septa and allowed to sit at room temperature for 12 h then at 4°C before GC analysis. Only filtration by gravity was applied.

The content of a sample tube was drawn into a plastic syringe and immediately injected into the sparging device through a Teflon faced septa. Samples were sparged with helium at 40 ml min<sup>-1</sup> for 10 min and cryotrapped on liquid nitrogen in a 1/8" (0.317 cm) diameter FEP-Teflon trap. Nafion dryer tubes were used to remove water after the gas stream had passed through a cold finger at +1°C. This kept water from entering the Nafion Dryer and increased its efficiency. The trap was heated at +95°C and its contents transferred for 5 min to the 1/16" (0.158 cm) FEP-Teflon loop (see above) immersed in liquid nitrogen. Cryofocussing was a necessary step to allow a rapid injection onto the capillary column.

DMSP-HCl standards were prepared in acidified Milli-Q water and treated the same way as the seawater samples. The PFPD response was quadratic. Detection limit was about 50 pg DMS, or 0.1 nM in an 8 ml sample. Precision was calculated from size-fractionated samples of the Mediterranean Sea. Four series of triplicates of the whole sample and the size fractions <10  $\mu$ m, <2  $\mu$ m and <GF/F (total dissolved DMSP) were prepared and analysed for their total DMSP+DMS ( $\Sigma$ DMS) concentration. The coefficients of variations (SD:mean) of each triplicate were in the range 2.9 to 4.6, 1.7 to 6.7, 3.9 to 8 and 1.5 to 8.9%, respectively, for  $\Sigma$ DMS levels of 10 to 25 nM. Precision of pDMSP concentrations calculated by difference between samples was typically 8 to 15%. Because dDMS(P) and pDMSP levels increased rapidly in spring 1995 we duplicated the analyses systematically.



## RESULTS

## Removal of dDMS(P)

Removal rates were calculated for bottles to which synthetic dDMS(P) was added (Expts A, B and D, each consisting of a control, a control + copepods, and 2 size fractions <10  $\mu\text{m}$  and <64  $\mu\text{m}$ ). Removal rates were calculated by subtracting dDMS(P) concentration at  $t_{24}$  from initial concentrations. Removal rates were not corrected for dDMS(P) production during incubation. Net dDMS(P) removal rates were not markedly different between the treatments and the controls. However, in Expt B, the removal rates observed in the size fractions <64  $\mu\text{m}$  and <10  $\mu\text{m}$  were distinctly lower than in the untreated water or copepod added bottles. Based on  $t_0$  measurements, the bottles containing the size-fractionated material received less dDMS(P) at the onset of the incubation than the untreated water or bottles to which copepods were added. These results, indicating an apparent relationship between initial concentrations and removal rates in Expt B, led us to consider such a relationship in the entire data set.

dDMS(P) removal rates are plotted versus dDMS(P) concentration in Fig. 1. A linear relationship was found (Fig. 1) with  $r^2 = 0.77$  ( $n = 12$ ), significant at the 0.1% level. The slope of the relationship was 0.48 and the intercept ( $-0.46$  nM) was not significantly different from 0 at the 95% level.

During the laboratory experiment, the net removal of added dDMS(P) was monitored with a higher resolution than during the field experiments. Levels of dDMS(P) that were initially about 10-fold higher in the bottles spiked with dDMS(P) (Pa and PCa) than in the controls

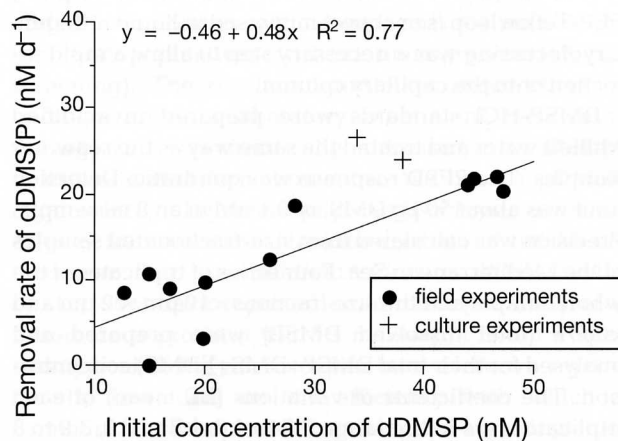


Fig. 1. Enrichment experiments with addition of dDMS(P) in incubation bottles containing different fractions of the planktonic food web. Removal rates of dDMS(P) versus initial concentrations. Regression considered only the values from the field experiments

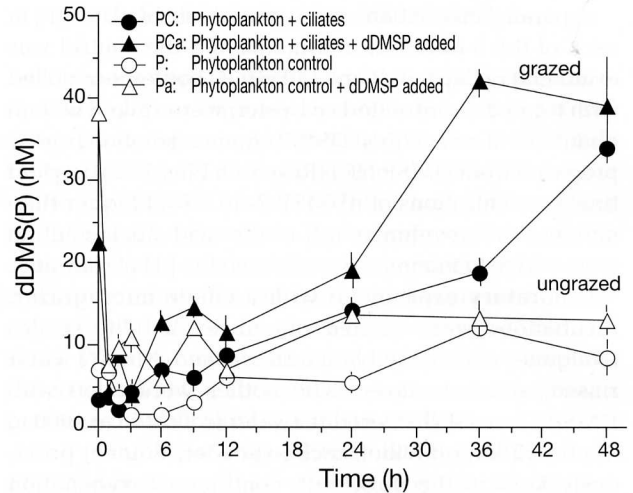


Fig. 2. Temporal variations of dDMS(P) concentration in bottles P (phytoplankton), Pa (phytoplankton + DSMP<sub>added</sub>), PC (ciliates + phytoplankton) and PCa (ciliates + phytoplankton + DSMP<sub>added</sub>). Vertical lines show the range of duplicate measurements

(P and PC) declined to the levels in P and PC unspiked solutions after only 1 h of incubation (Fig. 2). Thus, potential removal rates of dDMS(P) were high in the cultures of *Isochrysis galbana* both with and without *Strombidium sulcatum*. The dDMS(P) concentration in the ciliate-spiked bottle Ca also decreased about 5-fold but took twice as long (Fig. 3). Thus, there was also a large potential for removing dDMS(P) in the ciliate culture. However, in the Ca bottle the dDMS(P) concentration did not decline to the concentration of the unamended bottle C, but stabilised at a level that was about 7-fold higher (Fig. 3). Interestingly, in the exper-

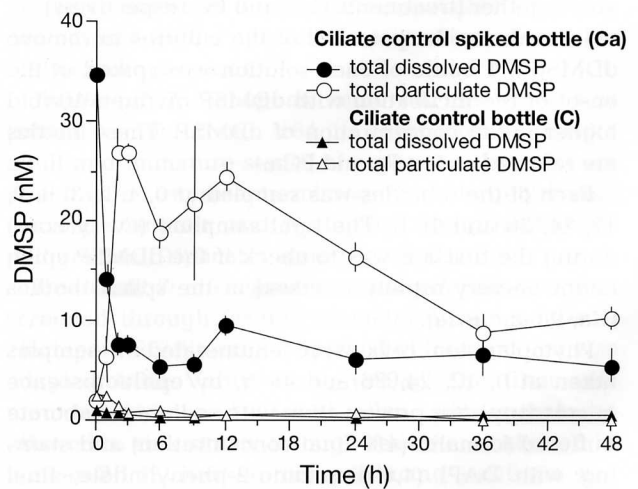


Fig. 3. Temporal variations of dDMS(P) and pDMS(P) concentrations in bottles C (ciliate control) and Ca (ciliate control + dDMS<sub>added</sub>). Vertical lines show the ranges of duplicate measurements

imental bottle Ca, containing only ciliates and bacteria spiked with dDMSP, the loss in dDMS(P) of about 26 nM during the first hours of incubation appeared in the particulate phase (Fig. 3). The total pool of DMS(P) remained rather stable during the first 12 h, then steadily decreased as the pDMSP pool until the end of the experiment.

### Copepods and dDMS(P) production in the Mediterranean Sea

To calculate the production rates of dDMS(P) in the treatments and the controls we used the changes in dDMS(P) concentrations after a 24 h period of incubation, corrected for dDMS(P) removal using the relationship we found between the net dDMS(P) removal rates and the dDMS(P) concentrations. The removal rate was roughly equivalent to 50% d<sup>-1</sup>. More precisely, dDMS(P) production was calculated as follows:

$$\text{Production} = \text{dDMS(P)}_{t_{24}} - 0.52 \times \text{dDMS(P)}_{t_0} \quad (1)$$

Overall, there was no consistent trend evident with the concentrations (added, *in situ*, removed) or simply presence/absence of copepods. The lowest average rates of dDMS(P) production were found in <10 µm size-fractionated water from which the micrograzers had been removed. However, we encountered a considerable amount of variability among replicates in all the experiments (Fig. 4). For example, in Expt B, the duplicates of the control were so different (14.6 and 0 nM d<sup>-1</sup>) that no treatment effects could be shown. In Expts A, C and D the coefficients of variation (SD:mean) of the controls were 36, 67 and 67% respectively.

### Ciliate grazing on phytoplankton

*Strombidium sulcatum* grazing on *Isochrysis galbana* was associated with a significant release of dDMS(P) (Fig. 2). The increase of dDMS(P) in the experimental bottles PC and PCa was pronounced after 12 h of incubation. The net production of dDMS(P) in bottles with grazers was 5 to 6-fold higher than in the bottles containing the phytoplankton alone. The results obtained for pDMSP in bottles with phytoplankton grazers showed an important decrease of the pDMSP in the size fraction <10 µm 9 h after the onset of the experiment, while the pDMSP in the phytoplankton controls (P and Pa) showed a steadily increasing trend (data not shown). These trends paralleled the *I. galbana* cell numbers (Fig. 5). In the presence of ciliates the dDMS(P):pDMSP ratio steadily increased from about 0.05 at the onset of the experiment to about 0.35 after

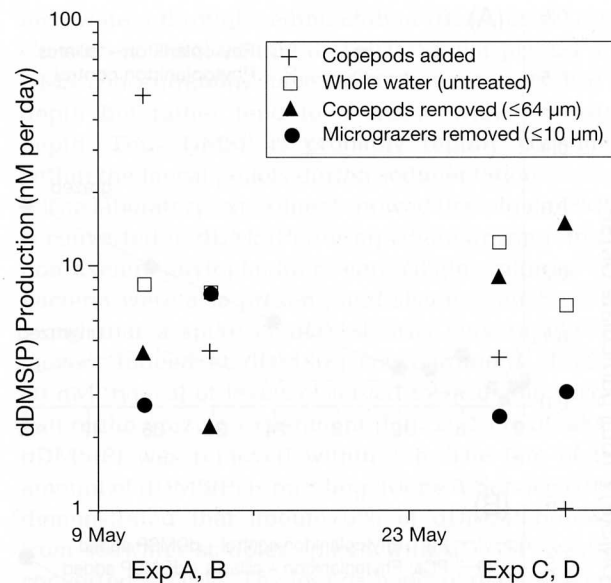


Fig. 4. Production rates of dDMS(P) [corrected for dDMS(P) removal] in different fractions of seawater. Only mean values of triplicates or duplicates are given. Data are plotted on a log scale only to allow separate data points to be distinguished. Pooling all 4 experiments, the overall mean values [nM dDMS(P) produced d<sup>-1</sup>] for the different treatments were: copepods added = 16.8 (SD = 22.77, n = 7), whole water = 8.9 (SD = 6.82, n = 11), copepods removed = 7.8 (SD = 5.96, n = 12), micrograzers removed = 3.8 (SD = 3.45, n = 8). Variability between replicates precluded demonstration of any treatment effects but based on average values, copepod concentration did not alter dDMS(P) production rates, while removal of micrograzers reduced production

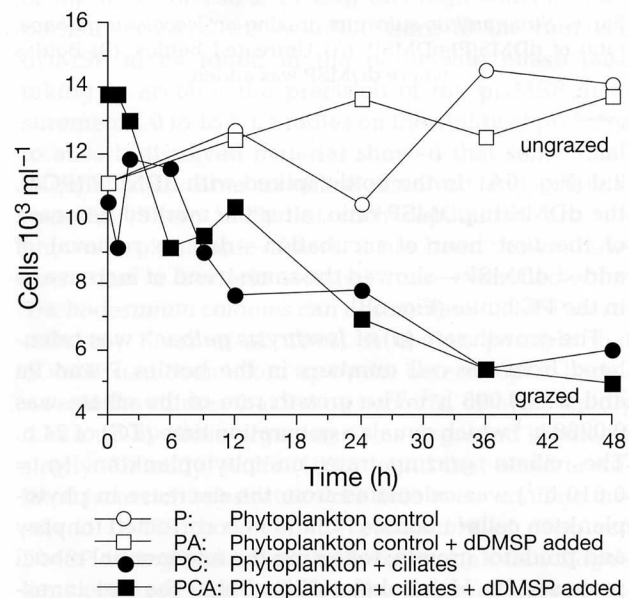


Fig. 5. *Isochrysis galbana* cell numbers in experimental bottles P (phytoplankton control), Pa (phytoplankton control + dDMSP<sub>added</sub>), PC (ciliates + phytoplankton) and PCa (ciliates + phytoplankton + dDMSP<sub>added</sub>)

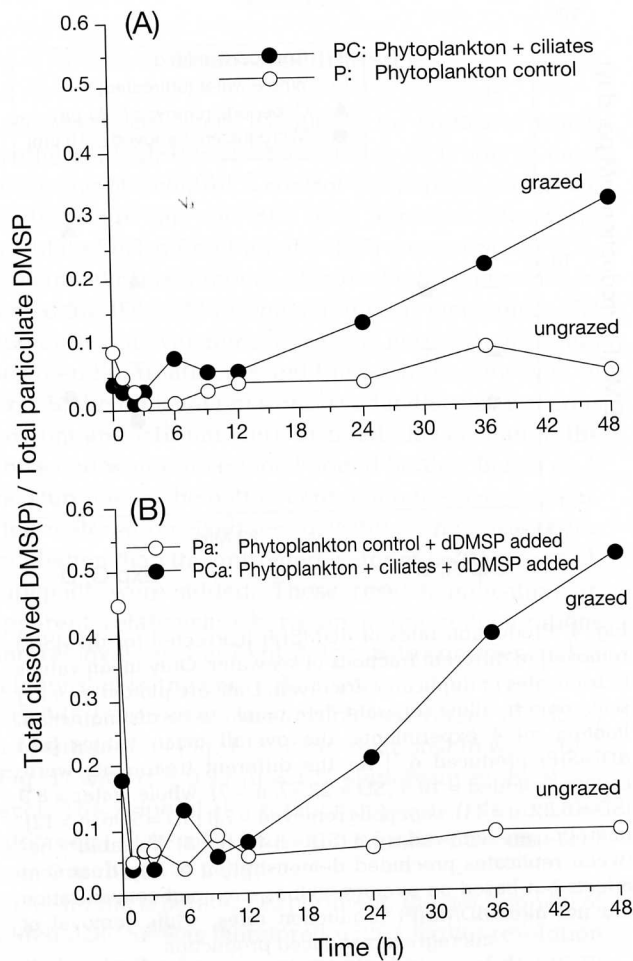


Fig. 6. *Strombidium sulcatum* grazing on *Isochrysis galbana*, ratio of dDMS(P):pDMSP. (A) Untreated bottles. (B) Bottles where dDMSP was added

2 d (Fig. 6A). In the bottle spiked with dDMSP (PCa), the dDMS(P):pDMSP ratio, after the marked decrease of the first hour of incubation—due to removal of added dDMSP—showed the same trend of increase as in the PC bottle (Fig. 6B).

The growth rate ( $\mu$ ) of *Isochrysis galbana* was calculated from the cell numbers in the bottles P and Pa and was  $0.005 \text{ h}^{-1}$ . The growth rate of the ciliate was  $0.0288 \text{ h}^{-1}$  which equals a generation time (TG) of 24 h. The ciliate grazing rate on phytoplankton ( $g = 0.010 \text{ h}^{-1}$ ) was calculated from the decrease in phytoplankton cells in bottles PC and PCa corrected for prey and predator growth, following the exponential model proposed by Heinbokel (1978). Thus, the net ingestion rate of *I. galbana* by *Strombidium sulcatum* was 3 to 4 cells ciliate $^{-1} \text{ h}^{-1}$  (microscopic examination also showed that 3 to 4 ingested cells were visible within 1 h of adding the prey).

## DISCUSSION

Dissolved DMSP (dDMSP) accounts for a significant fraction of the total DMSP pool (Turner et al. 1988). Field work has shown that the correlation of DMS with dDMSP is stronger than that of DMS with pDMSP (Turner et al. 1988). Because dDMSP is a potential source of DMS, it is reasonable to consider the production of dDMSP and DMS during grazing [total dissolved DMS(P)]. A budget of pDMSP consumption and conversion to dDMSP can be assembled provided that (1) all dDMSP is degraded to DMS and (2) DMS is not rapidly removed from the incubation bottles (naturally or using specific inhibitors). Conditions (1) and (2) were satisfied in the grazing experiments of Wolfe et al. (1994). However, there is compelling evidence that in most marine settings not all dDMSP is degraded by microbes to DMS and the DMS produced is degraded by bacteria (Kiene & Service 1991). A second approach is to use effective inhibitors of dDMSP removal, but there are very few available and they may have indirect effects (Kiene & Gerard 1995). In our experiments in the Mediterranean Sea where dDMSP concentrations were several-fold higher than DMS, we investigated potential removal rates of dDMSP by spiking with synthetic DMSP and by measuring the decrease in the total pool of dissolved compounds, dDMS(P), without discriminating between dDMSP and DMS. A relationship between dDMS(P) concentrations and removal rates was established this way and was used to assemble a budget of dDMS(P) production during zooplankton grazing on phytoplankton.

Combining the size-fractionation method with the spiking method was a novel way to approach the respective role of copepods and ciliates in the release to solution of DMSP. The turnover rates we calculated from dDMS(P) losses are different from classical kinetic parameterisation because they describe the activity of assemblages of diverse marine organisms rather than single species. During the dDMS(P) removal time courses, dDMSP may have been cleaved and/or demethylated and/or stored and DMS consumed and/or photo-oxidised. These processes are not expected to occur on the same time scale. Kiene (1992) showed that the 24 h time course of dDMSP concentrations in water samples from the southern Sargasso Sea spiked with dDMSP did not follow first-order kinetics but resembled zero-order kinetics. When the concentrations of dDMSP and DMS are summed, the removal of dDMS(P) definitely does not follow first-order kinetics. Moreover, because the central Ligurian Sea, where our study was carried out, is an oligotrophic area representative of the open ocean, we adopted a linear model for the time course of dDMS(P) removal. However, a rapid 'first-order' consumption of added dDMSP was ob-

served by Kiene & Gerard (1995) from samples collected from Mobile Bay, USA, and a beach on the Gulf of Mexico. Hence, dDMSP removal rates may be more rapid in coastal areas than in the open ocean. Rapid removal of added dDMSP was also observed during our grazing experiments (Fig. 2). Finally, using a linear model it may be possible that we underestimated removal rates, but the turnover time of dDMS(P) we measured was about 2 d. Our estimate is in agreement with the measurements of Ledyard & Dacey (1996) in another oligotrophic system, the Sargasso Sea, showing dDMSP turnover times to be in the range 0.4 to 2.8 d.

Bacteria are well-known agents of consumption or transformation of dDMSP and DMS (Kiene & Service 1991). However, not all bacteria are capable of cleaving DMSP (Visscher et al. 1992, Wolfe et al. 1994). Since a specific enumeration of such specialized bacteria was not carried out, we cannot affirm but only suppose that bacteria were responsible for dDMS(P) degradation. Our supposition is supported by the fact that similar removal rates were found in different seawater size fractions and cultures of planktonic organisms, where bacteria were always the common constituent.

Our experiments at sea showed the  $<10 \mu\text{m}$  filtrates to yield the lowest average rates of dDMS(P) production. In the NW Mediterranean we found that the majority (60 to 90%) of pDMSP was in the size fraction  $<10 \mu\text{m}$ . Hence, dDMS(P) production does not appear to be proportional to pDMSP abundance. Corn et al. (1996) showed that in the size range of picophytoplankton only picoeucaryotes are important carriers of DMSP, while prochlorophytes and cyanobacteria are not. Therefore, the production in the size fraction  $<10 \mu\text{m}$  could be attributed to grazing of picoeucaryotes by flagellates. Copepods were also shown to be comparatively unimportant, probably because the phytoplankton was composed of these small cells. The variable results with copepods in our experiments might be due to the low ability of adult copepods to feed on particles of size smaller than  $10 \mu\text{m}$  combined with their ability to feed on micrograzers (Stoecker & Capuzzo 1990, Turner & Granéli 1992, Christaki & Van Wambeke 1995). In any event, our results suggest that when pDMSP is found largely in small size fractions the role of copepod grazers in pDMSP transformation is probably small or indirect. In future experiments, it may be desirable to have concurrent measures of phytoplankton loss rates.

Most of the DMSP ingested by copepods appears to be compacted into faecal pellets that are rapidly removed from surface waters due to their fairly high settling rates of tens to hundreds of meters  $\text{d}^{-1}$  (Kwint et al. 1996). However, usually less than 0.1% of the particulate DMSP standing crop is exported from sur-

face waters through sedimentation (Bates et al. 1994, Corn et al. 1994) and neither dDMSP nor pDMSP nor DMS concentrations have been shown to increase at depth but rather tend to decrease with increasing depth. Thus DMSP is probably rapidly consumed within the faecal pellets during sedimentation.

The laboratory experiment showed that algal pDMSP is converted to dDMS(P) during ciliate grazing. In the non-axenic phytoplankton and ciliate cultures (i.e. bacteria were also present) and also in a mixture, we found that a spike of dDMSP was very rapidly removed. Indeed at dDMS(P) concentrations of 20 to 40 nM, typical of levels observed towards the second half of the grazing experiment (Fig. 2), 15 to 30 nM of dDMS(P) was removed within 1 h. The fate of this amount of dDMS(P) is puzzling. Kiene & Service (1991) demonstrated that about 70% of dDMSP removed from seawater samples spiked with dDMSP was not converted to DMS. The loss pathway of dDMSP would be through demethylation. In the non-axenic ciliate culture we observed that dDMS(P) was mostly transferred to the particulate phase (Fig. 3). Then pDMSP steadily decreased to a level that was far higher than the pDMSP levels of the control (Fig. 3). The transfer of removed dDMSP in the particulate phase could clearly be observed in the ciliate treatment (ciliates + dDMSP<sub>added</sub>) where the DMSP was almost zero in the particulate phase before addition of dDMSP. The concentration of pDMSP in treatments with phytoplankton (phytoplankton + ciliates + dDMSP<sub>added</sub>, phytoplankton + dDMSP<sub>added</sub>) was in the beginning of the experiment of the order of  $130 \pm 21 \text{ nM}$ ; this high concentration partially masked the eventual trace of the removed dDMSP to be found in the particulate phase (also taking in account the precision of the pDMSP measurements, 8 to 15%). Studies on the ability of protozoa to absorb dissolved material showed that some small flagellates are able to utilise dissolved and colloidal material (Sherr 1988) but such capabilities are unknown in planktonic ciliates. It has been shown, however, that a bacteria isolated from cyanobacterium *Trichodesmium* colonies can accumulate dDMSP intracellularly (Diaz et al. 1992). It is thus possible that dDMSP had been taken up by the heterotrophic bacteria and the ciliates that fed on bacteria. dDMSP rapidly taken up by these organisms was degraded relatively slowly. Recently, Wolfe (1996) found that bacteria isolated from rich substrates were able to accumulate dissolved DMSP, however the production of dissolved DMSP during bacterivory by heterotrophic nanoflagellates occurred only occasionally, and predators metabolized most of the dDMSP.

It should be noted that to sustain a concentration of dDMS(P) in the range 20 to 40 nM, typical of levels observed towards the second half of the laboratory



experiment (Fig. 2), a dDMS(P) production rate of 15 to 30 nM h<sup>-1</sup> equivalent to its removal rate would be required at steady state. Such a production rate would induce the exhaustion of the algal DMSP within a few hours unless conversion were balanced by a considerable increase of algal growth. Unknown also are the transformations of an ingested DMSP-containing cell within ciliate food vacuoles. This topic deserves further attention. Nevertheless, we can attempt a rough budget of DMS(P) consumption and conversion by ciliates. We began by considering that the removal rate of dDMS(P) calculated using the spiking method may not be representative. By increasing the dDMS(P) background 5- to 10-fold at the onset of the experiment we may have maximised dDMS(P) removal. To attempt a budget, we calculated dDMS(P) production during the grazing experiments from the dDMS(P) net production corrected for removal using the relationship in Fig. 1. Support for this approach can be found in the fact that the 2 removal rates calculated using dDMS(P) levels at  $t_0$  and  $t_0 + 24$  h in the bottles Pa- (Phytoplankton + dDMSP<sub>added</sub>) and Ca (ciliates + dDMSP<sub>added</sub>) (Fig. 2) fitted quite well with the *in situ* data (Fig. 1). Data from PCa (phytoplankton + ciliates + dDMSP<sub>added</sub>) were not used because the levels of dDMS(P) at  $t_0$  and  $t_0 + 24$  h were very close due to the increase in dDMS(P) release during grazing (Fig. 2). Production of dDMS(P) during the second half of the experiment was calculated using Eq. (1) and was equal to 26 and 29 nM d<sup>-1</sup> in PC (phytoplankton + ciliates) and PCa (phytoplankton + ciliates + dDMSP<sub>added</sub>) respectively. As far as we know, *Strombidium sulcatum* excretes indigestible contents of food vacuoles. This 'excretion' by ciliates likely represents release of intracellular phytoplankton DMSP into solution during grazing. This production of dDMS(P) was about 1 pmol ciliate<sup>-1</sup> d<sup>-1</sup> since the density of ciliates was 30 ml<sup>-1</sup>. During the same period, the amount of algal pDMSP (pDMSP in the size fraction <10 µm) lost from PC and PCa was 39 and 28 nM, respectively. We then conclude that the majority (66 to 100%) of lost algal DMSP was released to solution. This is in agreement with the budget proposed by Wolfe et al. (1994) for low predator concentrations, based on grazing experiments using a benthic dinoflagellate (*Oxyrrhis marina*) and *Emiliana huxleyi*. Can these results be extrapolated to open marine waters? We suggest that this is highly possible because (1) microzooplankton and autotrophic nanoflagellates including the prymnesiophytes are ubiquitous in open waters and (2) excreta of micrograzers are thought to have low sedimentation rates and thus can remain in surface waters (Mostajir et al. 1995, Stoecker et al. 1995). Excreta can undergo desegregation through which dDMS(P) would be released.

## CONCLUSION

In a series of shipboard experiments copepod concentrations did not markedly affect the production rate of dDMS(P) while removal of micrograzers, organisms 10 to 64 µm in size, appeared to reduce dDMS(P) production significantly. It is probable that as most of the pDMSP was in the size fraction <10 µm, it was unavailable for direct ingestion by copepods. In a laboratory experiment with a micrograzer, a ciliate isolated from the Mediterranean Sea (*Strombidium sulcatum*), it was estimated that the ciliate can convert pDMSP to dDMS(P) with an efficiency higher than 65%.

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