

Release and consumption of DMSP from *Emiliana huxleyi* during grazing by *Oxyrrhis marina*

Gordon V. Wolfe, Evelyn B. Sherr, Barry F. Sherr

College of Oceanic and Atmospheric Sciences, Oceanography Administration Bldg. 104, Oregon State University, Corvallis, Oregon 97331-5503, USA

ABSTRACT: Degradation and release to solution of intracellular dimethylsulfoniopropionate (DMSP) from *Emiliana huxleyi* 370 was observed during grazing by the heterotrophic dinoflagellate *Oxyrrhis marina* in 24 h bottle incubations. Between 30 and 70 % of the lost algal DMSP was metabolized by the grazers without production of dimethylsulfide (DMS) when grazer densities were 150 to 450 ml⁻¹. The rest was released to solution and about 30 % was converted to DMS by bacteria associated with the grazer culture. These experiments demonstrate that grazing by herbivorous protists may be an important sink for DMSP in marine waters, removing a potential source of DMS. Microzooplankton grazing may also indirectly increase the production of DMS by transferring algal DMSP to the dissolved pool, making it available for bacterial metabolism.

KEY WORDS: DMSP · DMS · Flagellate · Herbivory · Microbial loop

INTRODUCTION

Dimethylsulfide (DMS), a climatically active trace gas (Charlson et al. 1987), is thought to be formed in the marine environment primarily from algal dimethylsulfoniopropionate (DMSP). DMSP may function as a 'compatible solute' in some phytoplankton (Dickson & Kirst 1986) and can reach intracellular concentrations of hundreds of millimolar (Keller et al. 1989). Bulk DMSP abundances in seawater are usually 10 to 100 nM, much greater than typical DMS concentrations <10 nM (Cooper & Matrai 1989, Belviso et al. 1990, 1993). There is therefore a large potential for DMS production, and its regulation in part determines how much DMS is available for outgassing to the atmosphere.

Dissolved DMSP is consumed rapidly (hours) in natural seawater samples, with partial conversion to DMS and partial metabolism to other products such as 3-methylpropionate (Kiene & Service 1991, Kiene 1992, Taylor 1993). Bacteria which mediate these transformations have been enumerated, isolated, and characterized (Taylor & Gilchrist 1991, Ledyard et al. 1993, Visscher et al. 1993), and it is likely that DMSP-degrading bacteria are ubiquitous in surface waters.

Furthermore, at least 1 marine phytoplankter, *Phaeocystis pouchetti*, can also transform dissolved DMSP to DMS (Stefels & van Boekel 1993).

However, the majority of DMSP is usually associated with particulate material. Although most studies have not identified the exact source, it is usually assumed to be synthesized and stored inside living phytoplankton. Therefore, processes which release algal DMSP, such as grazing, may be critical to DMS production since they will influence the amount of dissolved DMSP available to bacteria which can transform it to DMS. Several field observations have shown correlations between zooplankton abundances and DMS concentrations (Nguyen et al. 1988, Leck et al. 1989, Belviso et al. 1990, 1993, Holligan et al. 1993), and bottle grazing experiments with 30 to 40 copepods l⁻¹ (Dacey & Wakeham 1986) increased DMS production by about 3-fold. At least some heterotrophic dinoflagellates may consume or convert DMSP, and Ishida & Kadota (1967) showed that the osmotroph *Gyrodinium cohnii* could synthesize DMSP and enzymatically convert it to DMS. However, the role of heterotrophic protists in the consumption and release of DMSP and the production of DMS is poorly understood, despite their importance as herbivores (Capriulo et al. 1991, Sherr & Sherr 1992),

and study of these processes has been limited to size-fractionation of natural water samples (Belviso et al. 1990).

Here we present results from batch culture experiments where we examined the role of the heterotrophic dinoflagellate *Oxyrrhis marina* in the consumption of algal DMSP from the coccolithophore *Emiliana huxleyi*, and the subsequent release of DMSP to solution followed by production of DMS. Our objective was to work with a highly simplified system, a monospecific, axenic, high-DMSP-titer prey and a monospecific (though non-axenic), low-DMSP-titer predator, at densities roughly comparable to those in natural waters.

METHODS

Bottle incubations. Incubations were carried out with 500 ml or 1000 ml polycarbonate bottles (Nalgene), acid-washed and autoclaved, filled completely with glass-fiber (0.7 μm) filtered, autoclaved seawater (FASW). *Oxyrrhis marina* (originally isolated from Danish coastal waters by H. Havskum) was grown in the light at 16°C on *Dunaliella tertiolecta* to densities of approximately 10^4 cells ml^{-1} , then transferred to the dark for several days to allow grazers to remove prey. Small aliquots of this culture were added to each experimental bottle to final densities of 150 to 400 *O. marina* ml^{-1} . Any chemical amendments, such as glycine betaine, were added and the bottles were allowed to sit at least 1 h before addition of prey.

Axenic *Emiliana huxleyi* 370 (Bigelow culture collection, West Boothbay Harbor, ME, USA) was maintained on *f/2* medium at 16°C in a 14:10 h light:dark cycle, and was added from log growth cultures to initial densities of 3000 ml^{-1} . Algal cultures were examined microscopically and periodically plated on rich heterotrophic marine agar plates to insure against contamination.

Duplicate bottles of *Emiliana huxleyi* only, *E. huxleyi* with *Oxyrrhis marina*, and *O. marina* only were prepared. Bottles were incubated in the dark at 16°C without agitation, and were sampled for DMS, DMSP (particulate and dissolved), and prey and predator densities over 24 h. We found that bottles without grazers showed stable cell and sulfur pools over this period (see 'Results'), but not during longer incubations, where 'bottle effects' became important.

Cell enumeration. Prey cells were enumerated by epifluorescence microscopy after preservation (sodium tetraborate-buffered formalin, 4% final concentration) and staining with acridine orange (AO). Actively swimming *Oxyrrhis marina* cells were enumerated with a dissecting microscope (Wild M3Z, Leica, Inc.)

in replicate 5 or 10 μl drops. Grazer cell numbers were also enumerated by epifluorescence microscopy after preservation and staining using AO or DAPI (4,6-diamidino-2-phenylindole). Samples (5 ml) were preserved with Lugol's (10 $\mu\text{l ml}^{-1}$ sample) to prevent ejection of grazer food vacuoles, and cleared with 3% sodium thiosulfate (Rassoulzadegan 1991, Sherr & Sherr 1993), then preserved with formalin. Samples were stained and filtered onto black 0.2 or 0.8 μm membrane filters (Poretics, Livermore, CA, USA; #11053, 11021) immediately after preservation. Samples stained with DAPI were used to examine prey inside grazer food vacuoles by observing algal chlorophyll autofluorescence under blue light, and samples stained with AO were used to count prey and grazer numbers.

Sulfur analyses. Sulfur analyses were made by gas chromatography (GC) using a Shimadzu GC-14 chromatograph equipped with a flame photometric detector. The column packing was Chromosil 330 (Supelco, Bellefonte, PA, USA), operated isothermally at 60°C. Helium was the carrier gas, and was also used for sample sparging.

Because DMS was frequently lost during filtration, whole-water samples (3 to 5 ml) were first pipetted directly into sparge tubes and stripped to remove DMS. A second 3 ml aliquot was then filtered gently using a syringe and 25 mm GF/C or GF/F glass-fiber filters into 3 ml of 10 N NaOH in a separate sparge tube, and stripped to measure DMS + dissolved DMSP (DMSP_d). DMSP_d was then calculated by difference. The filter was placed in 8 ml of 2 N NaOH, allowed to sit at room temperature for 6 to 12 h, and 1 to 2 ml subsamples were sparged for particulate DMSP (DMSP_p). Filtration was performed as slowly as possible, and injection into the sparge tube was done through a 1 mm Teflon tube rather than with a needle in order to minimize pressure across the filter.

Samples were sparged with helium at 85 to 90 ml min^{-1} for 3 to 6 min and cryotrapped on liquid nitrogen in Teflon traps, which were then heated to >80°C and injected onto the column. Nafion dryer tubes (#MD-050-72F, Permapure, Toms River, NJ, USA) were used to remove water vapor. DMSP-HCl standards were prepared in deionized water acidified to prevent microbial degradation, and were injected into sparge tubes pre-filled with NaOH. Detection limit was about 20 $\mu\text{g S}$, or approximately 0.1 nM DMS in a 5 ml sample. Sample-to-sample precision was typically 10 to 15%.

Size-fractionated production of DMS from DMSP. In 1 grazing experiment with 100 *Oxyrrhis marina* ml^{-1} , 50 ml subsamples taken at time zero and after 14 h of grazing were filtered sequentially by gentle positive pressure (syringe) through 25 mm filters to remove grazers (Whatman 540 qualitative filters), prey (Milli-

pore 3 μm membrane), and most bacteria (Millipore 0.45 μm membrane). Samples were examined microscopically before and after filtration to insure that qualitative removal of selected organisms took place. Subsamples (5 ml) of whole water, water without grazers but with prey and bacteria (<10 $\mu\text{m}'$), water without grazers or prey but with bacteria (<3 $\mu\text{m}'$), and water without all organisms (<0.45 $\mu\text{m}'$) were assayed for comparative DMS production rates from 50 μM DMSP_d additions.

Isolation of DMSP-lysing bacteria. A culture containing *Oxyrrhis marina* grown on *Dunaliella tertiolecta* was streaked onto plates made with FASW amended with 10 mM Tris, pH 7.4, and 0.1% DMSP-Cl, and incubated at room temperature in the dark. After 4 to 5 d, small colonies were visible and DMS odor was noted. Colonies were restreaked onto marine heterotrophic plates (1% glucose, peptone, 0.5% yeast extract). After several days, numerous uniform colonies formed. A large motile rod (~1 \times 2 μm) forming pale white colonies was the main strain found, and the only one which produced DMS from DMSP. The ability of this culture to degrade DMSP was studied by dispersing a loopful (ca 50 μl) of the plate culture in several ml ASW with 50 mM Tris, pH 7.4, and adding 100 μl to DMSP enzyme assays described below.

DMSP enzyme assays. DMSP lyase was assayed by observing the production of DMS from additions of DMSP in both whole-cell and sonicated cell extracts. Concentrated DMSP-Cl solution in FASW (titrated to pH 4 with NaOH) was added to a final concentration of 10 to 100 μM and the bottles were crimp-sealed with Teflon-lined septa and incubated in the dark at room temperature. Headspace samples (0.1 to 1 ml) were withdrawn by gas-tight syringe and injected onto the GC column to measure production of DMS.

Amendments and inhibitors were prepared as follows: glycine betaine (hydrochloride) was added to 100 mM from a 1 M stock in FASW/Tris, titrated with 10 N NaOH to pH 4.5. Chloramphenicol was added to final concentration of 400 $\mu\text{g ml}^{-1}$ from a stock solution of EtOH (100 mg ml^{-1} ; 20 μl stock per bottle). 20 μl of EtOH without chloramphenicol was added to the other bottles to account for any inhibition by the EtOH.

After incubations, 1 ml 10 N NaOH was added and DMS + DMSP was measured by headspace to estimate the amount of unconsumed DMSP. The difference between DMS + DMSP and the DMSP initially added was taken to represent the DMSP which was demethylated or metabolized to other products.

To test the ability of the protists to degrade DMSP, 60 ml of concentrated *Oxyrrhis marina* culture grown on *Dunaliella tertiolecta* was centrifuged at 2000 $\times g$ for 15 min to remove most bacteria. The supernatant was removed and the pink cell pellet resuspended in

4 ml FASW amended with 50 mM Tris, pH 7.4. Cells were then recentrifuged and resuspended a second time. Visual examination showed concentrated, motile *O. marina*. One-half of the cell suspension was sonicated for 15 s to lyse cells. Aliquots (1 ml) of the lysed and unlysed cell suspensions were added to 7 ml seawater buffer and DMSP-HCl was added to final concentration of 1 μM , and the samples were incubated in the dark at room temperature. Subsamples (50 μl) were sparged periodically for DMS and DMSP over 24 h. Control samples contained 8 ml seawater buffer with no cells.

Chemicals. DMSP-HCl was obtained from Research Plus (Bayonne, NJ, USA) and glycine betaine HCl from Aldrich (#14,793-1, Milwaukee, WI, USA). Stocks for bacterial growth were kept frozen until use. Stocks for GC standards were acidified with 10% HCl to prevent bacterial growth and stored at room temperature.

RESULTS

Removal of algal DMSP_p and production of DMSP_d during grazing

Four experiments employed similar prey densities (ca 3000 ml^{-1}), but initial grazer numbers varied from about 150 to 450 ml^{-1} . In bottles without grazers, *Emiliana huxleyi* numbers and DMSP and DMS pools were unchanged over the 24 h experiment periods (Figs. 1 & 2a). They showed little or no release of DMSP from the particulate to the dissolved pool, and little or no production of DMS, indicating low or no endogenous production of DMS from *E. huxleyi*. In 1 experiment, the bottles without grazers showed conversion of dissolved DMSP to DMS after about 12 h, apparently due to bacteria from the grazed bottles introduced during non-sterile sampling (see 'Discussion'). Internal *E. huxleyi* DMSP concentrations per cell were ca 30 to 100 mM, based on a cell diameter of 4 μm and assuming no vacuole space. DMSP_d and DMS in the algal culture were low. *Oxyrrhis marina* cultures raised on low-DMSP-titer prey (*Dunaliella tertiolecta*) contributed <5% of the total DMSP and DMS in the experiments.

The presence of *Oxyrrhis marina* caused a decline in the number of *Emiliana huxleyi*, but this did not result in any appreciable increase of *O. marina* cell numbers over 24 h (Fig. 1). Grazer numbers enumerated by epifluorescent microscopy were consistently lower than when enumerated by dissecting microscope, indicating lysis of protist cells during preservation, staining, and/or filtration. Prey were visible inside protist vacuoles within minutes of adding the prey and throughout the experiments.

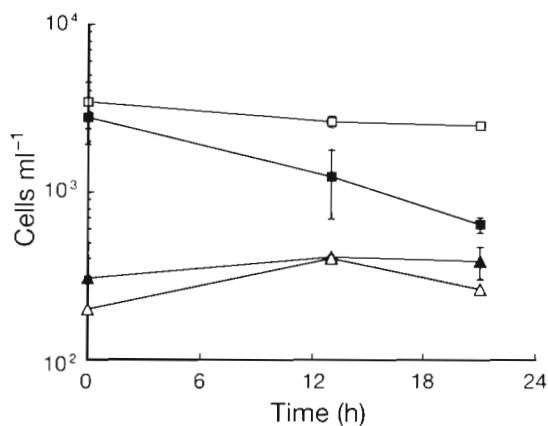


Fig. 1. *Oxyrrhis marina* grazing on *Emiliana huxleyi*. Grazing effect on cell numbers for experiment where *O. marina* density was about 350 ml⁻¹. *E. huxleyi* prey are shown for bottles without grazers (□) and with grazers (■). *O. marina* numbers are from bottles with prey (▲) and without prey (△). Numbers are means of duplicate bottles, with at least 10 individual counts of microscopic fields or actively swimming *O. marina* in 10 µl droplets, and error bars are 1 SD

In the predator+prey bottles, coincident with the decrease in prey, particulate DMSP also disappeared, and dissolved DMSP and DMS increased in the grazed bottles compared to those with out grazers (Fig. 2, Table 1). However, in all cases the decrease in DMSP_p was greater than the increase in DMS and DMSP_d. DMSP_p decreased more slowly than prey numbers (Fig. 3), suggesting that a portion of DMSP_p during the experiment was contained inside grazers, and this fraction increased as grazing continued. It is possible that part of the increase in DMSP_d observed was due to breakup of *O. marina* cells during filtration and release of undigested DMSP; this was not quantified but likely due to the fragility of the flagellates. The rate of fractional loss of DMSP_p [(change in DMSP_p)/(initial DMSP_p)/time] increased with increasing grazer density (data not shown).

Bacterial production of DMS from DMSP

The increase in DMS observed in all experiments coincided with an increase in bacterial numbers as determined by acridine orange direct count (AODC). These bacteria were probably introduced with the *Oxyrrhis marina* culture, since the other components of the experimental system were bacteria-free. Conversion of DMSP to DMS and consumption of DMS in prey and predator cultures was tested by addition of either compound to concentrated cultures used for the experiments. The *O. marina* culture, including *Dunaliella tertiolecta* prey and associated bacteria, showed rapid and linear conversion of DMSP to DMS, but

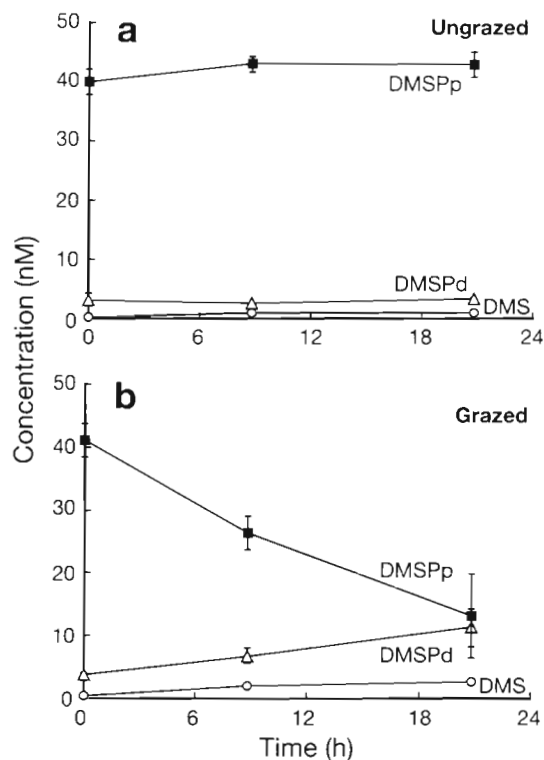


Fig. 2. *Oxyrrhis marina* grazing on *Emiliana huxleyi*. Grazing effect on DMS and DMSP for experiment where initial *O. marina* density was about 450 ml⁻¹. Shown are means of duplicate bottles (a) without grazers and (b) with grazers for DMS (○), DMSP_d (△), and DMSP_p (■). Error bars show ranges

no consumption of DMS (Fig. 4). The axenic *Emiliana huxleyi* culture showed little if any activity (data not shown). A sample of the *O. marina* culture was plated onto DMSP-FASW agar and a bacterium was isolated

Table 1. *Oxyrrhis marina* grazing on *Emiliana huxleyi*. Summary of DMSP and DMS pool changes in 3 grazing experiments

	Grazer density (cells ml ⁻¹)		
	159	366	476
Experiment period (h):	22.5	22.0	20.8
Initial pools (nM) ^a			
DMS	1.1 (0.2)	1.7 (0.1)	0.5 (0.1)
DMSP _d	5.7 (1.5)	2.4 (0.2)	3.8 (0.1)
DMSP _p	32.1 (5.2)	17.2 (2.5)	41.1 (2.7)
Change (nM)			
DMS	+ 3.4	+ 0.7	+ 2.2
DMSP _d	+ 6.0	+ 1.5	+ 7.6
DMSP _p	- 12.4	- 9.0	- 28.0
Net change in ΣDMS (nM) ^b	- 3.0	- 6.8	- 18.2

^aAverage with range in parentheses
^bΣDMS = DMS + DMSP_d + DMSP_p

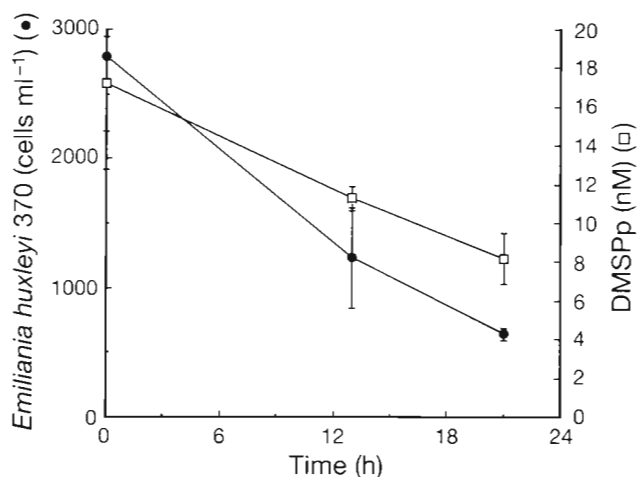


Fig. 3. *Oxyrrhis marina* grazing on *Emiliana huxleyi*. Comparative removal of prey and DMSP_p from experiment with about 350 *O. marina* cells ml⁻¹

that lysed DMSP to DMS. After growth on rich heterotrophic-marine agar plates, this organism showed a lag of about 1 to 3 h at room temperature before converting 20 μM DMSP (Fig. 5), and this lag period was greatly extended by 400 $\mu\text{g ml}^{-1}$ chloramphenicol, suggesting an inducible DMSP lyase enzyme. Addition of 100 mM glycine betaine had no effect on DMS production (Fig. 5). No other DMS-producing strains from the *O. marina* culture were isolated, although the presence of other strains with different DMSP metabolism (e.g. demethylation) cannot be ruled out. Addition of 100 μM glycine betaine in 1 grazing experiment with 150 *O. marina* ml⁻¹ did not result in any significant changes in DMS or DMSP concentra-

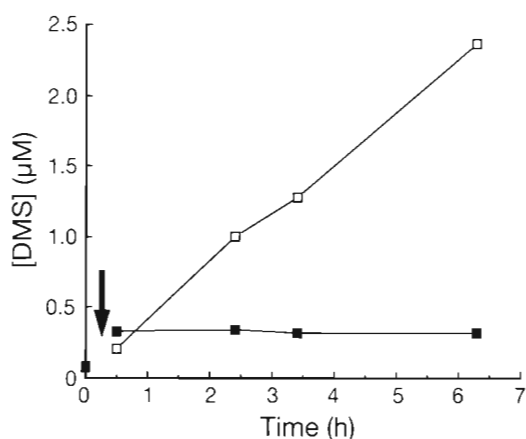


Fig. 4. Ability of concentrated, non-axenic *Oxyrrhis marina* culture to convert DMSP to DMS (□), but inability to consume DMS (■). Time zero shows endogenous DMS in culture; at 0.5 h 200 μM DMSP (□) or 0.3 μM DMS (■) was added (arrow) to 5 ml aliquots in sealed serum vials and DMS production or consumption was monitored by headspace analysis

tions in any bottles, with or without grazers (data not shown).

Degradation of DMSP without production of DMS by lysed *Oxyrrhis marina* cells

The lysed *Oxyrrhis marina* samples removed approximately 80% of the DMSP during the incubation without significant production of DMS while controls without cells were stable except for slight DMS production after 18 h (Fig. 6). Unlysed *O. marina* samples showed approximately 10% removal of DMSP with production of DMS (data not shown). After 24 h, unlysed samples were examined by dissecting microscope and motile *O. marina* were observed. AODC slides of the lysed samples showed concentrated cell debris as well as bacteria.

Size-fractionated production of DMS from DMSP in grazing experiments

At both time zero and after 14 h of grazing, the sample fractions from bottles without grazers did not show significant activity, consistent with the results of the *Emiliana huxleyi* culture alone. With addition of *Oxyrrhis marina* and associated bacteria, DMSP conversion activity was found at time zero in samples including grazers and prey and prey alone (Fig. 7a), while after 14 h activity was found in the bacterial fraction as well (Fig. 7b). The production of DMS in these bottles was initially weak and was noticeably greater after incubation for 6 h, suggesting bacterial growth.

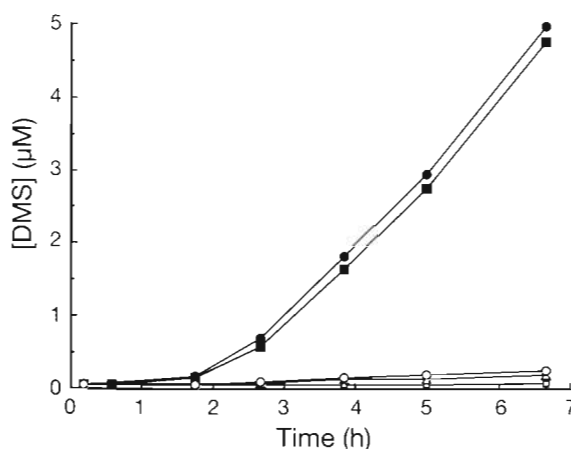


Fig. 5. Conversion of 20 μM DMSP to DMS by bacterium isolated from *Oxyrrhis marina* culture. Experiment was conducted after bacterial growth on heterotrophic media without DMSP. (■) No amendments; (●) with 100 mM glycine betaine; (Δ) with 400 $\mu\text{g ml}^{-1}$ chloramphenicol; (□) with betaine and chloramphenicol; (□) no bacteria

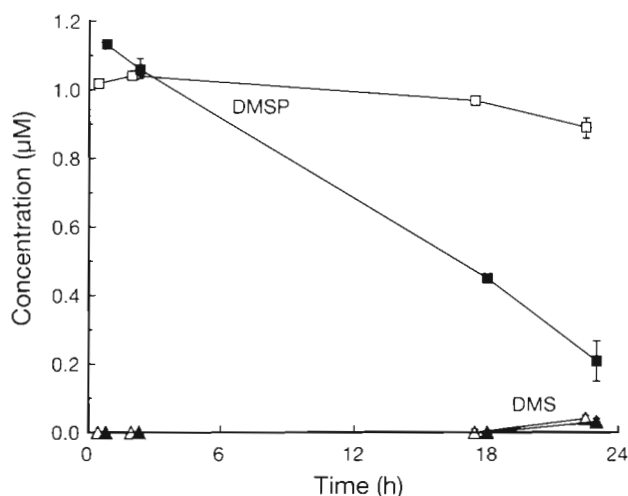


Fig. 6. Removal of DMSP_d (■) without production of DMS (▲) by lysed *Oxyrrhis marina* cells in Tris-buffered FASW (pH 7.4). (□, △) DMSP_d, DMS in controls without *O. marina*

DISCUSSION

These experiments show that protist grazing may be a sink for algal DMSP_p and may also convert it to dissolved or submicron form, making it available for metabolism by bacteria. Therefore, herbivorous protists may be a key *in situ* link between the production of algal DMSP and its conversion to climatically active DMS.

Although we employed an artificially simple system of a single prey and predator, cell densities were reasonably realistic and therefore the results may serve as a model for natural situations. We attempted to use the simplest possible system of a monospecific, axenic prey and a monospecific grazer. Because we did not have *Oxyrrhis marina* in axenic culture, we performed additional tests to determine whether removal and/or conversion of DMSP was due to the grazers or associated bacteria.

A key to understanding the production of DMS from DMSP lies in determining which organisms have the ability to express DMSP-lyase enzyme(s), and under what conditions these are activated. Although marine phytoplankton produce a large fraction of DMSP, it is not obvious that they are all capable of converting it to DMS, or that they do so under natural growth conditions. Reports of DMS production by living phytoplankton cultures are limited (Andreae et al. 1983, Vairavamurthy et al. 1985, Vetter & Sharp 1993) and it is not always clear that these were axenic cultures. Our observations that many phytoplankton cultures are easily contaminated by DMSP-consuming bacteria, and the reports of ubiquitous bacteria which either demethylate or cleave DMSP (Taylor & Gilchrist 1991,

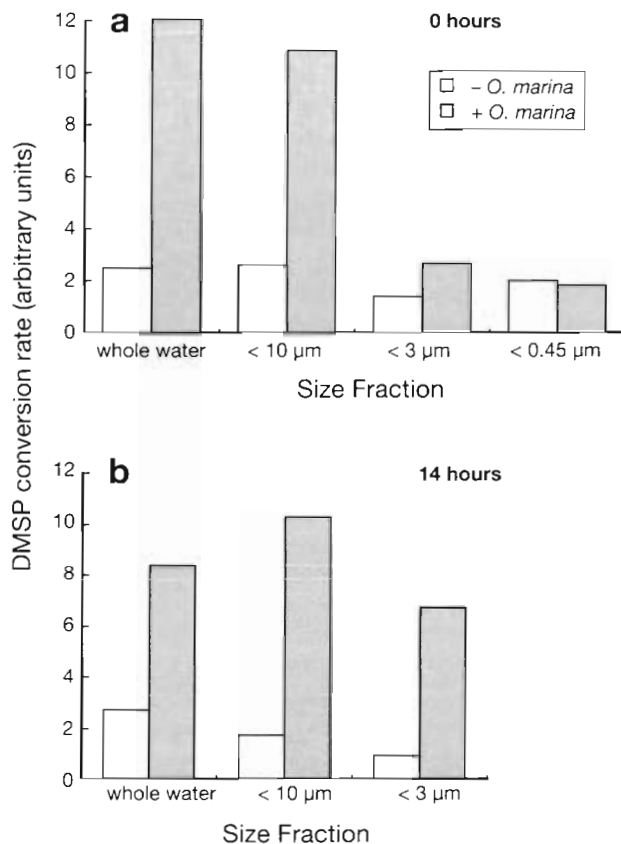


Fig. 7. *Oxyrrhis marina* grazing on *Emiliania huxleyi*. Conversion of DMSP to DMS by size-fractionated samples of an experiment with 100 *O. marina* ml⁻¹ taken at (a) experimental time zero and (b) after 14 h of grazing

Ledyard et al. 1993, Taylor 1993, Visscher et al. 1993), indicate that DMSP released from algal cells may be the most likely to be a source of DMS.

In our experiments, several observations indicate that the actual production of DMS was due to bacteria associated with the grazer cultures. In all cases, the major production of DMS in grazed bottles coincided with increase in bacterial numbers, and in one experiment, we observed this in non-grazed bottles after 12 h, which we believe was due to bacteria accidentally introduced from the grazer bottles during non-sterile sampling. In all experiments DMS rose by similar amounts (0.7 to 3.4 nM) over 21 to 23 h despite widely varying decreases in DMSP_p (9 to 28 nM; Table 1), suggesting a possible decoupling of DMSP-to-DMS conversion from DMSP_p consumption by grazers.

As grazing progressed, both prey and DMSP_p declined, and only a fraction of the lost DMSP_p appeared as DMSP_d or DMS (Figs. 1 & 2, Table 1). This suggests that *Oxyrrhis marina* may have metabolized a large fraction of the algal DMSP without production of DMS. The removal of DMSP in lysed *O. marina* samples

without production of DMS over 24 h also suggests this dinoflagellate may metabolize DMSP, possibly employing it as a methyl donor. Several studies have implicated DMSP in transmethylation reactions in a wide variety of marine heterotrophs (Ishida & Kadota 1968, Nakajima 1993), and this may be a common metabolic fate in many herbivores. The relatively slow removal of DMSP in the lysed samples may also have been due to the growth of DMSP-demethylating bacteria (Taylor & Gilchrist 1991, Visscher et al. 1993), although unlysed cells did not show significant DMSP degradation. However, the grazing experiments also suggested that DMSP may be degraded relatively slowly by *O. marina*. During grazing, phytoplankton prey numbers declined more rapidly than particulate DMSP (Fig. 3). We believe that DMSP production by the prey was low or zero during these unlit experiments; DMSP_p did not increase in samples without grazers (Fig. 2a) and there is some evidence that DMSP production by phytoplankton may be light-dependent (Karsten et al. 1991, Kiene pers. comm.). If prey DMSP_p were degraded rapidly in grazer guts, we would expect both prey numbers and DMSP_p to decline at the same rate. The more rapid removal of prey indicates the accumulation of unmetabolized prey DMSP either in protist food vacuoles or in feeding-associated detritus captured on our filters. By 21 h, this represented nearly 50% of the total DMSP_p, suggesting that turnover of DMSP_p inside grazer vacuoles may have been relatively slow.

Although microbial consumption of DMS is rapid in some marine environments (Kiene & Bates 1990, Kiene & Service 1991, Wolfe & Kiene 1993b), we have found no evidence to date for this process in our culture experiments. Additions of DMS to a concentrated *Oxyrrhis marina*/bacterial assemblage resulted in stable elevated DMS concentrations (Fig. 4), and in additional experiments with inhibitors of DMS consumption such as dimethyl ether (Wolfe & Kiene 1993a) or chloroform, DMS did not accumulate to any degree in incubations with amendments (data not shown). Therefore, we believe that DMS loss was probably dominated by outgassing, which was minimized by low headspace volumes and careful sampling handling. It is possible that microbial DMS consumption at 16 °C is slower than in warmer waters where it has been frequently measured, or it may be that our simplified experimental system simply did not have sufficient microbial diversity to include DMS-consuming bacteria.

If DMS loss were near zero, the accumulation of DMS observed in the grazed samples gives some indication of the rate of DMS production from either dissolved DMSP released during grazing or from the protists or prey themselves, and allows us to assemble a

budget of DMSP consumption and conversion (Fig. 8). Although our results do not exclude the possibility that *Oxyrrhis marina* might be able to take up and lyse dissolved DMSP, the production of DMS in the size-fractionated prey fraction taken from the grazing bottles suggests possible bacterial association with predator and prey (Fig. 7), and we assume that bacteria were the main utilizers of DMSP_d in our experiments.

We do not directly know the fraction of DMSP_d which was demethylated or metabolized to non-DMS products, but we believe it was low in these cultures since the only bacterial isolate which metabolized DMSP_d did not appear to form products other than DMS. Furthermore, addition of glycine betaine, which has been suggested to block transport of DMSP into bacterial cells (Kiene & Service 1993), did not inhibit DMS production in our bacterial isolate, nor did it affect DMSP_d pools in the grazing experiments. This suggests that organisms similar to our isolate may possibly have been the dominant sink for dissolved DMSP in these experiments.

If we assume that demethylation or other metabolism of DMSP_d was negligible, then the combined accumulation of DMSP_d and DMS gives the total DMSP_d produced during grazing. When grazer densities were high (>300 ml⁻¹), the majority of prey DMSP_p (about 70%; Fig. 8a) was consumed by grazers while only

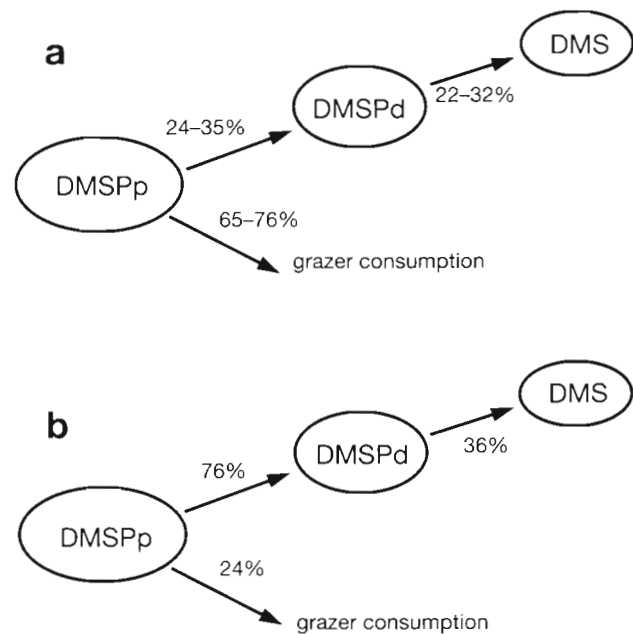


Fig. 8. *Oxyrrhis marina* grazing on *Emiliana huxleyi*. Effect of grazers on DMSP cycling for *O. marina* densities of (a) 350 to 450 ml⁻¹ and (b) 150 ml⁻¹. Percentages next to arrows are fractions of total change in DMSP_p or DMSP_d over 20 to 22 h incubation periods

about 30% was released to the dissolved pool. In the experiment where grazer density was lower, this pattern was reversed (Fig. 8b). In all cases, bacteria converted approximately 25 to 35% of the newly produced DMSP_d to DMS.

Although our evidence suggests that *Oxyrrhis marina* may metabolize DMSP without direct production of DMS, other heterotrophic flagellates and other microherbivores likely have different abilities. DMSP clearly accumulates to some degree in larger predators such as shellfish and fish (Motohiro 1962, Ackman et al. 1966, Ackman & Hingley 1968, Iida & Tokunaga 1986) and may bioconcentrate within marine food webs (Sieburth 1960). Measurements of DMSP in the >240 μm fraction in the NE Pacific showed approximately 10% of total DMSP associated with copepods and other metazoan grazers (Wolfe 1992). Therefore some heterotrophs probably do not rapidly metabolize DMSP. On the other hand, one of the few studies of a DMSP lyase enzyme was in a heterotrophic dinoflagellate, the osmotroph *Gyrodinium cohnii*, which synthesized and lysed DMSP when grown on acetate (Ishida & Kadota 1967). Additionally, Antarctic krill *Euphausia superba* accumulated DMSP from its prey and could enzymatically convert it to DMS (Tokunaga et al. 1977). These reports suggest that various marine heterotrophs probably can cleave, metabolize, and/or accumulate DMSP.

Because micrograzers comprise such a large fraction of marine herbivores and process a large fraction of primary production (Capriulo et al. 1991, Sherr & Sherr 1992), they are likely to be important to DMSP cycling regardless of their biochemical abilities. Only those predators which can lyse DMSP will form DMS directly, but those which excrete ingested DMSP in dissolved or submicron form may be more important to DMS production in the sea because they make it available to bacterial action. It is also possible that grazing stimulates associated bacterial activity by increasing availability of low molecular weight compounds like DMSP associated with egested detritus or dissolved waste. Furthermore, predators which metabolize algal DMSP in other ways, such as via transmethylation reactions, may remove a large fraction of 'potential DMS' and thereby limit the amount of DMS produced. The fraction of grazed DMSP that is metabolized by microzooplankton, or made available to water column bacteria, may be critical to regulating DMS production from marine microbial communities.

Acknowledgements. We thank Dr R. P. Kiene for helpful suggestions and for comments on the manuscript. This work was supported by NASA grant # NAGW-3737.

LITERATURE CITED

- Ackman, R. G., Dale, J., Hingley, J. (1966). Deposition of dimethyl-propiothetin in Atlantic cod during feeding experiments. *J. Fish. Res. Bd Can.* 23: 487–497
- Ackman, R. G., Hingley, H. J. (1968). The occurrence and retention of dimethyl-β-propiothetin in some filter-feeding organisms. *J. Fish. Res. Bd Can.* 25: 267–284
- Andreae, M. O., Barnard, W. R., Ammons, J. M. (1983). The biological production of dimethylsulfide in the ocean and its role in the global atmospheric sulfur budget. *Ecol. Bull.* 35: 167–177
- Belviso, S., Buat-Ménard, P., Putaud, J.-P., Nguyen, B. C., Claustre, H., Neveux, J. (1993). Size distribution of dimethylsulfoniopropionate (DMSP) in areas of the tropical northeastern Atlantic Ocean and the Mediterranean Sea. *Mar. Chem.* 44: 55–71
- Belviso, S., Kim, S.-K., Rassoulzadegan, F., Krajka, B., Nguyen, B. C., Mihalopoulos, N., Buat-Menard, P. (1990). Production of dimethylsulfonium propionate (DMSP) and dimethylsulfide (DMS) by a microbial food web. *Limnol. Oceanogr.* 35: 1810–1821
- Capriulo, G. M., Sherr, E. B., Sherr, B. F. (1991). Trophic behavior and related community feeding activities of heterotrophic marine protists. In: Reid, P. C., Turley, C. M., Burkill, P. H. (eds.) *Protozoa and their role in marine processes*. Springer-Verlag, Berlin, p. 219–265
- Charlson, R. J., Lovelock, J. E., Andreae, M. O., Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326: 655–661
- Cooper, W. J., Matrai, P. A. (1989). Distribution of dimethyl sulfide in the oceans. In: Saltzman, E. S., Cooper, W. J. (eds.) *Biogenic sulfur in the environment*. American Chemical Society, Washington, DC, p. 140–151
- Dacey, J. W. H., Wakeham, S. G. (1986). Oceanic dimethylsulfide: production during zooplankton grazing on phytoplankton. *Science* 233: 1314–1316
- Dickson, D. M., Kirst, G. O. (1986). The role of β-dimethylsulphoniopropionate, glycine betaine and homarine in the osmoacclimation of *Platymonas subcordiformis*. *Planta* 167: 536–543
- Holligan, P. M., Fernández, E., Aiken, J., Balch, W. M., Boyd, P., Burkill, P. H., Finch, M., Groom, S. B., Malin, G., Muller, K., Purdie, D. A., Robinson, C., Trees, C. C., Turner, S. M., van der Wal, P. (1993). A biogeochemical study of the coccolithophore, *Emiliana huxleyi*, in the North Atlantic. *Global biogeochem. Cycles* 7: 879–900
- Iida, H., Tokunaga, T. (1986). Dimethyl sulfide and dimethyl-β-propiothetin in shellfish. *Bull. Jap. Soc. scient. Fish.* 52: 557–563
- Ishida, Y., Kadota, H. (1967). Isolation and identification of dimethyl-β-propiothetin from *Gyrodinium cohnii*. *Agr. Biol. Chem.* 31: 756–757
- Ishida, Y., Kadota, H. (1968). Participation of dimethyl-β-propiothetin in transmethylation reaction in *Gyrodinium cohnii*. *Bull. Jap. Soc. scient. Fish.* 34: 699–705
- Karsten, U., Wiencke, C., Kirst, G. O. (1991). Growth pattern and β-dimethylsulphoniopropionate (DMSP) content of green macroalgae at different irradiances. *Mar. Biol.* 108: 151–155
- Keller, M. D., Bellows, W. K., Guillard, R. R. L. (1989). Dimethyl sulfide production in marine phytoplankton. In: Saltzman, E. S., Cooper, W. J. (eds.) *Biogenic sulfur in the environment*. American Chemical Society, Washington, DC, p. 167–183
- Kiene, R. P. (1992). Dynamics of dimethyl sulfide and dimethylsulfoniopropionate in oceanic water samples. *Mar. Chem.* 37: 29–52

- Kiene, R. P., Bates, T. S. (1990). Biological removal of dimethyl sulfide from seawater. *Nature* 345: 702–705
- Kiene, R. P., Service, S. K. (1991). Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration. *Mar. Ecol. Prog. Ser.* 76: 1–11
- Kiene, R. P., Service, S. K. (1993). The influence of glycine betaine on dimethyl sulfide and dimethylsulfoniopropionate concentrations in seawater. In: Oremland, R. S. (ed.) *The biogeochemistry of global change: radiatively active trace gases*. Chapman and Hall, New York, p. 654–671
- Leck, C., Larsson, U., Bågander, L. E., Johansson, S., Hajdu, S. (1989). DMS in the Baltic Sea — annual variability in relation to biological activity. *J. geophys. Res.* 95: 3353–3363
- Ledyard, K. M., DeLong, E. F., Dacey, J. W. H. (1993). Characterization of a DMSP-degrading bacterial isolate from the Sargasso Sea. *Arch. Microbiol.* 160: 312–318
- Motohiro, T. (1962). Studies on the petroleum odour in canned salmon. *Mem. Fac. Fish. Hokkaido Univ.* 10: 1–65
- Nakajima, K. (1993). Dimethylthetin- and betaine-homocysteine methyltransferase activities from livers of fish, chicken, and mammals. *Nippon Suisan Gakk.* 59: 1389–1393
- Nguyen, B. C., Belviso, S., Mihalopoulos, N., Gostan, J., Nival, P. (1988). Dimethyl sulfide production during natural phytoplankton blooms. *Mar. Chem.* 24: 133–141
- Rassoulzadegan, F. (1991). Methods for the study of marine microzooplankton — session summary. In: Reid, P. C., Turley, C. M., Burkill, P. H. (eds.) *Protozoa and their role in marine processes*. Springer-Verlag, Berlin, p. 39–57
- Sherr, E. B., Sherr, B. F. (1992). Trophic roles of pelagic protists: phagotrophic flagellates as herbivores. *Ergebn. Limnol.* 37: 165–172
- Sherr, E. B., Sherr, B. F. (1993). Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp, P. F., Sherr, B. F., Sherr, E. B., Cole, J. J. (eds.) *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton, p. 207–212
- Sieburth, J. M. (1960). Acrylic acid, an 'antibiotic' principle in *Phaeocystis* blooms in Antarctic waters. *Science* 132: 676–677
- Stefels, J., van Boekel, W. H. M. (1993). Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. *Mar. Ecol. Prog. Ser.* 97: 11–18
- Taylor, B. F. (1993). Bacterial transformations of organic sulfur compounds in marine environments. In: Oremland, R. S. (ed.) *The biogeochemistry of global change: radiatively active trace gases*. Chapman and Hall, New York, p. 745–781
- Taylor, B. F., Gilchrist, D. C. (1991). New routes for aerobic biodegradation of dimethylsulfoniopropionate. *Appl. environ. Microbiol.* 57: 3581–3584
- Tokunaga, T., Iida, H., Nakamura, K. (1977). Formation of dimethyl sulfide in Antarctic krill, *Euphausia superba*. *Bull. Jap. Soc. scient. Fish.* 43: 1209–1217
- Vairavamurthy, A., Andreae, M. O., Iverson, R. L. (1985). Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. *Limnol. Oceanogr.* 30: 59–70
- Vetter, Y.-A., Sharp, J. H. (1993). The influence of light intensity on dimethylsulfide production by a marine diatom. *Limnol. Oceanogr.* 38: 419–425
- Visscher, P. T., Diaz, M. R., Taylor, B. F. (1993). Enumeration of bacteria which cleave or demethylate dimethylsulfoniopropionate in the Caribbean Sea. *Mar. Ecol. Prog. Ser.* 89: 293–296
- Wolfe, G. V. (1992). The cycling of climatically active dimethyl sulfide (DMS) in the marine euphotic zone: biological and chemical constraints on the flux to the atmosphere. Ph.D. thesis, University of Washington
- Wolfe, G. V., Kiene, R. P. (1993a). Effects of methylated, organic, and inorganic substrates on microbial consumption of dimethyl sulfide in estuarine waters. *Appl. environ. Microbiol.* 59: 2723–2726
- Wolfe, G. V., Kiene, R. P. (1993b). Radioisotope and chemical inhibitor measurements of dimethyl sulfide consumption rates and kinetics in estuarine waters. *Mar. Ecol. Prog. Ser.* 99: 261–269

This article was submitted to the editor

Manuscript first received: February 23, 1994

Revised version accepted: May 30, 1994