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Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration*

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ABSTRACT: Dimethylsulfide (DMS) is an important trace gas which is emitted from seawater to the atmosphere. DMS is believed to be derived primarily from the plant osmolyte 3-(dimethylsulfonium)-propionate (DMSP). This study examined the decomposition of dissolved DMSP ($DMSP_{diss}$), the production of DMS from $DMSP_{diss}$, and the consumption of DMS in estuarine waters near Sapelo Island, Georgia, USA. Rate of $DMSP_{diss}$ disappearance from seawater was directly proportional to the concentration of $DMSP_{diss}$, over the range of concentrations tested (20 to 100 nM), and was a function of temperature; rates were very low at 4 °C and increased progressively at 16, 23 and 30 °C. At 49°C the rate of $DMSP_{diss}$ metabolism was substantially lower. The production of DMS from $DMSP_{diss}$ displayed similar concentration and temperature dependence. However, a mass balance of total DMSP during dark incubations indicated that < 30 % of the DMSP consumed during the experiments was converted to DMS, even when chloroform (500 µM) was included to prevent DMS consumption. Chloroform did not affect DMSP decomposition or DMS production from this compound. Thus, we conclude that DMS is not the major sulfur product of DMSP metabolism in estuarine waters. An alternative route for DMSP metabolism, possibly involving demethylation, is suggested. Rate of DMS consumption was directly dependent on DMS concentration and incubation temperature. DMS metabolism was strongly inhibited by chloroform (500 µM) and azide (0.25 %). Results suggest that DMS production and consumption may be closely coupled in natural seawater since both these processes displayed similar concentration and temperature dependence. The characteristics (kinetic parameters) of the enzyme systems involved in DMS production and consumption are likely to play a major role in controlling the concentration of DMS in seawater.

INTRODUCTION

The biogeochemical cycling of dimethyl sulfide (DMS) in seawater has come under close scrutiny in recent years because the oceans are a globally significant source of DMS to the atmosphere (Andreae 1986, 1990, Erickson 1990). The flux of DMS has important implications for the chemistry of the troposphere and, possibly, climate regulation (Bates et al. 1987, Charlson et al. 1987, Andreae 1990). Recent models of DMS cycling in seawater indicate that the distribution and concentration of DMS is governed to a significant extent by complex interactions in the food web involving phytoplankton and zooplankton, as well as bacteria, which metabolize both DMS and 3-(dimethylsulfonium)propionate (DMSP) (Wakeham & Dacey 1989, Kiene & Bates 1990, Kiene unpubl.).

Seawater DMS is believed to originate from DMSP, which is an osmolyte in certain marine plants (White 1982, Reed 1983, Vairavamurthy et al. 1985, Keller et al. 1989). In the open ocean, phytoplankton appear to be the major source of DMSP and several recent studies have shown that the distribution of DMS is related to the distribution of DMSP and the phytoplankton which produce this compound (Holligan et al. 1987, Turner et al. 1988, Iverson et al. 1989, Gibson et al. 1990). In coastal and estuarine areas, macroalgae and even rooted macrophytes such as *Spartina alterniflora* may also be sources of DMSP in the water column (Karsten et al. 1990, Pakulski & Kiene unpubl.).

DMSP can be degraded via an enzymatic elimination reaction to yield DMS and acrylic acid according to the following equation (Cantoni & Anderson 1956, Dacey & Blough 1987):

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$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow (CH_3)_2S + CH_2 = CHCOO^- + H^+$$

DMSP lyase activity has been found in algal extracts (Ishida 1968) and bacteria (Dacey & Blough 1987, Kiene 1990, Ledyard & Dacey 1990). In seawater, DMSP is found in a particulate pool ($DMSP_{part}$), which is presumably algae, algal grazers, fecal pellets and detritus; and in a dissolved or 'free' pool ($DMSP_{diss}$) (Turner et al. 1988, Iverson et al. 1989). The processes involved in conversion between $DMSP_{part}$ and $DMSP_{diss}$ and the production of DMS from these pools are still not well understood. However, recent studies indicate that microbial turnover of both $DMSP_{diss}$ and DMS occur at significant rates in seawater. These reactions are likely to be important factors which affect the concentration of DMS (Kiene & Bates 1990, Kiene 1990 unpubl.).

The aim of the present study was to examine the decomposition of dissolved DMSP and DMS in estuarine waters near Sapelo Island, Georgia, USA. We tested whether the metabolism of both $DMSP_{diss}$ and DMS was a function of the respective substrate concentrations and temperature. We also investigated the quantitative relationship between the decomposition of $DMSP_{diss}$ and the production of DMS from this pool. The influences of some inhibitors on these processes were also studied.

MATERIALS AND METHODS

Sample collection. Water samples used in this study were collected at Marsh Landing Dock, located on the Duplin River, Sapelo Island (31° 23' N, 81° 17' W). The Duplin River is a large tidal creek, with little direct freshwater input. Salinity in the region of sampling is generally 25 ‰, but varies between 15 and 32 ‰ depending on freshwater inputs from nearby coastal plain rivers. Water for experiments was collected directly into the incubation vessels from a small boat tied to the dock. While collecting, bottles were held several centimeters below the surface and always faced into the tidal current. Samples were immediately placed in the dark and transported to the laboratory within 10 min.

Incubation of samples. Experiments were carried out by incubating water samples and measuring the concentrations of DMS, $DMSP_{part}$ and $DMSP_{diss}$ over time. Not all pools were measured in each experiment. Incubation vessels were either 250 ml translucent Teflon (FEP) or 1 l polycarbonate bottles. All samples were incubated in the dark. With the exception of the temperature dependence experiments (see below), all samples were incubated within 2 °C of the in situ temperature. Samples were not agitated, except during sampling, when they were gently inverted before subsample removal. All incubation vessels were acid-rinsed (10 % HCl) and thoroughly rinsed with distilled water thereafter. The Teflon bottles were sterilized by autoclaving between experiments.

Experimental. Individual experiments were used to focus on the concentration and temperature dependence of $DMSP_{diss}$ and DMS metabolism as well as the effects of selected inhibitors on these processes. Inhibitors used included sodium azide (NaN_3, 0.25 % [w/v] final concentration), a general inhibitor of biological activity, and chloroform ($CHCl_3$, 500 µM), which has been shown to inhibit DMS metabolism in oceanic waters (Kiene & Bates 1990). Previous results indicated that this concentration of $CHCl_3$ did not affect DMS production from DMSP (Kiene 1990). Duplicate or triplicate bottles for each treatment were used where possible. In some experiments, single sample bottles for each treatment were used because either short time courses were desired or multiple treatments were required. This was necessary because changes in DMS and DMSP occurred over relatively short time scales (minutes) and DMS must be measured immediately, with each analysis requiring 5 to 7 min.

For the temperature dependence experiments, water samples were collected and then preincubated at the selected temperatures (4, 16, 23, 30 and 49 °C) for 1 h before additions were made and measurements begun. Separate experiments were carried out with additions of $DMSP_{diss}$ (45 nM) and with DMS (7 nM). In each experiment, controls which did not receive amendment were run at each temperature.

In further experiments, DMS and $DMSP_{diss}$ were added to selected samples at concentrations within the range of those observed in natural waters. DMS was added from a stock prepared in distilled water to give final added concentrations from 3 to 20 nM. DMSP was added from reagent stock solutions to give final concentrations of added $DMSP_{diss}$ ranging from 10 to 80 nM. Addition volumes were always less than 100 µl. Measurements of the initial concentrations of sulfur compounds in the samples were made immediately after the additions to determine the actual concentration resulting from the addition (amount added + endogenous pools). A separate experiment designed to examine the quantitative relationship between DMSP decomposition and DMS production involved the addition of 40 nM $DMSP_{diss}$ or 500 µM $CHCl_3$ or both.

Analytical. For analysis of DMS, a modification of the gas-stripping, cryotrapping method, used by other investigators, was employed (Andreae & Barnard 1983, Turner et al. 1988, 1990). A 4 ml sub-sample was withdrawn from each incubation vessel with a Teflon tube (1.5 mm dia.) which was attached to a glass-barreled syringe by means of a luer fitting. The Teflon

tube was removed from the syringe and a filter unit (Gelman), with a 2.5 cm glass fiber filter (Whatman GF/F or Gelman A/E, 0.7 and 1.0 µm nominal retention respectively) and an attached 22 gauge needle was connected. The volume in the syringe was carefully adjusted to 2 ml and the sample was then introduced via a septum into the gas-stripping system.

A diagram of the gas-stripping system is shown in Fig. 1. The sparging system consisted of a silanized glass tube with a porous glass frit on which the water

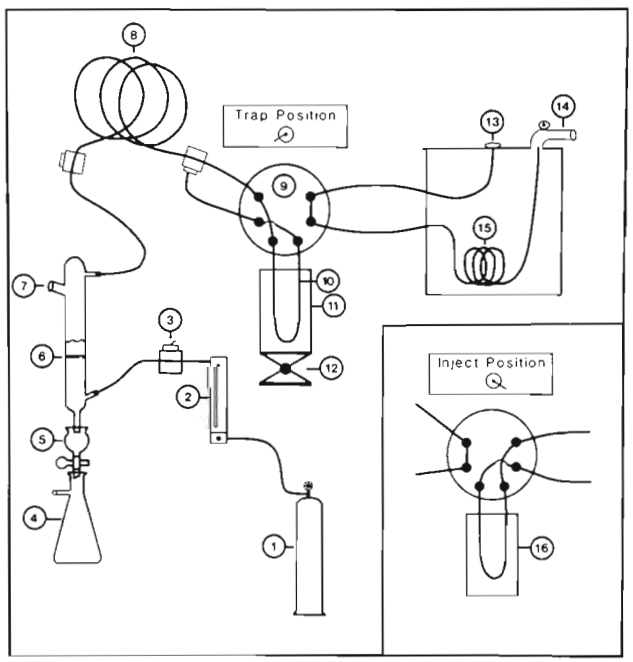

Fig. 1. Gas trapping and analysis system used in the present study. 1, Helium sparge gas; 2, rotometer; 3, toggle valve; 4, vacuum flask; 5, Omnifit 2-way valve; 6, porous glass frit; 7, crimp seal port; 8, Nafion dryer; 9, Valco 6-port valve; 10, sample loop of 1/8" (3.2 mm) Teflon tubing; 11, dewar with liquid N_2; 12, lab jack; 13, GC injector port; 14, flame photometric detector; 15, analytical column; 16, dewar with hot (> 70 °C) water

rested. The water sample was injected through a Teflon-faced septum on a side-port above the frit. The septum was held on by an aluminum crimp. Helium (ultra high purity) was used as the sparge gas and was introduced under the frit at a rate of 100 ml min^{-1}. Volatile sulfur compounds were stripped from the water sample and carried with the sparge gas through a Nafion dryer (a membrane selectively permeable to water vapor; Perma-Pure, Inc., Toms River, NJ, USA) and into a 6 port stainless steel valve (Valco). With the valve in the trap position, the sample gas stream passed through a 30 cm long (3 mm O.D.) Teflon loop immersed in liquid nitrogen. A small amount (0.2 cm^3) of Teflon wool was inserted in the middle of the Teflon loop to increase the surface area for gas trapping. When the sparging was completed (2.5 min), the valve was turned to the inject position which put the Teflon loop in line with the column in the gas chromatograph (GC). The loop was quickly placed in hot (> 70 °C) water and the carrier gas from the injector port of the GC was used to sweep the sample out of the Teflon loop and into the chromatographic column.

The GC was a Shimadzu GC-9A equipped with a flame photometric detector. The column was teflon [2 m × 1/8" (3.2 mm) dia.) filled with Carbopack BHT-100 (Supelco). The oven temperature was 100 °C and the carrier was He at 60 ml min^{-1}. Under these conditions, DMS eluted with a retention time of about 0.9 min, and was adequately separated from other sulfur gases such as CS_2. Peak areas were recorded with a Shimadzu CR-6A integrator. DMS standards were prepared gravimetrically in ethylene glycol as described by Andreae & Barnard (1983). Microliter quantities of standard were injected directly into the sparging system which held 2 ml of pre-sparged distilled water. The standard was then sparged and trapped in the same way as the samples. The square root of the peak area was a linear function of the amount of DMS injected. The minimum detectable amount of DMS ranged from 0.1 to 1 pmol per injection depending on the sensitivity of the detector, which varied during the course of the study. For typical water volumes analyzed (2 ml), this method gave detection limits for DMS of 50 to 500 pM. DMS standards prepared in glycol were compared to DMSP standards which were treated with base to give DMS (as described below). This comparison between the 2 different standards showed agreement to within 5 %.

Particulate and dissolved DMSP. Particulate DMSP was defined as that retained on a glass fiber filter (Whatman GF/F or Gelman A/E, nominal retention of 0.7 and 1.0 µm respectively). No differences between these types of filters were observed with respect to DMSP analysis in estuarine waters. Furthermore, submicrometer particles did not appear to contribute significantly to $DMSP_{part}$ since samples previously passed through glass fiber filters yielded insignificant amounts of DMSP on 0.2 µm filters. Incubation vessels were gently mixed by inverting the bottles before 10 to 20 ml was removed for analysis. The sample was filtered by gravity and the filters placed in 14 ml serum bottles. Two ml of 5 M NaOH was added and the bottles quickly capped with Teflon-faced septa. The samples were then allowed to react for at least 6 h; usually 12 to 24 h at 25 °C. DMSP is quantitatively and stoichiometrically decomposed in strong alkali to DMS and acrylate (White 1982, Dacey & Blough 1987). The DMS evolved into the headspace was measured by removing a subsample of the headspace gas and injecting it

directly into the GC column. DMSP standards were prepared by adding known amounts of DMSP to the vials and treating these exactly as the samples. Reagent DMS·HCl was obtained from Research Plus, Inc., and stock solutions were stored frozen.

Dissolved DMSP was defined as that which passed through the glass fiber filters. After the filtered water samples were sparged to remove DMS, they were pulled through the glass frit of the sparge tube into a glass side-arm flask. The sample was then poured into a small polyethylene vial and immediately frozen and stored for later analysis. For analysis, a subsample of 1 ml was injected into the sparging tube and 1 ml of 5 M NaOH added to produce DMS from the DMSP. The sample was sparged for 5 min after which time tests showed that > 95 % of the DMSP was reacted. The DMS was cryotrapped and quantified as described above. Precision of the dissolved DMSP determinations for natural water samples was 7 % (coefficient of variation; n = 5).

Statistical analyses. Rates (slopes) of consumption and production of DMS and DMSP were compared in the various experiments using SAS analysis of covariance procedures (SAS 1985). An alpha of 0.05 was used in all comparisons.

RESULTS

Decomposition of $DMSP_{diss}$ and production of DMS

Short-term (< 2.5 h) incubation experiments with Duplin River water indicated that net DMS production was immediately stimulated by the addition of $DMSP_{diss}$ (Fig. 2). Accumulation of DMS occurred at the same rate whether 40, 60 or 80 nM DMSP was added ($p > 0.05$) (Fig. 2A). The next day a similar experiment was conducted with 20, 40 and 80 nM concentrations of added DMSP. In this case, the 20 nM DMSP treatment produced a significantly lower ($p < 0.05$) DMS accumulation rate than did 40 nM, whereas accumulation with 80 nM DMSP was only slightly higher than at 40 nM and this result was not significant (Fig. 2B). These results suggested that the production of DMS from $DMSP_{diss}$ may have been saturated at concentrations above the 40 nM added level. However, simultaneous removal of DMS, along with its production, could also have limited the accumulation of DMS.

A subsequent DMSP-addition experiment was carried out in which DMS metabolism was inhibited by the inclusion of chloroform (500 µM) in the samples (Kiene & Bates 1990; see also below for data on the effectiveness of chloroform at preventing DMS removal). With DMS removal blocked, no apparent saturation of DMSP lyase activity was observed through the highest concentration tested (80 nM added DMSP) (Fig. 3A). The rate of DMS accumulation and the net loss rate of $DMSP_{diss}$ over the course of the incubation (< 4.5 h) were both directly related to the initial dissolved DMSP concentration measured in the samples (22 nM endogenous + added DMSP) (Fig. 3B). Despite the presence of chloroform, which blocked the biological removal of DMS, the loss of $DMSP_{diss}$ was not balanced by the accumulation of DMS. A comparison of the slopes of the 2 curves in Fig. 3B revealed that they were significantly different ($p < 0.05$) and only about 30 % of the lost DMSP was accounted for as DMS.

The quantitative relationship between changes in DMSP and DMS concentrations during incubations was investigated further (Fig. 4). In this experiment

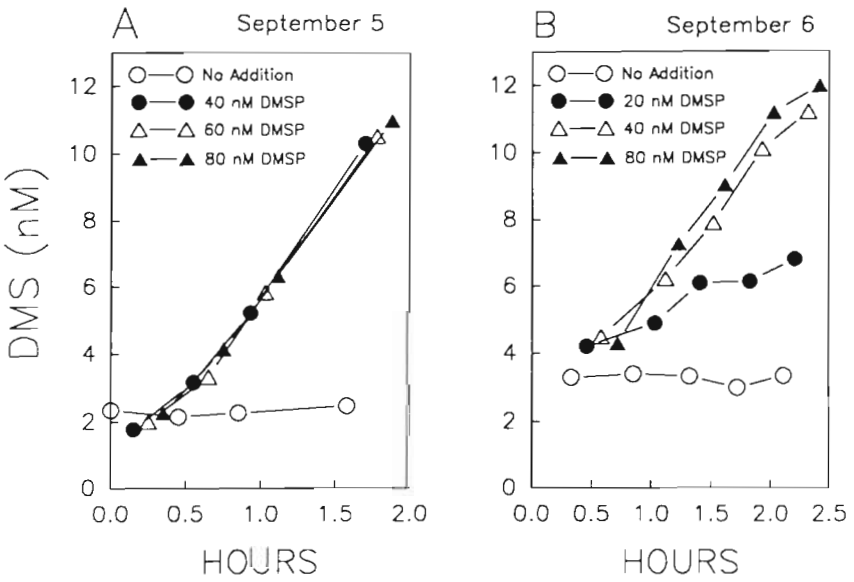

Fig. 2. Time courses of DMS concentrations in water samples treated with various levels of dissolved DMSP. Two experiments were carried out on successive days. Samples were incubated in the dark at the in situ temperature (28 °C). Results are from single sample bottles at each addition level

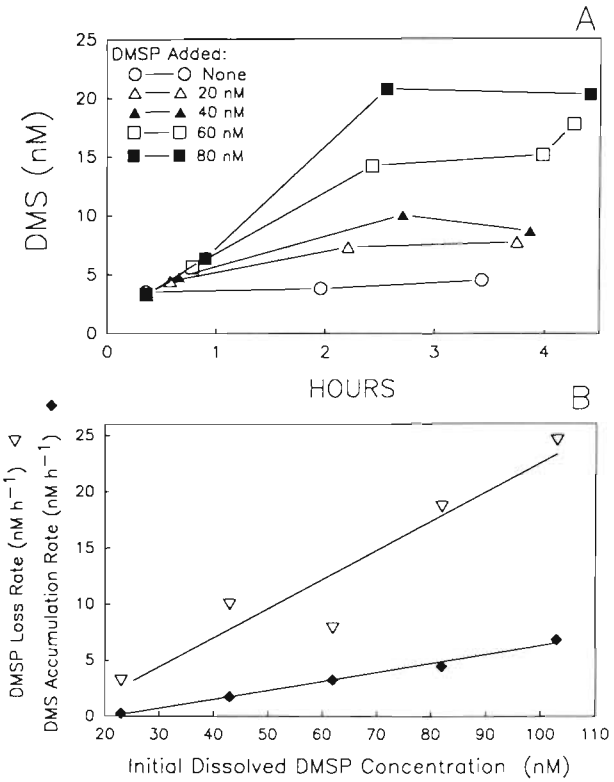

Fig. 3. (A) Time courses of DMS in water samples treated with various levels of dissolved DMSP and with 500 µM $CHCl_3$ to inhibit DMS consumption. Samples were incubated in the dark at 28 °C. Results are from single sample bottles at each addition level. (B) Plots of DMSP loss rates and DMS accumulation rates against the initial dissolved DMSP concentration measured in the sample bottles. Rates were calculated from the linear regression of DMS production (Fig. 3A) or DMSP loss (data not shown) vs time. The least squares fits for the 2 curves are: DMSP loss (nM h^{-1}) = 0.26 [nM DMSP] − 3.384, r^2 = 0.897; and DMS accumulation (nM h^{-1}) = 0.08 [nM DMSP] − 1.648, r^2 = 0.990

both $DMSP_{part}$ and $DMSP_{diss}$ as well as DMS were measured so that an accurate budget of these dimethyl sulfur compounds could be obtained. Particulate DMSP concentrations did not change significantly ($p > 0.05$) over the 6 h incubation period (Fig. 4A). Slightly higher $DMSP_{part}$ levels were seen in the $DMSP_{diss}$-amended samples by the end of the experiment, however these increases were not significant. Endogenous levels of $DMSP_{diss}$ did not change significantly during the experiment (Fig. 4B), and chloroform addition had no effect on this pool ($p > 0.05$). The concentration of $DMSP_{diss}$ decreased rapidly in the samples spiked with 40 nM $DMSP_{diss}$ and this decrease was unaffected by the presence of chloroform ($p > 0.05$) (Fig. 4B). The endogenous pool of DMS remained unchanged (slope = 0, $p > 0.05$) during the incubation (Fig. 4C), but chloroform addition caused DMS to increase at a steady rate (slope significantly greater than 0, $p < 0.05$). As expected, the DMSP-spiked samples had greater DMS production and the accumulation was greater when chloroform was present. However, the rate of increase in DMS for these 2 DMSP-treated samples was not significantly different ($p < 0.05$). The net changes in concentration which occurred in each of the dimethyl sulfur pools during the incubation are given in Table 1. For the DMSP-spiked samples, a net loss of 16 and 20 nM total DMSP ($DMSP_{part}$ + $DMSP_{diss}$ = $DMSP_{total}$) occurred in the uninhibited and chloroform-inhibited samples respectively. In the uninhibited samples with DMSP added, 2.3 nM DMS accumulated, which amounted to 14 % of the lost $DMSP_{total}$, while those with chloroform had 5.5 nM DMS accumulation, equivalent to 28 % of the lost $DMSP_{total}$.

$DMSP_{diss}$ disappeared very slowly when water samples were incubated at 4 °C (Fig. 5A), and DMS produc-

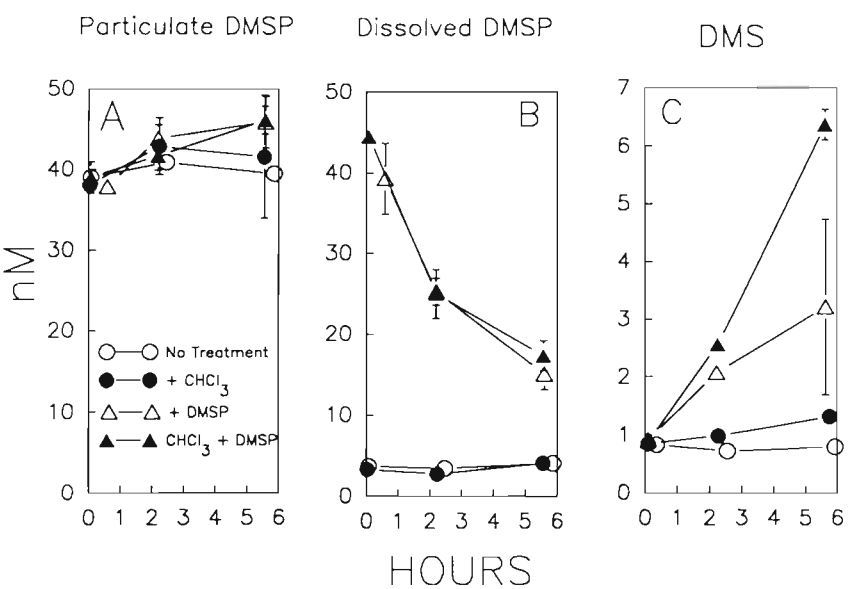

Fig. 4. Time courses of (A) particulate and (B) dissolved DMSP, and (C) DMS in water samples treated with either $CHCl_3$ (500 µM), 40 nM $DMSP_{diss}$, or both. Data represent the mean of duplicate bottles of each treatment with the bars indicating the range. Incubation was in the dark at 15 °C

Table 1. Net changes in $DMSP_{part}$, $DMSP_{diss}$ and DMS concentration during incubations of Duplin River water with various treatments. Incubation period was 6 h. Samples were incubated in 1 l bottles in the dark at the in situ temperature (15° C). Values represent the mean of duplicate bottles for each treatment. Data are taken from time course data in Fig. 3

Treatment	Net change during incubation in nmol l^{-1}			
	Particulate DMSP	Dissolved DMSP	Total DMSP (Diss. + Part.)	DMS
None	0.46	0.37	0.83	−0.04
$CHCl_3$	3.4	0.79	4.2	0.47
DMSP (40 nM)	8.0	−24	−16	2.3
DMSP (40 nM) + $CHCl_3$	7.0	−27	−20	5.5

tion was also slow (Fig. 5B). Despite the slow rates, the changes in both these variables were significant over time (slope $\neq 0$, $p < 0.05$). The rates of $DMSP_{diss}$ disappearance and DMS production at 16, 23 and 30 °C were significantly greater than at 4 °C and the rate increased progressively at these 3 temperatures. Despite the increasing trend, the rates at 16, 23 and 30 °C were not statistically different from each other. There was no significant loss of $DMSP_{diss}$ or production of DMS at 49 °C ($p > 0.05$) (Figs. 5 & 6). Although occurring at different rates, the loss of $DMSP_{diss}$ and the production of DMS had similar temperature dependence (Fig. 6). Rates increased up to 30 °C, but were significantly lower at 49 °C ($p < 0.05$). The initial $DMSP_{diss}$ and DMS concentrations in the 49 °C treatment were higher than other treatments, probably due to some release of $DMSP_{diss}$ from the particulate pool and production of DMS during the 1 h pre-incubation period. Endogenous pools of $DMSP_{diss}$ displayed similar patterns at these temperatures (data not shown), whereas endogenous DMS did not change significantly ($p > 0.05$) from the 1 nM initial level (or 3 nM level in the 49 °C treatment).

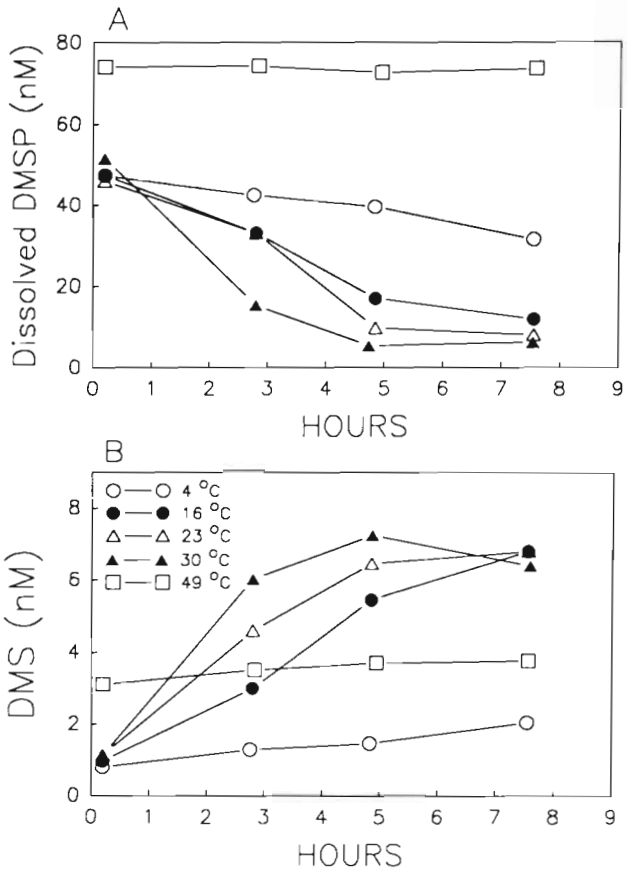

Fig. 5. Influence of temperature on (A) loss of 40 nM added dissolved DMSP and (B) production of DMS in estuarine water. In situ temperature at the time of collection was 15 °C

Consumption of DMS

DMS was readily consumed in estuarine water samples. This activity was strongly inhibited by azide and chloroform (Figs. 7 & 8). In the unspiked samples (no DMS added), both azide and chloroform caused DMS to accumulate at significantly faster rates than in uninhi-

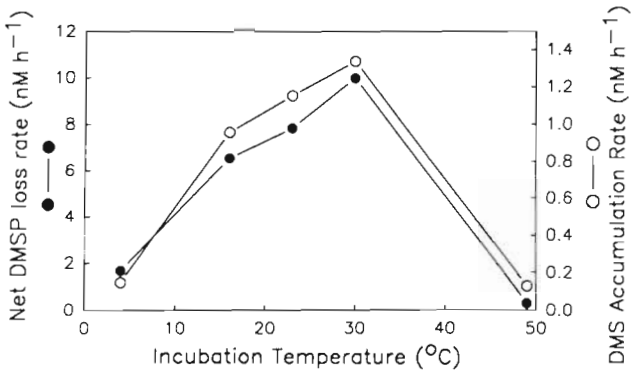

Fig. 6. Net $DMSP_{diss}$ loss rate and DMS accumulation rate vs incubation temperature. Rate data were obtained from time courses in Fig. 5. Only time points from less than 5 h were used for the rate estimates since $DMSP_{diss}$ had declined to endogenous levels by that time in some treatments

bited samples ($p < 0.05$). Net DMS consumption was at times evident from the very start of the incubation (Fig. 7), whereas in other experiments, some net production occurred during the incubation (e.g. Fig. 8). This net production was probably due to decomposition of endogenous pools of DMSP during the dark incubation.

DMS was consumed over a range of concentrations (5 to 20 nM added DMS) in estuarine water (Fig. 9). In this experiment, DMS concentrations were either steady or increased slightly over the first 14 h, and then decreased (Fig. 9A). The rate of DMS consumption over the time period 14 to 50 h was directly related to the concentration of the DMS concentration measured at ca 14 h (Fig. 9B).

The effect of incubation temperature on DMS consumption activity was investigated in a similar manner to that used to study DMSP decomposition. DMS consumption was very low at 4 °C but was progressively higher at 16 and 30 °C (Fig. 10). The rate at 23 °C was significantly lower than at 16 °C ($p < 0.05$), but this may have been due to an addition error resulting in a lower initial DMS concentration in this treatment. At 49 °C the rate of DMS disappearance was significantly lower than at 30 °C ($p < 0.05$). The endogenous levels of DMS (data not shown) were about 2 nM at the start of the experiment and declined to about 1 nM after 24 h at all temperatures except 49 °C. At this temperature, DMS was about 3 nM at the start and did not change significantly ($p > 0.05$) during the incubation.

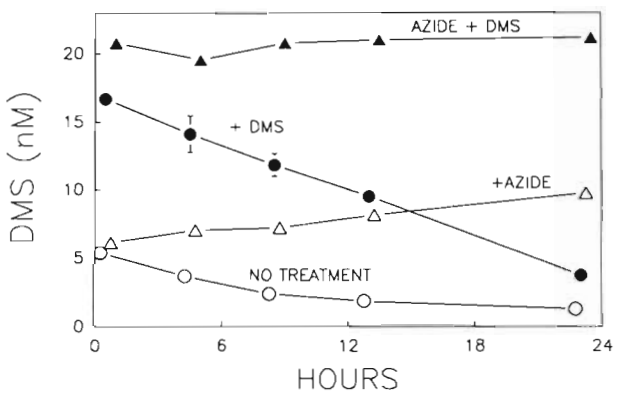

Fig. 7. Effects of azide (0.25 %) on DMS concentrations in DMS-spiked and unspiked estuarine water samples. Results are means of triplicate samples for each treatment, with bars indicating one standard deviation

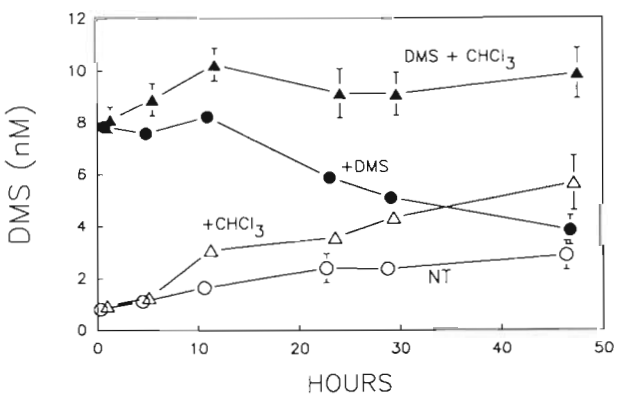

Fig. 8. Effects of chloroform (500 µM) on DMS concentrations in DMS-spiked and unspiked estuarine water samples. Results are means of triplicate samples for each treatment, with bars indicating one standard deviation. NT: samples received no treatment

DISCUSSION

The metabolism of DMSP and DMS are both important processes that affect the concentration of DMS in

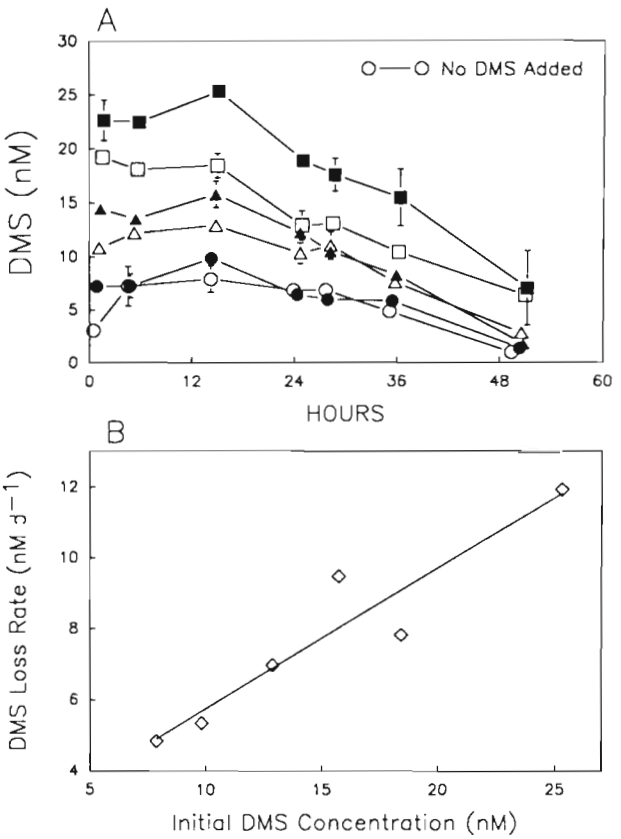

Fig. 9. (A) Time courses of DMS in water samples spiked with different levels of DMS. (○) Endogenous DMS levels; all other symbols reflect DMS added to the level shown. Incubation was in the dark at 25 °C. Data represent means of duplicate bottles for each treatment. Range of duplicates was less than 10 % of mean. (B) DMS consumption rate plotted against DMS concentration measured in samples at 14 h (from [A]). Rates were determined by linear regression analysis of DMS concentration vs time over the period 14 to 50 h. The least squares fit is: DMS loss rate (nM h^{-1}) = 0.395 [nM DMS] + 1.80, $r^2 = 0.891$

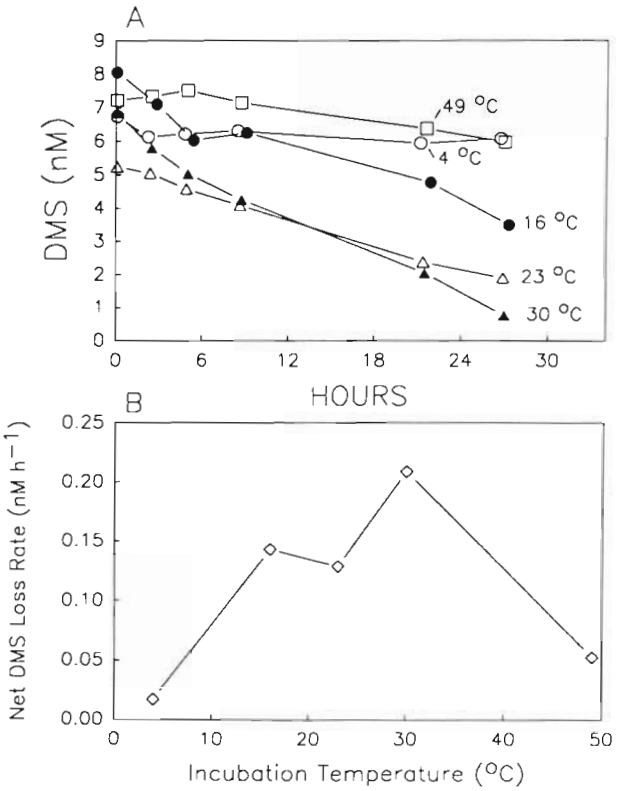

Fig. 10. (A) Effects of incubation temperature on consumption of DMS in estuarine water samples. DMS was added to these samples to give ca 7 nM initial concentration. (Due to an error in addition, the 23 °C treatment received only 5 nM DMS.) Samples were incubated in the dark. Data represent single sample bottles for each treatment. In situ temperature at the time of collection was 15 °C. (B) Temperature dependence of DMS consumption rates. Rates were obtained by linear regression analysis of the time courses in (A). Samples were incubated in the dark

seawater (Kiene & Bates 1990, Kiene unpubl.). Several recent studies have reported $DMSP_{diss}$ concentrations ranging from 2 to 180 nM in estuarine, shelf and oceanic waters (Turner et al. 1988, Iverson et al. 1989, Lee & Wakeham 1988, Kiene in press). DMS concentrations are generally lower than those of DMSP and usually fall in the range 1 to 30 nM (Andreae & Barnard 1984, Turner et al. 1988). Higher DMS concentrations (up to 290 nM) may be found in blooms of certain DMSP-producing phytoplankton such as *Phaeocystis pouchetii* (Gibson et al. 1990). Bacteria which utilize DMSP and produce DMS have been isolated from seawater (Dacey & Blough 1987, Ledyard & Dacey 1990, Kiene 1990). However, aerobic organisms which metabolize DMS have thus far only been isolated from non-marine habitats (Suylen et al. 1986, Kanagawa & Kelly 1986).

In this study, small (< 50 nM) spikes of $DMSP_{diss}$ were rapidly consumed in estuarine water samples (Figs. 4 & 5). Concentrations of $DMSP_{diss}$ decreased from 45 nM to about 18 nM in 5.5 h (Fig. 4B). This indicates a very high potential for $DMSP_{diss}$ turnover in these waters. The consumption of $DMSP_{diss}$ is biologically mediated since it is inhibited by autoclaving and 0.2 µm filtration (Kiene 1990) and displays a temperature maximum (Fig. 5). The production of DMS from $DMSP_{diss}$ displays similar characteristics. Unfortunately, the in situ turnover rate of DMSP in these estuarine waters is not known, and no estimates of $DMSP_{diss}$ turnover have been given elsewhere. The rapid consumption of the spike additions of $DMSP_{diss}$ suggest that the turnover of the endogenous pool must be very rapid, probably on the order of hours. This is also suggested by the fact that particulate DMSP concentrations in waters around Sapelo Island can range from 40 to 300 nM, yet at the same time, $DMSP_{diss}$ concentrations may only be 2 to 3 nM (R. P. Kiene unpubl.; see also Fig. 4). Either very little release of $DMSP_{diss}$ occurs or the turnover of the $DMSP_{diss}$ must be rapid.

The fact that the metabolism of $DMSP_{diss}$ is concentration dependent (Figs. 2 & 3) suggests that a kinetic approach could be used to estimate turnover rates. However, the plots of DMSP consumption and DMS production rates against the initial $DMSP_{diss}$ concentration yielded straight lines rather than the typical Michaelis-Menten type hyperbolic curves (Fig. 3). There are many possible reasons for this including the potential involvement of different enzyme systems for transport of DMSP into cells and subsequent degradation to DMS. In addition, our incubations were carried out over relatively long periods of time and the concentration of the substrate changed significantly during the incubation. Using the 4 to 6 h time courses we were unable to observe saturation of $DMSP_{diss}$ consumption activity even at 105 nM $DMSP_{diss}$ concentrations (80 nM added) (Fig. 3). Since it appeared from the DMS production data in Fig. 3 that the rate of DMS production may have been similar during the very early part of the incubation in several of the higher addition levels, we attempted several short-term (< 30 min) experiments with similar spikes of $DMSP_{diss}$ (data not shown). Even in these cases the DMS production rate vs the added $DMSP_{diss}$ concentration (10 to 80 nM) did not indicate saturation of DMS production activity. Ideally, radiolabeled (preferably ^{35}S) DMSP would be used to determine the in situ turnover rate of $DMSP_{diss}$. However, radiolabeled DMSP is not generally available and all the potential metabolic products of DMSP metabolism have not yet been identified (see below). Considerably more work will be needed to lay the groundwork for radiotracer methodology in this area.

The production of DMS from $DMSP_{diss}$ in estuarine waters is relatively insensitive to chemical inhibitors including chloroform (Kiene 1990). Furthermore, DMSP-utilizing bacteria are able to grow in the presence of 500 µM chloroform (Kiene 1990, Kiene

unpubl.). Here we showed that chloroform had little or no effect on $DMSP_{diss}$ consumption, nor did it affect endogenous pools of $DMSP_{diss}$ or $DMSP_{part}$ (Fig. 4). Chloroform is, however, a potent inhibitor of DMS metabolism (Fig. 8; see also Kiene & Bates 1990). This inhibitor was used to prevent DMS consumption in experiments designed to estimate how much of the DMSP degraded in water samples was converted to DMS. By measuring $DMSP_{diss}$ and $DMSP_{part}$ concentrations we could estimate the net change in total DMSP during the incubation (Table 1). When we compared the change in total DMSP concentration with the change in DMS concentration in the presence of chloroform, we found that only 28 % of the lost DMSP could be accounted for by DMS production. In the absence of chloroform the production of DMS accounted for only 14 % of the decrease in total DMSP. The doubling of the DMS production in the presence of chloroform suggests that about half of the total DMS produced was consumed during the incubation. Photochemical destruction of DMS (Brimblecombe & Shooter 1986) was precluded in this experiment by incubating samples in the dark. No significant chemical losses of DMS occur during these experiments (see Fig. 7). Therefore, in addition to the familiar lyase degradation of DMSP which yields equimolar amounts of DMS and acrylate (Cantoni & Anderson 1956), a major alternative pathway for DMSP decomposition must also occur in seawater. Similar conclusions have recently been reached for oceanic water samples (Kiene unpubl.). The observation that DMS is not the only, and perhaps not even the major, product of $DMSP_{diss}$ degradation is an important finding, since it is generally assumed that DMSP production and its ultimate decomposition will lead only to DMS.

At this time we can only speculate on the nature of the alternative DMSP degradation pathway(s). Mopper & Taylor (1986) proposed a demethylation pathway for DMSP decomposition leading to the production of 3-methiolpropionate and 3-mercaptopropionate in anoxic sediments. Kiene & Taylor (1988) later confirmed the operation of this demethylation pathway, which occurred along with the lyase pathway in anoxic sediments. No studies of aerobic DMSP demethylation have been reported. However, Taylor & Gilchrist (1991) have recently isolated aerobic marine bacteria which metabolize 3-methiolpropionate, an intermediate in the demethylation of DMSP. Marine bacteria that demethylate compounds which are structurally similar to DMSP, such as glycine betaine, have been isolated previously (Shieh 1966). Thus, it appears reasonable to speculate that demethylation may be responsible for removing $DMSP_{diss}$ from seawater. Other possibilities for DMSP removal include potential chemical demethylation by reaction with iodide, as proposed by White (1982) and Brinkman et al. (1985). However, this is not likely to be a major removal mechanism for $DMSP_{diss}$ because removal is inhibited at the relatively mild temperature of 49 °C (Fig. 5). Most chemical reactions would be expected to proceed faster at higher temperatures.

The DMS formed from DMSP may accumulate or it may be consumed by biological activity. DMS consumption was clearly biological since it was inhibited by azide and chloroform and it displayed a temperature maximum between 30 and 49 °C (Figs. 7, 8 & 10). DMS consumption rates increased at higher DMS concentrations (Fig. 9). The time course data in Fig. 9 show that DMS concentrations held steady or increased for the first 14 h of incubation, and declined thereafter. The initial steady or increasing concentration does not necessarily indicate a lag in DMS utilization, but rather a period of no net consumption. Some DMS is undoubtedly produced during the incubations from the endogenous pools of $DMSP_{part}$ and $DMSP_{diss}$ (these were not measured in this experiment). DMS production may balance or exceed consumption during certain periods. The time course behavior of DMS during bottle incubations is highly dependent on the characteristics of the water parcel sampled and the biological community within it. A variety of patterns for DMS vs time, including immediate increases, immediate decreases or no net change have been observed in estuarine and oceanic waters (Kiene unpubl.). These patterns reflect the dynamics of $DMSP_{part}$, $DMSP_{diss}$, and DMS.

In Fig. 9, the DMS concentrations at 14 h were used to plot the rate of DMS consumption against DMS concentration. This plot yielded a straight line over the range of 7 to 26 nM DMS (Fig. 9). As with the results from $DMSP_{diss}$ spike additions, discussed earlier, these results may not fit enzyme kinetic models because of the long incubation periods and constantly changing substrate concentrations. The rates of DMS consumption determined from the DMS addition experiments are similar to those obtained by Kiene & Bates (1990) using a chloroform inhibition technique with waters having 5 to 15 nM DMS concentrations.

Several recent studies suggested that DMS removal by biological mechanisms may be a very important factor controlling the concentration of DMS in seawater (Wakeham et al. 1987, Leck et al. 1990, Kiene & Bates 1990, Kiene unpubl.). The results presented here provide further evidence for this. In addition, our results suggest that the production and consumption of DMS may be closely coupled in estuarine waters. These processes displayed similar direct temperature and substrate concentration dependence within the natural range of these variables. Thus, if more DMS were to be produced, more would likely be consumed as well.

These interactions would tend to maintain the concentration of DMS within a narrow range. It may be premature to extend these conclusions to oceanic systems in general since relatively little experimental work has been done, especially in polar regions. However, interactions such as those described here may help to explain why regionally averaged DMS concentrations vary by only a factor of 2 or 3 from the highly productive upwelling zones to the oligotrophic oceans (Andreae 1990). Despite relatively low (2 to 5 nM) average DMS concentrations in the ocean, much higher concentrations have been observed in frontal regions and in blooms of certain phytoplankton (Barnard et al. 1984, Holligan et al. 1987, Gibson et al. 1990). Undoubtedly, certain factors or conditions, which remain poorly understood, must upset the balance between DMS production and consumption, resulting in substantially higher DMS concentrations.

The concentration of DMS is strongly influenced by biological processes including interactions between the phytoplankton, grazing organisms, and bacteria which metabolize both DMSP and DMS. For example, Dacey & Wakeham (1986) showed that grazing by zooplankton on DMSP-containing phytoplankton stimulated DMS production. The exact mechanism responsible for increased DMS production was not determined, but could include enhanced degradation of intracellular DMSP by the phytoplankton or enhanced release of $DMSP_{diss}$ and subsequent bacterial degradation to DMS. The most likely mechanism for $DMSP_{diss}$ production, as for most labile DOC (dissolved organic carbon), is through sloppy feeding by herbivores and leaching from their fecal material (Jumars et al. 1989). Whether DMS accumulates to any significant extent may depend on the relative capacities of microorganisms in the water to utilize $DMSP_{diss}$ and DMS. The V_{max}, or the maximum rate of a given reaction in a water sample, is an estimate of the amount of enzyme activity expressed in that water sample. If the V_{max} for DMSP-lyase activity is greater than the V_{max} for DMS consumption, and sufficient DMSP is available, then DMS consumption will become saturated and the DMS concentration would increase. The senescence phase of blooms might be a case where a large quantity of DMSP is released into the water. This could explain why Nguyen et al. (1988) observed substantial DMS accumulations only during the decline of phytoplankton blooms in flow-through microcosms. Eventually, a build-up in DMS may cause an increase in the DMS consuming organisms (or enzyme systems) and DMS would then decline. Physical factors such as advection or exchange with the atmosphere could also remove DMS. Knowledge of the relative values of V_{max}, as well as other kinetic parameters (e.g. half-saturation constants), for DMS production and consumption would be extremely useful in modeling the dynamics of these compounds in seawater The results from the present study suggest that, with the proper approach, these kinetic parameters can be estimated. The competition between the lyase pathway and the alternative degradation pathway(s) for $DMSP_{diss}$ needs to be considered as well.

It is becoming clear that only a small fraction of the total DMSP-sulfur in seawater is likely to escape to the atmosphere. The present results suggest that if all of the algal DMSP which is turned over in a given period of time passes through the $DMSP_{diss}$ pool, then < 30 % of this sulfur will be converted to DMS (based on Table 1 and the discussion above). Of the DMS formed, only 10 % or so (3 % of the original DMSP-S) is likely to escape to the atmosphere because the biological turnover time of DMS in surface seawater is generally 10 times faster than DMS exchange with the atmosphere (Kiene & Bates 1990). More DMS would escape if DMS were produced directly by phytoplankton and $DMSP_{diss}$ was not formed, but even with this scenario, 90 % of the DMS is likely to be consumed within the water column (Kiene & Bates 1990). Further work will be needed to compare the relative sources and sinks for DMSP and DMS in different regions of the ocean. The possibility that only a small percentage of the total dimethyl sulfur in seawater escapes to the atmosphere is interesting because it suggests that a small perturbation in the cycling of DMSP and DMS could significantly alter the amount of DMS ultimately emitted to the atmosphere.

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