

NOTE

Particulate dimethyl sulphoxide in seawater: production by microplankton

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ABSTRACT: Dimethyl sulphoxide (DMSO) represents a major pool of dissolved dimethylated sulphur in seawater. However, the origin and fate of this compound in the marine environment, and its role in the biogeochemical cycle of dimethyl sulphide (DMS), remain unclear. The only established route for the formation of DMSO in oxygenated seawater is photochemical oxidation of DMS. It is not known whether significant biotic production pathways exist. In a study of methylated sulphur speciation in coastal North Sea waters and cultures of marine unicellular algae, we measured pools of particulate DMSO (DMSO_p) at nanomolar and micromolar concentrations, respectively. Analyses of size-fractionated seawater particulates and incubation experiments provided evidence that DMSO_p was associated with microplanktonic organisms. Log-phase cultures of *Amphidinium carterae* and *Emiliana huxleyi* exhibited intracellular dimethylsulphoniopropionate (DMSP) to DMSO molar ratios of 25 and 8, respectively. Our results strongly suggest the existence of biological production and release of DMSO in eukaryotic microplankton.

KEY WORDS: Dimethyl sulphoxide · Phytoplankton · Dimethylsulphoniopropionate · Dimethyl sulphide · *Amphidinium carterae*

Just over a decade ago Charlson et al. (1987) brought attention to the possible role of dimethyl sulphide (DMS) in the global climate. Their hypothesis greatly stimulated research on all aspects of this biogenic trace gas, including the microbial ecology that leads to DMS production and cycling in marine waters and influences its emission to the atmosphere (Saltzman & Cooper 1989, Andreae 1990, Malin et al. 1992). It has generally been assumed that the oxidised form of

DMS, dimethyl sulphoxide (DMSO), would also be present in seawater and hence would play a role in the DMS cycle (Wakeham & Dacey 1989). However, this stable and soluble compound has proven difficult to analyse at the nanomolar concentration levels likely in marine aquatic environments. The first data were published by Andreae (1980), and it led him to suggest that phytoplankton were involved in DMSO production and release, but the analytical technique used is now known to be prone to interference from dimethylsulphoniopropionate (DMSP), which is considered the major precursor of DMS. Several methods are now available which overcome this problem (Ridgeway et al. 1992, Hatton et al. 1994, Kiene & Gerard 1994, Lee & de Mora 1996, Simó et al. 1996). These include specific reduction of DMSO with the enzyme DMSO-reductase (Hatton et al. 1994) or sequential hydrolysis of DMSP followed by reduction of DMSO with borohydride (Simó et al. 1996). In both cases the DMS evolved is preconcentrated using a purge-and-cryotrap technique prior to analysis by gas chromatography. As a consequence of the application of all these methods to oceanographic work in the last few years, much more is now known about the distribution of DMSO in marine waters. DMSO concentrations are generally higher than those of DMS in surface and euphotic waters, where they usually range between 2 and 20 nM (Bates et al. 1994, Simó et al. 1995, 1997, Hatton et al. 1996, Lee & de Mora 1996). DMSO is also found in deep marine waters, where DMS and DMSP are below usual detection limits (Hatton et al. 1996). Hence, a considerable pool of DMSO exists in the oceans.

Much less progress has been made regarding the production and turnover pathways of DMSO in the sea. The only routes for the formation of DMSO in oxygenated seawater demonstrated so far are abiotic: the photo-oxidation of aqueous DMS (Brimblecombe &

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Shooter 1986, Kieber et al. 1996) and the deposition of atmospheric DMSO generated by radical oxidation of DMS (e.g. Turnipseed & Ravishankara 1993). Although bacterial DMS-to-DMSO oxidation occurs in microbial cultures and anaerobic environments (Taylor & Kiene 1989, Zhang et al. 1991, Juliette et al. 1993, Taylor 1993), it is not known whether significant biotic production pathways exist in oxic marine waters. In this paper we present data from field and laboratory studies which show, for the first time, the occurrence of a significant pool of particulate DMSO associated with microplankton. Our results suggest that eukaryotic microorganisms, mainly phytoplankton, are involved in DMSO production and release.

Methods. Seawater sampling: Surface seawater was collected on 3 occasions (samples A, B and C in Table 1) at 07:30 h at an station 5.7 miles (9.2 km) offshore from Great Yarmouth (Norfolk, England). The water column depth was 29 m and seawater temperatures were 14°C (A), 15°C (B) and 18°C (C). 10 l water volumes were taken in glass bottles from just beneath the surface, transported to the laboratory in cool, dark conditions and analysed within 3 h of collection.

Size fractionation: Size fractionation of a seawater sample was achieved by gravitational filtration through Nutex mesh (200 µm) and Nucleopore 18, 5 and 1 µm filters. 50 ml aliquots of each size fraction were further filtered through GF/F filters, which were then analysed for the particulate species.

Seawater incubation experiment: Unfiltered seawater from sample B was distributed into acid-washed, 1 l glass bottles with Teflon-sided screw caps, and incubated in the dark for 48 h at the *in situ* temperature of 15°C. A microvolume of pure chloroform, used as an 'inhibitor', was added to one of the bottles to a final concentration of 500 µM. The water sample was carefully inverted to mix the contents both following chloroform addition and just before subsampling. Subsamples were withdrawn through Teflon tubing attached to a glass syringe.

Culture experiments: Cultures of *Amphidinium carterae* and *Pleurocysis carterae* were each grown in F/2-enriched natural seawater medium in a 2 l cylindrical glass culture vessel with a flat base and multi-socket flat-flange lid. The lid ports were sealed with cotton and muslin bungs. The cultures were slowly stirred using a Nalgene floating Teflon stir bar, and incubated at 12°C with a 12 h light/12 h dark cycle. A culture of *Emiliania huxleyi* was grown in a similar medium in a 300 ml glass vessel, and incubated at 18°C with the same light/dark cycle. Cells were counted using a light microscope and a haemocytometer.

Analyses: In freshly collected seawater and incubation experiments, DMS and dissolved DMSP (DMSP_d) and DMSO (DMSO_d) were determined sequentially

using GF/F filtrates, following an adaptation (Simó, Malin & Liss unpubl.) of the borohydride reduction method of Simó et al. (1996). Particulate DMSO (DMSO_p) was determined after removal and analysis of DMSP by cold NaOH hydrolysis of the GF/F filters for 24 h. DMSO and DMSP_p were not detected during routine blank analyses of filters. In the *Amphidinium carterae* culture experiment, sulphur species were determined using the methods of Hatton et al. (1994), involving DMSO analysis by enzymatic reduction. Dissolved compounds were measured after filtration through a Millipore AP25 depth filter. DMSO_p was analysed by adding the seawater sample to distilled water, so that cells would burst due to osmotic shock, thus enabling the DMSO reductase enzyme access to cellular DMSO. DMSO_p concentrations were then calculated by subtracting the DMSO_d value. Preliminary measurements in *Pleurocysis carterae* and *Emiliania huxleyi* cultures were done using this method and the borohydride reduction method, respectively. Phytoplankton speciation and abundance data for North Sea waters were obtained using an inverted microscope, and carbon biomass was estimated by applying appropriate carbon conversion factors.

Results and discussion. Surface seawater was collected at an offshore station in the North Sea on 3 occasions (Table 1). The first visit (A) coincided with a phytoplankton bloom, of which 98% of the biomass was accounted for by *Phaeocystis* sp. A month later (B) the planktonic biomass was substantially lower and dominated by heterotrophic dinoflagellates; and on the third visit (C) the population was dominated by heterotrophic dinoflagellates and diatoms. While carrying

Table 1. Concentrations of particulate and dissolved dimethylated sulphur species (nM, all values are means of duplicate measurements), chlorophyll *a* (mg m⁻³), total biomass and dominant eukaryotic microplankton species and group biomasses (mg C m⁻³) in North Sea waters

	A June 19, 1996	B July 18, 1996	C August 2, 1996
DMSO _p	16	3.9	2.7
DMSO _d	25	4.9	2.3
DMSP _p	340	5.2	7.1
DMSP _d	150	4.3	4.0
DMS _d	65	1.6	0.8
Chl <i>a</i>	13.26	1.33	2.74
<i>Phaeocystis</i> sp.	268	–	–
Diatoms	1.20	2.51	13.4
Flagellates	4.62	2.96	4.79
Ciliates	7.42	1.88	1.63
Heterotr. dinoflagellates	23.7	27.0	26.6
Other	0.04	0.10	0.33
Total biomass	305	34.5	46.8

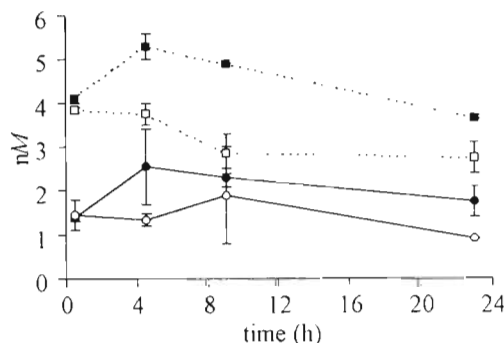


Fig. 1 Time course of the concentrations of particulate DMSP (squares) and DMSO (circles) during incubation of water sample B (see Table 1), with (open symbols) and without (solid symbols) addition of chloroform. Samples were incubated in the dark at the *in situ* temperature of 15°C. Error bars indicate the scatter of duplicate analyses

out DMS, DMSP_d and DMSP_p, and DMSO_d analyses, we found that DMSO could be detected on the GF/F filters which had been used to filter the seawater. Using the same criteria as those for DMSP analysis, it seemed likely that this non-filterable DMSO could be particulate in origin.

Supporting evidence for a pool of particulate DMSO was obtained during an incubation experiment, in which chloroform was used as an inhibitor of bacterial DMS metabolism. Chloroform is used to measure DMS consumption rates (Kiene & Bates 1990) with the assumptions that it has no effect on the production of DMS or the partitioning of the total DMSP pool. However, in some cases chloroform seems to cause DMSP_p-to-DMSP_d release from microbial cells, which may be due to induced cell mortality or effects on cell membrane properties (Wolfe & Kiene 1993). During the first 24 h of dark incubation of water sample B (Table 1), chloroform addition caused a 25 to 50% decrease of the DMSP_p and DMSO_p relative to the control series (Fig. 1), with a concomitant increase in the dissolved pools. It is well established that DMSP_p is mostly contained in the cells of certain types of marine phytoplankton (Keller et al. 1989). Thus, the observed parallel effects of chloroform on DMSO and DMSP partitioning between the particulate and dissolved phases suggests that a pool of DMSO exists in planktonic cells.

From Table 1 it can be seen that for all 3 seawater samples, concentrations of DMSO_p were of the same order as those of DMSO_d. This indicates that its concentration within cells (a minor portion of the total seawater volume) was orders of magnitude higher than its concentration in the dis-

solved pool. Hence, DMSO_p was unlikely to have derived from uptake of DMSO from seawater by diffusion. Although in-cell production seems more likely, to date there has been no definitive evidence for the biosynthesis and occurrence of DMSO in any marine microorganism. In the present study DMSO_p and DMSP_p occurred at similar moderate concentrations in samples B and C (Table 1), and at much higher levels during the bloom of *Phaeocystis* sp. (sample A), an organism that is known to contain high levels of DMSP (Liss et al. 1994).

In addition to phytoplankton, microzooplankton grazers and bacteria might also have contributed to the DMSO_p observed in the seawater samples. Herbivorous ciliates and heterotrophic dinoflagellates play a significant role in the seawater DMS cycle via processing of DMSP_p during grazing and digestion of phytoplankton cells (Belviso et al. 1990, Christaki et al. 1996, Wolfe et al. 1997). Bacteria could also accumulate DMSO either as a substrate for growth or as a product of DMS or DMSP metabolism (Taylor 1993). However, size fractionation of sample C showed that DMSO_p was mostly detectable in particles >5 μm (Fig. 2). This distribution indicates that DMSO_p was not largely confined to the free bacterial fraction but to eukaryotic organisms, though we cannot preclude that some of the DMSO_p could be associated with detritus and faecal pellets.

To test whether DMSP-containing phytoplankton can be a direct source for DMSO, we monitored DMSO_p, DMSO_d, DMS, DMSP_p, DMSP_d and cell numbers in a batch culture of the dinoflagellate *Amphidinium carterae* (Fig. 3). DMSO_p occurred at significant concentrations during the whole experiment, and increased exponentially with cell number and DMSP_p.

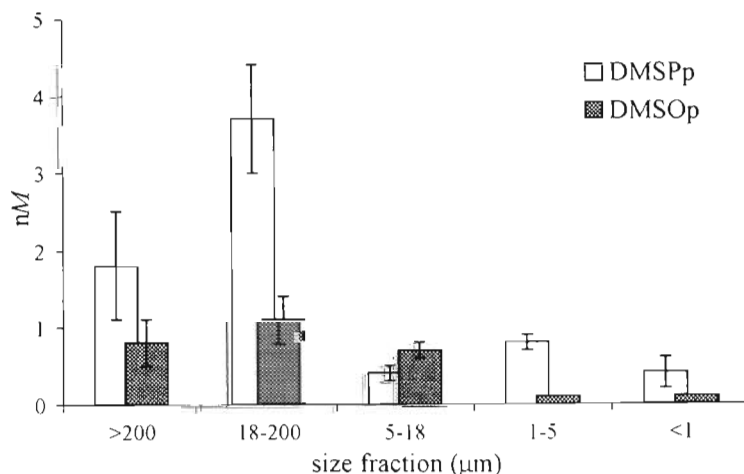


Fig. 2. Concentrations of DMSO_p and DMSP_p in size fractions of sample C. Error bars indicate the scatter of duplicate analyses. See Table 1 for the DMSO and DMSP concentrations and the microplankton composition of the whole water sample

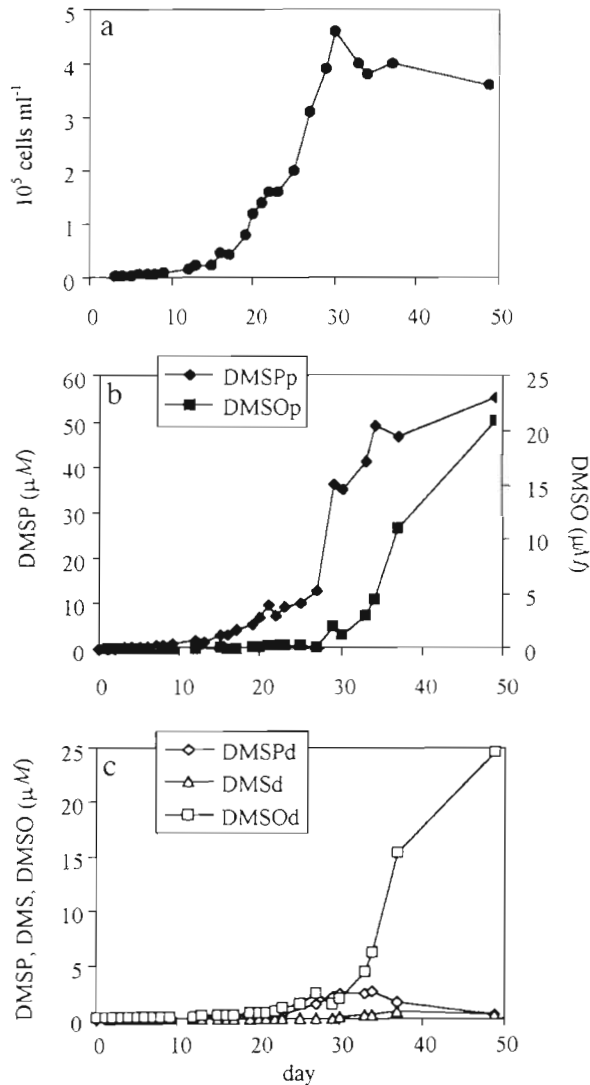


Fig. 3. *Amphidinium carterae*. Time course of the concentrations of (a) cells, (b) particulate DMSP and DMSO, and (c) dissolved DMSP, DMSO and DMS in a batch culture of the dinoflagellate

Intracellular contents of DMSO and DMSP during the log-phase averaged 0.3 ± 0.2 and $11 \pm 4 \text{ pg cell}^{-1}$, respectively ($n = 15$), with a mean value of ca 25 for the DMSP_p/DMSO_p molar ratio, which had a minimum value of approximately 3 during the stationary phase. DMSO_d was the major dissolved species, and its concentration paralleled that of DMSO_p throughout the experiment. This indicates that the major source for the dissolved DMSO was partitioning from the intracellular pool rather than transformation of dissolved DMS. We believe that these data provide strong evidence for the production and release of DMSO by dinoflagellate cells. We have also found submicromolar levels of DMSO_p in 2 preliminary experiments on cultures of the DMSP-producing prymnesiophytes *Emiliania huxleyi*

and *Pleurochrysis carterae* (data not shown). Log-phase *E. huxleyi* cells contained $0.1 \text{ pg DMSO cell}^{-1}$ and $1.4 \text{ pg DMSP cell}^{-1}$, and had a mean DMSP_p/DMSO_p molar ratio of 8 ($n = 4$). Hence, DMSO_p production does not appear to be restricted to dinoflagellates. It is worth stressing that 2 independent analytical methods were used for DMSO determination in coastal seawaters (Simó et al. 1996, Simó, Malin & Liss unpubl.) and algal cultures (Hatton et al. 1994, Simó, Malin & Liss unpubl.), and both revealed the existence of a particulate DMSO pool.

Our discovery of significant amounts of DMSO_p has implications for the complexity of the biogeochemical cycle of DMS in the marine environment (Malin et al. 1994). The results suggest that DMSO is not only produced via photo-oxidation of dissolved DMS (Kieber et al. 1996), but may also be a significant metabolite of biological methylated sulphur transformations within marine pelagic food webs. Ongoing investigation in our groups aims to ascertain the origin and pathways of DMSO_p production. All aerobic organisms have developed mechanisms to deal with reactive oxygen species, such as hydrogen peroxide and singlet oxygen, which are produced as by-products of photosynthesis, photo-oxidative stress and respiration. If the final steps of DMSP biosynthesis in unicellular algae occur in the chloroplast, as is the case in higher plants (Trossat et al. 1996), then it seems likely that DMSO_p could be generated via reaction of DMS with strong oxidants of this type. In the natural environment, another potential source for DMSO_p might be enzymatic reactions in the guts, feeding vacuoles or faecal pellets of grazers, where local anaerobic conditions appropriate for bacterial or enzymatic DMS oxidation (Taylor 1993) could occur. Grazing experiments with protists feeding on DMSP-containing prymnesiophytes and bacteria reveal an imbalance between DMSP_p ingested and DMSP_p processed through DMSP_d and DMS_d, thus indicating that a significant part of the algal/bacterial DMSP is metabolised by the grazer (Wolfe et al. 1994, Christaki et al. 1996, Wolfe 1996). Whether some of the metabolised DMSP is converted to DMSO is not yet known.

Once released by plankton in pelagic waters, DMSO may undergo loss processes such as transport to deeper waters (Hatton et al. 1996) and consumption by bacterioplankton capable of utilising it aerobically as carbon and energy source or anaerobically as an electron acceptor (Taylor 1993). If microbial reduction of aqueous DMSO is shown to occur in seawater, as observed in cultured bacteria (Taylor & Kiene 1989, Zhang et al. 1991, Taylor 1993), DMSO_p would represent a new planktonic DMS precursor.

Especially intriguing is the role of DMSO in phytoplankton, whether it is a by-product of sulphur metab-

olism or has any additional function. DMSP is known to act as a compatible solute, osmolyte and cryoprotectant, and is suggested to function as a methyl donor and act as a transient pool for excess of intracellular sulphur (Kiene et al. 1996). As a zwitterion, it can only be released from cells whose membranes are damaged, or by active transport. In contrast, DMSO is a dipolar aprotic hydroscopic substance which easily diffuses across intact membranes. This would explain why it appears to leak out of healthy dinoflagellate cells and increase in the dissolved phase faster than DMSP (Fig. 3). Because it is also nontoxic and does not cause irreversible damage to membranes, DMSO has been used extensively to protect algal, bacterial and protozoan cells against the adverse effects of freezing-thawing in cryogenic preservation methods (Kirsop & Doyle 1991). Hence, in view of its properties, we can speculate that DMSO might play diverse physiological roles in microplankton, such as protect cells against photo-generated oxidants and cryogenic damage, or serve as a sulphur and carbon transporter through membranes. Whether DMSO formation is involved in the control of DMSP and DMS production by plankton is something that should be investigated in future research. In the meantime, our findings suggest that it would be worthwhile to incorporate DMSO_p analyses alongside determinations of DMS_d, DMSP_d, DMSP_p, DMSO_d, methanethiol_d and methiolpropionate_d, in order to get a more complete picture of the cycling of methylated sulphur compounds in seawater.

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LITERATURE CITED

- Andreae MO (1980) Dimethylsulfoxide in marine and freshwaters. *Limnol Oceanogr* 25:1054–1063
- Andreae MO (1990) Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. *Mar Chem* 30:1–29
- Bates TS, Kiene RP, Wolfe GV, Matrai PA, Chavez FP, Buck KR, Blomquist BW, Cuhel RL (1994) The cycling of sulfur in surface seawater of the northeast Pacific. *J Geophys Res* 99:7835–7843
- Belviso S, Kim SK, Rassoulzadegan F, Krajka B, Nguyen BC, Mihalopoulos N, Buat-Menard P (1990) Production of dimethylsulfonium propionate (DMSP) and dimethylsulfide (DMS) by a microbial food web. *Limnol Oceanogr* 35:1810–1821
- Brimblecombe P, Shooter D (1986) Photo-oxidation of dimethylsulphide in aqueous solution. *Mar Chem* 19:343–353
- Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326:655–661
- Christaki U, Belviso S, Dolan JR, Corn M (1996) Assessment of the role of copepods and ciliates in the release to solution of particulate DMSP. *Mar Ecol Prog Ser* 141:119–127
- Hatton AD, Malin G, McEwan AG, Liss PS (1994) Determination of dimethyl sulfoxide in aqueous solution by an enzyme-linked method. *Anal Chem* 66:4093–4096
- Hatton AD, Malin G, Turner SM, Liss PS (1996) DMSO: a significant compound in the biogeochemical cycle of DMS. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, p 405–412
- Juliette LY, Hyman MR, Arp DJ (1993) Inhibition of ammonia oxidation in *Nitrosomonas europaea* by sulfur compounds: thioethers are oxidized to sulfoxides by ammonia monooxygenase. *Appl Environ Microbiol* 59:3718–3727
- Keller MD, Bellows WK, Guillard RRL (1989) Dimethyl sulfide production in marine phytoplankton. In: Saltzman E, Cooper WJ (eds) Biogenic sulfur in the environment. American Chemical Society, Washington, DC, p 167–182
- Kieber DJ, Jiao J, Kiene RP, Bates TS (1996) Impact of dimethylsulfide photochemistry on methyl sulfur cycling in the Equatorial Pacific Ocean. *J Geophys Res* 101:3715–3722
- Kiene RP, Bates TS (1990) Biological removal of dimethyl sulphide from sea water. *Nature* 345:702–705
- Kiene RP, Gerard G (1994) Determination of trace levels of dimethylsulfoxide (DMSO) in seawater and rainwater. *Mar Chem* 47:1–12
- Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) (1996) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York
- Kirsop BE, Doyle A (eds) (1991) Maintenance of microorganisms and cultured cells. Academic Press, London
- Lee PA, de Mora SJ (1996) DMSP, DMS and DMSO concentrations and temporal trends in marine surface waters at Leigh, New Zealand. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, p 391–404
- Liss PS, Malin G, Turner SM (1994) Dimethyl sulphide and *Phaeocystis*: a review. *J Mar Syst* 5: 41–53
- Malin G, Liss PS, Turner SM (1994) Dimethyl sulfide: production and atmospheric consequences. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. Clarendon Press, Oxford, p 303–320
- Malin G, Turner SM, Liss PS (1992) Sulfur: the plankton/climate connection. *J Phycol* 28:590–597
- Ridgeway R, Thornton D, Bandy A (1992) Determination of trace aqueous dimethylsulfoxide concentrations by isotope dilution gas chromatography/mass spectrometry: application to rain and sea water. *J Atmos Chem* 14:53–60
- Saltzman E, Cooper WJ (eds) (1989) Biogenic sulfur in the environment. American Chemical Society, Washington DC
- Simó R, Grimalt JO, Albaigés J (1996) Sequential method for the field determination of nanomolar concentrations of dimethyl sulfoxide in natural waters. *Anal Chem* 68:1493–1498
- Simó R, Grimalt JO, Albaigés J (1997) Dissolved dimethylsulphide, dimethylsulphonio-propionate and dimethylsulphoxide in western Mediterranean waters. *Deep Sea Res II* 44:929–950
- Simó R, Grimalt JO, Pedrós-Alió C, Albaigés J (1995) Occurrence and transformation of dissolved dimethyl sulphur species in stratified seawater (western Mediterranean Sea). *Mar Ecol Prog Ser* 127:291–299

- Taylor BF (1993) Bacterial transformations of organic sulfur compounds in marine environments. In: Oremland R (ed) *Biogeochemistry of global change—radiatively active trace gases*, Chapman and Hall, New York, p 745–781
- Taylor BF, Kiene RP (1989) Microbial metabolism of dimethyl sulfide. In: Saltzman E, Cooper WJ (eds) *Biogenic sulfur in the environment*. American Chemical Society, Washington, DC, p 202–221
- Trossat C, Nolte KD, Hanson AD (1996) Evidence that the pathway of dimethylsulfoniopropionate biosynthesis begins in the cytosol and ends in the chloroplast. *Plant Physiol* 111:965–973
- Turnipseed AA, Ravishankara AR (1993) The atmospheric oxidation of dimethylsulfide: elementary steps in a complex mechanism. In: Restelli G, Angeletti G (eds) *Dimethylsulphide: oceans, atmosphere and climate*. Kluwer Acad Publ, Dordrecht, p 185–195
- Wakeham SG, Dacey JWH (1989) Biogeochemical cycling of dimethyl sulfide in marine environments. In: Saltzman E, Cooper WJ (eds) *Biogenic sulfur in the environment*. American Chemical Society, Washington, DC, p 152–166
- Wolfe GV (1996) Accumulation of dissolved DMSP by marine bacteria and its degradation via bacterivory. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) *Biological and environmental chemistry of DMSP and related sulfonium compounds*. Plenum Press, New York, p 277–291
- Wolfe GV, Kiene RP (1993) Radioisotope and chemical inhibitor measurements of dimethyl sulfide consumption rates and kinetics in estuarine waters. *Mar Ecol Prog Ser* 99:261–269
- Wolfe GV, Sherr EB, Sherr BF (1994) Release and consumption of DMSP from *Emiliania huxleyi* during grazing by *Oxyrrhis marina*. *Mar Ecol Prog Ser* 111:111–119
- Wolfe GV, Steinke M, Kirst GO (1997) Grazing-activated chemical defence in a unicellular marine alga. *Nature* 387: 894–897
- Zhang L, Kuniyoshi I, Hirai M, Shoda M (1991) Oxidation of dimethyl sulfide by *Pseudomonas acidovorans* DMR-11 isolated from peat biofilter. *Biotechnol Lett* 13:223–228

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