Evaluation of the utility of chemotaxonomic pigments as a surrogate for particulate DMSP

Abstract—High-performance liquid chromatography (HPLC) analysis of accessory pigments, which provides a detailed description of a phytoplankton assemblage over the whole size range, was combined with size fractionation of particulate dimethylsulfoniopropionate (DMSP) to assess the origin of DMSP in the sea. More than 200 surface-water samples were collected over contrasting regions of the ocean. Concentrations of DMSP ranged between 6 and 190 nM. The size fraction <10 μm accounted for 65 ± 16% (1 σ) of DMSP on average. Concentrations of DMSP in this size fraction were strongly linearly correlated ($r^2 = 0.84, n = 189, \rho < 0.0001$) with the sum of concentrations of Hex-fuco (prymnesiophytes) and But-fuco (chrysophytes–pelagophytes), after excluding 17 samples from the Ligurian Sea (Northwestern Mediterranean Sea) taken during the spring and summer seasons of 1993 and 1994. These samples were unusual because of their high DMSP content. Concentrations of Hex-fuco + But-fuco appeared much better surrogates for DMSP than for DMS. In the size fraction >10 μm, DMSP was better correlated with peridinin (dinoflagellates) than fucoxanthin (diatoms) concentrations, but peridinin explained at most 25% of the variability in microplanktonic DMSP. Nearly peridinin-free (<0.01 mg m$^{-2}$) surface waters of the Ligurian Sea during spring and summer contained >15 nM of DMSP in the size fraction >10 μm, i.e., 44 ± 15% of total DMSP. Thus, the particulate material exhibited considerably more DMSP than expected from the levels of accessory pigments in the Ligurian Sea during the spring and summer seasons of 1993 and 1994. We suggest that this excess of DMSP was contributed by heterotrophic nano- and microorganisms.

Dimethylsulfoniopropionate (DMSP) has received considerable attention of late because it acts as a principal intermediate in the production of dimethylsulfide (DMS). In open-ocean surface waters, most DMSP is found in suspended particles (Turner et al. 1988), whose sizes range from tenths of to several hundreds of microns. The particulate DMSP (DMSPp) pool is a product of plankton that synthesizes DMSP (i.e., mainly phytoplankton), ingest it during grazing, or take it up from the dissolved DMSP pool (DMSPd). Phytoplanktonic DMSP production is highly species-specific. Diatoms, green algae, and autotrophic prokaryotes appear to produce much less DMSP than do prymnesiophytes, chrysophytes (pelagophytes), and dinoflagellates (Keller et al. 1989). Although phytoplanktonic DMSP is also sensitive to changes in environmental variables such as salinity, temperature, nutrients, and light (Groene 1995 and references therein), which vary dramatically with depth, how DMSP from phytoplankton may respond to changes in these variables is not well understood. The amount of DMSP contributed by zooplankton depends on the ability of zooplankton to accumulate ingested DMSP or to compact it into refractory fecal pellets that remain in suspension (see Tang et al. 1999 and references therein). Dissolved DMSP can be taken up by the heterotrophic bacteria, thus also contributing to the DMSPp pool (Diaz et al. 1992; Wolfe 1996). Since 1991, we have carried out systematic surveys in the surface waters of total DMSP and size-fractionated DMSPp, chlorophyll $a$ (Chl $a$), and the pigment biomarkers measured by high-performance liquid chromatography (HPLC). We expected that pigments would help assess the contribution of phytoplankton to the total DMSPp pool in the field. We have investigated the spatiotemporal variability of DMSPp in highly contrasted trophic regions within the framework of studies of JGOFS-France and EU projects (Table 1): the Atlantic Ocean central waters (MARATHON), the subtropical Northeastern Atlantic Ocean off Mauritania and Morocco (EUMELI and PROSOPE), the Ligurian Sea (Northwestern Mediterranean Sea, DYFAMED and PROSOPE), the Ionian Sea (Eastern Mediterranean Sea, PROSOPE), and the Indian Sector of the Southern Ocean (ANTARES). The changes in temperature, nutrients, phytoplankton abundance, and community structure we have encountered are typical of most open-ocean environments.

Over the 8 yr of data collection, different sampling and analytical methods have been used to quantify DMSP and DMS. During EUMELI, DYFAMED, ANTARES, and MARATHON, water samples were gravity filtered through either 10-μm pore-size Nuclepore polycarbonate membranes (47-mm diameter) or Whatman GF/F glass-fiber filters (47-mm diameter). Then, unfractionated whole water and filtrates were treated with 10 M cold alkali to obtain a final pH of 13. DMSP samples can be properly stored at room temperature over months under alkaline conditions in 60-ml glass, biochemical oxygen demand (BOD) bottles with plastic caps. In addition, some distilled water was put just above the glass stopper to prevent dehydration of the gas-tight seal. During EUMELI, ANTARES, and DYFAMED, samples were analyzed within days of their collection, whereas during MARATHON, they were stored up to 3 months. Alkali treatment of DMSP yields the volatile sulfur compound DMS. Sparged samples were cryotrapped on frozen ethanol (−100°C) in a 0.64-mm-diameter fluor ethylene propylene (FEP)-Teflon® trap filled with Tenax GC. We used a Varian gas chromatograph equipped with a double-flame photometric detector (FPD) to quantify DMS. DMS analyses were carried out either at sea or in the laboratory (Table 1). During PROSOPE, the method of size fractionation was slightly modified. We used 25-mm-diameter 10-μm pore-size Nuclepore PC membranes and 25-mm Whatman GF/F glass-fiber filters mounted on Teflon cylinders that were allowed to sink slowly through seawater contained in a clean 0.3-liter Teflon beaker, thus producing <10 μm and <GF/F filtrates by reverse filtration. An 8-ml aliquot of the filtrates was transferred to glass tubes, treated with cold alkali, sealed with a Teflon-faced septa, and allowed to sit at room temperature.
for at least 12 h. The content of a sample tube was drawn into a plastic syringe and immediately injected into the sparging device through a Teflon-faced septa. Sparged samples were cryotrapped on liquid nitrogen in a 0.16-mm-diameter FEP-Te¯on trap ®lled with Tenax GC. These more recent samples were analyzed using a Varian 3800 gas chromatograph equipped with a pulsed-¯ame photometric detector (PFPD). DMS analyses were carried out at sea.

DMS was calibrated either from DMS standards prepared by dissolving liquid DMS in degassed ethylene glycol (EUMELI) or from DMS standards (ANTARES, DYFAMED, MARATHON, and PROSOPE). The DMS content of particles over two size ranges (DMSPp >10 µm and DMSPp <10 µm) was indirectly measured by subtracting the total DMSP + DMS content of the ®ltrates (either <10 µm or <GF/F ®ltrates) from the total DMSP + DMS content of parent samples (either unfractionated whole water or <10-µm ®ltrate). Total DMSPp is obtained by subtracting the total DMSP + DMS content of the GF/F ®ltrate from the total DMSP + DMS content of unfractionated whole water.

Seawater DMS was analyzed always on board immediately after ®ltration through Whatman GF/F ®lters either by gravity (MARATHON, Belviso et al. 2000b) or by use of the reverse ¯ow technique described above (PROSOPE). Very few DMS measurements were performed during EUMELI and ANTARES (data not shown). Seawater DMS was not measured during DYFAMED.

Pigment samples were collected through gentle ®ltration of 1–5 liters of seawater onto 47- or 25-mm Whatman GF/F glass-®ber ®lters, which were subsequently analyzed on board or in the laboratory (after storage in liquid nitrogen). Unlike DMSPp, pigments were not size fractionated. Extraction was always performed in methanol. Over the 8 yr of data collection, different reverse phase HPLC methods have been used to separate the various pigments: C18 methods were used for DYFAMED and EUMELI, and a C8 method was used for MARATHON. For PROSOPE, the C8 method was modi®ed by using a 3-mm inside diameter (instead of 4 mm) column, by assigning a 0.5 ml min−1 ®ow rate (instead of 1 ml min−1), and by slightly modifying the solvent polarity at the beginning of the analysis. For all cruises, pigment identi®cation was performed using online diode array spectroscopy detection. HPLC detectors were always calibrated using authentic pigment standards. Measured diagnostic pigments included peridinin (Perid), fucoxanthin (Fuco), 19’-hexanoyloxyfucoxanthin (Hex-fuco), and 19’-butanoyloxyfucoxanthin (But-fuco), which generally characterize dinoflagellates, diatoms, prymnesiophytes, and chrysophytes (pelagophytes), respectively. Some deviation in this general pigment–taxa relationship is sometimes observed for fucoxanthin and its acyloxy derivatives (Hex-fuco and But-fuco) (e.g., Vaulot et al. 1994; van Leeuwe and Steefs 1998). Nevertheless, for the range of oceanic conditions investigated here, we consider such associations to be valid generally and that the sum Hex-fuco and But-fuco is a robust estimator of the biomass by autotrophic pico- and nanoplankton taxa except prochlorophytes. The data set consists of >200 measurements (Table 1). A plot of surface DMSPp versus surface Chl a concentrations shows that in upwelling areas of Western Africa (EUMELI and PROSOPE) where Chl a was >2 mg m−3, DMSPp concentrations ranged between 40 and 125 nM (Fig. 1a). Although dinoflagellates were considerably more abundant in the upwelling off Morocco than in the upwelling off Mauritania (data not shown), the DMSPp:Chl a ratios were roughly the same, i.e., 20 mmol g−1 (n = 2) and 24 ± 11
Fig. 1. Scatter diagram of DMSPp sea surface concentrations plotted against (a) Chl \(a\) sea surface concentrations, and (b) the sum of concentrations of Hex-fuco (from prymnesiophytes) and But-fuco (from pelagophytes). (c) Scatter diagram of DMSPp sea surface concentration in the size fraction <10 \(\mu m\) plotted against the sum of Hex-fuco and But-fuco. The 17 outliers corresponding to samples collected in the Ligurian Sea during spring and summer of 1993 and 1994 are not taken into account in the regression analysis. Arrows correspond to the seven samples surrounded by a hatched line in Fig. 1b.
mmol g⁻¹ (n = 6), respectively. When we subject our entire data set on DMSPp and Chl a concentrations to regression analysis, we obtain values of $r^2$ < 0.25, which, due to the number of data (>200), are significant at the level of 0.01%. The correlation explains ~50% of the variability when samples with Chl a concentrations < 2 mg m⁻³ are sorted, i.e., excluding the subtropical coastal upwellings (Table 2). Correlations between DMSPp and fucoxanthin or peridinin concentrations are worse (Table 2). A much clearer relationship is obtained when the samples are plotted as DMSPp against Hex-fuco + But-fuco (Fig. 1b; $r^2 = 0.58$ [Table 2]). When seven outliers are removed, the values of $r^2$ reach ~0.75 (Table 2). Hence, the utility of the pigments Hex-fuco and But-fuco as a surrogate for total DMSPp is clearly better than that of Chl a.

DMSPp levels in the <10-μm size fraction accounted for 65 ± 16% (1 σ) of total DMSPp. Linear regression analysis between DMSPp<10 μm and Hex-fuco + But-fuco shows $r^2 = 0.65$ (Table 2). It is noteworthy that the EUMELI and PROSOPE outliers in Fig. 1b now fall within other observations attesting to the nanophytoplanktonic size range of these Hex-fuco- and But-fuco-containing particles. At the DYFAMED site during the spring and summer seasons of 1993 and 1994, DMSPp levels in the size fraction <10 μm were surprisingly high according to the pigment levels (Fig. 1c). The DYFAMED samples are unusual because they either contain high levels of DMSPp or are relatively poor in Hex-fuco and But-fuco. *Phaeocystis* is a prymnesiophyte whose pigment composition varies over a wide range. In particular, fucoxanthin may represent up to 80% of total carotenoids (Vaulot et al. 1994). However, the pigment composition of the Mediterranean strain of the prymnesiophyte *Phaeocystis* sp. (Naples strain) appears to be dominated by Hex-fuco (56–66%) rather than fucoxanthin (3–6%). Thus, it is unlikely that the unusual behavior of the Ligurian Sea samples during the spring and summer of 1993 and 1994 results from the presence of *Phaeocystis*. Moreover, in the Southern Ocean samples where *Phaeocystis* has been frequently observed and where *Phaeocystis* exhibits a pigment composition similar to that of the Naples strain (Vaulot et al. 1994), there is no excess of DMSPp in the size fraction <10 μm (ANTARES; Fig. 1c). The contrast in DMSPp between the ANTARES and DYFAMED samples relative to the Hex-fuco + But-fuco levels may result from a nutrient-physiological link, because it is known that nitrate depletion stimulates phytoplanktonic DMSPp production (Keller and Korjeff-Bellows 1996). However, this controlling effect of nitrates on nanophytoplanktonic DMSPp is inconsistent with the observation of almost similar DMSPp <10-μm levels during PROSOPE (nitrate-deplete samples from warm waters) and ANTARES (nitrate-replete samples from cold waters) when Hex-fuco + But-fuco concentrations were in both cases in the range 0.02–0.03 mg m⁻³. The relationship between the sum of surface levels of Hex-fuco and But-fuco and DMSPp levels in the size fraction <10 μm is highly significant when the DYFAMED spring and summer samples are not taken into account in the regression analysis ($r^2 = 0.84$; Table 2). The slope of the relationship is 140 mmol g⁻¹, and the value of the intercept at the origin is 6 nM, both values being highly significant (P < 0.0001). Assuming that the positive value of the intercept at the origin supports the observation that heterotrophic organisms accumulate pools of DMSP (Wolfe 1996), a DMSPp excess of ~15 nM (i.e., ~20 nM at almost nil levels of Hex-fuco + But-fuco minus the intercept at the origin, i.e., 6 nM) still remains unaccounted for at the DYFAMED site during the spring and summer of 1993 and 1994. Thus, we suggest that DMSPp <10 μm at the DYFAMED site during the spring and summer of 1993 and 1994 was mainly contributed by nano- and microheterotrophs, because (1) it is unlikely that the Mediterranean prymnesiophytes are poor in Hex-fuco, and (2) the abundance of DMSPp <10 μm relative to the Hex-fuco + But-fuco content of surface waters appears inconsistent with the distribution of nitrates.

The utility of using the chemotaxonomic pigments Hex-fuco and But-fuco as surrogates for DMS appears considerably smaller (Fig. 2a). The MARATHON and PROSOPE data sets indeed show that DMS and Hex-fuco + But-fuco are not correlated, whereas the linear correlation between the latter and DMSPp <10 μm is striking (Fig. 2b).

### Table 2. Statistics of linear regressions.

<table>
<thead>
<tr>
<th></th>
<th>Slope* (mmol g⁻¹)</th>
<th>Intercept (nM)</th>
<th>r²</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSPp vs Chl a</td>
<td>26.2 (19.9–32.4)</td>
<td>23.0 (18.9–27.0)</td>
<td>0.25</td>
<td>207</td>
</tr>
<tr>
<td>DMSPp vs Chl a†</td>
<td>89.0 (77.3–100)</td>
<td>12.4 (8.8–16.0)</td>
<td>0.53</td>
<td>199†</td>
</tr>
<tr>
<td>DMSPp vs fuco</td>
<td>33.3 (20.2–46.4)</td>
<td>27.8 (23.6–32.0)</td>
<td>0.11</td>
<td>203</td>
</tr>
<tr>
<td>DMSPp vs perid</td>
<td>153 (103–203)</td>
<td>28.0 (24.0–32.0)</td>
<td>0.15</td>
<td>204</td>
</tr>
<tr>
<td>DMSPp vs Hex-fuco + But-fuco</td>
<td>226 (199–252)</td>
<td>13.9 (10.5–17.3)</td>
<td>0.58</td>
<td>204</td>
</tr>
<tr>
<td>DMSPp vs Hex-fuco + But-fuco‡</td>
<td>191 (175–207)</td>
<td>13.4 (11.4–15.4)</td>
<td>0.74</td>
<td>197‡</td>
</tr>
<tr>
<td>DMSPp &lt;10 μm vs Hex-fuco + But-fuco</td>
<td>134 (120–148)</td>
<td>8.6 (6.8–10.3)</td>
<td>0.65</td>
<td>206</td>
</tr>
<tr>
<td>DMSPp &lt;10 μm vs Hex-fuco + But-fuco§</td>
<td>141 (131–150)</td>
<td>6.0 (4.8–7.1)</td>
<td>0.84</td>
<td>189§</td>
</tr>
<tr>
<td>DMSPp &gt;10 μm vs fuco</td>
<td>22.5 (14.8–30.1)</td>
<td>10.0 (7.5–12.5)</td>
<td>0.14</td>
<td>200</td>
</tr>
<tr>
<td>DMSPp &gt;10 μm vs perid</td>
<td>108 (79–136)</td>
<td>10.1 (7.8–12.4)</td>
<td>0.22</td>
<td>201</td>
</tr>
</tbody>
</table>

* 95% confidence interval.
† Eight points with Chl a levels >2 mg m⁻³ have been removed (points surrounded by a hatched line in Fig. 1a).
‡ Seventeen points have been removed (but-fuco as a surrogate for total DMSPp is clearly better (Table 2). Hence, the utility of the pigments Hex-fuco and But-fuco as a surrogate for total DMSPp is clearly better than that of Chl a.
§ Seventeen points have been removed (Ligurian Sea, spring and summer, 1993 and 1994. The points are surrounded by a hatched line in Fig. 1c).
It is known from laboratory cultures (Keller et al. 1989) and several field studies that dinoflagellates produce DMSP. The relationship between surface levels of peridinin (dinoflagellates, but peridinin-lacking dinoflagellates exist, and certain dinoflagellates, such as *Gyrodinium aureolum*, even harbor prymnesiophyte plastids) and DMSPp levels in the size fraction $>10 \, \mu m$ is presented in Fig. 3. Such a relationship has never been published previously. DMSPp in the size fraction $>10 \, \mu m$ is poorly correlated with peridinin concentration ($r^2 = 0.22$ [Table 2]). The relationship between DMSPp $>10 \, \mu m$ and fucoxanthin (diatoms) is worse ($r^2 = 0.14$; Table 2). Surface samples in the Ligurian Sea during the spring and summer seasons of 1993 and 1994 exhibited DMSPp-to-peridinin ratios roughly equal to 4,000 mmol g$^{-1}$. Elsewhere, the ratios were considerably lower: $\sim 700$ mmol g$^{-1}$ in the central Atlantic Ocean (MARATHON) and 80 mmol g$^{-1}$ in the upwelling off Morocco. We highlight the high variability of the DMSPp $>10 \, \mu m$-to-peridinin ratio. Surface waters of the Ligurian Sea were nearly peridinin free ($<0.01 \, mg \, m^{-3}$) during spring and summer, but they contained $>15 \, nM$ of DMSPp in the size fraction $>10 \, \mu m$. That represents $44 \pm 15\%$ of total DMSPp. In the Ionian Sea in September 1999, peridinin was undetectable, and DMSPp in the size fraction $>10 \, \mu m$ was 4.6 nM on average. Thus, the microplanktonic DMSPp that remains unaccounted for by peridinin is roughly threefold higher in the Ligurian Sea than in the Eastern Mediterranean. The process studies carried out in May 1995 at the DYFAMED site (Bellino et al. 2000a) also pointed out the fact that peridinin was unable to account for the diel changing vertical distribution of DMSPp $>10 \, \mu m$. Better evidence of the role played by dinoflagellates and ciliates in the diel cycling of DMSPp $>10 \, \mu m$ was obtained from an enumeration of these organisms than from the use of pigment biomarkers. The possibility that DMSPp in the size fraction $>10 \, \mu m$ results from the aggregation of nanosized materials cannot be excluded. However, the colonial form of the DMSP-containing prymnesiophyte *Phaeocystis* has never been observed in the Ligurian Sea. The idea that DMSPp $>10 \, \mu m$ was in the form of aggregates of unpigmented DMSPp-containing nanoheterotrophs cannot be substantiated with the data shown here, although in the Ligurian Sea, most of the nanoplancktonic
DMSPp pool remains unaccounted for by accessory pigments. The importance of unpigmented nano- and microorganisms in the budget of DMSP, notably in the oligotrophic surface waters of the Mediterranean Sea, remains to be established experimentally.

This study is based on the presence of intracellular DMSP in some species of phytoplankton. Here, we determine cell biomass with pigment data rather than cell counts. A highly significant positive correlation between DMSPp and Hex-fuco was reported only once during a bloom of coccolithophores in June 1991 in the North Atlantic (Holligan et al. 1993). The study of Holligan et al. (1993) confirmed previous observations of Malin et al. (1993), who used cell counts to calculate the biomass of the motile Crystallolithus phase of Coccolithus pelagicus as well as other flagellates. In the works of DiTullio and Smith (1995) and Liss et al. (1997), the relationship between Hex-fuco and DMSPp was not specifically addressed. However, a clear relationship between Hex-fuco and total reduced sulfur (both dissolved and particulate DMSP plus DMS) was apparent in the work of Liss et al. (1997). Our study illustrates how important autotrophic nano- and picoplankton are in the budget of DMSPp. On a global scale, DMSP in the size fraction <10 μm (i.e., 65% of the whole DMSPp pool on average) is mainly of phytoplanktonic origin. Hence, it appears possible to predict nanoplanctonic DMSPp at the global scale from Hex-fuco + But-fuco. However, the same pigment combination is less useful as a surrogate for DMS. It is expected that this pigment combination would markedly underestimate DMSPp levels in North European waters, because in that region, the bloom-forming and strong DMSP-producing prymnesiophyte Phaeocystis produces fucoxanthin. Indeed, fucoxanthin represents 50–80% of total carotenoids in cultured strains of Phaeocystis from the North Sea (Vaulot et al. 1994). The cultured strains of Phaeocystis originating from the Antarctic did show the highest relative content of Hex-fuco (75–80% of total carotenoids) and very low fucoxanthin. Moreover, the effect of iron limitation on the pigment composition of Antarctic Phaeocystis is to induce synthesis of Hex-fuco and But-fuco at the expense of fucoxanthin (van Leeuwe and Stefels 1998). Hence, no underestimation of nanoplanctonic DMSPp is expected in the Southern Ocean, the largest of the three major oceanic high-nitrate, low chlorophyll systems.

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Chemical tracing of salinity sources in Lake Kinneret (Sea of Galilee), Israel

Our paper, which appeared in Vol. 44, p. 1035–1044, contains two kinds of errors: definition of variables and typographical mistakes. These were pointed out to us by our friend Dr. A. Ben-Zvi from the Israel Hydrological Service, and we are most grateful to him for his effort and generosity.

In Eq. 1 the input term of salts into the lake ($T_i$) should be split into two terms: the monitored and unmonitored inflow,

$$T_i = T_{US1} + T_{inm} \quad (1a)$$

where $T_{inm}$ is the monitored component of inflow. At steady state, the correct expression of Eq. 1 is

$$T_{US1} = T_{out} - T_{inm} \quad (1b)$$

A more rigorous substitute to Eq. 1 would consider deviations from steady state by adding a term for change in stored mass during the balance period, $\Delta T$,

$$T_{US1} = T_{out} + \Delta T - T_{inm} \quad (1c)$$

Fortunately, the values in Table 5 were calculated under the assumption of this definition without explicitly making it. When we defined $T_{inm}$ we erroneously confused “supply of chlorides” with “supply of salts.” Again, this confusion was made only in the text. In all tables and calculations, chloride masses were used properly.

Typographic errors: Water volume units in Table 3 should read “10⁶ m³.” The definition of $T_{out}$ toward the end of p. 1038 should read “the total annual Cl supply from the lake.” The final concentration in LK, at the bottom of p. 1040, $T^n$ column, should read “$f = 1997.$”

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