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Plankton origin of particulate dimethylsulfoniopropionate in a Mediterranean oligotrophic coastal and shallow ecosystem

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A B S T R A C T

We report here dimethylsulfide (DMS) and dimethylsulfoniopropionate (DMSP) levels as a function of plankton communities and abiotic factors over a 12-month cycle in the Mediterranean oligotrophic coastal and shallow ecosystem of Niel Bay (N.W. Mediterranean Sea, France). Total particulate DMSP ($DMSP_p$) and DMS concentrations were highly seasonal, peaking during a spring (April) bloom at 8.9 nM and 73.9 nM, respectively. Significant positive correlations were found between total $DMSP_p$ concentration and the abundance or biomass of the dinoflagellate *Prorocentrum compressum* (Spearman's rank correlation test: $r = 0.704$; $p = 0.011$). Similarly, DMS concentrations peaked during the development of blooms of *P. compressum and Gymnodinium* sp. There seemed to be a positive relationship between the chlorophyll *a* to pheopigment ratio and DMS concentrations, suggesting that DMS was released during phytoplankton growth. High DMS levels recorded in the shallow Niel Bay may also result from the activity of benthic macroalgae, and/or macrophytes such as *Posidonia* spp., or the resuspension of sulfur species accumulating in sediments. The fractionation of particulate DMSP into three size classes (>90 μm, 5–90 μm and 0.2–5 μm) revealed that 5–90 μm DMSP-containing particles made the greatest contribution to the total $DMSP_p$ pool (annual mean contribution = 62%), with a maximal contribution in April (96%). This size class consisted mainly of dinoflagellates (annual mean contribution = 68%), with *P. compressum* and *Gymnodinium* sp. the predominant species, together accounting for up to 44% of the phytoplankton present. The positive correlation between DMSP concentration in the 5–90 μm size class and the abundance of *P. compressum* (Spearman's rank correlation test: $r = 0.648$; $p = 0.023$) suggests that this phytoplankton species would be the major DMSP producer in Niel Bay. The DMSP collected in the >90 μm fraction was principally associated with zooplankton organisms, dominated by copepods (nauplii and copepodites). $DMSP_{>90}$, not due to a specific zooplankton production, resulted from the phytoplankton cells ingested during grazing. The concomitant peaks of DMS concentration and zooplankton abundance suggest that zooplankton may play a role in releasing DMSP and/or DMS through sloppy feeding.

1. Introduction

Dimethylsulfide (DMS) is found principally in ocean surface waters, in which it is the most abundant volatile sulfur compound. Lovelock et al. (1972) were the first to postulate that DMS was responsible for the transfer of sulfur from the seas, through the air, to land surfaces, a role originally assigned to H_2S. DMS is now widely thought to be the predominant natural source of atmospheric sulfur. Once emitted into the atmosphere, DMS is rapidly oxidized to generate methane sulfonic acid (MSA), SO_2 and SO_4^{2-}. The oxidation products of DMS can then be converted into sulfate aerosol particles. These particles may serve as condensation nuclei (CN) for water vapor to form cloud condensation nuclei (CCN) reflecting back the incoming solar radiation. Through their CLAW hypothesis, Charlson et al. (1987) have suggested that DMS may affect climate by regulating the greenhouse effect. Indeed, global warming, by inducing higher levels of primary production, would be expected to lead to an increase in DMS production. Gondwe et al. (2003) recently estimated the contribution of DMS to climate-relevant non-sea-salt sulfate (nss SO_4^{2-}) at 43% in the relatively pristine Southern Hemisphere, confirming the potential role of oceanic DMS in climate regulation.

DMS is generated by the enzymatic cleavage of ß-dimethylsulfoniopropionate (DMSP) by a bacterial or algal DMSP-lyase

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(Challenger, 1951). DMSP is produced by marine algae as an osmolyte and cryoprotectant (Kiene and Service, 1991; Kirst et al., 1991). DMSP and its degradation products have also been shown to have antioxidant properties in marine phytoplankton (Steinke et al., 2002; Sunda et al., 2002; Van Rijssel and Buma, 2002). Bacteria may use DMSP as a source of reduced sulfur, after demethylation or demethiolation of this biogenic compound (Kiene et al., 1999; Kiene et al., 2000). Howard et al. (2006) showed that "switch genes" were used by marine bacterioplankton to regulate the enzymatic conversion of DMSP into climate-active DMS. These authors demonstrated, in *Roseobacter* and SAR11 taxa, a glycine-cleaving T-family protein with DMSP methyltransferase activity involved in DMSP demethylation to generate methylmercaptopropionate. This discovery indicates that a substantial fraction of global marine primary production avoid DMS formation. A regulatory gene, *dddD*, was identified by Todd et al. (2007) in the marine bacterium *Marinomonas*. In its cloned form, this gene conferred the ability to generate DMS from DMSP on *Escherichia coli*, demonstrating the potential of this gene to regulate intracellular DMS production.

DMSP is differentially produced by phytoplankton species, the greatest DMSP producers being the coccolithophores, the genus *Phaeocystis* and the dinoflagellates (Liss et al., 1993). In nearshore waters, Turner et al. (1988) reported higher intracellular DMSP concentrations in flagellates (650 mM) than in the phytoplankton community as a whole (between 37 and 95 mM). Belviso et al. (2000) also recorded high DMSP concentrations, of 355–972 mM, in dinoflagellates. High DMS concentrations are generally associated with blooms and the growth of DMSP producers, such as *Phaeocystis* sp. (Leck et al., 1990; Kwint and Kramer, 1996). According to Stefels et al. (1995), these concentrations result from strong activities of the bacterial and/or algal DMSP-lyase, which converts DMSP into DMS. The production of dissolved DMSP and DMS is also associated with the senescence of phytoplankton blooms (Turner et al., 1988; Leck et al., 1990).

Significant correlations between DMS concentrations and zooplankton biomass have also been reported (Leck et al., 1990; Yang et al., 2000). Zooplankton grazing on algal cells may release DMS indirectly, by transferring DMSP to the dissolved compartment and making it available for conversion into DMS by bacteria (Belviso et al., 1990; Wolfe et al., 1994). Dacey and Wakeham (1986) found that a third of the DMSP originating from *Gymnodinium nelsoni* and *Prorocentrum micans* ingested by the copepods *Labidocera aestiva* and *Centropages hamatus* was released into the culture medium as DMS, confirming the role of zooplankton grazing in the DMS cycle. Viruses, by inducing the lysis of algal cells, also contribute to DMS release (Malin et al., 1998). Taking all these data into account, studies increasing our understanding of the biogeochemistry of DMS and DMSP by analyses of the temporal and/or spatial variations of their concentrations, appear to be particularly relevant. Over the last 30 years, a large database has been established, recording DMS and DMSP concentrations in all oceans worldwide (Kettle et al., 1999; Kettle and Andreae, 2000). However, as pointed out by Michaud et al. (2007), few studies have reported changes in DMS and DMSP concentrations during annual cycles in coastal areas. Besides, with the exception of Ledyard and Dacey (1996), few authors have provided data for DMS and DMSP levels in oligotrophic coastal waters.

Over the last 20 years, productivity in the Mediterranean Sea has increased considerably, due to the addition of large amounts of anthropogenic inputs (Duarte et al., 1999). This trend is particularly marked in coastal areas, which are the best sites for investigating the influence of increased trophic levels on DMS and DMSP production. In previous studies, we carried out such investigations in a coastal ecosystem: the eutrophicated Little Bay of Toulon (N.W. Mediterranean Sea, France) (Jean et al., 2005, 2006). In this ecosystem, which has high plankton abundances and biomasses, we found elevated particulate DMSP levels during dinoflagellate blooms, and high intracellular DMSP in the toxic and nitrophilic *Alexandrium minutum*. In this study, we sought to confirm the influence of eutrophication, by determining the conditions in which sulfur species are produced at a study site with a much lower trophic level. Niel Bay meets these criteria: it is more open to the sea than Little Bay of Toulon and has lower plankton abundances and biomasses, typical of oligotrophic marine coastal environments (Jamet et al., 2001, 2005). We report here DMS and DMSP concentrations over a 12-month period at this Mediterranean oligotrophic coastal study site, providing insight into the conditions in which sulfur species are produced by plankton communities in such an ecosystem.

2. Materials and methods

2.1. Study site

The study was carried out on the French N.W. Mediterranean coast, in a shallow station of Niel Bay (depth 5 m), located at the end of the Giens peninsula, 15 km east of the city of Toulon (Central point: 43°05′N and 6°00′E) (Fig. 1). Niel Bay is situated just 3 km north of Porquerolles Island, an official French conservation area. The East – West Liguro – Provençal stream influences the hydrodynamics of this bay, allowing exchanges between pelagic and coastal ecosystems all year. In summer, Niel Bay has limited tourist and fishing activity. This site, unpolluted and widely open to the sea, allows the development of large *Posidonia* spp. meadows (Paillard et al., 1993). In previous studies, we demonstrated that Niel Bay was oligotrophic, showing low levels of chlorophyll *a*, low zooplankton abundances and high zooplankton diversity (Jamet et al., 2001, 2005).

2.2. Field sampling

Seawater from Niel Bay was sampled for 12 months, from October 1999 to September 2000. Samples were systematically taken between 9.30 and 11.00 am, as the small tidal effects in the Mediterranean Sea (annual mean amplitude < 60 cm) had no influence on the study site. Samples were always taken from a depth of 2 m, using a Niskin 10-L sampling bottle (General Oceanic). $DMSP_p$ analysis was performed on an 8-L aliquot. Samples for DMS determination were collected specifically, by filling a 1-L polyethylene bottle, so as to prevent headspace and degassing in the sampling bottle. DMS analysis was carried out as soon as the sample arrived at the laboratory (a maximum of 2 h after the start of sampling). Finally, 20-L samples were taken to study the phytoplankton community and for chlorophyll *a* and protein determinations. Zooplankton were collected by vertical towing, using a net with a 90 μm mesh (0.5 m in diameter; 2.5 m in length) equipped with a flow meter.

2.3. Abiotic factors

Precipitation values in the Toulon area were obtained from Météo-France (the French meteorological service). Temperature and salinity were measured at the Niel Bay station, at a depth of 2 m, with a WTW LF 197 electronic multi-parametric sensor. For phosphate ($P\text{-}PO_4$) and nitrate ($N\text{-}NO_3$) determinations, seawater was first filtered through a polyamide membrane (Nytrel-TI, UGB) with 0.2 μm pores. Phosphate concentrations were then determined by a colorimetric method based on ammonium molybdate, as described by Murphy and Riley (1962). Nitrate concentrations

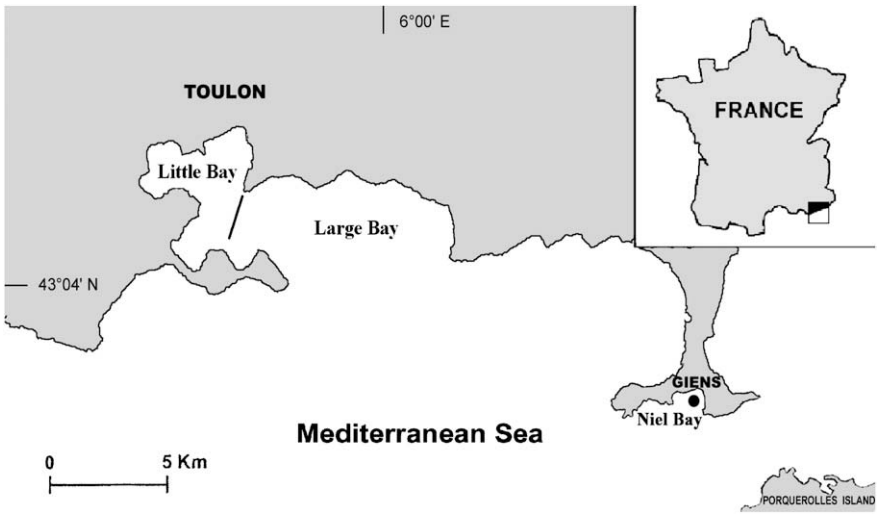


Fig. 1. Location of the sampling station in Niel Bay.

were determined with a Technicon II autoanalyzer (Diamond Diagnostics), using the cadmium reduction technique of Wood et al. (1967), as modified by Le Poupon (1994).

2.4. Biotic factors

2.4.1. Pigments

Particulate material was collected by filtering 20 L of seawater through glass fiber filters (Whatman GF/C). Pigments were then extracted from the particulate fraction in 90% acetone. The extracted chlorophyll *a* and pheopigments were analyzed by visible spectrophotometry (Uvikon Kontron Instruments), as described by Lorenzen (1967).

2.4.2. Protein analysis

The particulate material was fractionated into size classes by passing the 20 L of seawater successively through polyamide membranes with pore sizes of 90, 5 and 0.2 μm (Nytrel-TI, UGB). The proteins collected on the membranes were stored at −80 °C until analysis. The proteins of each class were resuspended in distilled water and analyzed with Folin phenol reagent (Lowry et al., 1951). Bovine serum albumin (BSA) was used as a standard.

2.4.3. Phytoplankton

A reverse filtration through a membrane with 0.2 μm pores was first carried out to concentrate phytoplankton cells. The phytoplankton samples were then preserved and stored in Lugol's reagent until examination. Phytoplankton cells >5 μm in diameter were identified and counted under an inverted Nikon Diaphot microscope, with a technique first described by Utermöhl (1958) and subsequently modified by Legendre and Watt (1971–1972) (total magnification ×400). As suspended material was present, we did not identify smaller cells (picoplankton). Cell biovolumes and biomasses were estimated in accordance with Lohman's (1908) calculation technique.

2.4.4. Zooplankton

Zooplankton samples collected with a net were stored in seawater-buffered 5% formol. Aliquots for counts were taken with a Hensen pipette from the total sample, the volume of which was adjusted to 250 mL.

2.4.5. Bacteria

Bacteria were counted by epifluorescence microscopy, as described by Porter and Feig (1980). Water samples were filtered through a black polycarbonate Nucleopore filter (mesh size 0.2 μm; 25 mm diameter). After filtration, the membrane was covered with 1 mL of DAPI solution (50 μg mL^{-1}) and left in the dark for staining. The stained filters were observed in a dark room, under an epifluorescence microscope (total magnification: ×1000), with immersion oil.

2.5. DMS and DMSP analysis

$DMSP_p$ was fractionated into size classes, by successively filtering seawater through filtration units with polyamide membranes (Nytrel-TI, UGB) having 90, 5 and 0.2 μm pores. Gravity filtration was applied only with 90 μm-pore membranes. Low pressure (<75 mm Hg) was used to separate the 5–90 μm and the 0.2–5 μm size classes. According to Kiene and Slezak (2006), the partitioning of DMSP between the particulate and dissolved compartments is affected by the filtration protocols commonly used, as cells may be lysed during the filtration procedure. As in a previous study by Jean et al. (2006), we estimated losses due to filtration by comparing the $DMSP_p$ measurements obtained for samples filtered (0.2 μm pore size) with and without pressure (<75 mm Hg). We estimated $DMSP_p$ losses at 37.3% ± 7.9% ($n = 4$).

Filters coated with particulate DMSP were stored at −80 °C, in 15 mL hermetic polyethylene flasks, until analysis. Preliminary controls showed that freezing at −80 °C had no significant effect on DMSP content (Jean, 2002). Immediately before DMSP analysis, a filter with particulate DMSP of a given size class (0.2–5, 5–90 or >90 μm) was placed in 250 mL cold distilled water for resuspension of the particles. DMSP was extracted from the particulate fraction by sonication. It was then converted into DMS by the cold alkali treatment method (Dacey and Blough, 1987; Turner et al., 1988), by adding NaOH (10 M) to pH ∼ 13 (White, 1982; Stefels and Van Boekel, 1993). The alkaline solution was rapidly and kindly transferred to a silanized glass bottle with a septum and no headspace. The full transformation of DMSP into DMS was achieved after 24 h in the dark at 2 °C. The DMS formed from DMSP was then analyzed as indicated below.

Seawater DMS concentrations were determined for a 250 mL sub sample of an unfiltered aliquot. DMS analysis was carried out with a modified version of a published method based on a cryo-

trapping gas chromatographic technique (Simo et al., 1993; Simo, 1998). The gas chromatograph (DELSI 330) was equipped with a flame photometric detector (FPD) and a 1/8 inch PTFE column filled with Chromosil 330 (Supelco). A 5 mL sub sample was taken with a polyethylene needle. DMS was stripped by bubbling helium (99.9996% quality) through the solution at a flow rate of 100 mL min^{-1}. The DMS was trapped on Tenax T.A. packed at −40 °C, with magnesium perchlorate used as the dryer (Despiau et al., 2002). The Tenax was heated at 180 °C and the released DMS was injected into the column. The flame photometric detector was supplied with a flow of air ($F_a = 150$ mL min^{-1}) and hydrogen ($F_{red} = 75$ mL min^{-1}; $F_{oxy} = 7$ mL min^{-1}). DMS solution was used for calibration by linear interpolation: Ln(Peak area)-Ln(DMS mass). The detection limit was 2 ng and the mean precision of DMS concentrations was 13% ($n = 9$). The DMS and DMSP measurements were carried out in triplicate and mean values and standard deviations are presented.

2.6. Statistical analysis

In this work, the non-parametric Spearman's rank correlation test (Statistica 7.1 version) was used to compare chronological data series. This test indicates the existence of correlations between temporal variations of two variables. Correlations, characterized by r and p coefficients, were regarded as significant if $p < 0.050$.

3. Results

3.1. Abiotic factors

More than 60% of the total annual precipitation occurred between October (288.0 mm) and November (169.0 mm) in the Toulon area, affecting Niel Bay water quality (Table 1). Salinity was particularly low from October (35.2) to December–January (36.2–36.3). Values typical of salinity in the Mediterranean (close to 38) were found again from February (38.1). Nutrient concentrations ranged from 2.0 μM (September) to 19.7 μM (December) (annual mean concentration = 5.8 μM) for nitrates, and from 53.5 nM (August) to 168.0 nM (November) (annual mean concentration = 98.3 nM) for phosphates (Table 1). These concentrations greatly increased in November (PO_4: 168.0 nM; NO_3: 13.1 μM) and December (PO_4: 149.0 nM; NO_3: 19.7 μM), coinciding with the heavy autumnal rains. Concentrations also rose in April for phosphate (107.0 nM) and in May for nitrate (9.2 μM), immediately after episodes of spring storms.

3.2. Plankton communities

3.2.1. Biomass of the particulate fraction

3.2.1.1. Chlorophyll a. Chlorophyll *a* concentration is regarded as a relevant indicator of phytoplankton biomass. Chlorophyll *a* concentrations remained low throughout the year (annual mean value = 0.39 μg L^{-1}), but displayed considerable fluctuations over time (min = 0.10 μg L^{-1}; max = 1.00 μg L^{-1}) (Table 2). The largest variations were observed during autumnal rains, when chlorophyll *a* concentrations dropped from 1.00 μg L^{-1} (October) to only 0.19 μg L^{-1} (November). Three other relative peaks of chlorophyll *a* concentration occurred later, reaching 0.72 (February), 0.42 μg L^{-1} (April) and 0.51 μg L^{-1} (September). The lowest concentrations, around 0.10 μg L^{-1}, were recorded in summer (June–July).

3.2.1.2. Proteins. We used protein analysis to determine the biomass of the total particulate fraction and the biomasses of the different particulate size classes. As described for chlorophyll *a*, total protein concentration decreased strongly in autumn, from 236.0 μg L^{-1} (November) to 71.0 μg L^{-1} (December) (Table 2). Biomass increased again in winter and spring, reaching more than 100 μg L^{-1} (May, January–March). Protein concentration then decreased again, stabilizing at about 100 μg L^{-1} in summer. The contributions of the three size classes (>90 μm, 5–90 μm and 0.2–5 μm) to the pool of total particulate biomass were estimated (Table 2). Throughout the year, the 5–90 μm class was found to make the largest contribution to biomass, with an annual mean contribution of 61%. The >90 μm fraction had the lowest protein concentrations, accounting for 5% (October, January and September) to 25% (April) of the total biomass (annual mean contribution = 28%). By contrast, the 0.2–5 μm size class collected much more biomass, ranging from 19% (June) to the highest value of 54% (April) (annual mean contribution = 11%).

3.2.2. Composition of the plankton communities

3.2.2.1. Zooplankton. Zooplankton community consisted principally of copepods, which accounted for 78–97% of all zooplankton (Fig. 2). The cyclopoids predominated in the copepod community, with abundances corresponding to up to 87% of the entire copepod community. The copepod community was composed of adults, but especially of nauplii and copepodites, which were always the most abundant (up to 50 times more numerous than the adults). The abundance of adults peaked in February, at 412 individuals m^{-3}, whereas the abundance of nauplii and copepodites peaked much later, in August, at around 2.8×10^4 individuals m^{-3}.

3.2.2.2. Phytoplankton. The biomass of different phytoplankton species was estimated from phytoplankton abundances, as explained in Section 2.4.3. Phytoplankton biomass was clearly dominated by dinoflagellates, with an annual mean biomass of 68% the total for phytoplankton, against only 32% for diatoms (Fig. 3). Dinoflagellate biomass followed a clear seasonal pattern, with a peak at the end of spring (26.6 μg L^{-1} in May) resulting from the large biovolumes of *Ceratium furca* and of diverse species of the *Protoperidinium* genus. Lower values (<5.0 μg L^{-1}) were observed during the rest of the year, except in September, when a moderate increase in dinoflagellate biomass (8.3 μg L^{-1}) occurred. Diatom biomass followed a different pattern, with moderate increases in December (8.6 μg L^{-1}) and May (6.6 μg L^{-1}), coinciding with peaks in nitrate concentration. Two other relative peaks in diatom levels also occurred in February–March (about 5.0 μg L^{-1}) and August (3.7 μg L^{-1}).

In the diverse phytoplankton community of Niel Bay, the most frequently represented dinoflagellates, in terms of abundance, were *Prorocentrum compressum* and *Gymnodinium* sp. (Fig. 4). Together, these two species accounted for 44% of the total phytoplankton community. *Prorocentrum compressum* was frequently the most abundant species present, at 29%, versus 15% for *Gymnodinium* sp. The populations of these dinoflagellates peaked at different periods: in April at 557 cells mL^{-1} for *P. compressum*, and later, in June, at 560 cells mL^{-1}, for *Gymnodinium* sp.

Table 1
Temporal variations in the abiotic factors in Toulon area (precipitation), and more specifically in Niel Bay (salinity, N-NO_3, P-PO_4).

Months	O	N	D	J	F	M	A	M	J	J	A	S
Precipitation (mm)	288.0	169.0	0.2	15.0	14.6	13.6	76.2	27.4	59.8	19.8	7.2	5.1
Salinity	35.2	35.6	36.3	36.2	38.1	37.9	37.8	37.8	38.0	38.0	38.2	38.3
N-NO_3 (μM)	5.9	13.1	19.7	5.5	2.9	2.1	2.2	9.2	2.6	2.2	2.1	2.0
P-PO_4 (nM)	108.0	168.0	149.0	108.0	99.4	78.7	107.0	62.9	59.5	126.0	53.5	59.1

Table 2
Temporal variations in the biological factors in Niel Bay (PC = contribution of a size class to the total protein biomass).

Months	O	N	D	J	F	M	A	M	J	J	A	S
Chl-*a* ($\mu g\,L^{-1}$)	1.00	0.19	0.24	0.50	0.72	0.12	0.42	0.38	0.13	0.10	0.40	0.51
Total Protein ($\mu g\,L^{-1}$)	178.0	236.0	71.0	173.0	128.0	176.0	53.2	199.0	86.9	107.0	82.4	84.0
Protein 0.2–5 ($\mu g\,L^{-1}$)	45.4	75.7	19.0	51.5	27.9	50.1	28.7	39.3	16.5	26.6	21.0	19.9
PC	*26%*	*32%*	*27%*	*30%*	*22%*	*28%*	*54%*	*20%*	*19%*	*25%*	*25%*	*24%*
Protein 5–90 ($\mu g\,L^{-1}$)	124.0	130.0	43.6	114.0	72.7	115.0	11.0	144.0	62.9	61.6	55.9	59.9
PC	*70%*	*55%*	*61%*	*66%*	*57%*	*65%*	*21%*	*72%*	*72%*	*57%*	*68%*	*71%*
Protein > 90 ($\mu g\,L^{-1}$)	8.6	30.0	8.4	7.9	27.8	10.9	13.5	15.5	7.5	19.0	5.5	4.2
PC	*5%*	*13%*	*12%*	*5%*	*22%*	*6%*	*25%*	*8%*	*9%*	*18%*	*7%*	*5%*

3.2.2.3. Bacteria. Bacterial abundance ranged from 9.2×10^4 cells mL^{-1} (February) to 2.9×10^5 cells mL^{-1} (April) (Fig. 5). Except for the peaks observed in autumn and in spring, bacterial abundance remained stable, at between 1.0 and 1.5×10^5 cells mL^{-1}.

3.3. DMS and DMSP concentrations

3.3.1. Total $DMSP_p$ concentrations

Total $DMSP_p$ concentrations, obtained by summing DMSP concentrations for the 0.2–5, 5–90 and >90 μm size classes, varied between 0.5 nM (November, July) and 8.9 nM (April) (annual mean concentration = 2.6 nM) (Fig. 6). These concentrations followed a clear seasonal pattern: after low values in the autumn, total $DMSP_p$ peaked in March–April. We then observed a decrease in concentrations (between May and July) followed by a slight recovery (August–September). No correlation was found between total $DMSP_p$ and chlorophyll *a* or protein concentrations. Similarly, $DMSP_p$ was not correlated with dinoflagellate abundance or biomass. However, significant positive correlations were found between total $DMSP_p$ concentration and the abundance or biomass of the dinoflagellate species *Prorocentrum compressum* (Spearman's rank correlation test: $r = 0.704$; $p = 0.011$).

3.3.2. DMS

DMS concentrations were recorded throughout the year in Niel Bay (annual mean concentration = 19.8 nM) (Fig. 7). DMS concentrations peaked at 73.9 nM in April, this peak coinciding with a peak in the total $DMSP_p$ concentration (Fig. 6), development of the dinoflagellate *Prorocentrum compressum* (Fig. 4) and a peak in bacterial abundance (Fig. 5). These concentrations also increased to 11.8 nM in February, during a moderate development of *Gymnodinium* sp. and a peak in zooplankton abundance (Figs. 2 and 4). Another DMS peak (64.9 nM) occurred in August, when zooplankton abundance (nauplii and copepodites) was maximal (Fig. 7).

The chlorophyll *a* to pheopigment ratio provides information about the health of phytoplankton cells (Shenoy et al., 2006). Throughout the year, temporal variations in DMS concentrations seemed to be positively related to this ratio (Fig. 8). However, when this ratio peaked (24.0) in February, DMS concentrations also peaked, but to a lesser extent (11.8 nM).

3.3.3. Contribution of the >90 μm size class to total $DMSP_p$ and to DMS

DMSP levels in the >90 μm size class were low throughout the year, with concentrations not exceeding 0.7 nM (Fig. 9). The highest DMSP levels in the >90 μm class occurred in February, June and August–September. This particulate fraction made a weak overall contribution to the total $DMSP_p$ pool (annual mean contribution = 18%).

In Niel Bay, $DMSP_{>90}$ resulted from the zooplankton community, with most zooplankton organisms belonging to the > 90 μm size class. $DMSP_{>90}$ levels were highest at the time at which zooplankton abundance peaked (Fig. 2), in February for all copepods, coinciding with the chlorophyll *a* peak corresponding to the development of diatoms and/or dinoflagellates (Fig. 3), and in August, for nauplii and copepodites only.

3.3.4. Contribution of the 5–90 μm size class to total $DMSP_p$

DMSP was much more abundant in the 5–90 μm size class than in larger particles, with concentrations ranging between 0.2 and 8.5 nM (Fig. 9). $DMSP_{5-90}$ concentrations increased strongly in March and April, at which times DMSP concentrations exceeded those in all the other fractions. As described for the total $DMSP_p$, no correlation was found between $DMSP_{5-90}$ and chlorophyll *a* or protein concentrations. Similarly, $DMSP_{5-90}$ was not correlated

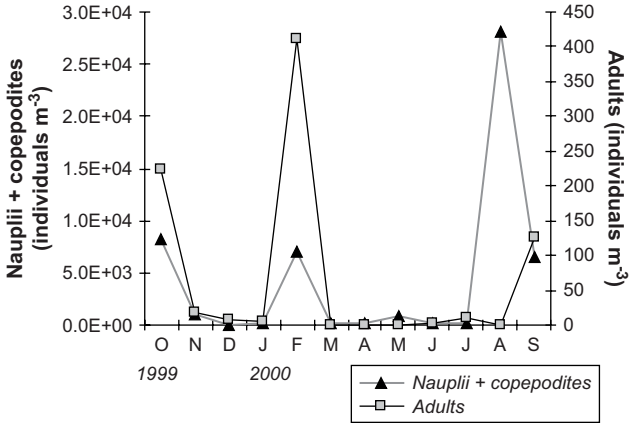


Fig. 2. Temporal variations in the abundance of the zooplankton community, consisting mostly of copepods (adults, nauplii + copepodites) in Niel Bay.

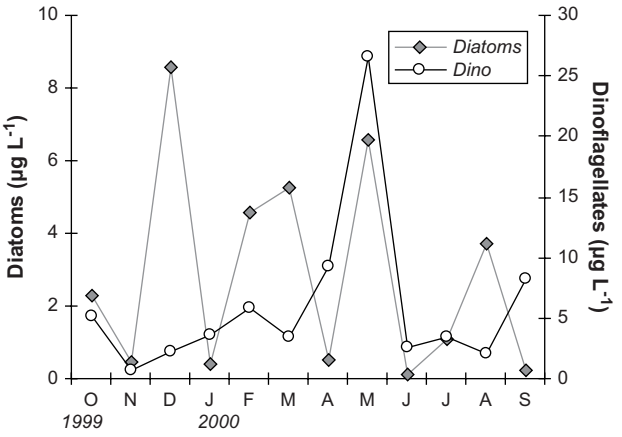


Fig. 3. Temporal variations in diatom and dinoflagellate biomasses in Niel Bay.

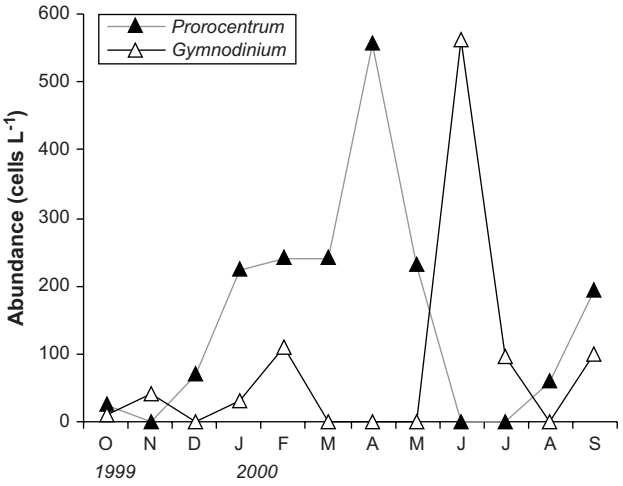


Fig. 4. Temporal variations in the abundance of the predominant dinoflagellate species, *Prorocentrum compressum* and *Gymnodinium* sp., in Niel Bay.

with dinoflagellate abundance or biomass. However, significant positive correlations existed between $DMSP_{5-90}$ and the abundance or biomass of the dinoflagellate species *Prorocentrum compressum* (Spearman's rank correlation test: $r = 0.648$; $p = 0.023$). The 5–90 μm fraction made the greatest contribution to the total $DMSP_p$ pool (annual mean contribution = 62%). This contribution was particularly large in April (96%), when total $DMSP_p$ concentration reached its maximal value, suggesting that the main DMSP producers belonged to the 5–90 μm size class.

3.3.5. Contribution of the 0.2–5 μm size class to total $DMSP_p$

DMSP was present in the same low proportions in this size class as in the >90 μm class. Like this fraction, the 0.2–5 μm size class made a small contribution to the total $DMSP_p$ pool (annual mean contribution = 20%), with $DMSP_{0.2-5}$ concentrations remaining below 1.0 nM (Fig. 9).

The highest DMSP levels in this size class were recorded in January (1.0 nM), March (0.9 nM) and September (0.7 nM). This fraction consisted of picoplankton, comprising bacteria and small phytoplankton cells, these phytoplankton cells not being counted in this study. However, no correlation was found between $DMSP_{0.2-5}$ and bacterial abundance, chlorophyll *a* or protein concentrations.

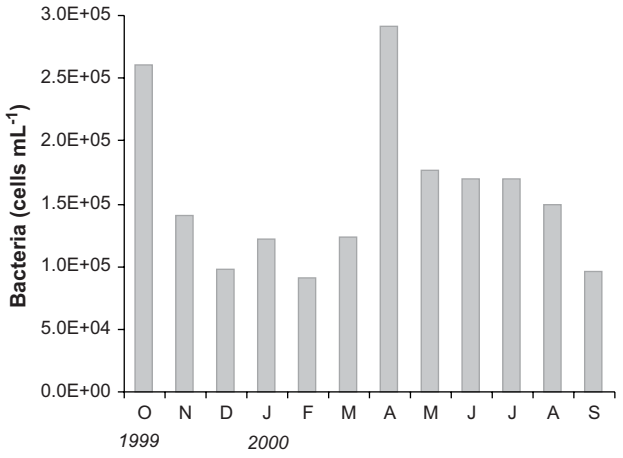


Fig. 5. Temporal variations in bacterial abundance in Niel Bay.

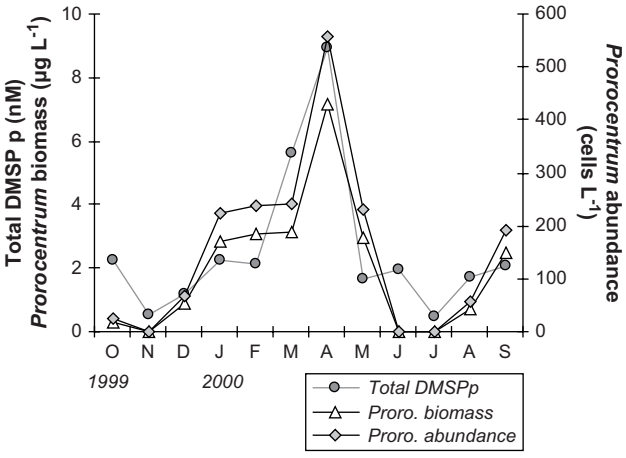


Fig. 6. Relationships between total $DMSP_p$ and *P. compressum* abundance and biomass in Niel Bay.

3.3.6. Intracellular DMSP concentrations

Intracellular DMSP concentrations were obtained for a particulate size class, by dividing DMSP values by the biomass contained in the size class, with biomass expressed as protein content.

3.3.6.1. >90 μm fraction. Intracellular $DMSP_{>90}$ concentrations remained below 0.16 nmol μg^{-1} throughout the year (Fig. 10). Intracellular $DMSP_{>90}$ was generally higher than intracellular $DMSP_{0.2-5}$, but lower than that calculated for the 5–90 μm fraction. The >90 μm size class seemed to produce more DMSP in January (0.07 nmol μg^{-1}), June (0.08 nmol μg^{-1}), and particularly in August (0.12 nmol μg^{-1}) and September (0.16 nmol μg^{-1}). The highest values recorded, in August–September coincided with high zooplankton abundances (Fig. 2).

3.3.6.2. 5–90 μm fraction. Intracellular $DMSP_{5-90}$ concentrations fluctuated strongly over time, with a large increase to 0.78 nmol μg^{-1} in April (Fig. 10). These concentrations were up to 50 times higher than those calculated for the other fractions, suggesting particularly active $DMSP_{5-90}$ production. The highest intracellular $DMSP_{5-90}$ concentrations coincided precisely with the peak abundance of *Prorocentrum compressum* (Spearman's rank correlation test: $r = 0.549$; $p = 0.064$). We compared temporal variations in intracellular $DMSP_{5-90}$ with temporal variations in

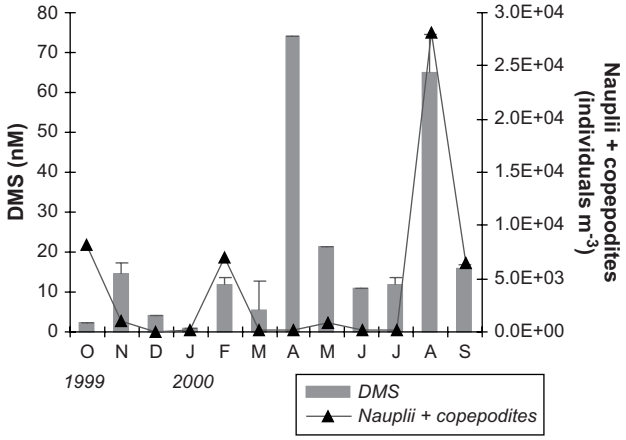


Fig. 7. Relationships between DMS concentrations and abundance of nauplii and copepodites in the zooplankton of Niel Bay.

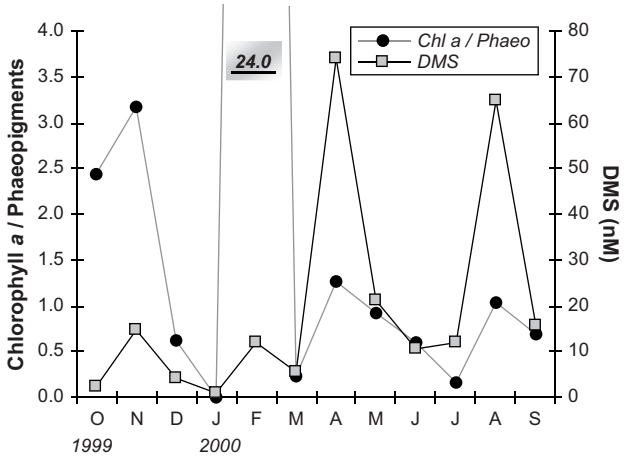


Fig. 8. Relationships between DMS concentrations and chlorophyll *a* to pheopigment ratio in Niel Bay.

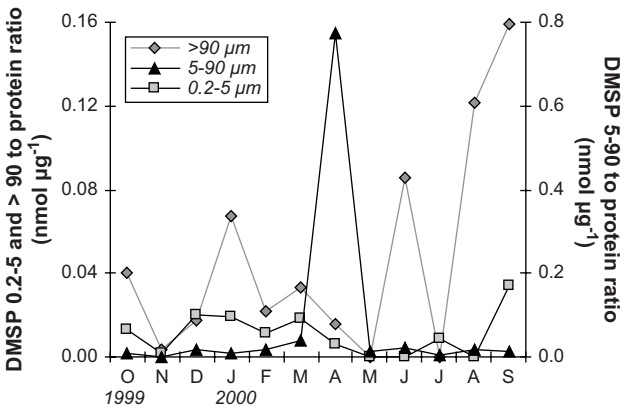


Fig. 10. Temporal variations of DMSP to protein ratio for the >90, 5–90 and 0.2–5 µm fractions in Niel Bay.

nitrate concentration. For most of the year (from October to May), these two concentrations varied in opposite manners: during the nitrate enrichment of seawater, from October to January, intracellular $DMSP_{5-90}$ levels remained low, rapidly increasing from February to April when nitrate was exhausted. This pattern was confirmed in May, when a secondary nitrate peak coincided with a decrease in intracellular $DMSP_{5-90}$. These observations suggest a possible negative relationship between intracellular DMSP and nitrate concentrations in seawater.

3.3.6.3. 0.2–5 µm fraction. The 0.2–5 µm size class produced the smallest amounts of DMSP, with low intracellular DMSP levels, not exceeding 0.03 nmol μg^{-1} in September (Fig. 10). Variations in intracellular $DMSP_{0.2-5}$ concentrations were characterized only by moderate increases between December and March, and then in July and September.

4. Discussion

According to Ignatiades (2005), there is no clear international consensus concerning the appropriate factors to be used to determine the trophic status of seawater. Using chlorophyll *a* concentration as a reliable indicator of water quality in the Aegean Sea (Eastern Mediterranean), Ignatiades estimated that oligotrophy was reached at chlorophyll *a* concentrations below 0.5 µg L^{-1}, whereas concentrations between 0.5 and 1.0 µg L^{-1}, and >1.0 µg L^{-1} indicated mesotrophy and eutrophy, respectively. In Niel Bay, the annual mean concentration of chlorophyll *a* was 0.4 µg L^{-1}, confirming the classification of Niel Bay as a Mediterranean oligotrophic study sites, as reported by Jamet et al. (2005).

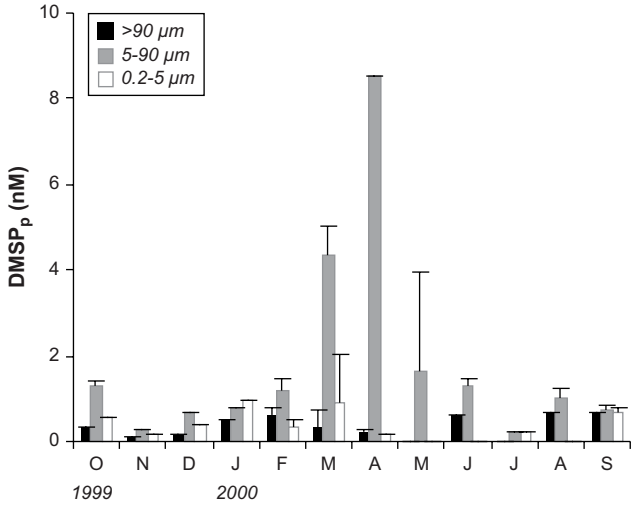


Fig. 9. Temporal variations of DMSP concentrations in the >90, 5–90 and 0.2–5 µm fractions in Niel Bay.

The annual mean total $DMSP_p$ concentration in Niel Bay was 2.6 nM (0.5–8.9 nM). This average is much lower than the total $DMSP_p$ levels recorded in the Little Bay of Toulon where a mean annual value of 16.1 nM (2.1–58.8 nM) was obtained. This finding probably results from differences in trophic levels, Niel Bay being an oligotrophic study site whereas Little Bay of Toulon displays eutrophication, with much higher nutrient concentrations and phytoplankton biomasses and abundances (Jean et al., 2006). Similarly, total $DMSP_p$ levels in Niel Bay were much lower than those in other coastal systems of the Mediterranean Sea (Barcelona harbor: 3.4–4.7 µM) (Belviso et al., 2000), in the Gulf of Maine (>200 nM) (Matrai and Keller, 1993; Townsend and Keller, 1996) or in the Gulf of St Lawrence (6–117 nM) (Cantin et al., 1996). As previously stated, it was difficult to compare our results with previous studies as few studies have been carried out on oligotrophic coastal systems. However, Ledyard and Dacey (1996) reported DMSP concentrations similar to those obtained in our study (2–9 nM) in the oligotrophic Sargasso Sea.

In Niel Bay, total $DMSP_p$ concentrations followed a strongly seasonal pattern, peaking in spring (March–April) at values 18 times higher than the autumnal minima. According to Liss et al. (1993), DMSP concentrations follow a strong seasonal pattern in temperate coastal waters. In most studies, DMSP levels are highest in spring, consistent with our results (Christaki et al., 1996; Kwint and Kramer, 1996; Townsend and Keller, 1996; Dacey et al., 1998; Van Duyl et al., 1998). Our lowest $DMSP_p$ concentrations were recorded in autumn, during the heavy rains affecting the Toulon area. Indeed, the subsequent drop in salinity of Niel Bay waters generated hypo-osmotic conditions that may have induced phytoplankton cell lysis, resulting in the release of large amounts of intracellular DMSP into seawater. The same phenomenon, based on the properties of DMSP as an osmolyte, has also been observed by Dickson and Kirst (1986) in cultures of *Tetraselmis subcordiformis*. In Niel Bay waters, a role for DMSP as an osmolyte was suggested by the peak in total $DMSP_p$ levels observed in March, coinciding with an increase in salinity.

In Niel Bay, particles of 0.2–5 µm, consisting of bacteria and picoplankton cells (which were not counted), contributed only 20%

of the total $DMSP_p$ pool. Other authors also considered the contributions of smaller particle fractions to be negligible: Meyerdierks et al. (1997) for particles <5 μm in the southern Atlantic Ocean, and Corn et al. (1996) for the picoplankton fraction, which accounted for only 10% of total $DMSP_p$ in the Ligurian Sea. By contrast, particles in the size range 5–90 μm accounted for a mean of 62% of the total $DMSP_p$. This contribution increased at the same time as total $DMSP_p$, indicating the key role played by the 5–90 μm fraction in Niel Bay DMSP production. In Barcelona harbor, Belviso et al. (2000) found that $DMSP_p$ in the >10 μm size fraction accounted for more than 90% of the total $DMSP_p$, consistent with our results. Microzooplankton organisms, such as ciliates and tintinids, may also be present (Belviso et al., 2000) in Niel Bay, but the DMSP-productive 5–90 μm fraction consisted principally of phytoplankton cells. In this ecosystem, most of the phytoplankton species counted were dinoflagellates or diatoms. However, dinoflagellates clearly dominated the phytoplankton community, with an annual mean biomass of 68%, versus only 32% for diatoms. The dinoflagellate population increased in the spring, in May, whereas several smaller diatom peaks were observed, in December, February–March, May and August. In December and May, peaks in the diatom population coincided precisely with high nitrate levels in seawater.

As previously reported, no correlation was found between the total $DMSP_p$ and biomass expressed as chlorophyll *a* or total protein concentrations (Jean et al., 2006). Similarly, $DMSP_{5-90}$ was not correlated with chlorophyll *a* or protein concentrations in the 5–90 μm fraction. This absence of correlation has been observed in many oceanic areas (Kwint and Kramer, 1996; Townsend and Keller, 1996; Belviso et al., 2000). Some authors agree that there is not necessarily a positive relation between chlorophyll *a* concentration and phytoplankton biomass, chlorophyll *a* being a better marker of photosynthetic activity (Bienfang and Szyper, 1981). To strengthen possible relationships between DMSP levels and the plankton communities of Niel Bay, we calculated specific phytoplankton biomasses, using the biovolumes determined by microscopy. However, DMSP concentrations were always not correlated with total phytoplankton biomass. According to Simo et al. (1997), such correlations occur only during monospecific blooms of phytoplankton producing DMSP. DMSP production is generally limited to particular groups of phytoplankton, such as dinoflagellates and prymnesiophytes (with coccolithophorids), whereas diatoms are thought to produce smaller amounts of DMSP (Belviso et al., 1990; Liss et al., 1993). We obtained similar results in a previous study using phytoplankton isolates, which showed DMSP contents to be five times higher in dinoflagellates (125 mM) than in diatoms (25 mM) (Jean et al., 2005).

However, in Niel Bay, where dinoflagellates dominated the phytoplankton community in terms of biomass and abundance, $DMSP_p$ and $DMSP_{5-90}$ were not correlated with this taxonomic group. Jean et al. (2005) demonstrated large differences in DMSP production among the various dinoflagellates isolated, with DMSP contents ranging from 37 mM in *Ceratium furca* to 3400 mM in *Alexandrium minutum*. In Niel Bay, dinoflagellates were more diverse, although some species, such as *Prorocentrum compressum* and *Gymnodinium* sp., were more abundant than others. The relatively high level of dinoflagellate diversity observed in this ecosystem may weaken the positive relationship generally found with DMSP. Indeed, when we investigated relationships with individual dinoflagellate species, some significant positive correlations were observed between total $DMSP_p$ and *P. compressum* abundance or biomass (Spearman's rank correlation test: $r = 0.704$; $p = 0.011$), and between $DMSP_{5-90}$ and *P. compressum* abundance or biomass (Spearman's rank correlation test: $r = 0.648$; $p = 0.023$), that were not revealed in the study by Jean et al. (2006). In the Mediterranean Bay of Villefranche-sur-mer, Belviso et al. (1990) also found that DMSP was predominantly associated with the population of *Prorocentrum* sp. This suggests that *P. compressum* may play an important role in the DMSP production of Niel Bay, similar to that of *A. minutum* in Little Bay of Toulon. The non-negligible intracellular DMSP contents previously measured in *Prorocentrum arcuatum* (442 mM) support this hypothesis (Jean et al., 2005). However, the DMSP contents found in *Prorocentrum* were (8 times) lower than those in *Alexandrium*, potentially accounting for the much lower (by a factor of 7) maximal DMSP levels in Niel Bay than in Toulon Bay. These differences were also amplified by differences in phytoplankton abundances and biomasses between the two study sites. The >90 μm particulate fraction contributed 18% annually to the total $DMSP_p$ pool. The highest $DMSP_{>90}$ concentrations occurred when zooplankton abundances were maximal, in February and August. Thus, DMSP may accumulate in zooplankton, but it does so to a lesser extent than in phytoplankton. The chlorophyll *a* peak associated with moderate developments of dinoflagellate and diatom populations during this period suggests that the DMSP in the zooplankton in Niel Bay probably came from phytoplankton cells ingested during grazing. Some authors have reported that the DMSP ingested by zooplankton is not metabolized (Kwint et al., 1996a; Tang, 2000). Taking this result into account, zooplankton organisms may be partly responsible for the bioaccumulation of DMSP in the trophic chain.

DMS concentrations in Niel Bay were found to be higher than those of its precursor, DMSP. The DMS to $DMSP_p$ ratio, which was high other than in October and January (below 1), had a mean value of 11.4. This situation is unusual in oligotrophic areas, in which most authors report a low DMS to $DMSP_p$ ratio, as exemplified by Belviso et al. (2004), who reported a ratio close to 0, and Dacey et al. (1998), who obtained a maximal ratio of 1.6 and a mean ratio of 0.57 during summer in the oligotrophic Sargasso Sea. Kiene and Slezak (2006) have demonstrated that pressure and the handling of large volumes of seawater influence the extraction of $DMSP_p$ as dissolved DMSP ($DMSP_d$), and consequently the release of DMS. As we suspected that the method used during the filtration procedure might account for our high DMS to $DMSP_p$ ratio, we used a corrective factor ($37.3\% \pm 7.9\%$) to take into account the potential losses of $DMSP_p$ and gains of DMS resulting from filtration artifacts. After the application of this corrective factor to DMS and $DMSP_p$ concentrations, we again obtained a high DMS to $DMSP_p$ ratio, of between 4.3 (applying the maximal corrective factor 45.2%) and 6.2 (applying the minimal corrective factor 29.4%), demonstrating that our results were not due to filtration artifacts. Without taking the filtration artifacts into account, we compared the ratio obtained at Niel Bay with that obtained in the Little Bay of Toulon. The annual mean DMS to $DMSP_p$ ratio in Niel Bay (11.4) was about three times higher than that obtained in the Little Bay of Toulon (4.2). However, DMS and $DMSP_p$ concentrations were three times and six times higher, respectively, in Little Bay than in Niel Bay (Jean et al., 2006). All these observations suggest that DMS levels in Niel Bay could have a source other than pelagic plankton, which are clearly less abundant in Niel Bay than in Little Bay. As benthic macroalgae, such as *Ulva lactuca*, *Polysiphonia fastigiata* and *Polysiphonia lanosa,* are able to produce DMSP (Challenger, 1951; Cantoni and Anderson, 1956; Greene, 1962), it is possible that $DMSP_d$ and DMS production by benthic macroalgae or by macrophytes, such as *Posidonia* spp., might account for high levels of DMS production by non-planktonic species. Few studies have considered the contribution of benthic macroalgae and/or macrophytes to DMS and DMSP concentrations, as most oceanographic studies generally take place in deep waters, in which the activity of these organisms is negligible relative to that of the pelagic plankton. However, as our study site in Niel Bay is shallow (depth 5 m), the seawater column is sufficiently clear for

high levels of photosynthetic activity at the benthic level and, thus, for high levels of DMSP and DMS production by benthic macroalgae and/or macrophytes. However, Nedwell et al. (1994) observed in the North Sea that concentrations of the precursor, DMSP, were higher in the surface sediments than in the seawater column, suggesting that, in the shallow waters of Niel Bay, high DMS concentrations may also result from the conversion of DMSP accumulated in sediments.

It has been suggested that DMS production may be indirectly increased by the sloppy feeding of zooplankton grazing on algal cells (Dacey and Wakeham, 1986; Belviso et al., 1990; Wolfe et al., 1994). Similarly, significant correlations have sometimes been observed between zooplankton biomass and DMS concentrations, confirming that zooplankton grazing on phytoplankton contributes to DMS production (Leck et al., 1990; Yang et al., 2000). In Niel Bay, we observed no such correlations, but the DMS peaks observed in February and in August coincided with higher zooplankton abundances. Simo and Pedros-Alio (1999) reported the existence of a "summer DMS paradox" in oligotrophic marine ecosystems. In these environments, high DMS levels may occur in summer, at precisely the times at which phytoplankton abundances are low. These authors suggested that this paradox may result from the poor degradation of DMS by some bacteria, the activity of which is decreased by nutrient limitation and strong UVB radiation. This may be the case in August in Niel Bay, with summer DMS concentrations in this ecosystem not exclusively dependent on zooplankton activity.

Similarly, zooplankton did not seem to be involved in the maximal DMS concentrations recorded in April, as suggested by the low abundance of zooplankton during this period. According to Kwint et al. (1996b), DMS release is particularly active during phytoplankton growth and during phytoplankton senescence. In April, total $DMSP_p$ concentrations were maximal, at the same time as *Prorocentrum compressum* and bacterial abundances peaked. Consequently, in April, high DMS concentrations are probably associated principally with growth of the dinoflagellate population, as this taxonomic group may contain the DMSP-lyase enzyme converting DMSP into DMS (Ishida, 1968; Niki et al., 2000). These DMS levels may also result from natural DMSP excretion by dinoflagellates, followed, as suggested by the higher bacterial abundances in spring, by conversion of the extracellular DMSP into DMS mediated by bacterial DMSP-lyase. The chlorophyll *a* to pheopigment ratio was used by Shenoy et al. (2006) as an indicator of the health of phytoplankton cells. In our study, the positive relationship that seems to exist between this ratio and DMS concentrations suggests that, in Niel Bay, DMS production was associated with phytoplankton growth. This seems to be the case in April in particular, when peak DMS concentrations coincided with a peak in the chlorophyll *a* to pheopigment ratio. This relationship even indicates that the high DMS concentrations measured in February and in August were probably due to zooplankton grazing on healthy phytoplankton cells.

Finally, our study suggests that intracellular DMSP concentrations in Niel Bay may be negatively related to the concentration of nitrate in seawater, with DMSP contents rapidly increasing when nitrate levels are low. Such a relationship has been observed in many marine ecosystems (Andreae and Barnard, 1984; Andreae et al., 1985; Curran et al., 1998; Yang, 2000). DMSP is structurally similar to glycine betaine (GBT), a well known osmolyte in phytoplankton. However, unlike GBT, DMSP does not contain nitrogen (Kwint, 1997). DMSP synthesis includes a transamination step which would encourage algae to synthesize DMSP rather than GBT in nitrogen-deficient conditions (Gage et al., 1997). According to Stefels (2000), nitrogen is released during DMSP production from methionine, allowing intracellular nitrogen regeneration and conferring resistance to nitrogen deficiency. This relationship may also account for the largest amounts of DMSP being produced by nitrophilic phytoplankton species, such as *Phaeocystis* sp. or *Alexandrium minutum,* blooming in eutrophicated ecosystems.

5. Conclusions

As previously observed in the eutrophicated Little Bay of Toulon, our data suggest that particulate DMSP in the oligotrophic Niel Bay originated principally from phytoplankton. The greatest producer of DMSP in the Niel Bay phytoplankton community seemed to be the dinoflagellate species *Prorocentrum compressum*, whereas *Alexandrium minutum* was the principal DMSP producer at Little Bay of Toulon. The lower intracellular DMSP contents in *Prorocentrum* than in the nitrophilic *A. minutum*, amplified by differences in phytoplankton abundances and biomasses between the two study sites, may account for the lower DMSP concentrations recorded in Niel Bay (oligotrophic) than in Little Bay of Toulon (eutrophicated). During the study, some of the total particulate DMSP pool was also stored in the zooplankton compartment, particularly in copepods (nauplii and copepodites), as a result of their grazing on phytoplankton. The high DMS levels recorded in Niel Bay do not seem to result purely from pelagic plankton, but instead may also be generated through the activity of benthic macroalgae or macrophytes, such as *Posidonia* spp., meadows of which are present in this ecosystem. The shallow configuration of Niel Bay, by increasing the photosynthetic activity of these macrophytes and favoring the resuspension of the DMSP accumulated in sediments, may also increase DMS levels.

Acknowledgments

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