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Comparison of β -dimethylsulfoniopropionate (DMSP) levels in two mediterranean ecosystems with different trophic levels

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Abstract

β -dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) concentrations were recorded from September 1999 to September 2000 in two geographically close ecosystems, differently affected by eutrophication: the Little Bay of Toulon and the Niel Bay (N.W. Mediterranean Sea, France). Little Bay had higher nutrient levels ($[\text{NO}_3^-]_{\text{max.}} = 30.3 \mu\text{M}$; $[\text{PO}_4^{3-}]_{\text{max.}} = 0.46 \mu\text{M}$) and higher chlorophyll *a* concentrations ($[\text{chl } a]_{\text{mean}} = 2.4 \mu\text{g/L}$) compared to Niel Bay ($[\text{NO}_3^-]_{\text{max.}} = 19.7 \mu\text{M}$; $[\text{PO}_4^{3-}]_{\text{max.}} = 0.17 \mu\text{M}$; $[\text{chl } a]_{\text{mean}} = 0.4 \mu\text{g/L}$). In the two sites, we measured dissolved ($\text{DMSP}_d < 0.2 \mu\text{m}$) and particulate DMSP ($\text{DMSP}_p > 0.2 \mu\text{m}$) concentrations. The DMSP_p was particularly analysed in the 0.2–5, 5–90 and $>90 \mu\text{m}$ fractions. In the eutrophicated Little Bay, DMSP_d concentrations showed a clear seasonality with high values from January to March (124–148 nM). The temporal profile of the DMSP_p concentrations was similar, peaking in February–March (38–59 nM). In the less eutrophic Niel Bay, DMSP_p concentrations were much lower (6–9 nM in March–April), whereas DMSP_d concentrations were relatively high (110–92 nM in February–March). DMS concentrations were elevated from the end of the winter to the spring in Little Bay, ranging from 3 nM in October to 134 nM in March. In the less eutrophic Niel Bay, lower DMS levels were observed, generally not exceeding 20 nM. Each particulate fraction (0.2–5; 5–90; $>90 \mu\text{m}$) contained less DMSP in Niel Bay than in Little Bay. At both sites, the 5–90 μm fraction made up most of the DMSP_p . This 5–90 μm fraction consisted of microphytoplankton, principally Dinophyceae and Bacillariophyceae. The 5–90 μm biomass calculated from cell biovolumes, was more abundant in Little Bay where the bloom at the end of the winter (165 $\mu\text{g/L}$ in March) occurred at the same time as the DMSP peaks. The estimated DMSP_p to biomass ratio for the 5–90 μm fraction was always higher in Little Bay than in Niel Bay. This suggests that the high DMSP levels recorded in Little Bay were not only due to a large Dinophyceae presence in this ecosystem. Indeed, the peak of DMSP_p to biomass ratio obtained from cell biovolumes (0.23 nmol/ μg in March) was consistent with the proliferation of *Alexandrium minutum*. This Dinophyceae species may account for between 50% (2894 cells/L) and 63% (4914 cells/L) of the total phytoplankton abundance in the Little Bay of Toulon.

Keywords: DMSP; DMS; Mediterranean Sea; *Alexandrium minutum*; Eutrophication

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2. Materials and methods

1. Introduction

Dimethylsulfide (DMS) is the main source of biogenic volatile sulfur compounds found in the atmosphere (Stefels, 1997). According to the CLAW hypothesis, DMS may influence the climate by regulating the increased greenhouse effect (Charlson et al., 1987). DMS is derived from enzymatic cleavage of β -dimethylsulfoniopropionate (DMSP) (Challenger, 1951), which is involved in the osmoregulation and the cryoprotection of marine algae (Andreae, 1990; Kiene and Service, 1991; Kirst et al., 1991). After demethylation or demethiolation, bacteria can use DMSP as a source of reduced sulfur (Kiene et al., 1999; Kiene and Linn, 2000). More recent studies have shown that DMSP and its degradation products have antioxidant properties for marine phytoplankton (Steinke et al., 2002; Sunda et al., 2002; Van Rijssel and Buma, 2002).

There have been very few investigations into the influence of eutrophication on the DMS(P) cycle, especially in shallow coastal systems. According to Kwint et al. (1993), experiments reflecting different trophic levels cannot be easily carried out in the laboratory. As a result, these experiments are difficult to transpose to the marine environment. The present study is a comparison of the DMSP concentrations measured in two coastal ecosystems of the N.W. Mediterranean: the eutrophic Little Bay of Toulon and the less eutrophic Niel Bay. We aim to describe the impact of eutrophication on the evolution of DMSP concentrations in the phytoplankton communities at the two different sites during a full year (September 1999 to September 2000).

2.1. Study sites

The study was carried out in two Mediterranean ecosystems: the Little Bay of Toulon (S_1 ; depth=13 m) and the Niel Bay (S_2 ; depth=4 m) (Fig. 1). Toulon Bay (Lat. $43^{\circ}05'N$ and Long. $6^{\circ}00'E$) is located on the French N.W. Mediterranean coast. Its seaport handles a large amount of military and commercial traffic. Toulon Bay is composed of two areas separated by a breakwater: the western basin, also called Little Bay is semi-enclosed, whereas the eastern basin, also called Large Bay, is open to the sea. According to Ifremer, Little Bay is contaminated by chemical pollutants, such as organic compounds (PCB, anti-fouling paints), heavy metals (Hg, Zn, Pb and Cu) and occasionally by toxic plankton species, such as *Alexandrium minutum* and *Dinophysis* spp. (Belin et al., 1995; Ifremer, 1997).

Niel Bay is widely open to the sea and is located 15 km from the city of Toulon, outside of Toulon Bay (Fig. 1). Niel, the port on the Giens peninsula, handles only small fishing and modest tourist traffic in the summer. Porquerolles Island, just in front of Niel Bay, represents a natural protected area. Niel Bay is characterized by a marine plant habitat of *Posidonia oceanica*. This species is protected at the international level and is still present in vast areas of Niel Bay. *P. oceanica* brings sufficient dioxygen concentrations to allow development of seventy fish species. While *P.*

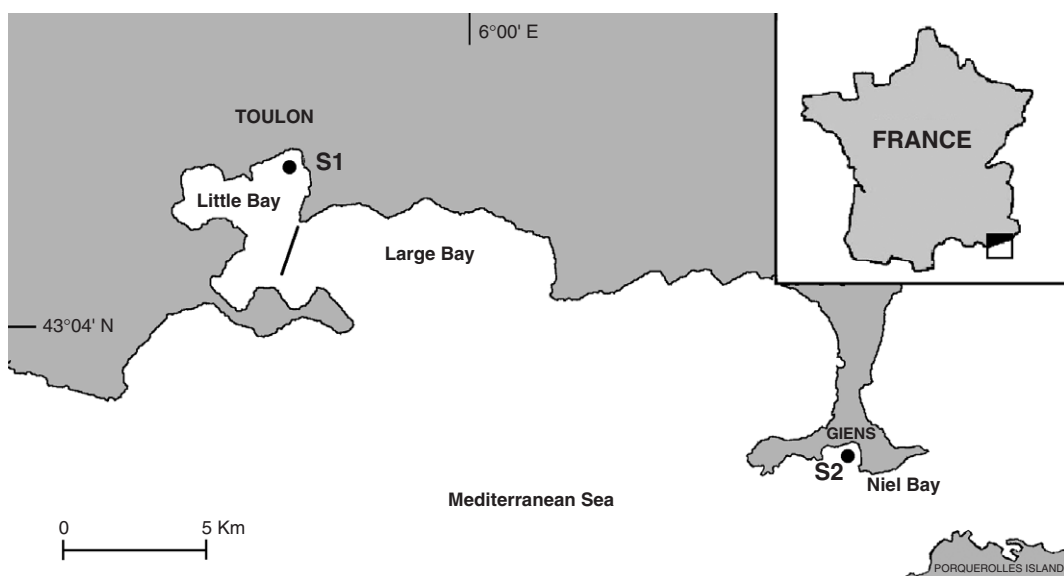


Fig. 1. Location of the sampling stations in Little Bay (S_1) and Niel Bay (S_2).

oceanica knows a strong regression in many mediterranean zones, its presence in Niel Bay shows evidence of a healthy ecosystem (Paillard et al., 1993).

2.2. Field sampling

Samples were collected monthly from September 1999 to September 2000, between 9:00 a.m. and 11:00 a.m., in the Little Bay of Toulon (S₁) and in Niel Bay (S₂). Water samples were systematically taken from a depth of 2 m, using a Niskin 10 L sampling bottle. DMSP_p (particulate DSMP) analysis was performed on a 4 L aliquot in S₁ and on a 8 L aliquot in S₂. At the two sites, DMSP_d (dissolved DSMP) concentrations were determined from a 250 mL subsample of filtered seawater. In S₁, several ten-litre samples were taken to study the phytoplankton community, and to measure chlorophyll *a* and protein concentrations, with 20 L samples being taken in S₂. Samples for DMS determination were carefully collected to avoid headspace and degassing in the sampling bottle. DMS analysis was performed from a 250 mL subsample of unfiltered seawater.

2.3. DMSP_p, DMSP_d and DMS analysis

DMSP_p was fractionated into size classes by successively filtering seawater samples through polyamide membranes (Nytrel-TI, UGB) with 90, 5 and 0.2 μm pore size. Gravity filtration was used for the 90 μm pore membranes, whereas gentle pressure was used to separate the 5–90 μm fraction and the 0.2–5 μm fraction. Each filter was put into 250 mL of cold distilled water, and then, resuspension and extraction of DMSP from the particulate was carried out using sonication. DMSP_d was isolated from the DMSP_p by filtering 250 mL seawater through a membrane with 0.2 μm pores. According to Bates et al. (1994), the distribution of DMSP between particulate and dissolved fractions may depend on filtering pressure. Cell lysis which occurs during filtration, may induce a decrease in DMSP_p and an increase in DMSP_d. In this study, we estimated the losses due to filtration by comparison of the DMSP_p concentrations coming from subsamples filtered through 0.2 μm pore size, respectively using gravity and gentle pressure. As a result, the losses of DMSP_p due to filtration were estimated at 37.3% ± 7.9% (*n* = 4).

The seawater DMSP determination requests the DMS analysis. In this work, DMSP_p (or DMSP_d) was therefore converted into DMS using the cold alkali treatment method (Dacey and Blough, 1987; Turner et

al., 1988). NaOH (10 M) was added until a pH of approximately 13 was attained (White, 1982; Stefels and Van Boekel, 1993). The alkaline solution was transferred to a silanized glass bottle with septum and no headspace. DMSP was fully transformed into DMS after 24 h in the dark at 2 °C. DMS was analysed according to a modified method based on a cryotrapping 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" PTFE column filled with Chromosil 330 (Supelco). Subsamples (5 mL) were taken from the alkaline solution with a polyethylene needle and the DMS was purged from the solution with 99.99% helium at a flow rate of 100 mL/min and trapped onto Tenax T.A. packed at -40 °C, and dried using magnesium perchlorate (Despiou et al., 2002). The Tenax was heated to 180 °C and DMS was transferred onto the column. The flame photometric detector was supplied with a flow of air ($F_a = 150$ mL/min) and hydrogen ($F_{red} = 75$ mL/min; $F_{oxy} = 7$ mL/min). Calibration was made with a DMS solution, using linear interpolation: $\ln(\text{Peak area}) - \ln(\text{DMS mass})$. The minimal quantity of detected DMS was equivalent to a peak amplitude two times higher than the baseline noise. The corresponding limit of detection was determined by analysing decreased DMS quantities. This limit of detection reached 2 ng DMS. The average precision of DMS concentrations, obtained by carrying out different injections (*n* = 9) of fixed DMS concentrations, was 13%. The DMSP measurements were carried out in triplicates and average DMSP values are presented with their standard deviations.

2.4. Biotic parameters

2.4.1. Phytoplankton counts and biomass

Samples of phytoplankton were preserved in an alkaline Lugol solution. Phytoplankton were identified to species level if possible and cells were counted under an inverted microscope (×400) according to Utermohl (1958) and the Lund Cell technique (Lund et al., 1958). Phytoplankton cells were counted in at least 40 fields, with at least 400 cells over all fields. At least 100 cells of the most abundant species were counted. Counts were then extrapolated to provide concentrations per litre (cells/L). The biomasses were then estimated according to Lohman's (1908) calculation technique.

2.4.2. Chlorophyll *a*

Seawater samples were filtered using glass fibre filters (Whatman GF/C). Cells collected on the filters

were extracted with 90% acetone to yield chlorophyll *a*. Chlorophyll *a* determination was carried out according to the visible spectrophotometric method described by Lorenzen (1967).

2.4.3. Protein analysis

Seawater samples were filtered through polyamide membranes (Nytrel-TI, UGB) to collect the 5–90 μm particulate fraction. Proteins collected on the filter were stored at $-80\text{ }^{\circ}\text{C}$ in 15 mL polyethylene bottles until analysis. After resuspension in distilled water, proteins were analysed with Folin phenol reagent (Lowry et al., 1951). Bovine serum albumin (BSA) was used as a standard. Each protein measurement was carried out in triplicate.

2.5. Abiotic parameters

Meteorological data were obtained from Météo-France. Seawater temperature and salinity were measured ($\pm 0.1\text{‰}$ and $\pm 0.1\text{ }^{\circ}\text{C}$) at a depth of 2 m with a WTW thermosalinometer (LF 197 model). Orthophosphate concentrations (P-PO_4) were determined according to the method of Murphy and Riley (1962). The technique of Wood et al. (1967) modified by Le Poupon (1994) was used to estimate the nitrate levels (N-NO_3) in seawater.

3. Results

3.1. Abiotic parameters

A large drop in salinity from 37.3‰ to 29.0‰ occurred in Little Bay (S_1), between September and October 1999 as a result of heavy autumnal rains

(288 mm in October) (Fig. 2A). The salinity then gradually increased and stabilized at around 38‰, from February. In Niel Bay (S_2), the decrease of salinity was less severe, with values reaching 35.9‰ in September and 35.2‰ in October 1999 (Fig. 2B).

In Little Bay, the annual mean nutrient concentration was 8.8 μM for nitrate and 0.13 μM for phosphate (Fig. 3A). In Niel Bay, these concentrations were lower and reached 5.5 μM for nitrate and 0.10 μM for phosphate (Fig. 3B). After the autumnal rains, the maximum nutrient concentrations were less important in Niel Bay ($[\text{NO}_3]_{\text{max}}=19.7\text{ }\mu\text{M}$ in December; $[\text{PO}_4]_{\text{max}}=0.17\text{ }\mu\text{M}$ in November) than in Little Bay ($[\text{NO}_3]_{\text{max}}=30.3\text{ }\mu\text{M}$ in October; $[\text{PO}_4]_{\text{max}}=0.35\text{ }\mu\text{M}$ in October).

3.2. Chlorophyll *a*

There were higher levels of chlorophyll *a* in Little Bay than in Niel Bay, throughout the year (Wilcoxon rank sum test: the *p*-value is the probability when the null hypothesis H_0 is true, it is generally accepted that the two populations are significantly different when $p < 0.05$, here, the Statview[®] software gives $p = 0.001$) (Fig. 4A). The annual mean value of chlorophyll *a* was of 2.4 $\mu\text{g/L}$ in Little Bay with peaks reaching 9.0 $\mu\text{g/L}$ (in September 1999), whereas the annual mean value in Niel Bay was only 0.4 $\mu\text{g/L}$.

3.3. Phytoplankton communities

The algal communities in the two bays were principally composed of microphytoplankton: Bacillariophyceae and Dinophyceae. The total microphytoplankton biomasses, calculated from cell biovolumes, were

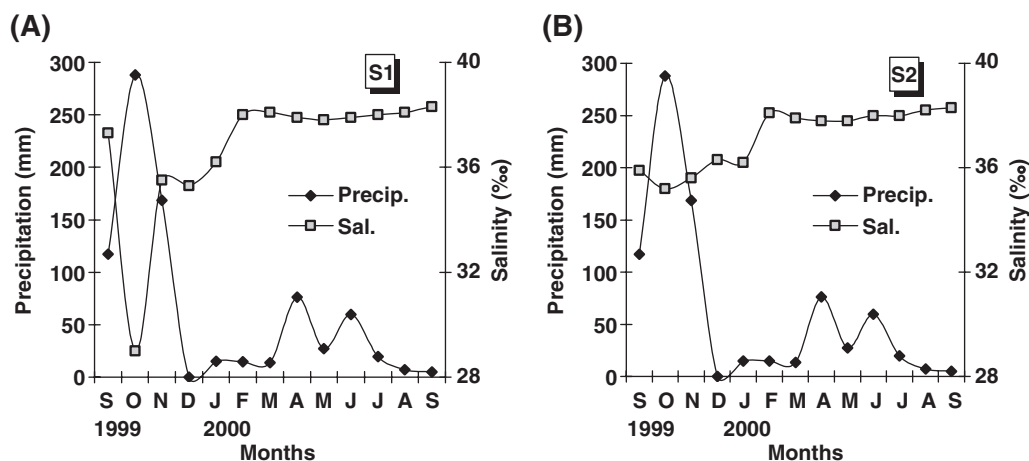


Fig. 2. Temporal variations in precipitation and salinity in Little Bay (S_1) (Fig. A) and Niel Bay (S_2) (Fig. B).

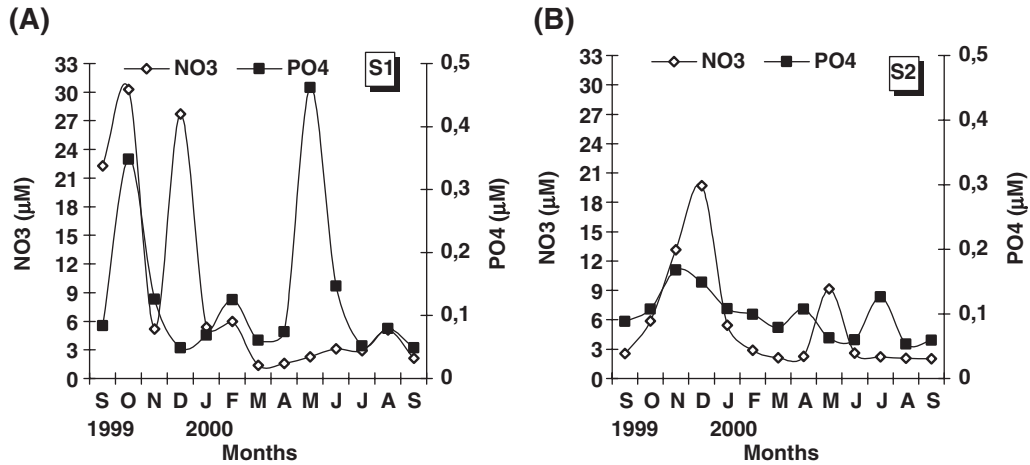


Fig. 3. Temporal variations in nitrate and orthophosphate concentrations in Little Bay (S₁) (Fig. A) and Niel Bay (S₂) (Fig. B).

always much higher in Little Bay than in Niel Bay (Wilcoxon rank sum test: $p=0.001$) (Fig. 4B). In Little Bay, the microphytoplankton biomass showed a sharp seasonal evolution, with a main bloom at the end of the winter (165 μg/L in March), whereas in Niel Bay, the maximum algal development occurred in spring (33 μg/L in May).

In Little Bay, Dinophyceae were responsible for most of the microphytoplankton biomass, contributing between 42% and 100% (respectively in December and March–June) of the biomass (Fig. 5A). By contrast, there was very little Bacillariophyceae, except in December (58%) and in August (30%). In Niel Bay, Dinophyceae were also the most predominant, however, their relative biomasses were less than in Little Bay (Wilcoxon rank sum test:

$p=0.002$) (Fig. 5B). Bacillariophyceae biomass was much higher in Niel Bay than in Little Bay ($p=0.033$). For example, in December, Bacillariophyceae made up 80% of the phytoplankton biomass in Niel Bay.

3.4. DMSP_p concentrations

We analysed DMSP concentrations in the particulate material collected on 0.2 μm pore filters (Fig. 6A). In Little Bay, the DMSP_p curve followed the same trend as for DMSP_d concentrations (Correlation with Spearman test: $r=0.687$; $p=0.017$). The DMSP_p concentrations were high and showed a marked seasonality. Throughout winter, a gradual increase in DMSP_p was observed, with a maximum through February and March (38–59 nM).

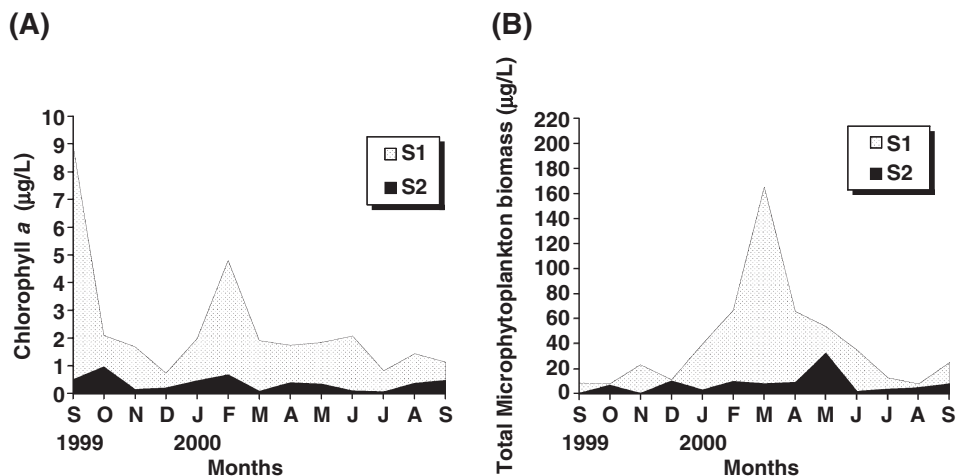


Fig. 4. Temporal variation in the chlorophyll *a* concentrations (Fig. A) and in the total microphytoplankton biomass (Fig. B) in Little Bay (S₁) and Niel Bay (S₂).

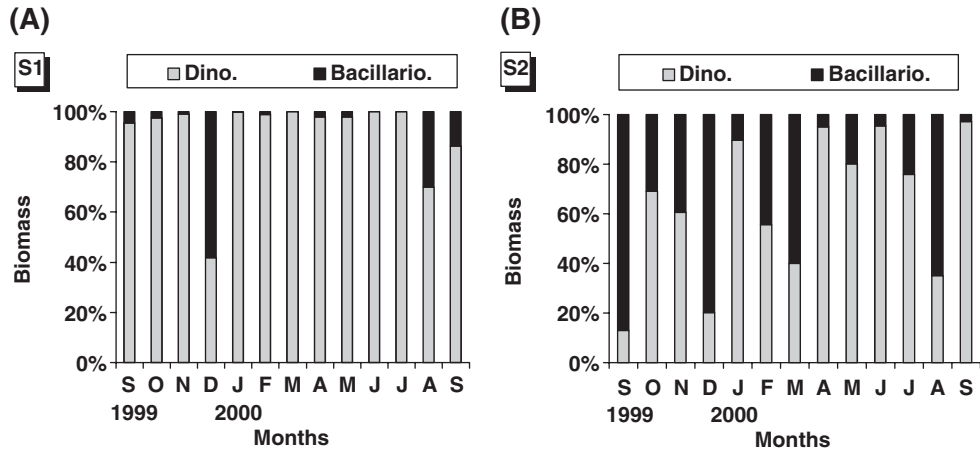


Fig. 5. Relative contributions of Dinophyceae and Bacillariophyceae to the total microphytoplankton biomass in Little Bay (S_1) (Fig. A) and Niel Bay (S_2) (Fig. B).

In spring and summer, the concentrations decreased and reached 21 nM in September 2000.

In Niel Bay, the $DMSP_p$ concentrations were generally much lower than in Toulon Bay (Wilcoxon rank sum test: $p=0.002$). Although temporal evolutions in the two sites were correlated (Correlation Spearman test: $r=0.610$; $p=0.035$), variations in $DMSP_p$ were less marked in Niel Bay. The main event was a relative peak of concentrations in March–April (6–9 nM), one month later than in Little Bay.

3.5. $DMSP_d$ concentrations

We found high concentrations of dissolved $DMSP$ in seawater filtered through 0.2 μm pore membranes (Fig. 6B). Besides, these concentrations were comparable in both Little Bay and Niel Bay.

In Little Bay, $DMSP_d$ concentrations peaked in winter, with the maxima stretching from January to March (124–148 nM). An additional peak of $DMSP_d$ was also recorded in November (123 nM). A similar pattern was observed for the $DMSP_d$ in Niel Bay, with the maximum through February and March (110–92 nM), and a peak in November (83 nM).

3.6. DMS concentrations

Most often, DMS concentrations were high in Little Bay, varying between 3 nM in October and 134 nM in March (Fig. 6C). Temporal evolution of the DMS concentrations was similar to the temporal evolution of the $DMSP$ concentrations in the 5–90 μm fraction (Correlation Spearman test: $r=0.560$; $p=0.052$). DMS concentrations gradually increased at the end of the winter

and stayed very elevated (higher than 100 nM) during the whole spring. A different pattern was observed in Niel Bay, where concentrations showed a non-seasonal variability. Except in April (74 nM) and in August (65 nM), DMS levels did not exceed 20 nM and were significantly lower than in Little Bay (Wilcoxon rank sum test: $p=0.023$). In both Little Bay and Niel Bay, $DMSP_d$ concentrations were often higher than DMS concentrations, except in April–May in S_1 and in July–August in S_2 .

3.7. $DMSP_d/DMSP_p$ ratio

At the two sites, the $DMSP_d$ concentrations were always higher than the $DMSP_p$ concentrations (Wilcoxon rank sum test: $p=0.011$ in Little Bay; $p=0.019$ in Niel Bay). Therefore, the $DMSP_d$ to $DMSP_p$ ratio was always high, especially in Niel Bay, where it reached an exceptional value of 99.0 in November after the autumnal rains (288 mm in October; 167 mm in November) (Fig. 7). In stable meteorological conditions, this ratio decreased and stabilised to between 6.9 (January) and 0.2 (September 2000).

3.8. Distribution of $DMSP_p$ between size classes

We measured the $DMSP$ concentrations in the 0.2–5, 5–90 and >90 μm fractions (Fig. 8) and estimated the relative contributions of these fractions to the total $DMSP_p$ (in %) (Fig. 9).

In Little Bay, the temporal profiles of the $DMSP$ concentrations were similar for all the three fractions (Correlation Spearman test: $r=0.626$ for >90 and 5–90 μm ; $r=0.779$ for >90 and 0.2–5 μm). For all fractions,

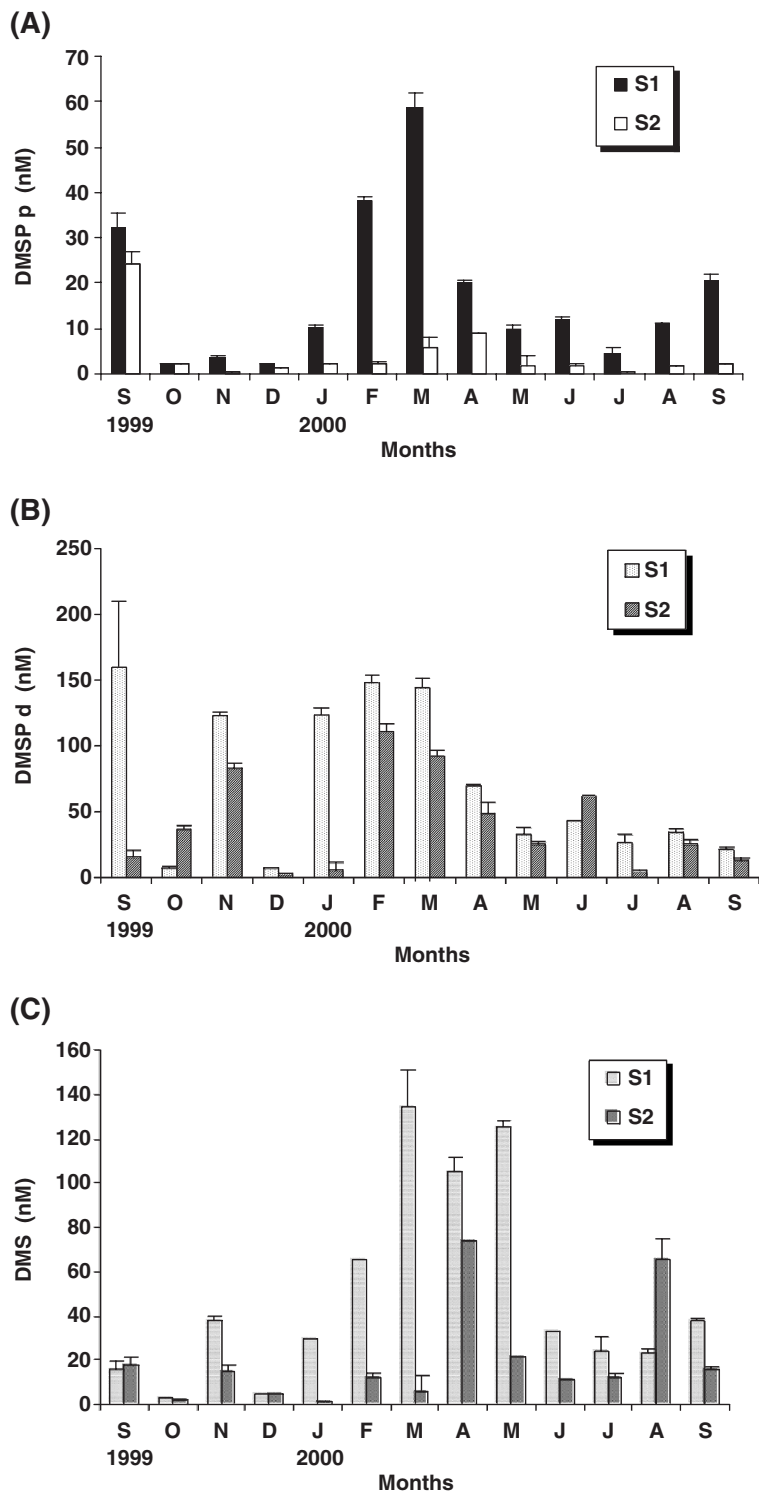


Fig. 6. Temporal variations in DMSP_p (Fig. A), DMSP_d (Fig. B) and DMS concentrations (Fig. C) in Little Bay (S₁) and Niel Bay (S₂).

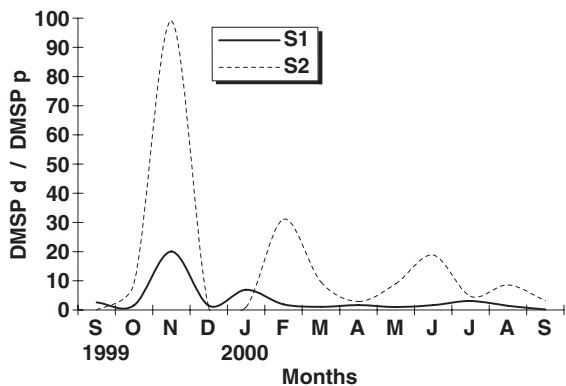


Fig. 7. Temporal variations in the $DMSP_d$ to $DMSP_p$ ratio in Little Bay (S_1) and Niel Bay (S_2).

the highest values were recorded in March (7 nM for the $>90 \mu m$ fraction, 40 nM for the $5-90 \mu m$ fraction and 11 nM for the $0.2-5 \mu m$ fraction). $DMSP_p$ was mostly present in the $5-90 \mu m$ size class, especially in the spring

(100% in May). In the $>90 \mu m$ and the $0.2-5 \mu m$ classes, the $DMSP_p$ levels were generally low and rarely contributed more than 20% of the total concentration, except in December (65%) for the $0.2-5 \mu m$ fraction.

In Niel Bay, the $DMSP$ concentrations for each fraction were much lower than in Little Bay, especially for the $5-90 \mu m$ size class (Wilcoxon rank sum test: $p=0.002$). In this class, the temporal profile was the same as in Little Bay (Correlation Spearman test: $r=0.566$; $p=0.050$). Again, the $5-90 \mu m$ fraction contained most of the $DMSP_p$, and generally contributed to more than 50% of the total $DMSP_p$ concentration, except in summer where there were high concentration in the $>90 \mu m$ fraction (39% in August) and the $0.2-5 \mu m$ fraction (50% in July).

3.9. Microphytoplanktonic $DMSP$ concentration

The $5-90 \mu m$ fraction, which is mainly composed of microphytoplankton, was identified as $DMSP$ -rich. The

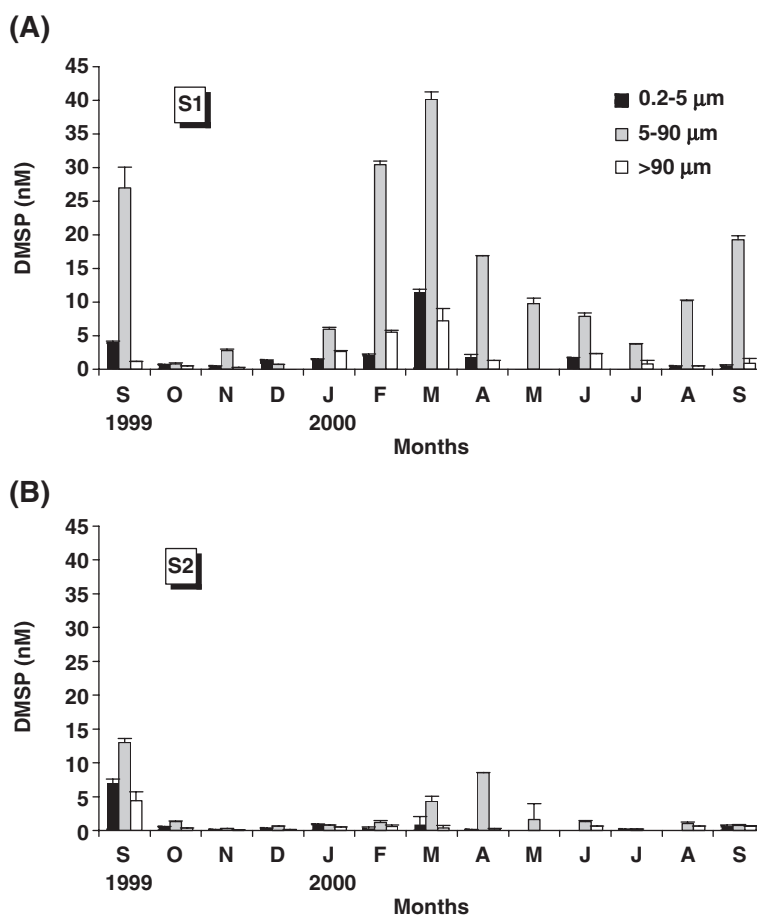


Fig. 8. Temporal variations in $DMSP$ concentrations in >90 , $5-90$ and $0.2-5 \mu m$ fractions of Little Bay (S_1) (Fig. A) and Niel Bay (S_2) (Fig. B).

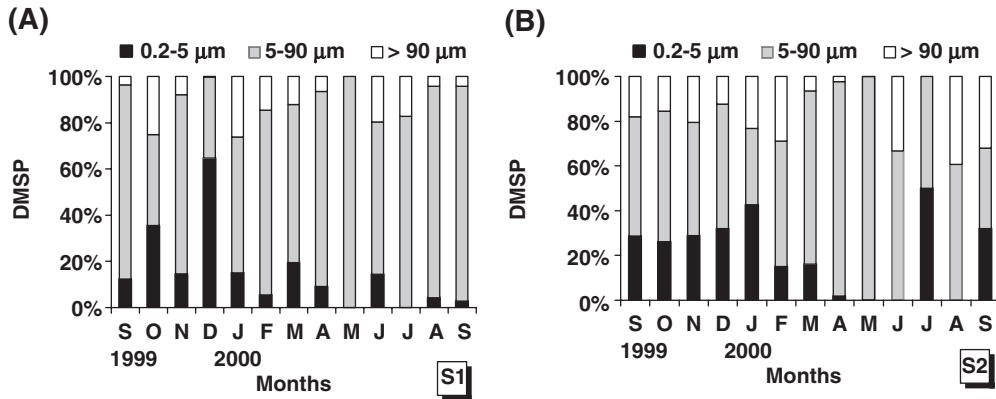


Fig. 9. Relative contributions of the >90, 5–90 and 0.2–5 μm fractions to the total DMSP_p (in %) Little Bay (S_1) (Fig. A) and Niel Bay (S_2) (Fig. B).

microphytoplanktonic DMSP_p concentration was calculated as the DMSP_p concentration to protein *ratio* for the 5–90 μm fraction. In Little Bay, these microphytoplankton concentrations ranged from 6.10^{-3} $\text{nmol}/\mu\text{g}$ in December to 0.28 $\text{nmol}/\mu\text{g}$ in September 2000 (Fig. 10). An increase was observed in winter, resulting in a peak in March (0.23 $\text{nmol}/\mu\text{g}$). In Niel Bay, this DMSP_p to protein *ratio* was mostly below 0.11 $\text{nmol}/\mu\text{g}$ and was much lower than in Little Bay (Wilcoxon rank sum test: $p=0.003$).

3.10. Relationships with phytoplankton species

No correlation was found between the DMSP concentrations in the 5–90 μm fraction and the phytoplankton community of Niel Bay. By contrast, in Little Bay, there was a significant correlation between this DMSP concentration and total Dinophyceae biomass

(Correlation Spearman test: $r=0.582$; $p=0.044$). Among these Dinophyceae, *Alexandrium minutum* may be very important in the DMS(P) cycle of Toulon Bay. Indeed, its abundance was much greater between March and April, when the DMSP concentrations increased, and accounted for between 50% (2894 cells/L) and 63% (4914 cells/L) of the total phytoplankton abundance (Fig. 11A). This role could be confirmed by the relationship between *A. minutum* biomass and the DMSP concentration in the 5–90 μm fraction and by the positive correlation between this biomass and the DMS concentration (Correlation Spearman test: $r=0.709$; $p=0.014$) (Fig. 11B).

4. Discussion

Over several decades, the productivity of the Mediterranean Sea has increased in response to intensified human intervention and a rise in temperature (Lacaze, 1993; Bethoux and Gentili, 1996; Duarte et al., 1999). This study aimed to describe the changes in DMS(P) concentrations due to eutrophication, to understand better the processes involved in the regulation of the sulfur species production.

Two sites close to each other were chosen in the Toulon area, where there are marked differences in the plankton abundance: the Little Bay of Toulon and Niel Bay. Previous studies have shown that Little Bay was more productive than outlying ecosystems (Jean, 2002; Jamet et al., 2005). Greater phytoplankton biomass and abundance, and increased nitrate, orthophosphate and chlorophyll *a* concentrations, confirm that Little Bay is more eutrophic than Niel Bay. Moreover, the semi-enclosed configuration of Little Bay slows down the nutrient dispersion, which it contributes to increase the productivity (Barth and Fegan, 1990). However, the

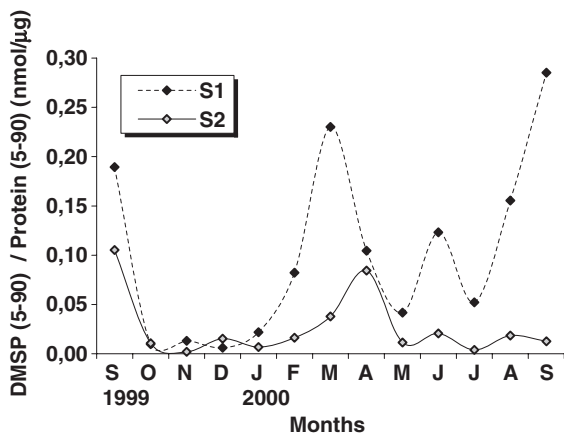


Fig. 10. Comparison of the DMSP_p to protein *ratio* (for the 5–90 μm fraction) between Little Bay (S_1) and Niel Bay (S_2).

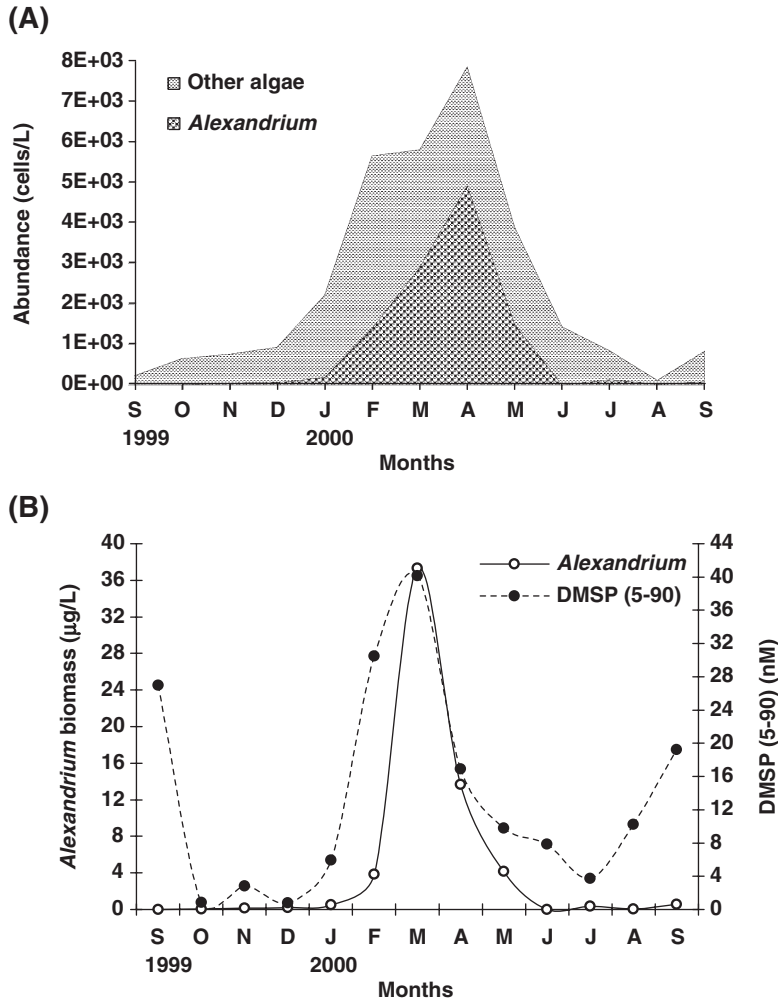


Fig. 11. Annual contribution of *Alexandrium minutum* to the total phytoplankton abundance (Fig. A) and relationship between DMSP concentration in the 5–90 μm fraction and *A. minutum* biomass (Fig. B) in Little Bay of Toulon.

absence of hypoxia or anoxia, indicates that Little Bay cannot be considered as severely eutrophic (Guillot and Souchu in Ifremer, 2001; Jean, 2002).

In Little Bay, DMSP_p concentrations were between 2 and 59 nM. In the less eutrophic Niel Bay, DMSP_p concentrations were significantly lower, varying between 0.5 and 24 nM. This confirms that DMSP_p concentrations increase in areas where algal abundance is greater. High DMSP concentrations (between 3.4 and 4.7 μM) have also been found in Barcelona harbour (Belviso et al., 2000), and in the Gulf of Mexico where DMSP_p concentrations are three times higher than in the oligotrophic ocean (Kiene and Linn, 2000). Hatton et al. (1999) also found higher DMSP_p values in the eutrophic Coast of Oman than in the Arabian Sea. In both sites of the Toulon area, dissolved and particulate DMSP showed a strong variability with time. In Little Bay, as in Niel Bay, the

highest dissolved, particulate DMSP, and DMS concentrations were recorded at the end of the winter. In colder regions of the world, the highest DMSP levels are often observed in springtime (Kwint and Kramer, 1996; Van Duyl et al., 1998). Unlike DMSP_p concentrations, DMSP_d concentrations were similar in the two sites, with values between 7 and 148 nM in Little Bay and between 2 and 110 nM in Niel Bay. These concentrations seem to be high compared to the average concentration of 14 nM generally found in the global oceans (Kettle et al., 1999). However, Townsend and Keller (1996) also mentioned high levels in the Gulf of Maine, where DMSP_d of these temperate waters can reach up to 101 nM. In Little Bay, high DMS levels were also recorded, with an average of 49 nM on the whole year. Similar observations were mentioned by Niki et al. (2000) in the Tokyo Bay, where elevated DMS concentrations are recorded, rising to 130 nM. DMS

concentrations measured in the less eutrophic Niel Bay, with an annual mean value of 20 nM, are closer to levels mentioned in the literature. However, these values are still higher than concentrations encountered in the global oceans (between 0.5 and 10 nM) (Kettle et al., 1999), in the Gulf of La Spezia and in the Venice Lagoon (around 16 nM) (Boniforti et al., 1993; Moret et al., 2000). At both sites, DMS concentrations were often lower than DMSP_d concentrations. Predominance of DMSP_d against DMS is mentioned by most of the authors (Turner et al., 1988). Such observations may suggest a slow enzymatic conversion by the DMSP-lyase of DMSP_d to DMS. The DMS to DMSP_d ratio observed in Toulon area also indicated a slow conversion to DMS, except in March–April in Little Bay, and in August–September in Niel Bay, may be on account of a rise in temperature which could increase the enzymatic activity.

DMSP_p usually represents the main part of the total DMSP (Turner et al., 1988; Andreae, 1990). In our study, DMSP_d concentrations were always higher than DMSP_p concentrations, especially in Niel Bay. Taking account of the losses by filtration, DMSP_p concentrations remained lower than DMSP_d concentrations. Therefore, the differences between DMSP_p and DMSP_d concentrations were not only due to an experimental artefact. In Niel Bay, which has a low algal biomass and low DMSP_p concentrations, phytoplankton activity cannot fully explain the high DMSP_d levels found there. It may be assumed that benthic macroalgae (possibly *Posidonia oceanica* present in this ecosystem), contribute to this DMSP_d pool. However, oceanographic studies are carried out in pelagic waters where the contribution of benthic algae metabolism to DMSP production cannot predominate compared to phytoplankton activity. However, marine macroalgae, such as *Ulva lactuca* (Greene, 1962), *Polysiphonia fastigiata* (Challenger, 1951) or *Polysiphonia lanosa* (Cantoni and Anderson, 1956) contain DMSP. This suggests that macroalgae can contribute to the DMSP_d pool, especially in shallow ecosystem such as Niel Bay. But, high DMSP_d concentrations found in this ecosystem, may also result from a resuspension of DMSP-rich sediment. Indeed, Nedwell et al. (1994), in the North Sea, and Gambaro et al. (2002) in the Venice Lagoon, measured DMSP concentrations a thousand times higher in the sediment than in the water column. High DMSP_d concentrations explain the large values in the DMSP_d to DMSP_p ratio. In the two ecosystems, this ratio greatly increased at the same time as a decrease in salinity after the heavy autumn rains. These hypo-osmotic conditions probably affected the algal metabolism, causing cell lysis and a release of DMSP in the

sea. Dickson and Kirst (1986) showed that *Tetraselmis subcordiformis* rapidly released intracellular DMSP after hypo-osmotic shock. Intracellular DMSP content generally increased once salinity rose again (Van Bergeijk and Stal, 2001). In our study, normal salinity recovered during October to February. At the same time, the DMSP_d to DMSP_p ratio decreased irregularly, suggesting a slow regulation of the algal osmotic potential, as described by Stefels (2000).

In the two study sites, the 5–90 µm fraction, which was principally composed of microphytoplankton, contained the greatest concentrations of DMSP_p. Belviso et al. (1993) observed at Villefranche-sur-mer that 16% of the total DMSP_p was present in the 10–200 µm size fraction, against 39% of the total DMSP_p observed at Banyuls. Here, the DMSP originated from heterotrophic organisms, such as microzooplankton present in the 10–200 µm fraction, rather than from phytoplankton. Meyerdierks et al. (1997) showed in the South Atlantic Ocean that most of the DMSP_p was present in the 5–20 µm fraction and the contribution from the <5 µm fraction was negligible, which is consistent with our results. The DMSP_p concentration of this 5–90 µm fraction was always higher in the more productive Little Bay. This was also true for the DMSP to protein concentration ratio in this fraction. Therefore, the high DMSP concentrations measured in Little Bay could not only be due to a higher biomass but also to DMSP rich algae. Little Bay is characterised by a higher abundance of Dinophyceae than Niel Bay, whereas Bacillariophyceae were more abundant in this less eutrophic ecosystem (Jamet et al., 2005). The positive correlation between the total Dinophyceae biomass and the DMSP of the 5–90 µm fraction observed in Little Bay, suggests that Dinophyceae may be largely responsible for this DMSP production.

It is widely accepted that DMSP comes primarily from Dinophyceae and Prymnesiophyceae (including coccolithophorids), with Bacillariophyceae being regarded as minor producers (Keller et al., 1989; Liss et al., 1993). We recently estimated the intracellular DMSP concentration after isolation of algal cells, and showed that, in Little Bay, Dinophyceae contained more DMSP than Bacillariophyceae. These measurements also revealed that *A. minutum* may contain up to 3388 ± 122 mM of DMSP (Jean et al., 2005). In the Little Bay of Toulon, the toxic *A. minutum* species was principally responsible for the algal bloom recorded in March and April 2000 (Ifremer, 2001). In Little Bay, high DMS(P) levels were observed when *A. minutum* contributed to more than 50% of the total algal abundance. Therefore, this species may be a sensitive bioindicator of high DMS

(P) content in highly eutrophic waters. In some eutrophic ecosystems of the North Sea, the non-toxic *Phaeocystis* microalga is also well known to be the main producer of DMS(P) (Liss et al., 1994; Stefels et al., 1995; Lancelot et al., 1987; Riegman et al., 1992).

In conclusion, our results confirm that DMS(P) concentrations are elevated in highly eutrophic waters. These high concentrations result from high phytoplankton biomass as well as the proliferation of size specific algal species such as that are high DMS(P) producers.

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