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Annual DMSP contribution to S and C fluxes through phytoplankton and bacterioplankton in a NW Mediterranean coastal site

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ABSTRACT: The contribution of dimethylsulfoniopropionate (DMSP) to the fluxes of carbon and sulfur through phytoplankton and bacterioplankton was investigated throughout an annual cycle in the Blanes Bay Microbial Observatory (coastal NW Mediterranean). DMSP accounted for 0.3 to 7% of biovolume-estimated phytoplankton carbon and 4 to 93% of calculated phytoplankton sulfur, with higher contributions in 'summer' (highly irradiated, oligotrophic waters, May to September) and lower in 'winter' (October to April). DMSP biosynthesis rates accounted for 0.8 to 7% of carbon fixation and 11 to 88% of sulfur assimilation through primary production, with slightly higher shares in summer. Upon release from the algal cells, DMSP supplied 0.5 to 6% of the total carbon demand of heterotrophic bacteria, and 3 to 100% of the sulfur demand over the year. Uncertainties associated with these calculations are due to a scarce knowledge of C:S ratios in marine bacteria. Bacterial DMSP-sulfur assimilation (measured with ^{35}S-DMSP) was positively correlated with bacterial heterotrophic production rates (measured with ^{3}H-leucine). In summer waters, characterized by higher ratios of particulate DMSP to chlorophyll *a* ($DMSP_p$:chl *a*), DMSP-sulfur assimilation by bacteria was higher and contributed a larger share of the bacterial sulfur demand. We propose that the DMSP:chl *a* ratio is a good indicator of the relative role of DMSP in the carbon and sulfur fluxes through the first levels of the planktonic food web.

KEY WORDS: Dimethylsulfide · Dimethylsulfoniopropionate · Sulfur · Food web · Primary production · Bacterial production · Respiration · Mediterranean

INTRODUCTION

Dimethylsulfoniopropionate (DMSP) is a ubiquitous organic sulfur compound produced in the euphotic zone by many species of marine phytoplankton, especially those belonging to the classes Dinophyceae (main class of dinoflagellates) and Prymnesiophyceae (Stefels et al. 2007). In addition to its well-known role as precursor of the relevant climatic gas dimethylsulfide (DMS), DMSP is also recognized as an important component of the fluxes of sulfur (S) and carbon (C) through marine microbial food webs (Kiene & Linn 2000). DMSP represents a source of reduced S and a potential source of C for marine heterotrophic bacteria (Kiene & Linn 2000, Simó et al. 2002, Zubkov et al. 2002), herbivorous protozoans (Burkill et al. 2002, Simó et al. 2002, Tang & Simó 2003, Simó 2004), and non-DMSP-producing phytoplankton (Vila-Costa et al. 2006a). The very few field studies that have quantified the contribution of DMSP to the fluxes of S and C through >1 level of the food web were conducted in blooms of high DMSP-producing phytoplankton

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(Burkill et al. 2002, Simó et al. 2002). Thus, the role of DMSP as carrier of S and C through food webs in other environmental and ecological settings still remains to be studied.

DMSP production is a widespread process in phytoplankton communities but its magnitude varies largely among taxa (Stefels et al. 2007). DMSP is believed to act as an osmoregulator, cryoprotector, and antioxidant in algal cells (Stefels et al. 2007). Other functions, such as a methyl donor in metabolic reactions and an overflow of reducing power under unbalanced growth conditions, have also been suggested (Stefels et al. 2007). The fraction of phytoplankton carbon that occurs as DMSP was estimated to range from 0.2 to 9% in a compilation work (Kiene et al. 2000). The fraction of primary production (PP) carbon invested in DMSP synthesis in blooms of the high-DMSP producer *Emiliana huxleyi* (7%) was similar to the upper end of this range (Archer et al. 2002, Simó et al. 2002). In terms of S, DMSP contributes the majority (>50%) of the pool of this element in cultured and field high-DMSP-producing phytoplankton (Matrai & Keller 1994, Simó et al. 2002).

The reduced S that is carried by phytoplankton in the form of DMSP enters and flows through the food web either by herbivore grazing or by algal release of dissolved DMSP ($DMSP_d$) and subsequent utilization by other phytoplankters and heterotrophic bacteria. Upon grazing, a fraction of the algal DMSP-S is thought to be assimilated (incorporated into biomass) by the herbivore or retained as particulate DMSP ($DMSP_p$) and transferred up the food chain (Tang & Simó 2003). Although this assimilated fraction may be significant, S budgeting exercises in predator-prey assemblages have shown that grazers mostly catalyze the release of $DMSP_d$ and DMS into seawater by breaking algal cells during grazing (Tang et al. 1999, Archer et al. 2001, Simó et al. 2002). Most of the $DMSP_d$ is eventually assimilated by heterotrophic bacteria and non-DMSP-producing phytoplankton (Vila-Costa et al. 2006a) to supply macromolecular S and relief from the energetic cost of sulfate reduction (Kiene et al. 1999).

Current knowledge is that $DMSP_d$ can supply 1 to 15% of the C demand and most of the S demand of heterotrophic bacterioplankton (Kiene & Linn 2000, Simó et al. 2002, Zubkov et al. 2002). DMSP degradation in the bacterial cell supplies the methanethiol (MeSH) moiety, which is incorporated primarily into the synthesis of sulfur amino acids and proteins (Kiene et al. 1999). This route, which involves the demethylation and subsequent demethiolation of DMSP, generally dominates DMSP degradation in the surface ocean. However, bacteria can also double-demethylate DMSP to produce non-volatile S compounds or cleave DMSP to produce DMS, either mediated by a DMSP lyase (Curson et al. 2008) or through the addition of an acyl-CoA moiety to DMSP (Todd et al. 2007). Kiene et al. (2000) suggested that, with enough available $DMSP_d$, the saturation of the S demands of bacteria would induce a switch from the demethylation to the cleavage route, increasing the production of DMS by bacteria.

A strong seasonality has been systematically found in sea-surface DMS concentrations in subtropical, temperate, and polar waters (e.g. Dacey et al. 1998, see a compilation in Simó & Pedrós-Alió 1999) with highest annual concentrations during the summer period. A recent study in the NW Mediterranean has shown that this seasonality is also observed in DMSP cycling dynamics (Vila-Costa et al. 2007, 2008). In particular, the rates of DMSP assimilation by microorganisms were significantly higher during summer, coinciding with a higher DMSP to chlorophyll *a* ratios (DMSP:chl *a*). The seasonal coupling between these 2 variables (DMSP assimilation and DMSP:chl *a* ratio) was seen as indicative that, in summer, DMSP contributed a larger share of the C and S fluxes through the microbial food web, but no elemental flux data were reported to support this suggestion.

Here we follow up from the former studies (Vila-Costa et al. 2007, 2008) to report on the annual variation of the contribution of DMSP to the fluxes of C and S through phytoplankton and heterotrophic bacterioplankton in a coastal oligo- to mesotrophic site, the Blanes Bay Microbial Observatory. For the first time, the variability of DMSP production rate by phytoplankton, and the variability of the bacterial S (BSD) and C demands (BCD) supplied by DMSP assimilation are described simultaneously with the evolution of the environmental and trophic conditions over an annual cycle.

MATERIALS AND METHODS

Sampling and environmental variables. We sampled monthly on 2 consecutive days at the Blanes Bay Microbial Observatory sampling site, NW Mediterranean, 41° 40′ N, 2° 48′ E, from January 2003 to March 2004. This site is located approximately 1 km offshore, over a 24 m deep water column. Surface seawater was collected, avoiding bubbling by carefully submerging 2 acid-cleaned amber glass bottles (2.5 l) to a depth of 0.5 m. Bottles were kept in the dark at *in situ* temperature until they were processed, within 2 h after sampling. Vertical temperature profiles were determined by collecting water from different depths (0, 5, 10, 15, and 20 m) using a Niskin bottle and measuring temperature with a mercury thermometer. A more detailed

vertical profile was performed by increasing the number of measured depths whenever the temperature difference between the 0 and 5 m measurements was >1°C. Salinity was measured using a YSI 556 MPS probe. Chl *a* concentration was measured by fluorometry (Turner Designs fluorometer) in extracts (90% acetone, 4°C, overnight) of 150 ml of seawater filtered through GF/F (Whatman). Parallel filtration onto polycarbonate 3 µm pore filters (Poretics) provided the chl *a* concentrations associated with larger particles. Sulfur compound concentrations, environmental variables, pico- and nanoplankton abundances, and rates of bacterial heterotrophic production (BHP) and bacterial consumption of DMS and DMSP were measured over the whole sampling period. PP and respiration rates were measured between March 2003 and February 2004, and microphytoplankton abundance was measured only between June 2003 and February 2004.

Analysis of total and particulate DMSP. Total DMSP ($DMSP_t$, i.e. the sum of dissolved and particulate DMSP) and $DMSP_p$ were measured as DMS after overnight alkaline hydrolysis (NaOH, 28 µM final concentration) of either 40 ml of whole seawater ($DMSP_t$) or GF/F-retained particles ($DMSP_p$) from 40 ml seawater subsamples (Vila-Costa et al. 2008). The evolved DMS was determined with the purge, cryotrapping, and sulfur-specific gas chromatography system described by Simó et al. (1996).

Determination of C and S content of phytoplankton. Concentrations of cyanobacteria (*Prochlorococcus* spp. and *Synechococcus* spp.) and autotrophic picoeukaryotes were determined by flow cytometry in a Becton Dickinson FACScalibur instrument following standard methods. Autotrophic nanoplankton were counted using epifluorescence microscopy. Abundances were converted to biomass or particulate organic carbon (POC) using average C:cell conversion factors from the literature: 51 ± 18 fgC $cell^{-1}$ for *Prochlorococcus* spp., 175 ± 73 fgC $cell^{-1}$ for *Synechococcus* spp., 1319 ± 813 fg C $cell^{-1}$ for picoeukaryotes (see references in Table 1), and 220 × (average cell vol.) fgC $cell^{-1}$ for nanophytoplankton (Børsheim & Bratbak 1987). Microphytoplankton were identified and counted with an inverted microscope. Width and length were measured for each cell. When possible, cell volumes were calculated applying the formulas provided by Hillebrand et al. (1999). When the corresponding formula included height, then the closest 2-parameter geometrical shape was used instead. Conversion to C was done by applying the formula $C = 0.109 \times (\text{cell vol.})^{0.991}$ fg C $cell^{-1}$ (Montagnes et al. 1994).

For the period March to May 2003, microphytoplankton abundance and biovolume data were not available, and microphytoplankton POC had to be estimated from size-fractionated chl *a* and C data. In the months with complete biomass data, we found a good linear relationship ($R^2 = 0.88$; $p < 0.05$) between the percentage of total chl *a* in the fraction >3 µm and the percentage of biomass comprised by microphytoplankton plus one-half of the nanophytoplankton and one-fourth of the picoeukaryotes. Thus, the chl *a* >3 µm fraction and the nano- and picophytoplankton biomass were used to estimate microphytoplankton biomass between March and May 2003.

To convert phytoplankton POC to phytoplankton particulate organic S (POS), we used a molar C:S ratio of 66 ± 27 (Table 1), which is the average ± SD of values for a number of cultured (Matrai & Keller 1994, Ho et al. 2003)

Table 1. Conversion factors extracted from the literature and used in the present study, and their associated variability. na: not applicable; V: cell volume

Conversion factor	Type of organism	Units	Literature mean or function	SD of the mean	Range	No. of source studies	Source[a]
C:cell	*Prochlorococcus* spp.	fg C $cell^{-1}$	51	18	14–92	22	1–22
C:cell	*Synechococcus* spp.	fg C $cell^{-1}$	175	73	69–294	28	1–28
C:cell	Picoeukaryotes	fg C $cell^{-1}$	1319	813	114–3110	24	1, 3–9, 11–13, 15, 16, 18 20–22, 24, 26–30
C:cell	Nanophytoplankton	fg C $cell^{-1}$	220·V	na	na	1	31
C:cell	Microphytoplankton	fg C $cell^{-1}$	$0.109 \cdot V^{0.991}$	na	na	1	32
C:S	Phytoplankton	mol:mol	66	27	39–90	3	33–35
C:S	Heterotrophic bacteria	mol:mol	75	24	32–141	1	36

[a]1. Li & Wood (1988); 2. Li et al. (1993a,b); 3. Campbell et al. (1994); 4. Sieracki et al. (1995); 5. Blanchot & Rodier (1996); 6. Chavez et al. (1996); 7. Buck et al. (1996); 8. Partensky et al. (1996); 9. Veldhuis et al. (1997); 10. Ishizaka et al. (1997); 11. Campbell et al. (1998); 12. Zubkov et al. (2000); 13. Li & Harrison (2001); 14. DuRand et al. (2001); 15. Blanchot et al. (2001); 16. Shalapyonok et al. (2001); 17. Landry & Kirchman (2002); 18. Brown et al. (2002); 19. Bertilsson et al. (2003); 20. Worden et al. (2004); 21. Grob et al. (2007a); 22. Zhang et al. (2008); 23. Booth (1988); 24. Kuosa (1991); 25. Ishizaka et al. (1994); 26. Hall et al. (2004); 27. Zabala (2004); 28. Grob et al. (2007b); 29. Kuuppo-Leinikki et al. (1994); 30. Tarran et al. (1999); 31. Børsheim & Bratbak (1987); 32. Montagnes et al. (1994); 33. Matrai & Keller (1994); 34. Ho et al. (2003); 35. Segura-Noguera (2007); 36. Fagerbakke et al. (1996)

and native (Segura-Noguera 2007) marine phytoplankton species belonging to the dinoflagellates, prymnesiophytes, prasinophytes, and diatoms. C:S ratios were averaged for taxonomic groups from each work, then averaged for taxonomic groups among works, and finally an overall (phytoplankton) average was calculated. The scatter was given by 1 SD of the mean obtained by propagation of SDs of the group means.

DMSP contribution to phytoplankton POC and POS. Phytoplankton-associated DMSP was calculated by comparing $DMSP_p$ concentrations with total phytoplankton C and S, considering that 1 mol DMSP contains 5 mol DMSP-C and 1 mol DMSP-S.

DMSP production rates. Based on the similarity of physical variables (temperature, salinity), chl *a*, and concentrations of DMS and DMSP between the 2 consecutive sampling days, we assumed that we were sampling the same water mass (see Vila-Costa et al. 2008 for more details). DMSP production rate was estimated by budgeting the concentrations of $DMSP_t$ from the 2 consecutive days, corrected by the measured $DMSP_t$ consumption as follows:

$$\text{DMSP production} = \{[\text{DMSP}]_2 - [\text{DMSP}]_1 - [(\text{DMSP consumption}) \times (t_2 - t_1)]\}/(t_2 - t_1)$$

where subscripts 1 and 2 refer to data from the first and second day.

$DMSP_t$ consumption was estimated as the net loss of $DMSP_t$ in dark incubations over 6 h (Simó et al. 2000, Vila-Costa et al. 2008).

PP rates. Incorporation of C into the particulate fraction was measured by the ^{14}C method (Steeman-Nielsen 1952) from *P-E* curves. Water for incubations was collected and dispensed in aliquots (70 ml) that were introduced in tissue culture bottles, which were then spiked with 10 µCi of ^{14}C-bicarbonate. Thirteen clear bottles and 1 dark bottle (covered with aluminum foil) were incubated in a temperature-controlled bath maintained at *in situ* temperature and in a gradient of irradiance (ca. 10 to 1000 µmol photons m^{-2} s^{-1}) generated by a quartz halogen lamp. Circulating water connected to a water bath maintained the temperature. Irradiance was measured with a small size spherical light meter (Illuminova) inside each of the tissue bottles. After 2 h incubation, samples were filtered through 0.2 µm pore size cellulose ester Millipore filters (GSWP02500). These filters were put into scintillation vials and left for 24 h in an HCl saturating atmosphere. Finally, 4 ml of scintillation cocktail (Optiphase Hisafe 2) were added in each vial and radioactivity was measured in a Beckman liquid scintillation counter. *In situ* particulate PP rate (PP_p) was determined from the *P-E* curve and the *in situ* (sampling time) irradiance obtained with a spherical quantum down-welling irradiance Li-Cor sensor (Li-193S).

Dissolved PP rate (PP_d) was measured together with PP_p only at 500 µmol photons m^{-2} s^{-1} during 2.5 h incubations as described in Alonso-Sáez et al. (2008). Filtrates (Millipore 0.2 µm cellulose ester) were acidified with 1 ml of 1 N HCl and left open in an orbital shaker for 12 h to remove inorganic ^{14}C. Scintillation cocktail was then added and radioactivity was measured as for filters. The percentage of PP_d over PP_d + PP_p under these conditions was then applied to *in situ* PP_p to calculate *in situ* PP_d. *In situ* total PP rate (PP_t) was calculated as the sum of PP_p and PP_d.

Bacterial DMSP consumption and incorporation rates. Rate constants for microbial $DMSP_d$ consumption were measured following the exponential disappearance of ^{35}S-DMSP added at trace concentrations (<0.01 nmol l^{-1}) to 30 ml of sample and incubated in the dark at the *in situ* temperature. Microbial $DMSP_d$ consumption rates were calculated as the product of $DMSP_d$ concentrations and the loss rate constants (Vila-Costa et al. 2008).

The percentage of DMSP incorporated into macromolecules was calculated as the proportion of ^{35}S-DMSP retained on the filter with TCA-precipitated particulate material versus the initial radioisotope added. Briefly, a 15 ml subsample of whole seawater was incubated in the dark at *in situ* temperature without headspace for 18 h with a trace addition of ^{35}S-DMSP (1000 dpm ml^{-1}, 5.79 Ci $pmol^{-1}$). Triplicate aliquots (5 ml) were filtered through nylon filters (GN, Millipore, 0.2 µm pore size) using a gentle vacuum (<5 cm Hg) and rinsed with 0.2 µm filtered seawater. Macromolecules were precipitated by treating filters with cold aliquots (5 ml) of trichloroacetic acid (TCA, 5%) for 5 min. Filters were then rinsed twice with MilliQ water and counted using a Beckman scintillation counter. The precision (coefficient of variation) of triplicate measurements averaged ~1%. Incorporation of ^{35}S-DMSP in formalin-killed controls was ≤1.5% that in live samples. DMSP incorporation rate was obtained by multiplying the percentage of the initially added ^{35}S-DMSP retained in the filters by the $DMSP_d$ consumption rate (Vila-Costa et al. 2007).

DMS consumption rates. Surface waters were incubated for approximately 6 h in the dark at the *in situ* temperature in acid-rinsed, amber glass bottles (2.5 l) without any amendment (control treatment) and, simultaneously, with dimethyldisulfide (DMDS) addition (260 nmol l^{-1} final conc., DMDS treatment). Bottles were sampled for DMS at times 0, 2, 4, and 6 h. Assuming that DMDS selectively inhibits DMS consumption (Wolfe & Kiene 1993), the DMS consumption rate was obtained by the difference between the slope of DMS accumulation in the DMDS treatment (gross DMS production) and the slope of the time course of DMS concentration in the control treatment (net DMS produc-

tion rate). For further details see Simó et al. (2000) and Vila-Costa et al. (2008).

BHP rates. BHP was determined from the incorporation of ^{3}H-leucine into protein using the method of Kirchman et al. (1985) with the modifications of Smith & Azam (1992). Briefly, 1.2 ml quadruplicate live and duplicate killed (5% TCA) subsamples were incubated with ^{3}H-leucine (40 nmol l^{-1} final conc.) for about 2 h, at the *in situ* temperature, in the dark. Incubations were stopped by addition of 120 µl of cold TCA 50% and then frozen (–20°C) until further processing by centrifugation and TCA rinsing. Leucine incorporation rates were converted to bacterial production rates with empirical conversion factors that ranged from 1.0 kg C mol^{-1} on average in summer to 1.9 kg C mol^{-1} on average in winter (details in Alonso-Sáez et al. 2008).

Bacterial respiration (BR) rates. BR rates were obtained by linear regression of oxygen concentration versus time in incubations (0 to 24 h) of ca. 130 ml of filtered seawater in boro-silicate glass bottles (Alonso-Sáez et al. 2008). Samples were filtered through 0.8 µm mixed cellulose ester filters to select exclusively the prokaryote fraction. The bottles were filled by siphon twice with sample seawater before they were closed with their stoppers. Five replicates of the initial-time samples were immediately fixed with Winkler reagents. The remaining 5 replicates were submerged inside dark coolers filled with tap water, which were maintained in a walk-in isothermal chamber. Previous to each determination, the water inside the coolers was stabilized to the correct temperature for at least 12 h. After 24 h, samples were fixed with Winkler reagents, and dissolved oxygen was determined with an automatic titrator, based on potentiometric endpoint detection (Oudot et al. 1988). The average standard error between replicate bottles was 0.53 µmol l^{-1} O_2. Oxygen consumption rates were transformed to carbon units assuming a respiratory quotient of 0.89 (Williams & del Giorgio 2005).

BCD and BSD. BCD was calculated as the sum of BHP and BR. BSD for production was calculated from BHP using the molar C:S ratio of 75 ± 24 (SE) determined by Fagerbakke et al. (1996) for marine bacteria.

RESULTS

Ecosystem and DMSP production rates

The marked seasonality of air temperature and heat flux in Blanes Bay caused a progressive stratification of the water column during half of the year, which resulted in a nutrient impoverishment of surface waters and the consequential decrease in chl *a* concentrations and phytoplankton biomass between May and September. From October through winter, the system was characterized by a well-mixed water column, high NO_3^- concentrations, and more phytoplankton (Table 2). The biomass of the phytoplankton assemblages was dominated by pico- and nanophototrophs all year round (Table 2). In 'summer' (May to September sampling dates), however, 66 ± 7% of the chl *a*

Table 2. Environmental variables and phytoplankton carbon biomass. Temp: temperature; MLD: mixed layer depth; Prochl: *Prochlorococcus* spp.; Synech: *Synechococcus* spp.; Picoeuk: autotrophic picoeukaryotes; Nano: autotrophic nanoplankton; Micro: autotrophic microplankton. The uncertainty derived from the use of average carbon:cell conversion factors from the literature is given as 1 SD in parentheses. Values in *italics* were estimated from size-fractionated chl *a* and carbon when microphytoplankton counts were not available

Date	Temp (°C)	MLD (m)	NO_3^- (µmol l^{-1})	Chl *a* (µg l^{-1})	Carbon biomass (pgC ml^{-1})				
					Prochl	Synech	Picoeuk	Nano	Micro
2003									
Mar 4	11	24	7.1	2.2	0	1167 (140)	29638 (889)	152361 (30472)	*96000*
Mar 25	13	15	1.5	1.2	145 (17)	11711 (1405)	9838 (295)	60717 (12143)	*11000*
Apr 22	14.5	7	0.6	0.6	0	26198 (3144)	18025 (5408)	104083 (20817)	*1600*
May 13	17	3	0.8	0.5	0	984 (118)	5876 (1763)	45793 (9159)	*2100*
Jun 25	25	2	0.1	0.3	0	2693 (323)	2912 (874)	13289 (2658)	2428 (711)
Jul 14	25.2	2	0.1	0.5	0	7205 (865)	4223 (1267)	14640 (2928)	6174 (1809)
Aug 4	25.2	3	0.0	0.3	86 (10)	13433 (1612)	4028 (1208)	17125 (3425)	2285 (669)
Sep 16	23	20	0.0	0.3	628 (75)	7709 (925)	2514 (754)	9086 (1817)	4106 (1203)
Oct 21	18	24	5.5	0.4	590 (71)	3098 (372)	1543 (463)	10141 (2028)	11351 (3326)
Nov 25	16	24	0.8	0.9	363 (44)	1912 (229)	11782 (3535)	42733 (8547)	31393 (9198)
Dec 16	14.5	24	3.9	2.8	455 (55)	2353 (282)	40252 (12075)	253283 (50657)	11507 (3371)
2004									
Jan 26	14	24	1.5	1.3	560 (67)	1299 (156)	13370 (4011)	70316 (14063)	37470 (10979)
Feb 23	12.9	24	1.6	1.0	36 (4)	365 (44)	5874 (1762)	30232 (6046)	32813 (9614)

occurred in organisms <3 µm (mostly contributed by prymnesiophytes and *Synechococcus* spp.), while in 'winter' (October through April sampling dates), 68 ± 7% of the chl *a* belonged to phototrophs >3 µm. The typical winter diatom bloom was observed on March 4, 2003. Another peak of chl *a* was recorded in December 2003, when the phytoplankton assemblage was dominated by small (<5 µm) flagellated prasinophytes, mainly *Micromonas* sp., and prymnesiophytes (Guadayol et al. 2009, M. Latasa & R. Massana pers. comm.).

The concentrations of $DMSP_p$ ranged between 7 and 72 nmol l^{-1}. High values were recorded in March 25, 2003 (72 nM) and December 2003 (56 nM). $DMSP_p$ concentrations decreased through summer to reach the lowest values in the period September to November (10 ± 1 nM). A detailed description of the time series of DMSP and other dimethylated S compound concentrations is given elsewhere (Vila-Costa et al. 2008). DMSP production rates ranged from 0.2 to 2.5 nmol l^{-1} h^{-1}, with the higher rates occurring in March 2003 and 2004, mid-summer 2003, and December 2003. Some degree of co-variation of DMSP production and PP_t rates was observed over the year (Fig. 1).

Bacterial abundance in Blanes Bay is typically quite constant at around 0.8×10^6 cells ml^{-1}. In 2003, it was slightly below that value in June and July (0.6×10^6 cells ml^{-1}) and slightly above it (1.3×10^6 cells ml^{-1}) in August (Alonso-Sáez et al. 2008). The coarse composition of the bacterial assemblage did not show major variations over the annual cycle. *Alphaproteobacteria* (mainly SAR11) dominated bacterial abundance overall, followed by *Bacteroidetes* and *Gammaproteobacteria*, the latter showing higher shares in summer. A singular situation occurred in July 2003, when the bacterial assemblage was clearly dominated by a single phylotype of *Gammaproteobacteria* (Alonso-Sáez et al. 2007). BHP, which ranged from 0.8 to 42.6 nmol C $l^{-1}h^{-1}$, exhibited its maxima during the summer, except for June, and in February and December 2003, concurrent with the maximum annual values of chl *a* (Fig. 2). BR was 23.5 to 151.4 nmol C 1^{-1} h^{-1} and co-varied with dissolved organic carbon (DOC, data not shown) much better than with BHP rates (see Alonso- Sáez et al. 2008).

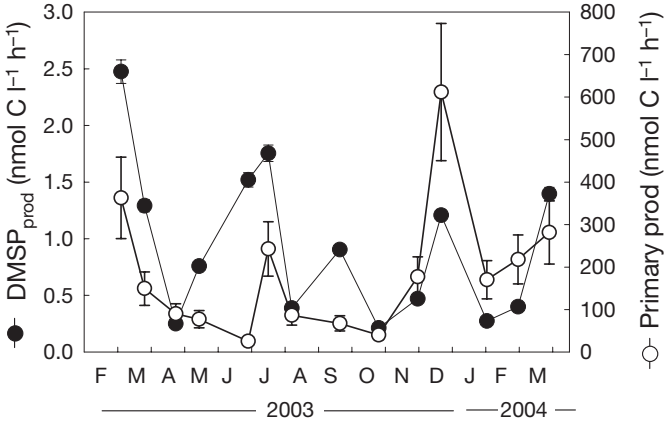

Fig. 1. Rates of dimethylsulfoniopropionate (DMSP) production (prod) and total (particulate + dissolved) primary production. Error bars are SD

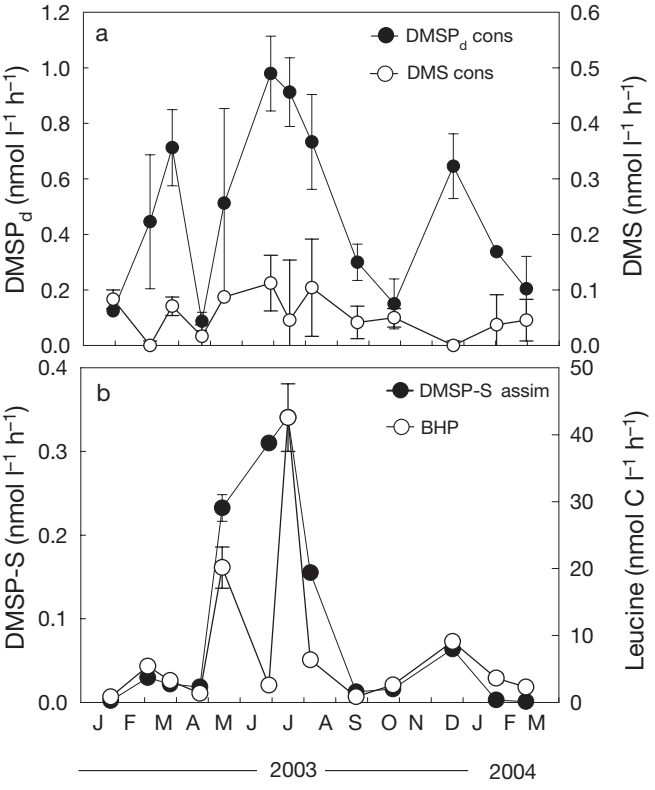

Fig. 2. (a) Microbial dissolved dimethylsulfoniopropionate ($DMSP_d$) and dimethylsulfide (DMS) consumption (cons) rates, and (b) microbial DMSP-S assimilation (assim) and bacterial heterotrophic production (BHP) rates. Note the different scales of the consumption rates of DMSP and DMS. Error bars are SD

DMSP contribution to phytoplankton C and S pools and fluxes

On annual average, DMSP accounted for a substantial fraction of organic C (2.7 ± 0.6%) and a large fraction of estimated organic S (35 ± 9%) of phytoplankton (Table 3). This elemental contribution of DMSP was higher in 'summer' (May to September, 4.6 ± 0.9% C, 61 ± 12% S) than in 'winter' (October to April, 1.4 ± 0.6% C, 19 ± 7% S), with the exception of March 2003 (4.6% C and 61% S). It closely paralleled the $DMSP_p$:chl *a* ratio (Fig. 3, Table 4).

The percentage of PP_t invested into DMSP production also showed a slight seasonal pattern (Table 3). On

Table 3. Contribution of dimethylsulfoniopropionate (DMSP) to carbon and sulfur pools and fluxes through phytoplankton. Phyto-POC: phytoplankton particulate organic carbon; phyto-POS: phytoplankton particulate organic sulfur; PP_t: total (particulate + dissolved) primary production rate. Uncertainty associated with the use of average C:cell and cellular C:S conversion factors from the literature is given as 1 SD in parentheses. SE: standard error of the annual and seasonal means

Date	DMSP-C:phyto-POC (%)	DMSP-S:phyto-POS (%)	DMSP-C production:PP_t (%)	DMSP-S production:PP_t-S (%)
2003				
Mar 4	0.8 (0.2)	11 (5)	3.4 (0.9)	45 (22)
Mar 25	4.6 (1.0)	61 (28)	4.3 (1.2)	57 (28)
Apr 22	0.6 (0.1)	8 (4)	1.4 (0.4)	18 (9)
May 13	4.3 (0.9)	57 (26)	4.9 (1.3)	65 (32)
Jun 25	7.1 (1.5)	93 (43)	29.7 (7.9)	392 (192)
Jul 14	5.8 (1.2)	76 (35)	3.6 (1.0)	48 (23)
Aug 4	2.8 (0.5)	37 (16)	2.2 (0.6)	30 (14)
Sep 16	3.1 (0.6)	41 (19)	6.7 (1.8)	88 (43)
Oct 21	2.7 (0.6)	35 (17)	2.7 (0.7)	35 (17)
Nov 25	0.5 (0.1)	7 (3)	1.3 (0.4)	18 (9)
Dec 16	1.1 (0.2)	14 (7)	1.0 (0.3)	13 (6)
2004				
Jan 26	0.3 (0.1)	4 (2)	0.8 (0.2)	11 (5)
Feb 23	0.8 (0.2)	11 (5)	0.9 (0.2)	12 (6)
Annual mean	2.7 (0.6)	35 (16)	2.8[a] (0.7)	37[a] (18)
SE	0.6	9	1.9	25
May–Sep mean	4.6 (1.0)	61 (28)	3.8[a] (1.0)	50[a] (25)
SE	0.9	12	1.0	13
Oct–Apr mean	1.4 (0.3)	19 (9)	2.1 (0.6)	28 (14)
SE	0.6	7	0.6	8

[a]Excluding the value for June

Table 4. Spearman coefficients (r_S values, n = 12) of the correlations (1–5 vs. a–e) between (1) total primary production (PP_t), (2) bacterial heterotrophic production (BHP), (3) bacterial carbon demand (BCD), (4) DMSP-C contribution to algal particulate organic carbon (DMSP-C:phyto-POC) and (5) DMSP:chl *a* ratio, and (a) DMSP production rate ($DMSP_{prod}$), (b) DMSP consumption rate ($DMSP_{cons}$), (c) DMSP assimilation rate ($DMSP_{assim}$), (d) DMS consumption rate (DMS_{cons}) and (e) DMSP:chl *a* ratio. Correlations significant at $p < 0.05$ are shown in **bold**. na: not applicable. DMSP: dimethylsulfoniopropionate; DMS: dimethylsulfide

	(a) $DMSP_{prod}$	(b) $DMSP_{cons}$	(c) $DMSP_{assim}$	(d) DMS_{cons}	(e) DMSP:chl *a*
(1) PP_t	0.36	0.05	−0.03	−0.35	−0.55
(2) BHP	0.34	0.57	**0.65**	0.42	0.22
(3) BCD	0.07	0.08	−0.06	0.00	0.03
(4) DMSP-C:phyto-POC	0.50	**0.71**	**0.66**	**0.78**	**0.92**
(5) DMSP:chl *a*	0.34	**0.64**	**0.76**	**0.8**	na

annual average, 2.8 ± 1.9% of the C fixed and 37 ± 25% of the S incorporated by phytoplankton were used to synthesize DMSP (Table 3). Note that these annual averages are very similar to those found for the contribution of DMSP to phytoplankton C and S contents, with 'summer' averages being significantly higher than 'winter' averages (Table 3). The contribution of DMSP production to PP in June 2003 was very different than what was seen in the other months. In the case of S production, it was well above 100%. This is a strong indication that PP_t was heavily underestimated on that sampling day, although we do not know what occurred that day to give us such data.

$DMSP_d$ and DMS consumption versus BHP rates

Peaks of $DMSP_d$ consumption were observed during the summer period and in March and December 2003, i.e. coinciding with peaks of DMSP concentration. It showed some co-variation with BHP (Fig. 2a), although the variables were not significantly correlated at the 95% confidence level (Table 4). In contrast, a significant correlation was found between DMSP-S assimilation into macromolecules and BHP (Spearman r_S = 0.65, n = 12, $p < 0.05$, Table 4, Fig. 2b). DMSP-S was more efficiently assimilated during the summer (range 21 to 46%) than over the rest of the year (1 to 22%).

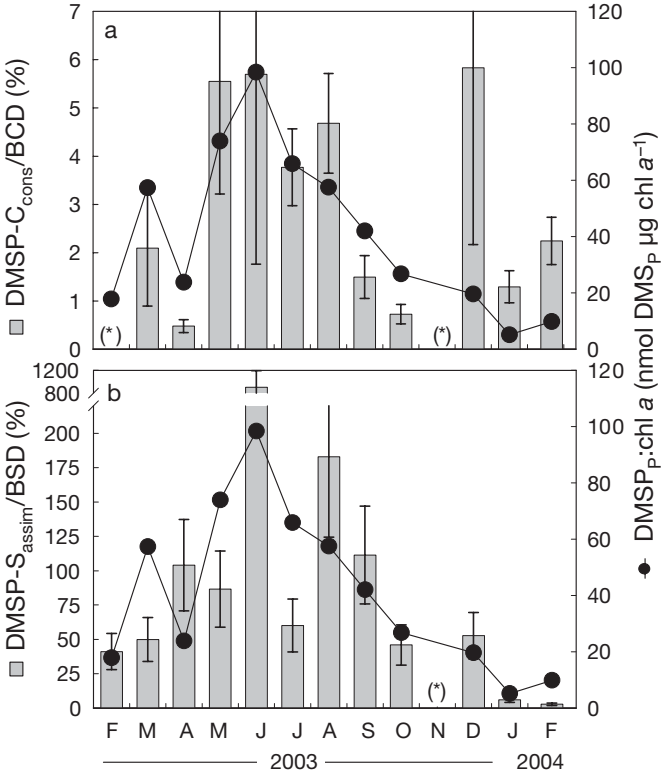

Fig. 3. (a) Contribution of dimethylsulfoniopropionate-carbon (DMSP-C) consumption (cons) to bacterial carbon demand (BCD). Error bars represent the SD associated with the measurements of DMSP consumption, bacterial heterotrophic production and bacterial respiration. (b) Contribution of DMSP-S assimilation (assim) to bacterial sulfur demand (BSD). Error bars show the uncertainty associated with the scatter of molar C:S ratios from the literature. The ratio of particulate DMSP to chl *a* (DMSP$_p$:chl *a*) is also plotted for comparison. (*) not determined

Bacterial DMS consumption rate did not show any clear seasonal pattern, nor did it show any co-variation with BHP (Fig. 2a).

DMSP contribution to C and S fluxes through bacterioplankton

BCD was calculated from empirical BHP and BR rates. Since most of the incorporated organic C was respired, the variability of BR drove most of the annual variability of BCD (Alonso-Sáez et al. 2008). Over the sampling year, the consumption of DMSP-C contributed from 0.5 to 6% of BCD (Fig. 3a). This contribution was higher in 'summer' (May to September, 4.3 ± 0.9%) than in 'winter' (October to April, 2.1 ± 0.9%). Accordingly, during the 'summer', DMSP was estimated to supply virtually all the S requirements of bacteria (110 ± 30%, Fig. 3b, excluding June), while in 'winter', DMSP contributed an average of 43 ± 14% of BSD, with values as low as 3 to 6% in January and February 2004. DMSP supported a remarkably high proportion of the BCD (6%) and the BSD (53%) in December 2003, coinciding with high chl *a* and DMSP$_p$ concentrations. The fact that in June 2003, the estimated contribution of DMSP to the BSD was much higher than 100% (900%) points to a severe underestimation of BSD on a sampling day during which PP had probably been underestimated too (see 'DMSP contribution to phytoplankton C and S pools and fluxes'). This could have occurred through an inaccurate measurement of the BHP rate, because BCD, which was mostly contributed by BR, did not fall far from the annual range (Fig. 3b).

DISCUSSION

The relative importance of specific organic compounds in the ocean can be determined by quantifying their contribution to the fluxes of matter and energy through the food web. In the present study, we showed that the contribution of DMSP to fluxes of C and S through phytoplankton and bacterioplankton were substantial and varied with time, not only because of the obvious connection with episodic higher abundances of DMSP-rich phytoplankton in winter but also with a tendency to higher values in summer.

DMSP role in phytoplankton C and S pools and fluxes

Different phytoplankton species produce different amounts of DMSP (Stefels et al. 2007). Over the year, the contribution of DMSP$_p$ to the total algal POC (0.3 to 7%) was within the range of values estimated from different oceanic sites (0.2 to 9%, Kiene et al. 2000) but at or below the lower limit of those roughly estimated in the oligotrophic Sargasso Sea (7.2 to 39%, Andreae 1990). The contribution of DMSP$_p$ to the phytoplankton S content was >30%, i.e. of the order of those measured for cultured species with high intracellular DMSP levels (Matrai & Keller 1994), over half of the year. In general, DMSP contributed more to the total algal particulate pools in the highly irradiated, nutrient-impoverished and less-productive summer months (May to September), and during the peak of chl *a* in March 2003 (Table 2). This monthly pattern can be mostly explained by the occurrence and succession of algal taxa that have been described for the site (M. Latasa pers. comm.). In March 2003, the relative proportion of prymnesiophytes (high-DMSP producers, Stefels et al. 2007) increased after the typical diatom

winter bloom (February 2003). In summer, the increasing share of prymnesiophytes in the phytoplankton assemblage could explain the higher contribution of DMSP to the phytoplankton particulate material, because *Synechococcus* spp. are very low-DMSP producers (Corn et al. 1996).

Physiological responses to environmental stressors are also thought to control DMSP production by phytoplankton communities. Sunda et al. (2002) suggested that DMSP biosynthesis could be stimulated by oxidative stress (e.g. high UV radiation, high H_2O_2) because of the role of DMSP and derivatives in scavenging stress-induced oxygen radicals in the algal cell. This could have added to the observed higher contribution to DMSP to phytoplankton biomass in summer and the lower contribution in winter (Table 3).

Another interesting finding is the apparent co-variation, which is not significantly correlated at the 95% confidence level (Table 4), between DMSP production and PP_t rates observed over the year (Fig. 1). Although it still is unknown whether DMSP production is a process strictly coupled to photosynthesis (Stefels et al. 2007), Simó et al. (2002) found excellent correlations between DMSP production rate and PP both over diel and multi-day scales in a study of North Atlantic waters dominated by high-DMSP producers. In the present study, we observed a comparable result at the annual scale for the first time. The fact that the co-variation through seasons was much weaker than that found previously over a week can be attributed to the successional and physiological differences in the phytoplankton described in 'Results: Ecosystem and DMSP production rates', which led to a seasonal pattern similar to that of DMSP contribution to algal POC and POS, i.e. a higher proportion of PP invested in DMSP production in the summer months (Table 3).

Is DMSP a quantitatively important compound for marine phytoplankton? Percentages of productivity directed to the synthesis of other organic compounds such as free amino acids (25%, inshore and offshore waters of Gokasho Bay, Hama et al. 1987) or particulate protein amino acids (5 to 15%, Salt Pond, Lohrenz et al. 1987) are higher than the annual averaged 3% of PP_t invested for DMSP synthesis in the present study. Proteins and free amino acids are the second most abundant group of molecules synthesized by algae after carbohydrates (Hama 2000). However, when compared with the rates of synthesis of single organic compounds, DMSP production rates (annual average of 1.3 ± 0.3 µg C l^{-1} d^{-1}) fall in the same range of those of alanine, glycine, ribose, or mannose, and are almost 1 order of magnitude higher than those of individual fatty acids (Hama 2000). These observations provide support to the idea that DMSP is indeed a very important S and C compound for many phytoplankters.

A significant source of uncertainty for our calculations lies in the use of average conversion factors and elemental ratios from the literature and their application throughout the annual cycle. Table 3 shows the effects of the variability in literature values on the DMSP share in biomass and production. Although the uncertainty is high, the seasonal differences are robust, and so is the large contribution of DMSP to phytoplankton S in the summer months and during the bloom of high-DMSP producers in late March 2003. It has to be noted, nonetheless, that the molar C:S ratio differs among algal species and varies over the growth cycle (Matrai & Keller 1994, Ho et al. 2003). It is thus plausible that it varies over the year with the succession of phytoplankton and their physiological acclimation to variable light and nutrient conditions. This however is a large unknown whose quantitative implications we cannot tackle with currently available information.

DMSP role in bacterioplankton C and S fluxes

The fact that the metabolism of DMSP by heterotrophic bacteria can follow diverse pathways (cleavage to DMS, acyl-CoA addition to produce DMS, double demethylation into non-volatile S compounds, or demethylation plus demethiolation to give rise to MeSH) explains the weak correlation encountered between $DMSP_d$ consumption rates and BHP as measured by ^{3}H-leucine incorporation (Table 4, Fig. 2), since the latter is strictly a measure of protein synthesis (Kirchman et al. 1985). Contrastingly, the strong positive correlation between DMSP-S assimilation and BHP confirms the role and efficiency of DMSP as a supplier of the MeSH moiety to be incorporated into proteins (Kiene et al. 2000). In this sense, both ^{3}H-leucine and ^{35}S-DMSP incorporation rates would be proxies for protein synthesis. Since a succession of bacterial assemblages over the year has been observed at the sampling site (Alonso-Sáez et al. 2007), the implication of this good match between DMSP-S assimilation and BHP is that DMSP would be as universal as leucine. This is in good agreement with the observation that DMSP assimilation is a widespread capability among different taxonomic groups of bacterioplankton in Blanes Bay (Vila-Costa et al. 2007) and other sites (Malmstrom et al. 2004, Vila et al. 2004). On the other hand, the lack of correlation between DMS consumption and BHP rates agrees with suggestions that DMS assimilation as a source of S for protein synthesis is a process of minor significance (Zubkov et al. 2002, Del Valle et al. 2007), and that bacterial DMS metabolism is not a widespread but a specialized process (Vila-Costa et al. 2006b).

The contribution of DMSP-C consumption to BCD averaged 3.1%. This average is very similar to those found in shelf (3.4%) and oceanic (3.1%) waters of the Gulf of Mexico (Kiene & Linn 2000). The highest values (up to ca. 6%), similar to those found during a DMSP-rich phytoplankton bloom (8%, Simó et al. 2002), were obtained in summer and during the December flagellate bloom, in both cases coinciding with higher DMSP-C to phytoplankton POC ratios. Except for December, the contribution of DMSP-C consumption to BCD followed an annual variability similar to that of the $DMSP_p$:chl *a* ratio (Fig. 3).

These values represent a significant contribution to total bacterial C demands by an individual substrate. Very few studies have focused on individual components of the dissolved organic pool; this issue has generally been assessed experimentally by adding groups of molecules in pools such as dissolved free amino acids (DFAA) or proteins, which have been seen to account for as much as 30% of C demands (Keil & Kirchman 1999, Rich et al. 1996). In Long Island Sound, a single DFAA (alanine) contributed C in values similar to the contribution we found for DMSP (8%, Fuhrman 1987). This lends support to the suggestion that DMSP is a very labile C source for bacteria, and is especially significant in oligotrophic conditions.

The contribution of DMSP-S assimilation to BSD was always higher than that of DMSP-C consumption to the C demand. Despite the fact that sulfate largely dominates the pool of available S in the oceans (ca. 28 mmol l^{-1} in dissolved salts), bacteria may be energetically favored by directly taking up S in a reduced form (such as DMSP) for satisfying their cellular S requirements (Kiene et al. 1999). Notably, a cultured member of SAR11, the taxonomic group that numerically dominates the bacterial assemblage in Blanes Bay, has been shown to lack the genes that allow for sulfate reduction, and relies on the uptake of exogenous reduced S (including DMSP) for growth (Tripp et al. 2008). The annual variability of the contribution of DMSP-S assimilation to BSD matched closely that of the $DMSP_p$:chl *a* ratio (Fig. 3), with higher values in the warmer months of the year (May to September). In August and September, estimated DMSP-S incorporation even exceeded 100% of bacterial S demands. That is, DMSP assimilation occurred at rates high enough to be the largest S source for bacterioplankton, far larger than sulfate reduction. We had previously shown that over the summer months the numbers of bacteria assimilating DMSP-S were higher, and additions of $DMSP_d$ and phosphate stimulated bacterial production over additions of glucose and phosphate; both features pointed to summer bacteria using DMSP as a preferred S source (Vila-Costa et al. 2007). Similar results were obtained by Zubkov et al. (2002) in summer waters of the North Sea and by Simó et al. (2002) in summer NW Atlantic waters, both dominated by blooms of the high-DMSP producer *Emiliana huxleyi*, and by Kiene & Linn (2000) in warm waters from the Gulf of Mexico. Contrastingly, during the 'winter' (October to April), DMSP accounted for a lower share of the S demand, so that other S sources were used.

As for phytoplankton, these calculations are subject to the uncertainty associated with the molar C:S ratio used. Among the scarce literature available, we chose the average ratio measured with native marine bacteria by X-ray microanalysis (Fagerbakke et al. 1996). We decided not to use the highly varying data by these same authors and others on C:S ratios of bacterial isolates from non-marine sources. Fig. 3 shows the effects of the uncertainty in the selected values on the DMSP share in BSD. Despite the uncertainty, the seasonality seems robust, and so is the evidence that DMSP acts as a major source of S for heterotrophic bacterioplankton during a considerable part of the year. However, we did not take into account that the C:S ratio could vary seasonally, but used a fixed average ratio of 75 to calculate BSD over the year. The only study we are aware of that has addressed this possibility is Fagerbakke et al. (1996). Bacterioplankton from a Norwegian fjord showed a molar C:S of 54 in June and 140 in October (Fagerbakke et al. 1996)—too few data to describe a seasonal pattern. There is a clear need to generate more stoichiometric data to better constrain the elemental composition of marine bacteria. Still another source of uncertainty comes with the assumption that DMSP-S assimilation was carried out by heterotrophic bacteria only (or, more accurately, by microorganisms that take up leucine). It has been shown that some marine phytoplankters, including the pico-cyanobacteria, are also able to take up and assimilate a significant (yet largely unknown) fraction of DMSP-S (Malmstrom et al. 2005, Vila-Costa et al. 2006b). The omission of this process might help explain why in some months DMSP apparently accounted for >100% of BSD.

CONCLUSIONS

The present study has shown that in an oligo- to mesotrophic coastal site, Blanes Bay, DMSP contributed significantly to C and S fluxes through phytoplankton and bacterioplankton throughout a year, with shares of the same order as those found in blooms of well-known DMSP producers. Both the biomass-specific DMSP content and the amount of PP invested into DMSP biosynthesis showed seasonality, with higher values in the May to September period. During this same period, bacteria assimilated a larger percentage of DMSP-S and obtained a larger share of their C and

S needs from this compound, to the extent that DMSP was the main source of bacterial biomass S. Since many studies of S biogeochemistry in the surface ocean do not have concurrent measurements of primary and bacterial productions, and bacterial DMSP-S assimilation, we propose the easily measurable $DMSP_p$:chl *a* ratio as a good proxy of the quantitative role of DMSP in the C and S fluxes through the first trophic levels of the food web.

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