The quantitative role of microzooplankton grazing in dimethylsulfide (DMS) production in the NW Mediterranean

Simó, Rafel; Saló, Violeta ; Almeda, Rodrigo; Movilla, Juancho; Trepat, Isabel ; Saiz, Enric; Calbet, Albert

Published in:
Biogeochemistry

Link to article, DOI:
10.1007/s10533-018-0506-2

Publication date:
2018

Document Version
Peer reviewed version

Biogeochemistry
General research article

The quantitative role of microzooplankton grazing in dimethylsulfide (DMS) production in the NW Mediterranean

Running head: Microzooplankton grazing and DMS production

Rafel Simó*[a], Violeta Saló[a], Rodrigo Almeda[†], Juancho Movilla[‡], Isabel Trepat, Enric Saiz, Albert Calbet

Institut de Ciències del Mar (ICM-CSIC). Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Catalonia, Spain

*Corresponding author: rsimo@icm.csic.es / +34 932309590
[a]These two authors contributed equally to the work
[†]Current address: Centre for Ocean Life, National Institute for Aquatic Resources, Technical University of Denmark, Lyngby, Denmark
[‡]Current address: Estación de Investigación Jaume Ferrer, Centro Oceanográfico de Baleares (Instituto Español de Oceanografía), La Mola, Menorca, Illes Balears, Spain

Keywords: dimethylsulfide; dimethylsulfoniopropionate; microzooplankton; grazing; dilution experiments, Mediterranean

ABSTRACT

The ubiquitous, biogenic trace gas dimethylsulfide (DMS) represents the largest natural source of atmospheric sulfur. Given DMS involvement in cloud formation and climate, understanding and parameterizing the oceanic DMS source and cycling processes is a necessary challenge. We report DMS cycling rates from microzooplankton dilution grazing experiments conducted monthly during one year in coastal northwestern Mediterranean waters. Concentrations of DMS, its algal precursor dimethylsulfoniopropionate (DMSPt) and chlorophyll *a* (Chl*a*) ranged 0.9-11 nmol L^{-1}, 10-71 nmol L^{-1}, and 0.2-1.5 μg L^{-1}, respectively. By comparing the growth and stock production rates of the DMSP-producing algae to those of total phytoplankton, we estimated that 3 ± 4% (range 0.4-12%) of the carbon primary production was invested in DMSP biosynthesis. Microzooplankton grazing rates on DMSP-producing phytoplankton (0.46-1.45 d^{-1}) were generally higher than those on the bulk assemblage (0.08- 0.99 d^{-1}), except in midsummer months. This could have been due to the smaller size of most DMSP producers. There was no indication of micrograzer selection against DMSP-containing phytoplankton, since they were not grazed at lower rates than the bulk phytoplankton assemblage. A proportion of 6-20% of the grazed DMSP was converted into DMS, and this grazing-derived production accounted for 32-96% of dark gross DMS production by the total community. Bacteria consumed daily ≤14-100% of the gross DMS production, which resulted in biological DMS turnover times of 1-≥10 days. Throughout the year, grazing-mediated DMS production explained 73% of the variance in the DMS concentration, implying that microzooplankton grazing plays a major role in controlling DMS concentration in surface waters across a broad range of environmental and productivity conditions in the Mediterranean Sea. These findings should help improve the representation of herbivore grazing in prognostic models to predict the distribution and dynamics of the global DMS emission and its feedback response to changing climate.

INTRODUCTION

Dimethylsulfide (DMS) is a climatically active trace gas that is found in the sunlit layer all over the world's oceans. DMS concentrations are supersaturated in surface waters relative to the atmosphere, driving a global net sea-air flux of *ca*. 16-28 Tg S y^{-1} (Lana et al. 2011; Galí et al. 2018), one of the largest amongst marine organic volatiles (Carpenter et al. 2012). In the atmosphere DMS is oxidized to molecules that either condense upon existing particles or nucleate to form new particles. Both newly born and growing aerosols have the capability to backscatter solar radiation and act as cloud condensation nuclei. The availability of condensation nuclei regulates cloud droplet number and size, hence cloud albedo, thereby contributing to regulate the global radiation budget (Charlson et al. 1987; Quinn et al. 2017). In addition to this climatic role, airborne DMS also acts as a foraging infochemical for marine birds, mammals and turtles (e.g., Nevitt 2011). The importance of DMS emissions for chemical ecology and climate has precipitated considerable research on its biological and biogeochemical cycling in the ocean (Simó et al. 2001; Stefels et al. 2007). Advances in process-level understanding, yet remarkable, have not been enough, and global ecosystem models still struggle to accurately reproduce macroscale and seasonal DMS patterns, especially at lower latitudes (Le Clainche et al. 2010).

DMS in marine environments is primarily formed from dimethylsulfoniopropionate (DMSP), a ubiquitous osmolyte in phytoplankton. Intracellular DMSP concentrations span from undetectable levels (<0.1 mmol L^{-1}) to as high as >1000 mmol L^{-1}, depending on taxon and growth conditions mediated by multiple environmental factors (Stefels et al. 2007). The taxonomic composition of phytoplankton assemblages plays the main role in determining DMSP production in natural waters (Keller et al. 1989). Algal inter-specific variations are thought to explain the poor correlations often found between chlorophyll *a* (Chl*a*) and particulate DMSP or DMS (e.g., Dacey et al. 1998; Vallina et al. 2007; Lizotte et al. 2012). Total DMSP concentrations in seawater are usually in the 10-200 nmol L^{-1} range, much higher than typical DMS concentrations (1-10 nmol L^{-1}; Kiene et al. 2000; Stefels et al. 2007; Galí et al. 2015). DMSP is a very labile compound produced inside the algal cell and released, transferred and transformed through the entire planktonic food web (Tang et al. 1999; Tang and Simó 2003) as a significant component of carbon and sulfur fluxes between trophic levels (Kiene et al. 2000; Simó et al. 2002, 2009). One of the byproducts of DMSP transformations

429 is DMS, most of which is degraded within the water column by microorganisms and solar
430 radiation, and only a small fraction is ventilated to the atmosphere and becomes climatically
431 active (Simó 2001; Stefels et al. 2007).
432
433 DMSP is released from phytoplankton cells to the water column through numerous
434 processes, namely algal senescence and physiological stress (Kwint and Kramer 1995; Sunda
435 et al. 2002), viral lysis (Malin et al. 1998), and zooplankton grazing (Dacey and Wakeham
436 1986; Christaki et al. 1996; Daly and Di Tullio 1996). DMSP exudation or excretion by
437 healthy algal cells seems to occur, but plays a secondary role (Laroche et al. 1999), whereas
438 lipophilic DMS, when produced intracellularly, easily crosses membranes and leaks out of the
439 cell (Spiese et al. 2015). A number of laboratory (e.g., Dacey and Wakeham 1986; Christaki
440 et al. 1996; Wolfe and Steinke 1996) and field studies (e.g., Daly and DiTullio 1996; Kwint et
441 al. 1996; Archer et al. 2003) have demonstrated that zooplankton grazing enhances DMS
442 production, probably by facilitating the mixing of algal DMSP with algal or bacterial DMSP
443 lyases. In spite of this line of evidence, few studies have attempted to assess the relative
444 importance of grazing within the cycle of dimethylated sulfur (Simó et al. 2002; Archer et al.
445 2001b, 2003, 2011).
446
447 Microzooplankton are major herbivores in most marine environments, channeling as
448 much as two thirds of daily phytoplankton production in both eutrophic and oligotrophic
449 pelagic systems worldwide (Calbet and Landry 2004; Schmoker et al. 2013).
450 Microzooplankton include heterotrophic and mixotrophic organisms: protists such as ciliates,
451 dinoflagellates, and foraminiferans, and small metazoans such as copepod nauplii,
452 meroplanktonic larvae, and rotifers (Sieburth et al. 1978). Microzooplankton are often the
453 same size as their prey, which poses operational difficulties for the quantification of their
454 grazing rates. To overcome this problem, Landry and Hassett (1982) proposed the dilution
455 technique, an assay that has since been widely used in various regions of the world's ocean.
456 The dilution technique involves incubation of a series of water samples diluted with
457 increasing amounts of filtered (organism-free) seawater to sequentially reduce grazer-prey
458 encounter rates and therefore the grazing of microzooplankton on phytoplankton. Changes in
459 Chl*a* concentration in the series of incubations yield an estimate of the growth and mortality
460 rates of the phytoplankton assemblage (Landry and Hassett 1982). The dilution technique has
461 also been used to calculate some biogeochemically relevant process rates, such as those of

462 nitrogen uptake, regeneration, and excretion (Andersen et al. 1991; Neuer and Franks 1993;
463 Lehrter et al. 1999).
464
465 As the dominant cause of algal mortality, microzooplankton grazing is expected to
466 play a central role in DMSP consumption and DMS production. In the last two decades, a few
467 studies have applied the dilution technique to estimate the growth and grazing-mediated
468 mortality rates of DMSP-producing phytoplankton (Wolfe et al. 2000; Archer et al. 2001b;
469 Fredrickson and Strom 2009; Archer et al. 2011) and the grazing-mediated rates of dissolved
470 DMSP and/or DMS production (Wolfe et al. 2000; Archer et al. 2001a, 2003, 2011; Park et
471 al. 2014) in temperate, subpolar and polar waters. Nothing is known about the role of
472 microzooplankton grazing in the DMS cycle at lower latitudes and across seasons, and how it
473 compares with rates of microbial DMS production and consumption. Moreover, a grazing
474 deterrent function has been suggested for DMSP. Initially, this was assigned to two of its
475 degradation products, acrylate as a toxic and DMS as an infochemical (Wolfe and Steinke
476 1997); later on, the hypothesis was revisited to suggest that DMSP itself would reduce protist
477 grazing rates (Strom et al. 2003). More recently, Seymour et al. (2010) showed that DMSP is
478 indeed an infochemical but a potent attractant, not a repellent. Thus, there is still controversy
479 about the inhibitory or stimulatory effects of DMSP on grazing in natural plankton
480 communities. One way to assess the validity of the deterrence hypothesis is testing for
481 reduced grazing rates on DMSP-containing phytoplankton with respect to grazing rates on the
482 bulk phytoplankton assemblage, even though this approach has limited reach since other
483 factors, such as prey size, morphology, motility and nutritious value have strong influence on
484 grazing rates (Verity 1991).
485
486 In the present study, we conducted monthly dilution experiments during a year in
487 oligo- to mesotrophic coastal waters of the north-western Mediterranean. We used a revised
488 version of the dilution technique (Saló et al. 2010) that includes measurements of DMSP (as
489 the specific biomarker for DMSP-producing algae) and aqueous DMS. Here we report the
490 results that refer to the cycling of DMS, whereas Chl*a* and cell count based results are fully
491 described in Calbet et al. (2008). For the first time, we compare the rates of grazing-mediated
492 DMS production with measured rates of DMS consumption by bacteria and gross DMS
493 production by the whole plankton community. Our goals were 1) to compare the growth and
494 mortality rates of the DMSP producers with those of the whole phytoplankton assemblages;
495 2) to explore if the grazing deterrence hypothesis could be tested in the field; and 3) to

quantify the role of microzooplankton grazing in DMS production and cycling across a broad range of plankton communities and environmental conditions within an annual cycle.

MATERIALS AND METHODS

Sampling, experimental setup and sub-sampling

The present study was designed as monthly sampling over a full year between September 2005 and September 2006 (Calbet et al. 2008). However, the sampling trips of December 2005 and February 2006 had to be cancelled due to technical problems with the Institute's boat. Furthermore, a bloom of colonial *Phaeocystis* sp. occurred in March 2006. Due to our sampling protocol at the time, which did not include pre-filtration of the samples upon subsampling for DMS (del Valle et al. 2009), no reliable values of DMS concentration could be obtained owing to continuous DMS production throughout the purging time, and the March experiment had to be cancelled too. In early April 2006, the receding bloom, now overtaken by mixotrophic ciliates, had left behind free living *Phaeocystis* sp. cells and a quite high DMS concentration (annual maximum at 11 nM), but the experiment could be conducted normally. Altogether, the annual study was constructed on the basis of 10 monthly samplings.

The water for the experiments was sampled 1.5 km offshore of the city of Barcelona (41.22° 775' N, 02.13° 150' E), at 11:00 h local time, over a water-column depth of 40 m. Seawater was collected from 5 m with a 15 L transparent hydrographic bottle, gently siphoned into 20-L carboys covered with back plastic bags (to avoid excessive exposure to sun-light), and rapidly transported to the laboratory. Temperature and light were measured *in situ* with a YSI 30 portable temperature meter and a LI-COR LI-1400 data logger, respectively.

Prior to each experiment, filter capsules, silicon tubing, carboys, and polycarbonate bottles were soaked in 10% HCl-Milli-Q water and rinsed thoroughly with Milli-Q water (> 10 L passed through filters and at least 3 rinses for the rest of material). Part of the sampled water was gently siphoned into a 50 L bucket and carefully mixed (named "whole water" thereafetr), and the rest was gravity filtered through 0.2 μm with a Pall Acropak 0.8:0.2 500 capsule (filtered water). As the whole water used for the experiments was not filtered through

530 a 200 μm mesh in order to avoid cell breakage of delicate microzooplankton organisms, it
531 might have contained some mesozooplankton. Visual examination of the water did not reveal
532 the presence of large organisms.
533
534 Whole water was added to 0.2 μm filtered water in duplicate 2.3 L polycarbonate
535 bottles, which were filled leaving minimal headspace and rapidly capped. Four levels of
536 dilution were prepared containing decreasing proportions of whole water: 100% (undiluted),
537 75%, 50%, and 25%, respectively. Nutrients were added to all the dilution bottles to final
538 concentrations of 15 μmol L^{-1} NH_4Cl and 1 μmol L^{-1} Na_2HPO_4. Two bottles of whole water
539 without nutrient addition were used as natural seawater controls. Four dark glass bottles with
540 undiluted seawater were incubated in parallel for the determination of community gross DMS
541 production and bacterial DMS consumption rates (see below).
542
543 Once all the experimental bottles had been prepared, incubations were carried out in a
544 large (600 L) outdoor incubator with a continuous flow-through system of water running from
545 the coastal sea-water intake of the laboratory. The incubator was covered with a neutral
546 density mesh that reduced ca. 40% of solar irradiance; this was meant to simulate the natural
547 attenuation of 33-50% of surface PAR irradiance observed at 5 meters depth in the sampling
548 site. Bottles were gently mixed at least three times through the 24 hours period in order to
549 minimize algal settling.
550
551 At the beginning of the experiment (t_0), whole and filtered waters were sub-sampled
552 from the buckets for Chl*a*, DMSP and DMS analyses before filling the dilution bottles. The
553 initial concentrations for each dilution level were obtained by calculations according to the
554 corresponding proportions of whole and filtered waters. Relevant tests had shown this method
555 was accurate within 3% (Saló et al. 2010). At the final time point after 24 h of incubation
556 (t_{24}), all the experimental bottles were sampled again for Chl*a*, DMSP and DMS. The dark
557 bottles used for measuring gross DMS production and bacterial DMS consumption were
558 sampled for DMS at times zero, 26 h, and two intermediate time points, typically 4-6 h and 20
559 h (Saló et al. 2010).
560
561
562
563

564 **Plankton community composition**

565

566 At the beginning each experiment, the composition of the plankton community in
567 whole seawater was determined. For nanoflagellates, 40 to 100 mL samples were fixed with
568 gluteraldehyde (1% final concentration), filtered onto 2 μm pore-size black polycarbonate
569 membrane filters and stained with 4',6-diamidino-2-phenylindole (DAPI, 5 μg mL^{-1} final
570 concentration) for 5 min. At least 200 cells (typically 20-30 fields) were counted and
571 classified as auto- or heterotrophic according to their chlorophyll fluorescence. Fifty cells
572 were sized and converted into carbon using a conversion factor of 0.22 pg C per μm^3 of cell
573 volume (Borsheim and Bratbak 1987). Two groups of algal flagellates, namely haptophytes
574 (typically DMSP producers) and cryptophytes (typically low-DMSP producers), were
575 differentiated according to their shape and fluorescence. To determine the concentration of
576 dinoflagellates, ciliates and diatoms, 250 mL subsamples were fixed with 1% acidic Lugol's
577 solution, and allowed to settle for 48 h in 100 mL Utermöhl chambers. The whole chamber
578 for ciliates and dinoflagellates, and at least 40 microscope fields (or 200 cells) for diatoms
579 were counted under an inverted microscope (Nikon Diaphot 200) at 200X magnification.
580 Fifty randomly-chosen cells for each group were sized and converted into carbon using the
581 conversion factors of 0.19 and 0.053 pg C μm^{-3} for oligotrich ciliates (Putt & Stoecker, 1989)
582 and tintinnids (Verity and Langdon 1984), respectively, and the equations of pg C_{Dino} $cell^{-1}$=
583 0.760 × $volume^{0.819}$ for dinoflagellates and pg C_{Diat} $cell^{-1}$= 0.288 x $volume^{0.811}$ for diatoms
584 (Menden-Deuer and Lessard 2000). Because microzooplankton samples were preserved with
585 acidic Lugol's solution, no distinction between strict heterotrophs and auto- or mixotrophs
586 was made for ciliates and dinoflagellates, with the exception of those genera easily
587 recognizable, such as *Laboea* spp. Samples (2 mL) for *Prochlorococcus* sp. and
588 *Synechococcus* sp. were preserved with paraformaldehyde + glutaraldehyde (1% + 0.05%
589 final concentration, respectively) and stored at –80°C for flow cytometry analysis with a
590 FACSCalibur (Becton and Dickinson) flow cytometer with a laser emitting at 488 nm.
591 *Prochlorococcus* and *Synechococcus* biomasses were determined after assuming a carbon
592 content of 0.123 pg C μm^{-3} and equivalent spherical diameters (ESD) of 0.60 and 1.0 µm,
593 respectively (Waterbury et al. 1986).

594

595

596

597

598 **DMSP and DMS analyses**

599

600 A purge-and-trap gas chromatography system was used to determine DMS
601 concentrations from 3-5 mL samples (Saló et al. 2010). Calibrations with DMS standards
602 from a DYNACAL (Vici Metronics) permeation tube were run every day (Simó et al. 1995).
603 Aliquots of 10-40 mL were sampled for total DMSP (DMSPt), placed in gas-tight vials and
604 hydrolyzed with 2 pellets of NaOH during 1 to 5 days, after which time the evolved DMS was
605 analyzed in small aliquots. The results were then corrected for pre-existing DMS. All analyses
606 were run in duplicate, and standard errors for both DMS and DMSP concentrations fell within
607 10% of the mean.

608

609 **Chl*a* analyses**

610

611 Concentrations of Chl*a* were determined at initial (t_0) and final (t_{24}) times by filtering
612 75 to 300 mL of water through a GF/F Whatman filter (0.7 μm nominal pore size) under
613 gentle vacuum. The filters were stored at -80ºC before being extracted in 90% acetone. Chla
614 fluorescence was measured on a Turner fluorometer with and without acidification to correct
615 for phaeopigments (Parsons et al. 1984).

616

617 **Calculation of growth and grazing rates**

618

619 Growth and grazing rates were calculated for the whole phytoplanktonic community
620 (Chl*a* data) and for DMSP producers (DMSPt data). Our intention was to use DMSPp instead
621 of DMSPt (Saló et al. 2010) because the former is more directly linked to DMSP-producing
622 cells. However, filtration for the separate determination of the dissolved and particulate pools
623 induced artefactual overestimation of DMSPd, most probably due to intracellular DMSP
624 release from fragile cells during syringe filtration (Kiene and Slezak 2006). We therefore used
625 DMSPt, with the assumption that most of it was actually DMSPp (Kiene and Slezak 2006)
626 and the production of DMSPd by grazing would be negligible in comparison with the fraction
627 consumed by grazers (Wolfe et al. 2000).

628

629 Net rates of change (r) of Chl*a* and DMSPt were determined from t_0 and t_{24}
630 concentrations (C_{t0}, C_{t24}) assuming an exponential model:
631 $r = \ln (C_{t24}/C_{t0})/t$

The r values of duplicate bottles were plotted against the level of dilution (fraction of whole water in the dilution treatment), and model I regression analysis was used to compute the specific growth rate of the algae (μ' = intercept) and the rate of mortality due to grazing (m = slope). Because the intercept of the equation would provide an overestimation of phytoplankton growth rates (nutrients were added to these bottles), gross growth rates (μ) were obtained from net growth in nutrient-unamended and undiluted bottles plus mortality rate m (Landry and Hassett 1982).

Estimates of primary and DMSP production

Chl*a*-based growth rates (μ_{chla}, d^{-1}) were converted into mass gross growth rates (μg Chl*a* L^{-1} d^{-1}) by multiplying them by the mean Chl*a* concentration in the non-diluted bottle without added nutrients ($<C_{Chla}>$), calculated according to the equation of Frost (1972):

$<C_{Chla}> = C_{to}\ [e^{(\mu-m)\ (t24-t0)}-1] / (t_{24}-t_0)\ (\mu-m)$

Mass growth rates were converted into carbon-based primary productivity rates by considering that the C:Chl*a* (mass:mass) ratio varies between 40 (mid-winter) and 120 (mid-summer) according to the month, as in the nearby study site of Blanes Bay (Gasol et al. 2016).

DMSP-based gross growth rates (μ_{DMSP}, d^{-1}) were converted into DMSP production rates (nmol DMSP L^{-1} d^{-1}) by multiplying them by the mean $DMSP_t$ concentration calculated as detailed for Chl*a*. The proportion of primary productivity invested in DMSP production was calculated by converting DMSP production into DMSP-C production by multiplying by 5, which is the number of C atoms in the DMSP molecule.

Calculation of grazing-mediated DMS production

The difference between t_0 and t_{24} concentrations of DMS was used to calculate net DMS production in duplicate bottles at each dilution level. In parallel, DMSPt grazing rates at each dilution were calculated by scaling DMSPt mortality rates m_{DMSP} to the dilution factor and multiplying them by the mean DMSPt concentration $<C_{DMSP}>$ in each dilution bottle calculated as: $<C_{DMSP}> = C_{to}\ [e^{(\mu-m)\ (t24-t0)}-1] / (t_{24}-t_0)\ (\mu-m)$

Net DMS production rate values were paired to the corresponding DMSPt grazing rates, and a model I regression analysis was conducted. The slope provided the daily DMS production per grazed DMSPt (Δnmol DMS L^{-1} / Δnmol DMSP L^{-1}), which was multiplied by the mean

DMSPt concentration in the control (nutrient-unamended and undiluted) bottles to obtain the rate of DMS production due to grazing (*Pg*). The error of *Pg* was obtained from those of the slope and the mean DMSPt concentration in replicate controls.

Measurements of gross DMS production and bacterial DMS consumption

Community gross DMS production and bacterial DMS consumption were estimated by the inhibitor method with dimethyl disulfide (DMDS; Wolfe and Kiene 1993; Simó et al. 2000; Saló et al. 2010) in parallel undiluted bottles incubated in the dark. DMS accumulation in duplicate DMDS amended bottles (final concentration of 200 nmol L^{-1}) provided the gross DMS production rate. The difference between gross DMS production and net DMS production in the non-DMDS-amended bottles provided an estimate of the bacterial DMS consumption rate. Rate errors were derived from the standard errors of the slopes.

RESULTS

Plankton community composition and dimethylated sulfur pools

The physicochemical conditions encountered in each sampling are reported in Calbet et al. (2008). In brief, seawater temperature at the sampling depth (5 m) was 23.5ºC at the beginning of the study in September 2005, decreased to 13.0ºC in January, and increased again to a maximum of 24.4ºC in July 2006. Nutrient concentrations varied almost the opposite to temperature, with highest concentrations in November and lowest levels in September 2006. Chl*a* concentrations ranged 0.5-1.7 μg L^{-1} between October and May, and 0.2-0.7 μg L^{-1} in the June-September period (Table 1 and Figure 1).

The phytoplankton assemblage, also partially reported in Calbet et al. (2008), was characterized by a clear dominance of organisms <10 μm in the period June-September, while in the rest of the year the larger cells contributed 40-50% of the total Chl*a*. Diatoms were present particularly in the colder months, contributing the largest share of phytoplankton biomass in November and May (Table 1). Autotrophic nanoflagellates occurred all year round, dominated by Haptophytes from June to September and by Cryptophytes from October

700 to January. *Synechococcus* sp. abounded throughout the warmer months, and even became the
701 largest contributor to phytoplankton biomass in midsummer (July-August). *Prochlorococcus*
702 sp. only occurred in September through January, though in low biomass. In April,
703 phytoplankton was dominated by the mixotrophic ciliate *Laboea* sp., and small dinoflagellates
704 (most of them <20 μm) took over in June.
705
706 The biomass of the microzooplankton assemblages spanned one order of magnitude
707 (from ca. 4 μg C L^{-1} in November to 43 μg C L^{-1} in April; Calbet et al. 2008), with alternate
708 dominance of nanoflagellates and ciliates plus dinoflagellates over the year (Table 1).
709 Remarkable features were the aforementioned large proportions of mixotrophic ciliates in
710 April and heterotrophic nanoflagellates in July. There was no evidence for any clear seasonal
711 pattern.
712
713 The initial concentrations of DMSPt and DMS in the waters used for the dilution
714 experiments are listed in Table 1 and graphically presented in Figure 1. DMSPt
715 concentrations ranged 10-71 nmol L^{-1}, with no clear seasonal pattern. Since Chl*a*
716 concentrations were typically higher in the colder months (October to May; 0.5-1.5 μg L^{-1})
717 than in the warmer months (June to September; 0.2-0.7 μg L^{-1}), DMSPt:Chl*a* ratios were
718 lower in the former (11-26 nmol μg^{-1}) and higher in the latter (44-145 nmol μg^{-1}). The far
719 highest DMSPt level and DMSPt:Chl*a* ratio were observed in June, coinciding with high
720 biomass of small dinoflagellates (Table 1). DMS concentrations roughly increased from late
721 fall and winter (ca. 1 nmol L^{-1}) to summer (5-8 nmol L^{-1}), with the exception of April, where
722 the maximum annual concentration (11 nmol L^{-1}) was recorded during the *Phaeocystis* post-
723 bloom.
724
725 **Dilution experiments**
726
727 Figure 2 shows two graphical examples of the results of the dilution experiments for DMSP
728 and DMS. They correspond to November 2005 and April 2006. Two more examples (June
729 and July 2006) can be found in Saló et al. (2010). As illustrated by the figure, regression
730 analysis of apparent DMSPt growth rates vs dilution level generally showed significant ($p <$
731 0.05) slopes and intercepts. The slope was taken as the grazing rate on DMSP, and the
732 intercept was corrected by the apparent growth in nutrient-unamended bottles to provide the
733 *in situ* DMSP growth rate. The results from all dilution experiments are presented in Table 2.

As shown in Table 2 and Figure 3, the growth rates of DMSP-producing phytoplankton (μ_{DMSP}) varied between 0.07 d^{-1} (August) and 1.49 d^{-1} (May), i.e., within a wider range than the Chl*a*-based growth (μ_{chla}, 0.30-1.08 d^{-1}). Nonetheless, the annual means were very similar (μ_{DMSP} = 0.74 ± 0.51 d^{-1}; μ_{chla} = 0.81 ± 0.25 d^{-1}). The DMSP-based growth rates were significantly higher in the colder months (October-May: 1.17 ± 0.07 d^{-1}) than in the warmer season (June-September: 0.31 ± 0.13 d^{-1}), despite higher DMSPt concentrations in the latter. As a result, mass production rates of DMSP were on average 2.5-fold higher in the colder months. When converted into carbon units, DMSP production represented a 0.4% to 12% share of carbon fixation (overall mean of 3 ± 4%).

Grazing rates on DMSP-producing phytoplankton (m_{DMSP}) ranged between 0.46 (August) and 1.45 d^{-1} (July), with an overall average (0.84 ± 0.31 d^{-1}) similar to the mean DMSP-based growth rate average (Table 2). These DMSP-based grazing rates were generally higher than the Chl*a*-based rates (m_{chla}), which ranged 0.08-0.99 d^{-1} (overall mean 0.50 ± 0.29 d^{-1}). DMSP-based mortality was higher than Chl*a*-based mortality during most of the studied period, except in June, August and September 2006 (Table 2 and Figure 3).

The rates of DMS production due to grazing (*Pg*) varied between not significantly different from zero in January to 6.3 nmol DMS $L^{-1}d^{-1}$ in April (Table 3). The estimated yield of DMSP conversion into DMS due to grazing was not significantly different from zero in January and varied between 6% and 20% during the rest of the year (overall mean 13 ± 6%, Table 3). Microzooplankton grazing accounted for 32-96% (overall mean 65 ± 9%) of the gross DMS production by the whole community in the dark (Table 3). Actually, *Pg* and gross DMS production were strongly correlated (r^2 = 0.86, n = 9, p < 0.01; Figure 4). Bacteria consumed daily ≤14-100% of the gross DMS production, which resulted in biological DMS turnover times of 1-≥10 days, with no significant difference between warm and cold months (Table 3).

DISCUSSION

Growth and mortality rates: is there evidence for grazing deterrence by DMSP?

During the study period, Chl*a* and DMSPt concentrations and the DMSPt:Chl*a* ratio in our sampling station off Barcelona followed monthly variations somewhat consistent with those found in the Blanes Bay Microbial Observatory located *ca*. 60 km northwards (Vila-Costa et al. 2008; Simó et al. 2009). Larger phytoplankton, mainly diatoms and cryptophytes, occurred in the colder months (October to May), associated with higher biomass and primary production rates, but with lower specific (Chl*a*-normalized) DMSP content. Indeed, diatoms and cryptophytes from temperate waters are amongst the phytoplankton phyla with lower intracellular DMSP concentrations (Stefels et al. 2007). With the onset of summer, characterized by stronger stratification, depleted nutrients and lower productivity (Gasol et al. 2016), plankton succession led to smaller cells (mainly haptophytes and *Synechococcus*), which are more efficient at nutrient uptake and overall have higher DMSP content (Table 1). *Synechococcus* are considered to contain little or no DMSP, but haptophytes are, along with dinoflagellates, the strongest DMSP producers, more so under high light and nitrogen limitation (Simó 2001; Stefels et al. 2007). The dilution experiments revealed that the DMSP-producing phytoplankton grew faster from October to May than in summer, while the growth rates of the bulk phytoplankton assemblage did not show any clear seasonal trend. The resulting proportion of total primary production invested in DMSP biosynthesis varied between 0.4 and 12%, which is consistent with the values (1-10%) obtained in Blanes Bay by Simó et al. (2009).

The growth rates of the bulk phytoplankton assemblage (μ_{chla}) were generally higher than the corresponding grazing-derived mortality rates (m_{chla}, Figure 3). This indicates that causes of phytoplankton loss (or Chl*a* stock renewal) other than microzooplankton grazing occurred, namely mesozooplankton grazing, viral infection, algal autolysis and sedimentation. As a matter of fact, mortality rates only caught up with growth rates in summer (July-September 2006). In these months the phytoplankton assemblage was dominated by *Synechococcus* sp., which are very inefficiently captured by mesozooplankton (during this season mostly ambush-feeding copepods and cladocerans; Atienza et al. 2006) and have low sinking rates. On annual average, microzooplankton consumed daily 58% of the phytoplankton growth.

As for the DMSP-producing phytoplankton, growth rates (μ_{DMSP}) were higher than mortality rates (m_{DMSP}) in 4 experiments (November to May), while the opposite occurred mainly in summer 2006 (Figure 3). On average, microzooplankton consumed daily 82 ± 30% of the DMSP stock at that depth (Table 3). This indicates that other DMSP sinks such as mesozooplankton grazing, algal autolysis, viral infection or intracellular DMSP turnover were likely insignificant. Several causes for such an apparent tight coupling between growth and micrograzing mortality can be invoked. On the one hand, the DMSP producers are generally small-sized algae such as small dinoflagellates and haptophytes (Belviso et al. 1993; Archer et al. 2011), i.e., those acting as target prey for herbivorous microzooplankton (Fenchel 1980). This would explain that microzooplankton consumed a larger proportion of the DMSP producers than of the total phytoplankton. On the other hand, there is the possibility that the experimental setup did not account for any intracellular turnover of DMSP that might be occurring due to high light and high nutrient exposure (Sunda et al. 2002) in the summer incubations, thus rendering underestimates of DMSP production or growth rates (Archer et al. 2011). This is very plausible, as measured μ_{DMSP} values were too low to sustain m_{DMSP} in 4 experiments in summer 2006. The possibility that grazing rates were overestimated is less likely since microzooplankton removed, during that period, the reasonable amount of 40-100% of the DMSPt stock, similarly to the findings of other authors in North Sea and sub-Antarctic waters (Archer et al. 2001b, 2011).

Overall, our results indicate that DMSP-containing phytoplankton were not grazed at lower rates than the bulk phytoplankton assemblage and, therefore, they do not support the hypothesis of DMSP as a grazing deterrent (Strom et al. 2003). According to these authors, release of DMSP by microalgae under grazing pressure would cause a decrease of feeding rates by herbivorous protists, as they demonstrated by adding dissolved DMSP to bottles with lab-prepared prey:predator mixtures. These deliberate additions caused significant reductions of the ingestion rates (Strom et al. 2003), in what was regarded as an evidence for a defense system in phytoplankton. DMSP additions to dilution experiments with natural communities, however, did not yield significant differences in the grazing rates with respect to controls in most of the cases (Fredrickson and Strom 2009). In a later work, Seymour et al. (2010) used microfluidics to investigate the response of bacterivore and herbivore protists to microscale pulses of dissolved DMSP, and concluded that this compound acts as a potent attractant rather than a repellent. Therefore, if anything, it should aid grazers to find their prey. Deliberate

DMSP additions like those used in the aforementioned lab experiments could have led to erroneous conclusions by disrupting the chemical gradients around the prey cells. Our results agree with those of Archer et al. (2011), who also measured higher grazing rates on DMSP-containing phytoplankton relative to the bulk assemblage. In recent years, therefore, observations in the field concur with laboratory-based experiments in not supporting the formulation of the defense hypothesis that proposes DMSP as a conspicuous grazing deterrent.

Microzooplankton grazing and DMS production and cycling

Unlike DMSPt concentrations, which showed no clear seasonality but an outstanding peak during a bloom of small dinoflagellates in June, DMS concentrations followed a general increase between winter and midsummer, broken by a peak derived from the *Phaeocystis* post-bloom in April. This seasonal pattern with a summer mode has been also found in Blanes Bay (Vila-Costa et al. 2007, 2008). Several other seasonal studies and data compilations in temperate to subtropical zones have also shown that maximum DMS concentrations occur in summer when the concentration of Chl*a* is at its annual minimum (e.g., Dacey et al. 1998; Lana et al. 2011). This phenomenon, named the "summer DMS paradox" by Simó and Pedrós-Alió (1999), is thought to be due to phytoplankton succession towards higher DMSP-producing phytoplankton in summer (confirmed by a higher DMSPt:Chl*a* ratio, Table 1) plus the seasonal shift in the environmental variables that drive DMS production and consumption by the whole plankton community. Among these variables, nutrient availability (Sunda et al. 2007; Archer et al. 2009; Polimene et al. 2012) and solar radiation effects on bacteria (Toole et al. 2006; Slezak et al. 2007; Ruiz-González et al. 2013), phytoplankton (Sunda et al. 2002; Archer et al. 2009) and photochemical reactions (Toole and Siegel 2004; Galí et al., 2016) are believed to play the main roles (Simó, 2004; Vallina et al., 2008; Lizotte et al. 2012; Galí and Simó 2015).

The series of dilution experiments revealed that microzooplankton grazing is a principal biotic factor influencing DMS production. Microzooplankton exerted a strong control on the size of the algal DMSP pool by consuming daily 39-141% of the stock, and also affected DMSP transformation rates into DMS and other breakdown products. Microzooplankton grazing has been shown to enhance DMS production (Archer et al. 2003) by 1) mixing up ingested DMSP and algal DMSP lyases in the grazer's vacuoles and

releasing the evolved DMS into the dissolved phase, and 2) releasing DMSP upon cell rupture and with detrital material, thus making DMSP readily available for either bacteria that will transform part of it into DMS (Wolfe et al. 1994; Wolfe and Steinke 1996; Archer et al. 2001b) or some phytoplankton that will take it up (Vila-Costa et al. 2006). Another fraction, estimated at approx. 1/3 of the ingested DMSP, is either assimilated by the micrograzer as a sulfur source for macromolecules (Saló et al. 2009) or accumulated as DMSP and transferred up the food chain (Tang and Simó 2003); in both cases it is diverted from DMS production in the short term. Overall, however, the net effect of grazing is to enhance DMS production.

Indeed, in all our dilution experiments but one, DMS production increased with increasing grazing pressure and proportionally to the DMSP ingested (Figure 2). As a result, the grazing-mediated DMS production (*Pg*) in the nutrient-unamended waters could be estimated. The yield of DMS production from the DMSP ingested ranged 6-20% (Table 3), which is similar to the range (3-23%) estimated by Archer et al. (2003) in the southern North Sea from Chl*a* ingestion and DMSP:Chl*a* ratios. *Pg* is the result of a number of processes mediated by grazing, including the direct action of algal DMSP lyases during prey capture, ingestion and digestion, but also the indirect action of bacteria after DMSP release by prey cell rupture (Saló et al. 2010). Bacteria generally convert only 5-10% of metabolized DMSP to DMS (Kiene et al. 2000); therefore, it must be algal lyases that increased these values, particularly in April and summer. Actually, the DMS yield of DMSP consumption by whole plankton communities can be anything between <5% and >90% (Simó and Pedrós-Alió 1999) depending on community composition and environmental conditions, yet they mostly fall in the range 7-28% (Galí and Simó 2015), being higher in shallow mixed, highly irradiated surface waters. Interestingly, the monthly community DMS yields estimated from dark gross DMS production and DMSP consumption in Blanes Bay ranged 5-25% over most of the year, with maximum values also in midsummer (Vila-Costa et al. 2008).

Pg represented on average 65 ± 9% of the dark gross DMS production by the whole community (Table 3), and both rates were strongly correlated (Figure 4). In other words, microzooplankton grazing provided a large proportion of DMS production in the dark. It should be noticed, however, that the removal of light, and specially UV radiation, from the DMDS-amended incubations may have led to underestimation of the gross DMS production rates (Galí et al. 2011) and, hence, the number above should be taken as an upper estimate. More interestingly, *Pg* accounted for 73% of the variance in the DMS concentration

899 throughout the time series (linear regression of the DMS and *Pg* series in Figure 4 yields a
900 coefficient of determination r^2=0.73), while community gross DMS production accounted for
901 64% (DMS *vs.* gross DMS prod. r^2=0.64). Bacterial consumption, conversely, only explained
902 16% of the variance in DMS (r^2=0.16). That is, biological production was more important
903 than biological consumption in determining DMS concentration. This is not an unexpected
904 result, since the only known sources of DMS are biological processes, whereas biological
905 metabolism only accounts for a fraction of total DMS loss, generally 50-80% (Simó 2004;
906 Galí and Simó 2015).
907
908 **Concluding remarks and implications for modeling**
909
910 We provide new estimates of the amount of carbon primary production invested in
911 DMSP biosynthesis by mixed phytoplankton assemblages, which was 0.4-12%. Our data
912 confirm that, in complex plankton communities, DMSP-containing phytoplankton generally
913 experience similar or higher grazing pressure than the bulk phytoplankton community, and
914 definitely not reduced grazing rates as the deterrence hypothesis would predict. Micrograzers
915 consumed daily 39-141% of the DMSP stock, and simultaneous estimates of DMS production
916 indicated that 6-20% of the grazed DMSP was converted into DMS. Our work points to
917 microzooplankton as a major driver of DMS production and concentration over seasonal time
918 scales.
919
920 The distribution of DMS concentration and emission fluxes and their dynamics over
921 seasons have been remarkably difficult to predict by numerical prognostic modeling (Le
922 Clainche et al. 2010). The difficulties arise mainly from the lack of an appropriate numerical
923 representation of both plankton ecophysiology and community interactions, the latter
924 including herbivore grazing and algal-bacterial mutualisms. Indeed, in most models of the
925 DMS cycle, DMSP loss from phytoplankton, which is the first gate towards DMS production,
926 is poorly parameterized. In the most complex models, cell DMSP content in phytoplankton is
927 either set according to phytoplankton functional types or made dependent on solar radiation;
928 herbivore grazing is set independent of the prey DMSP content, and DMSP release is set
929 proportional to overall grazing rate (e.g., Vallina et al. 2008; Toole et al. 2008; Vogt et al.
930 2010; Polimene et al. 2012).Then, bacteria act on released DMSP to produce DMS according
931 to their carbon and sulfur demands. Our findings indicate that grazing-mediated DMS
932 production has higher yields per DMSP lost (6-20%) than typical bacterial DMS production

933 (5-10%, Kiene et al. 2000), explaining the overall community DMS production yields
934 collected in a recent meta-analysis (7-28%, Galí and Simó 2015). Better representation of
935 grazing on DMSP-producing phytoplankton and its effects on DMS production is needed if
936 we are to improve DMS prediction.
937
938 Our findings have implications not only for DMS modeling but for food web modeling
939 as well. Feeding of heterotrophic protists depends on their searching, contact, capture,
940 processing, ingestion, and digestion abilities (Montagnes et al. 2008). Diffusive infochemicals
941 like DMSP are expected to influence prey encounter and selection either by attraction or
942 deterrence, with fundamental influence on phytoplankton abundance, assemblage composition
943 and carbon and energy fluxes (Strom 2008). Despite its potential to modulate grazing rates
944 and prey populations, however, prey selection is hardly implemented in models of the
945 planktonic food web (Davidson 2014). Changing the perception of DMSP as deterrent to that
946 of neutral or attractant fundamentally changes the way this implementation is to be conducted.
947 All in all, the challenge remains of improving population dynamics prediction for both
948 predators and prey by going beyond bitrophic interactions between single generalist predator
949 and prey, and incorporating the more specific roles of chemical communication between cells.
950

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministries of Science through the projects MICROROL (CTM2004-02575/MAR to A.C.), SUMMER (CTM2008-03309 to R.S.), BIOGAPS (CTM2016-81008-R to R.S.) and FERMI (CGL2014-59227-R to A.C. and E.S.), and PhD fellowships to V.S. and R.A.

The authors declare no conflict of interest.

REFERENCES

Andersen T, Schartau AKL, Paasche E (1991) Quantifying external and internal nitrogen and phosphorus pools, as well as nitrogen and phosphorous supplied through remineralisation, in coastal marine plankton by means of a dilution technique. Mar Ecol Prog Ser 69**:** 67-80

Archer SD, Stelfox-Widdicombe CE, Burkill PH, Malin G (2001a) A dilution approach to quantify the production of disolved dimethylsulphoniopropionate and dimethyl sulphide due to microzooplankton herbivory. Aquat Microb Ecol 23: 131-145

Archer SD, Widdicombe CE, Tarran GA, Rees AP, Burkill P (2001b) Production and turnover of particulate dimethylsulphoniopropionate during a coccolithophore bloom in the northern North Sea. Aquat Microb Ecol 24: 225-241

Archer SD, Stelfox-Widdicombe CE, Malin G, Burkill PH (2003) Is dimethyl sulphide production related to microzooplankton herbivory in the southern North Sea? J Plankton Res 25: 235-242

Archer SD, Cummings DG, Llewellyn CA, Fishwick JR (2009) Phytoplankton taxa, irradiance and nutrient availability determine the seasonal cycle of DMSP in temperate shelf seas. Mar Ecol Prog Ser 394: 111-124

Archer SD, Safi K, Hall A, Cummings DG, Harvey M (2011) Grazing suppression of dimethylsulphoniopropionate (DMSP) accumulation in iron-fertilised, sub-Antarctic waters. Deep Sea Res Part II 58: 839-850

Atienza D, Calbet A, Saiz E, Alcaraz M, Trepat I (2006) Trophic impact, metabolism, and biogeochemical role of the marine cladoceran *Penilia avirostri*s and the co-dominant copepod *Oithona nana* in NW Mediterranean coastal waters. Mar Biol 150: 221-235

Belviso S, Christaki U, Vidussi F, Marty JC, Vila M, Delgado M (1993) Size distribution of dimethisulfoniopropionate (DMSP) in areas of the tropical northeastern Atlantic Ocean and the Mediterranean Sea. Mar Chem 44: 55-71

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser 36: 171-175

Burkill PH, Edwards ES, Jon AWG, Sleigh MA (1993) Microzooplankton and their herbivorous activity in the northeastern Atlantic ocean. Deep Sea Res Part I 40: 479-493

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol Oceanogr 49: 51-57

Calbet A, Trepat I, Almeda R, Saló V, Saiz E, Movilla JI, Alcaraz M, Yebra L, Simó R (2008) Impact of micro- and nanograzers on phytoplankton assessed by standard and size-fractionated dilution experiments. Aquat Microb Ecol 50: 145-156

Carpenter LJ, Archer SD, Beale R (2012) Ocean-atmosphere trace gas exchange. Chem Soc Rev 41: 6473−6505

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326: 655-661

Christaki U, Belviso S, Dolan JR, Corn M (1996) Assessment of the role of copepods and ciliates in the release to solution of particulate DMSP. Mar Ecol Prog Ser 141: 119-127

Dacey JWH, Wakeham SG (1986) Oceanic dimethylsulfide: Production during zooplankton grazing on phytoplankton. Science 233: 1314-1316

Dacey JWH, Howse FA, Michaels AF, Wakeham SG (1998) Temporal variability of dimethylsulfide and dimethylsulfoniopropionate in the Sargaso Sea. Deep Sea Res Part I 45: 2085-2104

Daly KL, DiTullio GR (1996) Particulate dimethylsulfoniopropionate removal and dimethylsulfide production by zooplankton in the Southern Ocean. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, pp. 223-238

Davidson K (2014) The challenges of incorporating realistic simulations of marine protists in biogeochemically based mathematical models. Acta Protozool 53:129-138

del Valle DA, Kieber DJ, Kiene RP (2007) Depth-dependent fate of biologically-consumed dimethylsulfide in the Sargasso Sea. Mar Chem 103: 197-208

Fencehel T (1980) Suspension feeding in ciliated protozoa: functional response and particle size selection. Microb Ecol 6: 1-11

Fredrickson KA, Strom SL (2009) The algal osmolyte DMSP as a microzooplankton grazing deterrent in laboratory and field studies. J Plankton Res 31: 135-152

Frost BW (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17: 805-815

Galí M, Saló V, Almeda R, Calbet A, Simó R (2011) Stimulation of gross dimethylsulfide (DMS) production by solar radiation. Geophys Res Lett 38: L15612, doi:10.1029/2011GL048051

Galí M, Simó R (2015) A meta-analysis of oceanic DMS and DMSP cycling processes: disentangling the summer paradox. Global Biogeochem Cycles 29: doi:10.1002/2014GB004940.

Galí M, Devred E, Levasseur M, Royer S-J, Babin M (2015) A remote sensing algorithm for planktonic dimethylsulfoniopropionate (DMSP) and an analysis of global patterns. Remote Sensing Environ 171: 171–184

Galí M, Kieber DJ, Romera-Castillo C, Kinsey JD, Devred E, Pérez GL, Westby GR, Marrasé C, Babin M, Levasseur M, Duarte C, Agustí S, Simó R (2016) CDOM sources and photobleaching control quantum yields for oceanic DMS photolysis. Environ Sci Technol 50: 13361–13370; doi:10.1021/acs.est.6b04278

Galí M, Levasseur M, Devred E, Simó R, Babin M (2018) Sea-surface dimethylsulfide (DMS) concentration from satellite data at global and regional scales. Biogeosci 15: 3497–3519

Gasol JM, Cardelús C, Morán XAG, Simó R, Massana R, Marrasé C, Vaqué D, Balagué V, Forn I, Sala MM, Pedrós-Alió C, Estrada M (2016) Seasonal patterns in phytoplankton photosynthetic parameters and primary production at a coastal NW Mediterranean site. Sci Mar 80S1: 63-77, doi:http://dx.doi.org/10.3989/scimar.04480.06E

Keller MD, Bellows WK, Guillard RL (1989) Dimethyl sulfide production in marine phytoplankton. In Saltzman ES, Cooper WJ (eds) Biogenic Sulfur in the Environment. American Chemical Society, Washington, DC, pp. 183-200

Kettle AJ, Andreae MO, Amoroux D, Andreae TW and others (1999) A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude and month. Global Biogeochem Cycles 13: 399-444

Kiene RP, Linn LJ, Bruton JA (2000) New and important roles for DMSP in marine microbial communities. J Sea Res 43: 209-224

Kiene RP, Slezak D (2006) Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnol Oceanogr Methods, 4: 80-95

Kwint RLJ, Kramer KJM (1995) Dimethylsulphide production by plankton communities. Mar Ecol Prog Ser 121: 227-237

Kwint RLJ, Quist P, Hansen TA, Dijkhuizen L, Kramer KJM (1996) Turnover of dimethylsulfoniopropionate and dimethylsulfide in the marine environment: a mesocosm experiment. Mar Ecol Prog Ser 145: 223-232

Lana A, Bell TG, Simó R, Vallina SM, Ballabrera-Poy J, Kettle AJ, Dachs J, Bopp L, Saltzman ES, Stefels J, Johnson JE, Liss PS (2011) An updated climatology of surface dimethlysulfide concentrations and emission fluxes in the global ocean. Global Biogeochem Cycles 25: GB1004

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67: 283-288

Laroche D, Vezina AF, Levasseur M, Gosselin M, Stefels J, Keller MD, Matrai PA, Kwint RLJ (1999) DMSP synthesis and exudation in phytoplankton: a modelling approach. Mar Ecol Prog Ser 180: 37-49

Le Clainche Y, Vézina A, Levasseur M, Cropp RA, Gunson JR, Vallina SM, Vogt M, Lancelot C, Allen JI, Archer SD, Bopp L, Deal C, Elliott S, Jin M, Malin G, Schoemann V, Simó R, Six KD, Stefels J (2010) A first appraisal of prognostic ocean DMS models and prospects for their usein climate models. Global Biogeochem Cycles 24: GB3021

Lehrter JC, Pennock JR, McManus GB (1999) Microzooplankton grazing and nitrogen excretion across a surface estuarine-coastal interface. Estuaries 22: 113-125

Lizotte M, Levasseur M, Michaud S, Scarratt MG, Merzouk A, Gosselin M, Pommier J, Rivkin RB, Kiene RP (2012) Macroscale patterns of the biological cycling of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) in the North Atlantic. Biogeochem 110: 183-200

Malin G, Wilson WH, Bratbak G, Liss PS, Mann NH (1998) Elevated production of dimethylsulfide resulting from viral infection of cultures of *Phaeocystis pouchetii*. Limnol Oceanogr 43: 1389-1393

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45: 569-579

Montagnes DJS, Morgan G, Bissinger JE, Atkinson D, Weisse T (2008) Short-term temperature change may impact freshwa-ter carbon flux: a microbial perspective. Global Change Biol 14: 2823–2838

Neuer S, Franks PJS (1993) Determination of ammonium uptake and regeneration rates using the seawater dilution method. Mar Biol 116: 497-505

Nevitt GA (2011) The neuroecology of dimethyl sulfide: A global climate regulator turned marine infochemical. Integr Compar Biol 51: 819−825

Park K, Lee K, Shin K, Yang E-J, Hyun B, Kim J-M, Noh J-H, Kim M, Kong B, Choi DH, Choi S-J, Jang P-G, Jeong HJ (2014) Direct Linkage between dimethyl sulfide production and microzooplankton grazing, resulting from prey composition change under high partial pressure of carbon dioxide conditions. Environ Sci Technol 48: 4750−4756

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for sea water analysis. Pergamon, Oxford

Polimene L, Archer SD, Butenschön M, Allen JI (2012) A mechanistic explanation of the Sargasso Sea DMS ‘‘summer paradox’’. Biogeochem 110: 243–255

Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine “oligotrichous” ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097-1103

Quinn PK, Coffman DJ, Johnson JE, Upchurch LM, Bates TS (2017) Small fraction of marine cloud condensation nuclei made up of sea spray aerosol. Nature Geosci 10: 674-679

Ruiz-González C, Simó R, Sommaruga R, Gasol JM (2013) Away from darkness: A review on the effects of solar radiation on heterotrophic bacterioplankton activity. Front Microbiol, doi:10.3389/fmicb.2013.00131

Saló V, Simó R, Vila-Costa M, Calbet A (2009) Sulfur assimilation by *Oxyrrhis marina* feeding on a ^{35}S-DMSP radiolabelled prey. Environ Microbiol 11: 3063-3072

Saló V, Simó R, Calbet A (2010) Revisiting the dilution technique to quantify the role of microzooplankton in DMS(P) cycling: Laboratory and field tests. J Plankton Res 32: 1255-1267

Schmoker C, Hernandez-Leon S, Calbet A (2013) Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions. J Plankton Res 35: 691-706

Seymour JR, Simó R, Ahmed T, Stocker R (2010) Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. Science 329: 342-345

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antoine van Leeuwenhoek 81: 293-308

Sieburth JMcN, Smetacek V, Lenz J (1978) Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol Oceanogr 23: 1256-1263

Simó R (2001) Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol Evol 16: 287-294

Simó R (2004) From cells to globe: approaching the dynamics of DMS(P) in the ocean at multiple scales. Can J Fish Aquat Sci 61: 673-684

Simó R, Grimalt JO, Pedrós-Alió C, Albaigés J (1995) Occurrence and transformation of dissolved dimethyl sulfur species in stratified seawater (western Mediterranean Sea). Mar Ecol Prog Ser 127: 291-299

Simó R, Pedrós-Alió C (1999) Role of vertical mixing in controlling the oceanic production of dimethyl sulphide. Nature 402: 396-399

Simó R, Pedrós-Alió C, Malin G, Grimalt JO (2000) Biological turnover of DMS, DMSP and DMSO in contrasting open-sea waters. Mar Ecol Prog Ser 203: 1-11

Simó R, Archer S, Pedrós-Alió C, Gilpin L, Stelfox-Widdicombe C (2002) Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. Limnol Oceanogr 47: 53-61

Simó R, Vila-Costa M, Alonso-Sáez L, Cardelús C, Guadayol Ò, Vázquez-Domínguez E, Gasol JM (2009) Annual series of DMSP contribution to S and C fluxes through phytoplankton and bacterioplankton in a NW Mediterranean coastal site. Aquat Microb Ecol 57: 43-55

Slezak D, Kiene RP, Kieber DJ, Simó R, Toole DA (2007) Effects of solar radiation on the yield of DMS during consumption of DMSP. Aquat Sci 69: 377-393

Spiese CE, Le T, Zimmer RL, Kieber DJ (2015) Dimethylsulfide membrane permeability, cellular concentrations and implications for physiological functions in marine algae. J Plankton Res 38: 41–54

Stefels J, Steinke M, Turner S, Malin G, Belviso S (2007) Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling. Biogeochem 83: 245-275

Strom SL (2008) Microbial ecology of ocean biogeochemistry: A community perspective. Science 320: 1043-1045

Strom SL, Wolfe GV, Slajer A, Lambert S, Clough G (2003) Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethilsulfonipropionate. Limnol Oceanogr 48: 230-237

Sunda WG, Kieber DJ, Kiene RP, Huntsman S (2002) An antioxidant function for DMSP and DMS in marine algae. Nature 418: 317–320

Sunda WG, Hardison DR, Kiene RP, Bucciarelli E, Harada H (2007) The effect of nitrogen limitation on cellular DMSP and DMS release in marine phytoplankton: climate feedback implications. Aquat Sci 69: 341–351

Tang KW, Dam HG, Visscher PT, Fenn TD (1999) Dimethylsulfoniopropionate (DMSP) in marine copepods and its relation with diets and salinity. Mar Ecol Prog Ser 179: 71-79

Tang KW, Simó R (2003) Trophic uptake and transfer of DMSP in simple planktonic food chains. Aquat Microb Ecol 31: 193-202

Toole DA, Siegel DA (2004) Light-driven cycling of dimethylsulfide (DMS) in the Sargasso Sea: closing the loop. Geophys Res Lett 31: L09308

Toole DA, Slezak D, Kiene RP, Kieber DJ, Siegel DA (2006) Effects of solar radiation on dimethylsulfide cycling in the western Atlantic Ocean. Deep Sea Res Part I 53: 136–153

Toole DA, Siegel DA, Doney SC (2008) A light-driven, one-dimensional dimethylsulfide biogeochemical cycling model for the Sargasso Sea. J Geophys Res 113: G02009

Vallina SM, Simó R, Gassó S, de Boyer-Montégut C, del Rio E, Jurado E, Dachs J (2007) Analysis of a potential "solar radiation dose–dimethylsulfide–cloud condensation nuclei" link from globally mapped seasonal correlations. Global Biogeochem Cycles 21: GB2004, doi:10.1029/2006GB002787

Vallina SM, Simó R, Anderson TR, Gabric A, Cropp R, Pacheco JM (2008) A dynamic model of oceanic sulfur (DMOS) applied to the Sargasso Sea: Simulating the dimethylsulfide (DMS) summer paradox. J Geophys Res Biogeosci 113: G01009, doi:01010.01029/02007JG000415

Verity PG, Langdon C (1984) Relationships between lorica volume, carbon, nitrogen and ATP content in tintinnids in Narragansett Bay. J Plankton Res 6: 859-868

Verity PG (1991) Measurement and simulation of prey-uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol Oceanogr 36: 729-750

Vila-Costa M, Pinhassi J, Alonso C, Pernthaler J, Simó R (2007) An annual cycle of DMSP-sulfur assimilating bacterioplankton in the coastal NW Mediterranean. Environ Microbiol 9: 2451–2463

Vila-Costa M, Kiene RP, Simó R (2008) Seasonal variability of the dynamics of dimethylated sulfur compounds in a coastal northwest Mediterranean site. Limnol Oceanogr 53: 198-211

Waterbury JB, Watson SW, Valois FW, Franks DG (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In Platt T, Li WKI (eds) Photosynthetic Picoplankton. Canadian Department of Fisheries and Oceans, Ottawa, Ontario, Canada, pp. 71–120

Wolfe GV, Kiene RP (1993) Radioisotope and chemical inhibitor measurements of dimethyl sulfide consumption rates and kinetics in estuarine waters. Mar Ecol Prog Ser 99: 261-269

Wolfe GV, Sherr EB, Sherr BF (1994) Release and consumption of DMSP from *Emiliania huxleyi* during grazing by *Oxyrrhis marina*. Mar Ecol Prog Ser 111: 111-119

Wolfe GV, Steinke M (1996) Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliania huxleyi*. Limnol Oceanogr 41: 1151-1160

Wolfe GV, Steinke M (1997) Grazing-activated chemical defense in a unicellular marine alga. Nature 387: 864-867

Wolfe GV, Levasseur M, Cantin G, Michaud S (2000) DMSP and DMS dynamics and microzooplankton grazing in the Labrador Sea: application of the dilution technique. Deep Sea Res Part I 47: 2243-22

Table 1. Characteristics of the waters used for the dilution experiments, including biomass estimates of the dominant phytoplankton and microzooplankton (MZP) groups. Diat: diatoms; Crypto: cryptophytes; Hapto: haptophytes; Syn: *Synechococcus* sp.; Dino: dinoflagellates; HF: heterotrophic flagellates; C: ciliates. Numbers in parentheses are standard deviations of the means.

Experiment	Date (dd/mm/yy)	T (ºC)	Dominant phytoplankton	Dominant MZP	Chl*a* (μg L^{-1})	DMSPt (nmol L^{-1})	DMSPt:Chl*a* (nmol μg^{-1})	DMS (nmol L^{-1})
Sep05	14/09/05	23.5	Hapto>Crypto,Syn	HF>Dino,C	0.18	21.3	118	6.3
Oct05	17/10/05	21.5	Diat>Crypto>Hapto	C>HF>Dino	1.54	20.6	13	5.3
Nov05	29/11/05	16.1	Diat>>Crypto>Hapto	HF>Dino,C	0.97	11.4	12	1.5
Jan06	18/01/06	13.0	Crypto>Diat>Hapto	HF>C>Dino	0.47	12.4	26	0.9
Apr06	04/04/06	14.2	C>Diat>Hapto	C>>Dino>HF	1.13	23.2	21	11.0
May06	16/05/06	18.1	Diat>>Hapto,Crypto	HF>C>Dino	0.95	10.0	11	1.6
Jun06	14/06/06	21.1	Dino>Hapto,Crypto	Dino>HF,C	0.49	71.0	145	7.8
Jul06	31/07/06	24.4	Syn,Diat>Hapto	HF>>Dino,C	0.40	17.5	44	5.2
Aug06	29/08/06	24.4	Syn>Hapto>Crypto	C,HF>Dino	0.31	27.0	87	5.8
Sep06	28/09/06	22.2	Hapto>Syn>Crypto	C>Dino>HF	0.73	35.0	48	3.4
Mean (std dev)		*19.9 (4.2)*			*0.72 (0.43)*	*24.9 (17.9)*	*53 (48)*	*4.9 (3.2)*

Table 2. Results of the dilution experiments. μ and m are growth and mortality rates, respectively, and the subindices refer to the chlorophyll *a* (Chl*a*) and DMSP containing phytoplankton. DMSP prod.: rate of DMSP production calculated from μ_{DMSP}. PP: primary production calculated from μ_{chla}. DMSP-C prod:PP is the proportion of PP invested in DMSP production (in carbon units). Coefficients of the regression analyses of the dilutions are given with $^{*}p<0.05$; $^{**}p<0.01$. The comparison of the slopes (mortality rates) of DMSP- and Chl*a*-containing phytoplankton is expressed as not significantly (*ns*) or significantly ($^{*}p<0.05$, $^{**}p<0.01$) different. Numbers in parentheses are errors derived from the typical errors of the regression analyses.

Experiment	μ_{chla} (d^{-1})	m_{chla} (d^{-1})	r^2	μ_{DMSP} (d^{-1})	m_{DMSP} (d^{-1})	r^2	m_{DMSP} *vs.* m_{chla}	DMSP prod. (nmol L^{-1} d^{-1})	PP (nmol C L^{-1} d^{-1})	DMSP-C prod: PP (%)
Sep05	1.00 (0.09)	0.36 (0.12)	0.58*	0.44 (0.09)	0.56 (0.08)	0.91**	*ns*	9.5 (2.0)	1771	3
Oct05	1.03 (0.06)	0.72 (0.10)	0.92**	0.82 (0.21)	1.01 (0.40)	0.79*	*ns*	14.8 (3.8)	9332	0.8
Nov05	0.62 (0.05)	0.27 (0.07)	0.70**	1.10 (0.03)	0.80 (0.05)	0.98**	**	13.9 (0.4)	2700	3
Jan06	0.30 (0.03)	0.08 (0.03)	0.57*	1.08 (0.13)	0.78 (0.21)	0.70**	**	16.5 (2.0)	667	12
Apr06	0.95 (0.09)	0.38 (0.13)	0.61*	1.36 (0.07)	1.22 (0.15)	0.93**	**	33.2 (1.7)	7200	2
May06	0.86 (0.03)	0.21 (0.05)	0.78**	1.49 (0.11)	0.77 (0.16)	0.83**	**	21.5 (1.6)	5271	2
Jun06	1.08 (0.11)	0.82 (0.16)	0.83**	0.32 (0.04)	0.65 (0.06)	0.94**	*ns*	19.7 (2.5)	4000	3
Jul06	0.96 (0.16)	0.99 (0.24)	0.76**	0.59 (0.33)	1.45 (0.47)	0.74*	*ns*	7.4 (4.1)	3546	1
Aug06	0.57 (0.14)	0.56 (0.19)	0.63*	0.07 (0.07)	0.46 (0.18)	0.56*	*ns*	1.6 (1.6)	1490	1
Sep06	0.69 (0.09)	0.62 (0.14)	0.83**	0.11 (0.08)	0.67 (0.17)	0.84*	*ns*	2.8 (2.0)	3089	0.4
Mean (std dev)	*0.81 (0.25)*	*0.50 (0.29)*		*0.74 (0.51)*	*0.84 (0.31)*			*14.1 (9.5)*	*3907 (2694)*	*3 (4)*

Table 3. DMSP consumption and DMS production and consumption as estimated by the dilutions experiments. *Pg*: grazing-mediated DMS production. DMS yield: (*Pg* x 100)/DMSP grazed. Gross DMS prod.: gross DMS production by the whole community, as estimated with DMDS additions. Coefficient of the regression analyses of the DMS produced *vs* DMSP grazed plots are given with $^{*}p<0.05$; $^{**}p<0.01$. *ns*: not significant ($p=0.9$); *nd*: not determined. Numbers in parentheses are errors derived from the typical errors of the regression analyses.

Experiment	DMSP grazed (nmol L^{-1} d^{-1})	DMSP turnover (% d^{-1})	*Pg* (nmol L^{-1} d^{-1})	r^2	DMS yield (%)	Gross DMS prod. (nmol L^{-1} d^{-1})	*Pg*: gross prod (%)	Bacterial DMS cons. (nmol L^{-1} d^{-1})	Biol. DMS turnover time (d)
Sep05	12.1 (1.7)	57	1.1 (0.4)	0.93^{**}	9	2.4 (0.5)	44	2.4 (0.5)	2.6
Oct05	18.2 (7.2)	88	1.7 (0.9)	0.74^{**}	9	*nd*		*nd*	
Nov05	10.1 (0.6)	89	0.7 (0.1)	0.90^{**}	7	0.7 (0.1)	96	0.7 (0.1)	2.1
Jan06	11.9 (3.2)	96	*ns*	*ns*		0.5 (0.2)		0.5 (0.2)	1.8
Apr06	29.8 (3.7)	128	6.3 (0.8)	0.96^{**}	19	7.7 (0.7)	81	2.3 (1.5)	4.8
May06	11.1 (2.3)	111	0.7 (0.5)	0.37^{*}	6	2.2 (0.4)	32	≤0.3	≥5
Jun06	40.0 (3.7)	56	4.1 (0.9)	0.89^{**}	10	6.3 (0.8)	64	≤1.0	≥10
Jul06	18.1 (5.9)	104	3.7 (1.2)	0.93^{**}	20	5.0 (0.5)	74	4.0 (1.0)	1.3
Aug06	10.6 (4.1)	39	2.1 (0.9)	0.92^{**}	20	2.4 (0.5)	88	2.4 (0.5)	2.4
Sep06	16.8 (4.3)	48	2.1 (0.7)	0.86^{**}	13	5.5 (0.5)	39	3.5 (1.2)	1.0
Mean (std dev)	*17.9 (9.8)*	*82 (30)*	*2.5 (1.9)*		*13 (6)*	*3.6 (2.6)*	*65 (9)*		

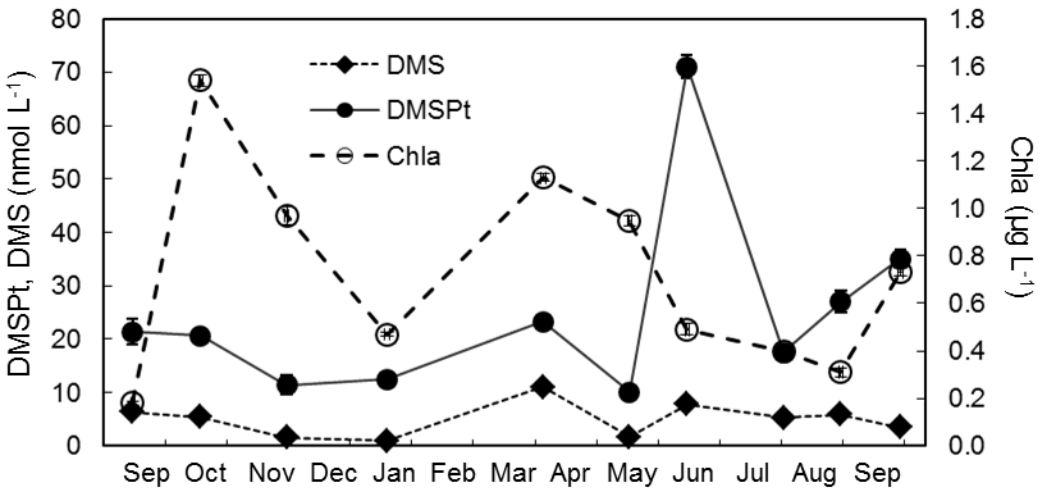
FIGURE LEGENDS

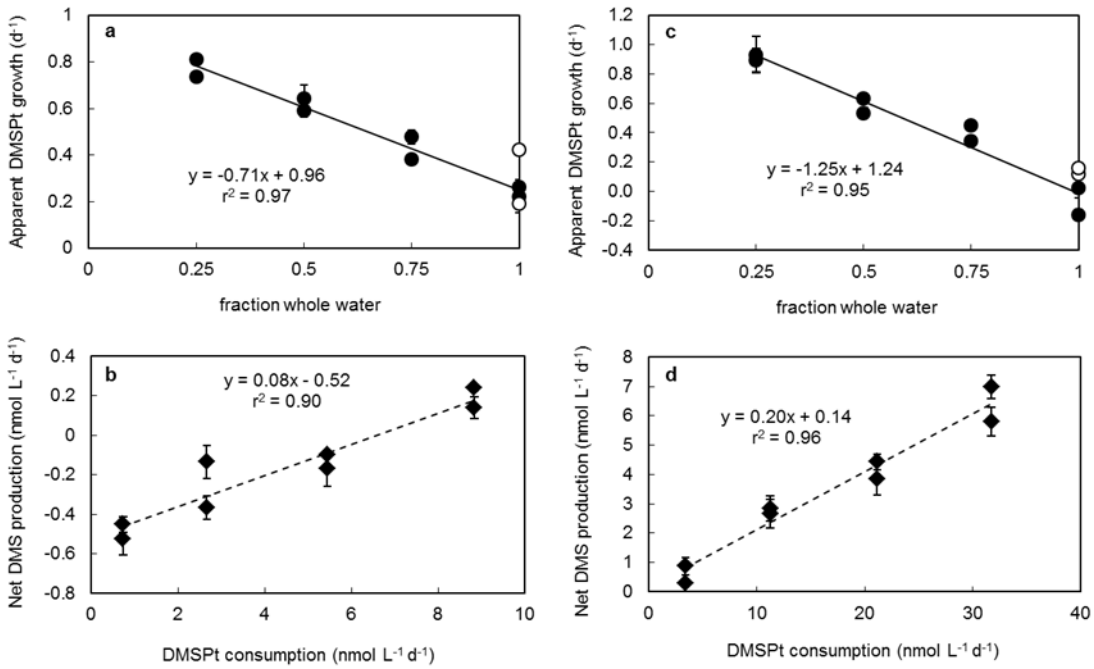
Figure 1. Concentrations of DMS, DMSPt and Chla in the waters sampled between September 2005 and September 2006, which correspond to the initial concentrations of the dilution experiments. Error bars represent one standard error (note that in most cases they are smaller than the marker).

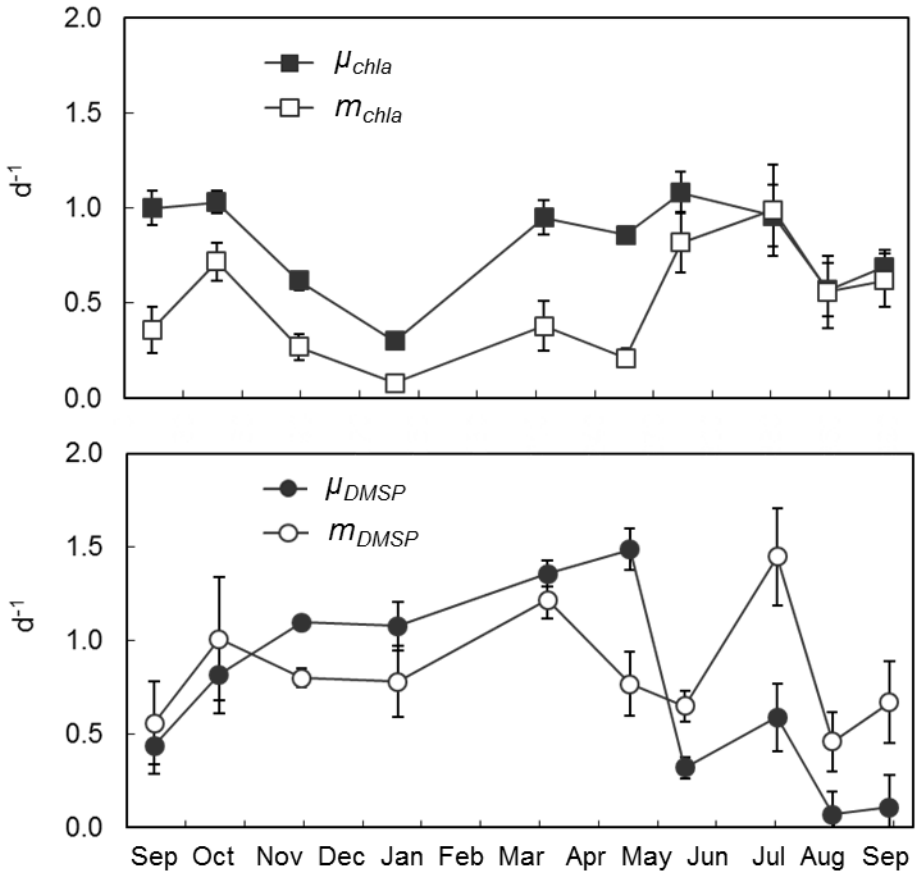
Figure 2. Data derived from two dilution experiments, those of 29 November 2005 (a, b) and 4 April 2006 (c, d). The upper plots (a, c) show the apparent growth of DMSP-producing phytoplankton vs the dilution fraction. The slopes provide the rates of microzooplankton grazing (m_{DMSP}), and the intercepts provide the algal growth rates (μ_{DMSP}). Empty circles show the incubations without nutrient additions. Parallel datapoints correspond to replicate

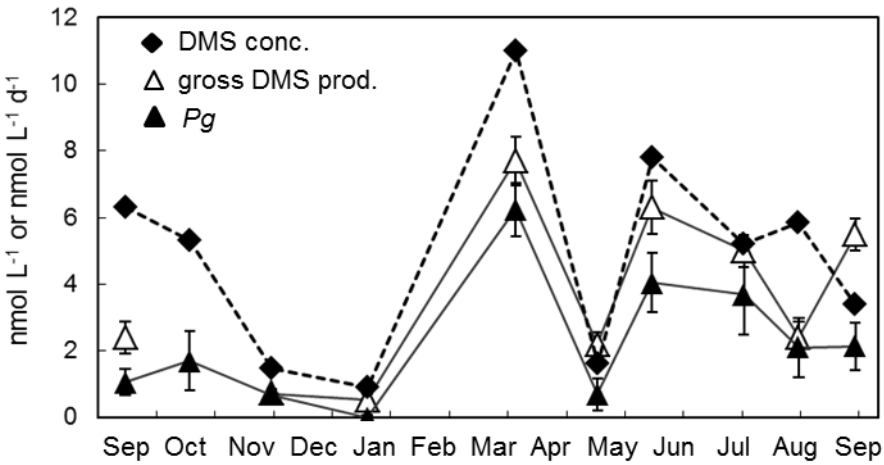
Figure 3. Comparison of growth (μ) and grazing (m) rates for Chla (top) and DMSP (bottom) containing phytoplankton. Error bars correspond to the typical errors derived from the regression analyses.

Figure 4. DMS concentrations (nmol L^{-1}), gross DMS production (nmol $L^{-1}d^{-1}$) and grazing-mediated DMS production rates (Pg; nmol $L^{-1}d^{-1}$).


DMS
DMSPt
Chla
DMSPt, DMS (nmol L-1)
Chla (µg L-1)
0
10
20
30
40
50
60
70
80
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep


a
Apparent DMSPt growth (d-1)
y = -0.71x + 0.96
r2 = 0.97
fraction whole water
c
Apparent DMSPt growth (d-1)
y = -1.25x + 1.24
r2 = 0.95
fraction whole water
b
Net DMS production (nmol L-1 d-1)
y = 0.08x - 0.52
r2 = 0.90
DMSPt consumption (nmol L-1 d-1)
d
Net DMS production (nmol L-1 d-1)
y = 0.20x + 0.14
r2 = 0.96
DMSPt consumption (nmol L-1 d-1)


2.0
1.5
1.0
0.5
0.0
d-1
μchla
mchla
2.0
1.5
1.0
0.5
0.0
d-1
μDMSP
mDMSP
Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep


DMS conc.
gross DMS prod.
Pg
nmol L-1 or nmol L-1 d-1
0
2
4
6
8
10
12
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep