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Published in:
Biogeochemistry

Link to article, DOI:
[10.1007/s10533-018-0506-2](https://doi.org/10.1007/s10533-018-0506-2)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Simó, R., Saló, V., Almeda, R., Movilla, J., Trepot, I., Saiz, E., & Calbet, A. (2018). The quantitative role of microzooplankton grazing in dimethylsulfide (DMS) production in the NW Mediterranean. *Biogeochemistry*, 141(2), 125-142. <https://doi.org/10.1007/s10533-018-0506-2>

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

Running head: Microzooplankton grazing and DMS production

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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* (Chla) 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.

397 **INTRODUCTION**

398

399 Dimethylsulfide (DMS) is a climatically active trace gas that is found in the sunlit
400 layer all over the world's oceans. DMS concentrations are supersaturated in surface waters
401 relative to the atmosphere, driving a global net sea-air flux of *ca.* 16–28 Tg S y^{-1} (Lana et al.
402 2011; Galí et al. 2018), one of the largest amongst marine organic volatiles (Carpenter et al.
403 2012). In the atmosphere DMS is oxidized to molecules that either condense upon existing
404 particles or nucleate to form new particles. Both newly born and growing aerosols have the
405 capability to backscatter solar radiation and act as cloud condensation nuclei. The availability
406 of condensation nuclei regulates cloud droplet number and size, hence cloud albedo, thereby
407 contributing to regulate the global radiation budget (Charlson et al. 1987; Quinn et al. 2017).
408 In addition to this climatic role, airborne DMS also acts as a foraging infochemical for marine
409 birds, mammals and turtles (e.g., Nevitt 2011). The importance of DMS emissions for
410 chemical ecology and climate has precipitated considerable research on its biological and
411 biogeochemical cycling in the ocean (Simó et al. 2001; Stefels et al. 2007). Advances in
412 process-level understanding, yet remarkable, have not been enough, and global ecosystem
413 models still struggle to accurately reproduce macroscale and seasonal DMS patterns,
414 especially at lower latitudes (Le Clainche et al. 2010).

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

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

498

499

500 **MATERIALS AND METHODS**

501

502 **Sampling, experimental setup and sub-sampling**

503

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

515

516 The water for the experiments was sampled 1.5 km offshore of the city of Barcelona
517 (41.22° 775' N, 02.13° 150' E), at 11:00 h local time, over a water-column depth of 40 m.
518 Seawater was collected from 5 m with a 15 L transparent hydrographic bottle, gently
519 siphoned into 20-L carboys covered with back plastic bags (to avoid excessive exposure to
520 sun-light), and rapidly transported to the laboratory. Temperature and light were measured *in*
521 *situ* with a YSI 30 portable temperature meter and a LI-COR LI-1400 data logger,
522 respectively.

523

524 Prior to each experiment, filter capsules, silicon tubing, carboys, and polycarbonate
525 bottles were soaked in 10% HCl-Milli-Q water and rinsed thoroughly with Milli-Q water (>
526 10 L passed through filters and at least 3 rinses for the rest of material). Part of the sampled
527 water was gently siphoned into a 50 L bucket and carefully mixed (named “whole water”
528 thereafetr), and the rest was gravity filtered through 0.2 µm with a Pall Acropak 0.8:0.2 500
529 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 NH₄Cl and 1 µmol L⁻¹ Na₂HPO₄. 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 Chla, 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 Chla, 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 $\mu\text{g mL}^{-1}$ 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 $\text{pg C}_{\text{Dino}} \text{cell}^{-1} =$
583 $0.760 \times \text{volume}^{0.819}$ for dinoflagellates and $\text{pg C}_{\text{Diat}} \text{cell}^{-1} = 0.288 \times \text{volume}^{0.811}$ 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 **Chla analyses**

610

611 Concentrations of Chla 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 -80°C 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 (Chla 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 Chla and DMSPt were determined from t_0 and t_{24}
630 concentrations (C_{t0} , C_{t24}) assuming an exponential model:

631
$$r = \ln(C_{t24}/C_{t0})/t$$

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

639

640 **Estimates of primary and DMSP production**

641

642 Chla-based growth rates (μ_{Chla} , d^{-1}) were converted into mass gross growth rates (μg
643 Chla $L^{-1} d^{-1}$) by multiplying them by the mean Chla concentration in the non-diluted bottle
644 without added nutrients ($\langle C_{Chla} \rangle$), calculated according to the equation of Frost (1972):
645
$$\langle C_{Chla} \rangle = C_{t_0} [e^{(\mu-m)(t_{24}-t_0)} - 1] / (t_{24}-t_0) (\mu-m)$$

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

649

650 DMSP-based gross growth rates (μ_{DMSP} , d^{-1}) were converted into DMSP production
651 rates ($nmol\ DMSP\ L^{-1}\ d^{-1}$) by multiplying them by the mean DMSP_t concentration calculated
652 as detailed for Chla. The proportion of primary productivity invested in DMSP production
653 was calculated by converting DMSP production into DMSP-C production by multiplying by
654 5, which is the number of C atoms in the DMSP molecule.

655

656 **Calculation of grazing-mediated DMS production**

657

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

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

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

669

670 Measurements of gross DMS production and bacterial DMS consumption

671

672 Community gross DMS production and bacterial DMS consumption were estimated
673 by the inhibitor method with dimethyl disulfide (DMDS; Wolfe and Kiene 1993; Simó et al.
674 2000; Saló et al. 2010) in parallel undiluted bottles incubated in the dark. DMS accumulation
675 in duplicate DMDS amended bottles (final concentration of 200 nmol L⁻¹) provided the gross
676 DMS production rate. The difference between gross DMS production and net DMS
677 production in the non-DMDS-amended bottles provided an estimate of the bacterial DMS
678 consumption rate. Rate errors were derived from the standard errors of the slopes.

679

680

681

682 RESULTS

683

684 Plankton community composition and dimethylated sulfur pools

685

686 The physicochemical conditions encountered in each sampling are reported in Calbet
687 et al. (2008). In brief, seawater temperature at the sampling depth (5 m) was 23.5°C at the
688 beginning of the study in September 2005, decreased to 13.0°C in January, and increased
689 again to a maximum of 24.4°C in July 2006. Nutrient concentrations varied almost the
690 opposite to temperature, with highest concentrations in November and lowest levels in
691 September 2006. Chla concentrations ranged 0.5-1.7 µg L⁻¹ between October and May, and
692 0.2-0.7 µg L⁻¹ in the June-September period (Table 1 and Figure 1).

693

694 The phytoplankton assemblage, also partially reported in Calbet et al. (2008), was
695 characterized by a clear dominance of organisms <10 µm in the period June-September, while
696 in the rest of the year the larger cells contributed 40-50% of the total Chla. Diatoms were
697 present particularly in the colder months, contributing the largest share of phytoplankton
698 biomass in November and May (Table 1). Autotrophic nanoflagellates occurred all year
699 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 $\mu\text{g C L}^{-1}$ in November to 43 $\mu\text{g C L}^{-1}$ 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 Chla
716 concentrations were typically higher in the colder months (October to May; 0.5-1.5 $\mu\text{g L}^{-1}$)
717 than in the warmer months (June to September; 0.2-0.7 $\mu\text{g L}^{-1}$), DMSPt:Chla 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:Chla 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.

734

735 As shown in Table 2 and Figure 3, the growth rates of DMSP-producing
736 phytoplankton (μ_{DMSP}) varied between 0.07 d^{-1} (August) and 1.49 d^{-1} (May), i.e., within a
737 wider range than the Chla-based growth (μ_{chla} , $0.30\text{-}1.08 \text{ d}^{-1}$). Nonetheless, the annual means
738 were very similar ($\mu_{DMSP} = 0.74 \pm 0.51 \text{ d}^{-1}$; $\mu_{chla} = 0.81 \pm 0.25 \text{ d}^{-1}$). The DMSP-based growth
739 rates were significantly higher in the colder months (October-May: $1.17 \pm 0.07 \text{ d}^{-1}$) than in the
740 warmer season (June-September: $0.31 \pm 0.13 \text{ d}^{-1}$), despite higher DMSPt concentrations in the
741 latter. As a result, mass production rates of DMSP were on average 2.5-fold higher in the
742 colder months. When converted into carbon units, DMSP production represented a 0.4% to
743 12% share of carbon fixation (overall mean of $3 \pm 4\%$).

744

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

751

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

762

763 **DISCUSSION**

764

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

766

767 During the study period, Chla and DMSPt concentrations and the DMSPt:Chla ratio in
768 our sampling station off Barcelona followed monthly variations somewhat consistent with
769 those found in the Blanes Bay Microbial Observatory located *ca.* 60 km northwards (Vila-
770 Costa et al. 2008; Simó et al. 2009). Larger phytoplankton, mainly diatoms and cryptophytes,
771 occurred in the colder months (October to May), associated with higher biomass and primary
772 production rates, but with lower specific (Chla-normalized) DMSP content. Indeed, diatoms
773 and cryptophytes from temperate waters are amongst the phytoplankton phyla with lower
774 intracellular DMSP concentrations (Stefels et al. 2007). With the onset of summer,
775 characterized by stronger stratification, depleted nutrients and lower productivity (Gasol et al.
776 2016), plankton succession led to smaller cells (mainly haptophytes and *Synechococcus*),
777 which are more efficient at nutrient uptake and overall have higher DMSP content (Table 1).
778 *Synechococcus* are considered to contain little or no DMSP, but haptophytes are, along with
779 dinoflagellates, the strongest DMSP producers, more so under high light and nitrogen
780 limitation (Simó 2001; Stefels et al. 2007). The dilution experiments revealed that the DMSP-
781 producing phytoplankton grew faster from October to May than in summer, while the growth
782 rates of the bulk phytoplankton assemblage did not show any clear seasonal trend. The
783 resulting proportion of total primary production invested in DMSP biosynthesis varied
784 between 0.4 and 12%, which is consistent with the values (1-10%) obtained in Blanes Bay by
785 Simó et al. (2009).

786

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

797

798 As for the DMSP-producing phytoplankton, growth rates (μ_{DMSP}) were higher than
799 mortality rates (m_{DMSP}) in 4 experiments (November to May), while the opposite occurred
800 mainly in summer 2006 (Figure 3). On average, microzooplankton consumed daily $82 \pm 30\%$
801 of the DMSP stock at that depth (Table 3). This indicates that other DMSP sinks such as
802 mesozooplankton grazing, algal autolysis, viral infection or intracellular DMSP turnover were
803 likely insignificant. Several causes for such an apparent tight coupling between growth and
804 micrograzing mortality can be invoked. On the one hand, the DMSP producers are generally
805 small-sized algae such as small dinoflagellates and haptophytes (Belviso et al. 1993; Archer et
806 al. 2011), i.e., those acting as target prey for herbivorous microzooplankton (Fenchel 1980).
807 This would explain that microzooplankton consumed a larger proportion of the DMSP
808 producers than of the total phytoplankton. On the other hand, there is the possibility that the
809 experimental setup did not account for any intracellular turnover of DMSP that might be
810 occurring due to high light and high nutrient exposure (Sunda et al. 2002) in the summer
811 incubations, thus rendering underestimates of DMSP production or growth rates (Archer et al.
812 2011). This is very plausible, as measured μ_{DMSP} values were too low to sustain m_{DMSP} in 4
813 experiments in summer 2006. The possibility that grazing rates were overestimated is less
814 likely since microzooplankton removed, during that period, the reasonable amount of 40-
815 100% of the DMSPt stock, similarly to the findings of other authors in North Sea and sub-
816 Antarctic waters (Archer et al. 2001b, 2011).

817

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

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

838

839 **Microzooplankton grazing and DMS production and cycling**

840

841 Unlike DMSPt concentrations, which showed no clear seasonality but an outstanding
842 peak during a bloom of small dinoflagellates in June, DMS concentrations followed a general
843 increase between winter and midsummer, broken by a peak derived from the *Phaeocystis*
844 post-bloom in April. This seasonal pattern with a summer mode has been also found in Blanes
845 Bay (Vila-Costa et al. 2007, 2008). Several other seasonal studies and data compilations in
846 temperate to subtropical zones have also shown that maximum DMS concentrations occur in
847 summer when the concentration of Chla is at its annual minimum (e.g., Dacey et al. 1998;
848 Lana et al. 2011). This phenomenon, named the “summer DMS paradox” by Simó and
849 Pedrós-Alió (1999), is thought to be due to phytoplankton succession towards higher DMSP-
850 producing phytoplankton in summer (confirmed by a higher DMSPt:Chla ratio, Table 1) plus
851 the seasonal shift in the environmental variables that drive DMS production and consumption
852 by the whole plankton community. Among these variables, nutrient availability (Sunda et al.
853 2007; Archer et al. 2009; Polimene et al. 2012) and solar radiation effects on bacteria (Toole
854 et al. 2006; Slezak et al. 2007; Ruiz-González et al. 2013), phytoplankton (Sunda et al. 2002;
855 Archer et al. 2009) and photochemical reactions (Toole and Siegel 2004; Galí et al., 2016) are
856 believed to play the main roles (Simó, 2004; Vallina et al., 2008; Lizotte et al. 2012; Galí and
857 Simó 2015).

858

859 The series of dilution experiments revealed that microzooplankton grazing is a
860 principal biotic factor influencing DMS production. Microzooplankton exerted a strong
861 control on the size of the algal DMSP pool by consuming daily 39-141% of the stock, and
862 also affected DMSP transformation rates into DMS and other breakdown products.
863 Microzooplankton grazing has been shown to enhance DMS production (Archer et al. 2003)
864 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.

873

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 (P_g) 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 Chla ingestion and DMSP:Chla ratios. P_g 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).

891

P_g represented on average $65 \pm 9\%$ 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, P_g accounted for 73% of the variance in the DMS concentration

throughout the time series (linear regression of the DMS and P_g series in Figure 4 yields a coefficient of determination $r^2=0.73$), while community gross DMS production accounted for 64% (DMS vs. gross DMS prod. $r^2=0.64$). Bacterial consumption, conversely, only explained 16% of the variance in DMS ($r^2=0.16$). That is, biological production was more important than biological consumption in determining DMS concentration. This is not an unexpected result, since the only known sources of DMS are biological processes, whereas biological metabolism only accounts for a fraction of total DMS loss, generally 50-80% (Simó 2004; 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.

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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 (°C)	Dominant phytoplankton	Dominant MZP	Chla ($\mu\text{g L}^{-1}$)	DMSPt (nmol L^{-1})	DMSPt:Chla ($\text{nmol } \mu\text{g}^{-1}$)	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
<i>Mean (std dev)</i>		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* (Chla) 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 Chla-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
<i>Mean (std dev)</i>	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)	<i>Pg</i> (nmol L ⁻¹ d ⁻¹)	<i>r</i> ²	DMS yield (%)	Gross DMS prod. (nmol L ⁻¹ d ⁻¹)	<i>Pg</i> : 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	<i>nd</i>		<i>nd</i>	
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	<i>ns</i>	<i>ns</i>		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
<i>Mean (std dev)</i>	<i>17.9 (9.8)</i>	<i>82 (30)</i>	<i>2.5 (1.9)</i>		<i>13 (6)</i>	<i>3.6 (2.6)</i>	<i>65 (9)</i>		

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 ($\text{nmol L}^{-1}\text{d}^{-1}$) and grazing-mediated DMS production rates (Pg; $\text{nmol L}^{-1}\text{d}^{-1}$).







