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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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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

is DMS, most of which is degraded within the water column by microorganisms and solar radiation, and only a small fraction is ventilated to the atmosphere and becomes climatically active (Simó 2001; Stefels et al. 2007).

DMSP is released from phytoplankton cells to the water column through numerous processes, namely algal senescence and physiological stress (Kwint and Kramer 1995; Sunda et al. 2002), viral lysis (Malin et al. 1998), and zooplankton grazing (Dacey and Wakeham 1986; Christaki et al. 1996; Daly and Di Tullio 1996). DMSP exudation or excretion by healthy algal cells seems to occur, but plays a secondary role (Laroche et al. 1999), whereas lipophilic DMS, when produced intracellularly, easily crosses membranes and leaks out of the cell (Spiese et al. 2015). A number of laboratory (e.g., Dacey and Wakeham 1986; Christaki et al. 1996; Wolfe and Steinke 1996) and field studies (e.g., Daly and DiTullio 1996; Kwint et al. 1996; Archer et al. 2003) have demonstrated that zooplankton grazing enhances DMS production, probably by facilitating the mixing of algal DMSP with algal or bacterial DMSP lyases. In spite of this line of evidence, few studies have attempted to assess the relative importance of grazing within the cycle of dimethylated sulfur (Simó et al. 2002; Archer et al. 2001b, 2003, 2011).

Microzooplankton are major herbivores in most marine environments, channeling as much as two thirds of daily phytoplankton production in both eutrophic and oligotrophic pelagic systems worldwide (Calbet and Landry 2004; Schmoker et al. 2013). Microzooplankton include heterotrophic and mixotrophic organisms: protists such as ciliates, dinoflagellates, and foraminiferans, and small metazoans such as copepod nauplii, meroplanktonic larvae, and rotifers (Sieburth et al. 1978). Microzooplankton are often the same size as their prey, which poses operational difficulties for the quantification of their grazing rates. To overcome this problem, Landry and Hassett (1982) proposed the dilution technique, an assay that has since been widely used in various regions of the world's ocean. The dilution technique involves incubation of a series of water samples diluted with increasing amounts of filtered (organism-free) seawater to sequentially reduce grazer-prey encounter rates and therefore the grazing of microzooplankton on phytoplankton. Changes in Chla concentration in the series of incubations yield an estimate of the growth and mortality rates of the phytoplankton assemblage (Landry and Hassett 1982). The dilution technique has also been used to calculate some biogeochemically relevant process rates, such as those of

nitrogen uptake, regeneration, and excretion (Andersen et al. 1991; Neuer and Franks 1993; Lehrter et al. 1999).

As the dominant cause of algal mortality, microzooplankton grazing is expected to play a central role in DMSP consumption and DMS production. In the last two decades, a few studies have applied the dilution technique to estimate the growth and grazing-mediated mortality rates of DMSP-producing phytoplankton (Wolfe et al. 2000; Archer et al. 2001b; Fredrickson and Strom 2009; Archer et al. 2011) and the grazing-mediated rates of dissolved DMSP and/or DMS production (Wolfe et al. 2000; Archer et al. 2001a, 2003, 2011; Park et al. 2014) in temperate, subpolar and polar waters. Nothing is known about the role of microzooplankton grazing in the DMS cycle at lower latitudes and across seasons, and how it compares with rates of microbial DMS production and consumption. Moreover, a grazing deterrent function has been suggested for DMSP. Initially, this was assigned to two of its degradation products, acrylate as a toxic and DMS as an infochemical (Wolfe and Steinke 1997); later on, the hypothesis was revisited to suggest that DMSP itself would reduce protist grazing rates (Strom et al. 2003). More recently, Seymour et al. (2010) showed that DMSP is indeed an infochemical but a potent attractant, not a repellent. Thus, there is still controversy about the inhibitory or stimulatory effects of DMSP on grazing in natural plankton communities. One way to assess the validity of the deterrence hypothesis is testing for reduced grazing rates on DMSP-containing phytoplankton with respect to grazing rates on the bulk phytoplankton assemblage, even though this approach has limited reach since other factors, such as prey size, morphology, motility and nutritious value have strong influence on grazing rates (Verity 1991).

In the present study, we conducted monthly dilution experiments during a year in oligo- to mesotrophic coastal waters of the north-western Mediterranean. We used a revised version of the dilution technique (Saló et al. 2010) that includes measurements of DMSP (as the specific biomarker for DMSP-producing algae) and aqueous DMS. Here we report the results that refer to the cycling of DMS, whereas *Chl a* and cell count based results are fully described in Calbet et al. (2008). For the first time, we compare the rates of grazing-mediated DMS production with measured rates of DMS consumption by bacteria and gross DMS production by the whole plankton community. Our goals were 1) to compare the growth and mortality rates of the DMSP producers with those of the whole phytoplankton assemblages; 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 black 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" thereafter), 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

a 200 μm mesh in order to avoid cell breakage of delicate microzooplankton organisms, it might have contained some mesozooplankton. Visual examination of the water did not reveal the presence of large organisms.

Whole water was added to 0.2 μm filtered water in duplicate 2.3 L polycarbonate bottles, which were filled leaving minimal headspace and rapidly capped. Four levels of dilution were prepared containing decreasing proportions of whole water: 100% (undiluted), 75%, 50%, and 25%, respectively. Nutrients were added to all the dilution bottles to final concentrations of 15 $\mu\text{mol L}^{-1}$ NH_4Cl and 1 $\mu\text{mol L}^{-1}$ Na_2HPO_4 . Two bottles of whole water without nutrient addition were used as natural seawater controls. Four dark glass bottles with undiluted seawater were incubated in parallel for the determination of community gross DMS production and bacterial DMS consumption rates (see below).

Once all the experimental bottles had been prepared, incubations were carried out in a large (600 L) outdoor incubator with a continuous flow-through system of water running from the coastal sea-water intake of the laboratory. The incubator was covered with a neutral density mesh that reduced ca. 40% of solar irradiance; this was meant to simulate the natural attenuation of 33-50% of surface PAR irradiance observed at 5 meters depth in the sampling site. Bottles were gently mixed at least three times through the 24 hours period in order to minimize algal settling.

At the beginning of the experiment (t_0), whole and filtered waters were sub-sampled from the buckets for $\text{Chl}a$, DMSP and DMS analyses before filling the dilution bottles. The initial concentrations for each dilution level were obtained by calculations according to the corresponding proportions of whole and filtered waters. Relevant tests had shown this method was accurate within 3% (Saló et al. 2010). At the final time point after 24 h of incubation (t_{24}), all the experimental bottles were sampled again for $\text{Chl}a$, DMSP and DMS. The dark bottles used for measuring gross DMS production and bacterial DMS consumption were sampled for DMS at times zero, 26 h, and two intermediate time points, typically 4-6 h and 20 h (Saló et al. 2010).

Plankton community composition

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

DMSP and DMS analyses

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

Chla analyses

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

Calculation of growth and grazing rates

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

Net rates of change (r) of Chla and DMSPt were determined from t_0 and t_{24} concentrations (C_{t_0} , $C_{t_{24}}$) assuming an exponential model:

$$r = \ln (C_{t_{24}}/C_{t_0})/t$$

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

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

$$\langle C_{Chla} \rangle = C_{to} [e^{(\mu-m)(t_{24}-t_0)} - 1] / (t_{24}-t_0) (\mu-m)$$

Mass growth rates were converted into carbon-based primary productivity rates by considering that the C:Chla (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 Chla. 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, DMSP_t grazing rates at each dilution were calculated by scaling DMSP_t mortality rates m_{DMSP} to the dilution factor and multiplying them by the mean DMSP_t concentration $\langle C_{DMSP} \rangle$ in each dilution bottle calculated as: $\langle C_{DMSP} \rangle = C_{to} [e^{(\mu-m)(t_{24}-t_0)} - 1] / (t_{24}-t_0) (\mu-m)$

Net DMS production rate values were paired to the corresponding DMSP_t grazing rates, and a model I regression analysis was conducted. The slope provided the daily DMS production per grazed DMSP_t (Δ nmol DMS L^{-1} / Δ nmol 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 (P_g). The error of P_g 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 23.5°C at the beginning of the study in September 2005, decreased to 13.0°C in January, and increased again to a maximum of 24.4°C 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

to January. *Synechococcus* sp. abounded throughout the warmer months, and even became the largest contributor to phytoplankton biomass in midsummer (July-August). *Prochlorococcus* sp. only occurred in September through January, though in low biomass. In April, phytoplankton was dominated by the mixotrophic ciliate *Laboea* sp., and small dinoflagellates (most of them $<20\ \mu\text{m}$) took over in June.

The biomass of the microzooplankton assemblages spanned one order of magnitude (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 dominance of nanoflagellates and ciliates plus dinoflagellates over the year (Table 1). Remarkable features were the aforementioned large proportions of mixotrophic ciliates in April and heterotrophic nanoflagellates in July. There was no evidence for any clear seasonal pattern.

The initial concentrations of DMSPt and DMS in the waters used for the dilution experiments are listed in Table 1 and graphically presented in Figure 1. DMSPt concentrations ranged $10\text{-}71\ \text{nmol L}^{-1}$, with no clear seasonal pattern. Since Chla concentrations were typically higher in the colder months (October to May; $0.5\text{-}1.5\ \mu\text{g L}^{-1}$) than in the warmer months (June to September; $0.2\text{-}0.7\ \mu\text{g L}^{-1}$), DMSPt:Chla ratios were lower in the former ($11\text{-}26\ \text{nmol}\ \mu\text{g}^{-1}$) and higher in the latter ($44\text{-}145\ \text{nmol}\ \mu\text{g}^{-1}$). The far highest DMSPt level and DMSPt:Chla ratio were observed in June, coinciding with high biomass of small dinoflagellates (Table 1). DMS concentrations roughly increased from late fall and winter (ca. $1\ \text{nmol L}^{-1}$) to summer ($5\text{-}8\ \text{nmol L}^{-1}$), with the exception of April, where the maximum annual concentration ($11\ \text{nmol L}^{-1}$) was recorded during the *Phaeocystis* post-bloom.

Dilution experiments

Figure 2 shows two graphical examples of the results of the dilution experiments for DMSP and DMS. They correspond to November 2005 and April 2006. Two more examples (June and July 2006) can be found in Saló et al. (2010). As illustrated by the figure, regression analysis of apparent DMSPt growth rates vs dilution level generally showed significant ($p < 0.05$) slopes and intercepts. The slope was taken as the grazing rate on DMSP, and the intercept was corrected by the apparent growth in nutrient-unamended bottles to provide the *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 Chla-based growth (μ_{chla} , 0.30-1.08 d⁻¹). Nonetheless, the annual means were very similar ($\mu_{DMSP} = 0.74 \pm 0.51$ d⁻¹; $\mu_{chla} = 0.81 \pm 0.25$ 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 DMSP 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 $3 \pm 4\%$).

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 Chla-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 Chla-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 (P_g) varied between not significantly different from zero in January to 6.3 nmol DMS L⁻¹d⁻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 $13 \pm 6\%$, Table 3). Microzooplankton grazing accounted for 32-96% (overall mean $65 \pm 9\%$) of the gross DMS production by the whole community in the dark (Table 3). Actually, P_g 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, Chla and DMSPt concentrations and the DMSPt:Chla 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 (Chla-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 Chla 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 $82 \pm 30\%$ 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 (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 Chl *a* ingestion and DMSP:Chl *a* 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).

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 *Pg* 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).

Concluding remarks and implications for modeling

We provide new estimates of the amount of carbon primary production invested in DMSP biosynthesis by mixed phytoplankton assemblages, which was 0.4-12%. Our data confirm that, in complex plankton communities, DMSP-containing phytoplankton generally experience similar or higher grazing pressure than the bulk phytoplankton community, and definitely not reduced grazing rates as the deterrence hypothesis would predict. Micrograzers consumed daily 39-141% of the DMSP stock, and simultaneous estimates of DMS production indicated that 6-20% of the grazed DMSP was converted into DMS. Our work points to microzooplankton as a major driver of DMS production and concentration over seasonal time scales.

The distribution of DMS concentration and emission fluxes and their dynamics over seasons have been remarkably difficult to predict by numerical prognostic modeling (Le Clainche et al. 2010). The difficulties arise mainly from the lack of an appropriate numerical representation of both plankton ecophysiology and community interactions, the latter including herbivore grazing and algal-bacterial mutualisms. Indeed, in most models of the DMS cycle, DMSP loss from phytoplankton, which is the first gate towards DMS production, is poorly parameterized. In the most complex models, cell DMSP content in phytoplankton is either set according to phytoplankton functional types or made dependent on solar radiation; herbivore grazing is set independent of the prey DMSP content, and DMSP release is set proportional to overall grazing rate (e.g., Vallina et al. 2008; Toole et al. 2008; Vogt et al. 2010; Polimene et al. 2012). Then, bacteria act on released DMSP to produce DMS according to their carbon and sulfur demands. Our findings indicate that grazing-mediated DMS production has higher yields per DMSP lost (6-20%) than typical bacterial DMS production

(5-10%, Kiene et al. 2000), explaining the overall community DMS production yields collected in a recent meta-analysis (7-28%, Galí and Simó 2015). Better representation of grazing on DMSP-producing phytoplankton and its effects on DMS production is needed if we are to improve DMS prediction.

Our findings have implications not only for DMS modeling but for food web modeling as well. Feeding of heterotrophic protists depends on their searching, contact, capture, processing, ingestion, and digestion abilities (Montagnes et al. 2008). Diffusive infochemicals like DMSP are expected to influence prey encounter and selection either by attraction or deterrence, with fundamental influence on phytoplankton abundance, assemblage composition and carbon and energy fluxes (Strom 2008). Despite its potential to modulate grazing rates and prey populations, however, prey selection is hardly implemented in models of the planktonic food web (Davidson 2014). Changing the perception of DMSP as deterrent to that of neutral or attractant fundamentally changes the way this implementation is to be conducted. All in all, the challenge remains of improving population dynamics prediction for both predators and prey by going beyond bitrophic interactions between single generalist predator and prey, and incorporating the more specific roles of chemical communication between cells.

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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>		<i>19.9 (4.2)</i>			<i>0.72 (0.43)</i>	<i>24.9 (17.9)</i>	<i>53 (48)</i>	<i>4.9 (3.2)</i>

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**	<i>ns</i>	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*	<i>ns</i>	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**	<i>ns</i>	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*	<i>ns</i>	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*	<i>ns</i>	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*	<i>ns</i>	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)	<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 ($\text{Pg; nmol L}^{-1}\text{d}^{-1}$).