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DIMETHYLATED SULFUR COMPOUNDS IN SYMBIOTIC PROTISTS: A
POTENTIALLY SIGNIFICANT SOURCE FOR MARINE DMS(P)

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ABSTRACT

Symbiosis with micro-algae (photosymbiosis) is a common feature among marine planktonic protists, but very little is known about the physiology and ecological significance of these associations. High concentrations of dimethylsulfoniopropionate (DMSP), a metabolite produced by marine microalgae, are commonly observed in coral-microalgae symbiosis, where DMS(P) is involved in multiple physiological functions. Knowledge on concentration and role of DMSP in analogous photosymbiosis in plankton is lacking. Here, we assess the total DMSP (DMSPt=DMSP+DMS) concentration and natural stable isotopes of sulfur across ecologically relevant symbiotic plankton groups, the Radiolaria and Foraminifera. We found that intracellular DMSPt concentrations in microalgal symbionts were among the highest recorded (range=170-702 mmol L⁻¹), while lower concentrations (range=0.1-23 mmol L⁻¹) were characteristic of the holobiont (i.e. host-microalgae). The contribution of symbiotic Radiolaria to the water column particulate DMSPt concentration ranged 0.1-8%. Sulfur isotopic composition (³⁴S) of DMSPt in the Collodaria holobionts was significantly higher than their symbiotic microalgae isolated in culture. Despite their high intracellular DMSPt content, SO₄²⁻ uptake in these holobionts throughout 3-day incubations was not detected. We observed a systematic ³⁴S depletion (~1.5‰) of DMS relative to DMSP in experimental incubations containing filtered seawater, which we hypothesize is related to the bacterial preference for the uptake of ³⁴S-depleted DMS. Overall, the results indicate that plankton symbiosis can, at times, represent a potentially important source of DMS(P). Specific differences in ³⁴S provided new insights into sulfur isotopic fractionation associated with DMS(P) biotransformation processes, with potential implications for current interpretations of isotopically tracked biogenic sources of marine aerosols.

INTRODUCTION

Dimethylsulfoniopropionate (DMSP) is a widespread metabolite in marine ecosystems, mainly produced by marine microalgae (Keller et al. 1989; Blunden et al. 1992; Raina et al. 2013). It is involved in multiple cross-scale processes, from cell physiology to ecosystem functioning. Physiologically, DMSP has been demonstrated to play an important role in osmotic acclimation (Vairavamurthy et al. 1985; Kirst 1990; Lyon et al. 2016) and cryoprotection in polar algae (Kirst et al. 1991; Karsten et al. 1996). DMSP production and breakdown have been also hypothesized to act as an overflow mechanism to get rid of excess reduced sulfur (Stefels 2000), to confer antioxidant protection by scavenging intracellular hydroxyl radical (Sunda et al. 2002; Bucciarelli et al. 2013; Deschaseaux et al. 2014), and to participate in the regulation of phytoplankton buoyancy through replacement of other organic solutes (Lavoie et al 2015, 2016). Beyond cellular limits, recent work suggests that DMSP and its cleavage product dimethylsulfide (DMS) act as efficient info-chemicals among plankton microorganisms (Seymour et al. 2010; Garces et al. 2013) and with higher trophic levels (Savoca and Nevitt 2014). DMS is a volatile compound that ventilates to the atmosphere and represents the main global biogenic source of atmospheric sulfur (Bates et al. 1992; Simó 2001). Several oxidation processes in the atmosphere transform DMS into sulfuric and methanesulfonic acids, which are key participants of cloud formation (Andreae 1997). Three decades after it was postulated, the hypothesis of a feedback loop between phytoplankton DMS production, cloud formation and climate regulation (Charlson et al. 1987), remains controversial. Issues like the relative contribution of DMS oxidation products, sea salt and organics to the number of cloud condensation nuclei are yet to be resolved (Quinn and Bates 2011; Lana et al. 2012).

In addition to phytoplankton, heterotrophic organisms containing endosymbiotic microalgae (i.e. photosymbiotic holobionts) can be an important source of DMS(P) (Hill et al. 2000; Broadbent et al. 2002; Van Alstyne et al. 2006). Indeed, coral reefs hold the highest natural concentrations of oceanic DMS(P) reported to date (Broadbent and Jones 2004). The production of DMSP in corals is seemingly associated with the endosymbiotic dinoflagellate *Symbiodinium* sp. (Van Alstyne et al. 2009), although a recent study showed capacity for the juvenile animal hosts to produce DMSP as well (Raina et al. 2013). Less studied than that of corals, photosymbiosis is a common feature in the planktonic realm, particularly among protists belonging to the Rhizaria eukaryotic super-group (e.g. Foraminifera and Radiolaria) (Stoecker et al. 2009; Nowack and Melkonian 2010; Decelle et al. 2015). Radiolaria are amoeboid protists exhibiting mineral skeletons that are abundant and widespread in modern oceans (Anderson 1983; Stemmann et al. 2008; Not et al. 2009; Biard et al. 2016). They include five major groups – Collodaria, Nassellaria, Spumellaria, Taxopodia and Acantharia – spanning a large size range, from a few micrometers for small solitary cells up to several centimeters for colonial forms of the Collodaria (Dennett et al. 2002; Caron et al. 2012; Suzuki and Not 2015), although the bulk of cell sizes range between 200-500 μm (Michaels 1988; Caron and Swanberg 1990). They are active predators, but many species dwelling in the surface layers harbor endosymbiotic microalgae in their cytoplasm, which allows them to thrive in ecological niches that otherwise would be less favorable (Decelle et al. 2015).

These symbiotic relationships involve essentially dinoflagellate microalgae such as *Brandtodinium nutricula* (Probert et al. 2014) and *Gymnoxanthella radiolariae* (Yuasa et al. 2016) in the case of Radiolaria, or *Pelagodinium beii* (Siano et al. 2010) in the case of Foraminifera. The Prymnesiophyceae species *Phaeocystis* sp., an abundant and widespread microalgal genus, has been recently described in symbiosis with acantharians (Decelle et al.

2012). Both Dinophyceae and Prymnesiophyceae classes are typical major DMSP producers (Keller et al. 1989; Caruana and Malin 2014), and include keystone species for the biogeochemical sulfur and carbon cycles (Malin and Steinke 2004; Schoemann et al. 2005). *Acantharia-Phaeocystis* sp. holobionts exhibit extremely high DMSP cellular content, with values significantly higher than those expected if all DMSP was contained in the endosymbiotic microalgae (Decelle et al. 2012). Should elevated cellular content of DMSP be a common feature not only in benthic, but also in planktonic photosymbiosis, this widespread but traditionally overlooked functional group of plankton (i.e. photosymbiotic) may constitute a relevant source of biogenic DMS(P) previously unaccounted by the standard microplankton oriented (*i.e.* <200 μm) sampling procedures.

The complexity of marine biogeochemistry makes it difficult to identify and assess the multiple biological sources and flows of DMS(P) from and through the different compartments of the pelagic ecosystem, and their links to the lower atmosphere (Simó, 2001). In this context, the sulfur isotope ratio ($^{34}\text{S}/^{32}\text{S}$; *i.e.* $\delta^{34}\text{S}$) in aerosol sulfate has been used to assess the contribution of different sources, mainly anthropogenic vs marine (Kaye 1987; Norman et al. 1999; Patris et al. 2002). Recent pioneering measurements in macroalgae and natural phytoplankton assemblages have provided similar $\delta^{34}\text{S}$ values for DMSP but have contradictory views of the isotopic fractionation associated with biotransformation processes in the formation of DMS (Oduro et al. 2012; Amrani et al. 2013). In natural planktonic systems, phytoplankton cells are often too small and too intermixed with other organisms to be isolated in sufficient quantities for analysis of sulfur isotopic composition of DMSP by conventional isotope ratio analysis methods. This hampers the characterization of the isotopic composition of specific phytoplankton taxa or even functional groups in the field, and little is known about the contribution of the different phytoplanktonic components to the bulk

community isotopic composition of DMSP. The new method of Compound Specific Sulfur Isotope Analysis (CSSIA) enables sub-nanogram level sensitivity (Amrani et al. 2009; Said-Ahmad and Amrani 2013) and opens the door for such single-cell level studies. The present study aims at opening this black box and refining our understanding of the different biogenic sources and transformations of dimethyl sulfur compounds in planktonic systems, with particular attention to the contribution of widespread photosymbiotic organisms. We have combined CSSIA with culture and field-based approaches for free-living phytoplankton and single-celled symbiotic Rhizaria (Radiolaria and Foraminifera) collected from different environments. The specific objectives of our study were to assess i) the cellular DMSP content, ii) the S-isotopic composition of DMSP and iii) the potential fractionation associated with DMSP biosynthesis and degradation to DMS in photosymbiotic Rhizaria and phytoplankton.

METHODS

Cultures of free-living microalgae and field sampling --- Monoclonal cultures of free-living strains of symbiotic dinoflagellates *Brandtodinium nutricula* (RCC3468) (Probert et al. 2014), *Gymnoxanthella radiolariae* (RCC3507) (Yuasa et al. 2016), and *Pelagodinium beii* (RCC1491) (Siano et al. 2010) have been previously obtained through single-cell isolation from their radiolarian and foraminiferan hosts, respectively. Cultures of *Phaeocystis* strain RCC1383, found in association with symbiotic Acantharia (Decelle et al. 2012), but originally isolated in its free-living stage, were chosen to represent the free-living symbiotic algae of the ubiquitous Acantharia-*Phaeocystis* symbiotic association. All cultures were maintained in 0.22 µm filter-sterilized (Stericup-GP, Millipore) seawater with K/2 (-Tris,-Si) medium supplements (Keller et al. 1987) at 18°C, ~ 80 µmol photon m⁻² s⁻¹ light intensity and 14:10 light:dark cycle in the lab. Samples for DMSP analysis, cell counts and image-based

analysis of biovolume (see below) were taken for cultures at exponential and stationary phase, during night and daytime (4 hours into each cycle; Supplementary information).

Field samples of symbiotic Radiolaria and Foraminifera were collected in coastal waters of the Red Sea in Eilat (29°33'N, 34°57'E) and the western Mediterranean in Villefranche-sur-Mer (43°42'N, 7°18'E) during March and June of 2014, respectively. Plankton community was sampled using plankton net with 220 µm mesh size towed obliquely (0-30 m) for 10 minutes from a boat or swimming at surface (0-5 m) for approximately 200 m. Collected samples were immediately diluted in buckets with freshly collected surface seawater, protected from direct sunlight, and transported to the lab within less than an hour. Individual specimens were then manually sorted under a stereomicroscope using a micropipette and transferred to Petri dishes in the case of single-celled acantharians and foraminiferans, and to larger beakers in the case of collodarians, where they were rinsed with surface 0.22 µm filtered seawater (fsw) before following DMSP/image analysis or experimental procedure. This single-cell approach allowed us to assess intracellular DMSP concentration and isotopic composition ($\delta^{34}\text{S}$ -DMSP) in ecologically relevant, uncultured specific symbiotic taxa. While large collodarians could be identified to genus level based on morphological characteristics, identification was not so reliable for Acantharia, and so related morphotypes of different species were used in subsequent measurements (Fig. 1). Samples manipulation and experimental work was conducted in the laboratory facilities of the Interuniversity Institute for Marine Sciences in Eilat (Israel) and the Observatoire Oceanologique de Villefranche-sur-Mer (France).

Image analysis and cellular biovolume assessment and microalgae cell counts ---

Cultured and uncultured organisms were imaged using a digital camera (Canon EOS 5D) coupled to an optical direct microscope (Olympus BX51 and Nikon Eclipse) or stereoscope

(Zeiss Stereo discovery V200). Microalgae and rhizarian cell and colonial size dimensions were assessed with the ImageJ open source image processing software (<https://imagej.nih.gov/ij/>) and organismal biovolume derived from minimum and maximum length dimensions and the formula of a prolate sphere, as described in Biard et al. (2016). For single-celled amoeboid acantharians and Foraminifera with highly variable space occupation of the cytoplasm the perimeter drawn to estimate the min/max lengths included the skeleton axis regardless of the position of the cytoplasm; while for colonial collodarians, the perimeter was given by the contour of the colony. Samples of cultured phytoplankton were fixed with 0.1 % glutaraldehyde (final concentration), for 15 min at room temperature in the dark, flash-freeze in liquid nitrogen and stored at -80 °C (Marie et al. 1997) until analysis. Cell abundance was determined using a FACS Aria (Becton Dickinson, San José, CA, USA) Flow Cytometer and raw files analyzed with the FlowJo software (TreeStar Data Analysis Software).

Dimethyl sulfur compounds concentration analysis --- DMSP was measured as the DMS evolved by alkaline hydrolysis using purge and trap coupled to gas chromatography (Shimadzu GC14A) with flame photometric detection (FPD) as described in Galí et al. (2011). We therefore measured total DMSP (hereafter DMSPt) that comprises mainly particulate DMSP+DMS in the case of individual symbiotic specimens, while DMSPt measurements in cultured microalgae comprise both particulate and dissolved forms. For cultured phytoplankton DMSP analysis, an aliquot of 0.5-1 mL from the culture was dispensed into 13 mL gas-tight vials previously filled with MilliQ water. For single-celled Radiolaria and Foraminifera, 2-4 specimens were transferred from the containers where they had been rinsed with fsw, to a 13 mL vial previously filled with MilliQ. For colonial Radiolaria, 1-2 specimens were transferred to the analytical vial, which was filled with fsw

189 instead, to minimize the potential contamination by DMSO in the MilliQ water system. We
190 then added two pellets of NaOH before sealing the vial with Teflon-capped lids. Samples
191 were stored at room temperature in the dark until analysis, within the next 2 months. To
192 account for dissolved DMSPt in solution, we preserved and analyzed blanks of the solutions
193 where the organisms were analyzed (MilliQ for microalgae and single-celled
194 Radiolaria/Foraminifera, and fsw for colonial Radiolaria). Dissolved DMSP measured in the
195 blanks was then subtracted from total DMSP in the corresponding organism samples to yield
196 the particulate total DMSP values presented in this study. The contribution of dissolved
197 DMSP to the total DMSP was minor (<5%).

198 Samples were sparged with 40 mL min⁻¹ of high-purity helium, with the volatiles
199 trapped in a Teflon loop tube submersed in liquid nitrogen for 3-5 min before re-volatizing
200 them by placing the Teflon tube in hot water. Sulfur compounds were separated using a
201 packed Carbopack® 60/80 mesh column (Sigma-Aldrich) maintained at 170°C. Intracellular
202 content and cell concentration of cultured microalgae and freshly-collected Radiolaria or
203 Foraminifera holobionts were assessed from the concentration of DMSPt measured in the
204 sample vial minus the corresponding blank, and normalized by the number and biovolume of
205 the specimens fixed in the vial. DMSP cell content in symbiotic microalgae within the
206 holobiont – *in hospite* – were calculated by dividing the DMSPt measured for the holobiont
207 by the mean number of host cells per colony surface, and also the mean number of microalgae
208 cells per host obtained from image analysis, assuming that the DMSP measured in the
209 holobiont was entirely confined in the symbionts.

210 *Analysis of sulfur isotopic composition of sulfate and dimethyl sulfur compounds ---*

211 The samples for sulfur isotope analysis of DMSP were prepared similarly to the concentration
212 measurements and therefore, they refer to DMSPt as well. For sulfur isotopic composition of

DMS, non-fixed samples were analyzed within 36 hours of collection (see below). Sulfur isotopic composition of DMS and cellular DMSP were measured by purge and trap system that was connected to a gas chromatograph (GC) coupled to a multicollector inductively coupled plasma mass spectrometer (MC-ICPMS) (Amrani et al. 2009; Said-Ahmad and Amrani 2013). Details for this method can be found in Said-Ahmad and Amrani (2013). Briefly, seawater samples diluted in anoxic MilliQ or fsw were collected from the original 13ml vial using a syringe with minimal disturbance, and injected gently into a new 40 mL sparging vial equipped with a Teflon septum. The vial was then sparged with He (40 mL/min) for 12 minutes. Water vapor was removed by a Nafion-membrane dryer (Perma pure LLC, NJ, USA) using dry N₂ as the counter flow. A Teflon sample loop was inserted in a dewar of liquid N₂ to trap DMS. After sparging, the 6-port valve (Valco Instrument Co, TX, USA; heated to 80°C) was turned to the inject position, and the sample loop transferred quickly from the liquid N₂ to hot water so that the trapped gases were injected into a Agilent J&W capillary column (DB-1, 60m x 0.32mm ID x 1.0µm), connected directly to the 6-port valve. At the same time the GC (Perkin Elmer 580) and the MC-ICPMS (Neptune Plus, ThermoFischer Scientific) were started. A standard DMS sample was introduced to the system for calibration every 3-4 samples and we used a bracketing technique to correct for instrumental mass bias and calibration of the SF₆ internal standard (Said-Ahmad and Amrani 2013). The results are expressed in conventional δ³⁴S notation as a per mil (‰) deviation from the international standard V-CDT (Vienna Canyon Diablo Troilite) according to the equation below.

$$\delta S^4 \left(R^4 / R^4_{std} \right)$$

where ³⁴R is the integrated ³⁴S/³²S ion-current ratio of the sample and standard peaks. Analytical precision of analysis of DMS and DMSP standards was usually in the range of 0.1-10

0.4‰ (1 σ standard deviation). The precision of sulfur isotope analysis for duplicate or triplicate samples of seawater and organism samples were usually less than 1‰. Accuracy as calculated by standards was in the range of 0.2 ‰ (Said-Ahmad and Amrani 2013) and estimated to be better than 1‰ for the seawater samples.

Dissolved sulfate (SO₄²⁻) sulfur isotope analysis was performed by a conventional elemental analyzer (EA) coupled to isotope ratio mass spectrometer (IRMS) method (Giesemann et al. 1994) using Delta Plus (Thermo) IRMS. Samples of BaSO₄ were prepared from diluted seawater samples by addition of 5% BaCl₂ solution and then analyzed for their $\delta^{34}\text{S}$ values by EA-IRMS. The sulfur isotope reference materials NBS-127 (BaSO₄; $\delta^{34}\text{S}$ = 21.1‰), IAEA-S-1 (Ag₂S; -0.3‰), and IAEA-SO-6 (BaSO₄; -34.1‰) were purchased from the National Institute of Standards and Technology (NIST) and used for calibration. Precision of this method for duplicates/triplicates was usually better than 0.3‰.

Sulfate assimilation and DMSP synthesis experiment --- For the biological sulfate assimilation experiment we incubated two cultured strains of microalgae (*Phaeocystis*_RCC1383 and *B.nutricula*_RCC3468) and freshly-collected specimens of solitary symbiotic *Thalassicolla* sp. (Collodaria-*Brandtodinium* holobiont) during three days in two different types of K/2 culture media prepared with isotopically distinct sulfate (Fig. 2a). One media ('Heavy') was prepared with filtered seawater amended with standard K/2 supplements to yield an average seawater sulfate $\delta^{34}\text{S}$ of $21.5 \pm 0.5\text{‰}$ at the beginning of the incubations. The second media ('Light') consisted of artificial seawater (Berges et al. 2001) prepared using isotopically depleted $\delta^{34}\text{S}$ -Sulfate relative to seawater sulfate, and mixed in 1:1 ratio with K/2 standard media, as described above, to give a sulfate $\delta^{34}\text{S}$ value of $7.6 \pm 0.4\text{‰}$. This procedure resulted in two 2-L batches of media with very distinct $\delta^{34}\text{S}$ -Sulfate composition but virtually identical nutrient concentrations. Experimental incubations for

each microalgae species were prepared by inoculating a 50 mL aliquot from a culture that had been maintained in K/2-based standard media for at least 50 generations, into 1 L of ‘Heavy’ and ‘Light’ media. After gentle homogenization initial samples (T_0) were taken from the two cultures and the remaining volume was subsequently aliquoted into 8 replicated 70 mL sterile tissue culture vessels (Fig. 2a). For incubations of Radiolaria holobionts freshly-collected specimens, collected and maintained in 0.2 μm fsw since the previous day, were transferred (2 ind./vessel) into 8 replicated tissue culture vessels previously filled with ‘Heavy’ or ‘Light’ media. Experimental design included therefore, three organismal types - two symbiotic microalgae strains in free-living stage, and one Radiolaria holobiont - each distributed on a series of replicated 8 vessels, half of which had been filled with ‘Heavy’ or ‘Light’ media, respectively (Fig. 2a). Organisms were incubated in parallel under constant temperature (19-20°C) and light (200 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$, 14:10 light:dark cycle) conditions in the lab. Replicated incubations from each organism and media type were sampled at time 0 and after 22, 46 and 70 hours of incubations for analysis of DMSPt concentration and sulfur isotopic composition.

DMSP→DMS cleavage fractionation experiment --- To assess potential differences in the isotopic signature of the DMS generated by symbiotic Radiolaria from that of the microbial community we determined the sulfur isotope composition of DMS resulting from the cleavage of DMSP produced during experimental incubations containing different planktonic biota (Fig. 2b). This was achieved by incubating a) the natural microbial assemblage < 200 μm (*i.e.* surface whole sea water, (*ws*)), b) a heterogeneous assemblage of Acantharia-microalgae holobionts (100 ind.) in 0.2 μm -filtered seawater obtained with sterile Stericup filtration device (Stericup-GP, Millipore) (*fsw+rads*), and a third control treatment containing only the same filtered seawater (*fsw-only*) (Fig. 2b). Organisms were manipulated

using sterile micropipette and petri dishes on the laboratory bench and incubations were prepared in 70 mL sterile polystyrene tissue culture vessels. Triplicates of each assemblage type were incubated for 4 hours (13:30-17:30) under ~30% incident light. Samples from *ws* for DMSP were preserved with NaOH in gas-tight vials, while those for DMS were maintained in the dark at similar temperature to that of the surface water, until analysis. In the “*fsw+rads*” treatments, 5 individuals from each incubation were sorted, imaged and preserved for DMSP concentration. The remaining ~95 specimens were transferred into 40 mL gas-tight vials with the same *fsw* where they were incubated and kept at room temperature until analysis.

Statistical analysis --- The statistical significance of the difference between organism groups in DMSP cell content, DMSP and DMS sulfur isotopic composition was tested with one-way ANOVA followed by Tukey’s post hoc test. All statistical analyses were conducted on experimental results using GraphPad 5.0 software (GraphPad Software, Inc, La Jolla, USA).

RESULTS

Intracellular DMSPt in cultured phytoplankton and freshly collected photosymbiotic organisms --- Table 1 summarizes cell size, intracellular DMSPt content and concentration in cultures of free-living phytoplankton and field-collected symbiotic Radiolaria and Foraminifera holobionts (Fig. 1). The highest values were observed in the large Collodaria-*Brandtodinium* holobiont with average DMSPt cellular content ranging from 2757 ± 750 to 3652 ± 2008 pmol specimen⁻¹ (Table 1). Colonial forms contained hundreds of radiolarian cells (i.e., estimated by the number of central capsules forming the colonies) per specimen ($N_{Sphaerzoum}=166 \pm 65$; $N_{Collozoum}= 509 \pm 72$ central capsules colony⁻¹). Each central capsule harbored a few endosymbiont cells ($N_{Collozoum}= 9.8 \pm 3.5$, $N_{Sphaerzoum}= 9.1 \pm 2.6$ symbionts

central capsule⁻¹). Solitary forms, consisting of millimeters-size single host cell, contained hundreds of endosymbiotic cells ($N_{\text{Thalassicolla}} = 723 \pm 703$ symbionts radiolarian cell⁻¹) (Fig. 1, Table 1). DMSPt content in different morphotypes of single-celled Acantharia and planktonic Foraminifera were much lower, according to their lower cell size, and varied within a relatively narrow range (15-38 pmol cell⁻¹). Foraminifera (37.8 ± 15.5 pmol cell⁻¹) and ‘Star’ morphotype acantharians (36.4 ± 10.4 pmol cell⁻¹) exhibited higher values than *A. elongata* (15.2 ± 5.4 pmol cell⁻¹) and ‘Translucid’ acantharians (20.9 ± 3.6 pmol cell⁻¹), yet differences were only significant between *A. elongata* and *Globigerinella* sp. ($p=0.03$, $F_{3,12}=4.1$, one-way ANOVA, Tukey’s post hoc test). Analysis of DMSP concentration per cellular biovolume yielded a different picture with *A. elongata* (17.1 ± 6.1 mmol L⁻¹) having significantly higher concentrations than Foraminifera (7.5 ± 3.1 mmol L⁻¹), ‘Star’ (2.4 ± 0.7 mmol L⁻¹) and ‘Translucid’ (0.3 ± 0.1 mmol L⁻¹) acantharian morphotypes, while both solitary and colonial collodarian species showed significantly lower concentrations ($p<0.001$, $F_{6,16}=14.8$) (Table 1).

Cellular DMSPt content in cultures of free-living algae was markedly lower (range=0.01-1.24 pmol cell⁻¹) according to their smaller size compared to their hosts (Table 1). Highest mean values were measured in *P. beii* (0.9 ± 0.2 pmol cell⁻¹); these were 3-4-fold higher than in *B. nutricula* (0.2 ± 0.1 pmol cell⁻¹) while *G. radiolariae* exhibited intermediate average cell DMSPt content (0.1 ± 0.1 pmol cell⁻¹). DMSPt concentration calculated from microscope-based estimates of cellular biovolume in free-living algae was one-to-three orders of magnitude higher than concentration in the radiolarian and foraminiferan holobionts (Table 1). *B. nutricula* (490 ± 107 mmol L⁻¹) and *G. radiolariae* (462 ± 168 mmol L⁻¹) showed higher values than *Phaeocystis* RCC1383 (307 ± 47 mmol L⁻¹) and *P. beii* (272 ± 49 mmol L⁻¹), although differences were only significant for *P. beii* ($p=0.0001$, $F_{3,35}=9.1$, one-way ANOVA, Tukey’s post hoc test). The large error associated with the mean values reflected

changes in the DMSPt cell content and concentration in relation to growth phase and photoperiod at the time of harvesting the culture (Supporting information).

DMSP sulfur isotopic composition ($\delta^{34}\text{S}$ -DMSP) --- The mean $\delta^{34}\text{S}$ -DMSP in isolated holobiont specimens of *Acantharia-Phaeocystis* sp. ($\delta^{34}\text{S}$ -DMSP = $19.8 \pm 0.4\text{‰}$) and Foraminifera-*P. beii* ($\delta^{34}\text{S}$ -DMSP = $20.1 \pm 0.4\text{‰}$) were very similar to each other, and also to the natural microbial assemblage coexisting in the same surface water ($\delta^{34}\text{S}$ -DMSP = $19.7 \pm 0.4\text{‰}$, Fig. 3). These values were slightly lower than $\delta^{34}\text{S}$ of local seawater sulfate ($\delta^{34}\text{S}$ - SO_4^{2-} = $21.5 \pm 0.5\text{‰}$). In contrast, the *Collodaria-Brandtodinium* holobiont showed a very distinct DMSP isotopic composition ($\delta^{34}\text{S}$ -DMSP = $23.5 \pm 0.8\text{‰}$), significantly enriched not only compared to other symbiotic groups (*Acantharia* and *Foraminifera*) and microbial assemblage, but also compared to $\delta^{34}\text{S}$ - SO_4^{2-} in seawater ($p < 0.0001$, $F_{7,26} = 46.9$, one-way ANOVA, Fig. 3).

Sulfur isotopic composition for cultured microalgae showed differences among species, with *Brandtodinium nutricula* ($18.2 \pm 0.3\text{‰}$) showing significantly lower $\delta^{34}\text{S}$ -DMSP values compared to *Phaeocystis* RCC1383 ($20.2 \pm 0.4\text{‰}$, Tukey comparison test, $p < 0.001$, Fig. 3). In the case of *B. nutricula* the $\delta^{34}\text{S}$ -DMSP values were significantly depleted relative to the *Collodaria-B. nutricula* holobiont (Tukey comparison test, $p < 0.0001$), whereas the $\delta^{34}\text{S}$ -DMSP values of free-living *Phaeocystis* RCC1383 was not significantly different from that measured in the *Acantharia-Phaeocystis* holobionts (Fig. 3).

Sulfate assimilation into DMSP and $\delta^{34}\text{S}$ fractionation --- Free-living microalgae growing in the 'Light' medium showed a progressive ^{34}S depletion of DMSP both with time and relative to microalgae growing in the 'Heavy' medium, indicating active sulfate assimilation from seawater for DMSP biosynthesis (Fig. 4a, b). Although relatively constant during the 3-day incubation, the $\delta^{34}\text{S}$ -DMSP of the microalgae growing in the 'Heavy' media

was depleted relative to the seawater sulfate. The depletion was more pronounced for *B. nutricula* (-3‰) than for *Phaeocystis* RCC1383 (-1‰) (Fig. 4a, b). Contrary to free-living microalgae, $\delta^{34}\text{S}$ -DMSP in symbiotic Radiolaria incubated in ‘Light’ and ‘Heavy’ media remained similar and substantially ^{34}S -enriched relative to available sulfate sources during the incubation (Fig. 4c).

DMSP to DMS conversion and sulfur isotope fractionation --- $\delta^{34}\text{S}$ of DMSP and derived DMS measured after daylight incubations showed differences between freshly-collected microbial assemblages and photosymbiotic radiolarians (Fig. 5). Sulfur isotopic composition of DMS measured from *ws*, which contained the bulk microbial assemblage (19.7 ± 0.3 ‰), was not significantly different from that measured in DMSP (19.4 ± 0.1 ‰, Fig. 5). Conversely, $\delta^{34}\text{S}$ -DMS measured from incubations containing photosymbiotic Radiolaria (*Acantharia-Phaeocystis* holobiont, *fsw+rad*) was significantly lower (18.4 ± 0.4 ‰; $p < 0.0001$, $F_{5,14}=12$, one-way ANOVA). Specifically, DMS- $\delta^{34}\text{S}$ values measured in the incubations with Radiolaria were significantly depleted relative to $\delta^{34}\text{S}$ -DMSP values in both *A. elongata* (1.2 ‰ mean difference) and ‘Star’ radiolarian morphotypes (1.5 ‰ mean difference). Similarly, $\delta^{34}\text{S}$ -DMS in *fsw-only* treatment incubated in parallel (18.0 ± 0.5 ‰) was depleted relative to $\delta^{34}\text{S}$ -DMSP in microbial assemblage and Radiolaria holobionts (Fig. 5).

DISCUSSION

DMSP cellular content in photosymbiotic plankton and partition among partners --- The role of DMSP in coral symbiosis has received considerable attention in recent years revealing high cell concentration and content of DMSP in the holobiont and different *Symbiodinium* sp. strains (Broadbent et al. 2002; Steinke et al. 2011; Deschaseaux et al. 2014) and DMS production associated to coral reefs (Broadbent and Jones 2004; Raina et al. 2010;

Exton et al. 2015; Frade et al. 2016). One of the objectives of this study was to determine whether photosymbiosis in planktonic organisms is systematically associated with high concentrations of cellular DMSP. The high concentration and cellular content of DMSP measured in symbiotic microalgal species cultured in their free-living stage but originally isolated from Radiolaria and Foraminifera hosts (Table 1) is consistent with this hypothesis. Highest DMSPt concentrations were observed among dinoflagellate strains (Table 1), which are within the upper range of previously reported values for this phytoplankton class (Caruana and Malin 2014). Although elevated DMSP concentrations are characteristic of Dinophyceae and Prymnesiophyceae (Keller et al. 1989), the extremely high concentrations associated with the symbiotic partners of Collodaria and planktonic Foraminifera, is suggestive of DMSP being an important attribute of the algal partner in rhizarian symbiotic associations.

The endosymbiotic nature of the Radiolarian-microalgae associations precludes direct measurements of the DMSP content in symbiotic microalgae cells within the radiolarian host (i.e. *in hospite*). However, the DMSP concentrations estimated for microalgae, based on the DMSP content in the holobiont and the number of microalgae cells hosted, yielded extremely high concentrations (Table 1) that matched the highest values estimated for photosymbiotic anemone and coral species (Broadbent et al. 2002; Van Alstyne et al. 2006). In the case of the colonial and solitary Radiolaria-microalgae holobiont, DMSP content per cell estimated for *B. nutricula in hospite* was 3-, 8-fold and 20-fold higher than for free-living cells, respectively. This difference was even larger for *Phaeocystis* sp. with 64-100-fold higher cellular content estimated for symbiotic algae in ‘*A. elongata*’ and ‘Translucid’ morphotypes of Acantharia, respectively, compared to the free-living algae, which would result in unrealistic (40-60 mM DMSP) cellular concentrations. In other words, if the 15-38 pmol DMSP cell⁻¹ measured in the Acantharia-*Phaeocystis* sp. partnership (Table 1) was allocated entirely to symbiotic algae

with similar concentrations to those measured in free-living cultures, the host should harbor 1250-3000 algal cells, which is a 100-fold more than the 10-20 cells commonly found in its cytoplasm. Acknowledging the limits of our data, these calculations suggest that DMSP could be present in the host as well. Whether the DMSP is translocated from the algae to the host (Van Alstyne et al. 2009) and/or produced by the latter (Raina et al. 2013) cannot be concluded from concentration measurements alone. Regardless of its origin, the moderate-to-high cellular content of DMSP estimated for the host and the algae are consistent with recent observations in Radiolaria (Decelle et al. 2012) and other photosymbiotic organisms (Hill et al. 2000; Stefels 2000; Broadbent et al. 2002; Van Alstyne et al. 2006), and suggest that both partners could mutually benefit from one or more of the multiple eco-physiological roles attributed to dimethyl sulfur compounds, e.g., osmoregulation, oxidant scavenger or info-chemical (Stefels 2000; Seymour et al. 2010; Raina et al. 2013).

Sulfate assimilation and DMSP production in photosymbiotic associations – Highly enriched $\delta^{34}\text{S}$ -DMSP observed in Collodaria-*Brandtodinium* sp. holobiont specimens (23.5 ± 0.8 ‰; Fig. 3) was particularly unexpected. This association showed significantly enriched $\delta^{34}\text{S}$ -DMSP not only relative to seawater SO_4^{2-} but also compared to other symbiotic radiolarians and all measurements previously reported for both macro- and microalgae, which showed a slight ^{34}S -depletion in DMSP compared to seawater SO_4^{2-} (Oduro et al. 2012; Amrani et al. 2013; Said-Ahmad and Amrani 2013)(Fig. 3). This distinctive sulfur isotopic composition could result from differential fractionation associated with the multi-step assimilation of sulfate into methionine (intracellular precursor to DMSP) and/or may reflect differences in the DMSP biosynthetic pathway. The 1-3‰ ^{34}S depletion in DMSP relative to sulfate during assimilation of sulfate is in agreement with previous reports (e.g. Kaplan & Rittenberg 1964). All three biosynthetic pathways for DMSP described to date for higher

plants and algae rely on assimilatory sulfate reduction (Gage et al. 1997; Summers et al. 1998). However, our incubation experiments with isotopically distinct sulfate solutions showed that while free-living microalgae did actively take up and assimilate sulfate into DMSP in time scales relevant for phytoplankton growth (Fig. 4a, b), collodarians with elevated cellular content of DMSP showed no isotopic evidence of assimilatory sulfate reduction into DMSP (Fig. 4c). This could be due to slower growth rates of these giant protists compared to microalgae, although the high photosynthetic rates of solitary Radiolaria (Caron et al. 1995) and abundance of endosymbiotic microalgae in incubated specimens (Table 1, Fig.1) could suggest additional explanations to the lack of sulfate uptake. The holobiont may for instance rely on inorganic or reduced sulfur species (e.g. methionine, cysteine) stored by the algae for DMSP synthesis. Radiolarians are active grazers (Anderson 1978; Swanberg and Anderson 1985; Suzuki and Not 2015), and captured prey and digestion products could provide such sulfur compounds to the symbiotic microalgae, reducing its dependence on sulfate uptake. It is worth noting however, that the assimilatory sulfate reduction only represents a small proportion of the total energetic cost (NADPH and ATP molecules) associated with *de novo* synthesis of DMSP (Lavoie et al. 2016).

In contrast to the collodarians, the $\delta^{34}\text{S}$ -DMSP measured in individual specimens of *Acantharia-Phaeocystis* ($19.8 \pm 0.4\text{‰}$) and *Globigerinella* sp.-*P. beii* ($20.1 \pm 0.4\text{‰}$), was very similar to the isotopic composition obtained from simultaneous ($19.7 \pm 0.4\text{‰}$) and previously reported bulk $\delta^{34}\text{S}$ -DMSP measurements of surface microbial assemblages ($19.7 \pm 0.5\text{‰}$, Amrani et al. 2013) (Fig. 3). In this regard, the $\delta^{34}\text{S}$ -DMSP of cultured *Phaeocystis* RCC1383 was similar to that measured in the *Acantharia-Phaeocystis* sp. holobiont (Fig. 3). The reasons for the different sulfur isotopic behavior between *Acantharia-Phaeocystis* sp. and *Collodaria-B. nutricula* associations is unclear to us, but given the phylogenetically distant

microalgal partners involved in these symbiotic association (*i.e.* Dinophyceae and Prymnesiophyceae), this isotopic variability could reflect differences in the metabolic capacities of the symbiotic algae and/or the holobiont. Differences in $\delta^{34}\text{S}$ -DMSP observed between *B. nutricula* and *Phaeocystis* RCC1383 (Fig. 3) were of similar magnitude to those reported between macro and microalgae species (Oduro et al. 2012). The observed differences between the two algal strains and the remarkably distinct $\delta^{34}\text{S}$ -DMSP observed in freshly collected Collodaria-*B. nutricula* associations (Fig. 3) contrast with the homogeneity in $\delta^{34}\text{S}$ -DMSP composition observed across diverse marine microbial communities (Amrani et al. 2013) and illustrate the isotopic variability potentially hidden in natural microbial communities. Little is known about the isotopic variability associated with taxonomic and functional diversity of major DMSP producers. Nonetheless, if adequately characterized, this variability offers an opportunity not only to investigate physiological differences between species in the lab, but also to refine our current ability to track specific sources of DMSP, and derived DMS, in highly intermixed and diverse natural microbial communities (Kaye 1987; Calhoun et al. 1991; Said-Ahmad and Amrani 2013).

Sulfur isotope fractionation associated with DMSP cleavage into DMS --- In addition to the sulfur isotopic composition of DMSP, it is important to constrain the isotopic fractionation associated with the cleavage of DMSP into DMS. The two previous studies assessing this fractionation reported contradictory patterns; Oduro et al. (2012) reported ^{34}S depletion for the DMS produced by macroalgae, while Amrani et al. (2013) reported small to negligible ^{34}S enrichment from parallel measurements of DMSP and DMS *in situ*. Our results from the experimental incubations conducted with natural microbial assemblages and symbiotic radiolarians (Fig. 5), although limited in data and scope, offer new insights that may contribute to reconcile contrasting previous observations. It is important to note that we

are not calculating fractionation factors (α), but rather apparent fractionation ($\Delta^{34}\text{S}$) from the difference between the product and the reactant. Consistent with Amrani et al. (2013), $\delta^{34}\text{S}$ -DMSP and $\delta^{34}\text{S}$ -DMS values were similar in whole seawater incubated from the Red Sea, suggesting negligible fractionation is associated with microbial cleavage of DMSP. However, the $\delta^{34}\text{S}$ value of DMS produced by symbiotic acantharians was lower (-1.2, -1.5‰) than that of DMSP (Fig. 5), resulting in a DMS fractionation similar in sign and magnitude to that reported for macroalgae by Oduro et al. (2012). These differences in the DMSP to DMS fractionation between the microbial assemblage, symbiotic radiolarians and macroalgae may reflect their taxonomic and functional diversity. However, similar ^{34}S depletion of DMS was observed between *fsw-only* and *fsw+rads* incubations (Fig. 5). Evidences exist that the filtration process can break phytoplankton cells and enrich the filtered seawater with dissolved DMSP and extracellular activity of DMSPlyase (Kiene and Slezak 2006) to the point that it is common to measure substantial DMS production in *fsw* (Galí et al. 2011). During the 4-hour experiments, DMS was produced in both *fsw-only* and *fsw+rads* incubations, although, the latter, containing approximately 100 specimens of Acantharia (see methods), produced larger amounts of DMS (Fig. 5). Overall, $\delta^{34}\text{S}$ values of DMS produced in natural microbial communities were similar or slightly higher than $\delta^{34}\text{S}$ values of DMSP (Amrani et al., 2013; this study), while cultures or incubations with isolated organisms in filtered seawater produced ^{34}S -depleted DMS (Oduro et al., 2012; this study). This may reflect the interplay among DMS sources and sinks: DMSPlyase selection for DMSP with lower $\delta^{34}\text{S}$ would produce ^{34}S depleted DMS, but preference of DMS-consumption processes (e.g. bacterial uptake, photochemistry, ventilation, and bacterial uptake) for ^{34}S -depleted DMS would buffer the overall result. This possibility was already discussed by Amrani et al. (2013), who invoked these DMS removal processes as potential mechanisms contributing to

‘re-enrich’ the DMS pool. To further constrain the contribution of each physical and biological process to this putative isotopic compensatory effect, we can compare their prevalence in the different incubations and natural measurements available from this and previous studies. The effect of ventilation can be ruled out because our incubation bottles did not allow for gas-exchange; also, the fact that the fractionation buffering effect had been observed at depths where ventilation is low further underscores the minor influence of ventilation (Amrani et al. 2013). The similarity in the light conditions for the three incubation types (*ws*, *fsw-only*, and *fsw+rads*), seem to argue against the effect of photochemistry upon DMS fractionation as well. Finally, although not directly measured, bacterial abundance, and likely bacterial DMS consumption, should have been largely suppressed in 0.2 μ m-filtered seawater during the short duration (4-hours) incubations. Filtered seawater showed ^{34}S -depletion of DMS relative to DMSP (Oduro et al. 2012; *fsw-only* incubation this study) while whole seawater, containing natural abundances of bacteria, showed similar $\delta^{34}\text{S}$ values for DMS and DMSP (Amrani et al. 2013; *ws* incubation this study, Fig. 2b). These observations are not sufficient to confirm, but are consistent with, a hypothetical scenario where bacterial uptake contributes the most to the homogenization of $\delta^{34}\text{S}$ DMS(P) in natural settings. Future experiments assessing 1) the fractionation towards ^{34}S depleted DMS preference of DMSP lyases, and 2) the magnitude and sign of the sulfur isotopic fractionation associated with bacterial uptake are needed to test this hypothesis.

Photosymbiosis contribution to the marine sulfur cycle --- Current understanding of DMS(P) cycling in pelagic ecosystems recognizes phytoplankton, particularly the nano-size fraction (2-20 μ m) with abundant representatives of high DMSP producer classes such as Prymnesiophyceae and Dinophyceae, and also diatoms (Bucciarelli et al. 2013), as the primary DMSP producers (Keller et al. 1989; Stefels et al. 2007). However, the concentration

of DMSP observed in symbiotic Radiolaria and Foraminifera, suggests that larger
 photosymbiotic plankton (50-2000 μm) that harbor photosynthetic endosymbionts from these
 same phytoplankton groups (Siano et al. 2010; Decelle et al. 2012; Probert et al. 2014; Yuasa
 et al. 2016), can represent a significant source of marine DMS(P) particularly in (sub)tropical
 latitudes where high abundances have been reported (Caron et al. 1995; Dennett et al. 2002;
 Biard et al. 2016). To evaluate this hypothesis we calculated the Radiolaria-associated
 contribution to total water column DMSP in the euphotic zone of a number of stations from
 the (sub)tropical-oriented *Tara* Oceans expedition track, using the mean group-specific
 DMSP concentration for Radiolaria (Table 1) and *in situ* abundance and biovolume obtained
 with the Underwater Video Profiler (UVP)(Biard et al. 2016), and micro- and nano-
 phytoplankton-associated DMSP estimated from chlorophyll-based empirical relationships
 (Belviso et al. 2004). The potential contribution of large symbiotic Radiolaria (UVP lower
 detection threshold = 600 μm) to water column DMSP was low on average ($1.1 \pm 2.2\%$, mean
 $\pm$ SD, $n=19$) although peaks of moderate contribution (8%) were inferred associated with
 Acantharia. Despite being inefficiently captured by the UVP – Acantharia are numerically
 dominated by <150 μm forms (Michaels 1988; Caron and Swanberg 1990) – these group of
 Radiolaria potentially contribute the most DMSP in virtue of their high DMSP intracellular
 concentration. Moreover, the Radiolaria-associated DMSPt may represent an important
 source of sulfur for higher trophic levels that cannot effectively prey on smaller
 nanophytoplankton. Considering all these caveats, this conservative approximation indicates
 that photosymbiotic plankton can represent a significant source of DMS(P), particularly in
 tropical and subtropical oceans, where the high DMSP producers that are typical of temperate
 and sub-polar latitudes (e.g. *E. huxleyi* or *Phaeocystis* sp.), are less abundant and Radiolaria
 and Foraminifera represent an important component of plankton communities (Caron et al.

1995; Decelle et al. 2015; Biard et al. 2016), and can account for up to 20% of primary production (Michaels 1988). In this regard, the distinct sulfur isotopic composition of DMSP in *Collodaria-Brandtodinium* holobiont, one of the most abundant and ecologically relevant group of photosymbiotic plankton (Dennett et al. 2002; de Vargas et al. 2015; Guidi et al. 2016), presents the possibility of assessing the contribution of photosymbiotic plankton to community DMSP production in the future. Beyond biogeochemical considerations, the highly ^{34}S enriched DMSP and the lack of apparent sulfate assimilation into DMSP observed in *Collodaria-Brandtodinium* associations suggest that DMSP biosynthetic pathways could be linked to the recycling of organic sulfur between symbiotic partners. If use of organic sulfur for DMSP synthesis also occurs among free-living phytoplankton, we may be able to discriminate between ‘new’ (from sulfate) vs ‘recycled’ (from organic S) DMSP production by their differential S-isotopic signature, in a similar way to approaches using nitrogen isotopes to apportion new and recycled nitrogen sources for primary production.

References

- Amrani, A., W. Said-Ahmad, Y. Shaked, and R. P. Kiene. 2013. Sulfur isotope homogeneity of oceanic DMSP and DMS. *Proc. Natl. Acad. Sci. U S A* **110**: 18413-18418.
- Amrani, A., A. L. Sessions, and J. F. Adkins. 2009. Compound-specific delta s-34 analysis of volatile organics by coupled GC/multicollector-ICPMS. *Anal. Chem.* **81**: 9027-9034.
- Anderson, O. R. 1978. Light and electron-microscopic observations of feeding-behavior, nutrition, and reproduction in laboratory cultures of *thalassicolla-nucleata*. *Tissue & Cell* **10**: 401-412.
- . 1983. *Radiolaria*. Springer.
- Andreae, M. O. 1997. Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry. *Science* **276**: 1052-1058.
- Bates, T. S., B. K. Lamb, A. Guenther, J. Dignon, and R. E. Stoiber. 1992. Sulfur emissions to the atmosphere from natural sources. *J. Atmos. Chem.* **14**: 315-337.
- Belviso, S., C. Moulin, L. Bopp, and J. Stefels. 2004. Assessment of a global climatology of oceanic dimethylsulfide (DMS) concentrations based on seawifs imagery (1998-2001). *Can. J. Fish. Aquat. Sci.* **61**: 804-816.
- Berges, J. A., D. J. Franklin, and P. J. Harrison. 2001. Evolution of an artificial seawater medium: Improvements in enriched seawater, artificial water over the last two decades. *J. Phycol.* **37**: 1138-1145.
- Biard, T., and others. Global in situ imaging observations reveal the biomass of rhizaria in the oceans. DOI 10.1038/nature17652
- Blunden, G., B. E. Smith, M. W. Irons, M. H. Yang, O. G. Roch, and A. V. Patel. 1992. Betaines and tertiary sulfonium compounds from 62 species of marine-algae. *Biochem. Syst. Ecol.* **20**: 373-388.

587 Broadbent, A. D., and G. B. Jones. 2004. DMS and DMSP in mucus ropes, coral mucus,
588 surface films and sediment pore waters from coral reefs in the great barrier reef. *Mar.*
589 *Freshwater Res.* **55**: 849-855.

590 Broadbent, A. D., G. B. Jones, and R. J. Jones. 2002. DMSP in corals and benthic algae from
591 the great barrier reef. *Estuar. Coast. Shelf Sci.* **55**: 547-555.

592 Bucciarelli, E., C. Ridame, W. G. Sunda, C. Dimier-Hugueney, M. Cheize, and S. Belviso.
593 2013. Increased intracellular concentrations of DMSP and DMSO in iron-limited
594 oceanic phytoplankton *thalassiosira oceanica* and *trichodesmium erythraeum*. *Limnol.*
595 *Oceanogr.* **58**: 1667-1679.

596 Calhoun, J. A., T. S. Bates, and R. J. Charlson. 1991. Sulfur isotope measurements of
597 submicrometer sulfate aerosol-particles over the pacific-ocean. *Geophys. Res. Lett.*
598 **18**: 1877-1880.

599 Caron, D. A., P. D. Countway, A. C. Jones, D. Y. Kim, and A. Schnetzer. 2012. Marine
600 protistan diversity, p. 467-493. *In* C. A. Carlson and S. J. Giovannoni [eds.], *Annual*
601 *review of marine science*, vol 4. *Ann. Rev. Mar. Sci.*

602 Caron, D. A., A. F. Michaels, N. R. Swanberg, and F. A. Howse. 1995. Primary productivity
603 by symbiont-bearing planktonic sarcodines (acantharia, radiolaria, foraminifera) in
604 surface waters near bermuda. *J. Plankton Res.* **17**: 103-129.

605 Caron, D. A., and N. R. Swanberg. 1990. The ecology of planktonic sarcodines. *Rev. Aquat.*
606 *Sci.* **3**: 147-180.

607 Caruana, A. M. N., and G. Malin. 2014. The variability in DMSP content and DMSP lyase
608 activity in marine dinoflagellates. *Prog. Oceanogr.* **120**: 410-424.

609 Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic
610 phytoplankton, atmospheric sulfur, cloud albedo and climate. *Nature* **326**: 655-661.

611 de Vargas, C., and others 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science*
612 **348**.

613 Decelle, J., S. Colin, and R. A. Foster. 2015. Photosymbiosis in marine planktonic protists. *In*
614 S. Ohtsuka and T. Suzaki, Horiguchi, T., Suzuki, N., Not, F. [eds.], *Marine protists*
615 *diversity and dynamics*. Springer.

616 Decelle, J., and others 2012. An original mode of symbiosis in open ocean plankton. *Proc.*
617 *Natl. Acad. Sci. U S A* **109**: 18000-18005.

618 Dennett, M. R., D. A. Caron, A. F. Michaels, S. M. Gallager, and C. S. Davis. 2002. Video
619 plankton recorder reveals high abundances of colonial radiolaria in surface waters of
620 the central north pacific. *J. Plankton Res.* **24**: 797-805.

621 Deschaseaux, E. S. M., and others 2014. Effects of environmental factors on dimethylated
622 sulfur compounds and their potential role in the antioxidant system of the coral
623 holobiont. *Limnol. Oceanogr.* **59**: 758-768.

624 Exton, D. A., T. J. McGenity, M. Steinke, D. J. Smith, and D. J. Suggett. 2015. Uncovering
625 the volatile nature of tropical coastal marine ecosystems in a changing world. *Glob.*
626 *Chang. Biol.* **21**: 1383-1394.

627 Frade, P., V. Schwaninger, B. Glasl, R. W. Hill, R. Simó, and G. J. Herndl. 2016.
628 Dimethylsulfoniopropionate in corals and its interrelations with bacterial assemblages
629 in coral surface mucus. *Environ. Chem.* **13**: 252-265.

630 Gage, D. A., D. Rhodes, K. D. Nolte, W. A. Hicks, T. Leustek, A. J. L. Cooper, and A. D.
631 Hanson. 1997. A new route for synthesis of dimethylsulphoniopropionate in marine
632 algae. *Nature* **387**: 891-894.

633 Gali, M., V. Salo, R. Almeda, A. Calbet, and R. Simó. 2011. Stimulation of gross
634 dimethylsulfide (DMS) production by solar radiation. *Geophys. Res. Lett.* **38**.

635 Garces, E., E. Alacid, A. Rene, K. Petrou, and R. Simó. 2013. Host-released dimethylsulphide
 636 activates the dinoflagellate parasitoid parvilucifera sinerae. ISME J. **7**: 1065-1068.
 637 Giesemann, A., H. J. Jager, A. L. Norman, H. P. Krouse, and W. A. Brand. 1994. Online
 638 sulfur-isotope determination using an elemental analyzer coupled to a mass-
 639 spectrometer. Anal. Chem. **66**: 2816-2819.
 640 Guidi, L., S. Chaffron, L. Bittner and others. 2016. Plankton networks driving carbon export
 641 in the oligotrophic ocean. Doi:10.108/nature16942
 642 Hill, R. W., J. W. H. Dacey, and A. Edward. 2000. Dimethylsulfoniopropionate in giant clams
 643 (tridacnidae). Biol. Bull. **199**: 108-115.
 644 Kaplan, I. R., and S. C. Rittenberg. 1964. Microbiological fractionation of sulphur isotopes. J.
 645 Gen. Microbiol. **34**: 195-212.
 646 Karsten, U., S. Koch, J. A. West, and G. O. Kirst. 1996. Physiological responses of the
 647 eulittoral macroalga stictosiphonia hookeri (rhodomelaceae, rhodophyta) from
 648 argentina and chile: Salinity, light and temperature acclimation. Eur. J. Phycol. **31**:
 649 361-368.
 650 Kaye, J. A. 1987. Mechanisms and observations for isotope fractionation of molecular-species
 651 in planetary-atmospheres. Rev Geophys **25**: 1609-1658.
 652 Keller, M. D., Selvin, R. C., Claus, W. & Guillard, R. R. L. 1987. Media for the culture of
 653 oceanic ultraphytoplankton. J. Phycol. 23:633–8.
 654 Keller, M. D., W. K. Bellows, and R. R. L. Guillard. 1989. Dimethyl sulfide production in
 655 marine phytoplankton Acs Symposium Series **393**: 167-182.
 656 Kiene, R., and D. Slezak. 2006. Low dissolved DMSP concentrations in seawater revealed by
 657 small-volume gravity filtration and dialysis sampling. Limnol. Oceanogr. Methods **4**:
 658 80-95.

659 Kirst, G. O. 1990. Salinity tolerance of eukaryotic marine-algae. *Annu. Rev. Plant. Biol.* **41**:
660 21-53.

661 Kirst, G. O., C. Thiel, H. Wolff, J. Nothnagel, M. Wanzek, and R. Ulmke. 1991.
662 Dimethylsulfoniopropionate (DMSP) in ice-algae and its possible biological role. *Mar.*
663 *Chem.* **35**: 381-388.

664 Lana, A., R. Simó, S. M. Vallina, and J. Dachs. 2012. Re-examination of global emerging
665 patterns of ocean DMS concentration. *Biogeochemistry* **110**: 173-182.

666 Lavoie, M., Levasseur, M., and M. Babin. 2015. Testing the potential ballast role for
667 dimethylsulfoniopropionate in marine phytoplankton: A modeling study. *J Plankton*
668 *Res.* **0**:1-13.

669 Lavoie, M., Raven, J. A., and M. Levasseur. 2016. Energy cost and putative benefits of
670 cellular mechanisms modulating buoyancy in a flagellate marine phytoplankton. *J.*
671 *Phycol.* **52**:239-51.

672 Lyon, B. R., J. M. Bennett, P. A. Lee, M. G. Janech, and G. R. DiTullio. 2016 Role of
673 dimethylsulfoniopropionate as an osmoprotectant following gradual salinity shifts in
674 the sea-ice diatom *fragilariopsis cylindrus*. *Environ. Chem.* **13**: 181-194.

675 Malin, G., and M. Steinke. 2004. Dimethyl sulfide production: What is the contribution of the
676 coccolithophores? In: Thierstein HR, Young JR (eds) *Coccolithophores: from*
677 *molecular processes to global impact*. Springer, Berlin, p 127–164

678 Marie, D., F. Partensky, S. Jacquet, and D. Vaulot. 1997. Enumeration and cell cycle analysis
679 of natural populations of marine picoplankton by flow cytometry using the nucleic
680 acid stain SYBR green I. *Applied and environmental microbiology* **63**: 186-193.

681 Michaels, A. F. 1988. Vertical-distribution and abundance of acantharia and their symbionts.
682 *Mar. Biol.* **97**: 559-569.

683 Norman, A. L., L. A. Barrie, D. Toom-Sauntry, A. Sirois, H. R. Krouse, S. M. Li, and S.
 684 Sharma. 1999. Sources of aerosol sulphate at alert: Apportionment using stable
 685 isotopes. *J. Geophys. Res.: Atmospheres* **104**: 11619-11631.
 686 Not, F., J. del Campo, V. Balague, C. de Vargas, and R. Massana. 2009. New insights into the
 687 diversity of marine picoeukaryotes. *Plos One* **4**: e7143
 688 Nowack, E. C., and M. Melkonian. 2010. Endosymbiotic associations within protists. *Phil.*
 689 *Trans. R. Soc. B, Biol. Sci.* **365**: 699-712.
 690 Oduro, H., K. L. Van Alstyne, and J. Farquhar. 2012. Sulfur isotope variability of oceanic
 691 DMSP generation and its contributions to marine biogenic sulfur emissions. *Proc.*
 692 *Natl. Acad. Sci. U S A* **109**: 9012-9016.
 693 Patris, N., R. Delmas, M. Legrand, M. De Angelis, F. A. Ferron, M. Stiévenard, and J. Jouzel.
 694 2002. First sulfur isotope measurements in central greenland ice cores along the
 695 preindustrial and industrial periods. *J. Geophys. Res: Atmos* **107**: ACH 6-1-ACH 6-
 696 11.
 697 Probert, I., R. Siano, C. Poirier and others 2014. Brandtodinium gen. Nov. And b. Nutricula
 698 comb. Nov. (dinophyceae), a dinoflagellate commonly found in symbiosis with
 699 polycystine radiolarians. *J. Phycol.* **50**: 388-399.
 700 Quinn, P. K., and T. S. Bates. 2011. The case against climate regulation via oceanic
 701 phytoplankton sulphur emissions. *Nature* **480**: 51-56.
 702 Raina, J. B., E. A. Dinsdale, B. L. Willis, and D. G. Bourne. 2010. Do the organic sulfur
 703 compounds DMSP and DMS drive coral microbial associations? *Trends Microbiol.*
 704 **18**: 101-108.
 705 Raina, J. B., D. M. Tapiolas, S. Foret and others 2013. DMSP biosynthesis by an animal and
 706 its role in coral thermal stress response. *Nature* **502**: 677-680.

707 Said-Ahmad, W., and A. Amrani. 2013. A sensitive method for the sulfur isotope analysis of
708 dimethyl sulfide and dimethylsulfoniopropionate in seawater. *Rapid Commun. Mass*
709 *Sp.: RCM* **27**: 2789-2796.

710 Savoca, M. S., and G. A. Nevitt. 2014. Evidence that dimethyl sulfide facilitates a tritrophic
711 mutualism between marine primary producers and top predators. *Proc. Natl. Acad.*
712 *Sci. U S A* **111**: 4157-4161.

713 Schoemann, V., S. Becquevort, J. Stefels, V. Rousseau, and C. Lancelot. 2005. Phaeocystis
714 blooms in the global ocean and their controlling mechanisms: A review. *J. Sea. Res.*
715 **53**: 43-66.

716 Seymour, J. R., R. Simó, T. Ahmed, and R. Stocker. 2010. Chemoattraction to
717 dimethylsulfoniopropionate throughout the marine microbial food web. *Science* **329**:
718 342-345.

719 Siano, R., M. Montresor, I. Probert, F. Not, and C. de Vargas. 2010. Pelagodinium gen. Nov.
720 And p. Beii comb. Nov., a dinoflagellate symbiont of planktonic foraminifera. *Protist*
721 **161**: 385-399.

722 Simó, R. 2001. Production of atmospheric sulfur by oceanic plankton: Biogeochemical,
723 ecological and evolutionary links. *Trends Ecol. Evol.* **16**: 287-294.

724 Stefels, J. 2000. Physiological aspects of the production and conversion of DMSP in marine
725 algae and higher plants. *J. Sea. Res.* **43**: 183-197.

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

729 Steinke, M., P. Brading, P. Kerrison, M. E. Warner, and D. J. Suggett. 2011. Concentrations
730 of dimethylsulfoniopropionate and dimethyl sulfide are strain-specific in symbiotic
731 dinoflagellates (*Symbiodinium* sp., dinophyceae). *J. Phycol.* **47**: 775-783.

732 Stemmann, L., M. Youngbluth, K. Robert and others 2008. Global zoogeography of fragile
733 macrozooplankton in the upper 100-1000 m inferred from the underwater video
734 profiler. *ICES J. Mar. Sci.* **65**: 433-442.

735 Stoecker, D. K., M. D. Johnson, C. deVargas, and F. Not. 2009. Acquired phototrophy in
736 aquatic protists. *Aquat. Microb. Ecol.* **57**: 279-310.

737 Summers, P., K. Nolte, A. Cooper, T. Leustek, D. Rhodes, and A. Hanson. 1998.
738 Identification and stereospecificity of the first three enzymes of 3-
739 dimethylsulfoniopropionate biosynthesis in a chlorophyte alga. *Plant. Physiol.* **116**:
740 369-378.

741 Sunda, W., D. J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for
742 DMSP and DMS in marine algae. *Nature* **418**: 317-320.

743 Suzuki, N., and F. Not. 2015. Biology and ecology of radiolaria. 179-222.

744 Swanberg, N. R., and O. R. Anderson. 1985. The nutrition of radiolarians: Trophic activity of
745 some solitary spurnellaria. *Limnol. Oceanogr.* **30**: 646-652.

746 Vairavamurthy, A., M. O. Andreae, and R. L. Iverson. 1985. Biosynthesis of dimethylsulfide
747 and dimethylpropiothetin by *hymenomonas-carterae* in relation to sulfur source and
748 salinity variations. *Limnol. Oceanogr.* **30**: 59-70.

749 Van Alstyne, K. L., V. J. Dominique, III, and G. Muller-Parker. 2009. Is
750 dimethylsulfoniopropionate (DMSP) produced by the symbionts or the host in an
751 anemone-zooxanthella symbiosis? *Coral Reefs* **28**: 167-176.

- 752 Van Alstyne, K. L., P. Schupp, and M. Slattery. 2006. The distribution of
753 dimethylsulfoniopropionate in tropical pacific coral reef invertebrates. *Coral Reefs* **25**:
754 321-327.
- 755 Yuasa, T., T. Horiguchi, S. Mayama, and O. Takahashi. 2016. *Gymnoxanthella radiolariae*
756 gen. Et sp. Nov. (dinophyceae), a dinoflagellate symbiont from solitary polycystine
757 radiolarians. *J. Phycol.* **52**: 89-104.

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Figure Captions

Figure 1. Symbiotic Radiolaria and Foraminifera specimens isolated from the NW Mediterranean and Red sea surface waters imaged under the binocular (a, b, d, e, f, g) and light microscopy (c, h, i). Single-celled acantharians *Amphilonche elongata* (a), ‘Star’ (b), ‘Translucid’ (c) morphotypes with *Phaeocystis* sp. endosymbiotic algae. Single-celled Foraminifera *Globigerinella* sp. with *Pelagodinium beii* endosymbionts (d), Solitary *Thalassicolla* sp. (e), and colonial *Sphaerouzoum* sp. (f, h) and *Collozoum* sp. (g, i) collodarian specimens with *Brandtodinium nutricula* endosymbionts.

Figure 2. Schematic representation of the experimental design and sequence of operations followed for (a) the sulfate assimilation and (b) the DMS production experiments described in the methods.

Figure 3. Sulfur isotopic composition of intracellular DMSP ($\delta^{34}\text{S}$ -DMSP) of freshly-collected symbiotic Radiolaria (Acantharia, solid-brown; Collodaria, green stars) and Foraminifera (red square), of cultured free-living symbionts isolated from the same radiolarian groups *B. nutricula* (green triangles) and *Phaeocystis* (empty-brown), and of natural microbial assemblages collected from surface waters. Seawater sulfate isotopic composition ($\delta^{34}\text{S}$ - SO_4^{2-}) (grey diamond). The range of $\delta^{34}\text{S}$ -DMSP from previous measurements in a bloom of *Prorocentrum minimum* and isolated macroalgae (Oduro et al. 2012) and surface seawater (Amrani et al. 2013, area between dash lines) are shown as reference.

Figure 4. Sulfur isotopic composition of sulfate ($\delta^{34}\text{S}$ - SO_4^{2-}) and intracellular DMSP ($\delta^{34}\text{S}$ -DMSP) in cultured free-living *Phaeocystis* RCC1383 (A), *Brandtodinium nutricula* (B), and freshly-collected Collodaria-*Brandtodinium* holobiont (C) incubated for 3-day in two different culture media containing sulfate with standard (‘Standard’) and light (‘Light’) $\delta^{34}\text{S}$ - SO_4^{2-} , respectively. Cell abundance for free-living cultured microalgae incubations is shown.

Figure 5. Sulfur isotopic composition of particulate DMSP ($\delta^{34}\text{S}$ -DMSP, black bars) and dissolved DMS ($\delta^{34}\text{S}$ -DMS, white bars) in freshly-collected symbiotic Radiolaria (Acantharia-*Phaeocystis*) and microbial assemblage sampled from the same waters in the Red Sea where the Radiolaria were collected, measured after 4 hours of incubation under natural sunlight conditions in surface waters. Squares with the error bars represent the concentration of dissolved DMSP (black) and DMS (white) at the end of the incubation. Error bars represent the standard error of the mean. Microbial assemblage (*wsw*), Radiolaria assemblage (*fsw+rads*), Filtered seawater (*fsw-only*).

810 TABLE 1. Cell and colony size, DMSPt cell content, and intracellular concentration in uncultured
811 symbiotic Radiolaria and Foraminifera (host+microalgae), and cultured free-living symbiotic
812 microalgae isolated from the same species of Radiolaria and Foraminifera. The abundance of
813 radiolarian cells per colony and the abundance of symbiont cells per holobiont were assessed from
814 image analysis of freshly-collected organisms. DMSPt cellular content per symbiont *in hospite*
815 estimated assuming all DMSPt measured in the holobiont is allocated in the symbiotic microalgae. Cc
816 refers to central capsule, *n/a* and *n/d* refer to not applicable and not determined, respectively. The
817 number of measurements are shown in brackets. The errors refer to the standard deviation of the mean.

TABLE 1 (All growth phases)			Size (um) Major axis (μm)	Size (um) Minor axis (μm)	DMSPt Content pmol cell ⁻¹	DMSPt Concentration mmol L ⁻¹	Number central capsules colony ⁻¹
Collodaria-Brandtodinium sp. (Radiolaria)							
<i>Collozoum</i> sp.	Colonial	Symbiotic	7267 ±1793(3)	3754 ±243(3)	3652 ±2008(3)	0.1 ±0.1(3)	509 ±72(3)
<i>Sphaerouzoum</i> sp.	Colonial	Symbiotic	8242 ±2041(4)	3463 ±1327(4)	3135 ±236(3)	0.1 ±0.01(3)	166 ±65(17)
<i>Thalassicolla</i> sp.	Solitary	Symbiotic	3972 ±1148(5)	3517 ±939(5)	2757 ±750(5)	0.2 ±0.1 (5)	<i>n/a</i>
Acantharia-Phaeocystis sp. (Radiolaria)							
<i>Amphilonche elongata</i>	Single- celled	Symbiotic	391 ±117(31)	64 ±30(31)	15.2 ±5.4(4)	17.1 ±6.1(4)	<i>n/a</i>
Translucid' morphotype	Single- celled	Symbiotic	483 ±188(12)	437 ±182(12)	20.9 ±3.6(3)	0.3 ±0.1(3)	<i>n/a</i>
Star shape' morphotype	Single- celled	Symbiotic	307 ±114(42)	242 ±119(42)	36.4 ±10.4(5)	2.4 ±0.7 (5)	<i>n/a</i>
Foraminifera-P. beii							
<i>Globigerinella</i> sp. (Planktonic)	Single- celled	Symbiotic	235 ±87(31)	209 ±58(31)	37.8 ±15.5(5)	7.5 ±3.1(5)	<i>n/a</i>
Free-living symbiotic							
<i>Brandtodinium nutricula</i> (RCC3468) (Dinophyceae)	Single- celled	Symbiotic	12 ±0.9(15)	8.6 ±0.7(15)	0.2 ±0.1(12)	490 ±107(12)	<i>n/a</i>
<i>Pelagodinium beii</i> (RCC1491) (Dinophyceae)	Single- celled	Symbiotic	20.5 ±1.8(18)	17.7 ±2.3(18)	0.9 ±0.2 (12)	272 ±49(12)	<i>n/a</i>
<i>Gymnoxanthella</i>	Single-	Symbiotic	9.1	7.9	0.1	462	<i>n/a</i>

<i>radiolariae</i> (RCC3507) (Dinophyceae)	celled		±1.0(15)	±0.6(15)	±0.1(12)	±168(12)	
Phaeocystis (RCC1383) (Prymnesiophyceae)	Single- celled	Symbiotic	4.0 nominal	4.0 nominal	0.01 ±0.001(3)	307 ±47(3)	<i>n/a</i>

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Figure 2

Figure 3

Figure 4

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846 SUPPORTING INFORMATION

847 **Intracellular DMSPt concentration of symbiotic microalgae as a function of growth phase and**
848 **diel cycle.** The large error associated to the mean DMSPt cell content and concentration values of
849 cultured symbiotic microalgae (Table 1) reflected the variability associated to growth phase and
850 photoperiod at the time of harvesting the culture. The photoperiod (day vs. night) had a significant
851 effect on DMSPt cellular concentration for *B. nutricula* and *G. radiolariae*, while this was only
852 marginally significant for *P. beii* (two-way ANOVA, $p=0.066$). The effect of growth phase
853 (exponential vs. stationary) on DMSPt content was only significant for *G. radiolariae* (two-way
854 ANOVA, $p<0.0001$), while the interaction between both photoperiod and growth phase was
855 significant for *B. nutricula* and *P. beii* ($p<0.001$). During exponential phase, DMSPt cell content was
856 slightly lower during the day than night for *B. nutricula* and *P. beii*, while the cellular DMSPt
857 concentrations decreased substantially at night for these strains. In *G. radiolariae* DMSPt content
858 decreased at night during both exponential and stationary growth phases ($p=0.003$, Supplementary
859 Figure 1).

Supplementary Figure 1. Mean intracellular DMSP concentration in free-living cultures of *Brandtodinium nutricula* (A), *Gymnoxanthella radiolariae* (B), and *Pelagodinium bei* (C) harvested at different growth phases (exponential vs. stationary) and times of the daily photoperiod (day vs night). Error bars represent the standard error of the mean. A) Growth $F_{1,8}=0.17$, $p=0.69$, photoperiod $F_{1,8}=16.6$ $p=0.0036$, interaction $F_{1,8}=32.1$, $p=0.0005$; B) Growth $F_{1,8}=304$, $p<0.0001$, photoperiod $F_{1,8}=17.7$, $p=0.0030$, interaction $F_{1,8}=2.7$, $p=0.14$; C) Growth $F_{1,8}=1.4$, $p=0.27$, photoperiod $F_{1,8}=4.5$, $p=0.066$, interaction $F_{1,8}=12.9$, $p=0.0071$. Two-way ANOVA not repeated measures.