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

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Running head: Plankton symbiosis and DMS(P) cycling

Key words: Radiolaria, Symbiosis, DMS(P)

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 (^{34}S) of DMSPt in the Collodaria holobionts was significantly higher than their symbiotic microalgae isolated in culture. Despite their high intracellular DMSPt content, SO_4^{2-} uptake in these holobionts throughout 3-day incubations was not detected. We observed a systematic ^{34}S 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 ^{34}S-depleted DMS. Overall, the results indicate that plankton symbiosis can, at times, represent a potentially important source of DMS(P). Specific differences in ^{34}S 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.

93 2012). Both Dinophyceae and Prymnesiophyceae classes are typical major DMSP producers
94 (Keller et al. 1989; Caruana and Malin 2014), and include keystone species for the
95 biogeochemical sulfur and carbon cycles (Malin and Steinke 2004; Schoemann et al. 2005).
96 Acantharia-*Phaeocystis* sp. holobionts exhibit extremely high DMSP cellular content, with
97 values significantly higher than those expected if all DMSP was contained in the
98 endosymbiotic microalgae (Decelle et al. 2012). Should elevated cellular content of DMSP
99 be a common feature not only in benthic, but also in planktonic photosymbiosis, this
100 widespread but traditionally overlooked functional group of plankton (i.e. photosymbiotic)
101 may constitute a relevant source of biogenic DMS(P) previously unaccounted by the standard
102 microplankton oriented (*i.e.* <200 μm) sampling procedures.

103 The complexity of marine biogeochemistry makes it difficult to identify and assess the
104 multiple biological sources and flows of DMS(P) from and through the different
105 compartments of the pelagic ecosystem, and their links to the lower atmosphere (Simó, 2001).
106 In this context, the sulfur isotope ratio ($^{34}S/^{32}S$; *i.e.* $\delta^{34}S$) in aerosol sulfate has been used to
107 assess the contribution of different sources, mainly anthropogenic *vs* marine (Kaye 1987;
108 Norman et al. 1999; Patris et al. 2002). Recent pioneering measurements in macroalgae and
109 natural phytoplankton assemblages have provided similar $\delta^{34}S$ values for DMSP but have
110 contradictory views of the isotopic fractionation associated with biotransformation processes
111 in the formation of DMS (Oduro et al. 2012; Amrani et al. 2013). In natural planktonic
112 systems, phytoplankton cells are often too small and too intermixed with other organisms to
113 be isolated in sufficient quantities for analysis of sulfur isotopic composition of DMSP by
114 conventional isotope ratio analysis methods. This hampers the characterization of the isotopic
115 composition of specific phytoplankton taxa or even functional groups in the field, and little is
116 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

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

143 Field samples of symbiotic Radiolaria and Foraminifera were collected in coastal
144 waters of the Red Sea in Eilat (29°33'N, 34°57'E) and the western Mediterranean in
145 Villefranche-sur-Mer (43°42'N, 7°18'E) during March and June of 2014, respectively.
146 Plankton community was sampled using plankton net with 220 μm mesh size towed obliquely
147 (0-30 m) for 10 minutes from a boat or swimming at surface (0-5 m) for approximately 200
148 m. Collected samples were immediately diluted in buckets with freshly collected surface
149 seawater, protected from direct sunlight, and transported to the lab within less than an hour.
150 Individual specimens were then manually sorted under a stereomicroscope using a
151 micropipette and transferred to Petri dishes in the case of single-celled acantharians and
152 foraminiferans, and to larger beakers in the case of collodarians, where they were rinsed with
153 surface 0.22 μm filtered seawater (fsw) before following DMSP/image analysis or
154 experimental procedure. This single-cell approach allowed us to assess intracellular DMSP
155 concentration and isotopic composition (δ^{34}S-DMSP) in ecologically relevant, uncultured
156 specific symbiotic taxa. While large collodarians could be identified to genus level based on
157 morphological characteristics, identification was not so reliable for Acantharia, and so related
158 morphotypes of different species were used in subsequent measurements (Fig. 1). Samples
159 manipulation and experimental work was conducted in the laboratory facilities of the
160 Interuniversity Institute for Marine Sciences in Eilat (Israel) and the Observatoire
161 Oceanologique de Villefranche-sur-Mer (France).

162 ***Image analysis and cellular biovolume assessment and microalgae cell counts ---***
163 Cultured and uncultured organisms were imaged using a digital camera (Canon EOS 5D)
164 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 FACSAria (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 N_2 as the counter flow. A Teflon sample loop was inserted in a dewar of liquid N_2 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 N_2 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 SF_6 internal standard (Said-Ahmad and Amrani 2013). The results are expressed in conventional $\delta^{34}S$ notation as a per mil (‰) deviation from the international standard V-CDT (Vienna Canyon Diablo Troilite) according to the equation below.

$$\delta^{34}S = \left({}^{34}R / {}^{32}R \right)$$

where ^{34}R is the integrated $^{34}S/^{32}S$ 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-

237 0.4‰ (1σ standard deviation). The precision of sulfur isotope analysis for duplicate or
238 triplicate samples of seawater and organism samples were usually less than 1‰. Accuracy as
239 calculated by standards was in the range of 0.2 ‰ (Said-Ahmad and Amrani 2013) and
240 estimated to be better than 1‰ for the seawater samples.

241 Dissolved sulfate (SO_4^{2-}) sulfur isotope analysis was performed by a conventional
242 elemental analyzer (EA) coupled to isotope ratio mass spectrometer (IRMS) method
243 (Giesemann et al. 1994) using Delta Plus (Thermo) IRMS. Samples of $BaSO_4$ were prepared
244 from diluted seawater samples by addition of 5% $BaCl_2$ solution and then analyzed for their
245 $\delta^{34}S$ values by EA-IRMS. The sulfur isotope reference materials NBS-127 ($BaSO_4$; $\delta^{34}S$ =
246 21.1‰), IAEA-S-1 (Ag_2S; -0.3‰), and IAEA-SO-6 ($BaSO_4$; -34.1‰) were purchased from
247 the National Institute of Standards and Technology (NIST) and used for calibration. Precision
248 of this method for duplicates/triplicates was usually better than 0.3‰.

249 ***Sulfate assimilation and DMSP synthesis experiment ---*** For the biological sulfate
250 assimilation experiment we incubated two cultured strains of microalgae
251 (*Phaeocystis*_RCC1383 and *B.nutricula*_RCC3468) and freshly-collected specimens of
252 solitary symbiotic *Thalasicolla* sp. (Collodaria-*Brandtodinium* holobiont) during three days in
253 two different types of K/2 culture media prepared with isotopically distinct sulfate (Fig. 2a).
254 One media ('Heavy') was prepared with filtered seawater amended with standard K/2
255 supplements to yield an average seawater sulfate $\delta^{34}S$ of 21.5 ± 0.5‰ at the beginning of the
256 incubations. The second media ('Light') consisted of artificial seawater (Berges et al. 2001)
257 prepared using isotopically depleted $\delta^{34}S$-Sulfate relative to seawater sulfate, and mixed in 1:1
258 ratio with K/2 standard media, as described above, to give a sulfate $\delta^{34}S$ value of 7.6 ± 0.4‰.
259 This procedure resulted in two 2-L batches of media with very distinct $\delta^{34}S$-Sulfate
260 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 µmol photon m^{-2} 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, (*wsw*)), 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 *wsw* 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 ($N_{Sphaerozoum}$=166 ± 65; $N_{Collozoum}$= 509 ± 72 central capsules colony^{-1}). Each central capsule harbored a few endosymbiont cells ($N_{Collozoum}$= 9.8 ± 3.5, $N_{Sphaerozoum}$ = 9.1 ± 2.6 symbionts

309 central capsule^{-1}). Solitary forms**,** consisting of millimeters-size single host cell, contained
310 hundreds of endosymbiotic cells ($N_{Thalassicolla}$ = 723 ± 703 symbionts radiolarian cell^{-1}) (Fig. 1,
311 Table 1). DMSPt content in different morphotypes of single-celled Acantharia and planktonic
312 Foraminifera were much lower, according to their lower cell size, and varied within a
313 relatively narrow range (15-38 pmol cell^{-1}). Foraminifera (37.8 ± 15.5 pmol cell^{-1}) and 'Star'
314 morphotype acantharians (36.4 ± 10.4 pmol cell^{-1}) exhibited higher values than *A. elongata*
315 (15.2 ± 5.4 pmol cell^{-1}) and 'Translucid' acantharians (20.9 ± 3.6 pmol cell^{-1}), yet differences
316 were only significant between *A. elongata* and *Globigerinella* sp. (p=0.03, $F_{3.12}$=4.1, one-way
317 ANOVA, Tukey's post hoc test). Analysis of DMSP concentration per cellular biovolume
318 yielded a different picture with *A. elongata* (17.1 ± 6.1 mmol L^{-1}) having significantly higher
319 concentrations than Foraminifera (7.5 ± 3.1 mmol L^{-1}), 'Star' (2.4 ± 0.7 mmol L^{-1}) and
320 'Translucid' (0.3 ± 0.1 mmol L^{-1}) acantharian morphotypes, while both solitary and colonial
321 collodarian species showed significantly lower concentrations (p<0.001, $F_{6,16}$=14.8) (Table 1).

322 Cellular DMSPt content in cultures of free-living algae was markedly lower
323 (range=0.01-1.24 pmol cell^{-1}) according to their smaller size compared to their hosts (Table
324 1). Highest mean values were measured in *P. beii* (0.9 ± 0.2 pmol cell^{-1}); these were 3-4-fold
325 higher than in *B. nutricula* (0.2 ± 0.1 pmol cell^{-1}) while *G. radiolariae* exhibited intermediate
326 average cell DMSPt content (0.1 ± 0.1 pmol cell^{-1}). DMSPt concentration calculated from
327 microscope-based estimates of cellular biovolume in free-living algae was one-to-three orders
328 of magnitude higher than concentration in the radiolarian and foraminiferan holobionts (Table
329 1). *B. nutricula* (490 ± 107 mmol L^{-1}) and *G. radiolariae* (462 ± 168 mmol L^{-1}) showed
330 higher values than *Phaeocystis* RCC1383 (307 ± 47 mmol L^{-1}) and *P. beii* (272 ± 49 mmol L^{-}
331 ^{1}), although differences were only significant for *P. beii* (p=0.0001, $F_{3,35}$=9.1, one-way
332 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 (δ^{34}S-DMSP) **---** The mean δ^{34}S-DMSP in isolated holobiont specimens of Acantharia-*Phaeocystis* sp. (δ^{34}S-DMSP = 19.8 ± 0.4‰) and Foraminifera-*P. beii* (δ^{34}S-DMSP = 20.1 ± 0.4‰) were very similar to each other, and also to the natural microbial assemblage coexisting in the same surface water (δ^{34}S-DMSP = 19.7 ± 0.4‰, Fig. 3). These values were slightly lower than δ^{34}S of local seawater sulfate (δ^{34}S-SO_4^{2-} =21.5 ± 0.5‰). In contrast, the Collodaria-*Brandtodinium* holobiont showed a very distinct DMSP isotopic composition (δ^{34}S-DMSP = 23.5±0.8‰), significantly enriched not only compared to other symbiotic groups (Acantharia and Foraminifera) and microbial assemblage, but also compared to δ^{34}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 ± 0.3‰) showing significantly lower δ^{34}S-DMSP values compared to *Phaeocystis* RCC1383 (20.2 ± 0.4‰, Tukey comparison test, p<0.001, Fig. 3). In the case of *B. nutricula* the δ^{34}S-DMSP values were significantly depleted relative to the Collodaria-*B. nutricula* holobiont (Tukey comparison test, p<0.0001), whereas the δ^{34}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 δ^{34}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 δ^{34}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, δ^{34}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 **---** δ^{34}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 *wsw*, 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, δ^{34}S-DMS measured from incubations containing photosymbiotic Radiolaria (Acantharia-*Phaeocystis* holobiont, *fsw+rads*) was significantly lower (18.4 ± 0.4 ‰; $p<0.0001$, $F_{5,14}=12$, one-way ANOVA). Specifically, DMS-δ^{34}S values measured in the incubations with Radiolaria were significantly depleted relative to δ^{34}S-DMSPt values in both *A. elongata* (1.2 ‰ mean difference) and 'Star' radiolarian morphotypes (1.5 ‰ mean difference). Similarly, δ^{34}S-DMS in *fsw-only* treatment incubated in parallel (18.0 ± 0.5 ‰) was depleted relative to δ^{34}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;

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

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

405 with similar concentrations to those measured in free-living cultures, the host should harbor
406 1250-3000 algal cells, which is a 100-fold more than the 10-20 cells commonly found in its
407 cytoplasm. Acknowledging the limits of our data, these calculations suggest that DMSP
408 could be present in the host as well. Whether the DMSP is translocated from the algae to the
409 host (Van Alstyne et al. 2009) and/or produced by the latter (Raina et al. 2013) cannot be
410 concluded from concentration measurements alone. Regardless of its origin, the moderate-to-
411 high cellular content of DMSP estimated for the host and the algae are consistent with recent
412 observations in Radiolaria (Decelle et al. 2012) and other photosymbiotic organisms (Hill et
413 al. 2000; Stefels 2000; Broadbent et al. 2002; Van Alstyne et al. 2006), and suggest that both
414 partners could mutually benefit from one or more of the multiple eco-physiological roles
415 attributed to dimethyl sulfur compounds, e.g., osmoregulation, oxidant scavenger or info-
416 chemical (Stefels 2000; Seymour et al. 2010; Raina et al. 2013).

417 ***Sulfate assimilation and DMSP production in photosymbiotic associations*** – Highly
418 enriched δ^{34}S-DMSP observed in Collodaria-*Brandtodinium* sp. holobiont specimens (23.5 ±
419 0.8 ‰; Fig. 3) was particularly unexpected. This association showed significantly enriched
420 δ^{34}S-DMSP not only relative to seawater SO_4^{2-} but also compared to other symbiotic
421 radiolarians and all measurements previously reported for both macro- and microalgae, which
422 showed a slight ^{34}S-depletion in DMSP compared to seawater SO_4^{2-} (Oduro et al. 2012;
423 Amrani et al. 2013; Said-Ahmad and Amrani 2013)(Fig. 3). This distinctive sulfur isotopic
424 composition could result from differential fractionation associated with the multi-step
425 assimilation of sulfate into methionine (intracellular precursor to DMSP) and/or may reflect
426 differences in the DMSP biosynthetic pathway. The 1-3‰ ^{34}S depletion in DMSP relative to
427 sulfate during assimilation of sulfate is in agreement with previous reports (e.g. Kaplan &
428 Rittenberg 1964). All three biosynthetic pathways for DMSP described to date for higher

429 plants and algae rely on assimilatory sulfate reduction (Gage et al. 1997; Summers et al.
430 1998). However, our incubation experiments with isotopically distinct sulfate solutions
431 showed that while free-living microalgae did actively take up and assimilate sulfate into
432 DMSP in time scales relevant for phytoplankton growth (Fig. 4a, b), collodarians with
433 elevated cellular content of DMSP showed no isotopic evidence of assimilatory sulfate
434 reduction into DMSP (Fig. 4c). This could be due to slower growth rates of these giant
435 protists compared to microalgae, although the high photosynthetic rates of solitary Radiolaria
436 (Caron et al. 1995) and abundance of endosymbiotic microalgae in incubated specimens
437 (Table 1, Fig.1) could suggest additional explanations to the lack of sulfate uptake. The
438 holobiont may for instance rely on inorganic or reduced sulfur species (e.g. methionine,
439 cysteine) stored by the algae for DMSP synthesis. Radiolarians are active grazers (Anderson
440 1978; Swanberg and Anderson 1985; Suzuki and Not 2015), and captured prey and digestion
441 products could provide such sulfur compounds to the symbiotic microalgae, reducing its
442 dependence on sulfate uptake. It is worth noting however, that the assimilatory sulfate
443 reduction only represents a small proportion of the total energetic cost (NADPH and ATP
444 molecules) associated with *de novo* synthesis of DMSP (Lavoie et al. 2016).

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

453 microalgal partners involved in these symbiotic association (*i.e.* Dinophyceae and
454 Prymnesiophyceae), this isotopic variability could reflect differences in the metabolic
455 capacities of the symbiotic algae and/or the holobiont. Differences in δ^{34}S-DMSP observed
456 between *B. nutricula* and *Phaeocystis* RCC1383 (Fig. 3) were of similar magnitude to those
457 reported between macro and microalgae species (Oduro et al. 2012). The observed differences
458 between the two algal strains and the remarkably distinct δ^{34}S-DMSP observed in freshly
459 collected Collodaria-*B. nutricula* associations (Fig. 3) contrast with the homogeneity in δ^{34}S-
460 DMSP composition observed across diverse marine microbial communities (Amrani et al.
461 2013) and illustrate the isotopic variability potentially hidden in natural microbial
462 communities. Little is known about the isotopic variability associated with taxonomic and
463 functional diversity of major DMSP producers. Nonetheless, if adequately characterized, this
464 variability offers an opportunity not only to investigate physiological differences between
465 species in the lab, but also to refine our current ability to track specific sources of DMSP, and
466 derived DMS, in highly intermixed and diverse natural microbial communities (Kaye 1987;
467 Calhoun et al. 1991; Said-Ahmad and Amrani 2013).

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

477 are not calculating fractionation factors (α), but rather apparent fractionation ($\Delta^{34}S$) from the
478 difference between the product and the reactant. Consistent with Amrani et al. (2013), $\delta^{34}S$-
479 DMSP and $\delta^{34}S$-DMS values were similar in whole seawater incubated from the Red Sea,
480 suggesting negligible fractionation is associated with microbial cleavage of DMSP. However,
481 the $\delta^{34}S$ value of DMS produced by symbiotic acantharians was lower (-1.2, -1.5‰) than that
482 of DMSP (Fig. 5), resulting in a DMS fractionation similar in sign and magnitude to that
483 reported for macroalgae by Oduro et al. (2012). These differences in the DMSP to DMS
484 fractionation between the microbial assemblage, symbiotic radiolarians and macroalgae may
485 reflect their taxonomic and functional diversity. However, similar ^{34}S depletion of DMS was
486 observed between *fsw-only* and *fsw+rads* incubations (Fig. 5). Evidences exist that the
487 filtration process can break phytoplankton cells and enrich the filtered seawater with
488 dissolved DMSP and extracellular activity of DMSPlyase (Kiene and Slezak 2006) to the
489 point that it is common to measure substantial DMS production in fsw (Galí et al. 2011).
490 During the 4-hour experiments, DMS was produced in both *fsw-only* and *fsw+rads*
491 incubations, although, the latter, containing approximately 100 specimens of Acantharia (see
492 methods), produced larger amounts of DMS (Fig. 5). Overall, $\delta^{34}S$ values of DMS produced
493 in natural microbial communities were similar or slightly higher than $\delta^{34}S$ values of DMSP
494 (Amrani et al., 2013; this study), while cultures or incubations with isolated organisms in
495 filtered seawater produced ^{34}S-depleted DMS (Oduro et al., 2012; this study). This may
496 reflect the interplay among DMS sources and sinks: DMSPlyase selection for DMSP with
497 lower $\delta^{34}S$ would produce ^{34}S depleted DMS, but preference of DMS-consumption processes
498 (e.g. bacterial uptake, photochemistry, ventilation, and bacterial uptake) for ^{34}S-depleted DMS
499 would buffer the overall result. This possibility was already discussed by Amrani et al.
500 (2013), who invoked these DMS removal processes as potential mechanisms contributing to

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

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

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

549 1995; Decelle et al. 2015; Biard et al. 2016), and can account for up to 20% of primary
550 production (Michaels 1988). In this regard, the distinct sulfur isotopic composition of DMSP
551 in Collodaria-*Brandtodinium* holobiont, one of the most abundant and ecologically relevant
552 group of photosymbiotic plankton (Dennett et al. 2002; de Vargas et al. 2015; Guidi et al.
553 2016), presents the possibility of assessing the contribution of photosymbiotic plankton to
554 community DMSP production in the future. Beyond biogeochemical considerations, the
555 highly ^{34}S enriched DMSP and the lack of apparent sulfate assimilation into DMSP observed
556 in Collodaria-*Brandtodinium* associations suggest that DMSP biosynthetic pathways could be
557 linked to the recycling of organic sulfur between symbiotic partners. If use of organic sulfur
558 for DMSP synthesis also occurs among free-living phytoplankton, we may be able to
559 discriminate between 'new' (from sulfate) vs 'recycled' (from organic S) DMSP production
560 by their differential S-isotopic signature, in a similar way to approaches using nitrogen
561 isotopes to apportion new and recycled nitrogen sources for primary production.

562

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758 ACKNOWLEDGMENTS

759 We thank the ASSEMBLE (Association of European Marine biological Laboratories)
760 European program for supporting the field campaigns conducted from the Interuniversity
761 Institute for Marine Science (IUI, Israel) and the Observatory Oceanologique de Villefranche
762 sur Mer (obs-vlfr, France). Particularly to Simon Berkowicz, Keren Zandbak and Yeala
763 Shaked for organizing our visit and help with the sampling and experimental logistics at the
764 IUI; John Dolan for sharing his laboratory facilities in obs-vlfr, Fabien Lombard and Sophie
765 Marro for their time and assistance with the sampling and logistic support. Many thanks to
766 Ian Probert and Estelle Bigeard for their invaluable contribution during the sampling and
767 experiments set up. We are in debt with Eva Bucciarelli for generously sharing her time and
768 analytical facilities at LEMAR (Brest, France) for DMSP analysis. We thank Cliff Law for his
769 comments and help copy editing the document. A.G.R. was supported by a Region Bretagne
770 postdoctoral fellowship SYMBIOX and by the National Institution for Water and
771 Atmospheric research (NIWA, New Zealand). T.B. was supported by the UMPC Emergence
772 program and L.P. by the SNF Seastar project. LP was supported by the Swiss National
773 Science Foundation postdoctoral grant P2GEP3_148800. A.A. thanks the Binational Science
774 Foundation Grant 2010407 for partial funding of this work. R.S. acknowledges support from
775 the Spanish Ministry of Science and Innovation through the SUMMER project (CTM2008-
776 03309).

777

778 **Figure Captions**

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

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

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

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

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

TABLE 1. Cell and colony size, DMSPt cell content, and intracellular concentration in uncultured symbiotic Radiolaria and Foraminifera (host+microalgae), and cultured free-living symbiotic microalgae isolated from the same species of Radiolaria and Foraminifera. The abundance of radiolarian cells per colony and the abundance of symbiont cells per holobiont were assessed from image analysis of freshly-collected organisms. DMSPt cellular content per symbiont *in hospite* estimated assuming all DMSPt measured in the holobiont is allocated in the symbiotic microalgae. Cc refers to central capsule, *n/a* and *n/d* refer to not applicable and not determined, respectively. The 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)							
Collozoum sp.	Colonial	Symbiotic	7267 ±1793(3)	3754 ±243(3)	3652 ±2008(3)	0.1 ±0.1(3)	509 ±72(3)
Sphaerozoum sp.	Colonial	Symbiotic	8242 ±2041(4)	3463 ±1327(4)	3135 ±236(3)	0.1 ±0.01(3)	166 ±65(17)
Thalassicolla sp.	Solitary	Symbiotic	3972 ±1148(5)	3517 ±939(5)	2757 ±750(5)	0.2 ±0.1 (5)	*n/a*
Acantharia-Phaeocystis sp. (Radiolaria)							
Amphilonche elongata	Single-celled	Symbiotic	391 ±117(31)	64 ±30(31)	15.2 ±5.4(4)	17.1 ±6.1(4)	*n/a*
Translucid' morphotype	Single-celled	Symbiotic	483 ±188(12)	437 ±182(12)	20.9 ±3.6(3)	0.3 ±0.1(3)	*n/a*
Star shape' morphotype	Single-celled	Symbiotic	307 ±114(42)	242 ±119(42)	36.4 ±10.4(5)	2.4 ±0.7 (5)	*n/a*
Foraminifera-P. beii							
Globigerinella sp. (Planktonic)	Single-celled	Symbiotic	235 ±87(31)	209 ±58(31)	37.8 ±15.5(5)	7.5 ±3.1(5)	*n/a*
Free-living symbiotic							
Brandtodinium nutricula (RCC3468) (Dinophyceae)	Single-celled	Symbiotic	12 ±0.9(15)	8.6 ±0.7(15)	0.2 ±0.1(12)	490 ±107(12)	*n/a*
Pelagodinium beii (RCC1491) (Dinophyceae)	Single-celled	Symbiotic	20.5 ±1.8(18)	17.7 ±2.3(18)	0.9 ±0.2 (12)	272 ±49(12)	*n/a*
Gymnoxanthella	Single-	Symbiotic	9.1	7.9	0.1	462	*n/a*

radiolariae (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)	*n/a*	

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820

821 *Figure 1*

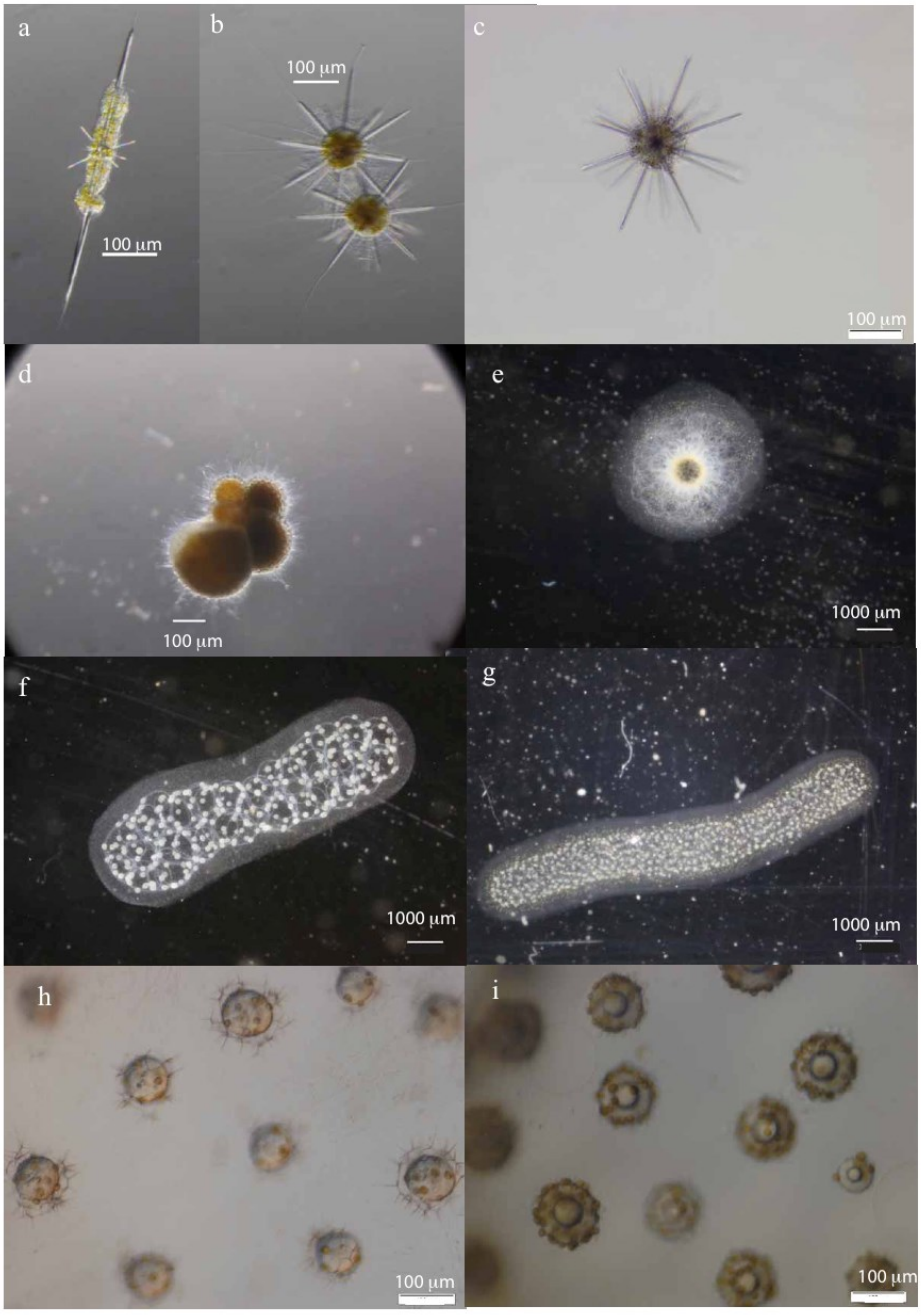

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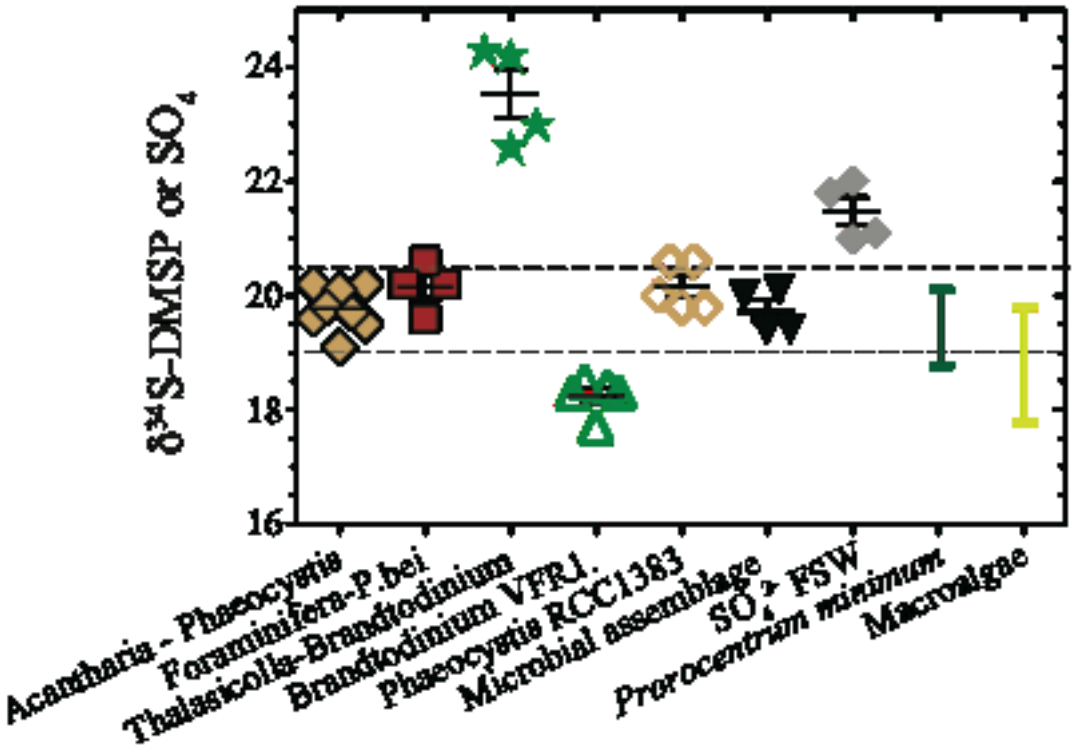
Figure 2


δ^{34}S-DMSP or SO_4
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Acantharia - Phaeocystis
Foraminifera-P.bei
Thalassiola-Brandtodinium
Brandtodinium VFRJ
Phaeocystis RCC1383
Microbial assemblage
SO_4 FSW
Prorocentrum minimum
Macroalgae

]

1

836 *Figure 3*

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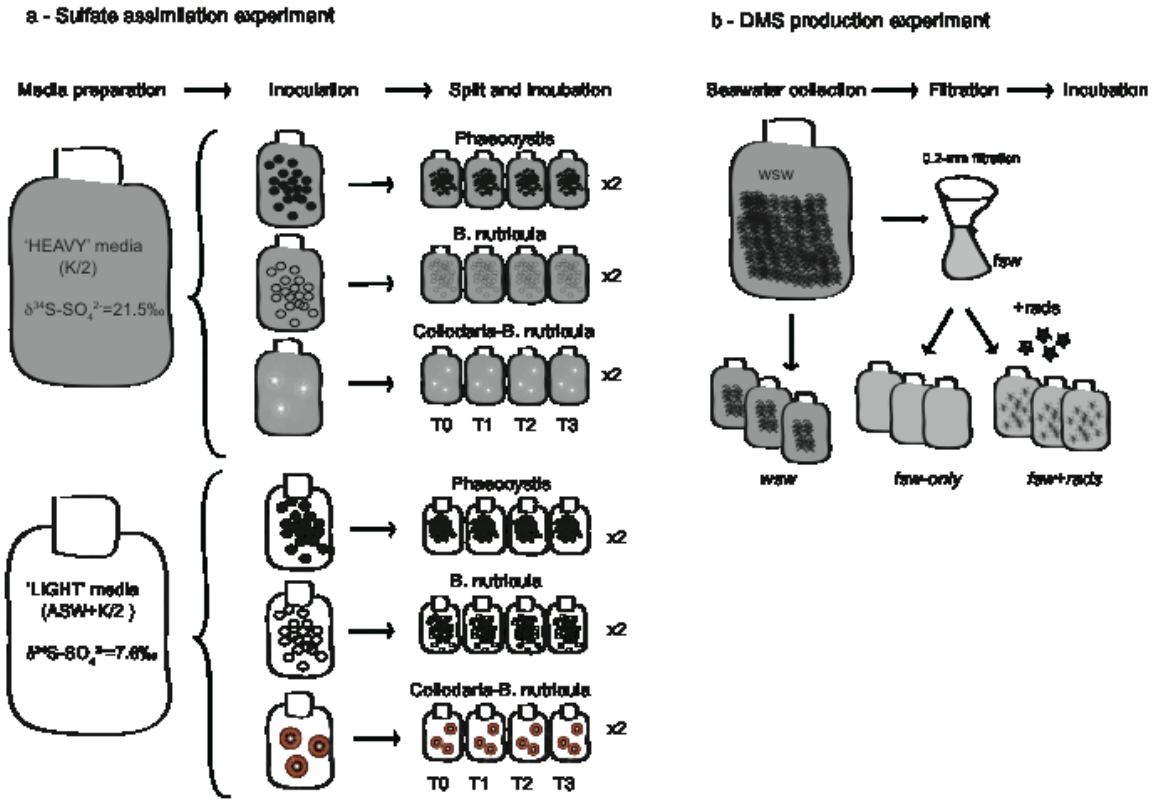
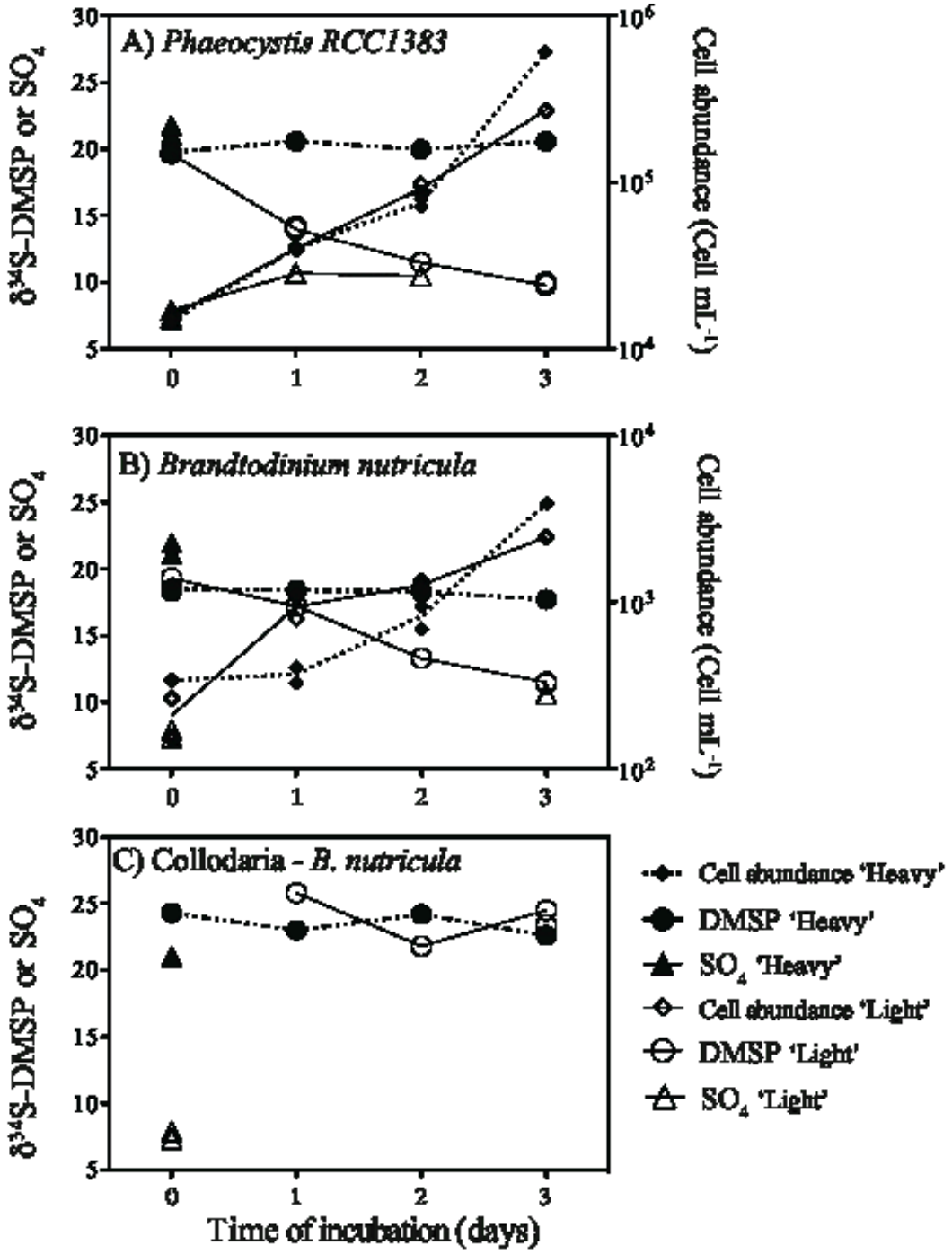


838

839 *Figure 4*


A) Phaeocystis RCC1383
B) Brandtodinium nutricula
C) Collodaria - B. nutricula
δ34S-DMSP or SO4
Cell abundance (Cell mL-1)
Time of incubation (days)
Cell abundance 'Heavy'
DMSP 'Heavy'
SO4 'Heavy'
Cell abundance 'Light'
DMSP 'Light'
SO4 'Light'

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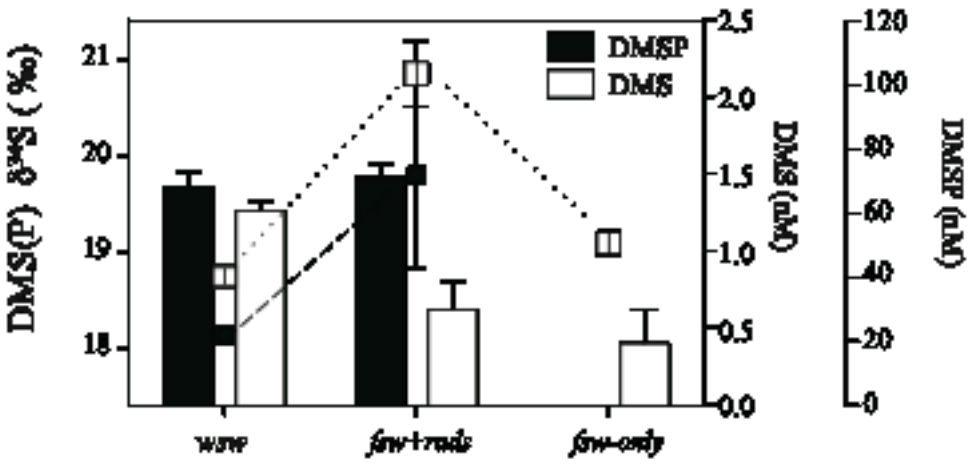
843 *Figure 5*



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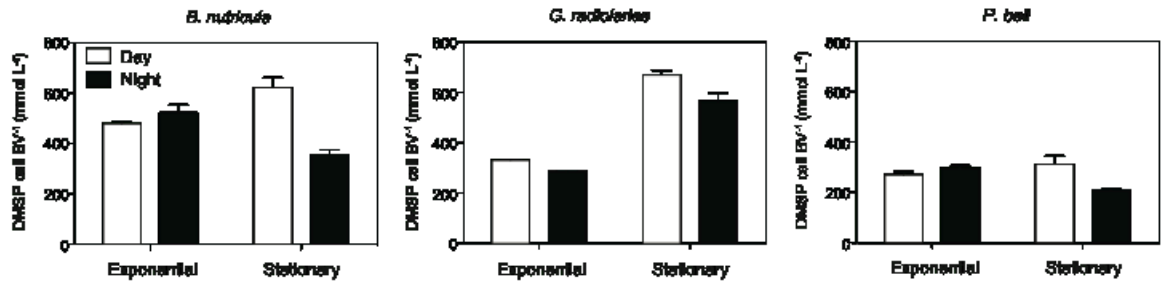
846 SUPPORTING INFORMATION

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 DMSP 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, DMSP cell content was
856 slightly lower during the day than night for *B. nutricula* and *P. beii*, while the cellular DMSP
857 concentrations decreased substantially at night for these strains. In *G. radiolariae* DMSP 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.