Dimethylated sulfur production in batch cultures of Southern Ocean phytoplankton

Cristin E Sheehan[1,2] and Katherina Petrou[1*]

1. School of Life Science, University of Technology Sydney, 15 Broadway, Ultimo, NSW, 2007 Australia
2. Climate Change Cluster, University of Technology Sydney, 15 Broadway, Ultimo, NSW, 2007 Australia

*Author for correspondence: Katherina Petrou

Phone: +61 2 95144159

Email: Katherina.Petrou@uts.edu.au

ORCID

Petrou: 0000-0002-2703-0694

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Abstract

Dimethylsulfoniopropionate (DMSP) is a ubiquitous organic sulfur compound that underpins sulfur cycling in the marine environment and is the precursor to the climatically active gas dimethylsulfide (DMS). Modelling studies have identified the Southern Ocean as a DMS hot spot during summer, yet except for the bloom forming haptophyte *Phaeocystis*, little is known about sulfur production by other important members of the marine microbial community. Here, we measured DMSP concentrations and DMSP lyase activity (DLA), with corresponding carbon, nitrogen and Chl *a* content, in 15 species of Antarctic phototrophic phytoplankton (14 microalgae species and one cyanobacterium) and one phagotrophic flagellate. We found that 11 of the 16 species were able to produce DMSP and eight possess DLA. DMSP content ranged from 0.06 – 73 fmol $cell^{-1}$ and estimated DMSP production rates ranged from 0.008 – 12.42 fmol $cell^{-1}$ day^{-1}. As expected, *Phaeocystis* was amongst the highest producers, however, contrary to expectation DMSP concentrations were high in several pennate diatom species, with intracellular concentrations between 1.85 and 46.6 mM. Here we present the first evidence that the cyanobacterium *Synechococcus* may be a DMSP producer, with the potential to contribute significantly to the DMSP pool. This study has provided the first analysis of DMSP production and DLA in a suite of phototrophic and phagotrophic species isolated from Antarctica, revealing the variability in DMSP concentrations across multiple strains and within genera and delivered new evidence for potential DLA in diatoms.

Introduction

Phytoplankton derived dimethylsulfoniopropionate (DMSP) is fundamental in the global sulfur cycle. It is the precursor of dimethylsulfide (DMS), the largest biological source of sulfur to the Earth's troposphere (Andreae and Crutzen 1997). The oxidation of DMS results in volatile sulfate aerosols that form cloud condensation nuclei (CCN) scattering solar radiation and influencing the Earth's radiative energy budget (Charlson et al. 1987). In the marine environment, both DMSP and DMS can form chemical cues that facilitate key inter-species interactions in the marine microbial food-web (Seymour et al. 2010; Garcés et al. 2013), making these compounds relevant for understanding atmospheric-ocean coupling and marine trophic interactions.

Ecologically, DMSP is a critical source of sulfur and an important carbon source for marine microorganisms (Kiene et al. 2000) accounting for the majority of organic sulfur fluxes from primary to secondary producers (Malin 1996; Simó 2001; Vila-Costa et al. 2006). As a zwitterion, DMSP can only be released from cells with damaged membranes or through active transport (Simó et al. 1998), meaning that for transfer and transport through the food web it has to be released into the surrounding waters through exudation, grazing or cell lysis. Alternatively, DMSP can leave the cell as DMS after conversion by DMSP lyase enzymes (Alcolombri et al. 2015). Once in the water column, DMSP can be assimilated into protein by bacteria, cleaved to DMS by bacterial or algal DMSP lyase or sink to ocean depths as faecal and detrital matter after grazing by zooplankton (Simo 2001). Dissolved DMS can either be taken up by bacterioplankton and used as a sulfur source (Alcolombri et al. 2015), photo-oxidised into non-volatile sulfur or ventilated into the atmosphere (Simo 2001). Despite some phytoplankton possessing the capacity to release DMS into the water column, the majority of the DMS pool in the ocean is believed to be derived from the lyase activity of bacteria (Curson et al. 2008; 2011), and as such, bacteria exert a controlling role over the production efficiency of DMS and sulfur fluxes in the marine environment.

While not all marine microalgae produce DMSP, research has revealed that the most prolific producers of DMSP can be found within two phytoplankton classes, the Dinophyceae (dinoflagellates) (Caruana and Malin 2014) and the Prymnesiophyceae, with studies focussed on two main taxa, the coccolithophore *Emiliana huxleyi* (Levasseur et al. 1996; Matrai and Keller 1993; Steinke et al. 1998) and species from the genus *Phaeocystis* (Liss et al. 1994; Mohapatra et al. 2013; Stefels and Van Boekel 1993). Both of these classes include common bloom-forming species and have been shown to possess DMSP lyase (Franklin et al. 2010; Stefels and Van Boekel 1993; Stefels and Dijkhuizen 1996).

The world's oceans contribute the majority of the biogenic sulfur to the atmosphere, however the flux of DMS from the oceans is regionally specific, being highly dependent on latitude and season (Lana et al. 2012; Yoch 2002). Antarctica is a recognised hot spot for DMSP (Galí et al. 2015) and DMS emissions (Kettle et al. 1999; Lana et al. 2012), yet species-specific data on DMSP production from these regions is poor (Fiddes et al. 2018). Species from the genus *Phaeocystis* are considered amongst the greatest DMSP producers in Antarctic waters, and indeed most studies on sulfur dynamics or cycling attribute the high DMSP concentrations and large fluxes of DMS in the region to the extensive *Phaeocystis* blooms that occur in coastal waters (DiTullio et al. 2000). However, high levels of DMSP have also been recorded at the sea ice margins (Carnat et al. 2016; Damm et al. 2016; Gabric et al. 2018; Stefels et al. 2018), where diatoms often dominate the community (Trevena and Jones 2006) and other studies have shown that Antarctic diatoms can produce substantial amounts of DMSP (Baumann et al. 1994; Tison et al. 2010). Therefore, while temperate diatoms are not generally considered prominent DMSP producers, there is some evidence to suggest that diatoms may make a significant contribution to sulfur cycling in high latitude regions.

The sea ice zone is an important source of DMS and DMSP in Polar Regions (Trevena and Jones 2006; Asher et al. 2011; Galindo et al. 2014, 2016; Damm et al. 2016; Gabric et al. 2018; Stefels et al. 2018). Each winter, Antarctic sea ice covers ~19 million square kilometres of the Southern Ocean entraining many microorganisms into its frozen matrix as it forms. This seasonal freeze and thaw cycle means that many Antarctic microalgae are acclimated to withstand extremely low temperatures (Morgan-Kiss et al. 2006), high salinity (Halsey and Jones 2015) and variable light and UV conditions (Vance et al. 2013). Production of DMSP along the sea ice edge is very high (Trevena and Jones 2006; Tison et al. 2010), and is often associated with the release of sea ice algal species as the ice melts seeding the water column, or directly from the algae which reside within the sea ice itself (Asher et al. 2011; Galindo et al. 2014, 2016). In these organisms, DMSP is suspected to act as a potential cryoprotectant or osmolyte (Kirst et al. 1991; Karsten et al. 1996) enabling these species to survive the freezing and hypersaline conditions over winter.

To accurately predict the influence of phytoplankton communities on DMSP concentrations and DMS emissions, we need to understand the individual contributions of the species that make up those communities, in particular those present in high abundance. Currently however, for Antarctic waters, which are often dominated by diatoms, limited knowledge exists on which taxa synthesise DMSP, what their intracellular concentrations are and whether they possess DMSP lyase capacity (Steiner et al. 2012). Here, we measured DMSP concentrations

and DMS-producing enzyme activity, with corresponding carbon, nitrogen and Chl *a* content, in 15 species of Antarctic phototrophic phytoplankton (14 microalgae species and one cyanobacterium) and one phagotrophic flagellate (*Telonema* sp.), with the aim to increase our understanding of the contribution of Southern Ocean phytoplankton to DMSP and DMS production in high latitude regions.

Materials and Methods

Cell culturing, experimentation, cell counts and growth rates

Thirteen phytoplankton strains were isolated from seawater collected in Prydz Bay, Davis Station, Antarctica (66°S, 77°E) during the Austral Summer (2014). Water was collected from the ice-free waters of Prydz Bay using the underway seawater line on the *RV Aurora Australis* from a depth of ~7 m. Since isolation in 2014, cultures have been maintained in 0.2 µm filtered natural seawater (salinity 35) enriched with nutrients (Table 1) at low irradiances and temperature (50 µmol photons m^{-2} s^{-1} on a 14:10 h light: dark cycle at 3 ± 1 °C) and transferred into new medium bi-monthly. These conditions were determined as optimal for cell growth and used in this study as cells had been acclimated to these light and temperature conditions for more than three years. Three additional phytoplankton cultures (*Dunaliella* sp., *Phaeocystis cf. pouchettii*, and *Synechococcus* sp.) were obtained from the CSIRO Australian National Algae Culture Collection (Table 1). Cultures (non-axenic) were acclimated over six generations to an irradiance of 50 µmol photons m^{-2} s^{-1} (14:10 h light: dark cycle) and maintained at 3 ± 1 °C.

For experimental sampling, batch cultures were grown in quadruplicate and aliquots of culture (1 mL) taken every second day and fixed in 1% glutaraldehyde for growth rate determination. Cell counts were performed using a Neubauer hemocytometer (Swastik Scientific, Mumbai, India) counting chamber (0.5 x 0.5 x 1 mm^3), and cell density estimated according to Guillard and Sieracki (2005), and specific growth rates (µ) calculated. Sampling for strain characterisation was undertaken at one time point during the exponential growth phase of a subsequent growth curve. This was done to ensure that all cells sampled were in balanced growth, avoiding any nutrient limitation. All sampling was performed mid-way through the photoperiod (~12 noon) to reduce physiological variation due to diel activity. Each replicate was subsampled for analyses of sulfur compounds, as well as Chl *a* and C:N ratio. For bacterial enumeration, a 2 mL aliquot was subsampled and fixed in 1% glutaraldehyde, snap frozen in liquid N_2 and stored at -80 °C until analysis. Bacterial cell counts were performed using flow cytometry (CytoFLEX S; Beckman Coulter, Inc., USA). The aliquot was rapidly thawed in hot water and cells were counted as both unstained (control) and stained (SYBR Green I Nucleic Acid Gel Stain (1:10,000),

15 min, Invitrogen, ThermoFisher Scientific, USA). The total bacterial density was calculated by subtracting the unstained cell count from the stained cell count and used to calculate DMSP lyase activity rates.

Chlorophyll a content and cell volume

Samples for chlorophyll *a* determination were filtered (6-15 mL - depending on culture density) onto GF/C filters, which were then snap frozen in liquid N_2 and stored at -80 °C until analysis. Pigments were extracted in 90% acetone and incubated at 4 °C in the dark for 24 h. Chlorophyll *a* content was determined using a spectrophotometer (Cary50: Varian, Santa Clara, CA, USA) and calculated using the equations of Jeffery and Humphrey (1975), modified by Ritchie (2006). To estimate cell volumes, fixed samples of cells in mid-exponential growth were imaged on a calibrated microscope (Nikon Eclipse Ci-L, Japan) and the length, width and height of ~10 cells determined using ImageJ (Schneider et al. 2012) software. Cell biovolume was then calculated according to the cell shape and corresponding equations as described in Hillebrand et al. (1999). In the case of *Synechococcus*, some cells may have passed through the filter due to their small size, resulting in a possible underestimation of chlorophyll *a* quota.

C:N analysis

For determination of cellular carbon and nitrogen, aliquots (5-20 mL) of culture were filtered onto GF/F filters (pre-combusted at 450°C for 4 h) and snap frozen in liquid N_2 until analysis. Prior to analysis, the sample and blank (filters with medium only) filters were dried at 35 °C for 48 h before being wrapped in tin foil and placed in ceramic boats with nickel boat liners (LECO Corporation, USA) and combusted at 1300 °C. Analyses were run on a Leco TruMac Carbon Nitrogen Analyser (LECO Corporation, USA). Concentrations were quantified using a series of soil reference material standards (LECO Corporation, USA) with calibration limits of 0.1 – 6 mg N and 1.2 – 223 mg C. Carbon and nitrogen concentrations were corrected against blanks and normalised to filtered volume and cell density.

Quantification of demethylated sulfur compounds

For quantification of dimethylsulphide (DMS), a 2 mL sample of culture was gravity filtered and placed into an amber vial, which was then sealed with a butyl rubber stopper, crimped capped, and analysed immediately. Care was taken during gravity filtration to ensure that the filter did not dry out and samples were manipulated gently to minimise any loss of DMS via ventilation. To measure total DMSP (DMSPt), 1 mL of culture was transferred

directly into a 20 mL amber vial containing 1 mL of 0.75 M NaOH (used to hydrolyse DMSP into DMS), sealed, crimp capped and left to react at room temperature. For dissolved DMSP (DMSPd), a maximum of 3 mL of culture was gravity filtered through a 2 µm filter and a 1 mL aliquot of filtrate pipetted into an amber vial containing 1 mL of 0.75 M NaOH, before the vial was immediately capped, crimped and stored at room temperature in the dark. For all DMSP samples, vials were left to equilibrate for a minimum of 12 h before analysis. Intracellular DMSP (DMSPp) used to determine DMSP quotas and cellular concentrations, was calculated by subtracting the dissolved DMSP (DMSPd) and DMS fractions from the total DMSP (DMSPt).

Analyses of all sulfur compounds were performed on a gas chromatograph (GC-2010 Plus, Shimadzu, Japan) coupled with a flame photometric detector (FPD) set at 160°C with hydrogen and air flow rates of 40 and 60 mL min^{-1}, respectively. Samples were analysed using a purge and trap system (Simó et al. 1993), where samples were sparged with helium, extracting all the volatile gas (including DMS) from the sample while trapping the DMS in a PTFE loop immersed in liquid nitrogen. After sparging, the sample was released from the cryotrap by heating the loop and allowing the volatiles to desorb and then injected into the GC. DMS was eluted onto a capillary column (30 m x 0.32 mm x 5 µm) set at 120°C, using high purity helium as the carrier gas with a flow rate of 12 mL min^{-1} and a split ratio of five. In instances of very high sulfur concentrations established during pilot tests (e.g. *Phaeocystis* spp.), the direct injection method was used, where a 500 µL sample of the DMS contained within the headspace of the vial was sampled using a gas tight syringe and injected directly into the GC. The peak area integration against a calibration curve allowed for the quantification of DMS. Each calibration curve was made of fresh standards prepared from DMSP chloride crystals (Sigma-Aldrich), hydrolysed to DMS using NaOH and loaded into the GC with the same injection mode as the samples. To estimate DMSP production rates, we used DMSPp per cell (pg $cell^{-1}$) multiplied by the specific growth rate (µ) of the culture.

DMSP lyase activity (DLA)

Estimates of DLA in the isolates were measured as described by Harada et al. (2004), while maintaining low incubation and measuring temperatures (~0°C) throughout the analysis to obtain ecologically relevant rates of lyase. Briefly, 2 mL of culture was gently filtered onto a 2.0 µm polycarbonate filter, rinsed with media, snap frozen in liquid N_2 and stored at -80 °C until analysis. Prior to analysis, filters were thawed slowly on ice and then transferred facedown into a glass vial in 1 mL of pH 8.2 TRIS buffer, capped with a rubber stopper and vortexed for 10 s. After 20 min incubation in iced water, 20 µL of DMSP-HCl (Sigma Aldrich, USA) was added to a final concentration of 5 mM, and the vial sealed and crimp capped. The vial was vortexed vigorously for 10 s, put back

in the iced water, the timer started and 100 µL of headspace immediately extracted using a gas tight syringe, which was then injected directly onto the GC for quantification of DMS. DMS production was monitored over time with 4-5 sequential measurements and the exact time of headspace removal recorded. DMS production if linear over time was corrected for the abiotic cleavage activity found in buffer controls. Bacterial DMSP lyase activity was measured by filtering the 2.0 µm filtrate from the culture and gently filtering it onto a 0.2 µm polycarbonate filter (SterliTech, USA), that was flash frozen liquid N_2 and stored at -80 °C until analysis. Enzyme activity was then measured as described above, with the assumption that no bacterial production of DMSP was occurring.

Data analysis

To compare distributions between taxa for dimethylated sulfur compounds, cell volume, carbon, nitrogen and Chl *a* content, a two sample Kolmogorov-Smirnov tests was used with a significance cut off of 0.05. When data were compared between two strains only, a t-test on the mean was used to verify significance at $p < 0.05$. All statistical analyses were run using the statistical package in R (R Core team, 2019). All plotting and curve fitting were performed in SigmaPlot v.12 (Systat Software Inc, UK).

Results

Growth rates across the 16 strains ranged from 0.12 to 0.49 day^{-1} with the green algae *Dunaliella* sp. and large centric diatom *Odontella weissflogii* exhibiting the slowest growth rates, while the diatom *Chaetoceros simplex* and cyanobacterium *Synechococcus* sp. exhibited the fastest growth rates (Figure 1; Table 2). Mean cell volume ranged several orders of magnitude between taxa (Table 2), where *Synechococcus* sp. was the smallest species (3 μm^3), and *Odontella weissflogii* was the largest (10,086 μm^3). Within diatoms there was a 100-fold difference in cell volume between the smallest (*Nitzschia acicularis*, 97 μm^3) and largest species (*Odontella weissflogii*). As with cell volume, particulate organic carbon (C), nitrogen (N) and Chl *a* per cell ranged three orders of magnitude across all species (Table 2; Figure 2A). However, when expressed per cell volume (CV), this variation was reduced (Figure 2B). Diatoms possessed much higher Chl *a*, C and N content than the other species, with an average of 6.11 ± 3.4 pg Chl *a* $cell^{-1}$, 294 ± 157 pg C $cell^{-1}$ and 51 ± 32 pg N $cell^{-1}$, respectively, but being larger cells, this pattern reversed when expressed per cell volume (Table 2). For C:N ratios, with the exception of *F. pseudonana* and *P. pouchetti*, most of the taxa had a C:N ratio between 0.5-6.4 g g^{-1} (Table 2).

Grouped data showed significant differences in N content (D = 0.64, $p = 0.0105$) and cell volume (D = 0.55, $p = 0.009$) between centric and pennate diatoms (Figure 3A), and when normalised to cell volume, only a significant difference in chlorophyll *a* (D = 0.64, $p = 0.0006$) was detected (Figure 3B). The two *Phaeocystis* species had significantly lower N ($t_{17} = -2.91$, $p = 0.0096$) and Chl *a* ($t_{15} = -3.50$, $p = 0.0032$) content compared with the 'other' group. However, *Synechococcus* sp. showed the lowest C ($t_{12} = -2.53$, $p = 0.0264$), N ($t_{15} = -3.59$, $p = 0.0265$) and Chl *a* ($t_{15} = -3.38$, $p = 0.004$) content, as well as cell volume ($t_{15} = -3.64$, $p = 0.0024$) of all three groups (Figure 3C), but a C:N ratio greater than 'other' ($t_{14} = 2.36$, $p = 0.0326$), but the same as the haptophytes. When expressed per cell volume however, N and chl *a* concentrations were significantly lower in the haptophytes ($t_{18} = -4.03$, $p = 0.0008$; $t_{16} = -8.24$, $p < 0.0001$, respectively) than the 'other' group (Figure 3D), while *Synechococcus* sp. had the highest mean C ($t_{15} = -5.15$, $p = 0.0001$) and chl *a* concentrations ($t_{17} = -6.82$, $p < 0.0001$) of all three groups.

DMSP was detected in 11 out of the 16 species (Table 3) ranging from 0.004 pg cell^{-1} in *Synechococcus* to 9.82 pg cell^{-1} in *Phaeocystis cf. pouchetii* (Table 3). As observed for the two *Chaetoceros* species, no detectable levels of DMSP were recorded for the chrysophyte, *Dunaliella* sp. and *Telonema* sp. (Table 3). Of all the DMSP producing species tested, *P. cf. pouchetii* and *Fragilariopsis pseudonana* had the highest amounts (73 and 59 fmol cell^{-1}, respectively), followed by *Nitzschia lecointei* (10.3 fmol cell^{-1}; Figure 4A). Interestingly, *Phaeocystis antarctica* was the fourth lowest producer within the 11 species tested (Figure 4A). Due to the difference in cell size however, when DMSPp was expressed per cell volume, the rank order changed (Figure 4B). While *P. cf. pouchetii* remained the species with the highest DMSP concentration (1460 mM), this was followed by *N. lecointei, F. pseudonana* and then *Phaeocystis antarctica*. This normalisation also altered positions of both *Synechococcus* sp., which exhibited higher intracellular concentrations than *P. gelidicola*, two pennate diatoms, and all three centric diatoms, *Odontella weissflogii* and the two *Thalassiosira* species (Figure 4B).

Grouping all 11 species, DMSPp per CV spanned four orders of magnitude (0.04 – 1460 mM) (Figure 5A) and variability of the interquartile range was lowest when DMSPp was normalised to carbon (Figure 5A). Separating the data into functional groups, large differences in all DMSP-related parameters between the centric and pennate diatoms were evident (Figure 5B), where pennate diatoms had significantly higher intracellular concentrations of DMSP ($t_{15} = 3.93$, $p = 0.001$) and more DMSP per cell ($t_{15} = 2.74$, $p = 0.015$), C ($t_{14} = 2.91$, $p = 0.011$), N ($t_{15} = 3.97$, $p = 0.001$) and Chl *a* ($t_{15} = 3.36$, $p = 0.004$; Figure 5B), highlighting a strong potential difference in the ecological and physiological role of DMSP in these two important groups of diatoms. There were equally large discrepancies between the two *Phaeocystis* species, with *P. cf. pouchettii* expressing much higher

DMSP concentrations than *P. antarctica* (DMSP:CV, $t_3 = -17.24$, $p = 0.0004$; DMSP:cell, $t_3 = -17.29$, $p = 0.0004$) and higher DMSP:C ($t_3 = -10.18$, $p = 0.002$) and DMSP:Chl *a* ($t_3 = -4.91$, $p = 0.016$) ratios (Figure 5C). Comparing *P. gelidicola* and *Synechococcus* sp., which both generally expressed low levels of DMSP, *Synechococcus* sp. showed significantly higher DMSP:CV ($t_4 = -3.78$, $p = 0.019$), per C ($t_4 = 13.69$, $p < 0.0001$), N ($t_4 = 5.51$, $p = 0.005$), and Chl *a* ($t_5 = 12.54$, $p < 0.0001$), but significantly lower values for DMSP per cell ($t_3 = 28.08$, $p = < 0.0001$; Figure 5D).

Of the 16 species screened, dissolved DMS was detected in nine of the cultures, ranging from 6 - 1527 pmol mL^{-1} (Table 4). The same cultures had detectable algal DMSP lyase activity (DLA), including both *Phaeocystis* cultures, *P. gelidicola* and four diatom species (Table 4). Unexpectedly, bacterial DMSP lyase activity was detected in only three of the cultures, but was consistently higher on a per cell basis than their respective microalgal cultures (Table 4). The highest DLA rate was measured in the bacterial fraction of *P. cf. pouchetti*, supporting the very high DMS concentrations measured in that culture.

Discussion

In the marine environment DMSP is one of the most important sources of sulfur for marine organisms (Kiene et al. 2000), including heterotrophic bacteria, many of which can metabolise DMSP to produce amino acids and/or cleave DMSP to DMS (Todd et al. 2009). Thus, knowledge on who produces DMSP is important for understanding the ecology and sulfur cycling in the environment. Of the 16 Antarctic strains tested in this study, 11, spanning four different taxonomic groups, had detectable levels of DMSP, suggesting that the production of DMSP in Antarctic waters is the domain of phytoplankton species from multiple functional groups.

Considerable DMSP levels were measured in all pennate diatoms in this study, where *Fragilariopsis* spp. and *Nitzschia* spp. had higher levels of DMSP per cell and cell volume than any of the DMSP-producing centric diatoms (*Odontella weissflogii* and both *Thalassiosira* spp.). This is consistent with a previous study that found Arctic pennate diatoms to have DMSPp/Chl *a* ratios two times higher than centric diatoms (Galindo et al. 2014). For the nine diatom strains tested, the two *Chaetoceros* species were the only diatoms that had no detectable DMSP. However, this general prevalence of DMSP production by Antarctic diatoms adds new weight to their potential role in sulfur cycling in high latitude waters. The two *Phaeocystis* strains in this study differed from one another, where the high intracellular concentrations in *P. cf. pouchetii* (1460 mM), while greater than those measured for temperate species (261 mM; Keller 1989) or North Sea isolates (71 - 150 mM; Stefels and Van Boekel 1993), did match the levels of DMSP observed previously in Antarctic *Phaeocystis* sp. (~1500 mM),

293 collected from Davis Station, Antarctica (Gibson et al. 1990). Interestingly, the *P. antarctica* strain in this study
294 had DMSP levels much lower than many lower latitudes isolates, which typically range from 2-13 fmol $cell^{-1}$
295 (Liss et al. 1994). These data reveal that high variability exists both inter- and intra-specifically for *Phaeocystis*,
296 with the possible ecological implication that some strains may only be partially responsible for high latitude
297 DMSP/DMS hot spots.

298 The green flagellate *Pyramimonas gelidicola*, which had DMSP concentrations in the mid-range (7.1
299 mM) and an estimated production rate equal to *P. antarctica*, has been seen to dominate under ice communities
300 alongside *Phaeocystis* spp. (Vance et al. 2013), thus it may support much of the DMSP production in the water
301 column at the marginal ice edge. As with the diatoms and *P. cf. pouchetti*, DMSP levels measured in the *P.*
302 *gelidicola* of this study were higher than those measured previously in a temperate strain of *Pyramimonas* sp.
303 (0.5mM; Keller 1989). Taken together, these latitudinal differences (seen in diatoms, *P. cf. pouchetti* and
304 *Pyramimonas*) propose that Antarctic isolates may commonly produce more DMSP than their temperate
305 counterparts and prompts some revision of the key producers across latitudes and ecological niches. The DMSP
306 lyase activity detected in *P. gelidicola* contrasts with a previous study on another Antarctic isolate of *Pyramimonas*
307 sp. in which no DLA was detected (Harada and Kiene 2011). In an ecological context, our data suggest that this
308 species, which can occur in high numbers (7×10^4 cells L^{-1}; Garibotti et al 2003; Vance et al. 2013), may contribute
309 substantially to DMSP production and possibly even DMS flux in polar waters, especially in the sea ice margins.

310 An important observation of this study was the detection of DMSP lyase activity in four of the diatom
311 cultures, supporting the DMS concentrations measured. With the exception of *N. lecointei*, DLA was not detected
312 in the bacterial fraction, which was unexpected, given the general prevalence of DMSP degradation genes in
313 bacteria (Todd et al. 2009). To our knowledge, these data represent the first measurements of DLA by diatoms
314 and suggest that diatoms may play a larger role in sulfur cycling than previously recognised. However, it is
315 important to note that these cultures were non-axenic and although attempts were made to separate the bacterial
316 component, potential contribution from attached bacteria to the DMSP lyase activity of the algal component
317 cannot be ruled out. Therefore, these findings offer a starting point from which to explore further the possibility
318 of DMSP lyase activity in diatoms and their potential role in DMS flux from polar systems.

319 The cyanobacterium, *Synechococcus*, is a known consumer of DMSP (Malmstrom et al. 2005; Vila-
320 Costa et al. 2006), and in the absence of any obvious external source of DMSP, the relatively high intracellular
321 DMSP in the *Synechococcus* in this study suggests that this organism may also be a DMSP producer, which to
322 our knowledge has not been reported previously. It is possible, however, that the DMSP may have originated from

other bacteria in the culture and was then subsequently taken up by the *Synechococcus.* We were able to confirm no conversion of DMSP to DMS via DLA, a finding supported by Malmstrom et al. (2005), who found axenic cultures of *Synechococcus* did not produce DMS.

The absence of lyase activity in the bacterial fraction (<2 µm) of the majority of the cultures is surprising, given their dominant role in DMS production (Curson et al 2008; 2011). This could be due to the low DMSPd concentrations in many of the cultures not meeting bacterial sulfur and carbon requirements. In marine ecosystems, DMSP plays a key role in bacterial cell metabolism satisfying up to 95% of the sulfur and 15% of the carbon demands (Zubkov et al. 2001). Thus, it is possible that the available DMSP in these cultures was preferentially taken up and utilised to produce protein for bacterial growth, rather than cleaved into DMS and acrylate. The cultures in which bacterial DLA was detected corresponded with relatively high DMSPd concentrations, supporting the idea of bacteria prioritising DMSP demethylation over DMS production (Kiene et al. 1999).

Assuming the data presented here are representative of Southern Ocean phytoplankton, using DMSP production rates (Table 3) we can start to estimate the contributions of certain species to the DMSP pool in the Southern Ocean. For example, a community dominated by *Fragilariopsis* sp., a ubiquitous pelagic genus in southern waters (Waters et al. 2000; Cefarelli et al. 2010; Petrou et al. 2016), would produce substantially more DMSP than one dominated by a bloom-forming species from the genus *Chaetoceros* sp.. Average cell densities for *Fragilariopsis* spp. (>20 µm) around East Antarctica have been recorded as 1.8×10^5 cell L^{-1} (Waters et al. 2000), and mean concentrations of *Fragilariopsis* spp. in the Weddell Sea recorded as ~1.1×10^5 cells L^{-1} of which up to 50% is *F. pseudonana* (Cefarelli et al. 2010). Using these numbers, DMSP production by *F. pseudonana* alone, could be in the order of 6.9 nmol L^{-1} day^{-1}. The prolific DMSP producer *Phaeocystis*, which can bloom in numbers exceeding 1×10^6 cells L^{-1} (Smith Jr et al. 2003), would have an estimated DMSP production rate ranging from 0.14 – 25 nmol L^{-1} day^{-1}. From these estimates, we can start to see the potentially significant contribution diatoms may make to the DMSP pool in Antarctic waters. If we then add to that the possibility for diatom driven conversion of DMSP to DMS, the potential influence of diatoms on sulfur cycling in the Southern Ocean could prove substantial.

A new potential contributor to DMSP production could come from the cyanobacterium, *Synechococcus* sp.. Although generally found in low abundance in Antarctic waters, if the DMSP concentrations in this study are representative of other *Synechococcus* strains, its contribution to DMSP production at lower latitudes could also be significant. *Synechococcus* abundances can be as high as 3.7×10^7 cells L^{-1} (Saito et al. 2005), which would

make the estimated DMSP production ~0.5 nmol L^{-1} day^{-1}. Furthermore, with warming and tropicalisation of oceans, the projected *Synechococcus* abundance and distribution for the end of this century predict cell densities exceeding 10,000 cell mL^{-1} as far as 60°S (Flombaum et al. 2013) making its future potential contribution to DMSP in polar regions considerably greater. Indeed, in the Arctic (79°N), *Synechococcus* abundance has already exceeded 21,000 cells mL^{-1} (Paulsen et al. 2016), suggesting that its geographical range may not be as limited by temperature as previously thought.

The combination of these varying contributions have implications for trophic interactions and DMSP availability, both for bacterial metabolism and through the act of grazing, whereby more grazable species such as *P. gelidicola*, may actually make a greater overall contribution to the dissolved DMSP pool than the silica walled diatoms that are harder to prey upon. This available pool has even wider reaching implications, as DMSP has also been shown to be available for uptake by other phytoplankton species (Vila-Costa et al. 2006), including diatoms (Petrou and Nielsen 2018), thereby linking the ecological role of DMSP into a potential physiological role, whereby uptake could assist with physiological adjustments.

Physiologically, DMSP is a secondary metabolite that has been shown to function as an osmoprotectant, cryoprotectant (Stefels 2000) and antioxidant (Sunda et al. 2002). The shift in species rank when data were normalised to intracellular concentrations of DMSP (Figure 5), could indicate differences in the physiological role DMSP has for those cells. We found pennate diatoms to possess much higher concentrations of DMSP than the centric diatoms in this study, reflecting potential differences in the needs for cryoprotection. Recent work in the Arctic showed clear differences in DMS and DMSP production in under ice blooms depending on whether the community was dominated by centric or pennate diatoms, with pennate diatoms recently released from the sea ice having higher intracellular DMSP concentrations (Galindo et al 2014). Alternatively, these differences in DMSP concentration could reflect the advantage of sea ice pennate diatoms to acclimate to sudden increases in UV and high light as sea ice breaks up (Vance et al. 2013; Galindo et al. 2016), by using DMSP as an antioxidant. The higher concentrations found in the *Phaeocystis* species and pennate diatoms may indicate that their cell physiology is more reliant on DMSP than in centric diatoms. The notably high intracellular concentrations found in *Synechococcus* is of particular interest, suggesting that perhaps this cyanobacterium has some dependence on this compound for cell maintenance. This is further supported by the fact that it is known to take up and assimilate DMSP from the environment as well (Malmstrom et al. 2005; Vila-Costa et al. 2006). It is important to note however, that this strain was isolated from Ace Lake, a meromictic lake in the Vestfold Hills, Antarctica, so while a potentially significant producer, it is not necessarily representative of truly pelagic species.

In addition to species and functional group comparisons, intra-genera comparisons can also be made. Between the two *Fragilariopsis* strains, *F. pseudonana* had much higher levels of DMSP (59 fmol cell^{-1}, 17 mM) than the smaller *Fragilariopsis* sp. (1.4 fmol cell^{-1}) and comparable to intracellular concentrations (16-18 mM) measured previously in the Antarctic diatom *Fragilariopsis cylindrus* (Lyon et al. 2016). *Fragilariopsis pseudonana* is found both in open waters and in the pack ice, suggesting that, like *F. cylindrus*, it has a broad ecological niche, suggesting that DMSP may play a role in their physiological plasticity. The two species of *Nitzschia* also varied greatly in DMSP production. Again, it was the larger of the two species, *N. lecointei* that had much higher DMSP levels, suggesting possible niche differentiation between the two species and thus environmentally driven differences in DMSP requirement. Given that *Nitzschia acicularis* is generally found in pelagic, offshore environments, whereas *N. lecointei* is commonly found on the under-surface of ice (tychopelagic) or near the sea ice edge (Scott and Marchant 2005), it is possible that the lower temperatures or more variable conditions experienced in the sea ice, means a greater physiological requirement for the cryoprotective or osmolytic properties of DMSP. Differences were also detected in the two *Thalassiosira* strains, where the larger strain also had the highest DMSP content and concentration. These data suggest that smaller cells may have reduced requirements for DMSP or reduced capacity to synthesise and store metabolites and together show that there is considerable strain variability even amongst genera.

To maximise the comparability of the data presented in this study, we have expressed DMSP not only per cell and cell volume, but also per C, N and Chl *a* content. This is because when measuring DMSP in the field, Chl *a* is often used for normalisation and as a proxy for phytoplankton biomass, and while it can be hard to determine the exact input of specific species, it is an easy and readily available parameter, making it especially attractive to modellers (Huot et al. 2007). Similarly, optical measurements of phytoplankton biomass can be expressed as carbon biomass (Behrenfeld and Boss 2006), with many global DMS/P models presented in units of carbon (Gali et al. 2015; Lana et al. 2011). This study has demonstrated the variability that exists in using different normalisation parameters (cell, Chl *a*, C and N) across functional groups and mixed communities, which is useful when standardising DMS/P at a regional scale (Figure 5).

This study has provided the first comprehensive data on DMSP production and DLA in a suite of phototrophic and phagotrophic species isolated from Antarctica. Among the species characterised, our species-specific results demonstrate the challenges in generalising across a genus or a community and the complexity of understanding sulfur dynamics in Antarctic waters. Our results highlight that DMSP concentration varies not only across species, but also among strains from the same genus, linking production with possible niche occupation or

environmental constraints and demonstrates how any conclusions or extrapolations to the environment are challenging. However, these data have started to reveal who the producers are within the Antarctic marine environment, how taxonomically broad DMSP and DMS production can be and provide a first insight into the species-specific variability that can be expected from mixed community samples.

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Conflict of interest

The authors declare no conflict of interest.

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607 **Tables**

608 **Table 1:** A summary table of the Antarctic phytoplankton cultures investigated. All cultures were grown at 50
609 µmol photons m^{-2} s^{-1} and 3°C ± 1°C. Grouping column shows species used for functional group comparisons. For
610 the diatoms, c = centric, p = pennate.
611

Species	Strain code	Collection site	Medium	Grouping
Chaetoceros castracanei	PZB010	Prydz Bay	L1	Diatom (c)
Chaetoceros simplex	-	Prydz Bay	L1	Diatom (c)
Chrysophyte sp.	PZB025	Prydz Bay	L1	Other
Dunaliella sp.	CS-635	Organic Lake	F2	Other
Fragilariopsis sp.	PZB060	Prydz Bay	L1	Diatom (p)
Fragilariopsis pseudonana	PZB009	Prydz Bay	L1	Diatom (p)
Nitzschia acicularis	PZB063	Prydz Bay	L1	Diatom (p)
Nitzschia lecointei	PZB001	Prydz Bay	L1	Diatom (p)
Odontella weissflogii	AAD015	Antarctica	L1	Diatom (c)
Phaeocystis antarctica	PZB016	Prydz Bay	L1	Haptophyte
Phaeocystis cf. pouchetti	CS-243	Antarctica	F2	Haptophyte
Pyramimonas gelidicola	PZB033	Prydz Bay	L1	Other
Synechococcus sp.	CS-601	Ace Lake	F2	Cyanobacterium
Telonema sp.	PZB013	Prydz Bay	L1	Other
Thalassiosira sp.	PZB048	Prydz Bay	L1	Diatom (c)
Thalassiosira sp.	PZB062	Prydz Bay	L1	Diatom (c)

612

Table 2: Specific growth rate (μ), mean cell volume, carbon, nitrogen and Chl *a* concentration measured in 16 Antarctic phytoplankton species, diatoms are in bold. Data are displayed as means (n=4) ± standard error in parentheses. Note: numbers in square brackets for *Thalassiosira* sp. indicate strain number from table 1.

Species	Specific growth rate μ		Volume		C				N				C:N ratio		Chl *a*			
	(div. day^{-1})		(μm^3)		pg cell^{-1}		pg μm^{-3}		pg cell^{-1}		pg μm^{-3}				pg cell^{-1}		fg μm^{-3}	
C. castracanei	0.20	(0.02)	334	(14)	78	(11.5)	0.23	(0.03)	24	(2.2)	0.07	(0.007)	3.4	(0.5)	0.52	(0.059)	1.6	(0.18)
C. simplex	0.49	(0.02)	17	(2)	1	(0.4)	0.04	(0.02)	-	-	-	-	-	-	0.16	(0.006)	9.2	(0.34)
Chrysophyte sp.	0.15	(0.02)	22	(3)	5	(3.3)	0.24	(0.15)	10	(0.7)	0.48	(0.034)	0.5	(0.3)	0.26	(0.014)	11.9	(0.66)
Dunaliella sp.	0.12	(0.00)	15	(1)	10	(0.8	0.67	(0.06)	5	(0.4)	0.31	(0.029)	2.2	(0.3)	0.32	(0.005)	20.8	(0.30)
***Fragilariopsis* sp.**	0.36	(0.01)	250	(15)	72	(14.7)	0.29	(0.06)	19	(0.9)	0.07	(0.004)	3.8	(0.6)	0.48	(0.028)	1.9	(0.11)
F. pseudonana	0.21	(0.05)	3392	(242)	650	(58.1)	0.19	(0.02)	49	(5.4)	0.01	(0.002)	13.5	(1.1)	8.16	(0.329)	2.4	(0.10)
N. acicularis	0.15	(0.01)	97	(11)	6	(2.5)	0.06	(0.03)	6	(0.9)	0.06	(0.009)	0.9	(0.3)	0.17	(0.004)	1.7	(0.05)
N. lecointei	0.23	(0.03)	221	(16)	18	(1.8)	0.08	(0.01)	7	(0.5)	0.03	(0.002)	2.8	(0.3)	0.33	(0.019)	1.5	(0.09)
O. weissflogii	0.12	(0.01)	10086	(930)	1426	(230)	0.14	(0.02)	240	(13)	0.02	(0.001)	5.9	(0.9)	31.75	(1.806)	3.1	(0.18)
P. antarctica	0.23	(0.01)	31	(2)	3	(1.7)	0.10	(0.06)	-	-	-	-	-	-	0.01	(0.002)	0.4	(0.05)
P. cf. pouchetti	0.16	(0.03)	50	(4)	38	(5.7)	0.76	(0.11)	4	(1.1)	0.08	(0.015)	12.7	(1.2)	0.08	(0.016)	1.5	(0.32)
P. gelidicola	0.14	(0.02)	75	(8)	26	(1.2)	0.35	(0.02)	4	(0.4)	0.06	(0.006)	6.4	(0.6)	0.49	(0.019)	6.5	(0.25)
Synechococcus sp.	0.45	(0.00)	3	(0.6)	3	(0.1)	0.89	(0.03)	1	(0.0)	0.16	(0.008)	5.5	(0.2)	0.07	(0.002)	23.2	(0.79)
Telonema sp.	0.14	(0.02)	238	(67)	139	(48.0)	0.58	(0.20)	31	(8.3)	0.13	(0.035)	3.6	(1.2)	2.48	(0.125)	10.4	(0.52)
***Thalassiosira* [48]**	0.14	(0.02)	2302	(225)	155	(67.4)	0.07	(0.03)	13	(13)	0.01	(0.006)	-	-	6.06	(0.876)	2.6	(0.38)
***Thalassiosira* [62]**	0.19	(0.03)	1765	(391)	239	(147)	0.14	(0.08)	-	-	-	-	-	-	7.36	(1.412)	4.2	(0.80)
Averages																		
Diatoms	0.23	(0.04)	2051	(1080)	294	(157)	0.14	(0.03)	51	(32)	0.04	(0.011)	5.0	(1.8)	6.11	(3.396)	3.1	(0.82)
All species	0.22	(0.03)	1181	(644)	179	(92.6)	0.30	(0.07)	32	(18)	0.12	(0.038)	5.1	(1.2)	3.67	(1.997)	6.4	(1.75)

Table 3: DMSP data for 16 Antarctic phytoplankton species, diatoms shown in bold. DMSP is expressed per cell, C, N and chl *a*. C-DMSP:C indicates proportion of the DMSP-carbon to total C (%), DMSP production is estimated as from DMSP content (fmol cell^{-1}) multiplied by the specific growth rate (μ) and expressed as DMSP fmol cell^{-1} day^{-1}. For each species, the mean value for all replicates (n=4) is shown ± standard error in parentheses. Note: numbers in square brackets for *Thalassiosira* sp. indicate strain number from table 1.

Species	DMSP:cell pg cell^{-1}		DMSP:C mmol mol^{-1}		DMSP:N mmol mol^{-1}		DMSP:Chl a mmol g^{-1}		C-DMSP:C %		DMSP production fmol cell^{-1} day^{-1}	
C. castracanei	BDL		-		-		-		-		-	
C. simplex	BDL		-		-		-		-		-	
Chrysophyte sp.	BDL		-		-		-		-		-	
Dunaliella sp.	BDL		-		-		-		-		-	
***Fragilariopsis* sp.**	0.19	(0.015)	0.26	(0.031)	1.07	(0.04)	3.01	(0.08)	0.13	(0.02)	0.517	(0.036)
F. pseudonana	8.03	(0.438)	1.12	(0.086)	17.76	(2.17)	7.37	(0.58)	0.56	(0.04)	12.42	(2.772)
N. acicularis	0.02	(0.001)	-		0.48	(0.08)	1.07	(0.00)	-		0.027	(0.002)
N. lecointei	1.38	(0.040)	7.04	(0.852)	22.07	(2.04)	31.81	(1.50)	3.52	(0.43)	2.335	(0.275)
O. weissflogii	0.15	(0.029)	0.01	(0.003)	0.06	(0.01)	0.03	(0.01)	0.01	(0.00)	0.139	(0.034)
P. antarctica	0.04	(0.004)	-		-		30.72	(5.73)	-		0.072	(0.007)
P. cf. pouchetti	9.82	(0.565)	23.89	(2.276)	302.1	(58.35)	1092	(216)	11.95	(1.14)	12.33	(2.691)
P. gelidicola	0.07	(0.002)	0.24	(0.004)	1.81	(0.16)	1.10	(0.05)	0.12	(0.00)	0.074	(0.006)
Synechococcus sp.	0.004	(0.000)	0.13	(0.007)	0.82	(0.07)	0.41	(0.03)	0.06	(0.00)	0.013	(0.001)
Telonema sp.	BDL		-		-		-		-		-	
***Thalassiosira* [48]**	0.05	(0.006)	-		-		0.06	(0.00)	-		0.047	(0.005)
***Thalassiosira* [62]**	0.01	(0.007)	-		-		0.01	(0.01)	-		0.008	(0.007)
Averages												
Diatoms	1.40	(1.119)	2.11	(1.662)	8.29	(4.80)	6.19	(4.39)	1.05	(0.74)	3.088	(2.731)
All species	1.80	(1.076)	4.67	(3.342)	305.9	(298.7)	106.1	(98.60)	2.34	(1.67)	2.544	(0.273)

**BDL = Below Detection Limit*

Table 4: Dissolved DMS concentrations and DMSP lyase activity measured at 0ºC for 16 Antarctic phytoplankton strains and associated bacterial consortia. Diatoms shown in bold. For DMS, the mean value for all replicates (n=4) is shown with standard error in parentheses. For DLA, the mean rate from replicates (n=3-4) is given with standard error in parentheses. Note: numbers in square brackets for *Thalassiosira* sp. indicate strain number from table 1.

Species	DMS pmol mL^{-1}		DLA_{algae} fmol cell h^{-1}		$DLA_{bacteria}$ fmol cell h^{-1}	
C. castracanei	BDL	-	ND		ND	
C. simplex	7.0	(0.37)	1.32	(0.10)	ND	
Chrysophyte sp.	BDL	-	ND		ND	
Dunaliella sp.	9.0	(0.21)	0.02	(0.005)	ND	
***Fragilariopsis* sp.**	6.0	(0.12)	0.05	(0.01)	ND	
F. pseudonana	BDL	-	ND		ND	
N. acicularis	BDL		ND		ND	
N. lecointei	26.0	(0.28)	0.04	(0.01)	0.17	(0.01)
O. weissflogii	BDL	-	ND		ND	
P. antarctica	12.0	(0.49)	0.002	(0.001)	ND	
P. cf. pouchetii	1527	(57.3)	0.56	(0.10)	272	(52)
P. gelidicola	11.0	(2.51)	0.025	(0.002)	3.3	(0.6)
Synechococcus sp.	BDL	-	ND		ND	
Telonema sp.	BDL	-	ND		ND	
***Thalassiosira* [48]**	BDL	-	ND		ND	
***Thalassiosira* [62]**	9.0	(0.41)	1.67	(0.52)	ND	

*BDL = Below detection limit. *ND = Not detected.

Figure legends

Fig. 1 Growth curves for 16 Antarctic phytoplankton species used in this study, listed alphabetically. Cell density is presented in cells mL^{-1} ($x10^4$) ± SE (n=4). Note: differences in X-axes. Sigmoidal curves (3 parameter) were fitted to the data using the equation f = a/(1+exp(-(x-x0)/b)). Samples for characterisation were taken during a subsequent growth curve. Red boxes indicate growth phase and cell density of sample

Fig. 2 A) The quantity of C, N, and Chl *a* in pg $cell^{-1}$ as well as range of C:N and cell volume in μm^3 for 16 Antarctic species and B) concentrations of C, N per cell volume in pg μm^{-3} and fg μm^{-3} for Chl *a*. Boxplots show the range of data, the 1st and 3rd quartile (box) and median (black horizontal line). Note: Y-axis is a log scale

Fig. 3 The quantity of C, N, and Chl *a* in pg $cell^{-1}$ as well as C:N and cell volume in μm^3 by functional group. A) Centric (grey) and pennate (white) diatoms; C) Haptophytes *Phaeocystis* (grey), other - consisting of Chrysophyte, *Dunaliella*, *Pyramimonas gelidicola* and *Telonema* (white) and *Synechococcus* sp. (yellow diamond). Concentrations of C and N per cell volume in pg μm^{-3} and Chl *a* fg μm^{-3} by functional group. B) Centric (grey) and pennate (white) diatoms; and D) Haptophytes *Phaeocystis* (grey), other - consisting of Chrysophyte, *Dunaliella*, *Pyramimonas gelidicola* and *Telonema* (white) and *Synechococcus* sp. (yellow diamond). Boxplots show the range of data, the 1st and 3rd quartile (box) and median (black horizontal line). Diamonds for *Synechococcus* sp. represent mean ± SE (n=4)

Fig. 4 DMSP per cell (DMSPp) and intracellular concentrations in 16 Antarctic species, data arranged in descending order. A) DMSP content in fmol per $cell^{-1}$ ± SE (n=4) by functional group. Diatoms – orange, centric (circles) pennate (triangles), haptophytes – pink (hexagon), *P. gelidicola* – green (square), *Synechococcus* sp. – yellow (diamond). B) Intracellular DMSP concentrations (mM ± SE, n=4) arranged by functional group. Diatoms – orange, centric (circles) pennate (triangles), haptophytes – pink (hexagon), *P. gelidicola* – green (square), *Synechococcus* sp. – yellow (diamond). BDL, below detection limit

Fig. 5 The DMSPp data for 11 species combined and by functional groupings. A) all 11 species, B) Diatoms - centric (grey) and pennate (white); C) Haptophytes – *P. cf. pouchetti* (black), *P. antarctica* (grey); D) *P. gelidicola*

(black) and *Synechococcus* sp. (grey). The data are presented in the following units: DMSP:CV – DMSP per cell volume (CV) in mM; DMSP:cell – DMSP per cell in pmol $cell^{-1}$; DMSP:C – DMSP per carbon in mmol mol^{-1}; DMSP:N – DMSP per nitrogen in mmol mol^{-1}; DMSP:Chl *a* - DMSP per chlorophyll *a* in mmol g^{-1}. Boxplots show the range of data, the 1st and 3rd quartile (box) and median (black horizontal line). Data in dot plots represent mean ± SE (n=4). Note: differences in Y-axes

Figures

Figure 1

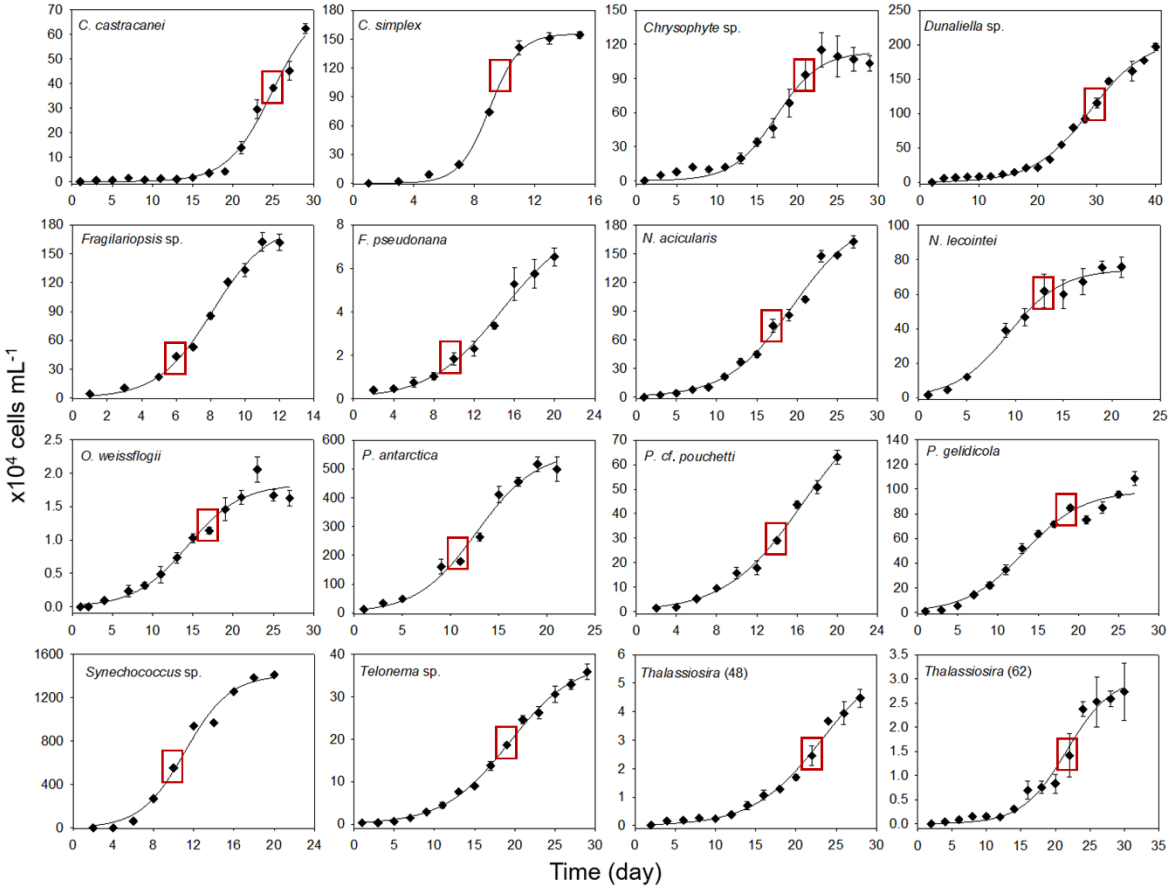
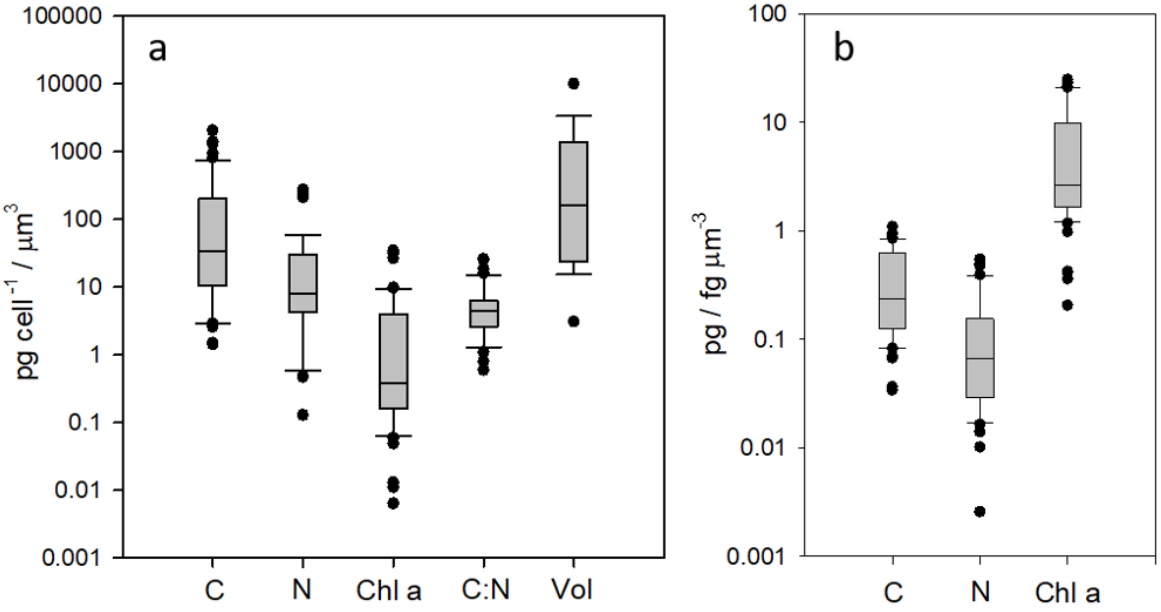


Figure 2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1000
100
10
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0.1
0.01
0.001
pg cell-1 / µm3
C
N
Chl a
C:N
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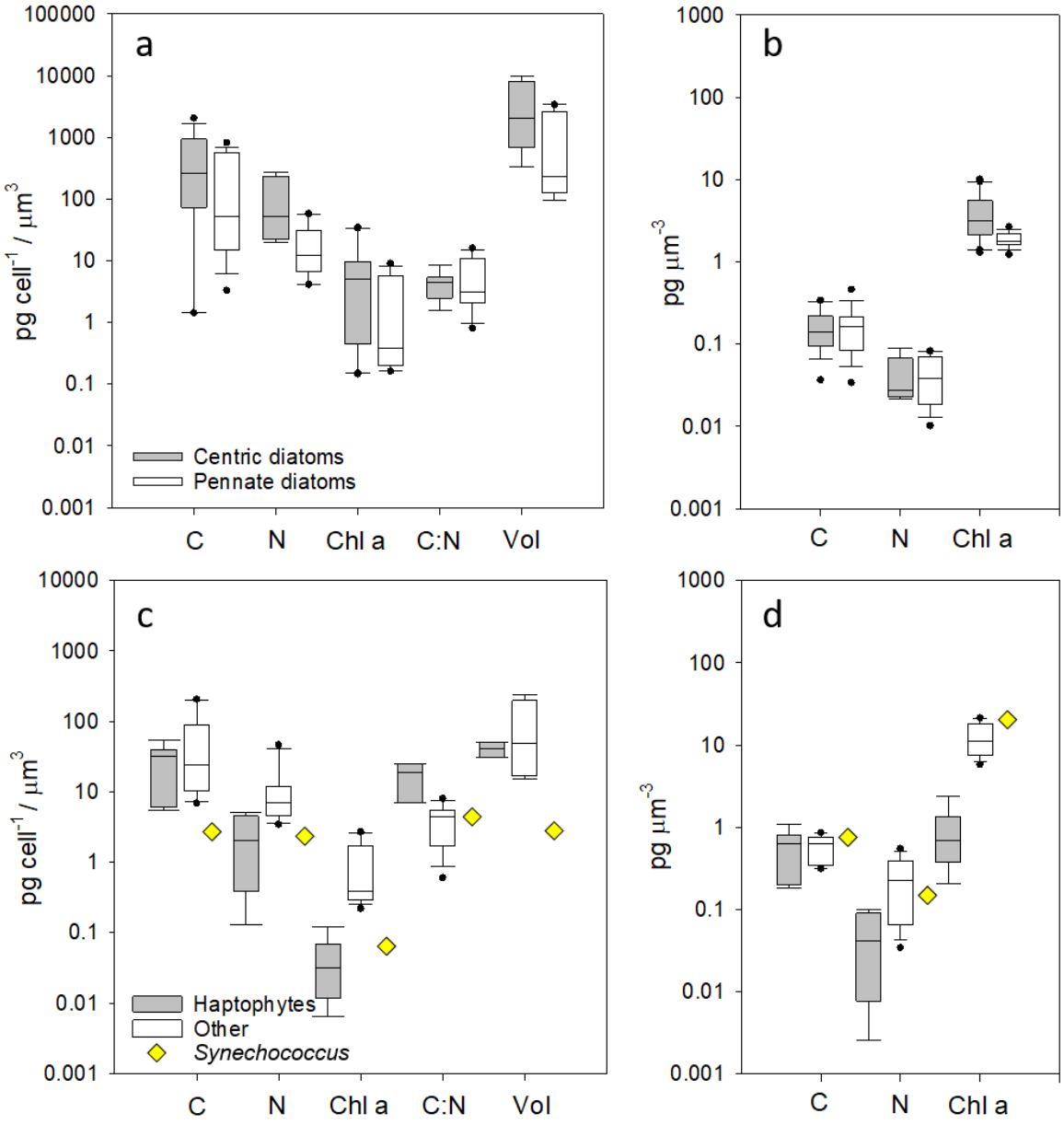
b
100
10
1
0.1
0.01
0.001
pg / fg µm-3
C
N
Chl a

Figure 3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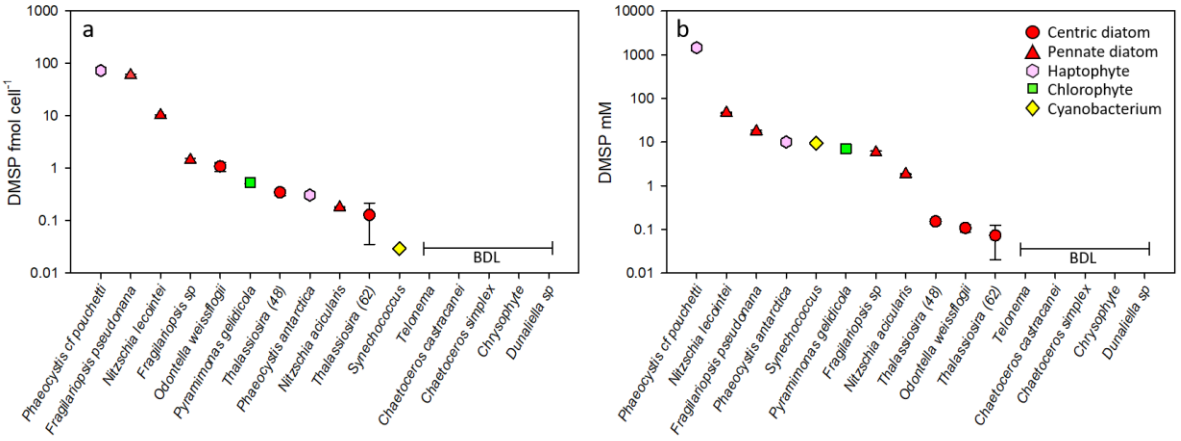
b
c
d
pg cell-1 / µm3
pg µm-3
Centric diatoms
Pennate diatoms
Haptophytes
Other
Synechococcus
C
N
Chl a
C:N
Vol

Figure 4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DMSP fmol cell-1
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BDL
Phaeocystis cf pouchetti
Fragilariopsis pseudonana
Nitzschia lecointei
Fragilariopsis sp
Odontella weissflogii
Pyramimonas gelidicola
Thalassiosira (48)
Phaeocystis antarctica
Nitzschia acicularis
Thalassiosira (62)
Synechococcus
Telonema
Chaetoceros castracanei
Chaetoceros simplex
Chrysophyte
Dunaliella sp
b
DMSP mM
10000
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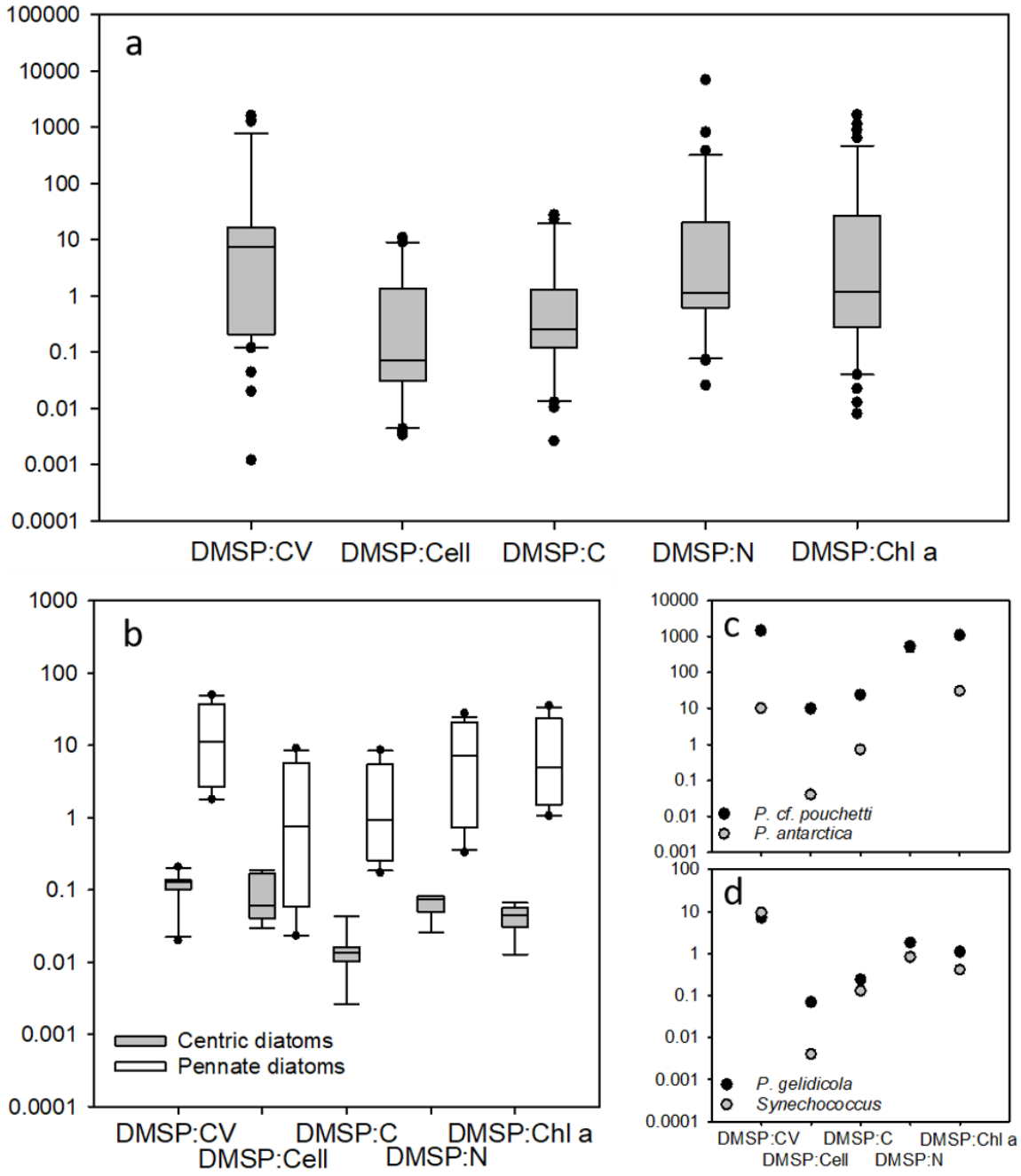
BDL
Centric diatom
Pennate diatom
Haptophyte
Chlorophyte
Cyanobacterium
Phaeocystis cf pouchetti
Nitzschia lecointei
Fragilariopsis pseudonana
Phaeocystis antarctica
Synechococcus
Pyramimonas gelidicola
Fragilariopsis sp
Nitzschia acicularis
Thalassiosira (48)
Odontella weissflogii
Thalassiosira (62)
Telonema
Chaetoceros castracanei
Chaetoceros simplex
Chrysophyte
Dunaliella sp

Figure 5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DMSP:CV
DMSP:Cell
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DMSP:Chl a
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Centric diatoms
Pennate diatoms
DMSP:CV
DMSP:Cell
DMSP:C
DMSP:N
DMSP:Chl a
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P. cf. pouchetti
P. antarctica
d
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1
0.1
0.01
0.001
0.0001
P. gelidicola
Synechococcus
DMSP:CV
DMSP:Cell
DMSP:C
DMSP:N
DMSP:Chl a