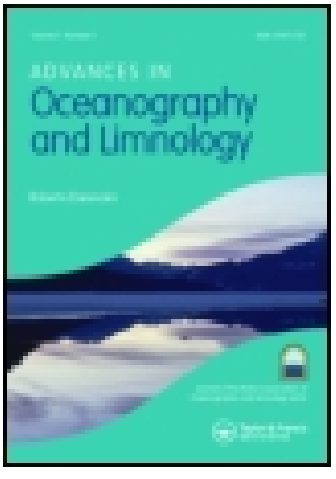

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In situ determination of cellular DMSP and pigment quotas in a *Prorocentrum minimum* bloom near the Falkland Islands

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Marine phytoplankton play critical roles in the biogeochemistry of open and coastal oceans. However, the impact that individual species have on an ecosystem-wide scale can strongly depend on the production of cellular compounds, especially those that are climatically active such as dimethylsulfide (DMS). Herein, we use sorting flow cytometry to separate a distinct phytoplankton population from four samples taken along the Patagonian shelf near the Falkland Islands. Morphological, genetic, and biochemical analyses demonstrated that three of the sorted samples were dominated by a bloom of the dinoflagellate *Prorocentrum minimum*. Cellular quotas of the DMS-precursor dimethylsulfoniopropionate (DMSP) ranged from 1.23−4.11 pg cell^{-1} in the same population at different sampling stations. Causes of this variability may be due to different growth stages of the *P. minimum* bloom or changes in other environmental variables. Overall, *in situ* intracellular DMSP concentrations were lower than what would be expected based on previous, culture-based measurements. We demonstrate the difficulties inherent in sorting individual phytoplankton species from natural samples in order to determine *in situ* species-specific cellular quotas of important biogeochemical compounds.

Keywords: dimethylsulfoniopropionate; Patagonian shelf; dinoflagellate; pigments; sorting flow cytometer

1. Introduction

Marine phytoplankton can modulate global biogeochemical cycles such as carbon sequestration to the deep ocean [1−2] and air-sea fluxes of important climate-controlling compounds such as CO_2 and dimethylsulfide (DMS) [3−5]. The impact that phytoplankton have on oceanic biogeochemical cycles can be strongly dependent on the interspecific variability in production rates of primary and secondary metabolites [6]. However, much of our understanding of the biogeochemical production and cycling of these compounds comes from laboratory studies involving unialgal cultures. By contrast, *in situ* conditions generally include multiple species simultaneously subjected to multiple environmental stressors. Recent reviews on the development of DMS ecosystem models and their incorporation into global climate models have highlighted the fact that our quantitative understanding of the drivers of DMS variability is severely limited [7−8]. These limitations are partly due to our lack of knowledge on the taxon-specific cellular quotas of dimethylsulfoniopropionate (DMSP), the precursor to DMS, in natural communities.

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DMS is a climatically active and volatile gas thought to play an important role in the formation of cloud condensation nuclei (CCN) [9]. Although the exact nature of the role played by DMS in CCN formation is a subject of much debate, a significant proportion of the sulfate in CCN is derived from DMS, making it a critical component of climate regulation mechanisms [10–11]. DMS in marine ecosystems largely comes from the breakdown of DMSP, which is produced by a broad range of eukaryotic phytoplankton [12]. Along with being a source of atmospheric DMS, DMSP is also important in microbial food webs and the oceanic carbon and sulfur cycles [13–14]. The cellular quotas of DMSP in marine phytoplankton taxa range by orders of magnitude between classes, genera, and species, and have mostly been determined in laboratory cultures [12,15–16]. A number of environmental conditions have also been shown to change intraspecific cellular DMSP concentrations, including temperature, salinity, light, and in some taxa nutrient limitation [8,17–21]. The differences in phytoplankton DMSP production under changing environmental conditions have been well established in the laboratory, but taxon-specific field measurements are rare [22]. This limitation has the potential to introduce bias into models when extrapolating laboratory-based cellular quotas to the environment due to, among other things, the nutrient enriched conditions of culture media and the age of the cultures.

In order to understand the coupling between the production of climatically active compounds by phytoplankton and their impact on the environment, it is critical to first determine taxon-specific cellular quotas under *in situ* conditions. While the influence of simultaneous multiple environmental stressors can make it difficult to constrain responses in natural samples, comparison between natural communities and laboratory cultures can provide context for the results from each situation. Sorting flow cytometry is a practical technique that can be used to separate marine phytoplankton populations *in situ* [22–24], and has the potential to better inform marine ecosystem models on this more fundamental level [6,8].

Previous work has used sorting flow cytometry to elucidate cellular DMSP production in groups of phytoplankton functional types (PFTs) in natural samples from the Mauritanian Upwelling in the eastern Atlantic Ocean [22]. However, the approach of Archer *et al*. [22] did not elucidate the contribution that individual species made *in situ* to the cellular DMSP production within each PFT class. Relationships of DMSP per cell in cytometrically-sorted PFT classes ignore taxon-specific cellular quotas of DMSP production, making it difficult to extrapolate any of the size class relationships to other regions of the world's oceans where species composition may differ within each PFT. Some field studies have shown correlations between different algal pigments and particulate DMSP ($DMSP_p$) concentrations in the environment [25–27]. For example, Sunda *et al*. [27] showed a strong correlation between the dinoflagellate-specific pigment peridinin and $DMSP_p$ in a coastal Belize lagoon, indicating that dinoflagellates were the predominant producer of DMSP in that system. This result demonstrates that variability in the phytoplankton population of pelagic ecosystems can exert strong control on the variability of DMSP through intracellular, species-specific production. However, it is well known that algal pigment concentrations can also vary with external factors such as light availability [28], which could affect any relationship between algal-specific pigments and intracellular DMSP concentrations. Therefore, it is important to determine both the *in situ* species-specific cellular quota of any taxon-specific pigments along with DMSP.

Herein, we report results from the flow cytometric sorting of a distinct phytoplankton population along the Patagonian shelf and subsequent analysis of important cell-specific compounds (DMSP and algal pigments). The phytoplankton population was sorted from

raw seawater using a high speed sorting flow cytometer (FCM) and identified using both morphological and genetic analyses. Combining flow cytometric sorting with other analyses may allow for the determination of cellular-specific quotas of important phytoplankton compounds (e.g. particulate organic carbon) and physiologically important metabolites throughout the world's oceans.

2. Materials and methods

2.1 Field site and sampling

All samples were obtained during the COPAS'08 expedition along the Patagonian shelf aboard the *R/V Roger Revelle* [29]. The expedition lasted from 04 December 2008 until 02 January 2009, and followed a sampling track from Montevideo, Uruguay to Punta Arenas, Chile. Salinity and temperature data from the ship's flow-through system along with satellite images of the sampling sites are given in Figure 1. One sample was taken at a profile station (S46) using a Niskin bottle, while the other three samples (U56, U59, and U63) were taken from the ship's underway system as it passed through areas of high surface chlorophyll concentrations just north of the Falkland Islands (Figure 1, Table 1). More details on the expedition including hydrographic data are reported elsewhere [29–30].

2.2 Flow cytometry

After collection, all samples were filtered through a 70 μm mesh and kept in the dark prior to FCM sorting, which was usually completed within a few hours of sampling. Seawater samples were first analyzed on a shipboard Beckman Coulter MoFlo Legacy in order to identify a distinct population in the cytograms suitable for sorting. For all sorting analyses, a 488 nm argon laser was used for excitation with the forward scatter (FSC) detector set to 488 ± 10 nm and a chlorophyll fluorescence detector set to detect emission wavelengths at 670 ± 15 nm. In all of the samples, the same distinct population of phytoplankton was separated according to its unique chlorophyll and FSC signature (Figure 2). All samples were sorted for morphological, genetic, pigment, and DMSP analyses, although some sorted samples were lost during processing (see below for more details; Table 1).

2.3 Scanning electron microscopy

Cells were sorted directly into 0.2 μm filtered seawater containing 1% paraformaldehyde and stored at -80°C until further analysis. In the laboratory, samples were gravity filtered onto a 1 μm polycarbonate membrane filter and ran through a series of salt water dilutions ending in 100% deionized water. The samples were then dehydrated through a graded series of ethanol and allowed to air dry. The filters were mounted onto stubs, sputter-coated with gold and imaged using a JEOL JSM5600-LV scanning electron microscope (SEM). Images of the phytoplankton cells were identified based on morphological characteristics [31].

2.4 Phylogenetics

Samples used for the phylogenetic analysis were sorted into 1 mL of sterilized seawater and frozen immediately at -80°C. Direct polymerase chain reaction (PCR) was used to

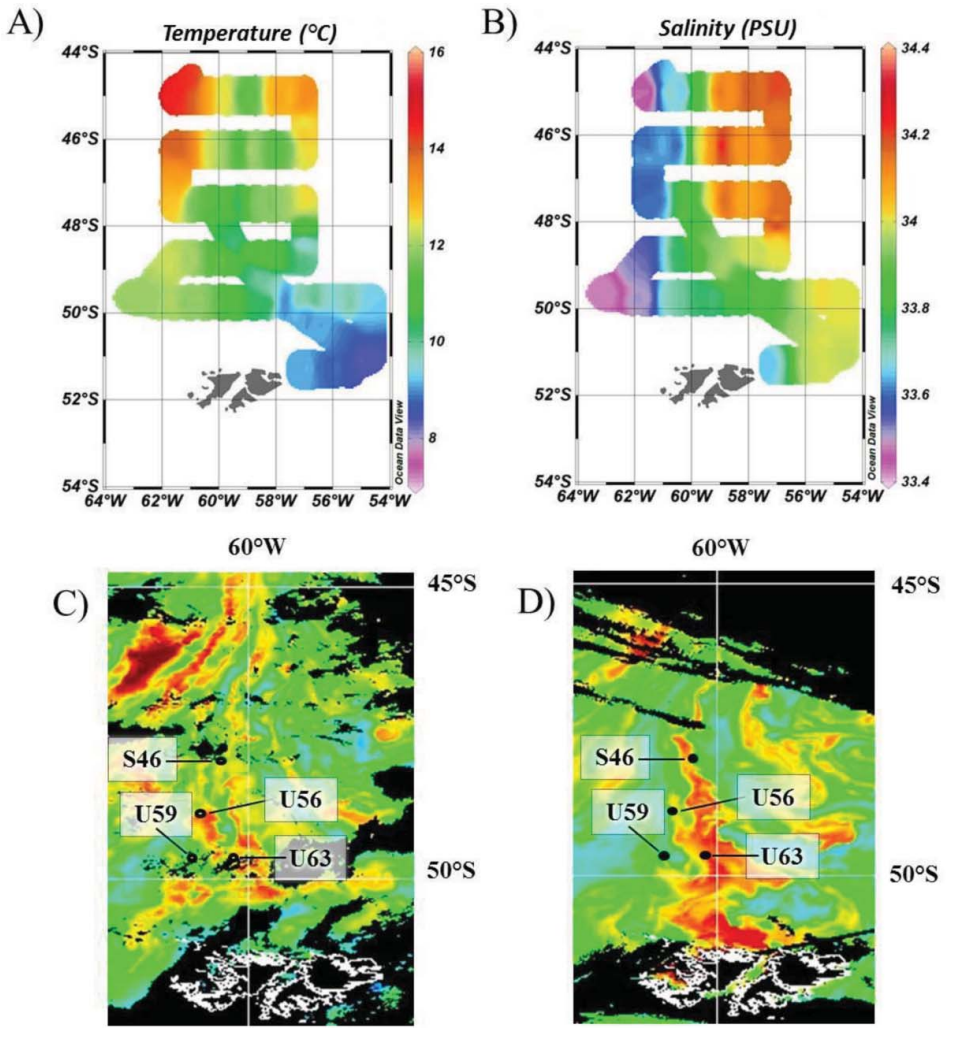

Figure 1. A) Sea surface temperature (measured using the ship's flow-through SBE 45 MicroTSG thermosalinograph) between 11 and 25 December 2008; B) Sea surface salinity; C) Chlorophyll *a* false color image (MODIS/Aqua satellite imagery collected 15 December 2008) showing the location of the four sampling stations S46, U56, U59, and U63 just north of the Falkland Islands; D) Chlorophyll *a* false color image (satellite imagery collected 25 December 2008).

amplify the 18S rDNA region of the sorted samples [32]. Each sample was spun down at 14,000 rpm and 4°C for 10 min. The supernatant was aspirated off and 1 mL of sterile water was added, after which the samples were subjected to two freeze-thaw cycles in liquid nitrogen in order to further lyse any cells. The samples were then spun down again (14,000 rpm, 4°C, 10 min) and the supernatant was aspirated off. GoTaq PCR mix with universal 18S primers (Table 2) [33] was pipetted directly onto the cells and transferred into PCR tubes after mixing. The sequences were amplified as follows: denaturing at 95°C for 2 min then 35 cycles of denaturing at 94°C for 45 s, annealing at 45°C for 45 s, extension at 72°C for 90 s with a final extension period at 72°C for 5 min [33]. The PCR products were visualized by gel electrophoresis using a 1.5% ethidium bromide agarose

Table 1. The date, depth, and location of water collection at each of the four sampling stations. The number of cells sorted for each analysis is shown along with the number of analyses (n) of each sort, n.s. means no sort was obtained.

			Location		Analyses and # of cells sorted (n)			
Station	Date	Depth	Latitude	Longitude	DMSP	Genetics	SEM	Pigments
S46	14-Dec-08	6 m	−47.498	−60.383	5,000 (4)	10,000 (1)	4,500 (1)	66,023 (1)
U56	18-Dec-08	Surface	−49.042	−60.829	500 (4)	n.s.	n.s.	132,462 (1)
U59	18-Dec-08	Surface	−49.750	−61.045	1,000 (3)	10,000 (1)	n.s.	n.s.
U63	19-Dec-08	Surface	−49.750	−60.163	500 (4) 1,000 (4)	10,000 (1)	? (1)	106,105 (1)

gel. DNA from weak bands was excised and cleansed using the Wizard SV Gel and PCR Cleanup System (Promega) and re-amplified as above.

Prior to cloning, all PCR products were purified using the Wizard SV Gel and PCR Cleanup System (Promega). The samples were cloned using a TOPO TA cloning kit for sequencing (Invitrogen). The vector was made with 4 μL of PCR product, 1 μL of a salt solution, and 1 μL of TOPO T4 vector. Two μL of this vector solution was added to TOPO TA Oneshot Top10 competent cells. The cells were incubated on ice for one hour and then heat shocked at 42°C for 30 s. Following the heat shock, 250 μL of room temperature Super Optimal Broth (SOC) medium was added to the cells and set to shake horizontally at 150 rpm and 37°C. After one hour, 100 μL of the cells were spread evenly

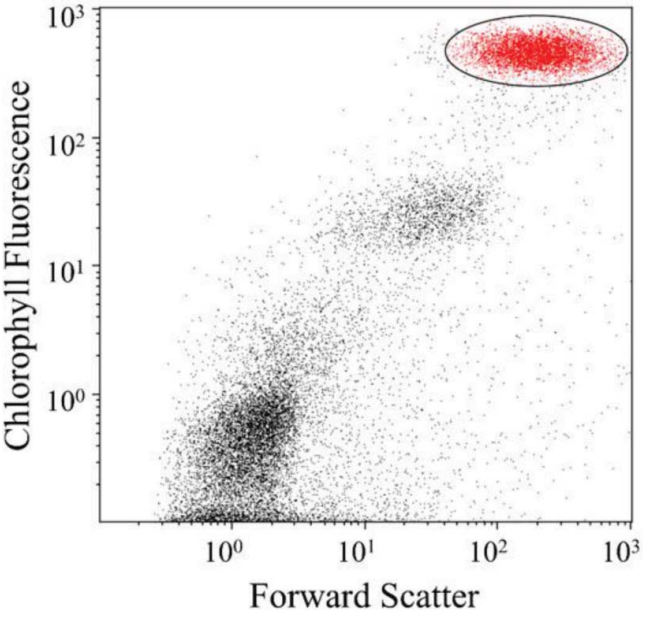

Figure 2. A representative cytogram from station U63 with the log of chlorophyll fluorescence plotted on the y-axis and the log of forward scattered light (488 nm) plotted on the x-axis. The sorted population was the same for all stations and is colored red and circled in the upper right corner.

Table 2. The primers used in the phylogenetic analysis of the sorted samples.

Name	Position	Sequence
UE18S1	18S universal eukaryote	5′-CGAATTCAACCTGGTTGATCCTGCCAGT-3′
UE18S2	18S universal eukaryote	5′-CCGGATCCTGATCCTTCTGCAGGTTCACCTAC-3′
T3	18S internal, forward	5′- ATTAACCCTCACTAAAG-3′
T7	18S internal, reverse	5′-TAATACGACTCACTATAGGG-3′
SR4	18S internal, forward	5′-AAACCAACAAAATAGAA-3′
SR6	18S internal, forward	5′-TGTTACGACTTTTACTT-3′
SR7	18S internal, reverse	5′-GTTCAACTACGAGCTTTTTAA-3′
SR10	18S internal, forward	5′-TTTGACTCAACACGGG-3′

onto a pre-warmed Kanamycin-resistant selective lysogeny broth (LB) plate (1 μg mL^{-1} Kanamycin) and incubated at 37°C for 48 h. Ten colonies from each sample were selected for further analysis. Each colony was inoculated into 5 mL of LB medium and incubated at 37°C for 18−20 h. The cultures were then spun down at 3000 g for 10 minutes and the resulting pellet was run through the QIAprep Spin Miniprep kit (QIAgen). The samples were digested with EcoRI and colonies with products in the proper size range were sequenced using the primers T3, T7, SR4, SR6, SR7, and SR10 (Table 2).

Sample sequences were assembled using Seqman (DNAStar). In order to reconstruct a phylogenetic tree, 18S rDNA sequences with a similar Blast score to the sorted samples were taken from Genbank and added to the analysis. The sequences consisted of 25 dinoflagellate and 7 diatom species from Genbank plus 10 sequences from each of the three unknown samples (S46, U59, and U63), for a total of 62 sequences. Sequences were aligned using Clustal W (European Bioinformatics Institute) and proofread by eye using BioEdit (Ibis Biosciences). A maximum parsimony analysis was performed on 1800 aligned base pairs using the phylogenetic software MEGA 4 [34].

2.5 *Pigment analysis*

Samples for high-performance liquid chromatography (HPLC) pigment analysis were sorted directly into 0.2 μm filtered seawater. Due to the large number of cells needed and subsequently long sort times (up to 8 h) for the pigment samples, the filtered seawater was kept in the dark at 2°C in order to reduce any pigment degradation. Directly after sorting, the samples were filtered onto glass microfiber filters (GF/F) (Whatman), wrapped in foil, and stored at −80°C until analysis in the laboratory. Bulk water samples were also filtered onto GF/F filters and stored and analyzed the same way as the sorted samples. Pigments were extracted by placing the filters in 100% acetone over night at −20°C. The extracted pigments were then injected into an Agilent 1100 HPLC with Diode Array and Fluorescence detectors, and separated according to the method of DiTullio and Geesey [35].

2.6 *DMSP analysis*

Cells for the determination of intracellular DMSP were sorted directly into 0.2 μm filtered seawater containing 2% sulfuric acid. Samples were then allowed to sit overnight before analysis. The number of cells that were sorted for each sample varied based on the cellular quota of the target population. Typically, between 500 cells

(for dinoflagellate-dominated populations) and 5000 cells (for diatom-dominated populations) were sorted during each run (Table 1). Samples for total DMSP ($DMSP_t$) and dissolved DMSP ($DMSP_d$) in bulk seawater were collected following a small-volume gravity filtration procedure and analyzed by conventional gas chromatography techniques [36]. Particulate DMSP ($DMSP_p$) was calculated as the difference between $DMSP_t$ and $DMSP_d$. A small aliquot ($\leq$20 mL) of each sample was immediately acidified with 50% sulfuric acid (10 μL mL^{-1}) then stored at 4°C for the determination of $DMSP_t$. A second aliquot ($\leq$20 mL) was gravity-filtered through a GF/F filter, collected and acidified with 50% sulfuric acid for the determination of $DMSP_d$. All sample preparation was conducted at room temperature. Upon analysis, all DMSP samples were base-hydrolyzed in strong alkali (2 mol L^{-1} sodium hydroxide) and analyzed for DMS using conventional cryogenic purge and trap gas chromatography [36]. Instrumental analysis was carried out on a Hewlett-Packard 5890 Series II gas chromatograph fitted with a flame photometric detector.

3. Results and discussion

3.1 Morphological and phylogenetic identification of the sorted population

SEM identification of the sorted population from stations S46 and U63 revealed that the population was dominated by the dinoflagellate species *Prorocentrum minimum*. Examination of SEM images from samples S46 and U63 revealed ovoid or triangular cells that were about 10 μm in diameter with regularly distributed small spines along the thecal plates (Figure 3). The periflagellar area was comprised of two different sized pores and an apical spine, which are all distinct characteristics of *P. minimum* [31]. While *P. dentatum* can be confused with *P. minimum*, morphological studies show *P. dentatum* cells to be larger ($\sim$17 μm) and more elongated with an asymmetrical and pointed end, unlike the rounded edge of *P. minimum* [31,37]. *P. dentatum* also has regularly distributed spines on the thecal plates, however, it does not have an apical spine like *P. minimum* and the sorted populations from S46 and U63.

A phylogenetic analysis was also conducted with the sorted populations from stations S46, U59, and U63. Clones from both S46 and U63 grouped mainly within the *Prorocentrum* clade (Figure 4). In both of the populations from S46 and U63, 7 of the 10 clones grouped strongly with *P. minimum*, while the remaining 3 clones grouped with other dinoflagellates. Only 1 of the 10 clones grouped closely with *P. minimum* in the sample from station U59, while 6 of the 10 clones grouped closely with *Thalassiosira* species of diatoms (Figure 4). The majority of diversity within each sample was likely due to the sorting of multiple species from a single population. When taken in context with the morphological and HPLC pigment (discussed in detail below) analyses, it can be concluded that the sorted populations from S46, U56, and U63 had a strong affinity to *P. minimum*. The population from U59, on the other hand, was most likely dominated by a diatom species. The high concentration of the diatom marker pigment fucoxanthin (up to 1.04 μg L^{-1}; unpublished data) in bulk water samples taken from a station near U59 supports this result, however, sorted pigment samples were unfortunately not obtained to confirm this. These findings demonstrate that while the sorted population was well separated from the majority of other phytoplankton using the FSC and chlorophyll detectors (Figure 2), it is often difficult to sort a taxon-specific eukaryotic population from a community of similar sized cells without using specific fluorescent markers in marine systems.

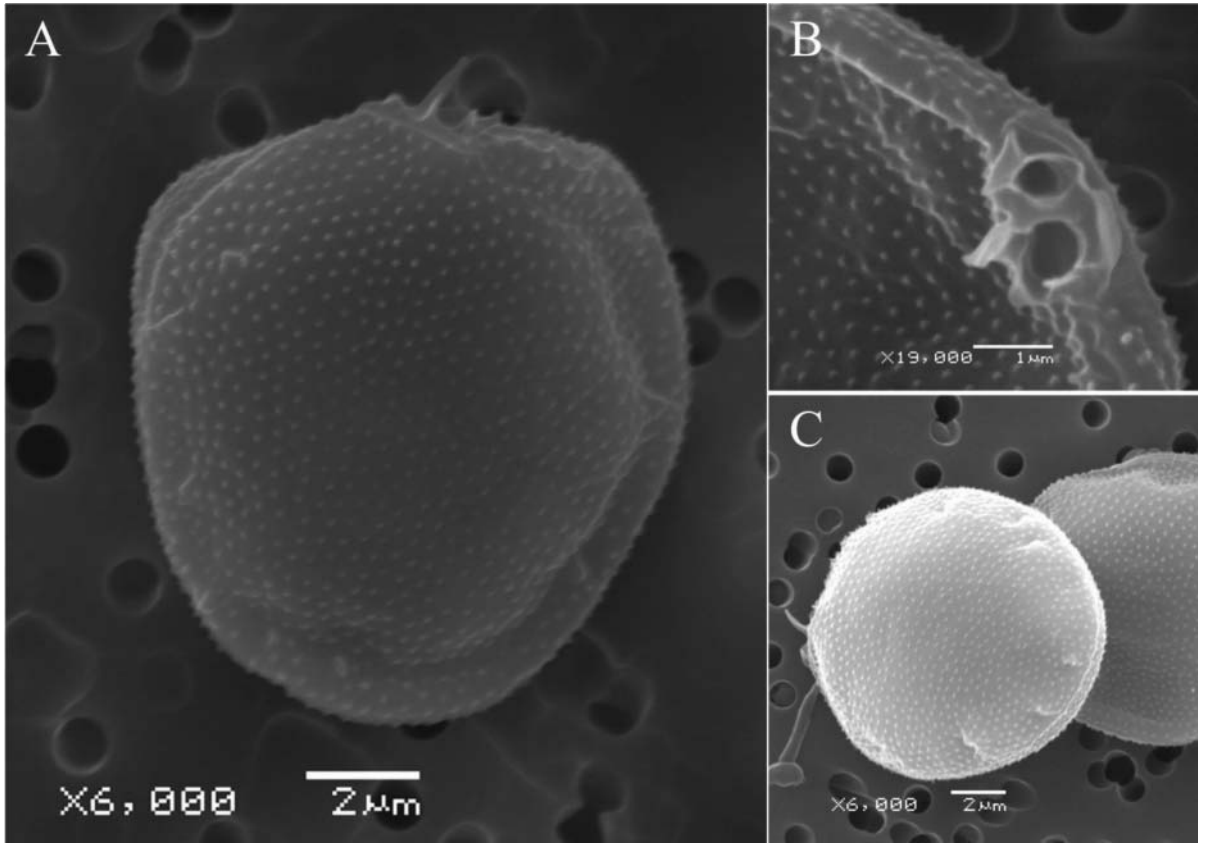

Figure 3. Representative SEM images from station S46 showing A) a full cell ~10 μm in diameter with an intact apical spine; B) a close up of the periflagellar area with two distinct pores and a prominent apical spine; C) SEM image from station U63 showing cells similar to those from S46.

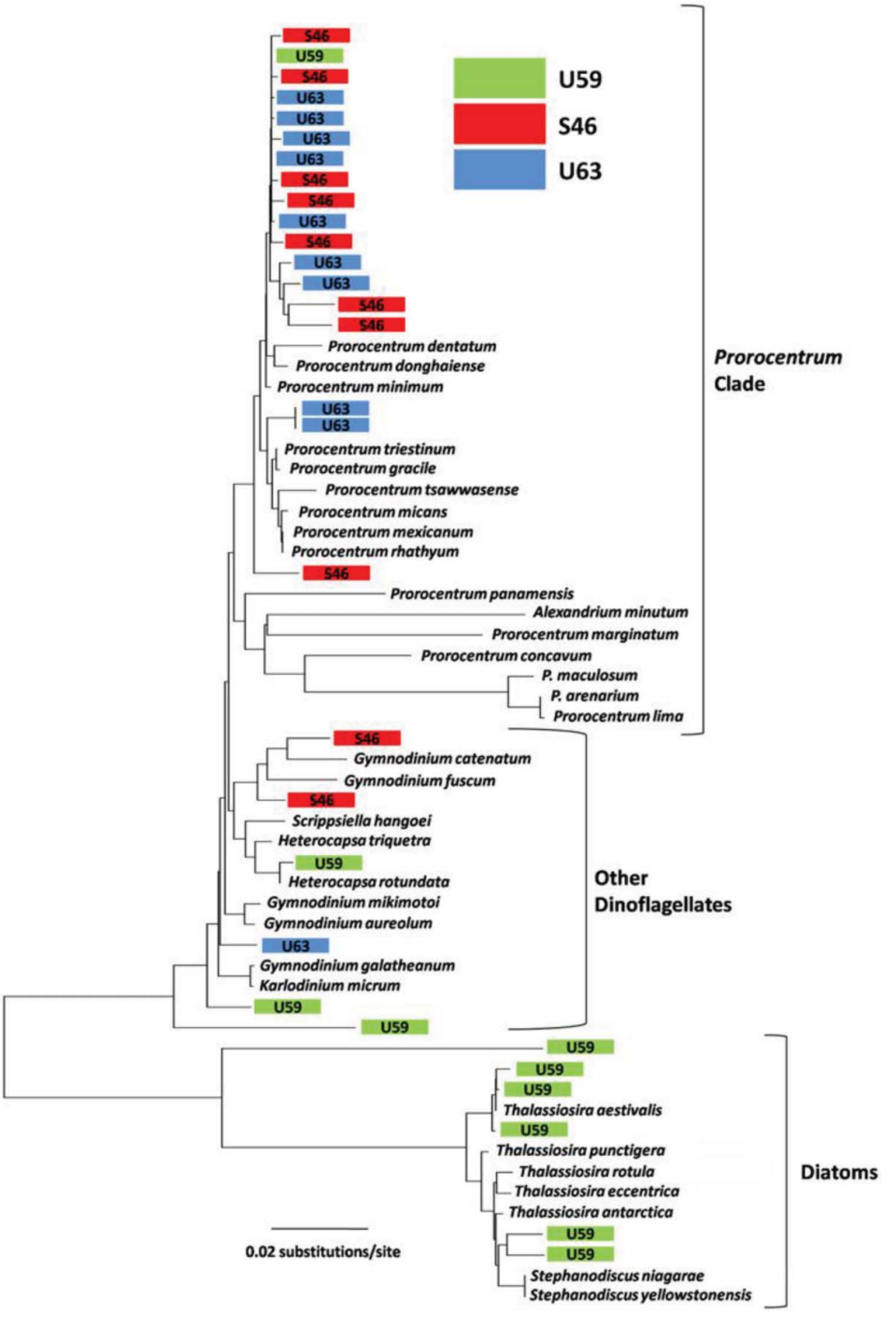

Figure 4. Phylogenetic tree derived from maximum parsimony analysis with the sorted populations from S46, U59, and U63 included. Stations U63 and S46 grouped mainly within the *Prorocentrum* clade while U59 grouped mainly with diatoms in the genera *Thalassiosira*.

3.2 Intracellular pigment concentrations

Bulk water samples that were collected for HPLC pigment samples throughout the COPAS'08 expedition can provide insight into the phytoplankton community composition. The dinoflagellate-specific pigment peridinin was elevated in the areas of high chlorophyll fluorescence from the satellite image (Figures 1, 5), indicating that the phytoplankton bloom contained a significant dinoflagellate population. The carotenoid

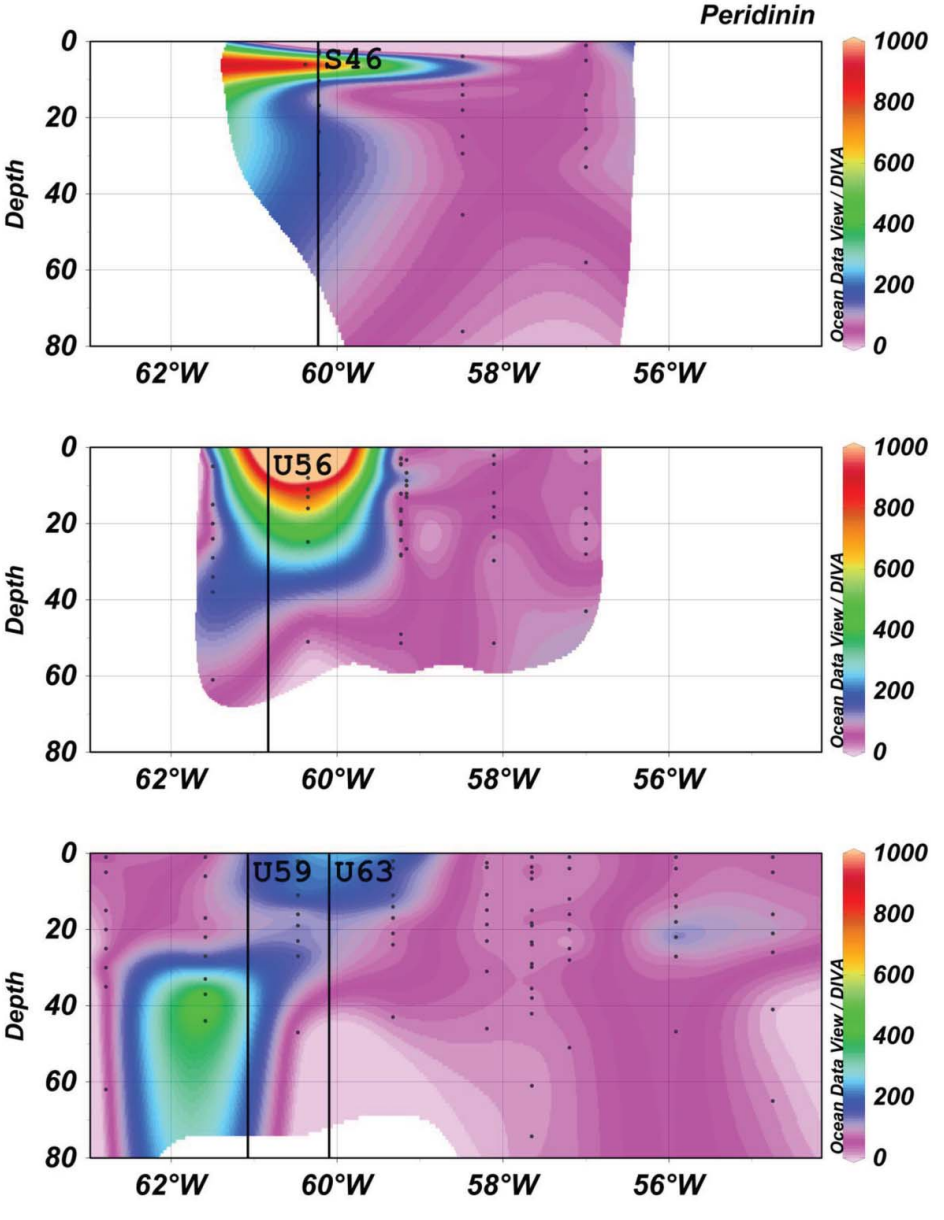

Figure 5. Depth profiles of peridinin concentrations (ng L^{-1}) across the three transects where the samples were sorted. The top graph corresponds to the transect containing station S46; the middle graph to the transect containing U56; and the bottom graph to the transect containing U59 and U63.

pigments in the sorted populations from S46, U56, and U63 were also dominated by peridinin, indicating that these populations were photoautotrophic dinoflagellates (Figure 6). These results agree well with both the morphological and phylogenetic analyses of the sorted population from those stations. FlowCAM and microscopic evidence confirmed the dominance of dinoflagellates in this region as well [29]. The locations where S46, U56 and U63 were sampled coincided with high peridinin concentrations in the bulk phytoplankton community, while U59 was taken from an area with lower peridinin (Figure 5). Although the sorted pigment sample was lost from U59, the lack of peridinin from the bulk community samples where U59 was taken from agrees well with the morphological and phylogenetic analyses of that sorted population (see section 3.1).

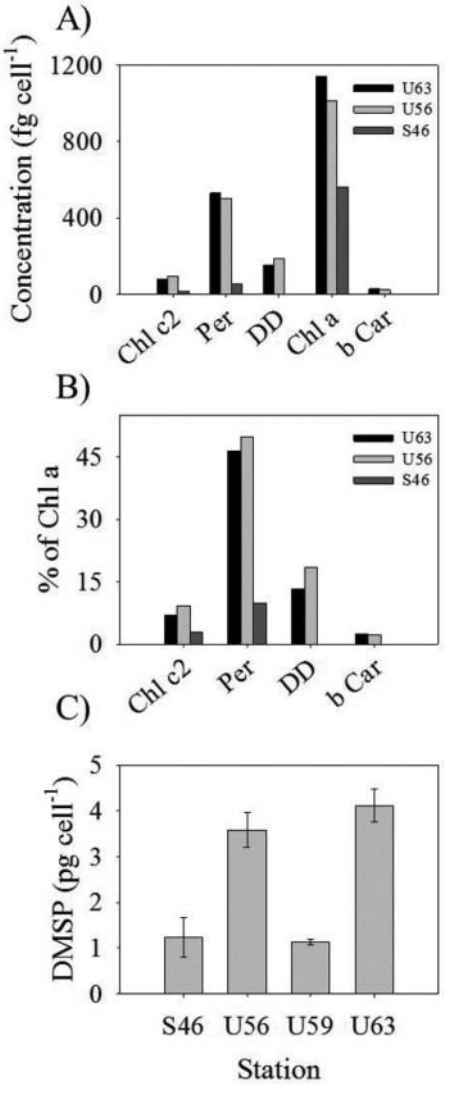

Figure 6. A) Cellular quotas of the algal pigments (fg cell^{-1}) chlorophyll c2 (Chl c2), peridinin (Per), diadinoxanthin (DD), chlorophyll a (Chl a), and β-carotene (b Car); B) percent of chlorophyll a for each pigment; C) DMSP quotas (pg cell^{-1}) in the sorted population from each station.

In the sorted populations of *P. minimum* the cellular-specific quota of chlorophyll *a* ranged from 562 to 1,141 fg cell^{-1}, while peridinin concentrations ranged from 55 to 529 fg cell^{-1} (Figure 6). These concentrations are within the range of concentrations reported in culture experiments of *P. minimum* [38]. The population from S46 had much lower intracellular pigment concentrations, which may be due to the low number of cells sorted (about half of U56 and U63) causing the sample to be near the limit of detection for the HPLC analysis (Table 1). However, using the average cellular quota of peridinin from U56 and U63 (516 $\pm$ 18.8 fg cell^{-1}) and community peridinin concentrations, an estimate of the *P. minimum* cell density can be made. A selection of surface stations along the sampling transects had peridinin concentrations ranging from 0.03 to 1.6 μg L^{-1}, which equate to cell densities of 0.05 to 3.1 $\times$ 10^6 cells L^{-1}, respectively (Table 3). If the cellular quota of peridinin from S46 is used (55 fg cell^{-1}) the cell densities become much greater (0.5 to 29.0 $\times$ 10^6 cells L^{-1}). Unfortunately, cell densities of the sorted population were not obtained from bulk samples due to problems with the calibration of the shipboard counting bead stock. Nonetheless, the cell densities calculated using the average peridinin quota from U56 and U63 fit well within the range of *P. minimum* densities (up to 8 $\times$ 10^6 cells L^{-1}) measured along the Patagonian shelf in a previous study [39]. Cell densities of *P. minimum* have been measured to be as high as 70 and 350 $\times$ 10^6 cells L^{-1} in other locations [40–41], demonstrating that the higher cell densities derived from the S46 quota are also realistic. The variability of the *in situ* cellular quotas of pigments, and subsequent estimates of *P. minimum* cell densities, suggests that the ability to analyze cell-specific pigment concentrations in the field could be important in modeling efforts that use pigment concentrations as proxies for the cellular abundance of algal taxa, as well as for the ground validation of satellite algorithms.

3.3 Intracellular DMSP concentrations

Intracellular DMSP concentrations derived from FCM sorting ranged from 1.14 to 4.11 pg cell^{-1}, with the lowest levels found in the sorted sample from U59. This low concentration may result from U59 being dominated by a species other than *P. minimum*, potentially a diatom of the *Thalassiosira* genera (Table 4, Figure 6). Previous studies have found that the Dinophyceae (dinoflagellates) have relatively large amounts of intracellular DMSP when compared to other classes of phytoplankton, especially the Bacillariophyceae (diatoms) [12,16,42–43]. Values of intracellular DMSP concentrations cover a broad range in dinoflagellates (0.68 to 593 pg cell^{-1}) and appear species-specific [12]. In the populations strongly associated with *P. minimum* (S46, U56, and U63), intracellular concentrations of DMSP were higher than in U59 and ranged from 1.23 to 4.11 pg cell^{-1}. Yet when compared to laboratory cultures of *P. minimum*, these intracellular concentrations were below the lower end of the reported range (8 to 43 pg cell^{-1}; Table 4). While this discrepancy could be due to contamination of the sorted sample with other phytoplankton species, we believe that this is unlikely as the populations from S46, U56, and U63 had a strong association with *P. minimum* as revealed by multiple lines of evidence including morphological, phylogenetic, and biochemical.

The population strongly associated with a diatom (U59; 1.14 pg DMSP cell^{-1}) had a higher intracellular DMSP concentration when compared to other *Thalassiosira* species (0.08–0.33 pg DMSP cell^{-1}), except for one reported in Keller *et al*. [12] to have 5.48 pg DMSP cell^{-1} that was designated as an unknown *Thalassiosira sp.* (Table 4). This result could be due to a high DMSP producing *Thalassiosira sp.* as observed by Keller *et al*. [12] or could have been caused by the concurrent sorting of *P. minimum* with any diatom

Table 3. Data from selected surface stations during the COPAS'08 cruise. Latitude (Lat), longitude (Long), depth, particulate and dissolved DMSP (DMSPp and DMSPd) concentrations, peridinin, and the ratio of peridinin to other accessory pigments (Per_{Acc}) in the bulk sea water samples. Cell densities of *P. minimum* estimated from the cellular peridinin quotas from the average of U56 and U63 and from S46, and the calculated contribution of *P. minimum* to bulk DMSPp concentrations estimated from cellular peridinin and DMSP quotas as described in the text.

Lat	Long	Depth (m)	DMSPp ($\mu g\ L^{-1}$)	DMSPd ($\mu g\ L^{-1}$)	Peridinin ($\mu g\ L^{-1}$)	Per_{Acc}	Cell Density (U56 &U63) (cells $L^{-1} \times 10^6$)	Cell Density (S46) (cells $L^{-1} \times 10^6$)	Calc DMSP (U56 &U63) ($\mu g\ L^{-1}$)	Calc DMSP (S46) ($\mu g\ L^{-1}$)
−47.498417	−60.383133	6	61.5	0.9	0.87	0.5	1.72	16.11	6.6	19.8
−47.498417	−60.383133	6	58.0	0.9	0.87	0.5	1.72	16.11	6.6	19.8
−48.750117	−57.000883	4	5.6	0.6	0.03	0.06	0.05	0.49	0.2	0.6
−48.717150	−58.111600	4.4	29.4	0.9	0.06	0.07	0.11	1.05	0.4	1.3
−48.717150	−58.111600	2	21.3	0.8	0.08	0.08	0.15	1.43	0.6	1.8
−48.749583	−59.163133	6.7	42.5	1.2	0.12	0.03	0.24	2.22	0.9	2.7
−48.749583	−59.163133	3.3	56.5	1.4	0.12	0.04	0.23	2.14	0.9	2.6
−48.748250	−60.351050	6	160.4	0.7	1.50	0.78	2.90	27.22	11.2	33.5
−48.748250	−60.351050	3	140.5	0.9	1.60	0.79	3.10	29.04	11.9	35.8
−48.749433	−61.498533	5	5.2	1.0	0.12	0.2	0.22	2.10	0.9	2.6
−49.750000	−61.588500	6	8.1	0.8	0.08	0.15	0.15	1.42	0.6	1.8
−49.750000	−61.588500	1	9.7	0.7	0.06	0.14	0.12	1.09	0.4	1.3
−49.750017	−60.470817	4	12.7	0.7	0.20	0.17	0.39	3.64	1.5	4.5
−49.750017	−60.470817	2	13.2	0.8	0.21	0.18	0.41	3.86	1.6	4.8
−49.749948	−59.325818	4	8.5	0.8	0.16	0.12	0.31	2.91	1.2	3.6

Table 4. Intracellular DMSP concentrations from this and other studies.

Species	Intracellular DMSP (pg cell^{-1})	Sample type	Reference
Prorocentrum spp.	16.4 - 593.0	Batch culture	Keller *et al*. 1989 [12]
P. minimum	21.4	Batch culture	Keller *et al*. 1989 [12]
P. minimum	43	Batch culture	Matrai and Keller 1994 [43]
P. minimum	8 - 32.6	Batch culture	Keller *et al*. 1999a [16]
P. minimum	8.9 - 12.5	Batch culture	Zhuang *et al*. 2011 [19]
P. minimum	1.23-4.11	Natural sample	This study
Thalassiosira spp.	0.08 - 5.48	Batch culture	Keller *et al*. 1989 [12]
T. pseudonana	0.16-0.33	Chemostat culture	Keller *et al*. 1999b [15]
Unknown (U59)	1.14	Natural sample	This study

species. Unfortunately, no pigment sample was obtained at U59, which may have helped to clarify what taxa dominated the sorted population. It must be also noted that some of the disparity observed between laboratory-based and flow-cytometrically-sorted results likely stems from the fact that many laboratory-based experiments use conditions (e.g. light levels, nutrients, growth temperatures) that are considerably more luxuriant as compared to *in situ* conditions. In addition, many laboratory phytoplankton species have been in culture for many years and may have genotypical and phenotypical variations from when they were first isolated.

Laboratory studies have demonstrated strong variations in phytoplankton intracellular DMSP concentrations due to changes in environmental conditions. Therefore, it is not surprising that variations were observed between the intracellular DMSP concentrations at the different stations (Figure 6). Multiple studies have demonstrated that intracellular DMSP concentrations of *P. minimum* are dependent on the growth phase, with up to 4-times greater intracellular DMSP concentrations observed during the early stages of the growth phase [16,43]. Some correlation has also been observed between nitrogen limitation and increased production of intracellular DMSP. Algae with relatively low-DMSP content tend to produce less nitrogen-based osmolytes and more sulfur-based DMSP as nitrogen availability decreases, although the same trend is not observed for high-DMSP producing species [7,15]. Other studies have also shown an increase in intracellular DMSP concentrations of phytoplankton in response to other types of nutrient limitation, which could be due to an overall increase in oxidative stress [20,21].

It is well known that salinity can alter the intracellular concentration of marine algae, and intracellular *P. minimum* DMSP concentrations were shown to increase from 8.9 to 12.5 pg cell^{-1} with an increase in salinity from 22 to 34 [19]. Changes in temperature have also been linked to greater intracellular DMSP concentrations [8,17], which may also be due to increases in oxidative stress. Therefore, the differences in intracellular DMSP concentrations between the sorted populations could also be explained by a number of confounding variables including salinity, temperature, general oxidative stress, growth phase of the algal bloom, and nutrient limitation. It is interesting to note that the *P. minimum* population with the lowest intracellular DMSP concentration (S46) was taken at a time when the bloom was starting to intensify (Figure 1c and 1d). However, S46 was also taken towards the edge of the bloom, which originated close to the Falkland Islands (Figure 1). The lower intracellular DMSP concentration in S46 may reflect that *P. minimum* populations in this region were approaching the senescent phase of

growth similar to DMSP quotas reported in culture studies [42−43]. Both nitrate and phosphate tended to decrease as the bloom moved further from the Falkland Islands (see Figure 11 in Painter *et al*. [30]) which may have resulted in nutrient limitation. However, nutrient depletion should have resulted in higher intracellular DMSP concentrations at station S46. Finally, the range of salinity was small throughout the area encompassing the bloom (Figure 1 and [29−30]), and therefore unlikely to have caused the observed differences in DMSP quotas. Independent of what caused the variation in intracellular DMSP concentrations between the stations, these results have important implications in extrapolating DMSP concentrations from culture-based methodology to the field.

The measurement of intracellular quotas of both pigments and DMSP allows us to estimate the contribution of *P. minimum* to the total amount of DMSPp measured in bulk water samples. Within the near surface stations, DMSPp varied from 5.2 to 160.4 μg L^{-1} (Table 3). Based on the estimates of cell densities from peridinin concentrations and cellular DMSP quotas, *P. minimum* could account for up to 50% of total community DMSPp concentrations (Table 3). If the average cellular ratio of DMSP to peridinin from stations U56 and U63 is used (7.4 pg DMSP:pg peridinin $cell^{-1}$), the percent contribution of *P. minimum* to total DMSPp ranges from 1.5 to 16.5%. However, if the ratio from S46 is used (22.4 pg DMSP:pg peridinin $cell^{-1}$) the percent contribution ranges from 4.4 to 49.5%. The ratio of peridinin to other accessory pigments (Per_{Acc}) in the bulk sea water samples ranged from 0.03−0.79, indicating that other phytoplankton groups could be responsible for the rest of the measured DMSPp concentration (Table 3). Therefore, it is important to use taxon-specific cellular quotas that match the true intracellular concentrations of marine phytoplankton when developing ecosystem level models.

4. Conclusions

The ability to measure intracellular, taxon-specific DMSP and pigment concentrations by sorting flow cytometry offers a valuable tool to oceanographers for developing biogeochemical and ecosystem level models. Furthermore, in order to successfully model the effects of climate change on phytoplankton community structure, it is necessary to measure the effects of physical parameters on the physiology and biochemistry of microalgae in their natural environment. While tightly controlled laboratory experiments can provide important information on relationships between DMSP and a stressor, these taxon-specific measurements only provide a 'snap-shot' of ecologically relevant species growing under modified environmental conditions. In this instance, *in situ* intracellular DMSP concentrations of *P. minimum* were on the order of 10-fold lower than would be expected based on previous, culture-based measurements. By knowing the amount of cellular DMSP produced in natural ecosystems, models can better predict the effect of algal blooms on local cloud cover and the radiation budget. While the flow cytometric sorting of specific eukaryotic microbial species in the field remains difficult without specific fluorescent markers, combining epifluorescent microscopy on sorted samples with a range of post-sort techniques will help to alleviate this problem and help to identify distinct populations for making biochemical measurements.

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References

[1] A.F. Michaels and M.W. Silver, *Primary production, sinking fluxes and the microbial food web*, Deep Sea Res. A 35 (1988), pp. 473–490.
[2] P. Boyd and P. Newton, *Evidence of the potential influence of planktonic community structure on the interannual variability of particulate organic-carbon flux*, Deep Sea Res. I 42 (1995), pp. 619–639.
[3] J.W. Murray, R.T. Barber, M.R. Roman, M.P. Bacon, and R.A. Feely, *Physical and biological controls on carbon cycling in the equatorial Pacific*, Science 266 (1994), pp. 58–65.
[4] P.W. Boyd, A.J. Watson, C.S. Law, E.R. Abraham, T. Trull, R. Murdoch, D.C.E. Bakker, A.R. Bowie, K.O. Buesseler, H. Chang, M. Charette, P. Croot, K. Downing, R. Frew, M. Gall, M. Hadfield, J. Hall, M. Harvey, G. Jameson, J. LaRoche, M. Liddicoat, R. Ling, M.T. Maldonado, R.M. McKay, S. Nodder, S. Pickmere, R. Pridmore, S. Rintoul, K. Safi, P. Sutton, R. Strzepek, K. Tanneberger, S. Turner, A. Waite, and J. Zeldis, *A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization*, Nature 407 (2000), pp. 695–702.
[5] P.W. Boyd, R. Strzepek, F. Fu, and D.A. Hutchins, *Environmental control of open-ocean phytoplankton groups: Now and in the future*, Limnol. Oceanogr. 55 (2010), pp. 1353–1376.
[6] M.W. Lomas, D.A. Bronk, and G. van den Engh, *Use of flow cytometry to measure biogeochemical rates and processes in the ocean*, Ann. Rev. Mar. Sci. 3 (2011), pp. 537–566.
[7] A.F. Vezina, *Ecosystem modelling of the cycling of marine dimethylsulfide: A review of current approaches and of the potential for extrapolation to global scales*, Can. J. Fish Aquat. Sci. 61 (2004), pp. 845–856.
[8] J. Stefels, M. Steinke, S. Turner, G. Malin, and S. Belviso, *Environmental constraints on the production and removal of the climatically active gas dimethylsulfide (DMS) and implications for ecosystem modeling*, Biogeochemistry 83 (2007), pp. 245–275.
[9] R.J. Charlson, J.E. Lovelock, M.O. Andreae, and S.G. Warren, *Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate*, Nature 326 (1987), pp. 655–661.
[10] P.K. Quinn and T.S. Bates, *The case against climate regulation via oceanic phytoplankton sulfur emissions*, Nature 480 (2011), pp. 51–56.
[11] M.O. Andreae and D. Rosenfeld, *Aerosol-cloud-precipitation. Part 1. The nature and sources of cloud-active aerosols*, Earth Sci. Rev. 89 (2008), pp.13–41.
[12] M.D. Keller, W.K. Bellows, and R.L. Guillard, *Dimethyl sulfide production in marine phytoplankton*, in *Biogenic Sulfur in the Environment*, E.S. Saltzman and W.J. Cooper, eds., American Chemical Society, New York, 1989, pp. 167–182.
[13] R.P. Kiene, L.J. Linn, and J.A. Bruton, *New and important roles for DMSP in marine microbial communities*, J. Sea Res. 43 (2000), pp. 209–224.
[14] R. Simó, S.D. Archer, L. Gilpin, and C.E. Stelfox-Widdicombe, *Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic*, Limnol. Oceanogr. 47 (2002), pp. 53–61.
[15] M. Keller, R. Kiene, P. Matrai, and W. Bellows, *Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. II. N-limited chemostat cultures*, Mar. Biol. 135 (1999), pp. 249–257.
[16] M.D. Keller, R.P. Kiene, P.A. Matrai, and W.K. Bellows, *Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. I. Batch cultures*, Mar. Biol. 135 (1999), pp. 237–248.

[17] M. van Rijssel and W.W.C. Gieskes, *Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of* Emiliania huxleyi *(Prymnesiophyceae)*, J. Sea Res. 48 (2002), pp. 17–27.
[18] J. Stefels, *Physiological aspects of the production and conversion of DMSP in marine algae and higher plants*, J. Sea Res. 43 (2000), pp. 183–197.
[19] G. Zhuang, G. Yang, J. Yu, and Y. Gao, *Production of DMS and DMSP in different physiological stages and salinity conditions in two marine algae*, Chin. J. Oceanol. Limnol. 29 (2011), pp. 369–377.
[20] W. Sunda, D. Kieber, R. Kiene, and S. Huntsman, *An antioxidant function for DMSP and DMS in marine algae*, Nature 418 (2002), pp. 317–320.
[21] E. Bucciarelli and W.G. Sunda, *Influence of CO_2, nitrate, phosphate, and silicate limitation on intracellular dimethylsulfoniopropionate in batch cultures of the coastal diatom* Thalassiosira pseudonana, Limnol. Oceanogr. 48 (2003), pp. 2256–2265.
[22] S. Archer, G. Tarran, J. Stephens, L. Butcher, and S. Kimmance, *Combining cell sorting with gas chromatography to determine phytoplankton group-specific intracellular dimethylsulfoniopropionate*, Aquat. Microb. Ecol. 62 (2011), pp. 109–121.
[23] W. Li, *Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting*, Limnol. Oceanogr. 39 (1994), pp. 169–175.
[24] M.D. DuRand, R.J. Olson, and S.W. Chisholm, *Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea*, Deep Sea Res. II 48 (2001), pp. 1983–2003.
[25] K.M. Crocker, M.E. Ondrusek, R.L. Petty, and R.C. Smith, *Dimethylsulfide, algal pigments and light in an Antarctic* Phaeocystis *sp. bloom*, Mar. Biol. 124 (1995), pp. 335–340.
[26] G.R. DiTullio and W.O. Smith Jr, *Relationship between dimethylsulfide and phytoplankton pigment concentrations in the Ross Sea, Antarctica*, Deep Sea Res. I 42 (1995), pp. 873–892.
[27] W.G. Sunda, R. Litaker, D. Hardison, and P.A. Tester, *Dimethylsulfoniopropionate (DMSP) and its relation to algal pigments in diverse waters of the Belize coastal lagoon and barrier reef system*, Mar. Ecol. Prog. Ser. 287 (2005), pp. 11–22.
[28] S.W. Jeffrey, *Algal pigment systems*, in *Primary Productivity in the Sea*, P. Falkowski, ed., Plenum Press, New York, 1980, pp. 33–58.
[29] W. Balch, D. Drapeau, B. Bowler, E. Lyczkowski, L. Lubelczyk, S. Painter, and A. Poulton, *Surface biological, chemical, and optical properties of the Patagonian shelf coccolithophore bloom, the brightest waters of the Great Calcite Belt*, Limnol. Oceanogr. 59 (2014), pp .1715–1732.
[30] S.C. Painter, A.J. Poulton, J.T Allen, R. Pidcock, and W.M. Balch, *The COPAS'08 expedition to the Patagonian shelf: Physical and environmental conditions during the 2008 coccolithophore bloom*, Cont. Shelf Res. 30 (2010), pp. 1907–1923.
[31] C.R. Tomas (Ed.), *Identifying marine phytoplankton*. Academic Press, San Diego, 1997.
[32] G. Wallner, B. Fuchs, S. Spring, W. Beisker, and R. Amann, *Flow sorting of microorganisms for molecular analysis*, Appl. Environ. Microbiol. 63 (1997), pp. 4223–4231.
[33] B.S. Leander, R.E. Clopton, and P.J. Keeling, *Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and β-tubulin*, Int. J. Syst. Evol. Microbiol. 53 (2003), pp. 345–354.
[34] K. Tamura, J. Dudley, M. Nei, and S. Kumar, *MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0*, Mol. Biol. Evol. 24 (2007), pp. 1596–1599.
[35] G.R. DiTullio and M.E. Geesey, *Photosynthetic pigments in marine algae and bacteria*, in *The Encyclopaedia of Environmental Microbiology*, G. Bitton, ed., John Wiley & Sons Inc., New York, 2002, pp. 2453–2470.
[36] P.A. Lee, J.R. Rudisill, A.R. Neeley, D.A. Hutchins, Y. Feng, C.E. Hare, K. Leblanc, J.M. Rose, S.W. Wilhelm, J.M. Rowe, and G.R. DiTullio, *Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. III. Dimethylsulfoniopropionate*, Mar. Ecol. Prog. Ser. 388 (2009), pp. 41–49.
[37] D. Lu, J. Goebel, Y. Qi, J. Zou, X. Han, Y. Gao, and Y. Li, *Morphological and genetic study of* Prorocentrum donghaiense *Lu from the East China Sea, and comparison with some related* Prorocentrum *species*, Harmful Algae 4 (2004), pp. 493–505.
[38] G. Johnsen, N.B. Nelson, R.V. Jovine, and B.B. Prezelin, *Chromoprotein- and pigment-dependent modeling of spectral light absorption in two dinoflagellates*, Prorocentrum minimum *and* Heterocapsa pygmaea, Mar. Ecol. Prog. Ser. 114 (1994), pp. 245–258.

[39] M.I. Gímez, A.R. Piola, G. Kattner, and V.A. Alder, *Biomass of autotrophic dinoflagellates under weak vertical stratification and contrasting chlorophyll levels in subantarctic shelf waters*, J. Plankton Res. 33 (2011), pp. 1304–1310.
[40] B. Witek and M. Pliński, *The first recorded bloom of* Prorocentrum minimum *(Pavillard) Schiller in the coastal zone of the Gulf of Gdańsk*, Oceanologia 42 (2000), pp. 29–36.
[41] S. Taş and E. Okuş, *A review on the bloom dynamics of a harmful dinoflagellate* Prorocentrum minimum *in the Golden Horn estuary*, Turkish J. Fish. Aquat. Sci. 11 (2011), pp. 673–681.
[42] M.D. Keller, *Dimethyl sulfide production and marine phytoplankton: The importance of species composition and cell size*, Biol. Oceanogr. 6 (1989), pp. 375–382.
[43] P. Matrai and M. Keller, *Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: Intracellular variations*, Mar. Biol. 119 (1994), pp. 61–68.