SAMS Research bursary report 2019

Abstract

Dimethylsulphoxide (DMSO) is the dominant dimethylated sulphur species in the oceans in the dissolved phase. Despite several known production pathways, oceanic DMSO concentrations are not continually increasing and the fate of DMSO in the ocean remains largely a mystery. Some of the highest open ocean surface DMS concentrations have been recorded in Northeast subarctic Pacific Ocean (NESAP) as well being one of the most extensive oxygen minimum zones (OMZs) in the world. As part of an international collaboration, a total of 100 discrete water samples for total DMSO and dissolved DMSO analysis were collected during a cruise in July 2016. This included 3 stations where low oxygen deep water (1000 m) was sampled and also incubations following a stable isotope tracer experiment. These were frozen for subsequent analysis at the Scottish Association for Marine Science (SAMS). This research bursary provided the means to transport these water samples and corresponding molecular filters back to Scotland for subsequent analysis. The analysis of these samples represents an important dataset as this will be the first investigation of DMSO and the bacteria involved in DMSO degradation to DMS along an oxygen gradient in the NESAP. In addition, there were also technical issues during the cruise so underway DMSO measurements were not taken as planned, as such these samples are important for our understanding of the dimethylated sulphur pool in the NESAP.

Background and the aims of the bursary

Susan Evans was invited to participate in a research cruise in 2016 as part of an international collaboration with Philippe Tortell (Director of the Peter Wall Institute, University of British Columbia, Canada). A total of 100 discrete water samples for total DMSO and dissolved DMSO analysis were collected along a cruise transect (Figure 1) in the northeast subarctic Pacific Ocean, including low oxygen deep water (1000 m) samples at 3 stations (Figure 1). Discrete water samples and corresponding molecular filters were frozen for subsequent analysis at the Scottish Association for Marine Science (SAMS). In addition water samples were frozen following a deck board tracer incubation study set up using water from 75 m and 1000 m.

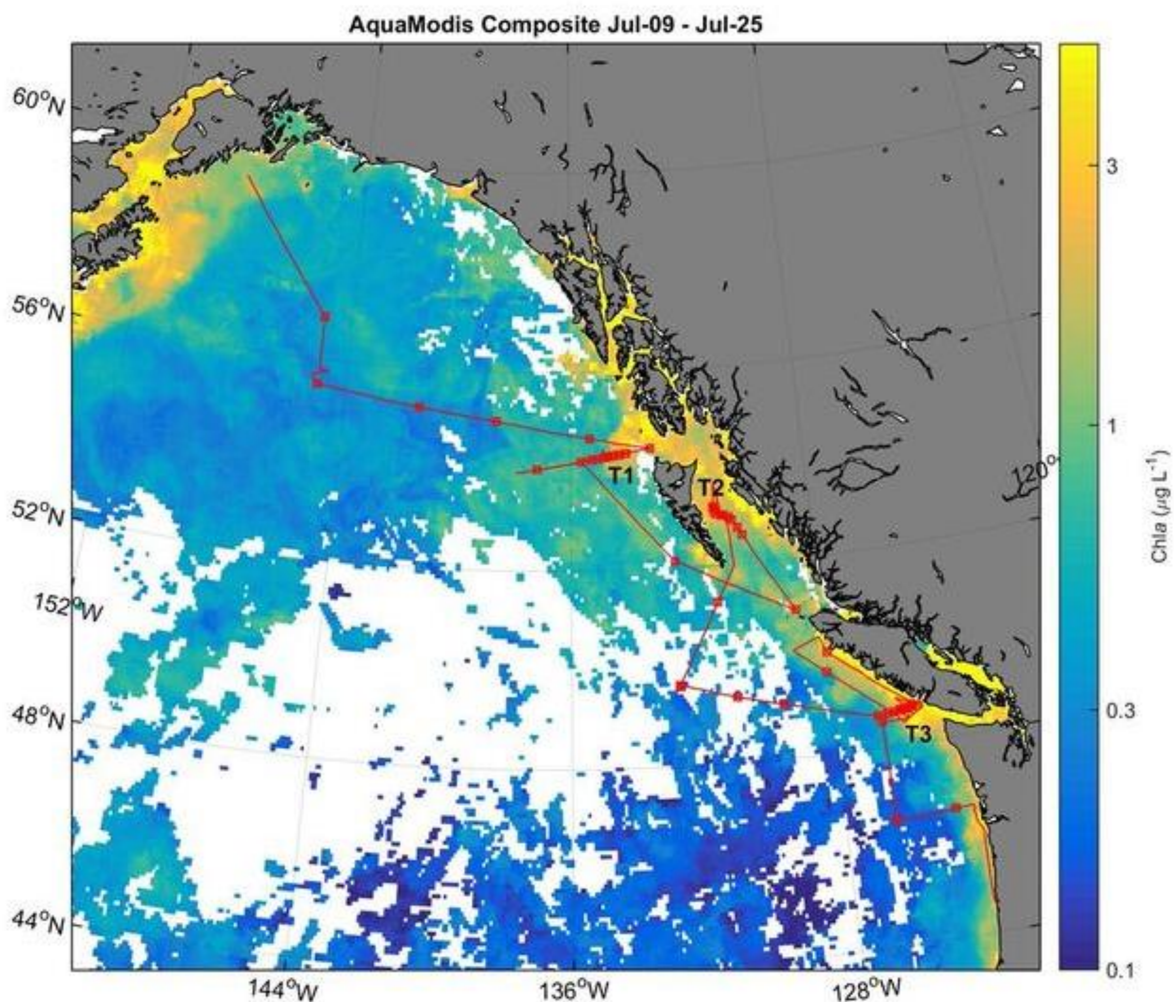

Figure 1. Cruise transect in the NESAP is shown in red with sampling stations shown as red squares. Chlorophyll-a concentration from AquaModis satellite data (Plotted by Philippe Tortell, UBC). Station 40 is located at T3.

Funding was requested to support the completion of the international collaborative project between SAMS and the University of British Columbia (UBC). £1000 was requested for the transport of frozen

water samples and molecular filters on dry ice from Vancouver to SAMS with Fedex. £700 of this was requested for the transport of samples using Fedex and the supply of dry ice required for this shipment. In addition, £300 was requested for subsistence for Susan Evans to allow her to analyse these samples using the gas chromatograph systems at SAMS.

The outcome (how the money was used and spent)

£600 of the research bursary was used to transport the water samples and Sterivex filters from the University of British Columbia to SAMS using Fedex International Priority. The remaining bursary was used as subsistence for Susan Evans allowing her to analyse the samples at SAMS. A total of 10 samples were analysed for DMS/O concentrations due to a unforeseen technical issue with the gas chromatograph. These samples were compared to DMSO concentrations measured at the sampling station RE5 in Loch Etive, which has experienced periodic hypoxia and also allowed some context to the deck board stable isotope tracer experiment conducted during the cruise.

Project background

Many marine bacteria are known to utilise DMSO as an alternative electron acceptor to oxygen via dissimilatory DMSO reduction to dimethylsulphide (DMS) (Zinder & Brock, 1978). This is an important pathway as a source of the climatically-active gas DMS as well as yielding energy. Despite this, very little is known about the environmental conditions required or the microorganisms involved in DMSO degradation. We hypothesised that in low dissolved oxygen environments such as oceanic oxygen minimum zones (OMZs), DMSO may be used as an alternative electron acceptor to oxygen and under these conditions, DMSO reduction is perhaps a more prevalent pathway in marine bacteria. As some of the highest open ocean surface DMS concentrations have been recorded in the NESAP (Asher *et al*, 2011), the NESAP could represent an area where the microbial reduction of DMSO is higher, or there is a high degree of cycling between DMS and DMSO. It was anticipated that the analysis of these water samples will have high impact as DMSO is not a well-studied compound in the ocean and knowledge of both global DMSO distribution and the prevalent pathways in the NESAP will greatly add to current knowledge of sulphur cycling. In addition, no underway DMSO measurements were collected during the cruise due to technical issues therefore the discrete DMSO measurements will add a great deal to the current dataset of DMS measurements.

Methodology

The cycling of DMS/P/O was simultaneously examined in discrete seawater samples using a stable isotope tracer method developed by Asher et al 2017. Seawater for rate experiments was collected during the cruise from 10th – 27th July 2016 from 1000 m and 75 m depth using Niskin bottles at 3 stations. Upon collection, water was immediately kept in the dark. 75 m was chosen as it was the bottom of the chlorophyll maximum. In addition to the water for tracer rate experiments, discrete water samples (100 ml) were collected from each sampling station along the cruise transect and frozen for subsequent DMSO and DMSOd analysis at SAMS.

Each sample for the tracer experiment was analysed on the ship for initial DMS and DMSPd concentrations, when possible, using the OSSCAR system (Asher et al.,2015) or discrete FPD-GC analysis. In triplicate, each sample from 1000 m and 75 m was spiked with D-3 deuterated DMS (CDN Isotopes, 99.9% purity), D-6 deuterated DMSP (produced using the method of Challenger and Simpson, 1948, from D-6 DMS Sigma Aldrich, 99% purity), and D-6 deuterated, 13C-labeled DMSO (ISOTEC, 99% purity) were added at a final concentration of 0.5 nM. Following addition of the stable isotopes, SW was gently mixed, and 3 L was dispensed into UV-transparent FEP bags (Welch Fluorocarbon) fitted with gas tight sampling ports. Bags were incubated at 4℃ in the dark at as close in-situ surface temperature as possible.

Every 120 minutes, a 5 ml subsample was withdrawn using a Hamilton gas-tight syringe with luer-lock and quickly transferred to glass vial and sealed with a rubber septum. These were attached to an octopodal valve sampler and analysed via PT-CIMS (pulse trace mass spectrometer). The concentration of isotopically labelled DMS species was determined using a SCIEX API 3200- series triple quadrapole mass spectrometer. Specifically, the change in m/z 63 signal of natural DMS (to measure net DMS production), labelled D-3 DMS (m/z 65; to measure gross DMS consumption), D-6 labelled DMS (m/z 69) produced through the cleavage of labelled DMSP, and m/z 71 DMS resulting from reduction of D-6 13C labelled DMSO were measured (Figure 2)

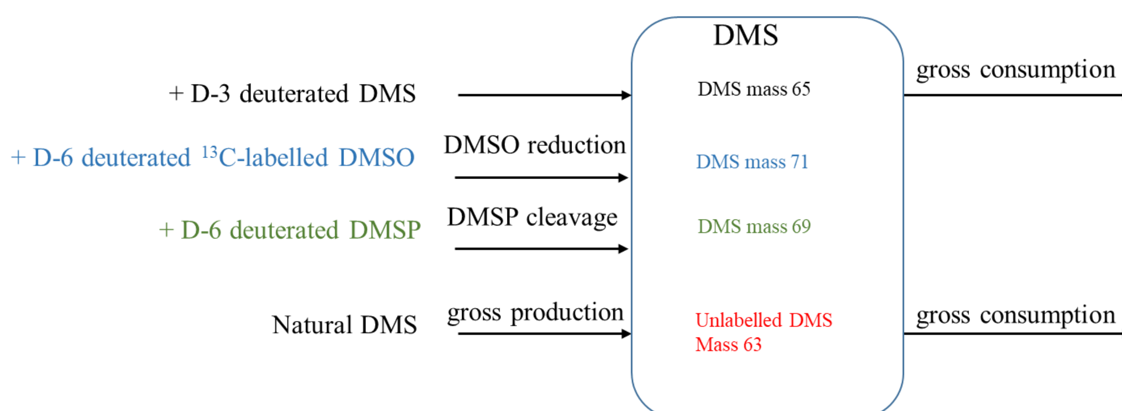

Figure 2. Schematic showing the origin and fate of isotopically labelled DMS species

Seawater (SW) samples were immediately frozen at -20ºC following sampling and PT-CIMS analysis. SW samples were shipped from University of British Columbia (UBC) to SAMS. The transport of these samples came from the direct receipt of this research bursary. Upon receipt, samples were slowly defrosted and equilibrated to room temperature.

DMS was measured by the purge and trap method as described by Turner et al. (1990). Aqueous samples were loaded through a 2-way injection port with luer connections into a 25 ml glass purge tube. Sulphur compounds were stripped from the sample using oxygen-free nitrogen gas (BOC, Oxygen Free Nitrogen-grade) (60 ml min^{-1}). The purge gas stream was directed through glass tubing containing glass wool (Alltech Associates Inc.) to remove water and prevent ice blockages in the sample loop. A Nafion® drier (International Science Consultants) filled with regenerated 13X molecular sieve (BDH Laboratory Supplies) removed residual water vapour. The gas stream then passed into a cryotrap dewar part filled with liquid nitrogen through a PTFE sample loop and into a 6-way injection valve (Alltech Associates Inc.). The sample loop was held in the dewar at approximately 2 cm above the liquid nitrogen to maintain a temperature of approximately -150°C. To inject a sample into the gas chromatograph (GC), the valve was turned to inject and the sample loop thawed by heating to > 80°C using freshly boiled water. Samples o were purged for a minimum of 16 minutes before being injected to allow the nitrogen carrier gas to bubble through the sample and purge all DMS from the solution. A Varian Star 3400 GC fitted with a pulse flame photometric detector (PFPD) and a Chromasil 330 packed column (Supelco Deutschland 8´ x 1/8" OD) (DMS has a retention time of approximately 2.3 minutes on this column) was used for the analysis of DMS. The detector oven was maintained at 300°C and the column oven at 40°C. N_2 was used as the carrier gas and maintained at a flow rate of 20 ml min^{-1}. Peak areas were recorded and analysed using the VarianStar package (Version 6.41) which was used for the analysis of peaks including the reintegration of selected peaks.

DMSO was measured indirectly using the enzymatic reduction to DMS followed by the quantification of DMS as described by Hatton et al., 1994. The reduction of DMSO to DMS was achieved by using a reducing solution containing 25 µg ml^{-1} DMSO reductase ($DMSO_r$) (purified in house) 30 mM ethylenediamine N, N, N´,N´,tetraacetic acid (EDTA) and 540 µM flavin mononucleotide (FMN). In the presence of light and under semi-anaerobic conditions required for $DMSO_r$, EDTA forms free radicals which reduce FMN to $FMNH_2$. $FMNH_2$ then acts as an electron donor to DMSO reductase catalysing the reduction of DMSO to DMS in a 1:1 ratio. The reducing solution was fast purged in a purge tube continually bubbled with oxygen-free nitrogen gas (100 ml min^{-1}) in front of 3 20 W bulbs. The reducing solution was then stored in the dark at 4°C for up to 14 days to avoid contamination.

Aliquots of DMS-free samples were injected into the purge tube containing 2 ml of reducing solution and bubbled with oxygen-free nitrogen to ensure the semi-anaerobic conditions required for EDTA to form free radicals. Prior to sample analysis and to ensure that the DMSO reductase method was working

correctly a 5 point calibration was carried out using DMSO standards prepared in house. DMSO calibration standards were prepared by adding 1 ml DMSO (99.5%, Sigma-Aldrich) into 500ml Milli-Q® water to give a primary stock of 28.2 mM. This was serially diluted with Milli-Q® water into a working stock solution of 3.16 ng S ml^{-1}. A range of volumes (1-10 ml) were used to produce a DMS standard curve through the reduction of DMSO.

Results & Discussion

The initial results from this study suggest that concentrations of DMSOd in the NESAP at 1000 m are approximately 2.1 nM (Table 1). However, replicate seawater samples need to be analysed from more than one station to give a meaningful insight as due to technical issues with the GC only 10 samples were analysed. However, this preliminary data corresponds to the concentration of DMSO at 1000 m in the Arabian Sea OMZ (Hatton et al., 1999) where concentrations of approximately 2 nM were detected. Typically, it is thought that DMSO is detected at greater depths in the ocean compared to DMS and DMSP, despite the lack of measurements of DMSO below 200 m. DMS concentrations were below the detection limit of the GC at 800 and 1000 m. This is perhaps unsurprising as DMS has a very short turnover with consumption rates ranging from <1 days – 2.1 days in the surface ocean (Simo et al., 2000) and as a labile source of carbon is likely rapidly scavenged in the deeper waters.

Table 1. DMS and DMSOd concentrations (nM) at Station 40 along the cruise transect.

Depth (m)	DMS concentration (nM)	$DMSO_d$ concentration (nM)
5	0.69	5.7
75	0.12	0.46
200	0.1	0.84
500	0.05	Not analysed
800	below detection limit	Not analysed
1000	below detection limit	2.4

The potential concentration of DMS (mass 71) derived from the reduction of DMSO in water taken from 75 m and 1000m in the NESAP is shown in Figure 3. At the start of the incubation, the amount of DMS derived from DMSO reduction was highest in the water from 1000 m. However, the amount of DMS (mass 71) derived from DMSO declines 4 hours into the tracer experiment and similar amounts of DMS are measured in both 75 m and 1000 m incubations. This could be because over time, oxygen is being introduced into the sampling bags as samples are taken for analysis. Initial results from the stable isotope tracer experiment (Figure 3) may suggest that DMSO reduction is greater in lower oxygen environments and that potentially any DMS produced is rapidly taken up or cycled. However, additional DMS and DMSO concentration measurements are required to calculate the rate of DMSO reduction associated with the stable isotope tracer experiments. Previous stable isotope tracer experiments in the

Southern Ocean suggest that DMSO is actively cycled in the mixed layer and can be a significant source of DMS (Herr, A. Personal communication).

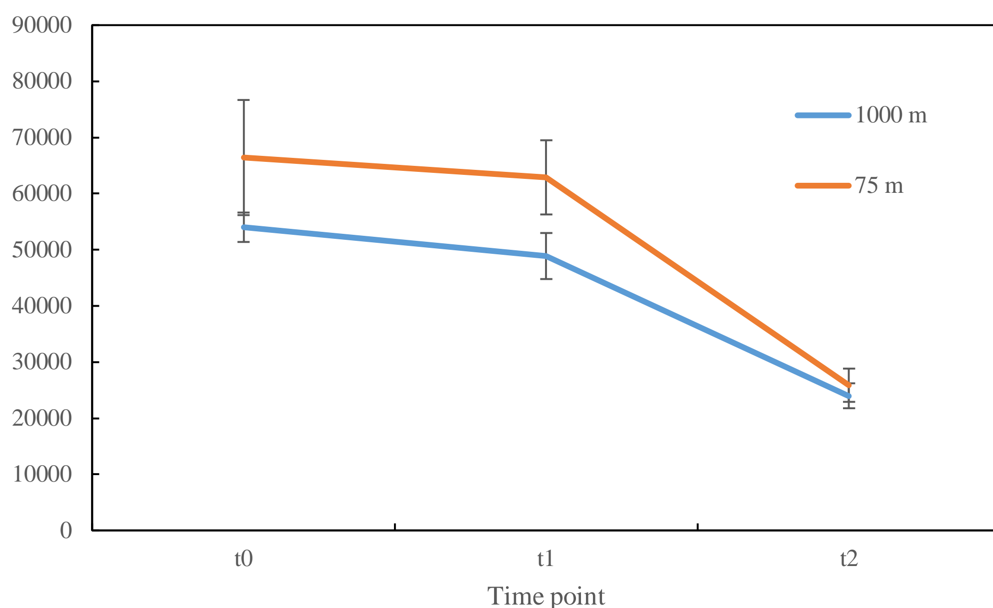

Figure 3. Peak integration of DMS 71 (from the addition of D-6 13C labelled DMSO) over time in 1000 m and 75 m tracer incubations. Samples were taken at 120 minute intervals. Error bars represent +/- SD of the mean (n=3).

These preliminary findings (Table 1 and Figure 3) are similar to those generated during a field study conducted in Loch Etive, West coast of Scotland (Evans, 2018). Restricted exchange (RE) station 5 (RE5) in Loch Etive, routinely experiences periodic hypoxia. DMS and DMSO concentrations were measured by purge and trap GC at station RE5 in Loch Etive. At the time of sampling, DMS at RE5 was below detection limit at all depths except at 142 m (3.1 nM) which corresponds to the depth with the lowest dissolved oxygen concentration (Figure 4). DMSO concentration was also highest at 142 m (12.2± 3.21 nM). DMSO can be transported out of the surface water in sedimenting particulate matter (Hatton et al., 2002). At RE5, the presence of both DMS and DMSO associated with a low oxygen environment could suggest the cycling of these two sulphur compounds as typically DMS is associated with phytoplankton in the surface waters.

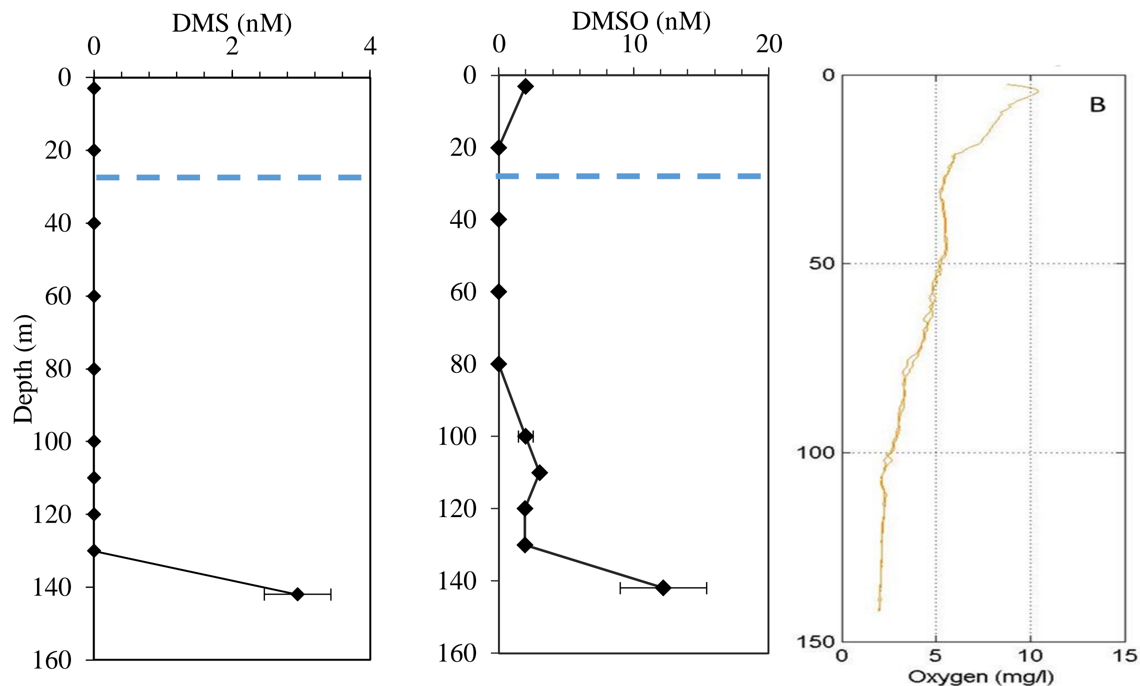

Figure 4. Vertical water column profiles for average A) DMS, B) DMSO, C) dissolved oxygen concentrations at Station RE5 as measured on 27.11.15. Horizontal error bars denote ± SD of the mean (n = 2). Dashed line indicated the depth of the oxycline (ca. 25 m).

Together, these finding suggest that DMSO reduction may be an important pathway in low oxygen environments. Further research is required to validate this preliminary data but the SAMS bursary has facilitated the initial analysis of important samples.

Future work

Several studies in OMZs suggest that a higher bacterial diversity often seem in these areas is typically linked to the use of a broader range of terminal electron acceptor compared to the surface depths where oxygen is the dominant electron acceptor (Stevens & Ulloa, 2008). To follow up on the preliminary results generated from this study, DNA will be extracted from the 0.2 µm Sterivex filters collected during the cruise and the 16S rRNA sequenced by Sanger sequencing. This will provide information about the bacterial community present at 75 m and 1000 m during the time of the tracer incubation. The presence/absence of bacterial species with known DMSO metabolism such as *Shewanella* (Xiao et al., 2007; Xiong et al, 2017) will be determined as well as bacteria with genes for DMSO reductase (*dmsABC*). In addition, the remaining seawater samples will be analysed for DMS/DMSO concentration by purge and trap GC analysis when future funding is available for an igniter coil.

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