Title
Marine Heterotrophic Bacterial Contribution to the Formation of Sulfur Volatile Organic Compounds

Permalink
https://escholarship.org/uc/item/0qb28558

Author
Price, Tyler

Publication Date
2021

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA SAN DIEGO

Marine Heterotrophic Bacterial Contribution to the Formation of Sulfur Volatile Organic

Compounds

A thesis submitted in the satisfaction of the requirements for the degree Master of Science

in

Biology

by

Tyler J. Price

Committee in charge:

Professor Kimberly Prather, Chair
Professor Eric Allen, Co-Chair
Professor Joseph Pogliano

2021

The thesis of Tyler J. Price is approved, and it is in acceptable quality and form for publication on microfilm and electronically.

University of California San Diego

2021

Dedication

This Thesis is dedicated to all who I know personally, thanks for making life memorable.

Table of Contents

List of Abbreviations

DMSP……………………………………….. Dimethylsulfoniopropionate

DMSPd… .... Dissolved Dimethylsulfoniopropionate

DMSPt .... Total Dimethylsulfoniopropionate

DMS .... Dimethyl Sulfide

UV…………………………………………Ultraviolet

VOC… .... Volatile Organic Compound

MeSH .... Methanethiol

CCN .... Cloud Condensation Nuclei

DMSO .... Dimethyl Sulfoxide

DOM .... Dissolved Organic Matter

Met .... Methionine

PCR… .... Polymerase Chain Reaction

qPCR… .... Quantitative Real-Time Polymerase Chain Reaction

CIMS .... Chemical Ionization Mass Spectrometery

CO_2 .... Carbon Dioxide

ESP .... Environmental Sample Processor

Chl.a ....Chlorophyll-a

SSML ....Sea Surface Microlyer

DNA .... …Deoxyribonucleic Acid

cDNA ....Complimentary Deoxyribonucleic Acid

RNA .... ….Ribonucleic Acid

BSA .... Bovine Serum Albumin

BSD .... Bacterial Sulfur Demand

ddd+…………………………..…..DMSP dependent DMS

dmd+……………………………...DMSP demethylase

CAICE…………………………….Center for Aerosol Impacts on the Chemistry of the Environment

ASW .... …..Artificial Seawater

N$_2$ …………………………………...Nitrogen

cps…………………………………..counts per second

List of Figures

List of Tables

Abstract of Thesis

Marine Heterotrophic Bacterial Contribution to the Formation of Sulfur Volatile Organic

Compounds

by

Tyler J. Price

Master of Science in Biology

University of California San Diego, 2021

Professor Kimberly Prather, Chair

Professor Eric Allen, Co-Chair

The globe is primarily made up of the Earth's oceans, which contribute biological, chemical, and physical influences towards global environments. However, many of the marine biological impacts, specifically on the chemistry of the atmosphere, are not well understood.

Compounds like Dimethylsulfoniopropionate (DMSP), and its derivative Dimethyl Sulfide (DMS), cycle through the marine environment as an exchange of carbon and sulfur among many organisms. DMSP derived DMS is also a major contributor towards irradiation of UV light and nucleation of clouds. Understanding the relationship between DMSP cycling and DMS production allows insight into biological impacts on climate.

This thesis addresses how heterotrophic bacteria in the marine environment degrade the organosulfur compound DMSP and produce sulfur containing VOCs. During SeaSCAPE 2019, an induced phytoplankton bloom resulted in an increased production of dissolved DMSP (DMSPd), leading to bacterial transcription of genes coding for the enzyme which cleaves DMSP into the volatile DMS. Following, an increase in dissolved DMS was measured in the wave flume. A mesocosm experiment was conducted to investigate the metabolic pathways and showed novel insights into simultaneous degradation of DMSP into DMS and MeSH by a model organism, the Rhodobacteraceae *Phaeobacter sp. La5*. From our findings, heterotrophic bacteria influence the formation of DMS through enzymatic cleavage of phytoplankton produced DMSP when bacterial sulfur demand is met with excess sulfur. With these novel findings, continued research on the DMSP cleavage pathway, the genes homologs, their rates of transcription and translation, and enzyme efficiency under varying environmental conditions will better our understanding of how microbes affect the composition of the atmosphere through sulfur cycling.

Introduction:

The interactions between the ocean and atmosphere influence climate and weather patterns around the globe. Emerging from the ocean, Volatile Organic Compounds (VOCs) can be climatically active molecules that originate from microbial primary production or from subsequent processing of primary produced organic compounds by heterotrophic organisms (Azam et al 1983). These VOCs emitted from the ocean can directly affect the chemistry of the atmosphere or can be precursors to secondary marine aerosols (Mayer et al 2020). In turn, these aerosols can act as cloud condensation nuclei (CCN) and impact cloud formation, albedo, and climate (Charlson et al 1987). Although there have been previous studies on VOCs and aerosols, little is known about the marine processes influencing the emission of VOCs and their impact on secondary marine aerosol formation. This thesis explores the role of marine microbial cycling of organosulfur molecules and how this can influence atmospheric chemistry and climate.

Decades-old studies addressing the composition and formation of the atmosphere continue to provide a basis for research in novel fields of atmospheric and biological sciences. Specifically, Charlson, Lovelock, Andreae, and Warren [of the CLAW hypothesis] are immortalized for their discussion of the impact of DMSP derived DMS on the chemistry of the atmosphere (Charlson et al 1987, Quinn et al 2011). DMS is the largest source of sulfur in the atmosphere and contributes to the formation of CCN in the form of DMS-oxidized products (Chin et al 1996, Mayer et al 2020). High concentrations of this CCN precursor were measured in tropical regions, regions defined by high UV light intensities, high salt levels, and warm weather (Liss et al 1986), leading Charlson et al to conceive the CLAW hypothesis. The CLAW hypothesis describes the release of DMS from microbial cleavage in these UV intense, warm, tropical conditions and how bacterial DMS production can unconsciously increase cloud coverage to reflect UV radiation and heat from the sun (Charlson et al 1987). The CLAW hypothesis proposes then, as this cloud coverage increases, the exposure of marine microbes to UV radiation and heat decreases, lowering the rate of DMSP and DMS production, leading to lower CCN concentration and decreasing

albedo (Charlson et al 1987). Lessening the release of DMS into the atmosphere would then allow UV radiation and heat to reach the Earth's surface again, defining this model as a negative feedback mechanism (Charlson et al 1987). The CLAW hypothesis gives insight into understanding mechanisms of global homeostasis, leading to more scientific questions about how biology affects atmospheric chemistry.

With the CLAW hypothesis describing how microbial communities may influence climate, research moved towards identifying the DMSP degradation pathways and how environmental and ecological conditions like light intensity and UV radiation impacted the selective degradation of DMSP into DMS. Biogeochemical and physical processes such as microbial lysis and marine snow dictate availability of nutrients, trace metals, nitrogen, carbon, and sulfur compounds, like DMSP, for primary producers and secondary consumers (Azam et al 1983, Ducklow et al 1992). For instance, Andreae et al 1986 discusses nitrogen availability as a driver for the production of DMSP as an osmoprotectant and another hypothesis states that DMSP formation may occur under higher light intensities in the water column (Gabric et al 1993). From these preliminary findings, Vallina and Símo then demonstrated a 'strong relationship' between the production of DMS and high doses of solar radiation, suggesting light and UV radiation encourage the cleavage of DMS from DMSP (Vallina and Símo 2000). Although research continues to probe for explanations for the CLAW hypothesis and this negative feedback system, a direct understanding between the relationship of DMSP production, DMS release, and nucleation of clouds has not been established.
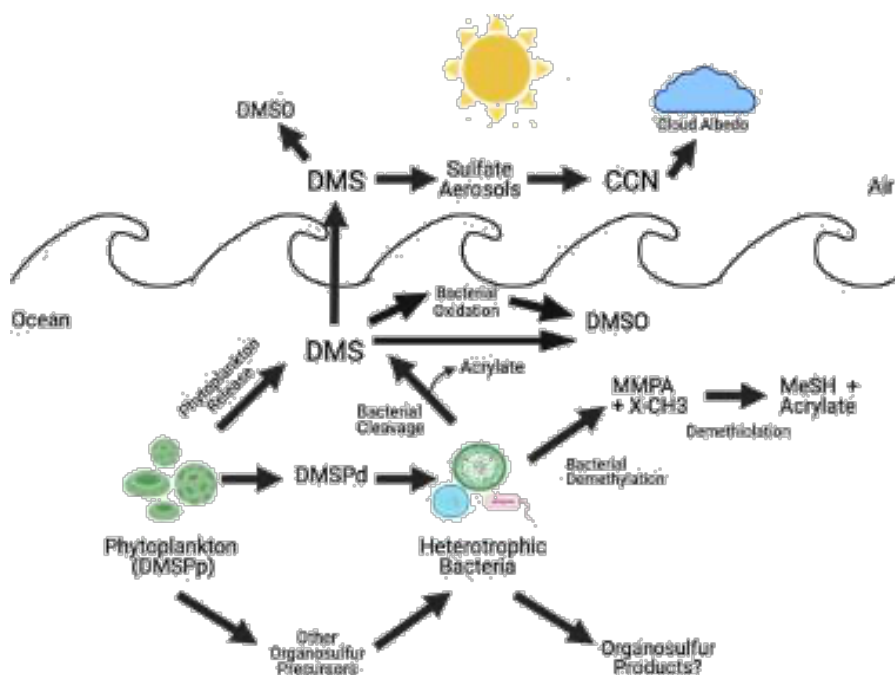
Figure 1: DMSP cycle in the marine environment. Illustration shows phytoplankton production of DMSP and its degradation by bacteria via the cleavage or the demethylation pathway.The cleavage by bacteria produces DMS and Acrylate. The demethylation pathway releases MeSH and Acrylate. DMS then reacts in the atmosphere and impacts atmospheric chemistry by e.g forming CCN.

Studying compounds like DMSP and DMS continues to narrow this understanding of the

relationship between microbial impacts on the chemistry of the atmosphere.

DMSP and DMS, among other organosulfur compounds such as methionine (Met) and methanethiol

(MeSH), are some of the most important molecules dissolved in the Dissolved Organic Matter (DOM)

pool. These molecules are directly bioavailable to bacteria and are channelled into the microbial loop

(Kiene et al 1990, Azam & Malfattil 2007).

DMSP produced at rates of up to 37Tg annually, is a major sulfur and carbon source in the marine ecosystem and is universally found in the DOM of seawater and freshwater aquatic ecosystems (Ksionzek et al 2016, Zhang et al 2019). The large amount of DMSP in the environment indicates its significance and emphasizes the importance in understanding its cycling throughout the ocean and atmosphere, especially as it is a precursor for CCN.

Phytoplankton, algaes, and some bacteria, form DMSP to fit numerous biological needs (Stefels et al 2007, Curson et al 2017). The formation of DMSP by phytoplankton deters grazing by predators, facilitates signal cascades between bacterial communities, and is a sink for excess carbon and sulfur from the environment (reviewed in Zhang et al 2019). Lysis of phytoplankton releases this DMSP and it dissolves into the water column, becoming available for use by other organisms (Bratbak et al 1995). Heterotrophic bacteria can use DMSP as a source of sulfur, through demethylation as a means to form MeSH, and a source of carbon, through cleavage to produce DMS (Zhang et al 2019). This formation of DMS and MeSH from DMSP may depend on the bacterial demands for carbon and sulfur (Símo et al 2001). Both gases are formed by bacteria through different metabolic pathways: DMS is formed through the cleavage pathway and MeSH through the demethylation pathway by bacteria (Fig. 1, Símo et al 2001).

The production of DMS or MeSH from DMSP by microbes is thought to be dependent on environmental factors. As discussed previously, heterotrophic bacteria can degrade DMSP by cleavage or by demethylation (Wirth et al 2020). The fate of DMSP degradation is known as the 'bacterial switch hypothesis,' first mentioned by Símo et al (2001). Bacteria switch the utilisation of DMSP between these pathways under a variety of conditions, such as temporal variation in temperature, UV radiation, and sulfur availability (Moran et al 2015, Cui et al 2015), suggesting seasonal changes in the environment, and phytoplankton bloom stages may drive pathway preference. The demethylation pathway translates demethylation enzymes, identified as the *dmd+* gene family, which act to ultimately produce methanethiol, CO_2 and acrylate (Wirth et al 2020).

The cleavage pathway, identified as *ddd+* genes, produces DMS through cleaving a carbon-sulfur bond in DMSP (Kiene and Linn 2000). DMSP transformation pathways are illustrated in Figure 2.

Marine bacteria, such as *Marinomonas* spp. and *Halomonas* spp. have either the cleavage pathway or the demethylation pathway, while other members of the Alphaproteobacteria such as the Roseobacter, SAR11, SAR116 clades are documented to have both (Curson et al 2011, Yoch et al 2002). The Roseobacter clades, specifically the well studied model *Ruegeria pomeroyi* str. DSS-3, possesses the *dmdA* gene coding for the demethylation pathway, along with several of the homologs of the cleavage genes *ddd+* (Curson et al 2011). Variations of the cleavage and demethylation pathways and their presence in a large portion of marine bacteria suggest the importance of DMSP as a source of carbon and sulfur (Curson et al 2011). The Roseobacter clade itself is ubiquitous in all marine ecosystems and is estimated to represent about 25% of the marine bacterial community (Lafay et al 1995). With over one quarter of the bacteria in the oceans being able to demethylate or cleave DMSP, the influence of marine heterotrophic bacteria on the cycling of these organosulfur molecules, and the consequent production of climatically important gases becomes evident.
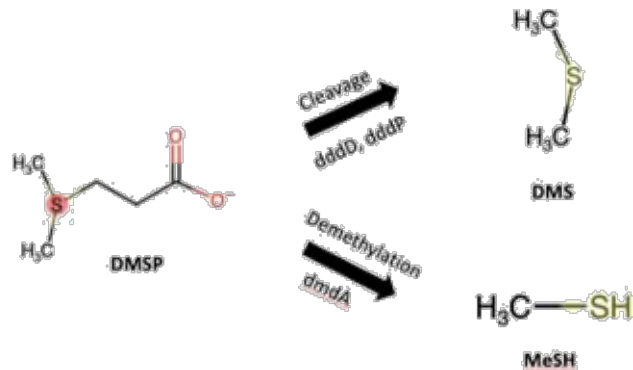
Figure 2: Degradation pathways for DMSP. DMSP can be cleaved into DMS by the DMSP dependent DMS gene family, or *ddd+*. DMSP is demethylated by *dmd+* or, dimethylsulfoniopropionate demethylase, to release MeSH.

Taking an integrative approach, this thesis will describe the production of DMS by DMSP cleavage from a coastal community during an induced phytoplankton bloom, in a model wave system (Chapter 1) and will further study the the ability of an abundant coastal heterotrophic bacteria from the Roseobacter clade to degrade DMSP simultaneously into DMS and MeSH (Chapter 2), shedding light on the complexity of the microbial organosulfur cycling. Combining chemistry and molecular biology through the characterization of gaseous molecules and the quantification of genes associated with their production, this thesis will bring more insights on how marine microbes contribute not only to the chemistry of the oceans, but also to the chemistry of the atmosphere.

Chapter 1: SeaSCAPE: Bacterial Cleavage of DMSP into DMS during an Induced Phytoplankton Bloom

Materials and Methods:

Experimental Summary:

A phytoplankton bloom was induced in a wave flume from July to August 2019 during the Sea Spray Chemistry and Particle Evolution (SeaSCAPE) experiment (Sauer and Mayer et al In prep). San Diego coastal seawater was brought into the wave flume to which nutrients were added to encourage a phytoplankton bloom. To follow phytoplankton growth chlorophyll-a (chl.a) was quantified using an Environmental Sample Processor (ESP) that measured fluorescence as chl.a. Bulk and Sea surface Microlayer (SSML) water measurements were taken to characterize the biology of the closed system. DMS and MeSH were measured using a Vocus proton transfer reaction time-of-flight mass spectrometer (PTR-ToF-MS) by detecting mass-to-charge (m/Q) ratios of gas-phase molecules in the headspace of the wave flume (Kilgour et al In prep). And dissolved DMSP and particulate DMSP were measured using a purge and trap system, described in Wurl et al 2009, that was connected to a chemical ionization mass spectrometer, which similarly measured the m/Q of volatilized DMSP.

Water Collection:

The seawater used in the wave flume was collected from the Scripps Pier (La Jolla, CA; 32*51'56.8"N: 117*15'38.48"W) and filtered first through an aluminum screen to remove debris (Sauer & Mayer et al In prep). The water was first pumped into 1,135L plastic tanks before being transported to the wave flume, where the water was filtered a 50uM Nitex nylon mesh (Flystuff; CAT # 57-106) that was fixed over the top of the wave flume.

Bulk and SSML Seawater Collection:

Bulk seawater was collected into Nalgene carboys daily, by submerging a ~2m long Teflon tube siphon about 20cm under the surface layer and then was collected. A glass plate was submerged into the water at a rate of 5-6 cm sec^{-1} and removed from the water at the same rate to collect the SSML (Carlson et al 1982; Cunliffe and Wurl, 2015). Following withdrawal of the plate from the water, the plate was suspended for 20 seconds above the flume to ensure the bulk water would drain from the plate. The SSML collected on the glass was scrapped off into a 250mL glass bottle with a Teflon scraper and a glass funnel until about 200mL was collected. The siphon, carboys, glass plate, funnel, and scrapper were cleaned as described in Sauer & Mayer et al 2021.

Bacterial Production:

Bacterial production was measured by [H_3]-leucine incorporation (Kirchman et al., 1985) modified for microcentrifugation (Azam and Smith, 1992). Triplicate 1.7 mL aliquots were incubated with [H_3]-leucine (20 nM final concentration) for 1 hour. Samples with 100% trichloroacetic acid added prior to [H_3]-leucine addition served as blanks. Leucine incorporation was converted to carbon production assuming 3.1 kg C (mol leucine)$_{-1}$ (Simon and Azam, 1989) The range of bacterial sulfur demand was estimated from bacterial carbon production using the cellular C:S ratios of 86 (Fagerbakke et al., 1996) and 248 as a range (Cuhel et al., 1982).

DNA and RNA filtration:

Two replicates of 500mL bulk water and one replicate of 50mL SSML were filtered onto separate 0.2 um Supor filters (Pall Life Science, Port Washington, New York, USA) . The filter towers were connected to a vacuum with a pressure no more than 10-15Hg to prevent microbial lysis. The filter was folded in half then in quarters, biological matter on the inside, and placed in 4mL Cryo tubes with 500uL of DNA/RNA shield for preservation and kept at -80*C until processing.

Sample Extraction:

The ZR Fungal/Bacterial DNA/RNA Miniprep Kit (ZYMO Research) was used to extract the 45 samples of DNA and RNA samples. Prior to extraction the filters were cut under sterile conditions and placed in the bead bashing tube with buffer and vortexed for 5 minutes. After the bead bashing, the samples were extracted following the manufacturers' instructions. All 45 RNA samples were reverse transcribed into complementary DNA (cDNA) from 1uL of RNA using Thermo Scientific Maxima H Minus Reverse Transcriptase (200 U/uL), following an optimized 30 minute DNAse treatment, prior to Polymerase Chain Reaction (PCR) and Real-time Polymerase Chain Reaction (qPCR) analysis.

PCR Optimization and qPCR Standard Preparation:

For DMSP lyase coding *dddD* gene and transcript (cDNA) quantification, PCR conditions were first optimized using the primer set dddD_1360F 5'-AACGTBATHGCHGGBCCBCAYTC-3' and dddD_1799R 5'-GTVCCGARRTGVGCGTGYTCYTC-3', described by Cui et al 2020. Optimized conditions were as follow for a 20uL reaction: 10uL of NEB Hot Start Taq DNA polymerase, 1.2uM of dddD_1360 forward primer, 1.2uM of dddD_1799 reverse primer, 0.8uL MgCl2, 2uL of sample, and 4.8uL of DNA/RNA free water. The PCR conditions were as follows: initial denaturation for 1 minute at 95*C, then 35 cycles of denaturation for 30 seconds at 95*C, annealing for 30 seconds at 61*C, and the extension at 72*C for 30 seconds.

For the DMSP lyase coding *dddP* gene and transcript quantification, a PCR was first optimized using the primer set described in Levine et al 2012, dddP_874F is 5'-AAYGAAATWGTTGCCTTTGA-3' and dddP_971R is 5'-GCATDGCRTAAATCATATC-3'. Optimized conditions were as follow for a 15uL reaction: 7.5uL of Dream Taq DNA Polymerase, 0.45uL of dddP_874 forward primer, 0.45uL of dddP_971 reverse primer, 0.9uL of bovine Serum Albumin (BSA), 0.6uL of MgCL2, 1uL of sample, and 4.1uL of DNA/RNA free water. The PCR conditions were as follows: initial denaturation for 3 minutes and 30 seconds at 95*C, then 35 cycles of denaturation for 35 seconds at 95*C, annealing for 30 seconds

at 53*C, and the extension at 72*C for 1 minute.

Once *dddD* and *dddP* genes and transcripts were amplified through PCR, the samples were run on a 1% Agarose gel to visualize the single band for each gene. A positive control, using *Phaeobacter* sp. La5, was also run. The *dddD* and *dddP* bands were cut from the gel using a sterile exacto knife and were purified using the Qiagen QIAquick Gel Extraction Kit. The concentration of standards for *dddD* and *dddP* were determined using the Qubit DNA Assay Kit.

qPCR conditions:

A series of qPCR were then performed in 96-well plates on a AriaMx Real-Time PCR system according to the optimized PCR reaction and conditions for *dddD* and *dddP* genes (Cui et al 2015, Levine et al 2012). SsoAdvanced Universal SYBR green supermix,was used for detection by fluorescence of PCR copies. Serially diluted 10^2 to 10^8 gene copies μL_{-1} standards and controls with no template were run simultaneously in duplicates. The relative levels of DNA in the standards were calculated based on the standard curve. Single amplifications were confirmed by a melt curve for each analysis.

DNA sequencing and analysis:

Extracted DNA was sequenced using 16S rDNA primers for the V4-V5 region following the Earth Microbiome Project protocol. All reads were processed using the QIIME 2 pipeline implemented in Qiita. Taxonomy assignments were made with the GreenGene 13_8 database.

Results:

Figure 3: Concentration of measured DMSP and dissolved DMS over the course of the phytoplankton bloom (top). Chlorophyll-a concentration (green), dissolved DMSP (blue), and dissolved DMS (yellow). DMSP cleavage genes dddP (green) and transcript (blue) dynamics during the bloom (middle). DMSP cleavage genes dddD (green), transcript (blue), and aerosol dddD (yellow), dynamics during the bloom (bottom).
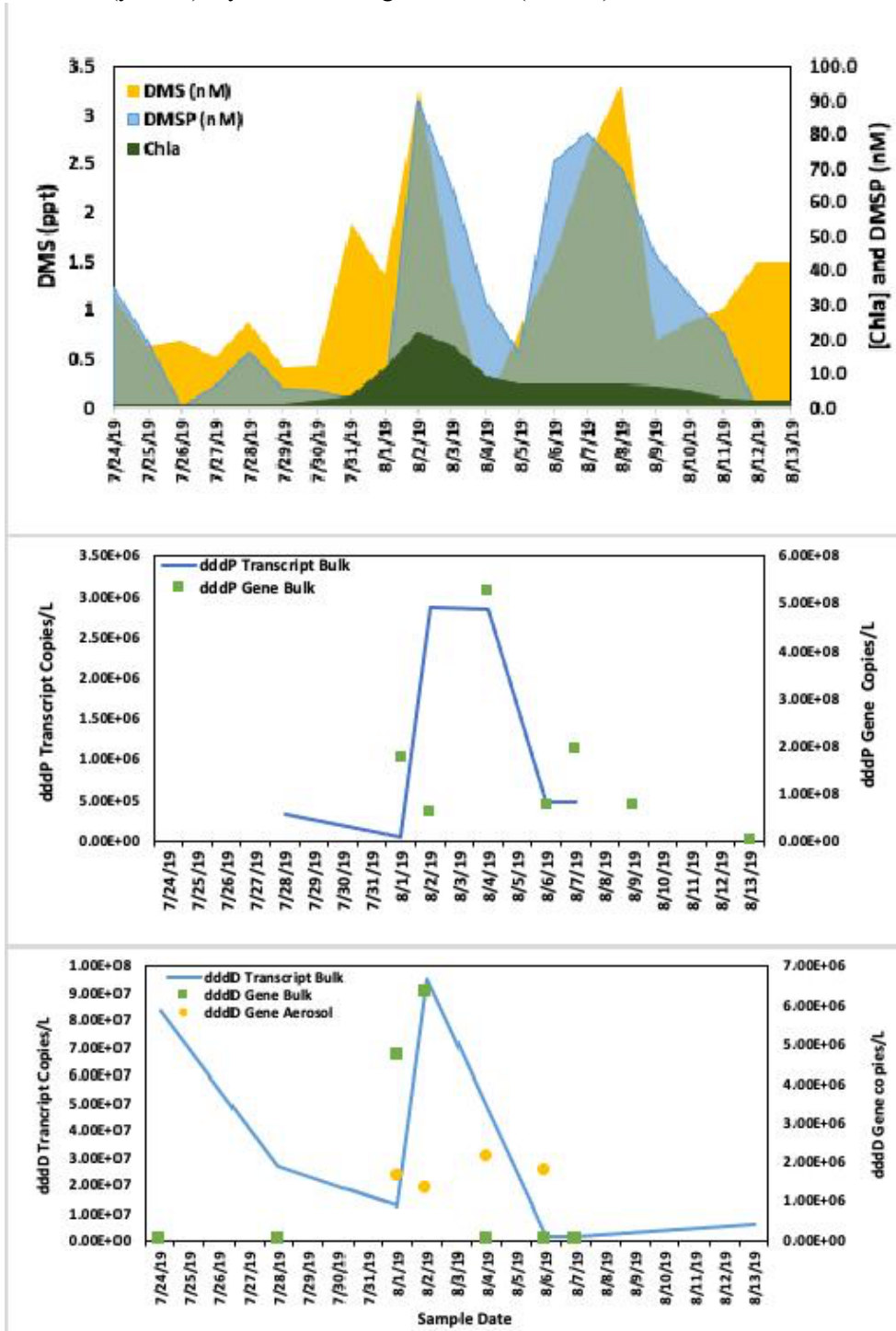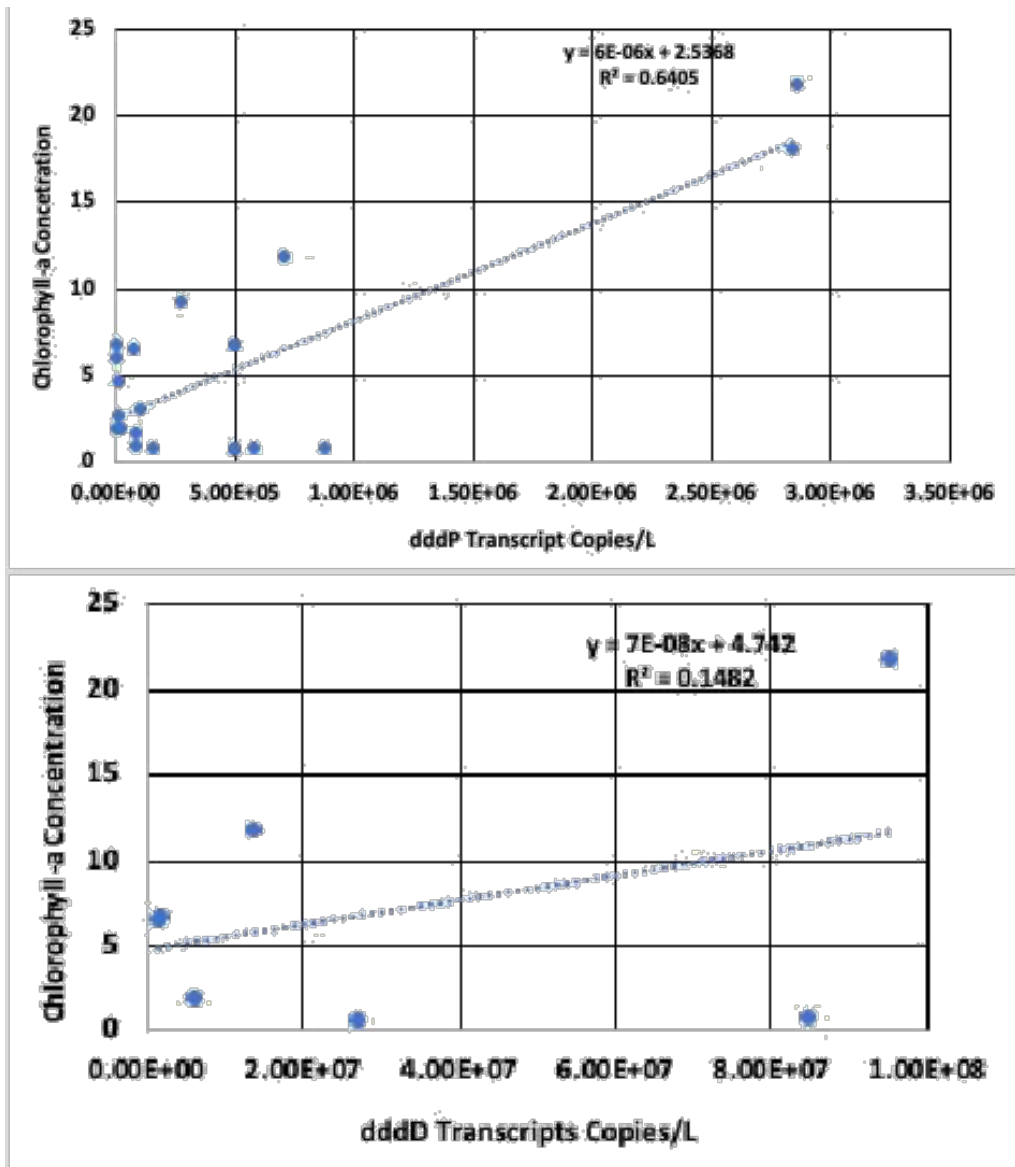
Figure 4: Correlation between cleavage gene transcript dddP (top) and dddD (bottom) with chlorophyll-a concentration over the course of the bloom shown as a R-squared value.
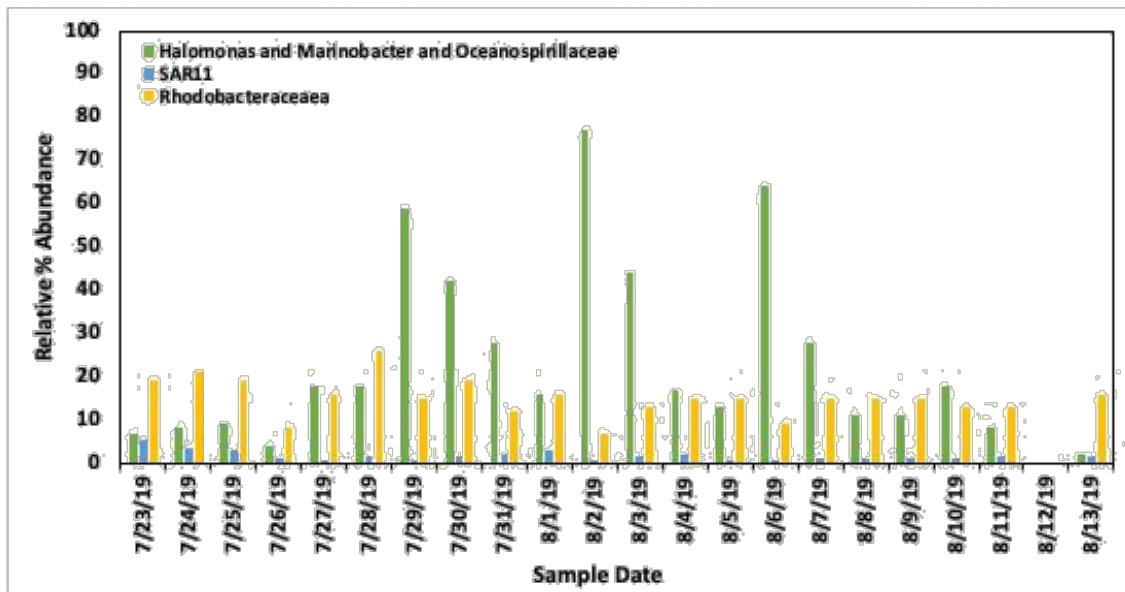
Figure 5: Relative abundance (%) of putative DMSP users over the course of the bloom in the bulk water.
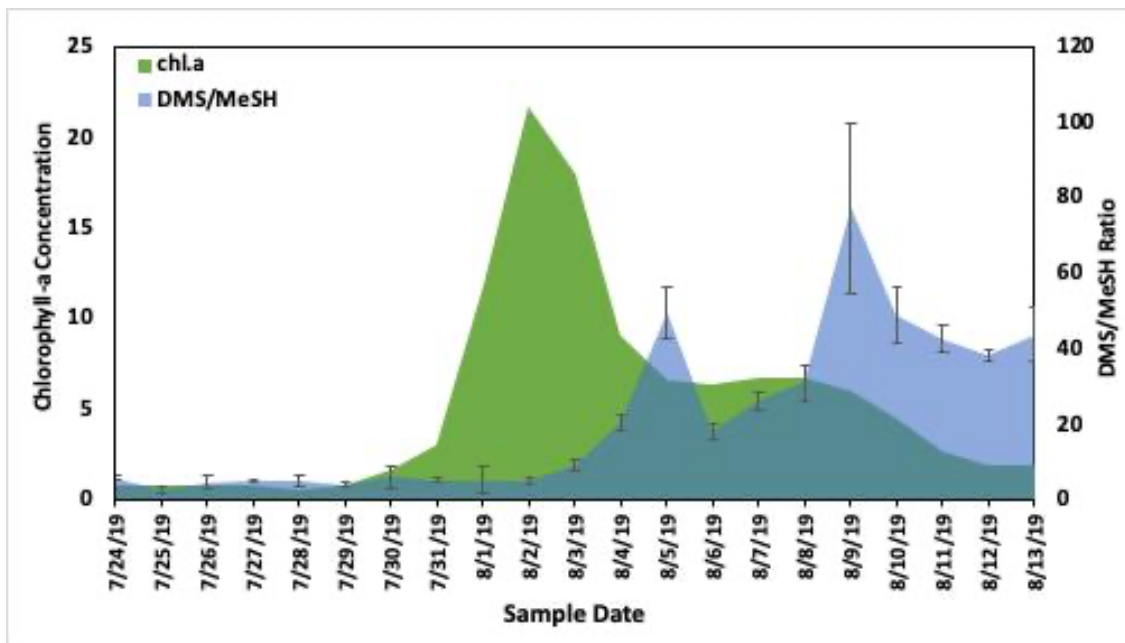
Figure 6: Comparison between chl.a concentration and DMS/MeSH over the course of the bloom. Error bars are presented for DMS/MeSH ratio (adapted from Kilgour et al in prep).
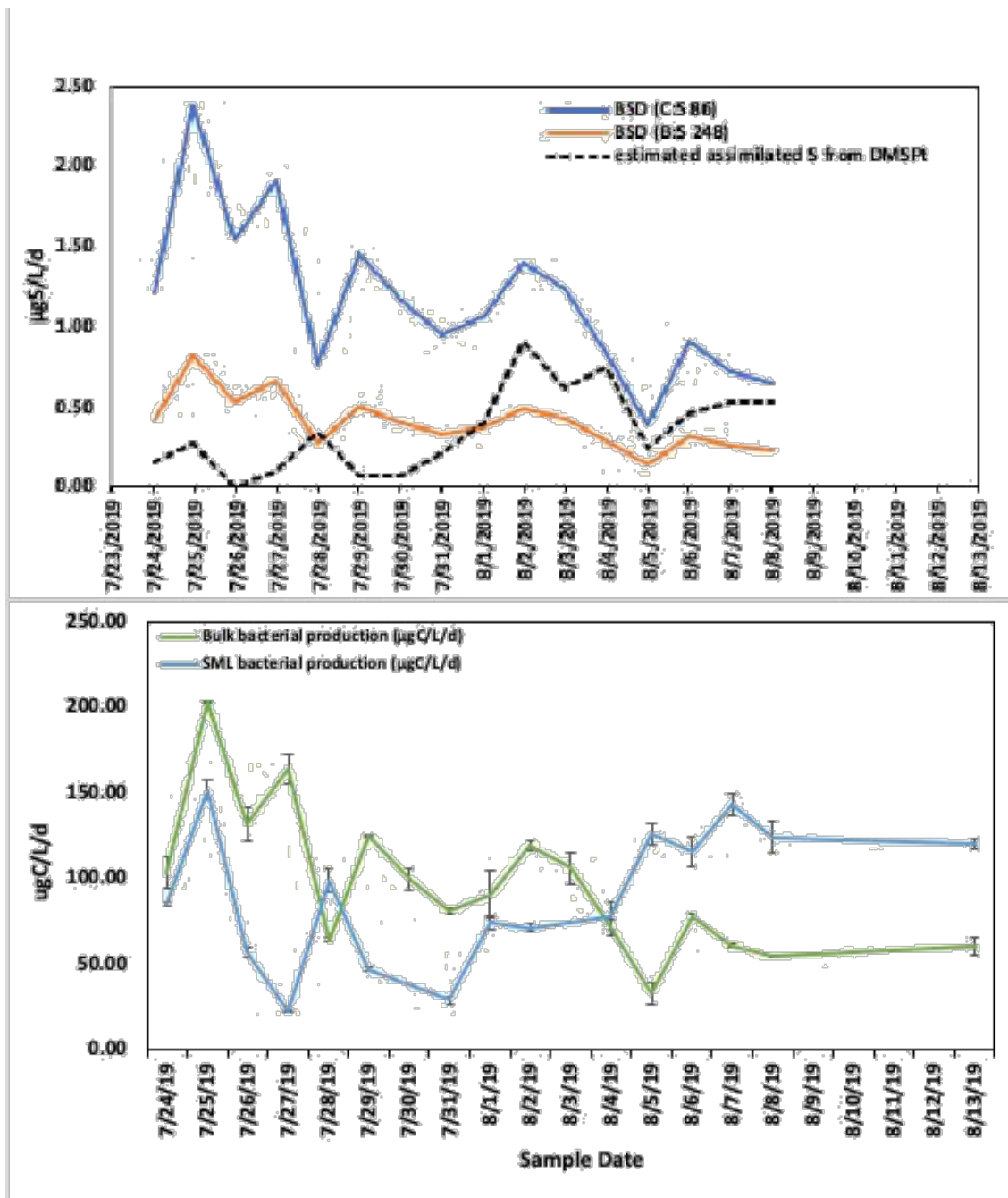
Figure 7: Bacterial sulfur demand (BSD) measured over the bloom (A). Bacterial sulfur demand for C:S 86 (blue) and bacterial sulfur demand C:S 258 (orange). Estimated assimilated sulfur from dissolved DML total is shown as black dotted line (adapted from Kilgour et al in prep). Bacterial production for bulk (green) and SML (blue) over the course of the bloom (B).

Table 1: The table presents the gene and transcript copy amounts for *dddP* and *dddD* cleavage genes at sampling points throughout the bloom. *dddP* genes and transcripts are from bulk water. *dddD* genes are from bulk water and aerosol, while *dddD* transcript was measured from only bulk water. SML and *dddP* aerosol data is not included. Blank dates show points where no genes or transcripts could be measured from the sample.

Date	dddP gene	dddP transcript	dddP Ratio (Transcript:Gene)	dddD Gene	dddD Transcript	dddD Ratio (Transcript:Gene)	dddD Gene aerosol
7/24/19	-	-	-	5.44E+04	8.38E+07	1541	-
7/28/19	-	3.32E+05	-	6.72E+04	2.71E+07	403	-
8/1/19	1.75E+08	4.11E+04	0.002	4.69E+06	1.25E+07	2.66	1.62E+06
8/2/19*	6.31E+07	7.05E+05	0.045	6.31E+06	9.52E+07	15.1	1.31E+06
8/4/19	5.28E+08	2.84E+06	0.005	1.62E+08	-		2.15E+06
8/6/19	7.47E+07	4.69E+05	0.006	3.29E+04	1.48E+06	45.0	1.77E+06
8/7/19	1.95E+08	4.72E+05	0.002	3.25E+04	1.89E+06	58.3	-
8/9/19	7.47E+07	-	-	-	-	-	-
8/13/21	3.29E+06	-	-	-	6.46E+06	-	-

During SeaSCAPE, conducted by the Center for Aerosol Impacts on the Chemistry of the Environment (CAICE) in summer of 2019, a phytoplankton bloom was induced in a 13000L wave channel. Phytoplankton growth was shown by the increase in chlorophyll-a, which peaked at 22.0 mg3 on August 2nd, and then decreased to 6.5mg3 on August 6th. DMSP peaked at 89.8 nM at the same time as the chl.a (Figure 3A). Emitted DMS, measured in the gas phase, peaked on August 5th and reached 3.24 nM (Fig. 3A).

As the bacterial population changed during the phytoplankton bloom, the sulfur demand and bacterial production changed with it. Prior to the induction of the bloom, the ratio of DMS to MeSH remained consistently low, around 5 for the ratio. After the phytoplankton bloom, the increase in dissolved DMS showed a change in the ratio between DMS and MeSH to 80 on August 9th. The increase in the ratio represents more dissolved DMS being measured than MeSH. The bacterial sulfur demand (C:S 86) and bacterial production for bulk samples were also highest prior to the induction of the bloom. Bacterial sulfur demand (C:S 86) steadily declined from a rate of nearly 2.50 ugS/L/d on July 25th to as low as 0.50ugS/L/d on August 5th, following the induction of the bloom on August 2nd. Additionally, before the bloom, bulk bacterial production was at a rate of 200 ugC/L/d then following the bloom the rate decreased to about 110.00 ugC/L/d. The bacterial production in theSML followed a different trend, where the production rate started around 150.00 ugC/L/d prior to the bloom, decreased until the induction, and then following the bloom increased to its peak rate of 150.00ugC/L/d.

The succession of putative DMSP users: the γ-Proteobacteria, Halomonas, Marinobacter, and Oceanospirillacea and the α-Proteobacteria, SAR11 clade, and Rhodobacter changed over time and was relatively similar between the bulk water and the SML Both bar graphs show a similar trend of an increase in DMSP degrading heterotrophic bacteria during peaks of chlorophyll-a and DMSP. Specifically, following peak of chl.a and DMSP on August 2nd, Halomonas, Marinobacter, and Oceanospirlliacea represented 74% of the relative abundance of the bacterial community, SAR11 and Rhodobacter populations stayed relatively stable over the course of the experiment.

The dddD and dddP transcripts abundance peaked at 9.52×10^{8} transcripts.L^{-1} and 2.86×10^{6} transcripts.L^{-1}, respectively, when chl.a and DMSP peaked (Fig. 3B). he dddD cleavage gene was transcribed up to 100-folds more than the dddP gene during the bloom. Interestingly, on July 24th, dddD transcript levels were measured at 8.48×10^{7} copies.L^{-1}. DMS appeared to increase after the transcription of the genes. dddD genes were found in an abundance of up to 2.15×10^{6} copies.L^{-1}

Discussion:

Phytoplankton abundance and production during blooms leads to the formation of many organic molecules, like DMSP. On August 1st, 2019, the induction of a bloom resulted in growth of phytoplankton, increasing DMSP concentrations up to 89.8nM, and further affecting the growth of heterotrophic bacteria and other microbes during SeaSCAPE. DMSP production during the bloom can be attributed to phytoplankton production; the measured concentration of DMSP here was within the range of other blooms (Gali et al 2015). Subsequent production of DMS is likely derived from bacterial degradation of DMSP, as observed in many studies (Kiene and Linn 2000, Lizotte et al et al 2012). We therefore followed the growth of phytoplankton to track the formation of DMSP and ultimately analyze its bacterial transformation into the climatically important gas DMS (Curson et al 2011, Zhang et al 2019), through enzymatic cleavage.

The low initial DMS production at the start of the bloom may be explained by the sulfur demand of heterotrophic bacteria compared to sulfur availability in the wave flume system. Before the introduction of nutrients to the system, the sulfur demand of bacteria was high, suggesting bacteria were channeling DMSP towards the demethylation pathway to meet their need for reduced sulfur in the form of DMSP derived MeSH (Kiene et al 2000). Kiene and Linn (2000) discuss the importance of MeSH as a precursor to amino acids and how DMSP demethylation provides two methyl groups and a reduced sulfur ion that is easily assimilated into Methionine and other significant biomolecules. Demethylation of DMSP into MeSH is preferred in conditions with restricted sulfur availability to prioritize use of limited sulfur for survival (Gonzales et al 1999, Kiene et al 2000), this was also reflected by the low DMS/MeSH ratio observed during the early phase of the bloom (Kilgour et al in prep).

Following the addition of nutrients to the wave flume, phytoplankton growth increased and led to the production of up to 89.8 nM of dissolved DMSP. At the point following the increase in dissolved DMSP, the ratio of DMS to MeSH (DMS/MeSH) increased (Kilgour et al in prep), suggesting a switch in the mechanism of bacterial utilisation of DMSP . The succession of potential DMSP users from

Rhodobacterales to Halomonas sp. over the course of the bloom may also reflect this switch in

bacterial strategy in DMSP utilization (Liu et al 2018, Todd et al 2010). With excess sulfur, heterotrophic

bacteria such as the putative DMSP users: α-Proteobacteria Rhodobacterales, SAR11 clade, and some γ-

proteobacteria, identified over the course of the bloom then used the excess DMSP as a source for the

carbon necessary for their survival (Curson et al 2011). The increase transcription of dddD and dddP

following the higher levels of total DMSP (DMSPt) reaffirms the shift from DMSP demethylation and

MeSH production to DMSP cleavage and DMS production, especially when looking at the dddP and

dddD transcript to gene ratios. Quantification of the DMSP demethylase gene dmd+ would strengthen our

observation of a 'bacterial switch,' however; we were unable to amplify dmdA genes at this time and are

therefore unable to say for sure when bacterial cleavage took over. Comparing dmd+ transcription to

ddd+ transcription would provide a more accurate and precise story of why and when bacteria shift from

DMSP demethylation to DMSP cleavage.

At the point of the bloom, when chl.a and DMSP were highest, the ratio of dddP transcript to

genes measured was 0.0045:1 and the ratio of dddD transcript to gene was 15:1. This active transcription

of dddD genes also suggest that the bacterial community present cleaved DMSP into DMS when the

sulfur demand of the community was met. Factors such as temperature and salinity are also thought to

contribute to the switch between bacterial demethylation and cleavage of DMSP in the marine

environment, specifically they were hypothesized to influence dddD transcription (Rusch et al 2007,

Levine et al 2012). The presence and increase of dddD genes and transcripts has not previously been

observed in coastal seawater; the use of this gene was only reported in environments such as lagoons

and mangroves with higher salinity (Rusch et al 2007, Todd et al 2009). The observation here of high

abundance of dddD transcripts at the beginning of the bloom and the trends of increasing transcription in

these experimental conditions, provides new insights into the potential distribution of ddd+ enzymes and

their role in DMS production in coastal ecosystems. Prior to the induction, the level of dddD transcripts

may suggest the summer coastal seawater community of bacteria may have higher preference for the use of

the dddD homolog. Transcription of dddD during this experiment could be explained by the range of temperatures observed in the wave flume, from 22 to 27*C, temperatures more similar to tropical environments where dddD transcription has been measured (Rusch et al 2007). Use of dddD in higher temperature waters could indicate temperature as an additional influence for DMSP cleavage. The importance of dddD transcript in our study and the fact that dddD appears to be more universally present in heterotrophic bacteria genome compared to other dddx genes suggests the significance of this gene in DMS production and the specific environmental drivers for its expression need to be further studied (Todd et al 2009).

Similarly, other environmental factors have been shown to potentially influence the preferential cleavage of DMSP into DMS. Research suggests the distribution of ddd+ homologs, like dddP, are influenced by chlorophyll-a presence (Cui et al 2015), however; the effects of chlorophyll-a concentration and its variation were not fully described and could be a reflection of the phytoplankton community. This correlation between DMSP cleavage and chlorophyll is observed during the bloom as both chlorophyll and DMSP increase at the same time (Figure 1).Therefore the hypothesis by Cui et al can be related back to sulfur availability as phytoplankton are the main producers of DMSP and then may dictate the bacterial switch through DMSP production (Stefels et al 2007, Curson et al 2011). It is also possible that the DDDP and DDDD enzymes are more or less efficient under certain environmental conditions. The conditions of SeaSCAPE could then have encouraged the use of DDDD for DMSP cleavage and explains its preference over the more ubiquitous DDDP enzyme (Todd et al 2009). DMSP derived DMS production may also go beyond the limits of 'ocean bound' bacteria and influences of phytoplankton and DOS, as dddD genes were quantified in the bulk water, SML, and aerosol samples. Analysis of aerosol samples collected during SeaSCAPE showed the presence of the dddD gene in the air. To our knowledge this is the first time that the presence of dddD is shown in sea spray aerosols (up to 2.15 x10^6 gene copies L$_{-1}$ of air). This could suggest the potential for aerosolized microbes to degrade DMSP and produce

DMS directly in the atmosphere, as DMSP has also been shown to be present in sea spray aerosols during coastal phytoplankton blooms (Chen et al 2020). Additionally, only genes were measured here and this is not a measure of direct activity of live bacteria in the aerosols.

The presence of the dddD and dddP genes and their active transcription over the course of the bloom brings new insights into coastal bacterial genetic capacities and potential preferential strategies for specific pathways over the course of a phytoplankton bloom. Some of the bacterial taxa present during this experiment, such as *Marinomonas* spp. and *Halomonas* spp. have also been shown to contain other ddd+ homologs, such as dddQ, dddW, and dddL (Curson et al 2011) which were not investigated in the present study. This suggests the potential succession of many ddd+ homologs over the course of the bloom. The environmental drivers associated with the changes in the population and DMS producers and the affinity for DMSP and efficiency to produce DMS needs to be further investigated to understand the feedback on atmospheric chemistry.

Chapter 2: Phaeobacter sp. La5 Simultaneously Produces DMS and MeSH from DMSP and grown on DMS

Materials and Methods:

Experimental Summary:

The model organism *Phaeobacter* sp. La5 was grown in closed systems to measure growth rate and gases production in the presence of DMSP and DMS as the sole source of carbon and sulfur. Controls without C and S addition were included as comparison. Bacterial counts were measured using Flow Cytometry and quantification of VOCs were measured with Chemical Ionization Mass Spectrometry (CIMS).

Mesocosm Set-Up:

The mesocosm experiment consisted of triplicates of model organism *Phaeobacter* sp. La5 was distributed into treatments of 4 µM DMSP and 4 µM DMS in 1-L of Artificial Seawater (ASW), in addition to triplicate controls with no C and S addition. About 10L of ASW was prepared, according to Cohen et al 1997, and then autoclaved in several 2L pyrex glass bottles. From the prepared ASW, 500mL was transferred to nine individual 1L polycarbonate plastic bottles. The glass and polycarbonate bottles were cleaned with detergent, 10% HCl and rinsed with ultrapure water before use.

Phaeobacter sp. La5 was inoculated in Zobell media, from a glycerol stock. Cell abundance of the inoculated *Phaeobacter* sp. La5 was measured using flow-cytometry, and an inoculum of x cells mL^{-1} was added to each bottle. The treatments were stored in a temperature controlled room, kept at 16*C, and were covered to prevent exposure to light.

Bacterial Abundance:

Bacterial abundance was measured by flow cytometry (Gasol and DelGiorgio, 2000), 500uL of each treatment was sub-sampled, at T0 and after 24, 48, and 72h. The samples were fixed with 2% final concentration of 0.2 µm filtered Formaldehyde. Once fixed, 1% (final concentration) of Sybr green was added to each sample. The samples were stored in the dark at room temperature for 10-15 minutes for staining. The abundance was measured on a Accuri BD flow cytometer. Fluorescent beads were used to calibrate the flow rate. Growth of the bacteria over the incubation was determined by averaging the cell count for the triplicates.

Sampling and Sample Preparation:

From the treatments, 4mL of each sample were pipetted into a glass scintillation vial using a glass pipette. The vial was then closed by crimping a rubber septa and metal cap to the top. The samples were placed in a dark container to prevent photochemical reactivity before gas measurements.

Purge and Trap and Chemical Ionization Mass Spectrometer:

A purge and trap system connected to a CIMS was used to quantify dissolved DMS and dissolved MeSH in the samples. The system consisted of a needle inserted into the sample, which allowed the inflow of gas to bubble the sample, and a second needle, which was inserted into the headspace of the vial, to sample gas outflow (Stefels 2009). The outflow runs through a tube submerged in liquid N_2 and the molecules are collected for 4 minutes before its arrival at the benzene CIMS (Stefels 2009). The CIMS measured gas-phase molecules as m/Q based on time of flight.

Standards:

Standards were used to calibrate the DMS measurements. A calibration method for MeSH has not been optimized yet, so MeSH readings are presented as relative counts per second (cps). The standards were prepared as instructed by Stefels at concentrations of 0.5nM, 5nM, and 10nM. A standard curve was derived from the m/Q measurements of the standards and then was used to calculate the concentration of dissolved DMS in the samples.
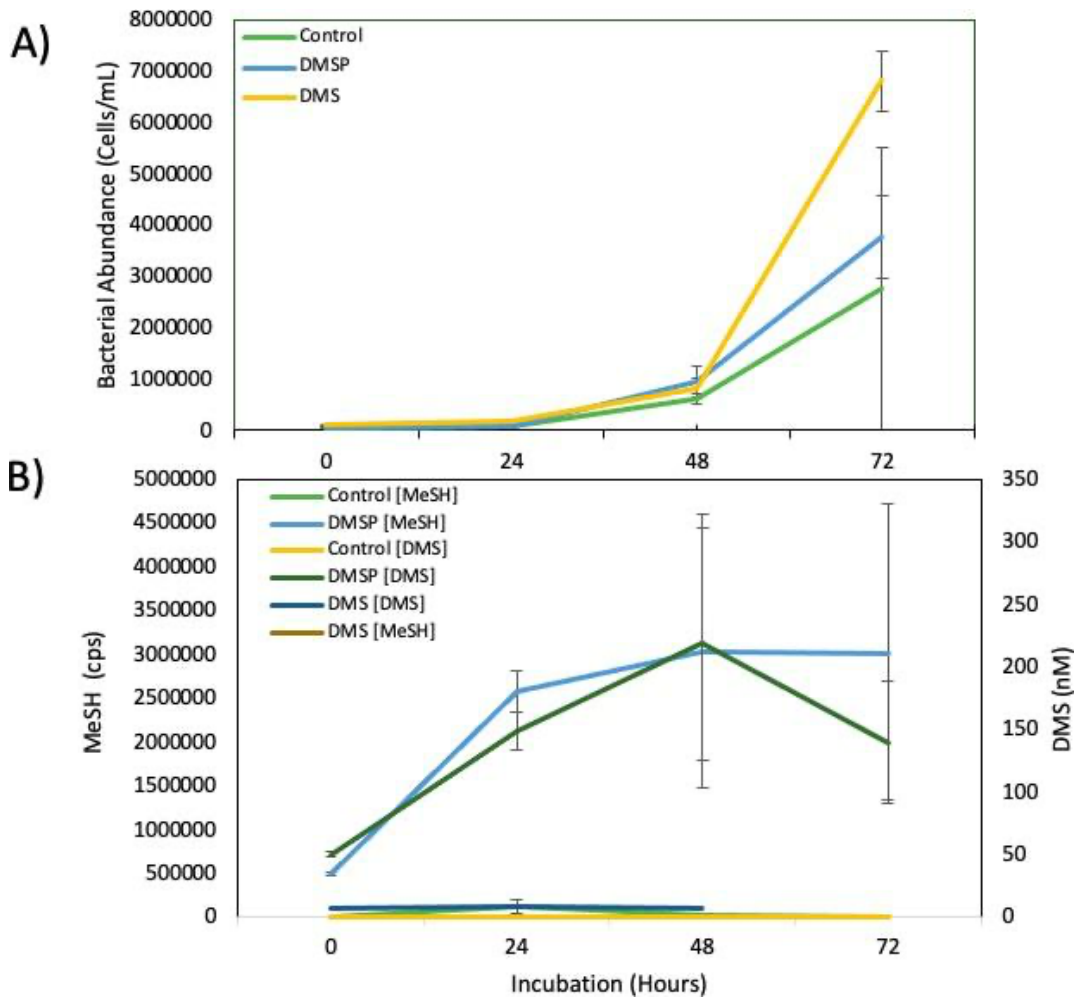
Results:

Figure 8: Bacterial abundance over the course of the experiment (A). DMS and MeSH concentration over the course of the experiment (B). DMS is presented in nanomolar (nM) and MeSH in counts per second (cps).

An incubation experiment with *Phaeobacter* sp. La5 was performed to characterize bacterial capacity to grow and degrade the organosulfur molecules DMSP and DMS. Upon inoculation in the closed systems, cell count concentration was at about $4.8 \times 10_2$ $2.9 \times 10_3$ cells mL$_{-1}$ for the control, 6.5×10^4 $1.6 \times 10_4$ cells/mL for DMSP, and $1.1 \times 10_5$ $1.1 \times 10_4$ cells/mL for DMS.

The cells grew exponentially during the 72h experiment (Figure 8A). The main difference between treatment was observed at 72 hours, 2.8 x 10_6 cells mL$_{-1}$ for control, 3.8 x 10_6 cells mL$_{-1}$ for DMSP, and 6.8 x 10_6 cells mL$_{-1}$ for DMS.

At the start of the experiment the concentration of DMSd was 0.2 0.06nM, 50nM 1.6, and 7.6nM for the control, DMSP, and DMS treatments respectively (Figure 8B). Initial MeSH relative counts measured 11,398 2921.4 cps for the control, 495,123 21768.7 cps for DMSP, and 14,184 cps for DMS. During the incubation period, the concentration of DMSd peaked for the control at 0.94 0.09nM after 72 hours, at 218 +/- 93.1nM for DMSP after 48 hours, and at 9.26nM for DMS after 24 hours. Highest MeSH relative counts for the control was 127,232 87064.0 cps after 24 hours, for DMSP was 3,042,320 1561808.5 cps after 48 hours, and was 168,743 cps at 24 hours for DMS.

Discussion:

Marine bacteria of the *Phaeobacter* genus, from the Roseobacter clade, are found across the globe in coastal waters and are known to associate with zooplankton and phytoplankton in the oceans (Lafay et al 1995, Freese et al 2017, Thole et al 2012). Present in the microbial community of many ecosystems, *Phaeobacter* sp. are not easily classified due to their large area of functions in biogeochemical cycling, they are widely studied because of their potential ecological impacts (Thole et al 2012). Specifically, this genus has been shown to be a key player in the sulfur cycle in the Pacific during phytoplankton blooms (Martinez et al 2010, Thole et al 2012, Moran et al 2007). Bacteria of the Roseobacter clade associate with phytoplankton to acquire freshly produced sources of sulfur and carbon for growth (Moran et al 2007, Kiene and Linn 2000). The Roseobacter clade, and the order Rhodobacterales at large, are often observed in association with phytoplankton bloom (Buchan et al 2005) and were abundant during the SeaSCAPE induced phytoplankton bloom (see Chapter 1 figure 1). The presence of Roseobacter clade taxa, closely related to *Phaeobacter* sp., shows the relevance of studying *Phaobacter* to better understand DMSP cycling.

The Roseobacter clade utilizes DMSP for carbon and sulfur for growth and replication (Howard et al 2006, Kiene and Linn 200). Use of DMSP by these bacteria produces DMS and MeSH. DMS and MeSH are products Roseobacter strains can further transform to produce important biomolecules and amino acids (Soda et al 1987, Kanzaki et al 1987, Hatton et al 2012). DMS is generally oxidized by bacteria as a source of carbon and further transformed into DMSO when MeSH is assimilated as a source of reduced sulfur to form methionine (Hatton et al 2012, Kiene and Linn 2000). Formation of DMSO by bacteria creates a reservoir of available carbon and sulfur to bacteria when nutrients are scarce (Hatton et al 2004). The degradation of DMSP into DMS and ultimately into DMSO by bacteria demonstrates the possibility of cross feeding interactions in the bacterial community. Here, we show the ability of a single bacterium to cross feed on the degradation product of DMSP in a closed system. *Phaeobacter* sp. La5 can grow on DMS produced DMSP, and further on DMSO produced from DMS utilization.

La5 is not the only bacteria known to possess both DMSP cleavage and DMSP degradation pathways. *Ruegeria pomeroyi* and the SAR11 clade also show preferential degradation of DMSP into DMS or MeSH depending on environmental conditions like salinity or DMSP concentration (Salgado et al 2014, Moran et al 2012, Sun et al 2016, Giovannoni et al 2008). Marine heterotrophic bacteria, with the ability to demethylate or cleave DMSP, may have a larger influence on the release of sulfur-containing VOCs than previously understood. From these findings, we can hypothesize that bacterial sulfur utilization and demand affects bacterial degradation of DMSP. The formation of DMS and MeSH from bacterial DMSP metabolism emphasizes the importance in studying bacterial effects on atmospheric formation.

Conclusion and Perspectives:

The research presented in this thesis describes the impact heterotrophic bacteria have on the formation of sulfur containing VOCs DMS and MeSH produced from DMSP utilization, and ultimately the significance of these compounds' potential impact on the atmosphere. During an induced phytoplankton bloom, we report for the first time, to our knowledge, the importance of the transcription of *dddD* cleavage pathway in coastal ecosystems, as well as the presence of *dddD* genes in marine aerosols, shedding light on the diversity of genetic pathways associated to DMSP degradation and that microbial strategies associated to the production of climatically active VOCs is still widely understudied. Additionally, a mesocosm experiment measuring DMSP and DMS consumption by *Phaeobacter* sp. La5 resulted in novel insights into the bacterial switch, as *Phaeobacter* sp. La5 was observed to simultaneously produce DMS and MeSH in a closed system.

These processes illustrate the necessity to further understand molecular processes and their environmental drivers enabling the prediction of the role of marine bacteria in the production of atmospheric relevant gases. Specifically, other factors like microbial community dynamics and functionality, metal cofactor availability, and enzyme efficiency should be further studied to determine holistic influences on the 'bacterial switch'. Research should continue to consider what favors one cleavage gene homolog over another, as well as continue efforts to characterize which taxa of microbes contain which family of genes and describe new molecular pathways associated to production of sulfur containing VOCs.. In considering these questions, research can attempt to understand the impact microbes have on the climate and atmosphere through degradation of not only organosulfur molecules, like DMSP, but other climatically significant compounds as well.

References:

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Thingstad, F. (1983). The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series*, *10*, 257–263. https://doi.org/10.3354/meps010257

Azam, F., & Malfatti, F. (2007). Erratum: Microbial structuring of marine ecosystems. *Nature Reviews Microbiology,5*(12), 966-966. doi:10.1038/nrmicro1798

Bratbak, G., Levasseur, M., Michaud, S., Cantin, G., Fernández, E., Heimdal, B. R., & Heldal, M. (1995). Viral activity in relation to Emiliania huxleyi blooms:a mechanism of DMSP release? *Marine Ecology Progress Series*, *128*, 133–142. https://doi.org/10.3354/meps128133

Carlson, D. J. (1982). Surface microlayer phenolic enrichments indicate sea surface slicks. *Nature*, *296*(5856), 426–429. https://doi.org/10.1038/296426a0

Charlson, R. J., Lovelock, J. E., Andreae, M. O., & Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature, 326*(6114), 655-661. doi:10.1038/326655a0

Chen, L., Kieber, D. J., Amato, F., Vila, M., Viure, L., Alastuey, A., Querol, X., & Berdalet, E. (2020, February 17). *Dimethylsulfoniopropionate concentrations in Mediterranean coastal water and ambient aerosols during Ostreopsiscf. ovata blooms*. AGU. https://agu.confex.com/agu/osm20/meetingapp.cgi/Paper/649841.

Chin, M., Jacob, D. J., Gardner, G. M., Foreman-Fowler, M. S., Spiro, P. A., & Savoie, D. L. (1996). A global three-dimensional model of tropospheric sulfate. *Journal of Geophysical Research: Atmospheres*, *101*(D13), 18667–18690. https://doi.org/10.1029/96jd01221

Cuhel, R. L., Taylor, C. D., and Jannasch, H. W.: Assimilatory Sulfur Metabolism in Marine Microorganisms: Sulfur Metabolism, Protein Synthesis, and Growth of Alteromonas luteo-violaceus and Pseudomonas halodurans During Perturbed Batch Growth, APPL Env. MICROBIOL, 43, 9, 1982.

Curson, A. R., Todd, J. D., Sullivan, M. J., & Johnston, A. W. (2011). Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nature Reviews Microbiology*, *9*(12), 849–859. https://doi.org/10.1038/nrmicro2653

Cui, Y., Suzuki, S., Omori, Y., Wong, S.-K., Ijichi, M., Kaneko, R., Kameyama, S., Tanimoto, H., & Hamasaki, K. (2015). Abundance and Distribution of Dimethylsulfoniopropionate Degradation Genes and the Corresponding Bacterial Community Structure at Dimethyl Sulfide Hot Spots in the Tropical and Subtropical Pacific Ocean. *Applied and Environmental Microbiology*, *81*(12), 4184–4194. https://doi.org/10.1128/aem.03873-14

Cunliffe, M., & Wurl, O. (2015). Sampling the Sea Surface Microlayer. *Springer Protocols Handbooks*, 255–261. https://doi.org/10.1007/8623_2015_83

Ducklow, H. W. & Carlson, C. A. Oceanic bacterial production. Adv. Microb. Ecol. 12, 113–181 (1992).

Fagerbakke, K., Heldal, M., and Norland, S.: Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria, Aquat. Microb. Ecol., 10, 15–27, https://doi.org/10.3354/ame010015, 1996.

Freese, H. M., Methner, A., & Overmann, J. (2017). Adaptation of Surface -Associated Bacteria to the Open Ocean: A Genomically Distinct Subpopulation of Phaeobacter gallaeciensis Colonizes Pacific Mesozooplankton. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.01659

Gabric, A., Murray, C. N., Stone, L., & Kohl, M. (1993). A Model of Dimethylsulphide Production during a Phytoplankton Bloom. *Dimethylsulphide: Oceans, Atmosphere and Climate*, 63–81. https://doi.org/10.1007/978-94-017-1261-3_8

Gasol, J. M., & Del Giorgio, P. A. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Scientia Marina*, *64*(2), 197–224. https://doi.org/10.3989/scimar.2000.64n2197

González, J. M., Kiene, R. P., & Moran, M. A. (1999). Transformation of Sulfur Compounds by an Abundant Lineage of Marine Bacteria in the α-Subclass of the Class Proteobacteria. *Applied and Environmental Microbiology*, *65*(9), 3810–3819. https://doi.org/10.1128/aem.65.9.3810-3819.1999

Hatton, A., Darroch, L., & Malin, G. (2004). The Role of Dimethylsulphoxide in the Marine Biogeochemical Cycle of Dimethylsulphide. *Oceanography and Marine Biology*, 29–56. https://doi.org/10.1201/9780203507810.ch2

Kiene, R. P. (1990). Dimethyl Sulfide Production from Dimethylsulfoniopropionate in Coastal Seawater Samples and Bacterial Cultures †. *Applied and Environmental Microbiology*, *56*(11), 3292–3297. https://doi.org/10.1128/aem.56.11.3292-3297.1990

Kiene, R. P., Linn, L. J., González, J., Moran, M. A., & Bruton, J. A. (1999). Dimethylsulfoniopropionate and Methanethiol are Important precursors of methionine and protein-sulfur in Marine Bacterioplankton. *Applied and Environmental Microbiology, 65*(10), 4549-4558. doi:10.1128/aem.65.10.4549-4558.1999

Kiene, R. P., & Linn, L. J. (2000). The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: tracer studies using 35S-DMSP. *Geochimica Et Cosmochimica Acta*, *64*(16), 2797–2810. https://doi.org/10.1016/s0016-7037(00)00399-9

Kilgour, D. B., Novak, G. A., Sauer, J. S., Moore, A. N., Dinasquet, J., Amiri, S., Barnes, E. B., Mayer, K., Winter, M., Morris, C. K., Price, T., Malfatti, F., Crocker, D. R., Lee, C., Cappa, C. D.,Goldstein, A. H., Prather, K. A., & Bertram, T. H. (2021). *Marine gas-phase sulfur emissions during an induced phytoplankton bloom*. Unpublished Manuscript.

Kirchman, D., K'nees, E., & Hodson, R. (1985). Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology*, *49*(3), 599–607. https://doi.org/10.1128/aem.49.3.599-607.1985

Ksionzek, K.B., Lechtenfeld, O.J., McCallister, S.L., Schmitt-Kopplin, P., Geuer, J.K., Geibert, W., and Koch, B.P. (2016). Dissolved organic sulfur in the ocean: Biogeochemistry of a petagram inventory. Science 354, 456–459.

Lafay B, Ruimy R, de Traubenberg CR, Breittmayer V, Gauthier MJ, Christen R. Roseobacter algicola sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate Prorocentrum lima. Int J Syst Bacteriol. 1995 Apr;45(2):290-6. doi: 10.1099/00207713-45-2-290. PMID: 7537061.

Levine, N. M., Varaljay, V. A., Toole, D. A., Dacey, J. W., Doney, S. C., & Moran, M. A. (2012). Environmental, biochemical and genetic drivers of DMSP degradation and DMS production in the Sargasso Sea. *Environmental Microbiology, 14*(5), 1210-1223. doi:10.1111/j.1462-2920.2012.02700.x

Liss, P., & Merlivat, L. (1986). Air-Sea Gas Exchange Rates. *The Role of Air-Sea Exchange in Geochemical Cycling*, 113–127. https://doi.org/10.1007/978-94-009-4738-2

Liu, J., Liu, J., Zhang, S.-H., Liang, J., Lin, H., Song, D., Yang, G.-P., Todd, J. D., & Zhang, X.-H. (2018). Novel Insights Into Bacterial Dimethylsulfoniopropionate Catabolism in the East China Sea. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.03206

Moran, M. A., Reisch, C. R., Kiene, R. P., & Whitman, W. B. (2012). Genomic insights into bacterial dmsp transformations. *Annual Review of Marine Science, 4*(1), 523-542. doi:10.1146/annurev-marine-120710-100827

Quinn, P. K., & Bates, T. S. (2011). The case against climate regulation via oceanic phytoplankton sulphur emissions. *Nature*, *480*(7375), 51–56. https://doi.org/10.1038/nature10580

Sauer, J. S., Mayer, K. J., Lee, C., Alves, M. R., Amiri, S., Bahaveolos, C., Barnes, E. B., Crocker, D. R.,
Dinasquet, J., Garofalo, L. A., Kaluarachchi, C. P., Dang, D., Kilgour, D., Mael, L., Mitts, B. A.,
Moon, D. R., Morris, C. K., Moore, A. N., Ni, C.-M., Prather, K.
A. (n.d.). The Sea Spray Chemistry and Particle Evolution Study (SeaSCAPE): Overview and
Experimental Methods . https://doi.org/2021

Simó, R. (2001). Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and
evolutionary links. *Trends in Ecology & Evolution*, *16*(6), 287–294.
https://doi.org/10.1016/s0169-5347(01)02152-8

Simon, M., & Azam, F. (1989). Protein content and protein synthesis rates of planktonic marine
bacteria. *Marine Ecology Progress Series*, *51*, 201–213. https://doi.org/10.3354/meps051201

Simon, M., & Azam, F. (1989). Protein content and protein synthesis rates of planktonic marine
bacteria. *Marine Ecology Progress Series*, *51*, 201–213. https://doi.org/10.3354/meps051201

Stefels, J. (2009). Determination of DMS, DMSP, and DMSO in Seawater. *Practical Guidelines for the
Analysis of Seawater*. https://doi.org/10.1201/9781420073072.ch11

Thole, S., Kalhoefer, D., Voget, S., Berger, M., Engelhardt, T., Liesegang, H., Wollherr, A., Kjelleberg,
S., Daniel, R., Simon, M., Thomas, T., & Brinkhoff, T. (2012). Phaeobacter gallaeciensis genomes
from globally opposite locations reveal high similarity of adaptation to surface life. *The ISME
Journal*, *6*(12), 2229–2244. https://doi.org/10.1038/ismej.2012.62

Todd, J. D., Rogers, R., Li, Y. G., Wexler, M., Bond, P. L., Sun, L., Curson, A. R., Malin, G., Steinke, M., &
Johnston, A. W. (2007). Structural and Regulatory Genes Required to Make the Gas Dimethyl Sulfide
in Bacteria. *Science*, *315*(5812), 666–669. https://doi.org/10.1126/science.1135370

Todd, J. D., Curson, A. R., Dupont, C. L., Nicholson, P., & Johnston, A. W. (2009). The dddP gene,
encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is
widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete
fungi. *Environmental Microbiology*, *11*(6), 1376–1385. https://doi.org/10.1111/j.1462-
2920.2009.01864.x

Vallina, S. M., & Simo, R. (2007). Strong Relationship Between DMS and the Solar Radiation
Dose over the Global Surface Ocean. *Science*, *315*(5811), 506–508.
https://doi.org/10.1126/science.1133680

Wirth, J. S., Wang, T., Huang, Q., White, R. H., & Whitman, W. B. (2020). Dimethylsulfoniopropionate
sulfur and Methyl carbon assimilation in Ruegeria Species. *MBio, 11*(2). doi:10.1128/mbio.00329-
20

Yoch, D. C. (2002). Dimethylsulfoniopropionate: Its Sources, Role in the Marine Food
Web, and Biological Degradation to Dimethylsulfide. *Applied and Environmental Microbiology*, *68*(12), 5804–5815. https://doi.org/10.1128/aem.68.12.5804-5815.2002

Zhang, X., Liu, J., Liu, J., Yang, G., Xue, C., Curson, A. R., & Todd, J. D. (2019). Biogenic production of DMSP and its degradation to DMS—their roles in the global sulfur cycle. *Science China Life Sciences, 62*(10), 1296-1319. doi:10.1007/s11427-018-952