Sulfur Metabolites in the North Pacific Subtropical Gyre and Transition Zone

Leland Wood

ljwood@uw.edu

Senior Thesis

University of Washington

School of Oceanography, Box 357940

Seattle, WA 98195

Abstract

Sulfur is an essential nutrient for life and a constituent of many important biomolecules. Phytoplankton in the surface ocean use assimilatory reduction pathways to reduce inorganic sulfur, primarily in the form of sulfate (SO_4^{2-}), into the necessary organic compounds. Many marine microbes including *Pelagibacter*, the most abundant and ubiquitous heterotrophic bacteria, do not have these pathways and are not able to reduce their own sulfur. These organisms therefore require exogenous sources of reduced sulfur. While some sulfur compounds, such as DMSP or DHPS, have been well studied, the entire composition of sulfur and the roles of these compounds is poorly understood. Metabolomics were used to characterize the composition of sulfur metabolites from four particulate depth profiles in the North Pacific. This study quantified 12 sulfur metabolites within particulate matter of the North Pacific. I identified gonyol as the most abundant of these sulfur metabolites. This sulfonium likely plays an important role in sulfur cycling as a source of reduced sulfur for the heterotrophic bacteria. Sulfonates, the more oxidized form of sulfur, likely play a key role in communities north of the North Pacific Transition Zone as they are the most abundant form of sulfur in this area. This research highlights the importance of these understudied compounds and provides a foundation for future research on sulfur cycling in the marine environment.

Plain Language Summary

All living organisms require sulfur as it is an essential nutrient for growth. This element is incorporated into a large variety of biological compounds that serve specific functions. Phytoplankton in the surface ocean use the sun’s energy to produce the necessary sulfur molecules for growth. Sulfoniums, a group of sulfur compounds characterized by the sulfur having three carbon bonds, are very important as they are used in compounds with cellular functions. However, there are some organisms that are not able to make these compounds on their own and rely on taking up sulfur compounds produced by other organisms in the water column. Most of the available sulfur compounds for these organisms are poorly understood. The aim of this project was to characterize this pool of sulfur compounds to build an understanding of the role of these compounds in microbial communities. Twelve sulfur compounds were identified and quantified within cells. Of these, a compound called gonyol was found to be the most abundant overall. This suggests that this compound, of which very little is known, may be an important source of sulfur for organisms not able to make sulfur compounds. Analysis of major functional groups revealed that sulfonates, which are compounds that have sulfur bonded to three oxygen atoms and one carbon atom, were the major form of sulfur observed in organic molecules in the northernmost stations. Sulfonates have only recently been discovered to play key roles in community wide cycling of sulfur and carbon. This group of sulfur compounds is likely more important in sulfur cycling than previously thought. This study brings to light the importance of these sulfur metabolites and suggests an intriguing avenue for future research.

Introduction

Sulfur is an element required for life as it is a part of many small organic molecules with cellular functions, known as metabolites, and larger biomolecules such as proteins. Metabolomics uses state-of-the-art instruments to identify and quantify organic molecules produced by cells to study the physiology of organisms and chemical interactions within a community (Roessner and Bowne 2009). Sulfur containing metabolites are an abundant and diverse group of compounds (Moran and Durham 2019). The expanded Redfield ratio, $C_{124}N_{16}P_1S_{1.3}$, showcases the high cellular quota for sulfur and a requirement similar to that of phosphorus (Ho et al. 2003). In order for the annual primary production of carbon to be fixed, 48.5 Pg year^{-1}, cells require the assimilation of 1.3 Pg year^{-1} of sulfur (Koch et al. 2017). This coupling of the carbon and sulfur cycles presents a large inventory of diverse sulfur metabolites which is highly labile with short turnover times (Landa et al. 2019). The oxidation state, or relative charge of the atom, is important as sulfur is assimilated into cellular components at specific oxidation states (Giordano and Raven 2014). For example, amino acids and sulfoniums contain sulfur with the lowest oxidation state, -2, while sulfolipids and sulfonates contain sulfur at the higher oxidation states, +4 (Moran and Durham 2019). Phytoplankton use assimilatory reduction pathways to convert inorganic sulfur, which has a +6 oxidation state, into the necessary organic compounds for life (Landa et al. 2019). This reduced form of sulfur can be made into a variety of small organic compounds, such amino acids, sulfolipids, osmolytes, and other biomolecules. However, unlike inorganic sulfur, the distribution and roles of many organic sulfur compounds remains unknown.

The high concentrations of inorganic sulfur in seawater originally suggested its availability was not a factor controlling growth of marine microbial communities. However, this is not the case for all organisms. While many phytoplankton are capable of reducing sulfur, some bacterioplankton do not have the necessary genes required for these pathways (Tripp et al. 2008). *Pelagibacter*, the most abundant and ubiquitous heterotrophic marine bacteria, do not have such genes and are not able to produce their own sulfur metabolites (Tripp et al. 2008). This clade, along with many others in the surface ocean, rely on scavenging the organic sulfur produced by other plankton for growth (Tripp et al. 2008). These organisms assimilate organic sulfur compounds from metabolites dissolved in seawater. The metabolite DMSP is one of the few well studied sulfur metabolites as it is a substrate for these heterotrophic bacteria and is produced in large quantities by phytoplankton (Ledyard and Dacey 1996; Thume et al. 2018; Landa et al. 2019). DMSP has also been well studied as its degradation product, DMS, was found to enter the atmosphere where it regulates global climate (Charlson et al. 1987). Recent studies have expanded our understanding of sulfur metabolites by describing the roles of sulfonate metabolites, the more oxidized group of sulfur metabolites (Celik et al. 2017; Durham et al. 2019). This group of metabolites has been found to act as valuable energy and nutrient substrates and help maintain redox balance within cells (Durham et al. 2019). Although these metabolites are very important pieces of the sulfur cycle, the community wide distribution of sulfur metabolites remains understudied. What other metabolites are available for uptake by organisms? What is the most abundant sulfur metabolite in the marine environment? Are the proportions of sulfur metabolites similar across communities? More research needs to be done in order to fully understand the sulfur cycle, metabolite production and consumption, and how these compounds influence community structure.

This study evaluated the composition of sulfur metabolites within particulate matter. Although heterotrophic bacteria assimilate sulfur through the dissolved pool, dissolved metabolites are challenging to quantify. Developing methods to measure dissolved metabolites is an area of active research (Widner et al. 2020), but current methods are limited in the number of molecules that can be detected (Johnson et al. 2017). This is due to the high amount of inorganic salts in seawater and the wide range of structures present in metabolites. Particulate metabolites, those which are still within cells, have well developed methods and a greater proportion of metabolites are able to be measured (Boysen et al. 2018). This particulate fraction can be used to make inferences about dissolved metabolites, as it is the source of what becomes dissolved through mechanisms such as sloppy feeding, cell lysis, and exudation (Landa et al. 2019). This study will use metabolomics to characterize the composition and distribution of sulfur metabolites within depth profiles from the North Pacific. By understanding the community metabolite profile, this research will provide the foundation for future studies on metabolite uptake and utilization and sulfur metabolites.

Proposed Research

Research Location

To capture the diversity of metabolites and understand influence of community on composition, metabolites from 4 depth profiles in the North Pacific were analyzed (Figure 1). One depth profile is from Station ALOHA in the oligotrophic North Pacific Subtropical Gyre. This area is dominated by organisms adapted to low nutrient conditions, such as the cyanobacteria *Prochlorococcus* (Rii et al. 2016). The three other depth profiles are from across the North Pacific Transition Zone, an area of strong environmental and ecological gradients (Roden 1971). The convergence of the currents in the Transition Zone facilitates high productivity and diverse microbial communities which have higher counts of diatoms, dinoflagellates, and haptophytes (Clayton et al. 2014). Diatoms are known to produce large amounts of the important sulfur metabolites taurine and DHPS, while dinoflagellates produce large amounts of DMSP (Moran and Durham 2019).

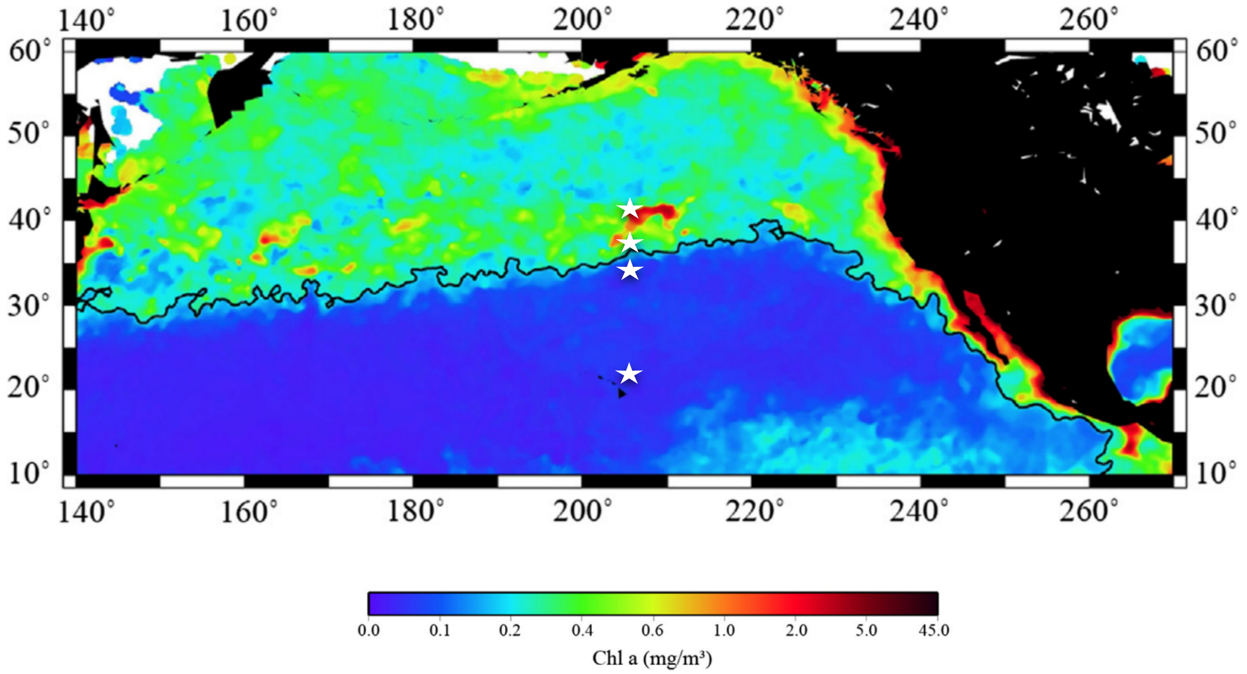


Figure 1. Adapted from (Polovina et al. 2017). *Sea surface chlorophyll of the North Pacific in March. Black line denotes the southern boundary of the North Pacific Transition Zone. Stars in white denote locations of depth profiles.*

Methods

Samples were collected by members of the Ingalls lab. Particulate matter from a known volume of seawater was collected onto 0.2 µm filters, which was then used to extract metabolites following previously described methods (Boysen et al. 2018; Dawson et al. 2020). Extracted metabolites, along with blanks and standards, were then processed using liquid-chromatography mass spectrometry (Boysen et al. 2018). Metabolite extraction and processing was conducted during the summer of 2019 by members of the Ingalls lab, including myself. I used MSDial (Tsugawa et al. 2015) and Skyline (Adams et al. 2019) for analysis of LC-MS data (Boysen et al. 2018). This gave relative abundance of compounds in peak area, which can be compared to standards and annotated using mass to charge ratio and retention time. Peak areas of compounds can be normalized using Best-Matched Internal Standard Normalization (B-MIS), subject to in-house quality control, and quantified by comparing to standards (Boysen et al. 2018). This process ultimately yielded the concentration of sulfur metabolites within particulate per liter of seawater.

Results

This study measured the concentrations of particulate sulfur metabolites within four depth profiles from the North Pacific. I identified and quantified a total of 12 sulfur metabolites from the particulate fraction. The measured concentrations of each metabolite from all profiles are shown in Figure 2. Gonyol was found to be the overall most abundant sulfur metabolite within cells (Figure 2). With depth, dimethylsulfonioacetate (DMS-Ac) accounts for a larger proportion of the sulfur metabolites at depth (Figure 2.C).

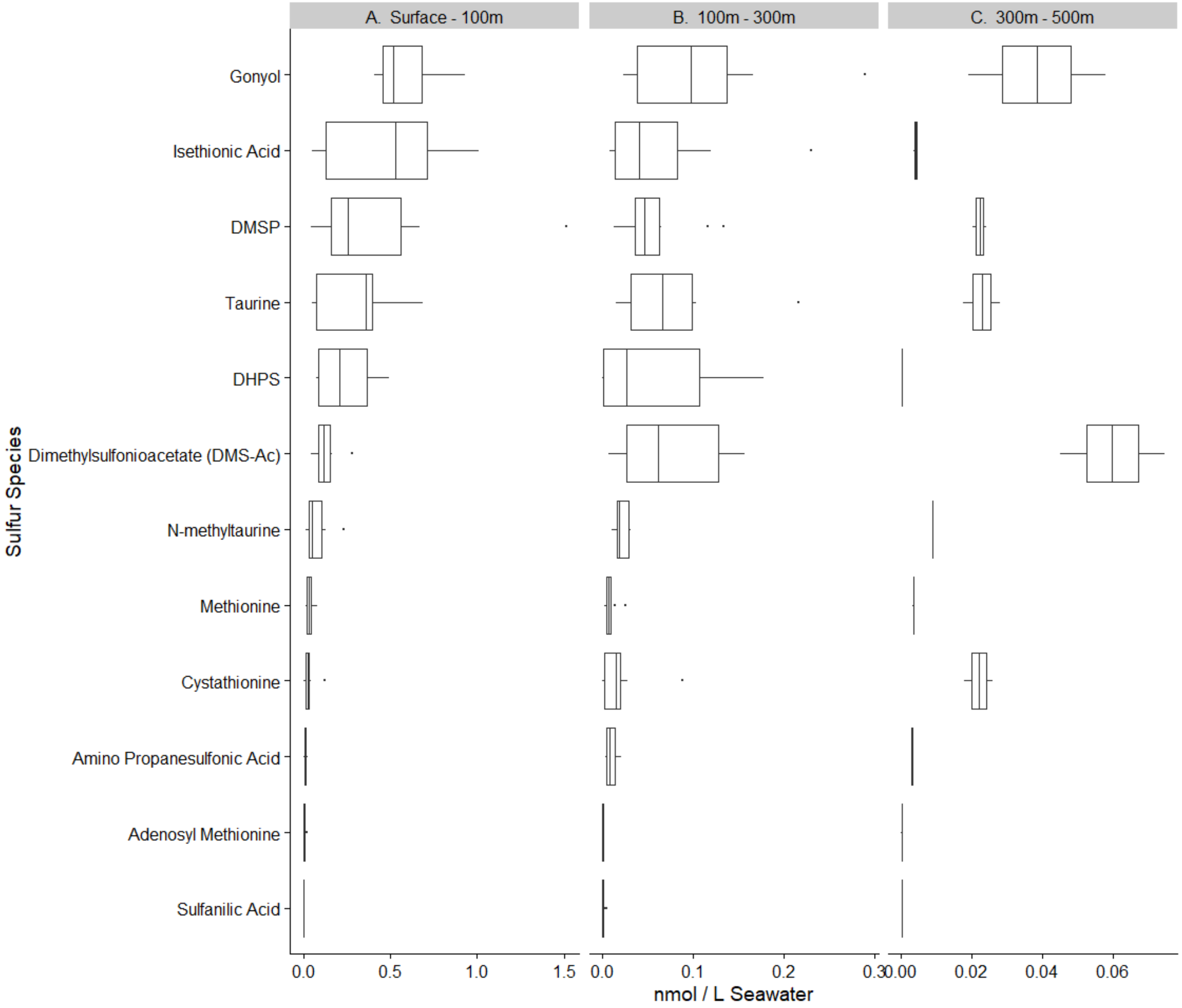


Figure 2. Concentrations of particulate sulfur metabolites for all locations for depths between (A) the surface and 100 meters, (B) 100m – 300m, and (C) 300m – 500m.

The average concentration of each metabolite was found to quantitively shift with latitude (Figure 3). Total concentration of sulfur metabolites was highest in the middle of the North Pacific Transition Zone (Figure 3.C) and lowest at the oligotrophic station ALOHA (Figure 3.A). Gonyol was found to have the highest overall average concentration of the sulfur metabolites (Figure 2 and 3). Although gonyol was the highest overall, some surface samples have higher concentrations of other sulfur metabolites in the surface, such as isethionic acid or DMSP (Figure 3.C). Isethionic acid, taurine, and DMSP increase in concentration in the northernmost latitudes (Figure 3.C-D). By plotting the concentrations of metabolites based on their functional group, this trend leads to a shift in the major sulfur oxidation state of the entire pool. In the southernmost depth profile (Figure 4.A), sulfoniums make up most of the sulfur pool. This shifts in the northern latitudes (Figure 4.C-D) where sulfonates are the major functional group. Therefore, most of the sulfur in the southern station has an oxidation state of -2, while most have an oxidation state of +4 in the north.

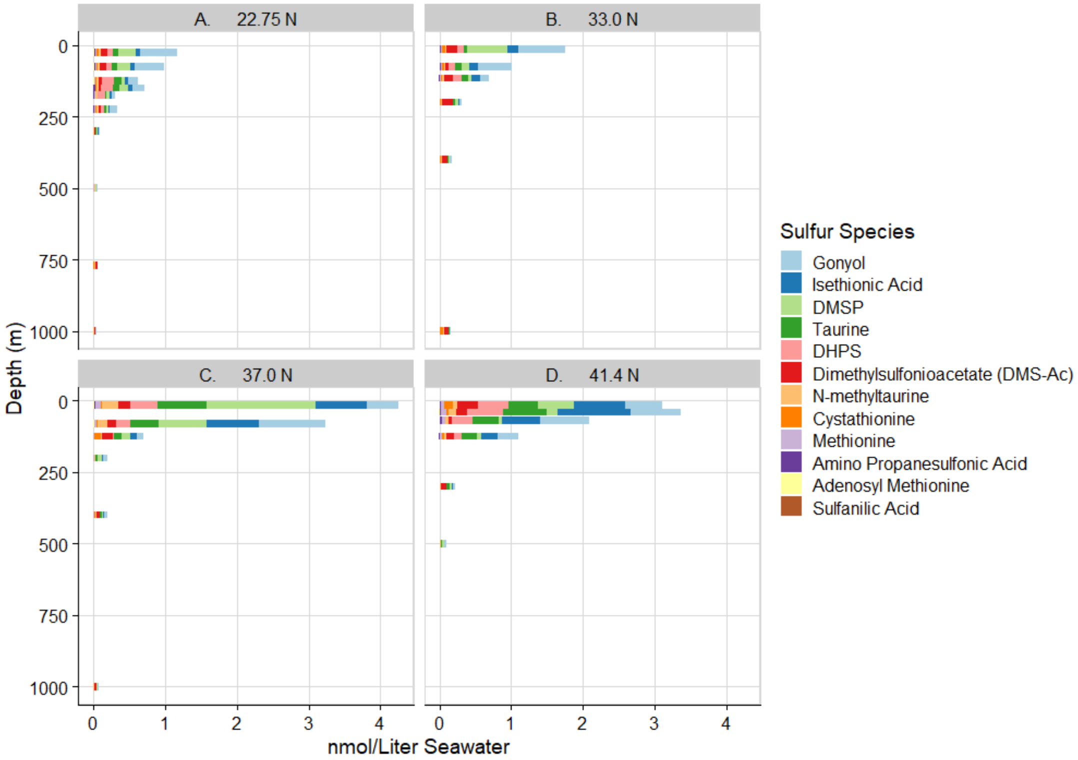


Figure 3. Average concentration for each sulfur metabolite. Each panel represents a depth from the North Pacific, in the Subtropical Gyre (A) 22.75°N, and the North Pacific Transition Zone, (B) 33.9°N, (C) 37.0°N, and (D) 41.4°N.

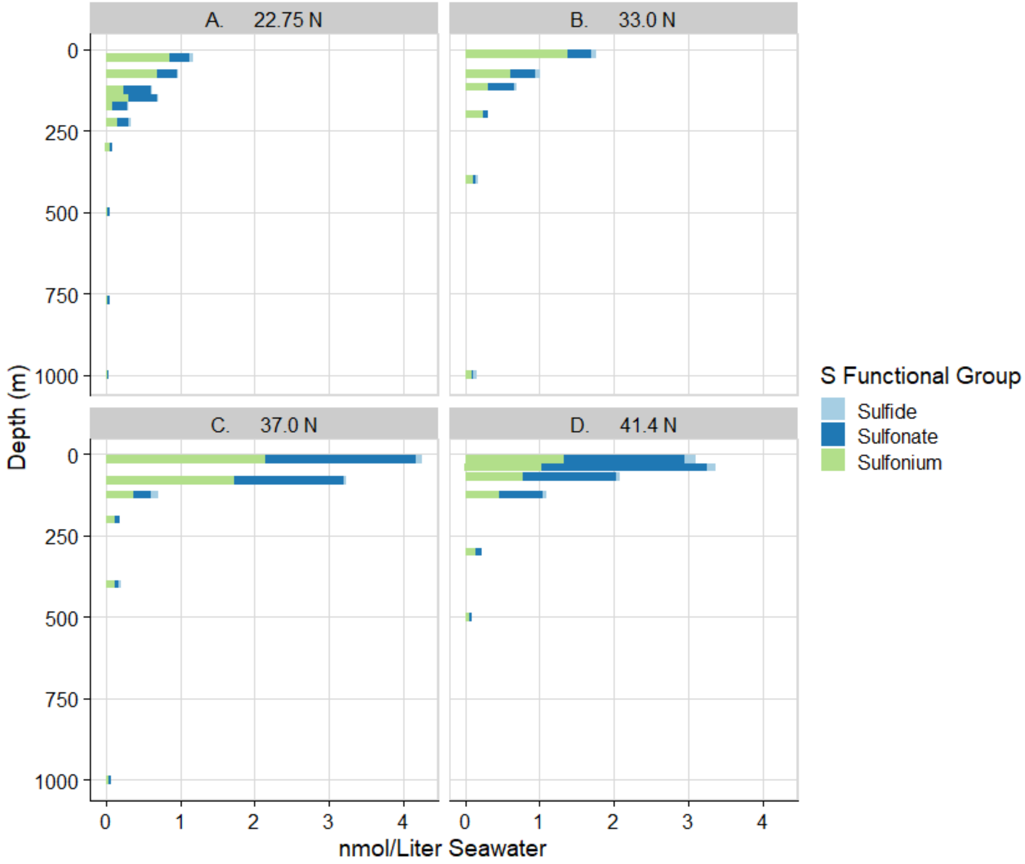


Figure 4. Average concentration for sulfur metabolites grouped by the functional group of the sulfur species at (A) 22.57°N, (B) 33.0°N, (C) 37.0°N, and (D) 41.4°N. Sulfides [R_2S] contain sulfur with an oxidation state of -2 are in light blue, while sulfonates [$R-SO_3$]$^-$, in dark blue, have +4 sulfur, and sulfoniums [R_3S]$^+$, in green, contain -2 sulfur.

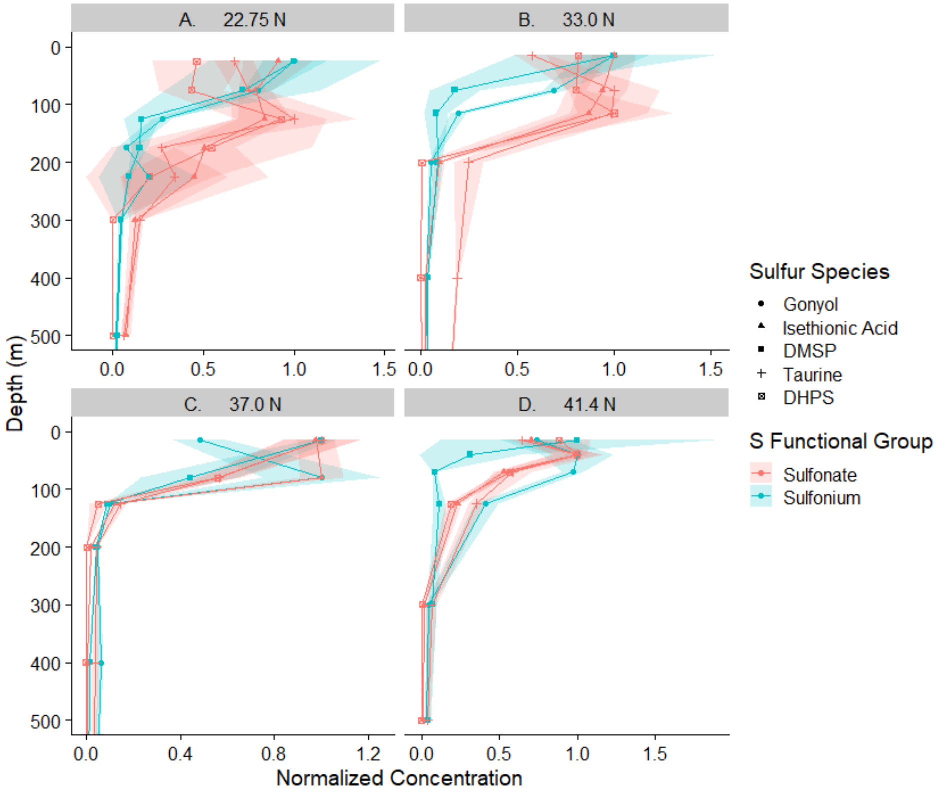


Figure 5. The normalized concentration of the five most abundant sulfur metabolites at (A) 22.57°N, (B) 33.0°N, (C) 37.0°N, and (D) 41.4°N. Line color denotes the functional group while line point shape separates species. Shaded area around line represents the normalized standard deviation.

Finally, I explored the patterns of individual depth profiles for the five most abundant metabolites that were measured. By plotting the normalized concentration, I can compare the shapes of metabolite attenuation with depth (Figure 5). This analysis yields an intriguing trend between the two main functional groups of sulfur, sulfoniums and sulfonates. In the southernmost station, Figure 5.A, the two sulfoniums, DMSP and gonyol, have very close profiles shapes. The three sulfonates, isethionic acid, taurine, and DHPS, also have similar profile shapes to the others, but the sulfonate profiles are different than those of the sulfoniums. The sulfoniums are greatest at the surface and attenuate sharply with depth. Meanwhile the sulfonates begin to increase with depth to reach a subsurface maximum, and then attenuate with depth. This observation continues north into the southern transition zone, Figure 5.B, and the two groups have different profile shapes. In the middle of the transition zone, Figure 5.C, the profiles between sulfoniums and sulfonates do not appear to be different. However, in the northernmost station, Figure 5.D, this separation returns with one key change. The sulfonates again have very similar profiles and retain the subsurface maximum. The sulfonium DMSP has a similar shape to its shape in the southern stations and the shallower attenuation can be attributed to the shoaling of the euphotic zone (Supplemental Figure 1). But, the sulfonium gonyol has now changed and its profile appears to be similar to the sulfonates, where gonyol has a subsurface maximum before it attenuates with depth.

Discussion

This study builds on recent work to understand the roles of sulfur metabolites in marine microbial communities. Gonyol was found to have the highest average concentrations within cells of the sulfur metabolites measured (Figure 2 and 3). As a sulfonium, similar to DMSP, gonyol has the potential to be used as source of reduced sulfur. Although the degradation pathway has yet to be fully revealed, gonyol is known to be utilized by some heterotrophic bacteria (Gebser et al. 2020). As gonyol was found to be the most abundant sulfur metabolite that was measured, these data suggests this compound is likely an important substrate for organisms in the surface ocean and a valuable source of reduced sulfur for heterotrophic bacteria. My analysis also revealed an interesting observation with the profile shape of gonyol (Figure 5). The two groups of sulfur metabolites, sulfonates and sulfoniums, appear to have different profile shapes in the southern, oligotrophic stations. Gonyol then shifts in the northernmost latitude to follow closely to the profile shapes of the sulfonates. This observation suggests the sources or sinks for this molecule are changing. Not enough is known about which organisms are the producers of this compound, nor is enough yet known about the degradation of this compound. This trend could be due to the community shift between the northern and southern latitudes and the profile is a reflection of a different community metabolomic fingerprint (Heal et al. 2020). A second hypothesis for this observation could be due to different physiological stressors causing a change in the production of gonyol. Alternatively, this could also be due to microbes using the substrate differently or having different degradation pathways. More research would need to be conducted on the production and fate of gonyol to understand its role in sulfur and carbon cycling in the ocean. Nonetheless, these data suggest gonyol is an important substrate of reduced sulfur for heterotrophic bacteria in the marine environment.

Although the sulfonium gonyol was the single most abundant sulfur metabolite, the major functional group of sulfur metabolites shifts with latitude (Figure 4). In the southern latitudes, the oligotrophic stations were found to have sulfoniums as the major sulfur metabolite class (Figure 4.A-B). This means majority of the sulfur is in the reduced form of sulfur. Contrasting these southern stations, the northern stations (Figure 4.C-D) were found to have sulfonates as the major sulfur functional group. This shift is primarily due to the accumulation of the sulfonates DHPS, isethionic acid, and taurine within cells. A shift in microbial community is thought to drive this observation. Diatoms, which are more prevalent in the northern latitudes, are known to produce these compounds in large amounts (Durham et al. 2019). These sulfonates are known to be the basis for close community networks and are a valuable carbon and energy substrate (Celik et al. 2017; Durham et al. 2019). These data suggests sulfonates could be a more important group of metabolites for organisms in the North Pacific. Additionally, of these sulfonate metabolites, isethionic acid was found to be the most abundant of this group. Previous studies on the roles of sulfonates have centered around the compound DHPS (Celik et al. 2017). The abundance of isethionic acid suggests it could be just as important, if not more important, than DHPS and the other sulfonate compounds. As the major functional group of sulfur, these sulfonates could have a larger role in biogeochemical cycling than previously thought.

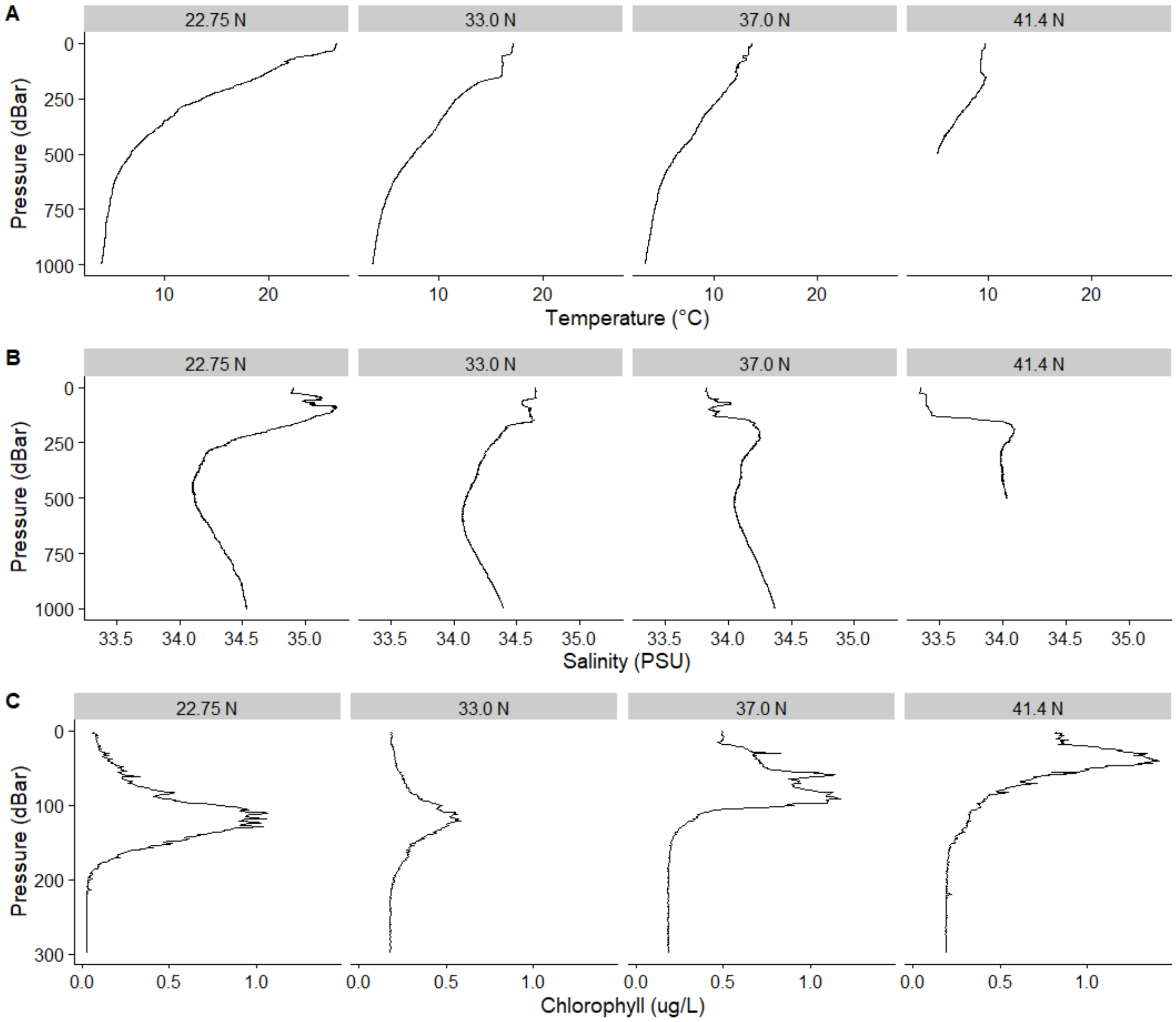
Conclusion

Sulfur is essential to life and needed in large amounts to facilitate growth. This creates a large inventory of sulfur metabolites that exist in a broad range of oxidation states. Understanding the function of these compounds and their role in biogeochemical cycling is an area of active research. This study provides foundational measurements of sulfur metabolites within particulate matter from the North Pacific Subtropical Gyre and the North Pacific Transition Zone. My results suggest that gonyol is likely an important source of reduced sulfur and of carbon, as it was found to be the overall most abundant sulfur metabolite. Very little is currently known about gonyol and its production or fate. Gonyol exhibits two different attenuation profiles between the oligotrophic and eutrophic North Pacific, suggesting a change in its sources or sinks. More research would need to be done on the sulfur cycle, especially gonyol to understand what is driving these observations. Future research could include pairing culture experiments to understand the production and fate of gonyol with more diverse environmental measurements to improve our understanding of its distribution. In addition to gonyol, analysis suggests sulfonates, the more oxidized form of sulfur, are more important than previously thought. As the most abundant group of sulfur in the northern latitudes of the North Pacific Transition Zone, sulfonates, especially isethionic acid, are likely playing a key role in the sulfur cycle. These sulfonate networks have only recently been identified and more research should be conducted on this group of metabolites. Studying how organisms catabolize and exchange these metabolites would aid in determining how sulfonates influence community composition. This study has shown that our understanding of the sulfur cycle is still limited and there is much to uncover. Gonyol and sulfonate metabolites offer an intriguing avenue for future research as they have the potential to be very important in global biogeochemical cycling of sulfur.

Acknowledgments

A very special thank you to Dr. Katherine Heal for her guidance through each stage of this project. Her expert opinion, instruction, and assistance were instrumental to the success of this study. I would like to thank my advisor, Dr. Anitra Ingalls, for her continued support and mentorship through this project and my undergraduate education. Thank you for the many opportunities and involvement that allowed me to grow as an academic and individual. I would like to thank the thesis advisors, especially Dr. Rick Keil, for their support and encouragement in finishing this capstone to my undergraduate education. I would like to thank my friends and peers, especially Kennedy Cameron and Marissa Leatherman, for their encouragement, collaboration, and sympathetic ears. Finally, I would like to thank my parents and family for supporting me through my education at the University of Washington. Thank you for the freedom to pursue higher education and follow my dreams of becoming an oceanographer.

Supplemental Figures



Supplemental Figure 1. Temperature (A), salinity (B), and chlorophyll (C) profiles from the CTD downcast for each station. Note:

References

Adams, K., B. Pratt, N. Bose, L. Dubois, L. John-Williams, K. Perrott, K. Ky, and B. MacLean. 2019. Skyline for Small Molecules: A Unifying Software Package for Quantitative Metabolomics. Physiology & behavior **176**: 139–148. doi:10.1021/acs.jproteome.9b00640.Skyline

Boysen, A. K., K. R. Heal, L. T. Carlson, and A. E. Ingalls. 2018. Best-Matched Internal Standard Normalization in Liquid Chromatography-Mass Spectrometry Metabolomics Applied to Environmental Samples. Analytical Chemistry **90**: 1363–1369. doi:10.1021/acs.analchem.7b04400

Celik, E., M. Maczka, N. Bergen, T. Brinkhoff, S. Schulz, and J. S. Dickschat. 2017. Metabolism of 2,3-dihydroxypropane-1-sulfonate by marine bacteria. Organic and Biomolecular Chemistry **15**: 2919–2922. doi:10.1039/c7ob00357a

Charlson, R. J., J. E. Lovelock, M. O. Andreaei, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature **330**: 1987.

Clayton, S., T. Nagai, and M. J. Follows. 2014. Fine scale phytoplankton community structure across the Kuroshio Front. Journal of Plankton Research **36**: 1017–1030. doi:10.1093/plankt/fbu020

Dawson, H. M., K. R. Heal, A. K. Boysen, L. T. Carlson, A. E. Ingalls, J. N. Young, D. Helmig, and K. Arrigo. 2020. Potential of temperature- and salinity-driven shifts in diatom compatible solute concentrations to impact biogeochemical cycling within sea ice. Elem Sci Anth **8**. doi:10.1525/elementa.421

Durham, B. P., A. K. Boysen, L. T. Carlson, and others. 2019. Sulfonate-based networks between eukaryotic phytoplankton and heterotrophic bacteria in the surface ocean. Nature Microbiology **4**: 1706–1715. doi:10.1038/s41564-019-0507-5

Gebser, B., K. Thume, M. Steinke, and G. Pohnert. 2020. Phytoplankton-derived zwitterionic gonyol and dimethylsulfonioacetate interfere with microbial dimethylsulfoniopropionate sulfur cycling. MicrobiologyOpen **9**. doi:10.1002/mbo3.1014

Giordano, M., and J. A. Raven. 2014. Nitrogen and sulfur assimilation in plants and algae. Aquatic Botany **118**: 45–61. doi:10.1016/j.aquabot.2014.06.012

Heal, K. R., B. P. Durham, A. K. Boysen, and L. T. Carlson. 2020. Community metabolomes reflect taxon-specific fingerprints of phytoplankton in the ocean. BioRxiv doi:https://doi.org/10.1101/2020.12.22.424086

Ho, T. Y., A. Quigg, Z. v. Finkel, A. J. Milligan, K. Wyman, P. G. Falkowski, and F. M. M. Morel. 2003. The elemental composition of some marine phytoplankton. Journal of Phycology **39**: 1145–1159. doi:10.1111/j.0022-3646.2003.03-090.x

Johnson, W. M., M. C. Kido Soule, and E. B. Kujawinski. 2017. Extraction efficiency and quantification of dissolved metabolites in targeted marine metabolomics. Limnology and Oceanography: Methods **15**: 417–428. doi:10.1002/lom3.10181

Koch, B. P., K. B. Ksionzek, O. J. Lechtenfeld, S. L. McCallister, P. Schmitt-Kopplin, J. K. Geuer, and W. Geibert. 2017. Dissolved organic sulfur in the ocean: Biogeochemistry of a petagram inventory. Science **356**: 456–460. doi:10.1126/science.aam6328

Landa, M., A. S. Burns, B. P. Durham, and others. 2019. Sulfur metabolites that facilitate oceanic phytoplankton – bacteria carbon flux. 2536–2550. doi:10.1038/s41396-019-0455-3

Ledyard, K. M., and J. W. H. Dacey. 1996. Microbial cycling of DMSP and DMS in coastal and oligotrophic seawater. Limnology and Oceanography **41**: 33–40. doi:10.4319/lo.1996.41.1.0033

Moran, M. A., and B. P. Durham. 2019. Sulfur metabolites in the pelagic ocean. Nature Reviews Microbiology **17**: 665–678. doi:10.1038/s41579-019-0250-1

Polovina, J. J., E. A. Howell, D. R. Kobayashi, and M. P. Seki. 2017. The Transition Zone Chlorophyll Front updated: Advances from a decade of research. Progress in Oceanography **150**: 79–85. doi:10.1016/j.pocean.2015.01.006

Rii, Y. M., D. M. Karl, and M. J. Church. 2016. Temporal and vertical variability in picophytoplankton primary productivity in the North Pacific Subtropical Gyre. Marine Ecology Progress Series **562**: 1–18. doi:10.3354/meps11954

Roden, G. 1971. Aspects of the transition zone in the Northeastern Pacific. J Geophys Res **76**: 3462–3475. doi:10.1029/jc076i015p03462

Roessner, U., and J. Bowne. 2009. What is metabolomics all about? BioTechniques **46**: 363–365. doi:10.2144/000113133

Thume, K., B. Gebser, L. Chen, N. Meyer, D. J. Kieber, and G. Pohnert. 2018. The metabolite dimethylsulfoxonium propionate extends the marine organosulfur cycle. Nature **563**: 412–415. doi:10.1038/s41586-018-0675-0

Tripp, H. J., J. B. Kitner, M. S. Schwalbach, J. W. H. Dacey, L. J. Wilhelm, and S. J. Giovannoni. 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. Nature **452**: 741–744. doi:10.1038/nature06776

Tsugawa, H., T. Cajka, T. Kind, and others. 2015. MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nature Methods **12**: 523–526. doi:10.1038/nmeth.3393

Widner, B., M. C. Kido Soule, F. X. Ferrer-González, M. A. Moran, and E. B. Kujawinski. 2020. Superior and novel detection of small, polar metabolites in saline samples using pre-extraction benzoyl chloride derivatization and ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC MS/MS). ChemRxiv. doi:10.26434/chemrxiv.12915488.v1