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Plankton biomass decomposition enriches for methanogenic archaea in near-shore waters of Puget Sound

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Plankton Biomass Decomposition Enriches for Methanogenic Archaea in Near-Shore Waters of Puget Sound

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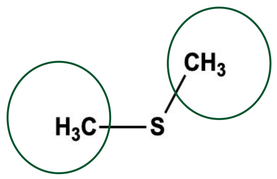
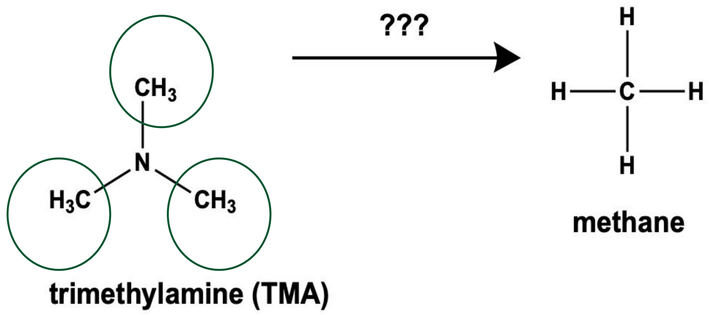
The ocean emits large quantities of methane to the atmosphere, yet the biological pathways and marine organisms responsible are not fully understood.[1] Marine surface waters are typically oversaturated with methane, a phenomenon coined the ocean methane paradox because of the existing paradigm that methanogenesis is restricted to anaerobic environments. Trimethylamine (TMA) and dimethyl sulfide (DMS) are both known to promote methanogenesis.[2,3] **It is possible that anaerobic microniches, present within aerobic waters, harbor the organism(s) necessary for methanogenesis, and can be promoted with TMA or DMS enrichment.**

Objectives

1. Monitor CH_4 concentration during plankton decomposition
2. Enrich for TMA and DMS degraders
3. Use metagenomic analysis to identify dominant species



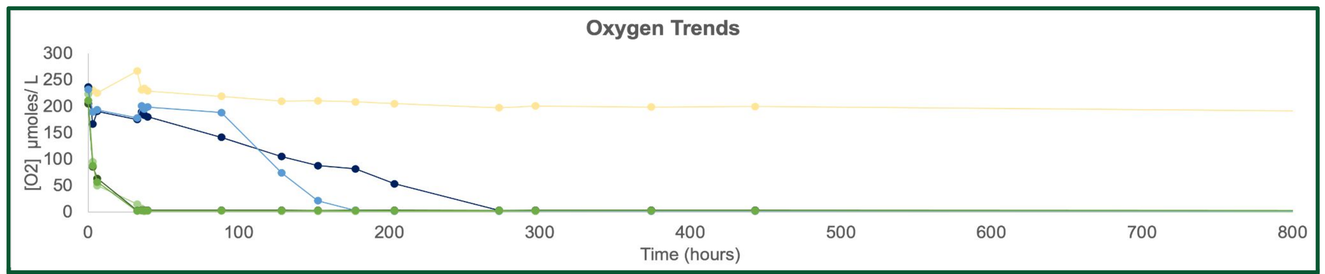
dimethyl sulfide (DMS)

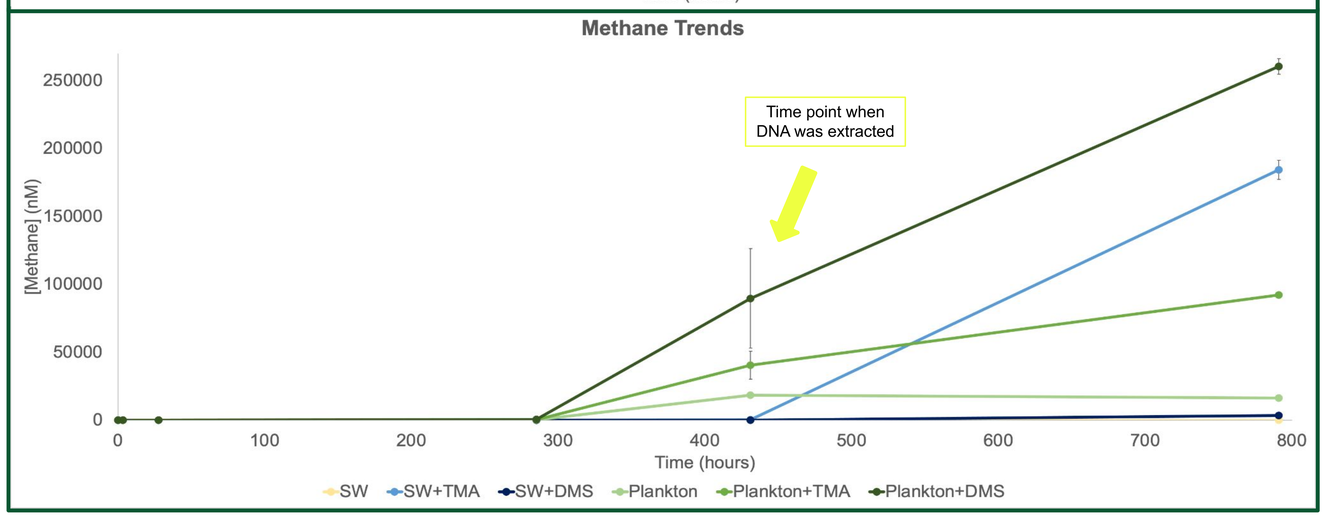


trimethylamine (TMA) **methane**

Results

Monitoring $[CH_4]$ During Anaerobic Decomposition: $[O_2]$ vs $[CH_4]$

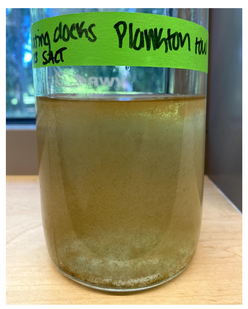




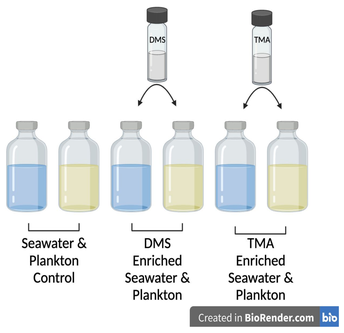
Methods

Collection & Characterization → DMS & TMA Enrichments → $[O_2]$ & $[CH_4]$ Measurements → DNA Extraction & Sequencing → Metagenomics → Contig Binning

- Surface seawater and plankton were collected from the Puget Sound (Old Town Floating Docks) (Fig. 1)
- Collected plankton was then characterized using microscopy



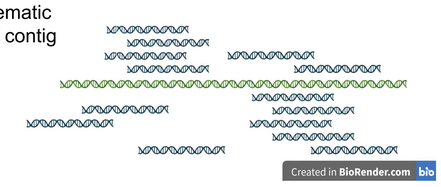
- Samples were enriched with trimethylamine (TMA) and dimethyl sulfide (DMS) (Fig. 2)
- Samples were sealed in airtight glass vials and incubated in the dark



- $[O_2]$ measurements were collected constantly with an oxygen probe and sensors to track decomposition
- Gas chromatography with known calibrations was used to obtain $[CH_4]$ at various time stamps

Fig 1. (far left) Concentrated plankton collect from PS
Fig 2. (left) Sample schematic
Fig 3. (right) Diagram of contig assembly

- DNA was extracted and purified from samples of interest
- Sent DNA out for Illumina next generation sequencing



- Sequences were compared to reference database of marine organisms for taxonomic analysis
- Began metagenome reassembly via contig construction with programs: SPAdes and Tadpole (bbmaps) (Fig.3)

- Use bioinformatics to cluster contigs
- Group clustered contigs into genome bins representative of main taxa
- Begin characterizing MAGs (metagenome assembled genomes).
- Identify de-replicated MAGs (Table 1)

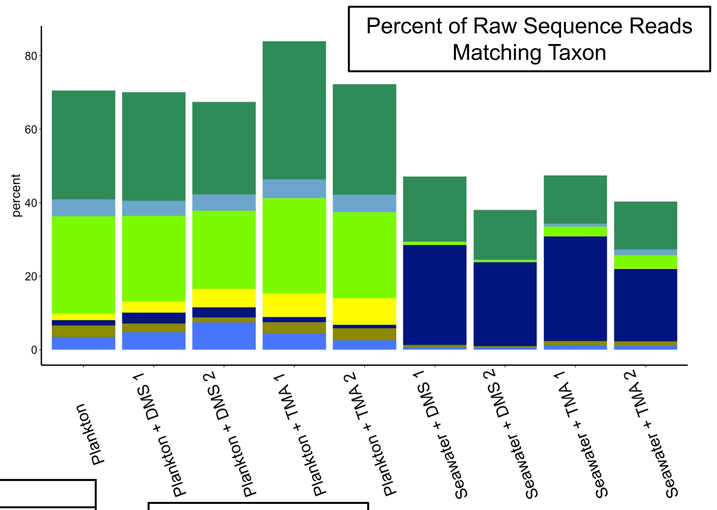


Table 1. De-replicated MAGs and Sample Location

MAG	Genus	Sample	Completeness	Contamination
1	*Methanolobus*	Plank+DMS A	82.68%	0.70
2	*Paraglaciecola*	SW+TMA A	92.43%	0.51
3	*Lentibacter*	SW+TMA A	97.19%	0.17
4	*Lacinutrix*	SW+TMA A	93.23%	0.21
5	*Nereida*	SW+TMA A	86.57%	0.80
6	*Anaerosporobacter*	SW+TMA B	80.17%	24.61
7	*Planktotalea*	SW+DMS A	85.80%	0.00
8	*Methylophaga*	SW+DMS A	96.98%	0.42
9	*Polaribacter*	SW+DMS B	95.93%	1.35
10	*Colwellia*	SW+DMS B	99.97%	2.27

Summary of Results: At the time of DNA extraction, methanogens were enriched for during plankton decomposition in surface waters of the Puget Sound as is seen by the large quantity of methane product in comparison to just seawater. At the time of DNA sampling, more methane was found in samples enriched with either DMS or TMA suggesting they act as methyl sources for methane emission. Comparing raw sequences to a database of marine organisms identifies *Methanosarcinaceae*, a known archaea methanogen, only within the plankton samples.

Conclusion: Archaea of the family *Methanosarcinaceae* might be one of the microbial groups responsible for the methane produced. The diverse set of bacteria enriched in our samples indicates that these may also play a role in supporting plankton decomposition and methanogenesis. We will use metagenomics to explore the involvement of these bacteria in methanogenesis and how this differs when TMA or DMS are present.

References

1.Weber, Thomas et al. 2019. "Global Ocean Methane Emissions Dominated by Shallow Coastal Waters." Nature Communications 10 (1): 4584.
2.Schafer et al. 2010. "Microbial Degradation of Dimethylsulphide and Related C1-Sulphur Compounds: Organisms and Pathways Controlling Fluxes of Sulphur in the Biosphere." Journal of Experimental Botany 61 (2): 315–34.
3.Zhou et al. 2021. "Microbial Drivers of Methane Emissions from Unrestored Industrial Salt Ponds." The ISME Journal 16: 284–295

Next Steps...

Genome Mapping

- Compare the raw sequence reads to the assembled MAGs to determine abundance of identified species across all samples
- Compare organism abundances across samples in attempts to identify organism involved in pathway of methanogenesis
- explore other ways to assemble MAGs to improve assemblies

Application

- Compare found organism(s) to known benthic methanogens
- Identify genes used in TMA and DMS degradation and methanogenesis
- Use identified methanogenic genes to quantify their expression in the environment.