evergreen

Master of Environmental Studies

Thesis Prospectus 2022-23

Name: Serena West

1) Working title of your thesis[1].

   Population dynamics of deep marine sediment microbial communities: indicated by active and inactive DNA

2) In 250 words or less, summarize the key background information needed to understand your research problem and question.

   Deep marine sediments act as a time capsule of the biotic and abiotic materials present on the ocean floors that slowly became buried. The environmental conditions of subsea sediments are anoxic with very little nutrients or energy available leading to very slow microbial growth rates and little diversity (Starnawski et al., 2017). Non-living DNA is also preserved in the sediment, the origins of which can be as old as sedimentation or excretions from metabolically active microbes. This non-living or extracellular DNA (eDNA) is then subject to an abiotic process known as deamination, the removal of an amino group from an amino acid, changing the structure of base pairs, for example converting cytosine to uracil (Briggs et al., 2007). The pattern and rate of deamination on eDNA can reveal when it was deposited in the sediment and if it is older or younger than the surrounding abiotic materials. This process does not occur in metabolically active microbes making it impossible to determine if that lineage is original to the time of sedimentation or ingrown from another location.

3) State your research question(s).

   What does the deamination proportion of microbial DNA isolated from deep marine sediments reveal about metabolically active and inactive microbial assemblages at different sediment depths?

4) Situate your research problem within the relevant literature. What is the theoretical and/or practical framework of your research problem?

   Deep ocean sediment is a relatively young field of research only receiving major funding 12 years ago (C-DEBI, 2021). Researchers are trying to determine the basics of microbial ecology in these sediments, including, energy sources, generation times, evolutionary pressures, etc. A task made much more difficult by location

[1] You are not locked into this title; we want you to identify the main point or topic of your thesis.

inaccessibility and the impracticality of recreating conditions in a laboratory. Ascertaining the trends of microorganisms towards metabolic stability or decay will add further understanding to sediment ecology.

5) Explain the significance of this research problem. Why is this research important? What are the potential contributions of your work? How might your work advance scholarship?

   Very little is known about the structure of deep marine sediment microbial communities and how they affect the wider global ecology (Ye et al., 2022). It is estimated that subsea sediment contains half of all the microbes in the oceans, approximately $2.9 \cdot 10^{29}$ cells (Kallmeyer et al., 2012). Additionally, eDNA makes up a massive amount of physical material in marine sediments approximately 0.45 Pg (0.45×10^{15} g) (Lennon et al., 2018). Many questions remain on the longevity of eDNA in sediment, the rate of gene transfer within living cells, and the role of eDNA as an energy source. By comparing genomic data at different sediment depths and determining the proportion of decayed DNA a greater understanding can be obtained about these communities and their impact on the wider global ecology.

6) Summarize your study design[2]. If applicable, identify the key variables in your study. What is their relationship to each other? For example, which variables are you considering as independent (explanatory) and dependent (response)?

   The bulk of the thesis will be statistical analysis of microbial taxa abundance at different sediment locations and depths, comparing paired samples of metapolicy active DNA and samples of both active & inactive DNA. This analysis will identify which microbes are active at each depth and at what depths DNA decay becomes dominant.

7) Describe the data that will be the foundation of your thesis. Will you use existing data, or gather new data (or both)? Describe the process of acquiring or collecting data[3].

   Deep ocean sediment samples were collected during several research cruises from 2013-2015. The samples were then processed to identify the bacterial and archaea taxa and abundance at different sediment depths. The data is based on microbial DNA amplification and metagenomic sequencing. No new data will be collected for this thesis.

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[2] You might discuss selection of case studies, sampling methods, experimental design, and/or specific hypotheses you will test. You should also address any specialized knowledge or skills that are necessary to complete the research.

[3] If you are planning to use existing data, explain the specific source, contact information, arrangement with collaborating agencies, and expectations about use of data and final products of your research. If you are planning to gather new data, describe specific methods, time, place, and equipment that will be required.

8) Summarize your methods of data analysis. If applicable, discuss any specific techniques, tests, or approaches that you will use to answer your research question.

In the theoretical example below is a pairwise analysis between two match samples, sample one contains only metabolically active DNA, and sample 2 contains metabolically active & inactive DNA. A larger number of reads in sample 1 indicates the microorganism is active, fewer reads indicate the DNA is degrading and not active.

	A	B	C	D	E	F	G	H	I	J	K	L
1	This is going to be an example of how to compare different sites with different numbers of sequences and different taxa											
2	**Sample 1**	**TOT**	**Taxa 1**	**Taxa 2**	**Taxa 3**	**Taxa 4**						
3	# reads	326059	13951	12108	0	300000						
4	# reads, transformed		13952	12109	1	300001						
5	rel.reads		4.28%	3.71%	0.00%	92.01%						
6												
7	**Sample 2**	**TOT**	**Taxa 1**	**Taxa 2**	**Taxa 3**	**Taxa 4**						
8	# reads	126000	0	0	28000	98000						
9	# reads, transformed		1	1	28001	98001						
10			0.00%	0.00%	22.22%	77.78%						
11												
12	Comparison:		5391.52	4679.32	0.00	1.18	Note: any number > 1, means enriched in sample 1					
13	='1/2						Note: any number < 1, means enriched in sample 2					
14												
15	Is enriched in samp1?		Yes	Yes		Yes						
16	Sample name if enriched		Taxa 1	Taxa 2		Taxa 4						
17	(shortcut)		Taxa 1									
18												
19	This is an example of a pairwise analysis (between two matched samples), using a taxonomy table.											
20	To analyze differences between multiple (>2) samples using a taxonomy table, we'd want to use ordination.											
21												

(Conversation with John Kirkpatrick, September 27, 2022)

Each sediment sample will have a corresponding taxonomy table identifying archaea and bacteria, these tables will be added as an appendix. The taxonomy tables will be generated by the Visualization and Analysis of Microbial Population Structures website (VAMPS, 2014). At each site location taxa will be compared at different sediment depths, as a proxy for sediment age. If the work is to be published, the data will be made publicly available in a free database such as that of the NCBI (National Center for Biotechnology Information)

9) Address the ethical issues[4] raised by your thesis work. Include issues such as risks to anyone involved in the research, as well as specific people or groups that might benefit from or be harmed by your thesis work, perhaps depending on your results. List any specific reviews you must complete first (e.g., Human Subjects Review or Animal Use Protocol Form).

The operation of large drilling ships carries an environmental impact, including

[4] If you're not sure where to start, consult a 'Code of Ethics' or other similar document from an academic society in an applicable field of study.

damage to the seafloor and pollution. Locations, where sediment is removed from the seafloor and the surrounding areas, will be forever altered. However, these disruptions are extremely localized and have a negatable impact on the seafloor globally.

In some cases, drilling sites are located within the borders of developing nations without robust research institutions. Extra care must be taken to ensure the full support of these nations. Also, any resulting knowledge and financial profits should benefit the nations that contributed their natural resources. However, the samples used in this study were collected from within the territorial waters of the USA.

10) List specific research permits[5] or permissions you need to obtain before you begin collecting data (e.g. landowner permissions, agency permits).

None will be needed for this thesis project.

11) Reflect on how your positionality as a researcher could affect your results and how you will account for this in the research process[6].

Humans are hardwired to see patterns even when none are there, working with noisy data increases this likelihood. Using an established methodology recognized by other researchers in this field with pre-selected p-values reduces the risk of type II errors. Also, working with a faculty advisor to determine what data is reasonable to exclude from analysis removes the risk of 'p-hacking' the results.

12) Provide at least a rough estimate of the costs associated with conducting your research, if any. Provide details about each budget item so that the breakdown of the final cost is clear.

None, the cost of collecting and processing the samples has been paid by other organizations that benefited from the research. No special equipment or software is needed to finish the analysis.

13) Provide a detailed working outline of your thesis.

I. Introduction
II. Literature Review
III. Methods
    a. Sample Locations
    b. Sample Collection & Preparation
    c. Data Selection

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[5] If you are collecting ANY samples or data, even observational data, on public lands (city, county, state and/or federal) it is your responsibility to find out the permit requirements BEFORE you collect data. Conducting research with tribal members/on tribal lands will have different and additional requirements.

[6] Your *positionality as a researcher* refers to the fact that one's "…beliefs, values systems, and moral stances are as fundamentally present and inseparable from the research process as [one]'s physical, virtual, or metaphorical presence when facilitating, participating and/or leading the research project…" (The Weingarten Blog 2017).

    d. Data Analysis
IV. Results
    a. Comparison of Each Location
        i. Figures
    b. Comparison Across Locations
        i. Figures
V. Discussion
VI. Conclusion

14) Provide a specific work plan and a timeline for each of the major tasks in the work plan. Be as realistic and specific as you can at this point, including the deadlines for Spring quarter.

- Winter Break
    - Improve literature review
- Winter Quarter
    - Week 1: Work with John K. plan for data analysis & methods
    - Week 3: Complete literature review
    - Week 5: Methods complete
    - Week 6: Complete data analysis
    - Week 7: Figures generation
    - Week 8: Complete results
    - Week 9: Complete discussion & conclusion
    - Week 10: Revision of sections as needed
- Spring Quarter
    - Week 1: Review & formatting of thesis
    - Week 2: Turn in complete draft
    - Week 4: Work trip
    - Week 5: Request to present
    - Week 6-8: rework thesis based on feedback
    - Week 9: Present thesis & turn in final draft

15) Who (if anyone), beyond your MES thesis reader, will support your thesis (in or outside of Evergreen)? Be specific about who they are and in what capacity they will support your thesis. If you are working with an outside agency or expert, be specific about their expectations for your data analysis or publication of results.

Steven D'Hondt, Professor of Oceanography at The University of Rhode Island was the principal investigator of the International Ocean Discovery Program (IODP) cruises that generated the data used in this thesis. Any publication using the data would list him as a co-author.

16) Provide the 5 most important references you have used to identify the specific questions and context of your topic, help with issues of research design and analysis, and/or provide a basis for interpretation. Annotate these references with notes on how they relate to/will be helpful for your thesis. For any other sources cited in your

prospectus in other answers, provide a complete bibliographic citation here as well.

Briggs, A. W., Stenzel, U., Johnson, P. L. F., Green, R. E., Kelso, J., Prüfer, K., Meyer, M., Krause, J., Ronan, M. T., Lachmann, M., & Pääbo, S. (2007). Patterns of damage in genomic DNA sequences from a Neandertal. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(37), 14616–14621. https://doi.org/10.1073/pnas.0704665104

C-DEBI. (2021). Resolving the extent, function, dynamics and implications of the subseafloor biosphere. Center for Dark Energy Biosphere Investigations. https://www.darkenergybiosphere.org/

Frederico, L. A., Kunkel, T. A., & Shaw, B. R. (1990). A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy. *Biochemistry*, *29*(10), 2532–2537. https://doi.org/10.1021/bi00462a015

This paper establishes that deamination of microbial DNA can be accelerated under different laboratory conditions and consistent rates of deamination calculated. Experiments were conducted on both single and double strained DNA; double stranded DNA was much more resistant to deamination and had a much lower mutation rate. Foundational to establishing deamination as a reliable method of determining mutation rates in living cells and DNA strand age.

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., & D'Hondt, S. (2012). Global distribution of microbial abundance and biomass in subseafloor sediment. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), 16213–16216. https://doi.org/10.1073/pnas.1203849109

Kirkpatrick, J. B., Walsh, E. A., & D'Hondt, S. (2019). Microbial selection and survival in subseafloor sediment. *Frontiers in Microbiology*, *10*, 956. https://doi.org/10.3389/fmicb.2019.00956

Deep ocean sediment samples were taken from the Bearing Sea and Bay of Bengal and microbial DNA isolated. Generally, as sediment depth increases the environment becomes anoxic with less available energy greatly reducing the metabolic activities of microbes. Sediment depth also correlates to time, the deeper the sample the older it is, in this case samples ranged from 660 to 1,300,000 years in age. The focus of the paper was to determine the bacterial and archaeal populations as depth increased at each location. From that data trends in microbial selection and population dynamics can be determined. A comparison of trends from both locations was conducted to determine the universality or lack thereof of microbe populations on opposite side of the globe.

Lennon, J. T., Muscarella, M. E., Placella, S. A., & Lehmkuhl, B. K. (2018). How, when, and where relic DNA affects microbial diversity. *MBio*, *9*(3). https://doi.org/10.1128/mBio.00637-18

Starnawski, P., Bataillon, T., Ettema, T. J. G., Jochum, L. M., Schreiber, L., Chen, X., Lever, M. A., Polz, M. F., Jørgensen, B. B., Schramm, A., & Kjeldsen, K. U. (2017). Microbial community assembly and evolution in subseafloor sediment. *Proceedings of the National*

Academy of Sciences of the United States of America, *114*(11), 2940–2945. https://doi.org/10.1073/pnas.1614190114

Subsea sediment samples were taken from 5 locations in Aarhus Bay, Denmark to determine the microbial assemblages at increasing depths. From these samples four separate taxonomic lineages were identified as persisting throughout the samples from near surface to deep sediments, representing 8,700 years of time. Single cell DNA amplifications and sequencing was conducted on each of the four taxonomic lineages at two separate depths, near surface and deep. By comparing base pair changes over time, it was found that very few changes had occurred in the DNA sequences, indicating microbial survival rather than growth and adaptation.

Torti, A., Lever, M. A., & Jørgensen, B. B. (2015). Origin, dynamics, and implications of extracellular DNA pools in marine sediments. *Marine Genomics*, *24 Pt 3*, 185–196. https://doi.org/10.1016/j.margen.2015.08.007

This review identifies the sources of both extracellular DNA (eDNA) and intracellular DNA (iDNA) in marine sediments, how they interact, techniques for study, and the need for future research. iDNA consists of DNA contained within an intact cellular envelope either metabolically active (alive) or inactive (dead), revealing the structure of the living microbial community in a sample. eDNA is everything else, free DNA found in the environment, in water, bond to minerals, or organic matter. The sources of eDNA range from DNA recently excreted from living cells, the remainder of an organism that died after sedimentation, or terrestrial DNA deposited by marine snow before burial. Once eDNA has been sampled and sequenced abiotic degradation can be measured and the age of DNA estimated, providing a record of organisms and potential environmental conditions go back hundreds of thousands of years.

VAMPS. (2014). Mbl.edu. https://vamps2.mbl.edu/

Ye, M., Zhang, Z., Sun, M., & Shi, Y. (2022). Dynamics, gene transfer, and ecological function of intracellular and extracellular DNA in environmental microbiome. *IMeta*, *1*(3). https://doi.org/10.1002/imt2.34

A summary of the importance of extracellular DNA (eDNA) and it's roles in gene flow, biofilm formation, nutrient cycling, and environmental interactions. Marine sediment eDNA makes up the planets largest genetic revisor, understanding how eDNA and intracellular DNA (iDNA) interact with each other and the environment can provide incites in to past environmental conditions an future prospects.