eDNA metabarcoding measures biodiversity in Kachemak Bay

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Introduction

Environmental DNA (eDNA) is an emerging science that allows researchers to examine biodiversity by looking at the DNA found in samples of water, soil, sediment, etc. For this project, I used an eDNA sampling scheme to look at biodiversity in five different bays within Kachemak Bay (Fig. 1). Each site has a different level of glaciation, ranging from 0 – 60% glacial coverage, and I was interested to see how that affects the biodiversity of coastal species.

Methods

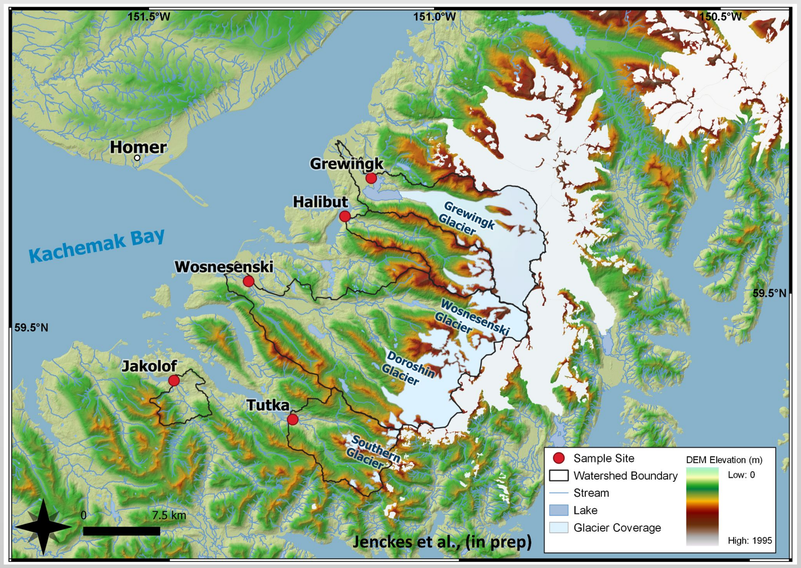
Field Methods: During September 2022, I collected four, 1L bottles of water for eDNA sampling, corresponding with beach seining, at each of the five sites (Fig. 1). I also collected 1 negative control (drinking water) from each site.

Field Lab Methods: Using sanitized equipment, the 1L bottles of water were filtered on a .45 µm filter. The filter was then stored in ethanol, frozen, and shipped to Fairbanks.

Genetics Lab Methods: Using sanitized equipment, I extracted DNA from each filter. The filter was cut up, placed in a lysis solution, incubated, and extracted using the Qiagen DNeasy kit to isolate the DNA.

Sequencing Methods: Samples were sent to the company Jonah Ventures for two rounds of PCR and to sequence the Co1 barcoding gene region (Leray et al. 2013). Jonah Ventures did one PCR replicate for this project, and samples were sequenced on an Illumina NovaSeq 6000. For bioinformatics, raw sequenced data was demultiplexed using *pheniqs* v2.1.0 (Galanti et al. 2021), enforcing strict matching of sample barcode indices. Counts of the resulting exact sequence variants (ESVs) were compiled using uchime3. We kept ESVs with a >90% match and performed a Principal Component Analysis.

Figure 1: Map of Kachemak Bay sampling sites. The sites, Grewingk, Wosnesenski, Jakalof, Halibut, and Tutka all have different levels of glacial input, ranging from 0-60%.



Results

- In total, using an 90% accurate match rate for ESVs, we detected 44 species representing 23 phyla and 54 genera.
- For phyla of interest (arthropods, vertebrates, cnidarians and molluscs), we detected 7 species and 8 genera (Table 1).
- Grewingk, the most glaciated site, had no detections for taxa of interest.
- The PCA of all 23 phyla distinguished Tutka from the remaining sites.
- For the least glaciated sites, Tutka contained several Cnidarians (jellyfishes) whereas Jakalof was distinguished by Mollusca (molluscs).

Table 1. Presence and absence of taxa by site for the following phyla: Arthropoda, Chordata, Cnidaria, and Mollusca

Phylum	Class	Order	Family	Genus	Species	hal_4	hal_3	hal_1	tut_4	tut_3	tut_2	tut_1	wos_3	jak_4	jak_3	jak_2	jak_1
Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	*Pseudocalanus*	*Pseudocalanus newmani*	0	8	8	0	0	0	0	0	0	0	0	0
Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	*Pseudocalanus*	*Pseudocalanus newmani*	9	0	0	0	0	0	0	0	0	0	0	0
Chordata	Actinopteri	Salmoniformes	Salmonidae	*Salvelinus*	*Salvelinus namaycush*	0	0	0	0	0	0	0	31	0	0	0	0
Chordata	Actinopteri	Salmoniformes	Salmonidae	*Oncorhynchus*	*Oncorhynchus gorbuscha*	0	0	0	0	0	16	0	0	0	0	0	0
Cnidaria	Hydrozoa	Limnomedusae	Olindiidae	*Craspedacusta*	*Craspedacusta sowerbii*	0	0	0	53	0	0	0	0	0	0	0	0
Cnidaria	Scyphozoa	Semaeostomeae	Ulmaridae	*Aurelia*		0	0	0	0	13	0	8	0	0	0	0	0
Cnidaria	Hydrozoa	Leptothecata	Clytiidae	*Clytia*	*Clytia gregaria*	0	0	0	0	0	13	0	0	0	0	0	0
Cnidaria	Hydrozoa	Leptothecata	Clytiidae	*Clytia*	*Clytia gregaria*	0	0	0	0	0	11	0	0	0	0	0	0
Cnidaria	Scyphozoa	Semaeostomeae	Cyaneidae	*Cyanea*		9	0	0	0	0	0	0	0	0	0	0	0
Cnidaria	Scyphozoa	Semaeostomeae	Cyaneidae	*Cyanea*		0	8	0	0	0	0	0	0	0	0	0	0
Mollusca	Bivalvia	Cardiida	Tellinidae	*Macoma*	*Macoma nasuta*	0	0	0	0	0	0	0	0	0	0	24	0
Mollusca	Bivalvia	Mytilida	Mytilidae	*Mytilus*	*Mytilus trossulus*	0	0	0	0	0	0	0	0	9	9	0	0
Mollusca	Bivalvia	Mytilida	Mytilidae	*Mytilus*	*Mytilus trossulus*	0	0	0	0	0	0	12	0	0	56	36	49

Objectives

My objectives were to use eDNA metabarcoding to:

1) Conduct a presence/absence analysis of species at each site
2) Relate diversity estimates to environmental factors (e.g., glaciation)
3) Learn new laboratory techniques and practice with data analysis (Fig. 2)

Figure 2. A) Extracting DNA in the lab by cutting up the filters and adding a lysis buffer before using the Qiagen Kit.

B) Collecting eDNA samples in Kachemak Bay required wading out into ice cold water and taking 1L water samples by hand.

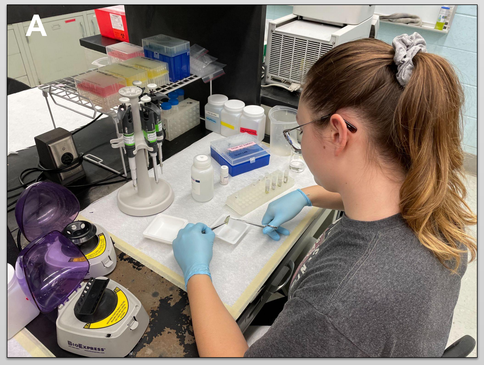
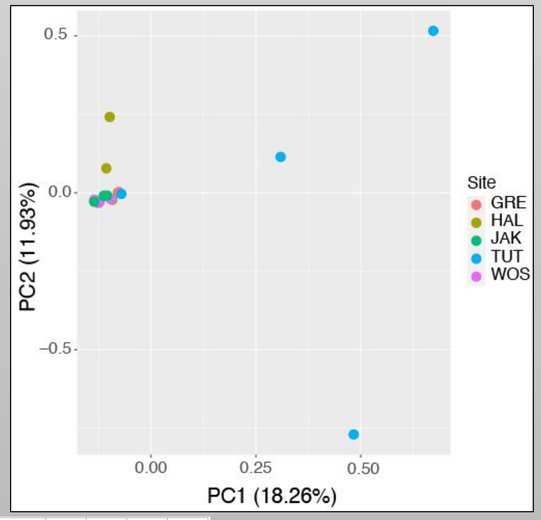


Figure 3. Principal component analysis showing similarities between sites: GRE (Grewingk), HAL (Halibut), JAK (Jakalof), TUT (Tutka) and WOS (Wosnesenski). Tutka is distinguished from the remaining sites.



Future Work

I plan to look at samples from additional sampling months and years (2021 and 2022) to get an estimate of how species composition changes across year and seasons.

Acknowledgements

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References: (1) Leray et al. 2013. Front Zool 10:34. (2) Galanti et al. 2021. BMC Bioinformatics 22.