Identifying the Factors that Influence the Detection of Benthic Macroinvertebrates using eDNA in the Subarctic

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Background

- Caddisflies (Trichoptera) are often used as indicator species because they are sensitive to pollution during their aquatic larval stage (Medeiros et al. *Arctic*. 2011).
- qPCR is more sensitive to low concentrations of DNA but what affects eDNA detection is still relatively unclear (Goldberg et al. *Methods Ecol. Evol.* 2016).

Figure 1: *Philarctus bergrothi* with their typical mineral-based case

Study System

- Churchill, Manitoba's fauna has been extensively barcoded so it's an ideal system for investigating the potential of eDNA analysis for arthropods.
- We targeted a particularly widespread and abundant species (*Philarctus bergrothi*).
- 24 bluff ponds were sampled based on historical data on the target species' distribution.
- Bluff ponds are naturally occurring mesocosm along the coast that are fed by rainwater, snowmelt and spray from the bay.

Figure 2: Examples of bluff ponds that were sampled in Churchill.

Figure 3: A picture of the laboratory setup that allowed us to obtain qPCR results in the field. To the left is the pump and manifold where the water samples were filtered, then a space where extractions were performed using the Biomeme extraction kit, our minimal PCR reaction setup station and finally the Open qPCR, a 16 well qPCR machine that is very portable.

Methods

eDNA Sampling: 250mL surface water samples were first collected at 4 extremities of each pond before being filtered together using a pump and manifold with 5µm cellulose-nitrate filters back at the research station. Water samples were kept on ice and processed within a couple hours. Filters were extracted using the Biomeme extraction kit and the DNA was kept frozen until analysis.

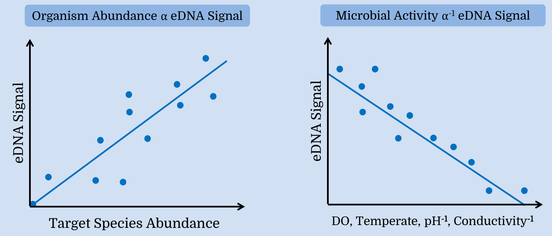
Water Chemistry: Using a YSI water probe, we gathered data on temperature, conductivity, pH and dissolved oxygen (DO).

Traditional Sampling: Caddisflies were sampled using a combination of kick-netting and rock-washing (Boyle and Adamowicz. *PLoS ONE*. 2015) . Up to 20 individuals from each morphospecies were collected but in most cases abundances were lower than that.

qPCR Analysis: The resulting eDNA extract was analyzed on an Open qPCR machine using custom species-specific primer and probe sets. We were thus able to obtain presence/absence data at a field station shortly after sampling. By comparing the proportion of positive qPCR replicates between samples and considering the variation in water chemistry and abundance of target organisms between ponds, we can determine which factors most affect the detection of macroinvertebrates using eDNA in this environment.

qPCR Assay Design: a 148 bp region of the COI gene of *P. bergrothi* was used to develop a novel, geographic-specific probe-based qPCR assay.

Hypotheses and Predictions



- We hypothesize that one of the main mechanisms that hinders eDNA detection is **microbial activity** (Strickler et al. *Biol. Conserv.* 2015) and therefore eDNA signal will become weaker as water conditions become more favourable for microbial life (**DO and temperature increase/pH and conductivity decrease**).
- Also, we expect to see signal increase with the abundance of the target species in the ecosystem and are interested in estimating the **effect of density** on this relationship.

Preliminary Data

- Preliminary data suggests a negative relationship between the amount of dissolved oxygen in a pond and eDNA signal.
- They also suggest that the relationship between signal and target species abundance and density may not be as straightforward as thought. This may be due to the ecology of the target organisms (benthic macroinvertebrates) and the methods of eDNA sampling (surface samples).

Implications and Next Steps

- With more data we are excited to see which factors impact detection via eDNA the most.
- We are also contemplating quantifying the amount of recovered eDNA and seeing the relationship between this and the abiotic factors of interest.
- Making our own lab-based mesocosm experiment would be an interesting way to control for one parameter at a time.
- Comparing the detection rates of benthic and pelagic macroinvertebrates would also be ideal in order to make more general conclusions from the results of this study.

Conclusion

- The successful development of **standardized**, **validated** eDNA assays will enhance our ability to survey Arctic biodiversity, detect **invasive species**, and track **shifts in distribution** caused by climate change.
- eDNA research can tremendously alter how we perform arthropod surveys, and could result in research that is **cheaper**, more **sensitive**, **accessible** to people without taxa-specific identification skills and increase our knowledge of poorly studied species.

Figure 4: Kamil Chatila-Amos sampling one of the bluff ponds in this study.

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