What state is environmental DNA found and does that affect detection rates in the North American river otter?

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Background

- Threatened and endangered species are challenging to manage due to difficulties associated with sampling small populations.
  - Results in uncertainty when attempting to conserve populations[1].
- Animals in all populations release trace amounts of DNA known as environmental DNA (eDNA) [2].
- eDNA sampling methods can be used to improve conservation management efforts by improving sampling efficiency[2].
  - eDNA is used to detect mammals, fishes, and amphibians[3].
  - For example, eDNA has been used to characterize spatial trends in keynote species like the sea otters (*Enhydra lutris*) that are vital to the health of kelp forest ecosystems[4].
- However, much about the 'ecology' (i.e. the origin, state, fate, and transport) of eDNA is poorly understood[5].
  - Relative concentrations between intracellular and extracellular DNA (i.e., the state of eDNA) are currently unknown.
    - Based on the microbial community literature, the state of eDNA may vary by several orders of magnitude depending on sampling location[6,7].
  - Species detections may be affected by the state of eDNA.
  - Studying the ecology of eDNA can be used to optimize sampling efforts.
- North American river otters (*Lontra canadensis*) currently have a stable population (Figure 1).
  - Exhibits many of the same behaviors and uses similar habitats as the endangered species (e.g., sea otter).
  - eDNA assay has already been developed[8] and was studied as part of Ms. Waloven's Capstone research project in the Fall of 2019.
  - Could be used as a model to study the ecology of eDNA of aquatic mammals to improve detection rates and sampling methodology.

Objectives

- Determine the state (intracellular or extracellular) at which eDNA is collected in water samples.
  - Quantify the relative concentration of intracellular to extracellular DNA extracted from water DNA samples collected.
- Determine whether eDNA collection state affects detection rates.
  - Compare the detection rate (via end-point and quantitative Polymerase Chain Reaction (PCR) assays for the river otter using intracellular and extracellular DNA extracts per sample).

Figure 2. Study Map of sampling locations throughout Rice Creek Pond and Stream.

Figure 1. North American river otter
https://www.animalsportal.net/wp-content/uploads/2016/09/North-American-river-otter.jpg

General Methods

- Collected 15 mL samples (n = 25) from each of the following locations at Rice Creek Field Station (Figure 2)
  - Locations consist of sampling upstream, downstream, and near a known river otter den.
- Centrifuged samples at 5,000 g for 10 minutes to pellet animal cells.
- Decanted supernatant (containing extracellular DNA) into another 15 mL tube, precipitated external DNA, and centrifuged samples at 14,000 g for 20 minutes.
- DNA concentration ([DNA]) and quality quantified with NanoDrop.
- Double stranded [DNA] quantified with PicoGreen assay.
- Converted published qPCR assay[8] to end-point PCR.
  - Final volumes for PCRs was 20 µl and consisted of 1X Gold PCR Buffer, 2 mM $MgCl_2$, 0.24 mM dNTPs, 1 µg/µl BSA, 0.3 mM each primer, and 1.25 U of Dream TAQ polymerase.
- Test PCR conductions using known river otter, fisher (*Martes pennanti*), and marten (*Martes americana*) DNA samples.

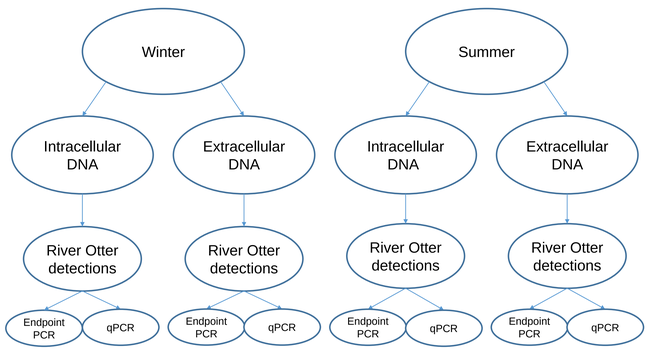

Figure 4. Schematic depiction of summer plan and the statistical comparisons we would be calculating.

Preliminary Results

- No eDNA differential centrifugation protocol currently published.
- We created a protocol to separate intracellular and extracellular DNA components of water samples.
- Used eDNA from a captive spotted turtle (*Clemmys guttata*) named Jerry to develop protocol.
- Used 15 mL water samples to test variations of the protocol now described in general methods.
- Method is successful at extracting high quality eDNA (Table 1).

Table 1: DNA concentration ([DNA] – ng/µl) results from eDNA samples collected from Jerry's Tank.

Sample	[DNA]	A260/A280
Jerry 1	78.2	2.18
Jerry 2	173.1	2.09

- The *in silico* analysis suggested published assay would produce a 147 base pairs PCR product with river otter eDNA.
  - Also, suggested that fishers eDNA would not amplify, but marten eDNA may amplify.
- The *in vitro* analysis confirmed amplification of non-target species (Figure 3).

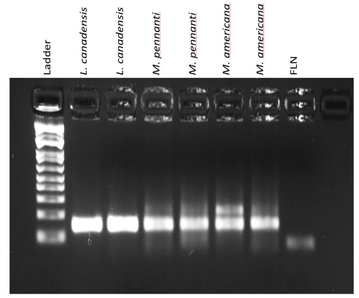

Figure 3. eDNA assay shows evidence of non-specific amplification.

Future Work

- Ms. Waloven submitted a proposal to the Rice Creek Small Grants Association to continue this project this summer (Figure 4).
  - Intends to expand sampling to capture summer eDNA variation.
- To reduce non-specific amplification, PCR conditions will be altered.

Acknowledgments

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