Testing and refinement of eDNA methodology to support ecological assessment within the St. Louis River esturary

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Overview

Environmental DNA (eDNA) is rapidly and easily collected and potentially yields high organism detectability but understanding of the collection mechanics and utility for specific environmental assessments is still evolving. During the summer of 2020, we conducted two eDNA-related sampling efforts aimed at characterizing species assemblages via metabarcoding within the SLR

Survey 1 – eDNA method optimization for assessing fish communities *efforts described below*

Survey 2 – evaluate utility of eDNA data in establishing pre & post-restoration biological conditions at St. Louis River AOC sites *efforts described below*

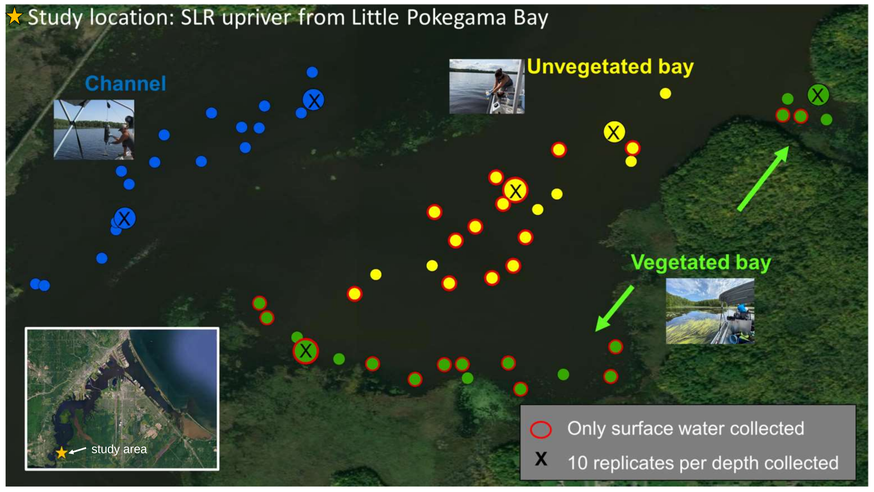
1) Method optimization for assessing fish communities

Factors related to water and fish movement may impact the density and distribution patterns of fish eDNA. Accounting for these factors in sampling designs may improve effectiveness and efficiency. We conducted a pilot study in late August 2020 to better understand how and where to sample eDNA to improve species detection.

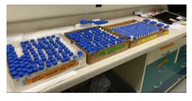
Research questions:

- How does species detection and eDNA quantity vary between habitats?
- How does spatial resolution (sampling density) in different habitats affect species detection?
- How does increasing sample replication affect detection in different habitats or water strata?
- How does species detection vary between surface and bottom water samples?

- Sampling done in one sub-region of SLR – upriver from Little Pokegama Bay to minimize spatial gradients in fish assemblages present in the study area – range of water depths, vegetation cover, little habitat disturbance
- Contrast 3 habitats of equal area (100,000 m^2) with 20 sites each for consistent site density between habitats
- Sites randomly assigned to habitats using a probabilistic design



Samples filtered within 16 hrs of collection, Filters frozen at -80C then dried and sent to sequencing lab (USEPA Cincinnati, OH) for amplicon library prep and metabarcoding



Total samples collected = 352

- **Sampling methods**
- All surface water collected by hand or with pole grab
- < 2m depth (veg & unvegetated) – Bottom water/pole grab
- > 2m depth (channel) - Niskin deployed 0.5m above benthos
- Hydrolab water quality measurements (0.5m below surface & above benthos)
- 3 replicates were collected at all sampled depths except for two sites/habitat where we increased replication to 10 reps per depth

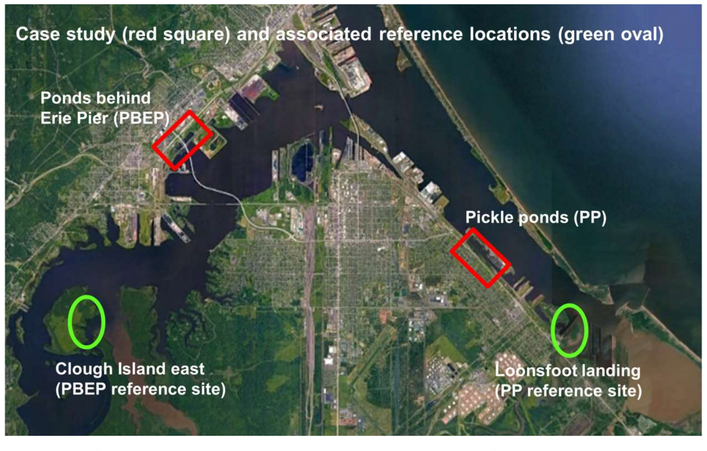
What's next? Comparisons will be made using DNA concentration and species richness data from metabarcoded samples. Results will help inform general eDNA sampling for a broad range of applications, including early detection monitoring for invasive fish

2) Remedy and restoration effectiveness assessment

Understanding conditions both before and after remediation and restoration is essential for assessing project outcomes. Research to improve methods for the assessment of outcomes is being conducted at two project locations in the SLR AOC.

Collecting water for eDNA metabarcoding to assess biological communities including fish & macroinvertebrates is one of many metrics being used to evaluate the outcomes and effectiveness of remediation and restoration projects

(see companion poster by Peterson et al. titled "Remedy Effectiveness and Restoration Effectiveness at Erie Pier Ponds and Pickle Ponds in the SLR AOC" for in-depth look at additional metrics used in this research).



5 sites sampled for eDNA at each location 2x per year

total samples collected in 2020 = 120

Pre-restoration assessment conducted for PBEP & reference in 2018, 2019, 2020; PP & reference sampled in 2020 (Both case study locations slated for restoration summer 2021)

Sampling methods

- Sample within 1 week prior to deploying and retrieving passive colonization gear (early Aug. & late Sept. 2020)
- Surface water grab, 3 replicates/site
- Sample processing & sequencing methods same as above survey

What's next? Fish and invertebrate composition data resulting from metabarcoding eDNA samples will be compared to data obtained from direct organism collections to evaluate if eDNA has the necessary specificity to resolve differences between impacted and reference sites

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