Building a Molecular Algal Classification Index for Stream Assessments in New Hampshire

Alison Watts UNH, David Neils NHDES, Devin Thomas, Aaron Kearnan and many others!
Can molecular analysis support biotic assessments in streams?

New Hampshire DES Pilot for determining the possibility of using eDNA as a response indicator to stressors in rivers and streams.
Methods being developed in Europe, and even EPA

**Taxonomy eDNA bio**

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**Development of High-Throughput DNA Sequencing Techniques to Improve and Advance Environmental Monitoring and Bioassessment**

Scientists can learn about the health of rivers, streams, lakes, and other aquatic ecosystems by looking at the species that live in them—the populations of insects, snails, and worms that can be found in different aquatic ecosystems can indicate the overall health of the areas they live in. However, finding and evaluating these species can be difficult, costly, and time-consuming, resulting in delays in decisions related to the protection of these ecosystems.

Currently, biological monitoring programs for rivers, streams and other aquatic areas focus primarily on identifying aquatic macroinvertebrates such as insects, snails and worms. These efforts can take 6 to 12 months. However, because these aquatic organisms leave molecular-level DNA 'footprints' in the environments where they live, scientists can use molecular methods, such as DNA barcoding, to obtain reliable information about the species in a given area. DNA barcoding uses short genetic markers in an organism's DNA to identify it as belonging to a particular species. It is also possible to use these DNA-based techniques to accurately identify much smaller aquatic organisms, such as algae and bacteria.
What is Metabarcoding?

‘Barcoding’ amplifies a very short section of DNA, and matches it to a database.

Only works if that short section of DNA is present in the organism.

Much cheaper, much less detail than full genomics.
What is eDNA?

Environmental DNA – collected from an environmental sample (water, air, biomass, etc)
That’s nifty, what use is it?

Identifying (macro) species in the vicinity from trace DNA
-Fish, animals, insects etc.

Distinguishing (micro) species present in a mixed sample
-Larval tows, algae, invertebrates
Sampling: Summer 2019

Sites selected from NHDES Trend sites based on:

- Nutrient levels
- Geographic distribution
- Substrate and depth (wadeable, rocky)

30 streams in New Hampshire & (southern) Maine sampled
- 60 water samples
- 40 composite algae samples
Sampling Algae

Algae collection methods adapted from Maine DEP

Thank you Tom Danielson!

18 rocks, 1in circle scrubbed, frozen until extracted
Sampling Water

Water eDNA collection methods adapted from USDA

2x1 liter samples, filtered onsite

Samples collected for nutrients - sent to NHDES

pH, T, DO, Conductivity measured onsite
Extraction & Analysis:

Filters (water)
- 12S Fish
- 18S broad eukaryote

Biomass
- 18S broad eukaryote
- rbcl (plants)

Sample extracts are stored (-80°C) can be rerun for other parameters.
Identifying species sequence variants

Several thousand unique sequences per sample:

CATCACTGCTGGTAATATGGACGAAGTTATCAAACGTGCAGACTATGCTAAAGCAGTAGGTTCTGTAATCGTTATGCTGATTTA
GTTATGGGTTACACAGGAATTCAAAGTGCAGCTATTTTGGGCTCGTGAAAACGATATGATTTTACATTACACCGTGCAGTTAACT
CACTTACGCGCGCTAAAAATCATGGTATCAACTTCCCGTGTATCTGTAATGGATGGGCGTATTGCTCTTGGTAGATCATATCCAC
GCTGGTA

CATTACTGCTGTACTATGGAAAGAAGTGTACAAACGTGCAGAATACGCTAAAACAATTGGTTCTGTAATCGTTATGATCGATTTA
GTTATGGGTTACACAGCAATTCAAAGTATTGCTCTTTGGTCTCGTGACAACGACATGATTTTACACTTACACCGTGCTGGTAACTC
TACTTACGCTCAAAAGAATCATGGTATTAACTTCCGTGTTATCTGTAAATGGATGCGTATGTCTGGTGTAGACCACATCCACGCTG
GTA

Eukaryota;Euglenozoa;Euglenida;Euglenales;Euglenaceae;Euglena. Euglena archaeoplastidiata;__
Eukaryota;Rhodophyta;Compsopogonophyceae;Compsopogonales;Boldia erythrosiphon;__;__
Eukaryota;Rhodophyta;Compsopogonophyceae;Erythropeltidales;Madagascaria erythrocladioides;__;__
Eukaryota;Rhodophyta;Florideophyceae;Nemaliophycidae;Virescentia helminthosa;__;__
Eukaryota;Rhodophyta;Florideophyceae;Nemaliophycidae;__;__;__;__
Eukaryota;Rhodophyta;Florideophyceae;Rhodymeniophycidae;Gayliella dawsonii;__;__
Eukaryota;Rhodophyta;Florideophyceae;Rhodymeniophycidae;Hydropuntia cornea;__;__
Eukaryota;Rhodophyta;Florideophyceae;Rhodymeniophycidae;Hypnea cornuta;__;__
Eukaryota;Rhodophyta;Florideophyceae;Rhodymeniophycidae;Tsengia pulchra;__;__
Eukaryota;Rhodophyta;Florideophyceae;Rhodymeniophycidae;__;__;__;__
Eukaryota;Rhodophyta;Florideophyceae;__;__;__;__
Eukaryota;Stramenopiles;Bacillariophyta;Bacillariophyceae;Achnanthidium digitatum;__
We use the taxonomic units to look for relationships between stressors and:

- Phylogenetic diversity (Faith)
- Community (Unweighted UniFrac) Includes rough phylogeny, not counts

Does the biodiversity change when stressors change?
Does the algae community change when stressors are present?
Are there specific sequences that can be used as indicators of stream condition?
### Analysis - Algae

Starting with indicators already used by NH DES river Monitoring Network

#### Table 1. River Monitoring Network water quality condition indicators.

<table>
<thead>
<tr>
<th>Indicator Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Conductance</td>
<td>A measurement of the water’s ability to conduct electricity. Compounds such as road salts, fertilizers and other chemical compounds increase the specific conductance of water.</td>
</tr>
<tr>
<td>pH</td>
<td>A measure of the water’s acidity. In addition to natural processes, the pH of surface water is affected by the precipitation of acidic compounds, such as sulfuric or nitric acid, released into the atmosphere as a result of industrial processes.</td>
</tr>
<tr>
<td>Nutrients (Phosphorus and Nitrogen)</td>
<td>The concentration of compounds that facilitate plant and animal growth. Some nutrients are deposited in surface water via atmospheric deposition, however, an excessive build up is often a result of improper use of fertilizers, human waste products, and improper agriculture practices.</td>
</tr>
<tr>
<td>Biological Condition (Macroinvertebrates)</td>
<td>An estimate of the composition and diversity of the aquatic community. Aquatic communities, such as macroinvertebrates, are expected to balanced and adapted to natural conditions.</td>
</tr>
<tr>
<td>Biological Condition (Dissolved Oxygen)</td>
<td>The concentration of oxygen in water used by plants and animals. Low or highly variable dissolved oxygen concentrations can result from excessive biological activity such as decomposition of organic material.</td>
</tr>
<tr>
<td>Water Temperature</td>
<td>Aquatic communities are adapted to specific water temperature conditions. Water temperatures are affected by the amount vegetation that shades surface waters, industrial discharges, upstream damming, and global climate patterns.</td>
</tr>
</tbody>
</table>
## Analysis – Algae/biomass

Starting with indicators already used by NH DES river Monitoring Network

<table>
<thead>
<tr>
<th>Indicator Parameter</th>
<th>Bio 18S</th>
<th>Bio rbcl</th>
<th>Water 18S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Conductance</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrients - TP</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrients - TN</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-IBI</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Temperature</td>
<td>N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Algae rbcl water temp

Phylogenetic Diversity vs Total phosphorus (TP) category

- High
- Med
- Low
Nutrients - Total Phosphorus

Phylogenetic Diversity

Nutrients - Total Phosphorus

Algae biomass – rbcl primer

Total phosphorus category (5-55 ug/l)
Nutrients - Total Phosphorus

Unweighted UniFRac analysis
Algae biomass – rbcl primer
Nutrients - Total Nitrogen

Phylogenetic Diversity

Total Nitrogen category (150-800 ug/l)

Algae biomass – 18S primer
Nutrients - Total Nitrogen

Unweighted UniFRac analysis
Algae biomass – 18S primer, rbcl is similar
Benthic IBI

Algae biomass – 18S primer
Benthic IBI

Unweighted UniFRac analysis
Algae biomass – 18S primer
Next steps – Linear Response Models

Model: relative abundances ~ Multinomial(alr^{-1}(Total Phosphorus category * weights))
Baseline: relative abundances ~ weights*1
Implemented by Songbird

We have a tiny bit of predictive power for the selected training samples.
Identifying (macro) species

Fish in NH streams
41 Fish species, 27 other animals

Example: Souhegan River, Milford, NH (09-SHG) ~1L of Water

- Freshwater Fish Species Detected
  - American Eel
  - Atlantic Salmon
  - Blacknose Dace
  - Bluegill Sunfish
  - Brook Trout
  - Brown Bullhead
  - Chain Pickerel
  - Golden Shiner
  - Longnose Dace
  - Longnose Sucker
  - Pumpkinseed Sunfish
  - Rainbow Trout

- Other Detections
  - Redbreast Sunfish
  - White Sucker
  - Yellow Bullhead
  - Yellow Perch
  - American Beaver
  - Mallard
  - Muskrat
  - Racoon
  - Red-Spotted Newt
  - Two-Lined Salamander
  - Wood Duck
## Identifying (fish) species

<table>
<thead>
<tr>
<th>eDNA (water) species - 2019</th>
<th>Electrofishing species 2014-2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-CLD 070919-1</td>
<td>9/8/2014</td>
</tr>
<tr>
<td><strong>American beaver, blacknose dace, bluegill sunfish, brook trout, common merganser, creek chub, longnose dace, longnose sucker, rainbow trout, robin, slimy sculpin, sp. shiner, two-lined salamander, white sucker, white-tailed deer, yellow perch</strong></td>
<td>Blacknose Dace, Creek Chub, Longnose Dace, Rainbow Trout, Slimy Sculpin</td>
</tr>
<tr>
<td>06-EBS 081519-1</td>
<td>9/16/2015</td>
</tr>
<tr>
<td>blacknose dace, slimy sculpin</td>
<td>Blacknose Dace, Brown Trout, Eastern Brook Trout, Fallfish, Slimy Sculpin</td>
</tr>
<tr>
<td>15-EXT 062519-1</td>
<td>10/13/2016, 6/18/99</td>
</tr>
<tr>
<td>Rock Bass, American eel, bluegill sunfish, brown bullhead, chain pickerel, creek chubsucker, golden shiner, <strong>gray squirrel, mallard ducks, pumpkinseedsunfish, rainbow trout, redbreast sunfish, sp. shiner, white sucker, yellow bullhead, yellow perch</strong></td>
<td>American Eel, Bluegill, Brown Trout, Common Shiner, Common White Sucker, Eastern Chain Pickerel, Fallfish, Redbreasted Sunfish, Sea Lamprey</td>
</tr>
<tr>
<td>01-MSC 071119-1</td>
<td>8/25/2015</td>
</tr>
<tr>
<td><strong>Rock Bass, American beaver, blacknose dace, bluegill sunfish, brook trout, brown bullhead, common merganser, creek chub, longnose dace, robin, sea trout, slimy sculpin, smallmouth blackbass, striped shiner, white sucker</strong></td>
<td>Blacknose Dace, Common Shiner, Common White Sucker, Creek Chub, Eastern Brook Trout, Fallfish, Longnose Dace, Rock Bass, Smallmouth Bass, Tessellated Darter</td>
</tr>
</tbody>
</table>
Fish community by cold water classification

Phylogenetic Diversity

Cold water | Transitional | Warm

Fish Community Characterization

Water samples – 12S fish primer
Do sites have characteristic sequences in water samples???
Can molecular analysis support biotic assessments in streams?

Does the biodiversity change when stressors change? Yes – for categories

Does the algae community change when stressors are present? Yes – for categories

Can we build predictive models from sequence data? Probably – working on it!
Questions?

Thank you to our partners:
UNH Hubbard Center for Genome studies: Kelley Thomas, Devin Thomas, Jeff Hall, Jessica Haskins, Aaron Kearnon

NH Department of Environmental Services: David Neils
Maine Department of Environmental Protection: Tom Danielson
Algae rbcl TKN