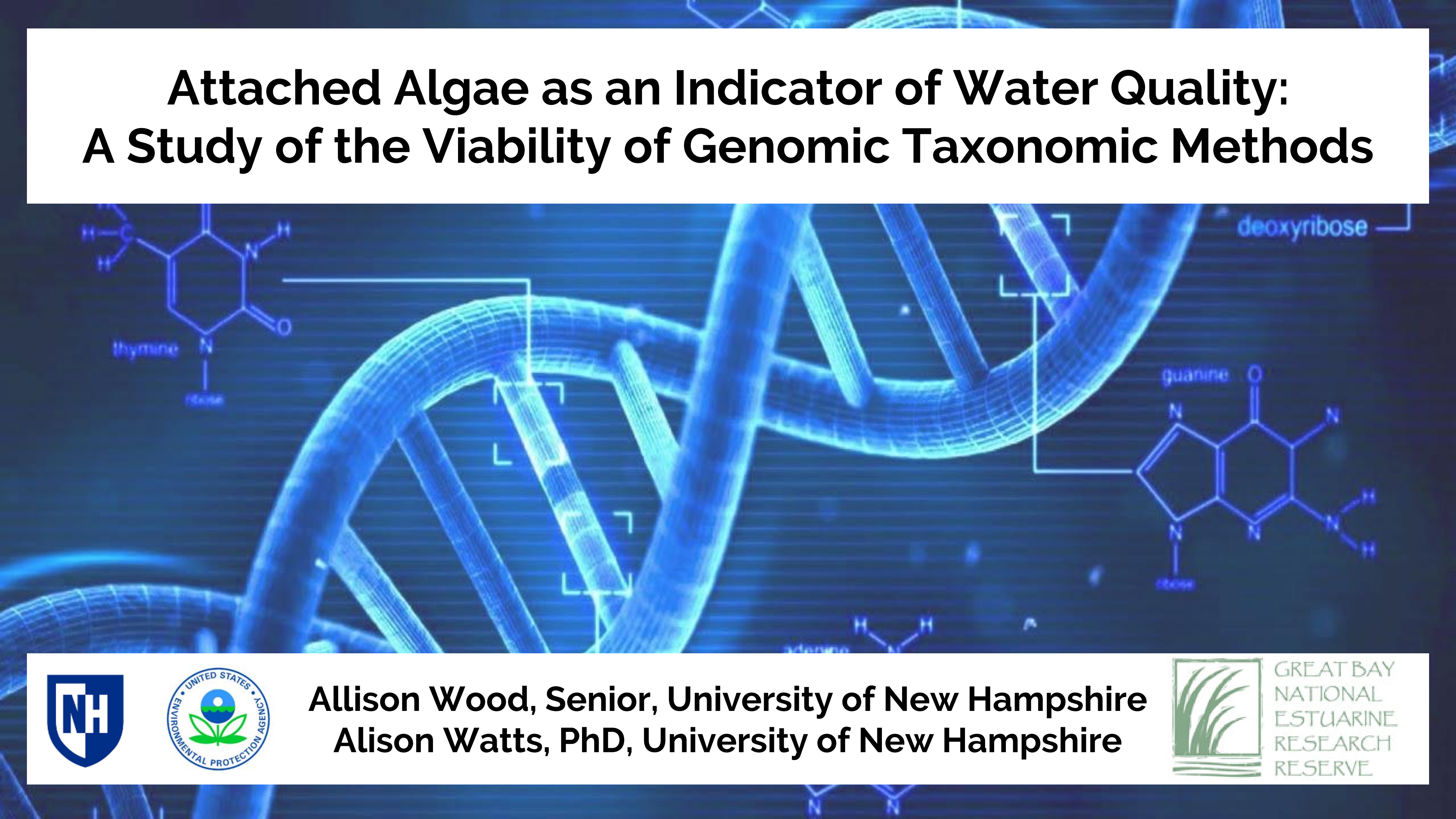
Attached Algae as an Indicator of Water Quality: A Study of the Viability of Genomic Taxonomic Methods



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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

GREAT BAY
NATIONAL
ESTUARINE
RESEARCH
RESERVE

Special Thanks:

- Paul Stacey, Great Bay National Estuarine Research Reserve
- Jeff Hall, Kelley Thomas and the Hubbard Genome Center
- US EPA Greater Research Opportunities Fellowship Program

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Hubbard Center for Genome Studies
Linking Genomics to Organisms and the Environment

EPA United States
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Outline:

- Project Goals
- Background
- Sampling Methodology
- Data Analysis & Results
- Next Steps

Project Goals

#1 Verify that algae constitute a viable ecosystem indicator for Great Bay

#2 Compare traditional microscopic methods to genomic analysis

- Microscope **$450/sample** (100-1,000 species)
- Genomic **$50/sample** (100,000 species)

#3 Further explore genomic methods

Why Algae?

- Sensitive to nutrient enrichment
- Short life cycles, react quickly
  - Variety of species
  - Population density
- Estuarine & freshwater
- Easy to collect

Dr. Ruth Patrick 1973

http://www.stroudcenter.org/newsletters/2013/issue6/img/ruth-patrick.jpg

http://www.microscopy-uk.org.uk/mag/imagsmall/netrium.jpg

hhttp://static01.nyt.com/images/2013/09/24/us/patrick-obit2/patrick-obit2-master675.jpg

Current Applications:

United States Geological Survey (USGS)

science for a changing world

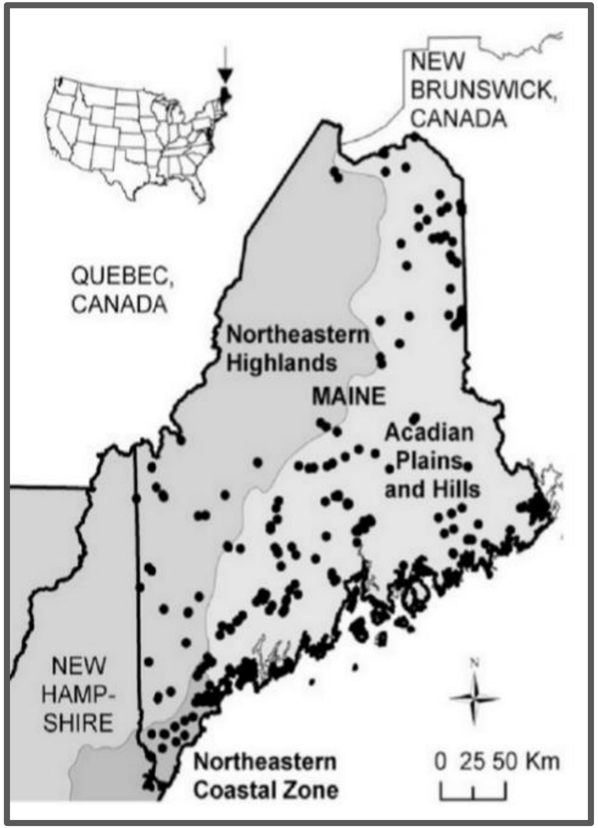
- Porter et al., 2008
- National database of algal - metric indicators
    - Including regional indicators
- Categories include:

  Trophic condition, Salinity, Chloride, Organic enrichment, pH, Calcium, etc.

Current Applications:

State monitoring programs

- Maine Dept. of Environmental Protection
  - Biomonitoring Program
  - Developed model for assessment using specific indicators for Maine ecosystems with success
  - TP, TN, % Impervious cover, etc.

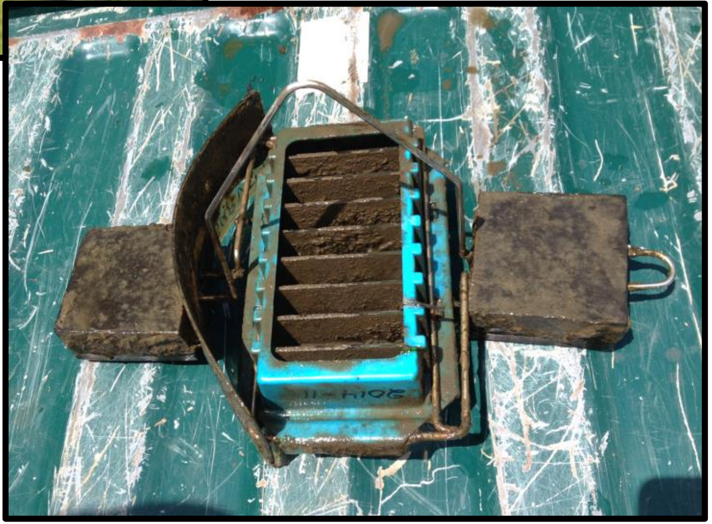


Danielson et al., 2011

Sampling Method

- Periphytometer
- Controlled for substrate, time, light, flow, depth
- Glass slides submerged for 2-week intervals

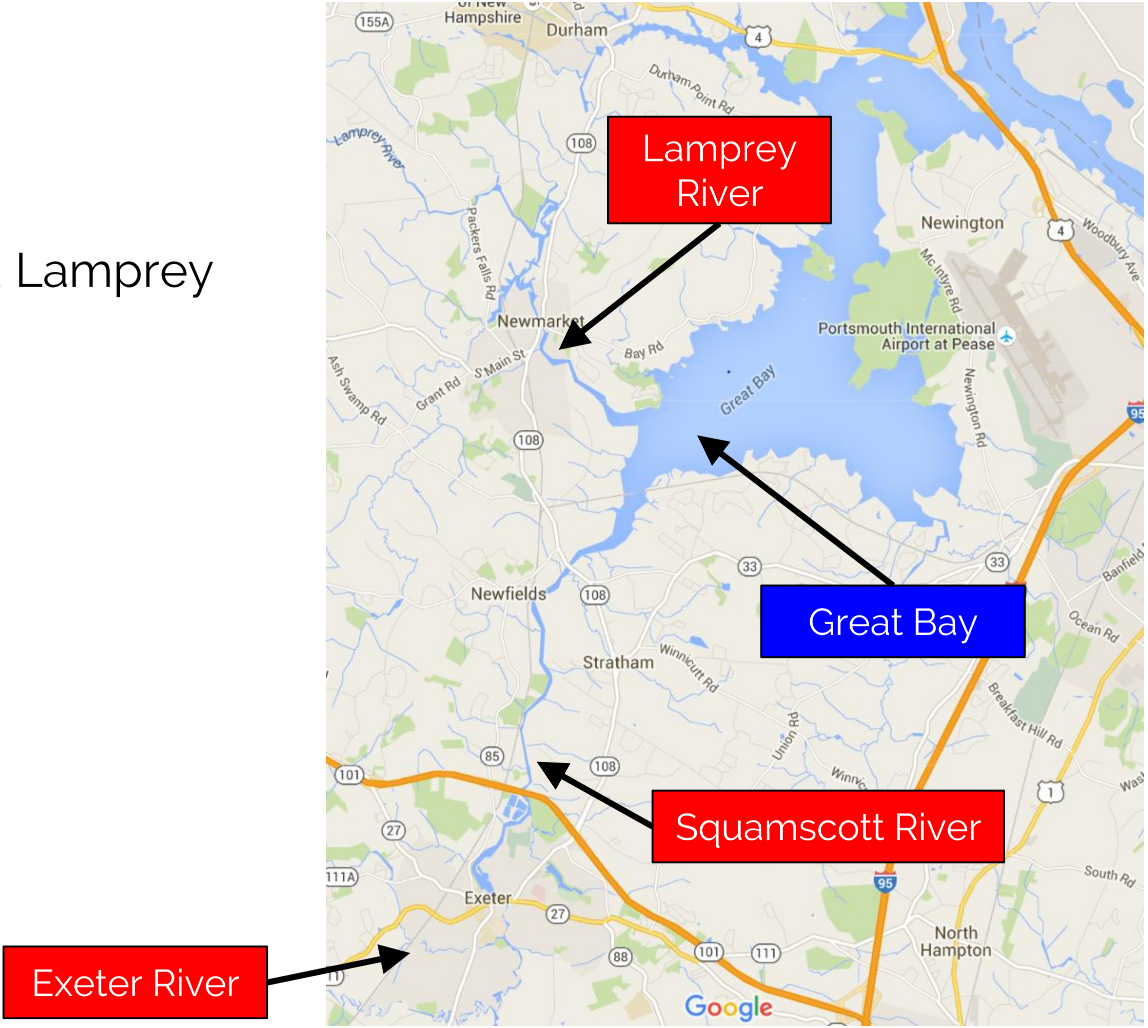
Maine DEP:
Surface Collection



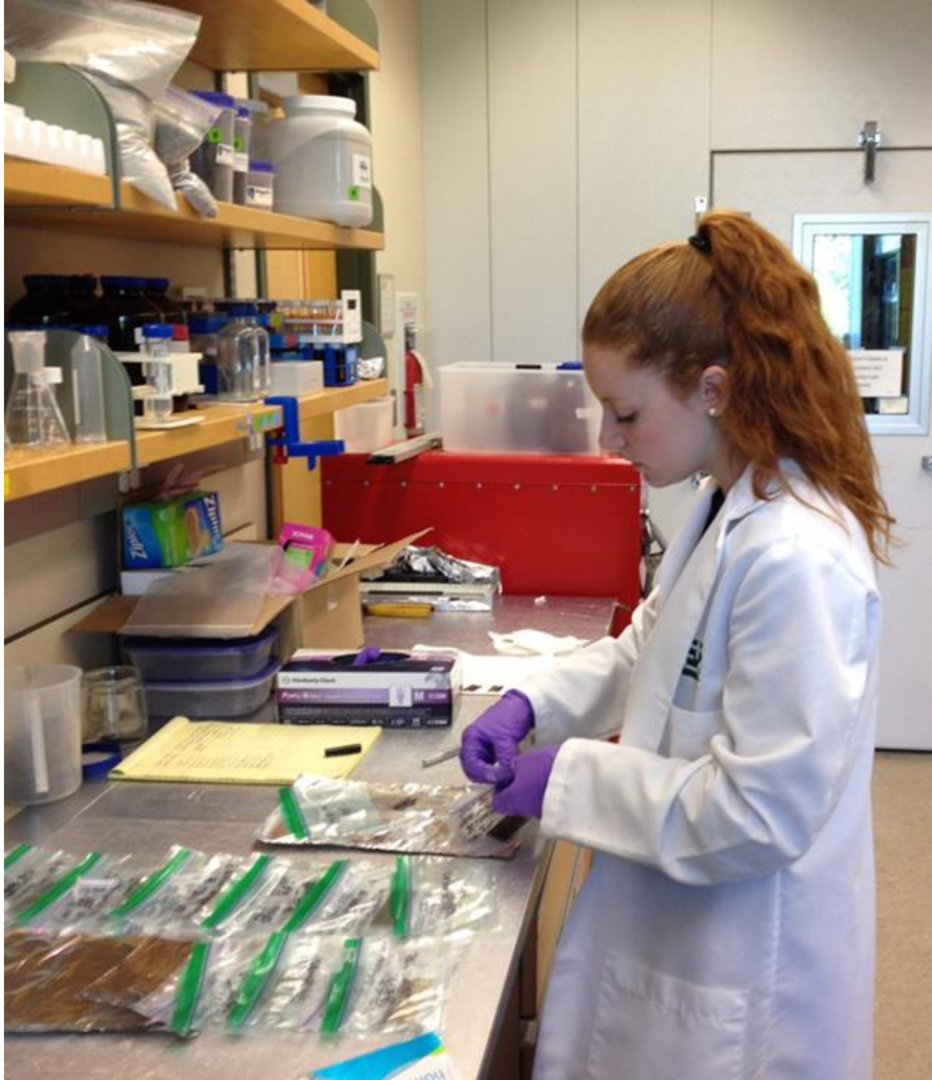
Great Bay:
Periphytometer

Sample Sites

- Exeter, Squamscott and Lamprey Rivers; Great Bay
  - Attached algae
  - Water chemistry
  - Nutrient content



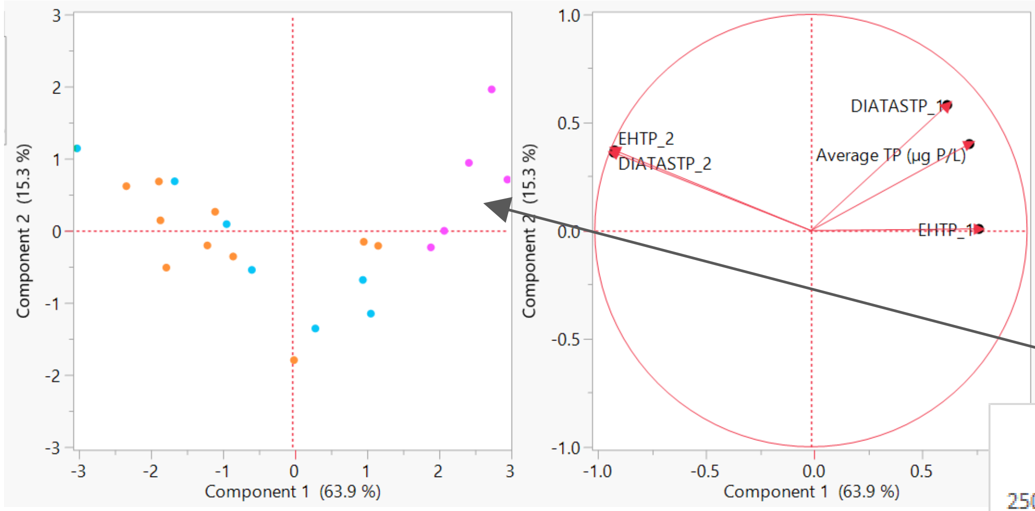
ANALYSIS



Goal #1: Traditional Microscopic Analysis

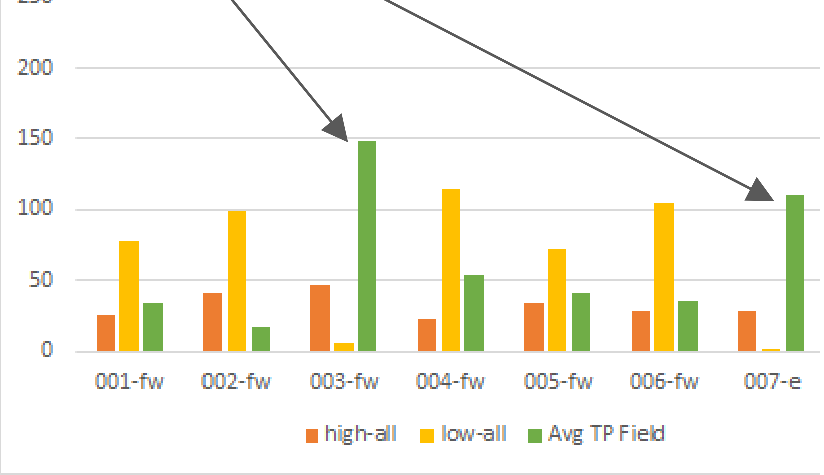
- Algae were identified and % abundance of each species was obtained from each sample site
- Porter et. al, USGS, 2009
  - 64 Algae classifications (mostly diatoms
  - Species classified by attributes;
  - Nutrients, pH, salinity, etc.
- Analyzed in combination with site data
- Principal components analysis in JMP

http://clipartfreefor.com/cliparts/microscope-clip-art/cliparti1_microscope-clip-art_03.jpg



Sites 3 & 7 had **HIGH** Phosphorus Levels

Phophorus Indicators



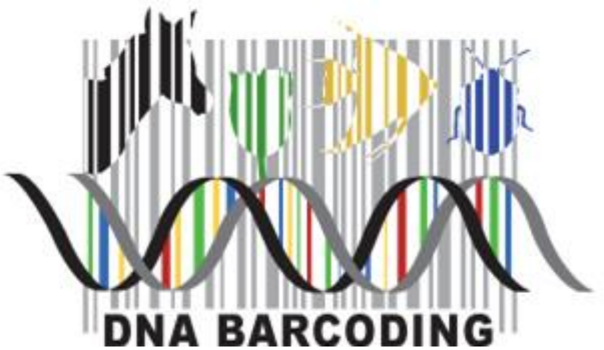
Principal components analysis in JMP

Pink sites had greater than 75 µg/L TP

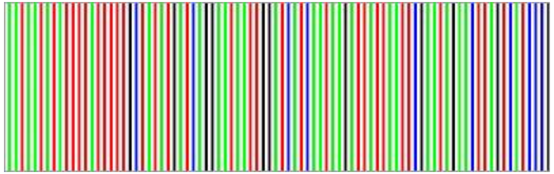
Goal # 2: Genomic Analysis Comparison

- **Microscope methods**
  - Searching for known target
- **Genomic methods**
  - Can identify hundreds of thousands of individual organisms, even in small samples
  - Unless we can match that DNA/RNA to a known database, can't identify species
- **Full DNA sequencing**
  - Technology available, very expensive / time consuming
- **Barcoding**
  - Identifies species based on only a small segment of DNA sequence

Method: *Next-Generation Sequencing*



http://www.dnabarcoding101.org/images/dnabarcoding101_logo.jpg



http://www.nata.com.au/nata/images/e-news/march2014/dna-barcode.jpg

- Short pieces of DNA/RNA are washed across a flow cell with selected primers
- Those that stick are amplified, forming clusters
- Strands are tagged with indicators one base at a time
- Indicators are hit with a laser, activating a corresponding fluorescent color and read with a camera
- Produces millions of highly accurate reads

Challenges thus far (Goal # 2)

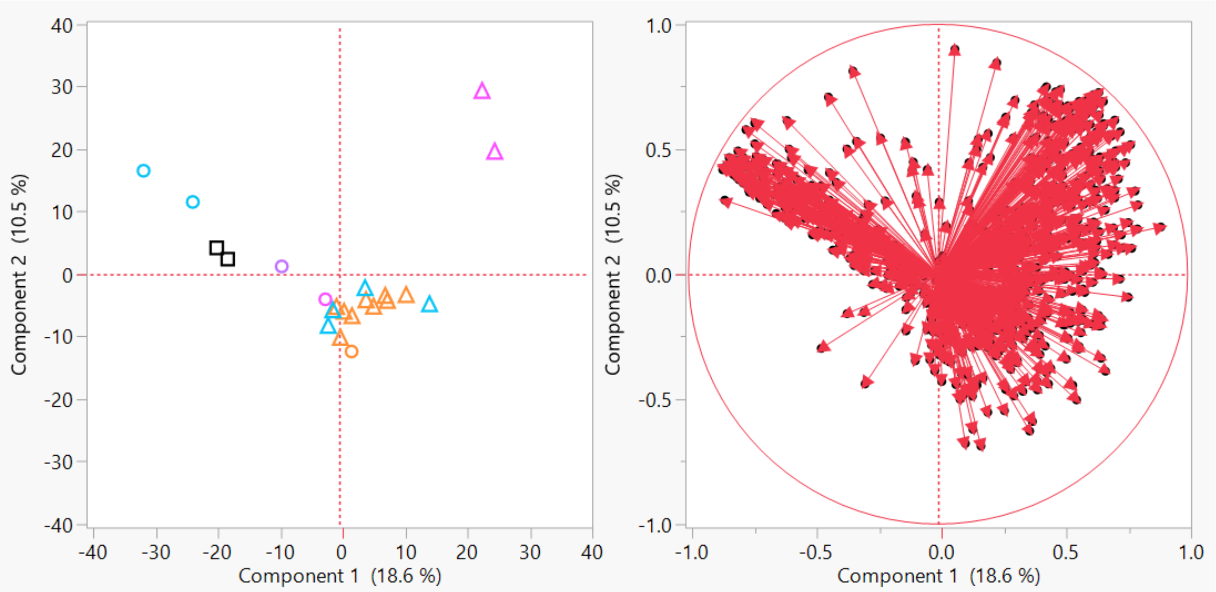
- Algae database are incomplete
- Results not as specific as microscope
  - Unable to resolve most taxa past “family”, we need “species” to relate to indicators
- Not sure if database issue or method issue
  - DNA/RNA segment might not provide enough detail
- **NO DIRECT COMPARISON POSSIBLE CURRENTLY**
- *BUT we have thousands of other microorganisms, such as **BACTERIA**…*

Goal # 3: Further explore genomic analysis

- Using the genomic data
  - Trying to identify other potential indicators from the microorganism information we do have
  - Such as BACTERIA

https://i.ytimg.com/vi/Eghle1k14S8/maxresdefault.jpg

Bacterial Results



- Pink triangles are both upstream sites w/ very high Phosphorus
- Blue circles are sites downstream in the tidal rivers
- Black squares are sites in Great Bay

Next Steps

- Research
  - Determine if there are additional reference database for algae samples/decide whether to build our own
  - Identify additional indicator species from genomic data
- Regulation
  - Determine if this method holds value for NH stream monitoring programs
    - Early indication of changes in water quality
    - Targeted management efforts

Questions?

http://www.fws.gov/uploadedImages/Region_5/NWRS/North_Zone/Great_Bay_Complex/Great_Bay/Images/StationPhoto2.jpg