UNIVERSITY OF SASKATCHEWAN

Environmental DNA: A novel approach in support of environmental monitoring

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Surveying/Monitoring of Aquatic Organisms and Communities

- **Purpose**
  - ✓ Increased understanding of structure and functions of ecosystems
  - ✓ Evaluate species establishment (invasive, native, endangered)
  - ✓ Inform environmental management actions (e.g. Environmental Effects Monitoring [EEM])

https://response.restoration.noaa.gov

https://slideplayer.com/slide/5735080/

Surveying/Monitoring of Aquatic Organisms and Communities

- **Current approach**
  - ✓ Monitoring through field surveys by physical collection and visual confirmation of organisms

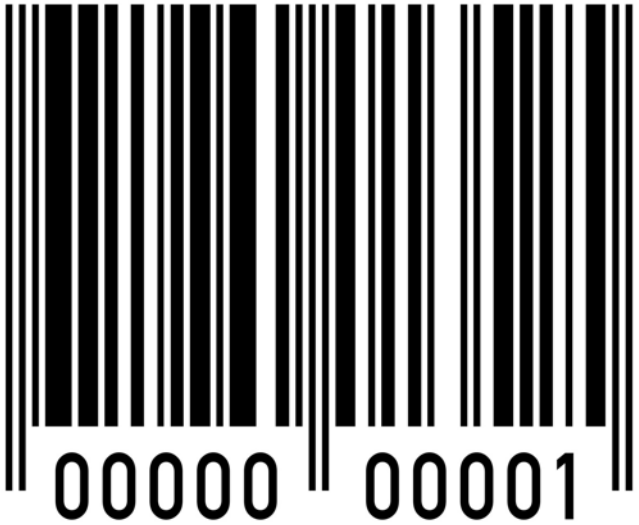
http://www.elr.ca/index.php/fisheries-and-aquatics

Current Monitoring Methods - Issues

- Taxonomic misidentification (requires expert taxonomists)
- Expensive, time consuming, labor intensive
- Observer bias
- Bias in collection methods
- Rare and endangered species
- Invasive/influence on species or ecosystem

What is eDNA?

http://fishbio.com/field-notes/conservation/traces-left-behind



- All organisms leave DNA behind in their environment
- DNA is unique to each organisms -> compare to a fingerprint or barcode
- This DNA can be extracted from an environmental matrix (e.g. water, sediment) and then identified in the laboratory

eDNA Analyses

- DNA is extracted from environmental samples without first isolating/identifying the organisms or their parts (e.g. Ogram et al. 1987; Lodgeet al. 2012; Taberlet et al. 2012)





Spectrum of size & integrity

Small/degraded → Large/intact

Free DNA molecules
- Microbes: extra-cellular DNA
- Macrobes: extra-organellar
  extra-nuclear
  extra-cellular DNA

Whole living organisms
- Microbes: bacteria
- Macrobes: larvae/pollen







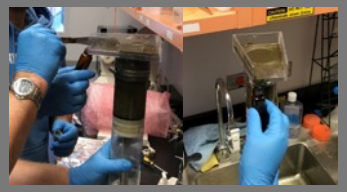

eDNA Analyses

Environmental sampling

eDNA Extraction

Water

Sediment



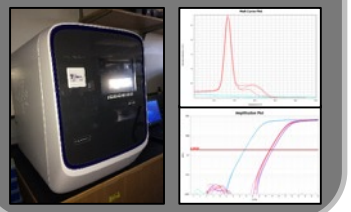
eDNA Analysis

How does eDNA analysis work?

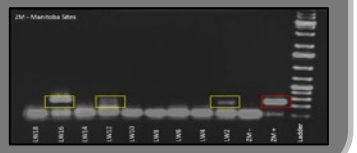
- Two approaches

1. Targeted eDNA analysis using quantitative Polymerase Chain Reaction (qPCR)
   - ✓ Used for individual species such as endangered or invasive species
   - ✓ Small targeted assemblies of organisms
   - ✓ Need to design specific primers for each organism
2. eDNA barcoding/metabarcoding using sequence-by-synthesis analysis (RNAseq)
   - ✓ Used for whole communities
   - ✓ Non-target screening (shotgun approach)
   - ✓ Requires advanced bioinformatics capacities and databases that contain sufficient genetic information on the communities of interest

qPCR



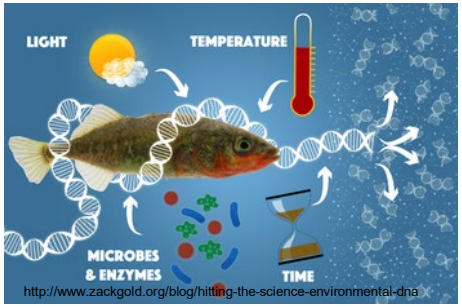
Confirmation

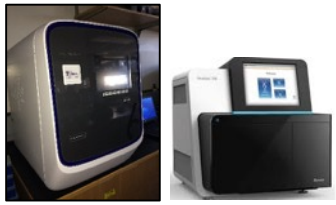


Next generation sequencing

Bioinformatics

Processes Affecting eDNA Detection





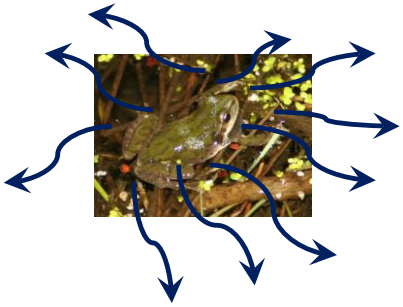
Production

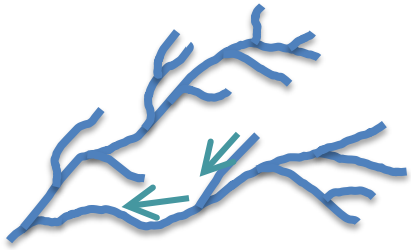
Degradation

Diffusion/Transport

Detection

Contamination





Objectives of the project

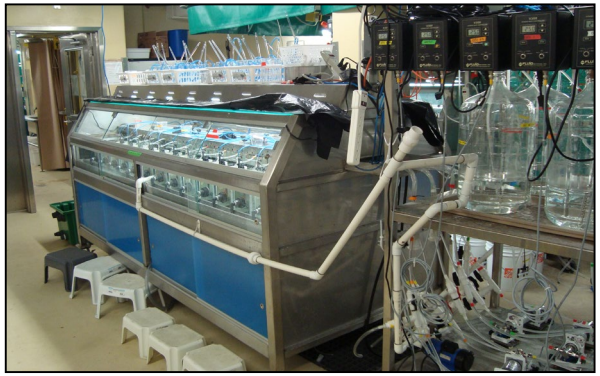
- Adapt and optimize eDNA approaches to:
  - **a)** **Assess absolute and relative changes in populations and communities** in aquatic environments of northern and urbanized Canadian watersheds
  - b) Characterize these communities in surface waters and sediments as **indicators of ecosystem health** under varying degrees of natural and anthropogenic stressors
  - **c)** **Identify invasive, endangered and rare species**

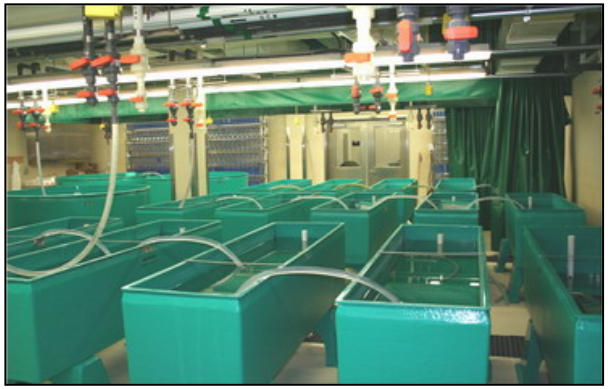
Approach

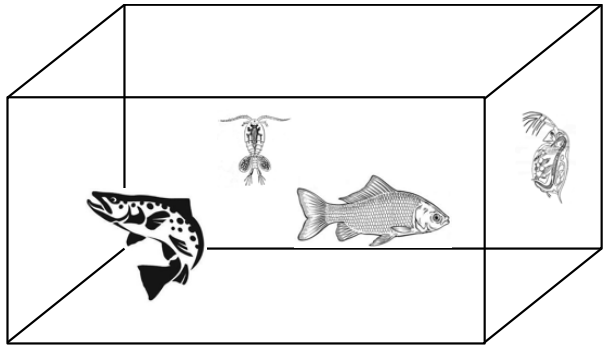
1. Calibrate eDNA approach using controlled laboratory studies
2. Conduct mesocosm studies at the Experimental Lakes Area (dilbit and Selenomethionine limnocoral studies) to test methods under controlled conditions in the field
3. Apply eDNA methods to support field monitoring efforts

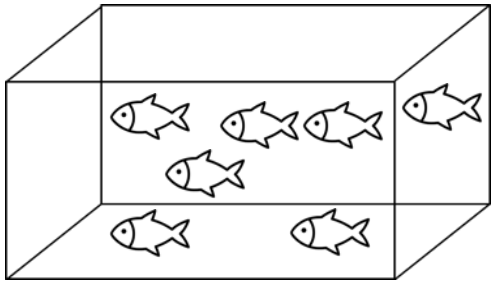
Approach

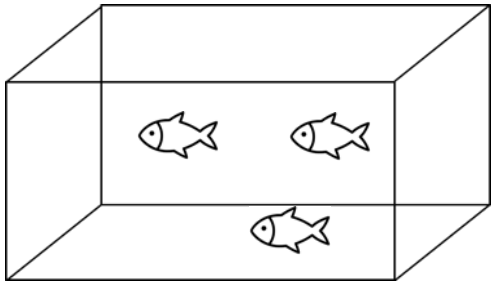
1. Calibrate eDNA approach using controlled laboratory studies
   - ✓ Mock communities of vertebrates and invertebrates
   - ✓ Test sensitivity of method

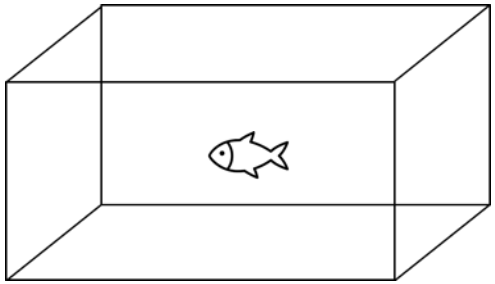












Approach

2. Conduct mesocosm studies at the Experimental Lakes Area (dilbit and Selenomethionine limnocoral studies) to test methods under controlled conditions in the field
   - ✓ Confirm microbial communities estimated by eDNA method with parallel traditional taxonomic assessments
   - ✓ Identify the utility of different eDNA methods to estimate shifts in aquatic community due to exposure to stressors

Approach

3. Apply eDNA methods to support field monitoring efforts
   - ✓ Parallel sampling of water and sediment samples during Orano's EEM monitoring campaigns to compare eDNA outcomes to taxonomic assessments of local fish and invertebrate communities
   - ✓ Joint sampling efforts in the Grand River (ON) for fish community monitoring (McMaster U)

https://www.canada.ca

Anticipated Outcomes/Benefits

- Reduce field survey time/costs
- Have little or no impact on ecosystems (non-invasive)
- Species identification from DNA sequences is often easier and more accurate than identification by observation of external morphology
- Variety of aquatic species may be detected from a single sample of Water or Sediment

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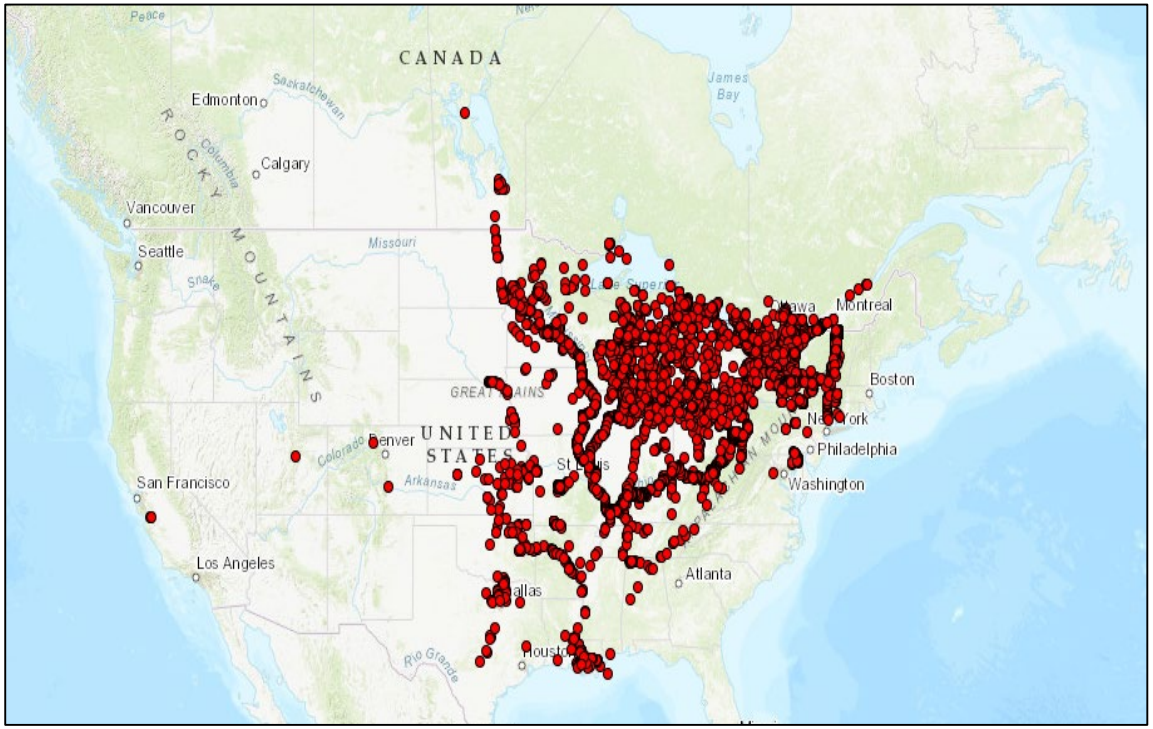
eDNA as an Early Detection Tool for the Potential Spread of Zebra Mussels (*Dreissena polymorpha*) to Saskatchewan Lakes.

Jenna Zee, Allyson Gerhart, Jon Doering, Timothy Jardine, Markus Hecker

Spread of Zebra Mussels (*Dreissena polymorpha*)

- First detected in Lake Eerie, ON in 1986
- Established in Lake Winnipeg, MB by 2013
- 2015 a single veliger was found in Cedar Lake, MB threatening the Saskatchewan River basin



Map of Zebra Mussel Invasion as of 2017
https://nas.er.usgs.gov/queries/SpeciesAnimatedMap.aspx?speciesID=5

Ecological and Economical Impact of Zebra Mussel Invasion

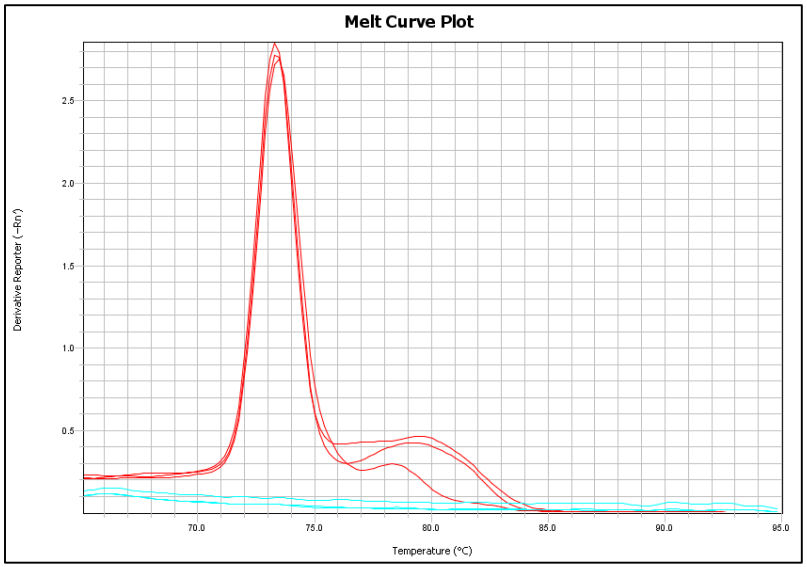
- Cost Ontario $75-91 million/year
- Mandatory boat check stops in Alberta and BC
- Drastically alter the ecosystem
  a) Rapid filter feeding
  b) Mat like colonies
- Current detection methods include seine netting for veligers or physical sighting of adult mussels
- eDNA could be a useful early detection technique

Positive Control Sampling – Lake Winnipeg

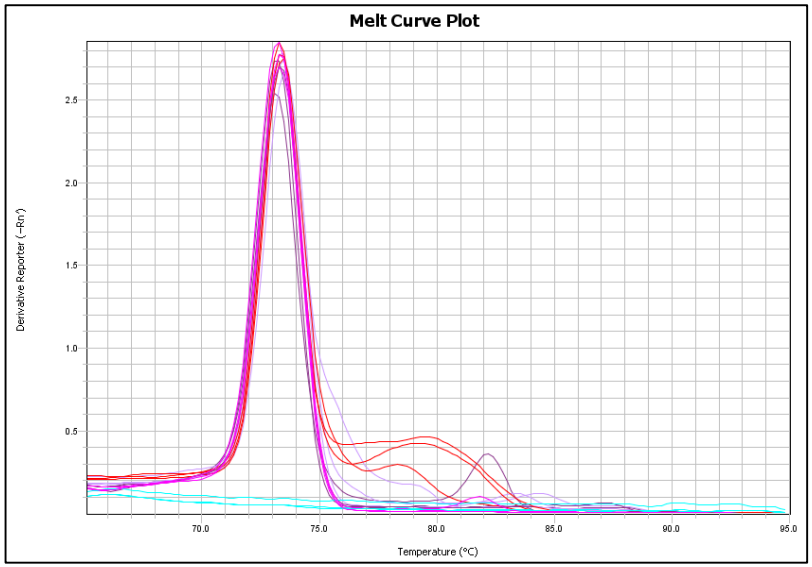
1. Collected 500 mL water
   a) 3 reps per site
2. Sterile 0.45 um, cellulose nitrate filters
   a) Snap freeze on dry ice of store in ethanol
3. Isolate DNA from filter
4. qPCR analysis
   a) Previously published primers for the Cytochrome oxidase subunit 1 (CO1) gene
   b) Zebra mussel tissue positive control
5. Follow up electrophoresis and sequencing if necessary

Results – qPCR Detection

Controls



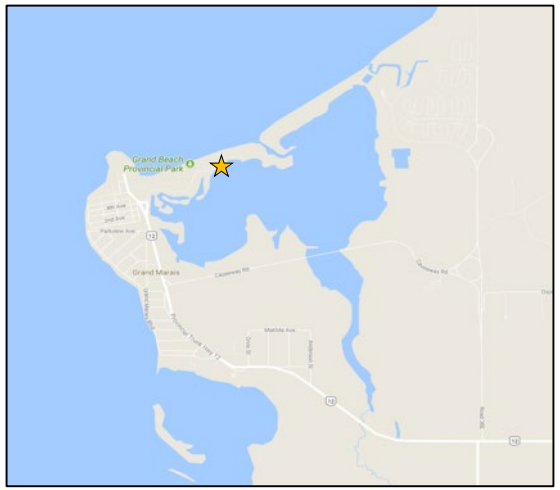
Controls + Hnausa Dock "hit"

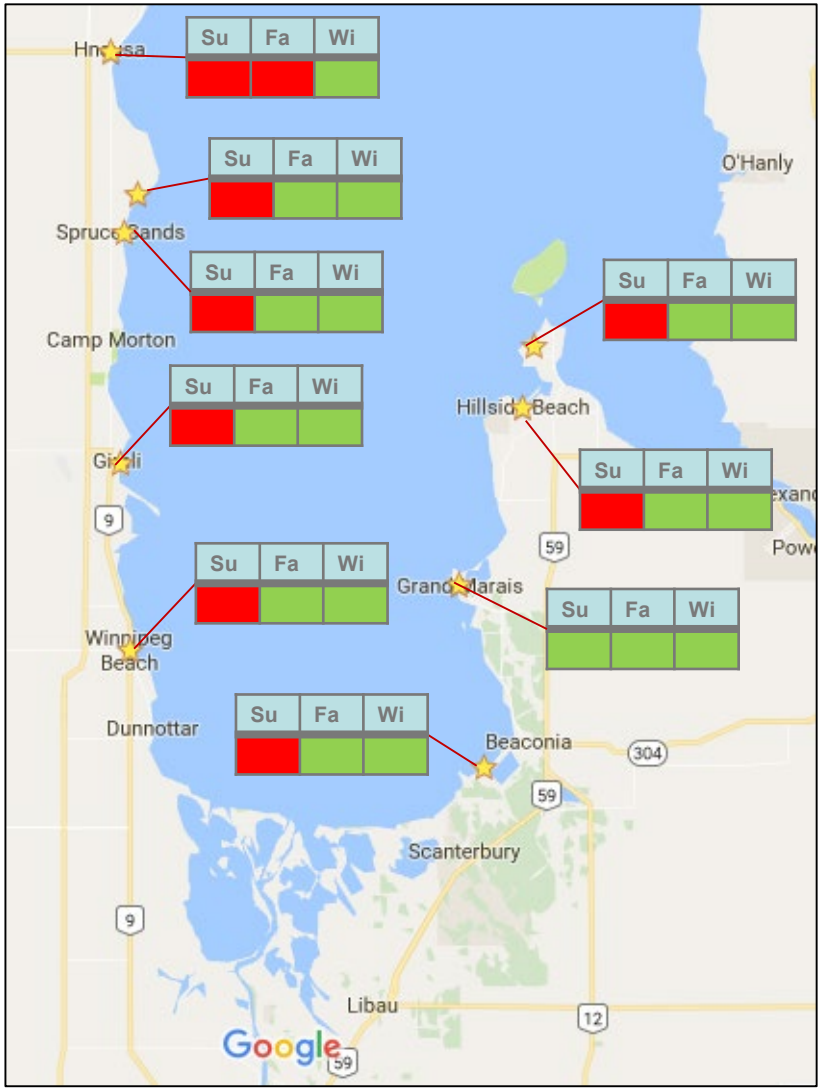


Positive Control = red,
Negative Control = Blue,
Hnausa Dock, Lake Winnipeg = Purple

Results – qPCR Seasonal Differences 2016

- "Blind" analysis of sample results
- Lake Winnipeg 2016 sites
  a) Summer (Su) (Aug 8, 2016) = 8/9 "hits"
  b) Fall (Fa) (Oct 26, 2016) = 1/9 "hits"
  c) Winter (Wi) (Jan 24, 2017) = 0/9 "hits"





Results - qPCR

- Other studies have found a drop off of free swimming veligers by late summer/early fall
- Veliger DNA is most important for detection of zebra mussels?

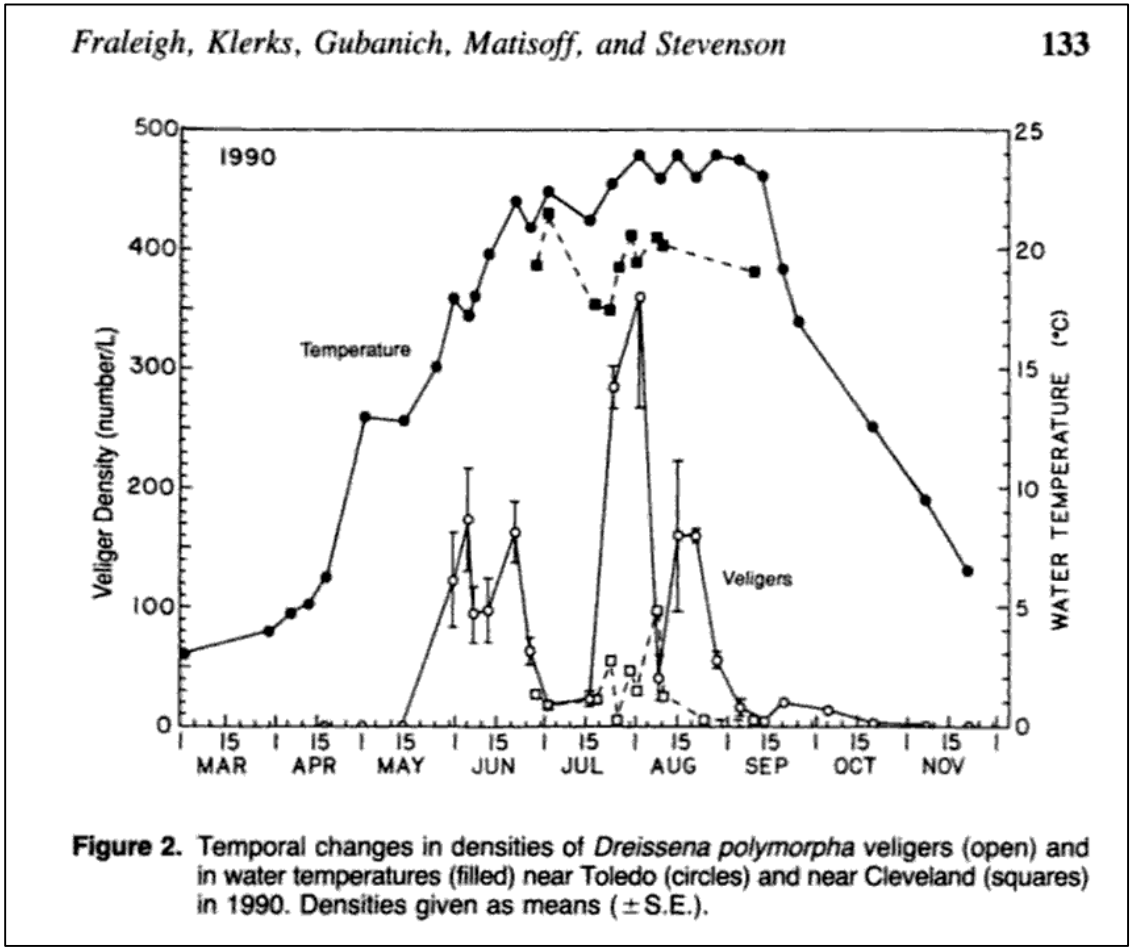
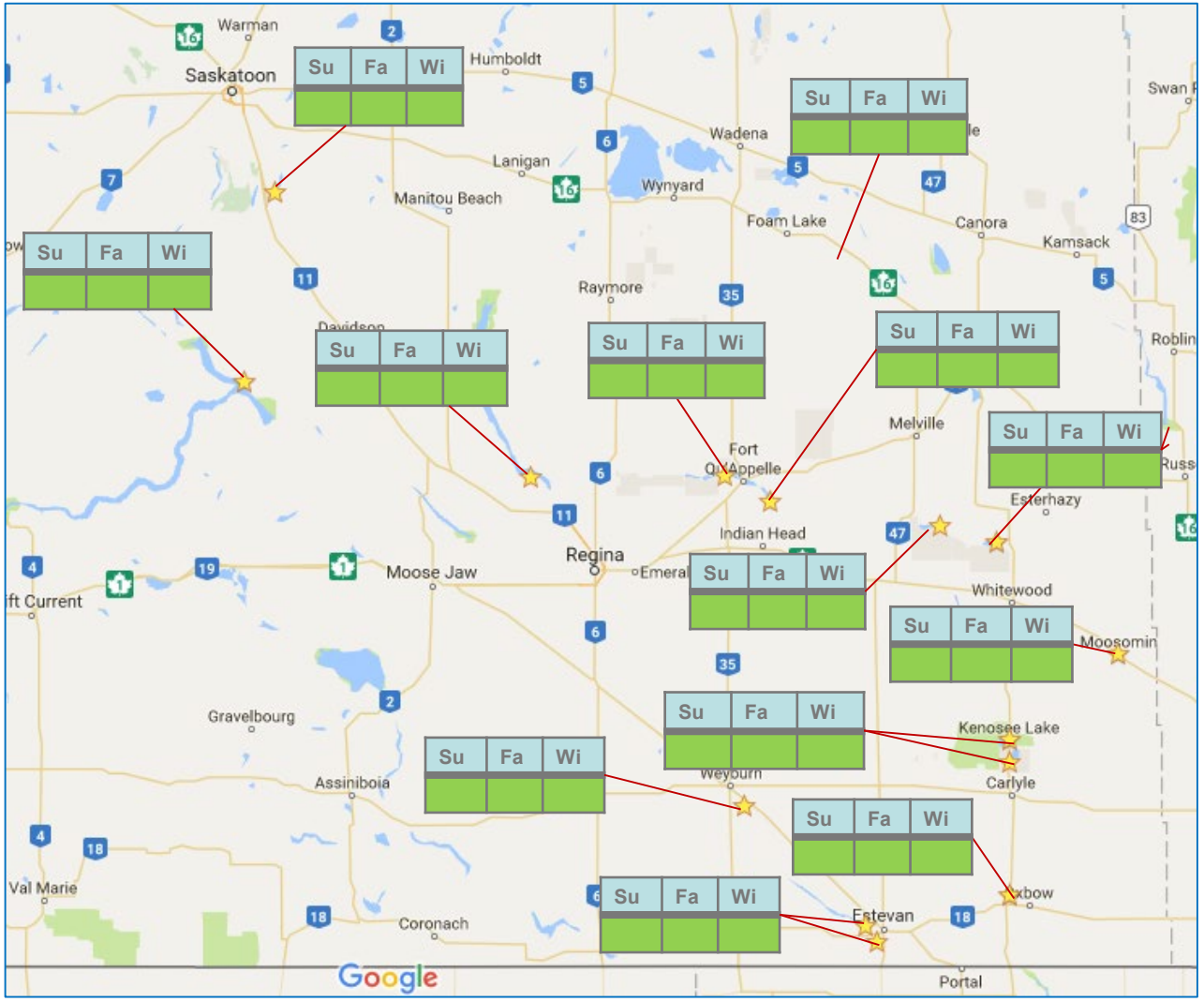


Figure 2. Temporal changes in densities of *Dreissena polymorpha* veligers (open) and in water temperatures (filled) near Toledo (circles) and near Cleveland (squares) in 1990. Densities given as means (±S.E.).

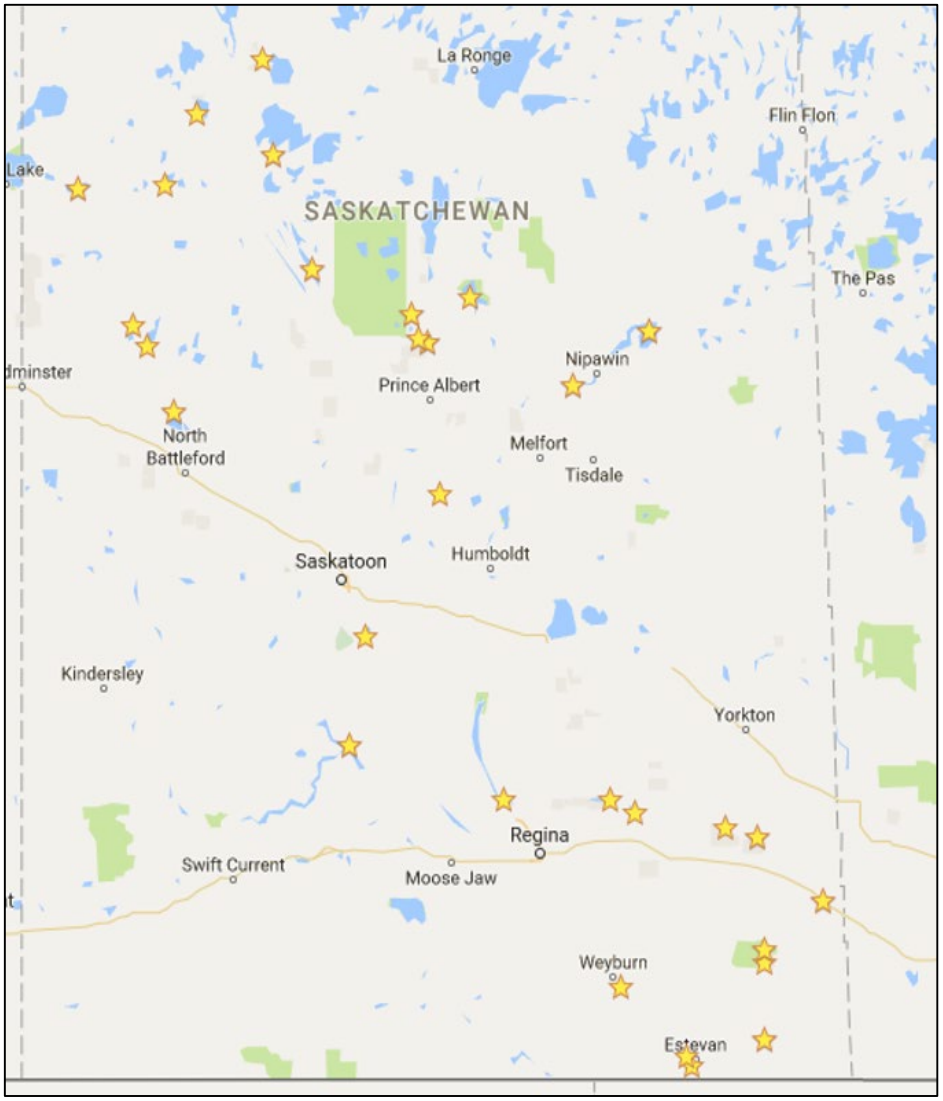
Results – qPCR Saskatchewan 2016

- "Blind" analysis of sample results based on "acceptable hit" parameters
- No hits in sampled Saskatchewan Lakes (0/12)



Ongoing Work

- Non target metagenomics method calibration
- Targeted approach focusing on additional species
  a) Carp
  b) Water milfoil
  c) Spiny waterflee
  d) Rusty crayfish
  e) Other
- Adapt technology to make sampling and analyses more user-friendly
  a) Simplify sampling
  b) In situ PCR system



Acknowledgements

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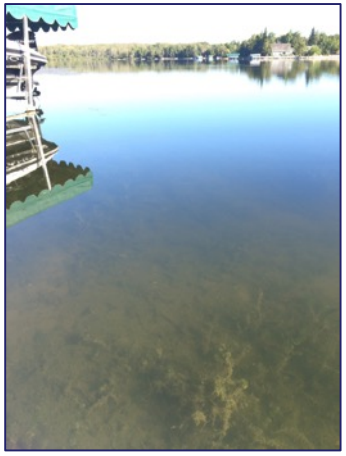
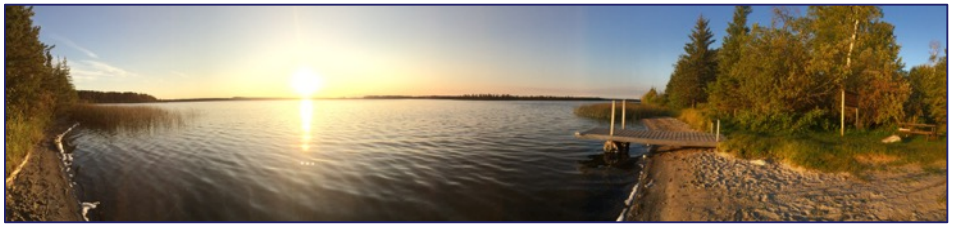
- McMaster University






ANY
QUESTIONS
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THANK YOU!
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