Environmental DNA surveillance for aquatic plants: a 21st century tool for species detection, biodiversity monitoring and management

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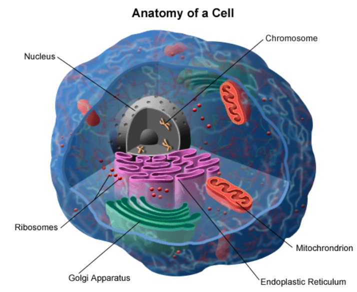
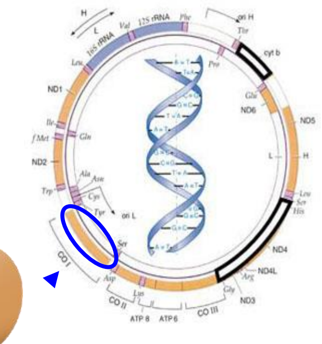
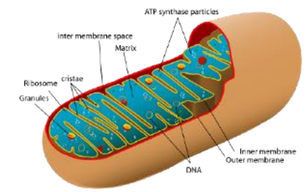
Environmental DNA (eDNA)







cells / DNA shed from living or dead organisms into surrounding environment

"molecular smoke alarm"

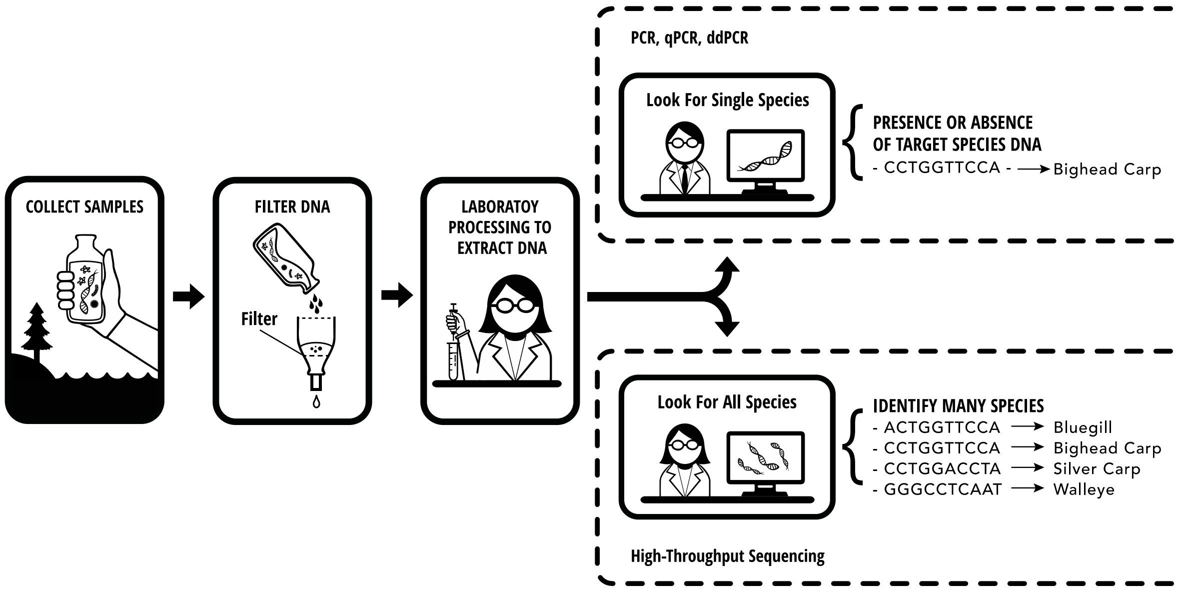
doesn't say what the source was

- living / dead; body parts; fluids, cells, free molecules, etc.

doesn't tell re: age, sex, size, reproductive status, population info

➢ ***positive detection*** = *DNA from the species was present at that location when the sample was collected*

Complementary approaches: targeted and community eDNA assays

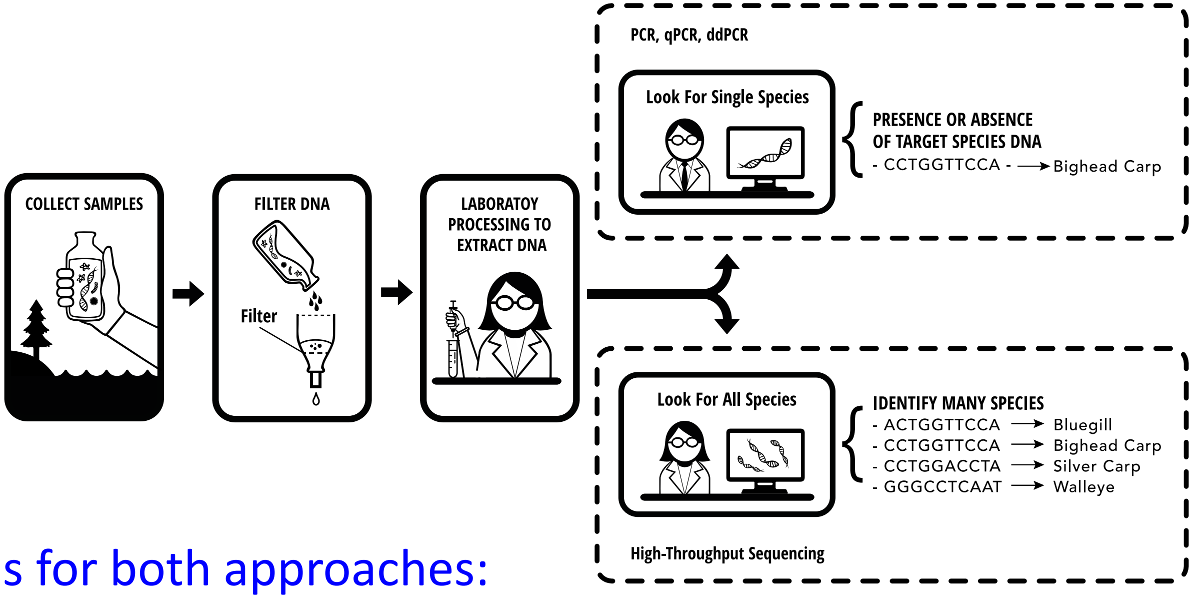


Active surveillance: **targeted single species** eDNA testing to evaluate if a species is present (bighead carp, tench)

Passive surveillance: use **community eDNA** or **eDNA metabarcoding** to characterize aquatic communities / local species assemblages (fish, invertebrates, plants, etc.) using high-throughput sequencing

(www.glfc.org/science-transfer-toolkit.php)

Complementary approaches: targeted and community eDNA assays



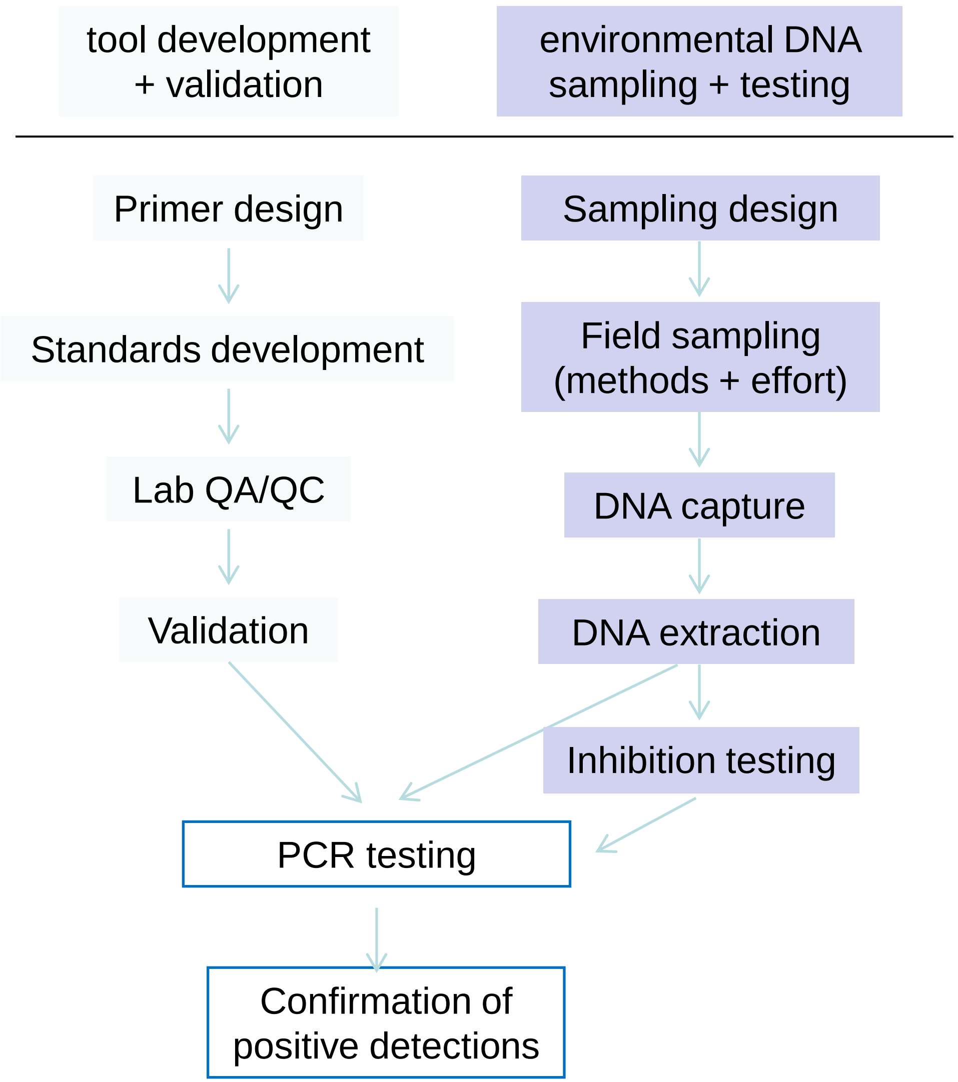
Considerations for both approaches:

- species specificity and sensitivity
- spatial sensitivity
- temporal sensitivity
- quantitative sensitivity

(www.glfc.org/science-transfer-toolkit.php)

Information needs for eDNA data acceptance

- "Invisible Man" scenario
- habitat description + characteristics
- sampling design + effort
- spatial + temporal metadata
- assay metadata
    - technology used
    - test conditions (details)
    - assay sensitivity + specificity
    - replicates + controls



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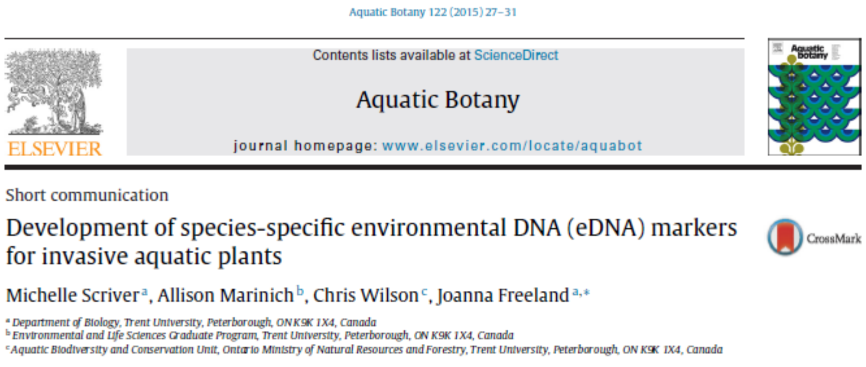
(www.csagroup.org)

Challenges for aquatic plant eDNA

- more challenging DNA barcoding for assay design
- less available baseline sequence data
- lower shedding rate vs animals (movement, excretion, temperature-related activity levels, vegetative reproduction)
- seasonal variation in shedding (growth vs. senescence)
- long-distance drifting of plant remains (rafting, etc.)
- passive dispersal by boats, trailers, propellors
- dormant life stages

Targeted eDNA assays for "least wanted" aquatic plants



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journal homepage: www.elsevier.com/locate/aquabot

Short communication

Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants

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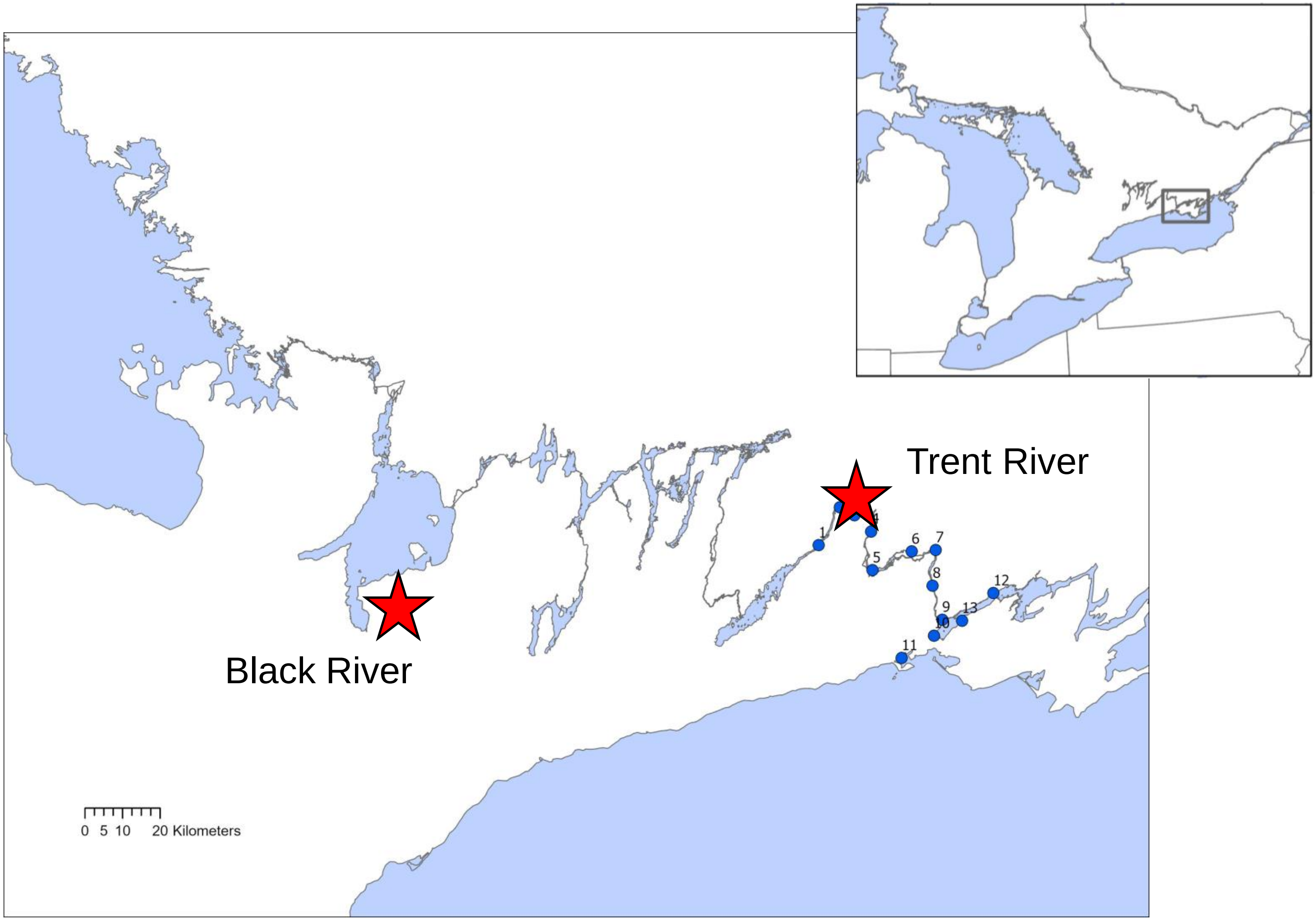
matK, rbcL, trnH-bsbA as target sequences:

- Water soldier
- Eurasian water milfoil
- Carolina Fanwort
- Parrotfeather
- Water hyacinth
- Water lettuce
- Yellow floating heart

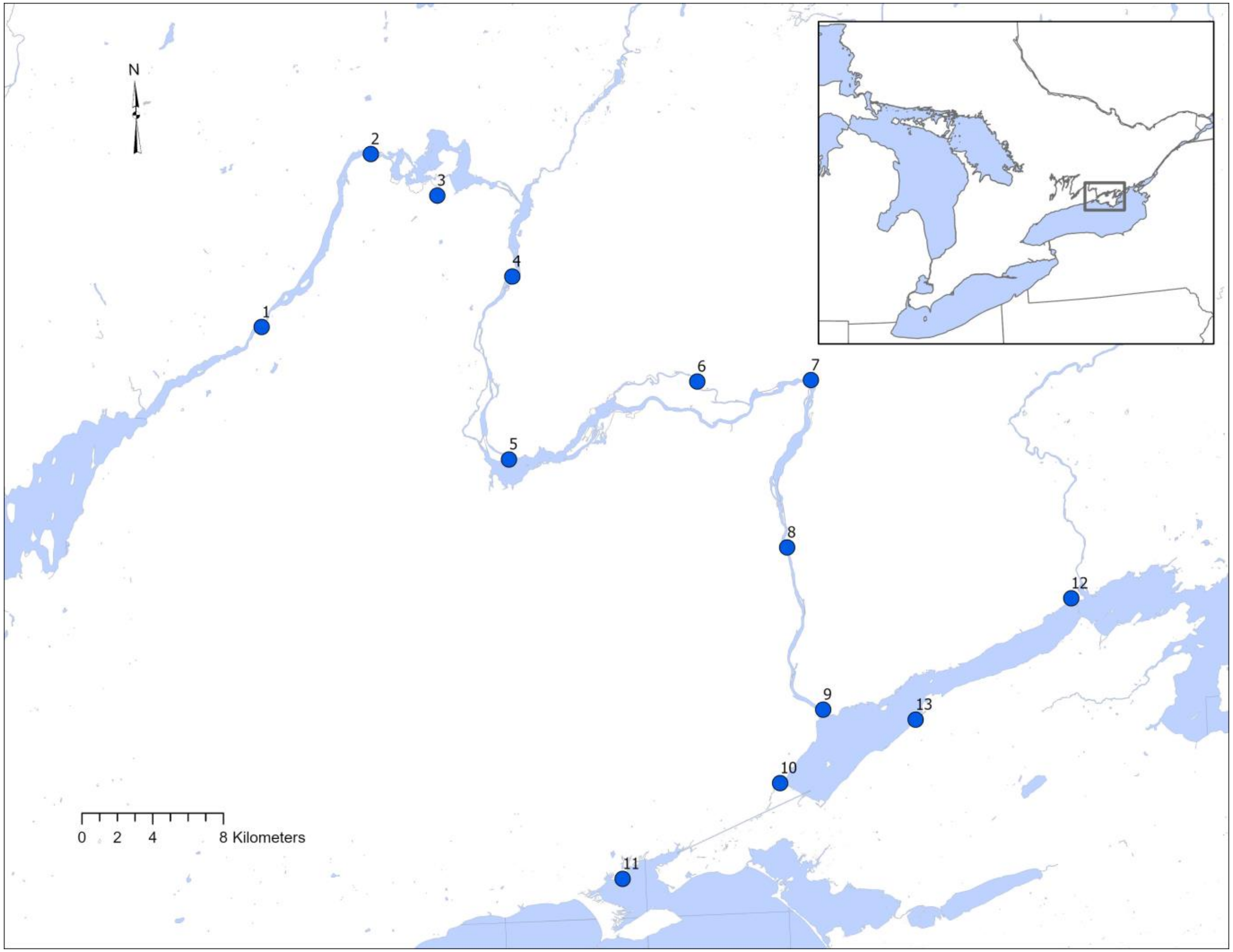
(Scriver et al. 2015)

Ontario

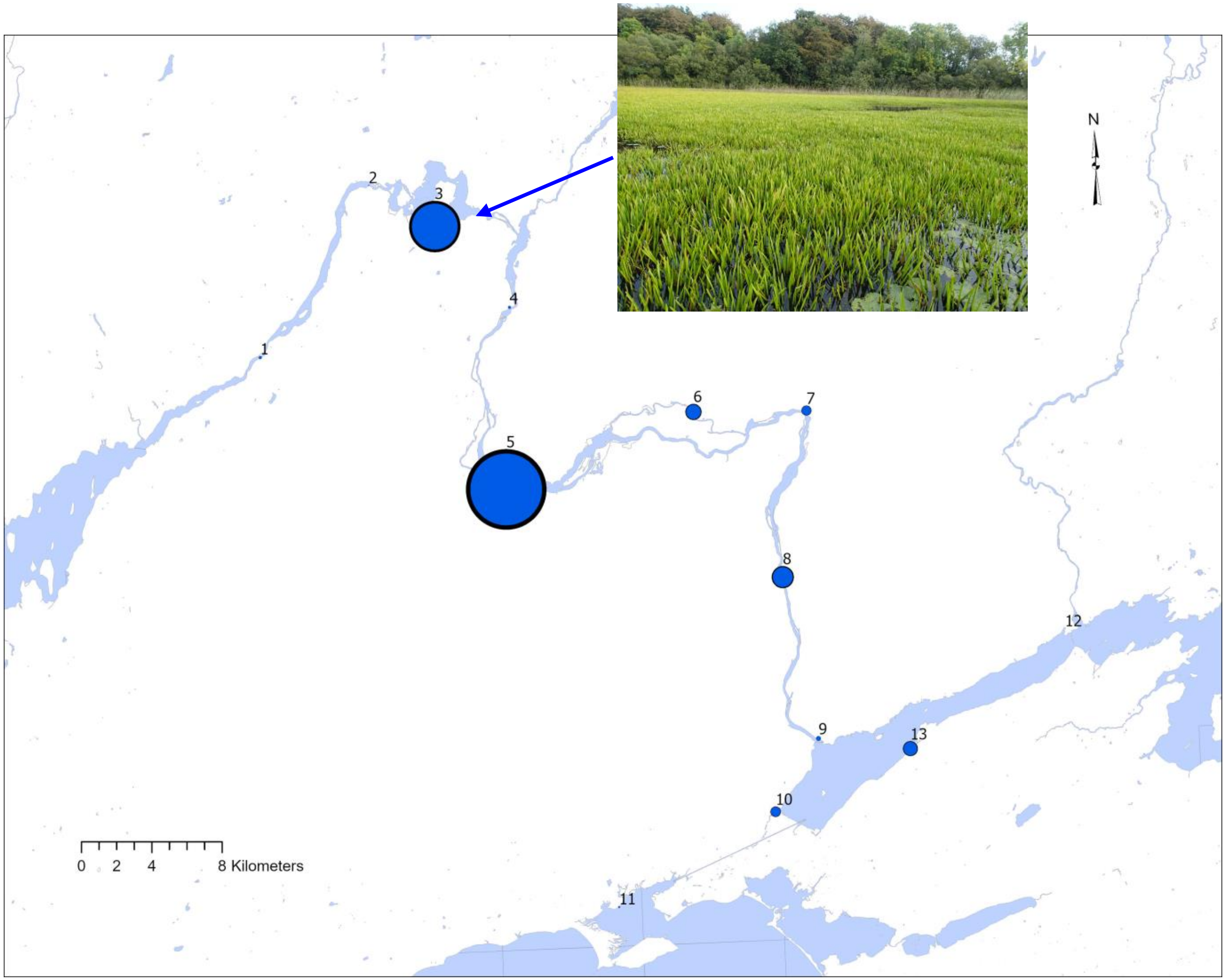
Water soldier infestations in Ontario



Water soldier eDNA sampling in the Trent-Severn Waterway



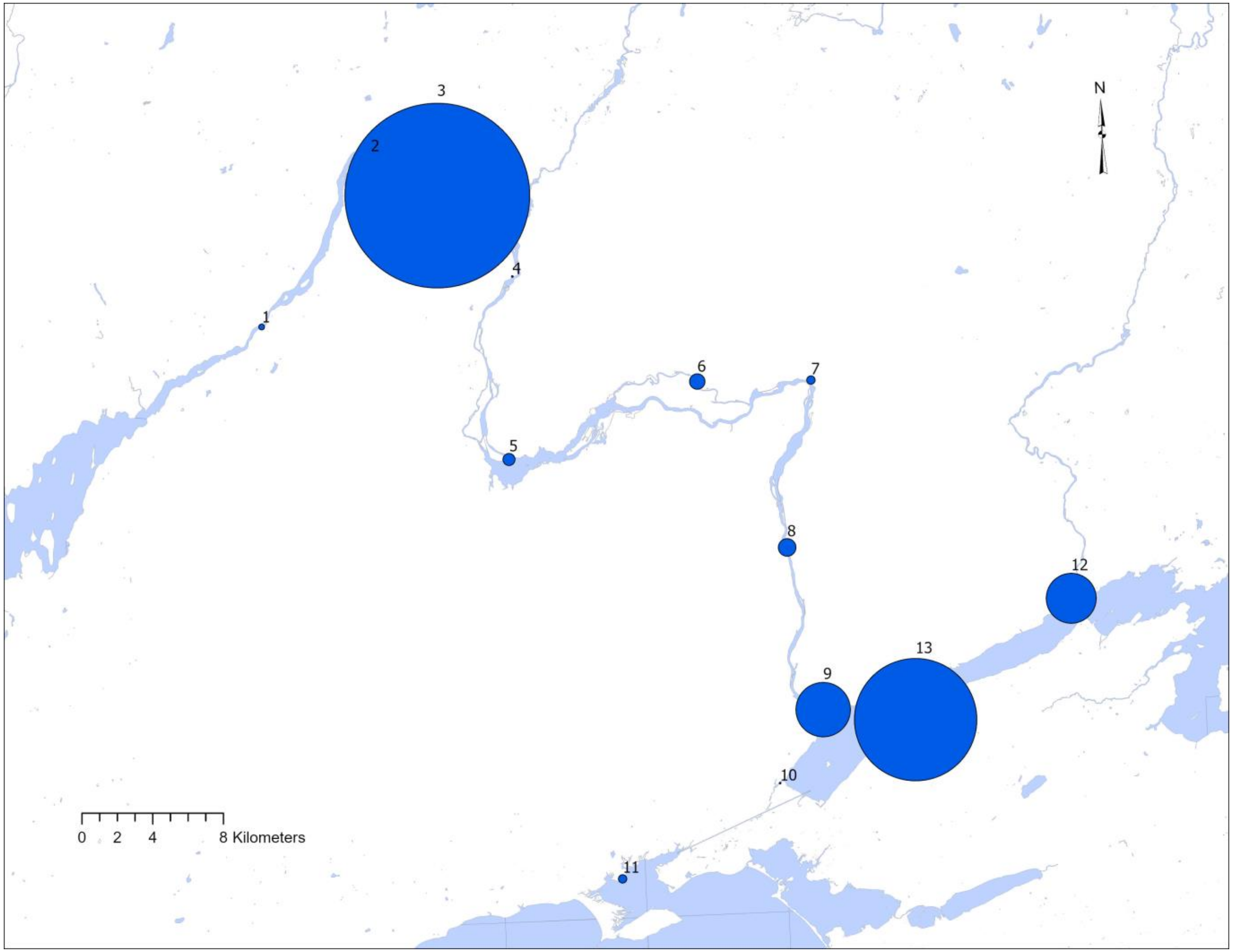
September 2021 eDNA detection results (pre-herbicide treatment)



- strong detections at known established populations
- weak detections at sampling sites without known species presence, including Bay of Quinte in Lake Ontario

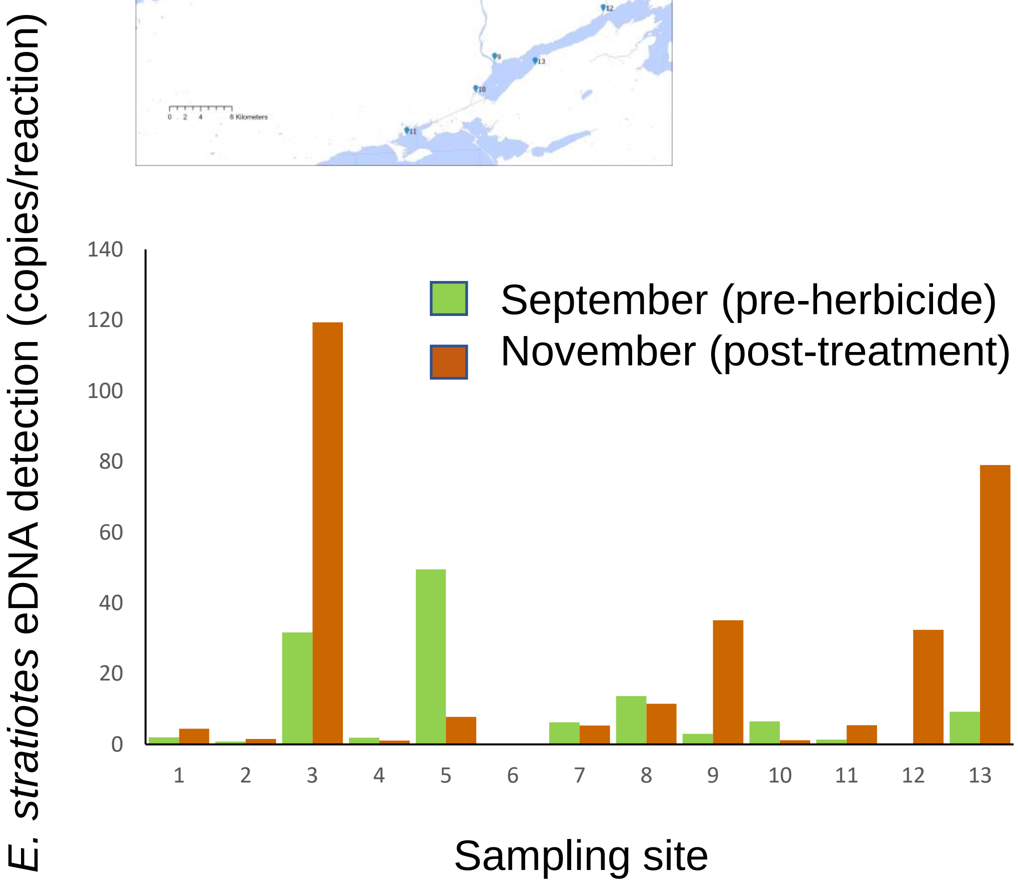
➢ *presence confirmed in 2022*

November 2021 eDNA detection results (post-herbicide treatment)



- very strong detection at treatment site
- strong detections also at untreated sites in Bay of Quinte
- weak detections at sites without known species presence at the time

➢ *presence confirmed in 2022*



Herbicide treatment substantially affected eDNA results

Pre-herbicide treatment

- weak detections upstream of established population (site 3) before herbicide treatment
- weak detections in Bay of Quinte and Presqu'ile Bay (Lake Ontario)

Post-treatment

- strong detections in treatment area
- Strong detections downstream of treatment area most likely reflect flushing of dead material

Targeted eDNA efforts for invasive aquatic plants

Water soldier control / eradication

- 2022 detections in Bay of Quinte visually confirmed by plant surveys
  - Local removal efforts
  - Expanded survey in 2023 incorporating hydrodynamic modelling
- Black River eradication considered successful after 3 years of no positive detections

Other species

- Water chestnut (Kingston area, Lake Ontario) strong detections in treatment area
- Hydrilla (Niagara River)

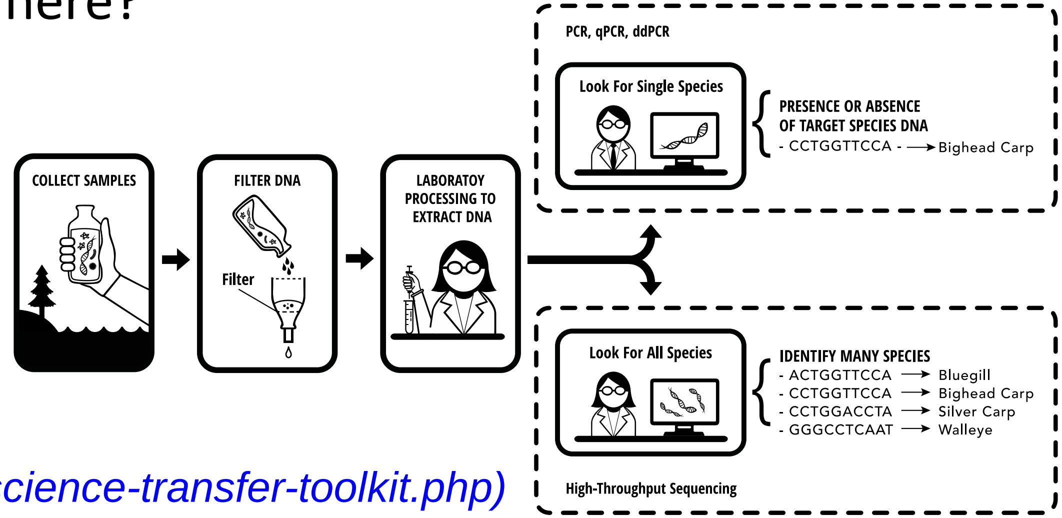
Stakeholders / citizen science

- Invasive Species Centre: eDNA sampling for community AIS detection in Ontario www.invasivespeciescentre.ca/eDNA

Invasive Species Centre

Why eDNA metabarcoding?

- Large areas to cover – don't need to locate individual plants
- Experts required to ID physical specimens
- Species-specific eDNA solves some of these, but requires prior knowledge of expected species/species of interest
- Metabarcoding allows us to gain comprehensive knowledge of what species are present
  - Instead of "is water soldier here?" we're asking "what plants are here?"



(www.glfc.org/science-transfer-toolkit.php)

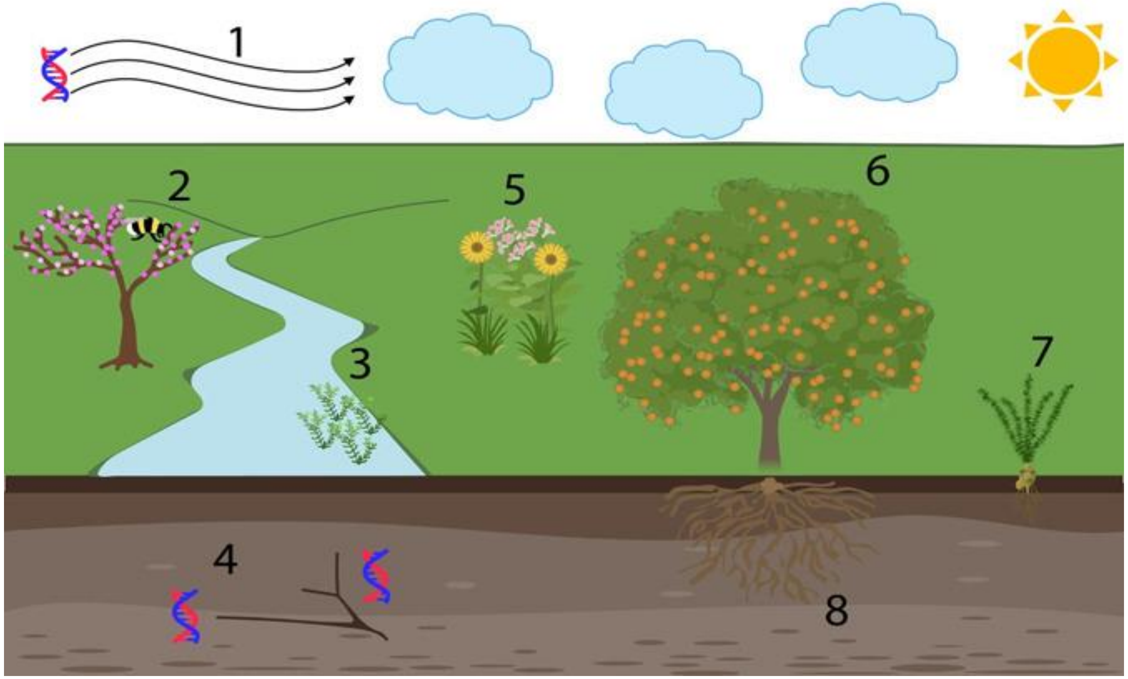
Metabarcoding

Well-developed assays and databases for other organisms

- Animals, invertebrates, microbes

Sources:

- Water
- Soil
- Pollen
- Air



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My MSc thesis

- Dr. Joanna Freeland & Dr. Aaron Shafer

- Develop aquatic plant metabarcoding assays
- Developed assays for 3 gene regions
  - Well-represented in database and appropriate for metabarcoding
  - matK, rbcL, and ITS2
- Tested against metabarcoding assays (rbcL and ITS2) developed for terrestrial plant metabarcoding from soil samples (Fahner et al. 2016)

Mock community

- eDNA sample of known composition
- Provides baseline of what to expect in results

omatK2: 9 species-level, 11 genus-level

orbcL2: 7 species-level, 17 genus-level

omatK2 + orbcL2 + oITS2: 14 species-level, 10 genus-level

Coghlan et al. 2021

Mock community

AIS/species of concern in Ontario:

Carolina fanwort

Frogbit

Water soldier

Water hyacinth

Yellow iris

Water lettuce

Parrot feather

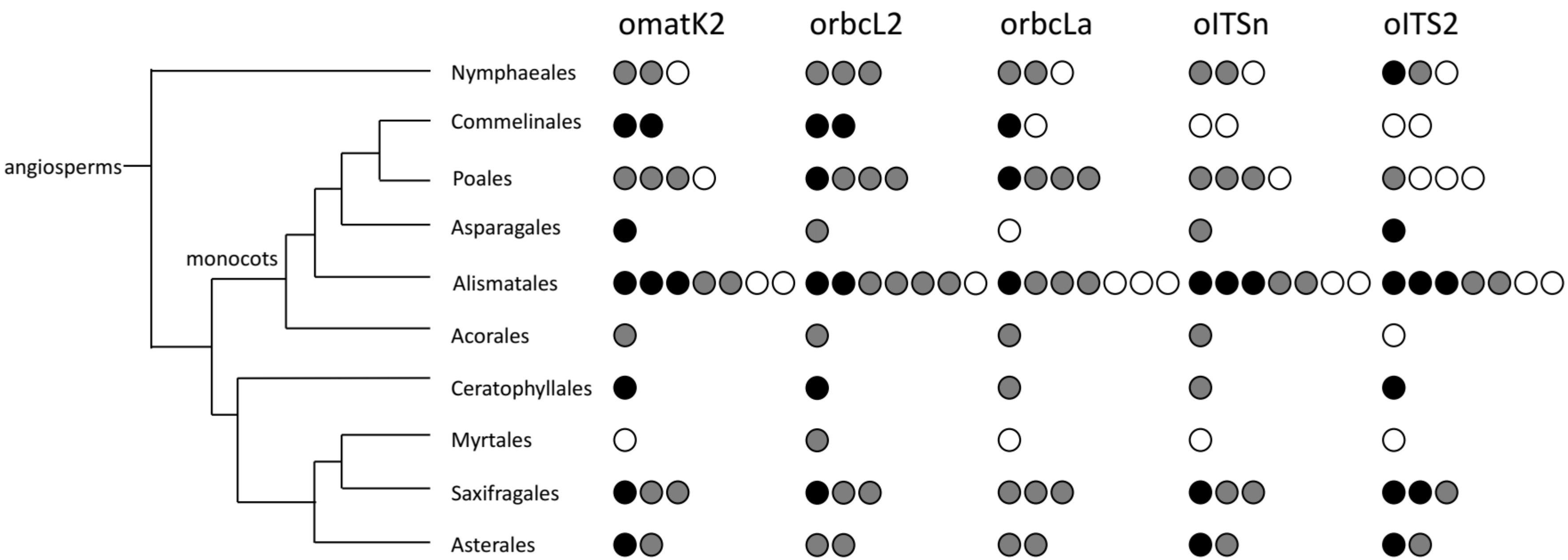
Eurasian watermilfoil

Yellow floating heart

European water chestnut

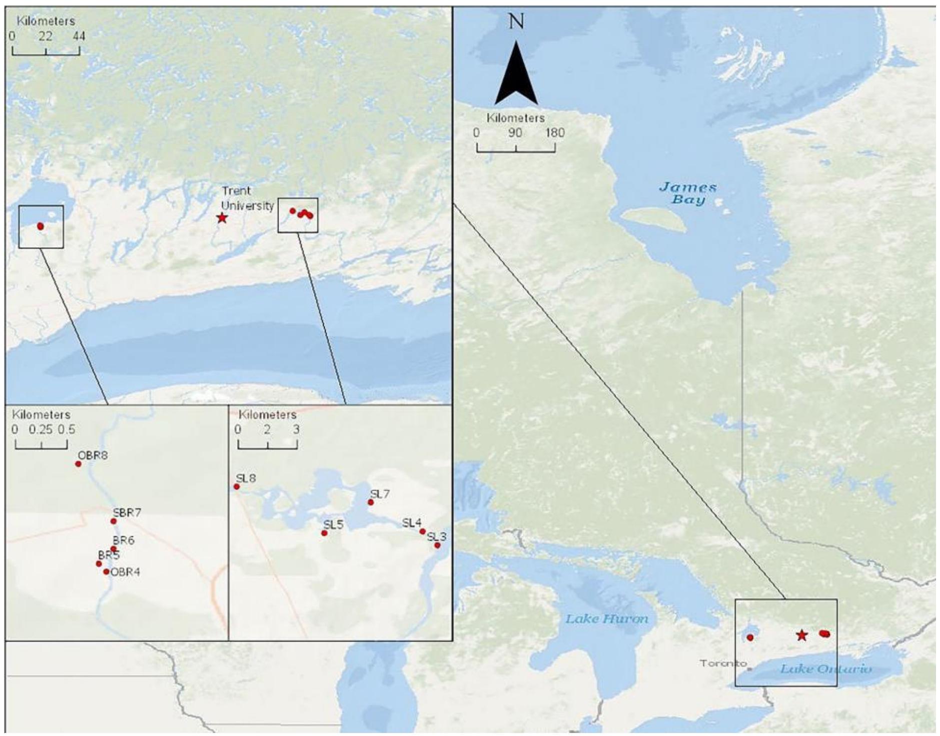
Coghlan et al. 2021

Diversity of identified species



Coghlan et al. 2021

Pilot sites



Coghlan et al. 2021

- Black River
- 17 species
- 24 genera

- Seymour Lake
- 14 species
- 27 genera

Noteworthy detections:

Flowering rush (*B. umbellatus*)
Frogbit (*H. morsus-ranae*)
Yellow iris (*I. pseudacorus*)
Parrot feather (*M. aquaticum*)
Yellow floating heart (*N. peltata*)
Phragmites (*P. australis*)
Water lettuce (*P. stratiotes*)
Water soldier (*S. aloides*)

Work since MSc

Testing orbcL2 on more sites and more replicates

- 44 sites
- orbcL2 identified all but one species from mock community to at least genus-level, and identified largest number of species from pilot sites

Monitor water soldier presence following removal/herbicide treatments

General results

- Noteworthy aquatics
  - AIS: European frogbit (*Hydrocharis morsus-ranae*), flowering rush (*Butomus umbellatus*), water soldier (*Stratiotes aloides*)

Limitations

Challenges with plant DNA in general

- Assay design: interspecific variation does not consistently exceed intraspecific variation; multi-assay approach might yield best results
  - Hybrids
- Taxonomic resolution – often genus-level ID

- Doesn't offer insight to abundance
- Relies on complete, error-free reference database

Strengths and next steps

Monitor biodiversity over time

- Incl. species occurrences before and after AIS invasions, patterns of co-occurring organisms
- Beyond plants – same samples can be used for any organisms
- Establish a baseline for routine monitoring

Early signs of invasions, particularly when you don't know what species you expect to find

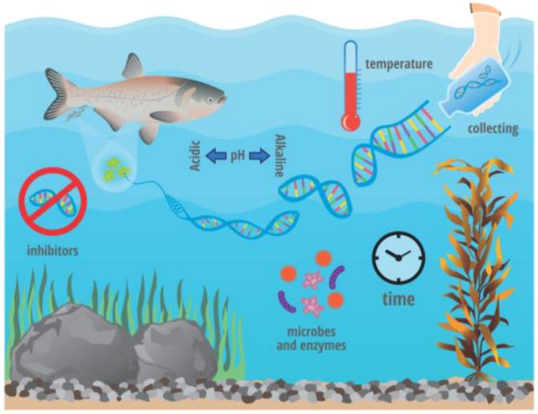
May lead to targeted inquiries

eDNA resources

CSA GROUP www.csagroup.org

Uses and Limitations of Environmental DNA (eDNA) in Fisheries Management

Project leader: Welsh, A.



GLFC Science Transfer eDNA

http://www.glfc.org/
science-transfer-toolkit.php

GEN-FISH
Genomic Network for Fish Identification, Stress and Health

www.gen-fish.ca

eDNAtlas (USDA and USFS):

www.fs.usda.gov/rmrs/projects/aquatic-ednatlas-project