eDNA metabarcoding for invasive fish detection in British Columbia

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Outline

- State of invasive fish in BC
- What is eDNA?
- Terminology
- 2 eDNA methods
- When to use eDNA vs other methods?
- AIS FW project
Invasive species

- One of the principal drivers of changes to global biodiversity and ecosystem function (Sala et al., 2000)
- In Canada, aquatic invasive species (AIS) have been identified as the second biggest threat to ‘at-risk’ freshwater fish (Dextrase & Mandrak, 2006)

Terminology around invasive species often confused
- Exotic
- Alien
- Weed
- Pest
- Nuisance
- Naturalized
- Non-native
BC’s freshwater AIS database includes 145 species of flora and fauna.

- Plants: 101
- Fish: 30
- Invertebrate: 5
- Amphibian: 5
- Turtle: 2
- Algae: 1

Number of non-native species
Analysis for Vancouver Island

• Distance of all invasive fish occurrences on Vancouver Island to municipalities was calculated
• Results divided into 5km, 10km and 25km from nearest municipality
• 132 of 135 invasive fish reports (98%) are within 25 km of a municipal area
Vectors of fish introductions into BC

- Illegal stocking (bucket brigade) is the most important vector, however several species like carp were historic introductions.

Vector analysis was done for fish where good data is available.
Ecological Consequences of Invasive Fish

<table>
<thead>
<tr>
<th>Species</th>
<th>Ecological Consequences</th>
<th>Small water bodies</th>
<th>Large water bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Largemouth bass</td>
<td></td>
<td>Very High</td>
<td>Moderate</td>
</tr>
<tr>
<td>Smallmouth bass</td>
<td></td>
<td>Very High</td>
<td>High</td>
</tr>
<tr>
<td>Yellow perch</td>
<td></td>
<td>Very High</td>
<td>Moderate</td>
</tr>
<tr>
<td>Northern pike</td>
<td></td>
<td>Very High</td>
<td>Very High</td>
</tr>
</tbody>
</table>

Results from a DFO MoE risk assessment conducted in 2008
Impact definitions

**Moderate** A measurable decrease in abundance of native populations is likely to occur in most locations or genetic exchange with native populations may occur in some instances and cause harm.

**High** The invasive species becomes a dominant component of the food web and causes significant reductions in existing biota or genetic exchange with native populations likely to occur in some circumstances and cause harm.

**Very High** Extirpation of native populations likely. Food webs are highly altered or genetic exchange is likely to be widespread or seriously deleterious.
Comparison with US Drainages

- Based on our current knowledge BC still has low numbers of non-native fish
- Nearby AIS source populations: potential for illegal introductions
- Natural dispersal within the Columbia drainage poses a risk

Sanderson et al. Bioscience 2009
Invasive fish in BC freshwater systems

- AIS: major threat to FW aquatic ecosystems and fisheries in BC

- eDNA detection offers:
  - Early detection, increases chance for effective management intervention
  - Increased speed, cost-efficiency & sensitivity over traditional methods
  - Possibility of increased spatial scale of monitoring
Next-generation monitoring of aquatic biodiversity using environmental **DNA metabarcoding**

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Abstract

Global biodiversity in freshwater and the oceans is declining at high rates. Reliable tools for assessing and monitoring aquatic biodiversity, especially for rare and secretive species, are important for efficient and timely management. Recent advances in DNA sequencing have provided a new tool for species detection from DNA present in the environment. In this study, we tested whether an environmental DNA (eDNA) metabarcoding approach, using water samples, can be used for addressing significant questions in ecology and conservation. Two key aquatic vertebrate groups were targeted: amphibians and bony fish. The reliability of this method was cautiously validated in silico, in vitro and in situ. When compared with traditional surveys or historical data, eDNA metabarcoding showed a much better detection probability overall. For amphibians, the detection probability with eDNA metabarcoding was 0.97 (CI = 0.90–0.99) vs. 0.58 (CI = 0.50–0.63) for traditional surveys. For fish, in 89% of the studied sites, the number of taxa detected using the eDNA metabarcoding approach was higher or identical to the number detected using traditional methods. We argue that the proposed DNA-based approach **has the potential to become the next-generation tool for ecological studies and standardized biodiversity monitoring in a wide range of aquatic ecosystems**.
Ecosystems

- Rich diversity of species
- Rich diversity of eDNA
- Naturally occurring genetic material
  - Sloughed cells/mucous, excreted, reproduction
DNA Barcoding

Barcodes
• Short standardized DNA sequences
• Discriminate among species
• Broad taxonomic range

Reference library
• Sequence morphologically identified specimens
  – Validate DNA ‘barcode’
• Store reference sequences in ‘Barcode library’
DNA Metabarcoding

Next generation sequence technology
Generate millions of DNA barcodes
‘Massively Parallel Sequencing’
Traditional *versus* next generation sequencing

**DNA barcoding**
- sampling and DNA extraction
- DNA amplification

**DNA metabarcoding**
- sequencing

Results

Adapted from Taberlet
Adapted from Taberlet eDNA metabarcoding

bioinformatics

ACGTTA ACGTAA ACGTTG ACATTA ACGCTA

Canada
eDNA Detection – 2 pathways

• Community Approach
  – ID all species in a given community
    • Biosurveillance
    • Reveal presence of unexpected invaders
    • Monitor secondary spread

• Species-specific Approach
  – Is Sp X here?
  – Presence/Absence (not detected)
    • Targeted sampling for specific species
    • Monitor secondary spread

NGS eDNA Metabarcoding
qPCR targeted detection
eDNA Detection – 2 pathways

- Community Approach
  - ID all species in a given community
    - Biosurveillance
    - Reveal presence of unexpected invaders
    - Monitor secondary spread
  - Biosurveillance
  - 3 month turn-around
  - Lower per sample cost

- Species-specific Approach
  - Is Sp X here?
  - Presence/Absence (not detected)
    - Targeted sampling for specific species
    - Monitor secondary spread
  - 1-∞ samples
  - ~1 week turn-around
  - Higher per sample cost
eDNA

• Bulk, complex, samples
  – Ethanol from insect traps
  – Gut contents
  – Leech’s blood
  – Soil/benthos
  – Water

qPCR
High population density
Large body size

Field sampling more cost effective

Effort

Detection

eDNA sampling more cost effective
AIS early detection!

Low population density
Small body size

Adapted from Goldberg
Conventional vs eDNA

1. Standardised sampling
2. High sensitivity
3. Non invasive
4. No permits required
5. ID all stages
6. No observation/detection bias
7. Multi species detection
8. Retroactive addition of taxa
9. Low pathogen transfer risk
10. Unrestricted timing

• BUT: no info about abundance, proximity, age, sex
Aim 1: Biomonitoring tool FW fish in BC

- Compare effectiveness of tool with existing biodiversity data from lakes with well-characterized species assemblages
- Apply tool in 2017 to assess finfish biodiversity in key BC lakes
  - Develop baseline biodiversity data and monitor changes
  - Detect new incursions
  - Monitor 2° spread of known invasives

Water is easier to catch than fish
Aim 1: Biomonitoring tool FW fish in BC

- 2017 Fish biodiversity in the Lower Fraser
  - High population density in urban areas
  - High probability of invasives
  - Undetected invasions?
Aim 2: Develop rapid response, targeted detection tool

- Targeted species detection
  - Yellow Perch — Small Mouth Bass — ZQM
  - Northern Pike — Large Mouth Bass

- Investigate AIS reports, monitor 2° spread
- Asses eradication success
Smallmouth bass
Beaver Creek

- Assess effectiveness of SMB management efforts in Beaver Creek drainage system
- Sample upstream and downstream of installed fish barriers
- Sample Big Lake to assess Bass presence/absence in watershed
Smallmouth bass
Beaver Creek

Non-native species
- Smallmouth bass
- Largemouth bass
- Ecological Drainage Units

Beaver Creek Watershed Showing 4 Treatment Zones:
- Lakes, streams and wetlands to be treated:
  - Wetland
  - Riparian
  - Stream
- Streams Lakes, wetlands (Big Lk area) to be treated:
  - Boundary of treat area at garrison (no treatment)
Northern Pike Columbia

- Assess spread of NP in Okanagan
- Sample in Columbia River upstream and downstream of HLK dam
- Sample in the Salmo River above and below fish barriers
Northern Pike
Columbia
Development of methods

1. DNA capture methods
   - Syringe, vacuum, capsule

2. Seasonal detection
   - Summer, autumn, winter

3. Sampling location
   - Shore, open water

1. Ease of collection, contamination risk, probability of detection

2. Animal behaviour, UV, temperature, water level…

3. DNA distribution in environment
Filter
Where to sample?
Collect water → Filter eDNA from water → Extract eDNA from filter → Amplify extracted DNA

Species-specific primers → qPCR → Presence/Absence 1 species @ a time

Community primers → NGS → Bioinformatics → Community ID taxonomy using reference library
Contamination

Site

Field

Laboratory

Analysis
Contamination Controls

- Field sampling control
- Filtration control
- Extraction control
- Amplification control
Increasing detection probability

- Replication
  - Field sampling
    - How many samples
    - What volume of water for samples
  - PCR replicates
    - 3-12
  - Sequencing depth
    - NGS, aim for 20k-300k reads per sample
Aquatic biodiversity assessment for the lazy
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