eDNA Science & Application
Detecting Species Sight Unseen

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Indirect, DNA-based Species Detection

Breathtaking discovery:

1. Bamboo ring covered with nylon stocking is held near blow.
2. Exhaled air or blow contains mucus and cells from whale’s lungs.
3. Sample collected on nylon contains whale hormones.
4. Method could be used to determine sex and reproductive health.

Image of whale and samples.
Asian Carps: Imminent Threat to Great Lakes

1975

USGS data

Bighead Carp

Silver Carp
Asian Carps: Imminent Threat to Great Lakes

2013

USGS data

Bighead Carp

Silver Carp
Chicago Sanitary & Ship Canal

Canal opened ~1900
Silver and bighead carps CPUE: Electroshocking vs. eDNA

Potential Applications for eDNA

1. Species that are rare
   • Incipient invasions—early detection-rapid response
   • Threatened, endangered species

2. Species that are difficult to sample with traditional tools

3. Species for which handling causes harm

4. Habitats in which traditional tools are difficult to deploy
   • Limited access for boats and sampling gear
   • Strong currents
   • Interference with navigation, fishing, or other uses

5. Where/when “integrative” samples are cost effective
   • “contaminant” species in bait trade, fish stocks, imports
Environmental DNA Overview

1. Collect water sample
2. Filter water sample
3. Extract all DNA
4. Amplify target DNA
5. Visualize DNA presence
Environmental DNA Overview

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Design primers for target DNA; Polymerase Chain Reaction (PCR)

PCR
Quantitative PCR
Digital droplet PCR
Environmental DNA Overview

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Gel electrophoresis

Sequencing for metabarcoding

Digital readout for qPCR and ddPCR, Laser Transmission Spectroscopy
Environmental DNA Overview

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The more sensitive an assay, the greater the risk for false positives from contamination.

Meticulous care is required to reduce risk of contamination at every step in the field and laboratory.
• Laboratory design to separate different processes
THE ECOLOGY of eDNA

A. ORIGIN
- Reproduction: [eDNA] over time
- Decomposition: [eDNA] over time

B. STATE
- Particle size range
- Filter type A
- Filter type B

C. TRANSPORT
- Water sample: true
- Water sample: false

D. FATE
- Slow decay: [eDNA]
- Fast decay: [eDNA]

Barnes & Turner 2015
Sources of Asian Carp DNA in Canal (in the absence of live fish)?

- Ballast or bilge water from barges?
- Dead fish kicked off of barges?
- Sewage discharge from carp eating humans?
- Feces from carp eating birds?
- Overflow from Chicago ponds?

None of these possibilities were plausible explanations for the overall temporal and spatial pattern of eDNA results.
## Interpretations of eDNA results and implications for management

### Strong

- Multiple eDNA detections on multiple occasions, invasion pathway exists, historical capture record
- Multiple eDNA detections on multiple occasions, invasion pathway exists
- Multiple eDNA detections from one sample event, invasion pathway exists
- One or two eDNA detections from one sample event, invasion pathway exists
- Limited number of eDNA detections, no known invasion pathways

### Weak
Interpretations of eDNA results and implications for management

<table>
<thead>
<tr>
<th>Weight of eDNA evidence</th>
<th>Strong</th>
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<tbody>
<tr>
<td>Multiple eDNA detections on multiple occasions, invasion pathway exists, historical capture record</td>
<td></td>
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<table>
<thead>
<tr>
<th>Weak</th>
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<tbody>
<tr>
<td>Localized range expansion</td>
</tr>
<tr>
<td>Or limited potential for further spread</td>
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<table>
<thead>
<tr>
<th>Low risk</th>
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<tbody>
<tr>
<td>Response options: Confirm presence using alternative detection methods</td>
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</table>

<table>
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<tr>
<th>Moderate Risk if response delayed</th>
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</thead>
<tbody>
<tr>
<td>Response options: Increased eDNA surveys coupled with conventional methods (Consider containment measures)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Risk if response delayed</th>
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</thead>
<tbody>
<tr>
<td>Response options: Contain, delimit, control and eradicate where possible</td>
</tr>
</tbody>
</table>

Tucker et al. 2016
Current & Future eDNA R&D

1. Degradation rates of eDNA
2. Assay sensitivity & capture efficiency of eDNA
3. Vertical transport of eDNA
4. Horizontal transport of eDNA
5. Differences among taxa in ecology of eDNA
6. eDNA concentration re population size
7. Intrapopulation variation (population genetics)
8. Metabarcoding for community detection
9. Faster, portable, automated analysis analysis
Automated sampling, detection
eDNA DETECTION

MOTIVATES OPPORTUNITY TO MANAGE

CREATES
END
Future general directions for eDNA R&D

1. Degradation rates of eDNA
2. Assay sensitivity & capture efficiency of eDNA
3. Vertical transport of eDNA
4. Horizontal transport of eDNA
5. Differences among taxa in ecology of eDNA
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**Current/future management applications?**

1. Statewide fisheries surveys (e.g., more efficient, cheaper than electrofishing & nets?) to prioritize systems for follow up.

2. Statewide invasive or imperiled species surveillance (e.g., pathway hotspots like ports).

3. Post management monitoring program for invasive (e.g. hydrilla) or imperiled species (e.g. hellbender, cisco).
Tension points at research-management interface

1. More humility often needed from academics.
   • Be open to questions from management;
   • Be sensitive to limited resources, political context, rapidly changing priorities;
   • Communicate clearly about uncertainty.

2. More understanding of academic environment often needed from managers.
   • Creativity and cheap labor come with slower, episodic progress;
   • Interest in novelty in addition to problem solving.

3. Data management, sharing protocols essential.

4. Communications protocols are essential.
END
Degradation of eDNA: How long ago was the animal present?

Experimental results:
- eDNA undetectable after hours-days
- Degradation affected by biological activity

(Barnes et al 2014. Environmental Science & Technology)
eDNA collection:
sequential filtration of field water samples

(Turner et al. 2014 Methods in Ecology & Evolution)
Common carp eDNA collection: sequential filtration of field water samples

For optimal eDNA capture:
- 0.2 um filtration or
- a combination of larger pore size and larger water volume that captures the same amount of eDNA (i.e., exceeds the 0.2 um isocline)

(Turner et al. 2014 Methods in Ecology & Evolution)
Differences among taxa?

Differences among taxa in:
• Production rate?
• Degradation rate?
• Particle size?
• Location in water column?
  • Vertical transport?
  • Horizontal transport?
eDNA concentration correlates to pop size

Asian carp biomass density (g/m³)

eDNA concentration (copies/mL)

R² = 0.35

(Turner et al. unpublished)
Metagenetic Analysis (Ultrasequencing): Kansas ponds

<table>
<thead>
<tr>
<th>Fish Type</th>
<th>Cytb (bp)</th>
<th>12S (bp)</th>
<th>16S (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass carp</td>
<td>464</td>
<td></td>
<td>392</td>
</tr>
<tr>
<td>Common Carp</td>
<td></td>
<td></td>
<td>366</td>
</tr>
<tr>
<td>Silver carp</td>
<td></td>
<td></td>
<td>250</td>
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<tr>
<td>Bighead carp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluegill</td>
<td>464</td>
<td>270</td>
<td>392</td>
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<tr>
<td>Redear sunfish</td>
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<td></td>
<td>392</td>
</tr>
<tr>
<td>White crappie</td>
<td></td>
<td></td>
<td>366</td>
</tr>
<tr>
<td>Bullfrog</td>
<td></td>
<td></td>
<td>250</td>
</tr>
</tbody>
</table>

(Olds et al. unpublished)
Metagenetic Analysis (Ultrasequencing): Juday Creek, Notre Dame

Species richness detected with traditional tools

Chao estimated species richness

eDNA species richness

\[ \hat{S} = S(KK) + S(KU) + S(UU) \]
\[ \hat{S} = 12 + 7 + ? = 19 + \epsilon \]

Year Sampled

2000 2005 2010

(Jerde et al. unpublished)
Example of bioinformatics workflow

Data Prep Step

- Raw reads from Miseq
  - Cytb #1
  - 12s #1
  - 12s #2
  - 16s #1
  - 16s #2
  - 16s #3

  Filtering of reads

Analysis Step

- Known species
  - Map to reference
  - Unmapped reads

- Unknown species
  - Create OTUs
  - Compare to Genbank
  - Report of species
eDNA detection using Laser Transmission Spectroscopy (LTS)

Recent LTS results:
- Very high sensitivity (picomolar range)
- Detects invertebrate species in “ballast” water with 100% accuracy, and no false positives

LightSprite (http://www.m3dev.com/)