Molecular detection of aquatic species in water samples using environmental DNA

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Introduction

• Early detection is essential for managing invasive species
• Aquatic species are often difficult to detect
• Water samples can be used to detect aquatic species using DNA
• Highly efficient, sensitive, and cost-effective
Outline

• Identification of species using DNA
• DNA in the environment (eDNA)
• 3 studies using eDNA
  – Bullfrog detection
  – Asian carp detection
  – Headwater amphibian detection
• Potential for eDNA detection of mudsnails
Identification of species using DNA

- Every species has unique DNA sequences

NZ mudsnail (mtDNA COI):
...TATTCTATTTTGGTATATGATCTGGGACTAGT...

Pebblesnail (mtDNA COI):
...TATCCTATTTTGGGATATGATCAGGACTTTGT...
Identification of species using DNA

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DNA in the environment
DNA in the environment

Up to ~1 million years old

Frozen:
- Frozen tissues
- Permafrost sediments

Dry:
- Mummies
- Dry cave sediments
DNA in the aquatic environment
DNA in the aquatic environment

- DNA of 400 bp can persist for up to 1 week in lake water at 18°C (Matsui et al. 2001)
DNA in the aquatic environment

• To be useful for species detection:
  – DNA must remain for long enough to be detected
  – Species can be detected at low densities where they are difficult to detect otherwise
  – No false positives
eDNA study #1: Bullfrogs (Ficetola et al. 2008)

- Invasive in Europe and Western N. America

- Developed PCR test for 79 bp fragment

- Aquaria experiment
  - 3L of water in each
  - 15 mL sample after 24 hours
  - Detected: 1 tadpole in 3L water

- Wetlands
  - 1 or 2 adults in 1 km² surface area
  - 3 15-mL samples (2+ positive)
eDNA study #1: Bullfrogs (Ficetola et al. 2008)

• No false positives:
  – in clean aquaria
  – in wetlands without bullfrogs
eDNA study #2: Asian carp
(Jerde et al. 2011)
eDNA study #2: Asian carp
(Jerde et al. 2011)

• 1,000 2L samples from surface water
  – Run through filter to capture DNA in lab

• Developed test for 312 bp and 191 bp fragments

[Graph showing CPUE: Catch per person day for Illinois waterway pools.]

93 person-days
eDNA study #2: Asian carp
(Jerde et al. 2011)

• 1,000 2L samples from surface water
  – Run through filter to capture DNA in lab
• Developed test for 312 bp and 191 bp fragments
• No false positives:
  – In rivers without Asian carp
  – In water run through filter apparatus between samples after sterilization (1L)
  – When tested with other fish species
eDNA study #3: Headwater amphibians
(Goldberg et al. in review)
Methods:

- Identified a short fragment of DNA unique to each species (78, 85 bp)
- Created species-specific PCR test with known samples
- Collected field samples (peristaltic pump)
- Conducted PCR test on field samples
- Sequenced resulting products

eDNA study #3: Headwater amphibians
(Goldberg et al. in review)
Field methods

- 1 10-L, 2 5-L stream water samples
  - Cellulose nitrate filters
  - Stored in ethanol

- Field densities:
  - Salamanders: 0.04 individuals/m\(^2\), 2.75 person hours to detect
  - Frogs: 0.16 individuals/m\(^2\), 1.5 person hours to detect

eDNA study #3: Headwater amphibians
(Goldberg et al. in review)
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Lab optimization

• Ficetola method for extraction and PCR
  – 1 positive for tailed frogs only

• Soil kit method for extraction
  – 0 positives

• Qiagen method for extraction and PCR
  – 3/3 filters positive for both species
eDNA study #3: Headwater amphibians
(Goldberg et al. in review)

• No false positives:
  – With co-occurring amphibian species
    • *Ambystoma macrodactylum*
    • *Bufo boreas*
    • *Pseudacris sierra*
    • *Rana luteiventris*
  – In negative extraction controls
eDNA study #3: Headwater amphibians
(Goldberg et al. in review)

- Combined PCRs into one test for both species
eDNA study #3: Headwater amphibians
(Goldberg et al. in review)

Costs

• $2000 for development

• Estimated cost per sample: $35

• If sequencing is necessary to confirm a new location: $60 for that sample
Future directions:

- Use quantitative PCR to relate amount of DNA to species density
- Test outside of range of species to confirm no false positives
- Test lower limits of detection

eDNA study #3: Headwater amphibians
(Goldberg et al. in review)
eDNA for detection of mudsnails

• Potential sources of mudsnail DNA:
  – Dead mudsnails
  – Waste products
  – Mucous
eDNA for detection of mudsnails

- To create an eDNA test for mudsnails:
  1. Identify unique DNA sequence
  2. Create and verify PCR test
  3. Collect water samples from a range of densities
  4. Run PCR test
  5. Sequence resulting fragment
eDNA for detection of mudsnails

• Ensuring no false positives:
  – Sterilize collecting supplies between samples
  – Clean room for extractions and PCR set up (no PCR product exposure)
  – Negative controls

• Interested in collaboration
Future of eDNA

• eDNA could have a huge impact on studies of aquatic systems
• Efficient multi-species detection and monitoring
• Detection and monitoring of elusive aquatic species
• Efficient early detection of invasive species
Thank you