Detection of Elusive Pool-Breeding Amphibians with Environmental DNA Analysis

Samara Davis
GENIUS OLYMPIAD 2022
41% of amphibian species are currently threatened with extinction. Lepofsky et al. compared physiological risks with climate projections. Climate change severely threatens amphibians at an increasing rate.

Amphibians are more vulnerable to the effects of climate change.

Vernal Pools

No single inflow of water $\rightarrow$ no fish

amphibian breeding and development

shorter hydroperiods, longer dry periods

Less reproduction of pool-breeding amphibians


More accurate, inexpensive, and expeditious biomonitoring is needed

Environmental DNA analysis is a new non-invasive biomonitoring technique

- eDNA metabarcoding determines the presence of multiple species

Ambystoma jeffersonianum and Ambystoma laterale are...

- Vernal pool breeding in the lower Hudson Valley region

- Special Concern by the NYSDEC

- Severe Concern by NEPARC

- Harbingers of climate change

- Naturally sedentary and elusive

Previous research has yet to apply eDNA metabarcoding to census vernal pool-breeding amphibians.

**Purpose**

Detect two threatened pool-breeding amphibian species using eDNA analysis.

Compare local historical data to recent temperature data.

**Hypotheses**

eDNA analysis will reveal the presence of Jefferson and Blue-spotted salamanders, and wood frogs.

Historical data will support a local warming trend.
Environmental Data

Collect data on env. variables to control for eDNA variation

Methods

Collected at the time of sampling

Sampled from 8 wetlands total

4 vernal pools

Results

Sample Site 4 → only site with full 10-week data

Air Temperatures and Water Temperatures of Sample Site 4 Over 10-week Breeding Season

Date of Data Collection

- Air temp
- Water temp
Historical Data

Compare local historical data to recent temperature data.

Methods

- Compiled weekly average surface temperatures
- LHV, NY, February - May 2001-2021

Results

0.84°C increase over 21 years

Finding Study Sites

In Lower Hudson valley region with vernal pools

Acquired sampling permissions and permits

NYSDEC Environmental Resource Mapper and Google Maps

Methods
Water Samples

500ml samples from bodies of water
Four vernal pools, eight wetlands total

Spring water – negative control

Methods

Stored frozen → thawed → filtered

Filters frozen → stored

DNA extracted, washed, and purified
eDNA analysis

Vert. 12S mitochondrial rRNA

Amplified through PCR

Riaz or MiFish primers

Next generation sequencing

GENEWIZ
eDNA analysis

BLASTed against GenBank nucleotide data

>96% identity
DNA Yields

DNA products after PCR, stained with SyberSafe dye, under UV transilluminator

200nM primers; 52C annealing; 40 cycles
Positive eDNA Detections

eDNA detections increase as breeding season progresses

≥96% identity threshold

Conservative ≥98% identity threshold

<table>
<thead>
<tr>
<th>Amphibian Detections Across All Sample Sites (96% identity, 92% coverage)</th>
<th>Number of Amphibian eDNA Detections in Vernal Pool Samples Over the Amphibian Breeding Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lithobates catesbeianus -1.4</td>
</tr>
<tr>
<td>2</td>
<td>Lithobates catesbeianus -2.4</td>
</tr>
<tr>
<td>3</td>
<td>Lithobates catesbeianus -4.2</td>
</tr>
<tr>
<td>4</td>
<td>Lithobates catesbeianus -4.4</td>
</tr>
<tr>
<td>5</td>
<td>Lithobates catesbeianus -3.1</td>
</tr>
<tr>
<td>6</td>
<td>Lithobates catesbeianus -3.3</td>
</tr>
<tr>
<td>7</td>
<td>Lithobates catesbeianus -3.4</td>
</tr>
<tr>
<td>8</td>
<td>Lithobates catesbeianus -4.5</td>
</tr>
</tbody>
</table>

Dates of Water Sample Collection:
- 2/28/2021
- 3/13/2021
- 3/27/2021
- 4/10/2021
- 4/25/2021
- 5/7/2021
eDNA Detections

Results

Less common

More common

Positive detection

Seq. alignment did not meet ID range (≥96%)

Negative detection

eDNA Detections of Vernal Pool-Breeding Amphibian Species Across All Sample Sites

- Amphibian Species
  - Ambystoma mexicanum
  - Ambystoma maculatum
  - Plethodon cinereus
  - Eurycea bislineata
  - Pseudacris crucifer
  - Rana sylvatica
  - Lithobates catesbeianus

Dates of Sample Collection

- 28-Feb
- 13-Mar
- 27-Mar
- 10-Apr
- 25-Apr
- 7-May
<table>
<thead>
<tr>
<th>eDNA Detections</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 25, 2021</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>5.0</th>
<th>5.1</th>
<th>5.3</th>
<th>5.6</th>
<th>5.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- **Amphibian species**
- **Fish species**
- **Negative control**
- **eDNA detections locality-specific**

- **Brook**
- **Vernal pool**

Results/Discussion
Detection of pool-breeding amphibians with eDNA metabarcoding

eDNA may be preferable for elusive amphibian species
There are no vernal pool protections in New York.

Increase of eDNA detections over breeding season

More holistic amphibian census when amphibians are most active in the pools

0.84°C warming trend over 21 years

Increased threat to amphibian populations

*www.fs.fed.us/ne/newtown_square/publications/other_publishers/OCR/ne_2004brooks01.pdf*. 
eDNA analysis will reveal the presence of Jefferson and Blue-spotted salamanders, and other vernal pool breeding amphibians.

eDNA analysis revealed the presence of multiple elusive pool-breeding amphibian species and two mole salamander species.
Limitations

- Contamination between samples
- DNA degradation in storage
- Incomplete database
- 1 sample per wetland -> Inconsistent detections
Applications

- Effect of climate change on vernal pools
- Population status of threatened mole salamanders
- eDNA analysis on elusive amphibian species

Discussion

conservation planning

Increased/more effective biomonitoring
Environmental DNA analysis effectively censuses pool-breeding amphibians in a threatened vernal pool habitat.
Acknowledgements

I would like to thank...
- Dr. Mark Stoeckle at The Rockefeller University for providing excellent guidance and supplies for this project.
- My friends and family for all their encouragement.
- Ms. Valerie Holmes and Mr. Angelo Piccirillo for providing endless support and resources.
Detection of Elusive Pool-Breeding Amphibians with Environmental DNA Analysis

Samara Davis
GENIUS OLYMPIAD 2022