Detection of Elusive Pool-Breeding Amphibians with Environmental DNA Analysis

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GENIUS OLYMPIAD 2022

41% of amphibian species are currently threatened with extinction [1]

Lepofsky et al. compared physiological risks with climate projections [1]

Climate change **severely threatens amphibians** at an increasing rate [1]

Amphibians are **more vulnerable** to the effects of **climate change** [2, 3, 4]

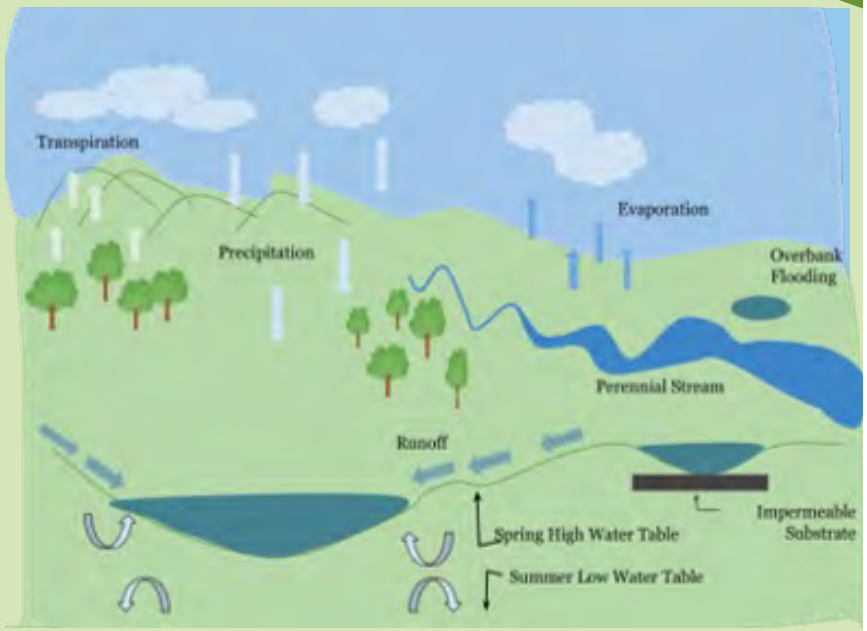
1. "The IUCN Red List of Threatened Species." IUCN Red List of Threatened Species, www.iucnredlist.org/resources/summary-statistics.
2. Lertzman-Lepofsky, Gavia F., et al. "Water Loss and Temperature Interact to Compound Amphibian Vulnerability to Climate Change." *Global Change Biology*, vol. 26, no. 9, 2020, pp. 4868–4879., doi:10.1111/gcb.15231.
3. Blaustein, Andrew R., et al. "Direct and Indirect Effects of Climate Change on Amphibian Populations." *Diversity*, vol. 2, no. 2, 2010, pp. 281–313., doi:10.3390/d2020281.
4. Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of USA*, 105, 11466–11473.

Vernal Pools

Fig. 2 Vernal pool at Teatown Nature Reservation. Photo by S. Davis

No single inflow of water → no fish [1]

amphibian breeding and development [1]



shorter hydroperiods, longer dry periods [2]

Less reproduction of pool-breeding amphibians [2]

1. "Woodland Pool Conservation." *Woodland Pool Conservation - NYS Dept. of Environmental Conservation*, www.dec.ny.gov/lands/52325.html.

2. Brooks, Robert T. "Weather-Related Effects on Woodland Vernal Pool Hydrology and Hydroperiod." *Wetlands*, vol. 24, no. 1, 2004, pp. 104–114., doi:10.1672/0277-5212(2004)024[0104:weowvp]2.0.co;2.

More accurate, inexpensive, and expeditious biomonitoring is needed

Environmental DNA analysis is a **new non-invasive biomonitoring** technique[1]

photo link

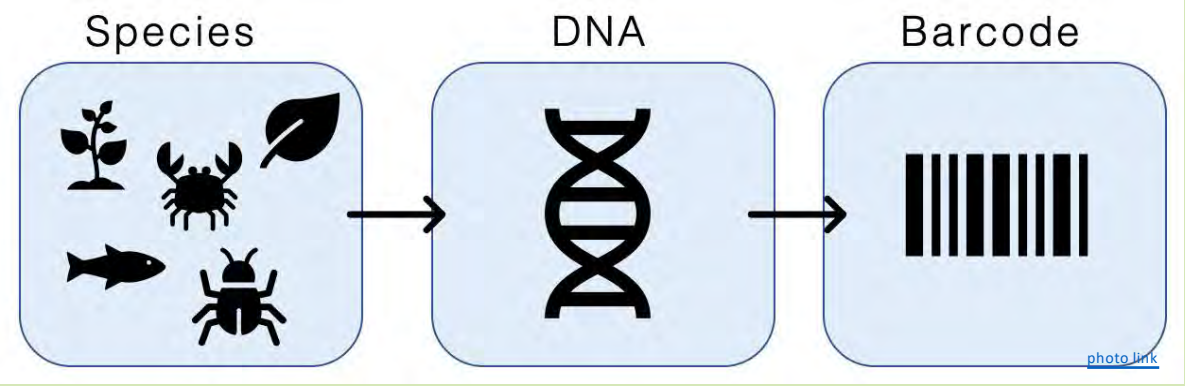


photo link

- eDNA metabarcoding determines the presence of **multiple species** [1]

1. Taberlet, Pierre, et al. "Soil Sampling and Isolation of Extracellular DNA from Large Amount of Starting Material Suitable for Metabarcoding Studies." Molecular Ecology, vol. 21, no. 8, 2012, pp. 1816–1820., doi:10.1111/j.1365-294x.2011.05317.x.
2. Dejean, Tony, et al. "Persistence of Environmental DNA in Freshwater Ecosystems." PLoS ONE, vol. 6, no. 8, 2011, doi:10.1371/journal.pone.0023398.

Ambystoma jeffersonianum and *Ambystoma laterale* are...

- Vernal pool breeding in the lower Hudson Valley region [2]

- **Special Concern** by the NYSDEC [1]

- **Severe Concern** by NEPARC [5]

-Harbingers of **climate change** [3]

- Naturally sedentary and **elusive** [4]

Jefferson salamander

Blue-spotted salamander

1. "List of Endangered, Threatened and Special Concern Fish & Wildlife Species of New York State." *List of Endangered, Threatened and Special Concern Fish & Wildlife Species of New York State - NYS Dept. of Environmental Conservation*, www.dec.ny.gov/animals/7494.html,
2. Gibbs, James P. *The Amphibians and Reptiles of New York State: Identification, Natural History, and Conservation*. Oxford University Press, 2007.
3. Bucciarelli, Gary M., et al. "Amphibian Responses in the Aftermath of Extreme Climate Events." *Scientific Reports*, vol. 10, no. 1, 2020, doi:10.1038/s41598-020-60122-2.
4. Petranka, James W. *Salamanders of the United States and Canada*. Smithsonian Books, 2010.
5. NEPARC. 2010. Northeast Amphibian and Reptile Species of Regional Responsibility and Conservation Concern. Northeast Partners in Amphibian and Reptile Conservation (NEPARC). Publication 2010-1.

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.

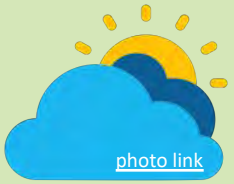
Hypotheses

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

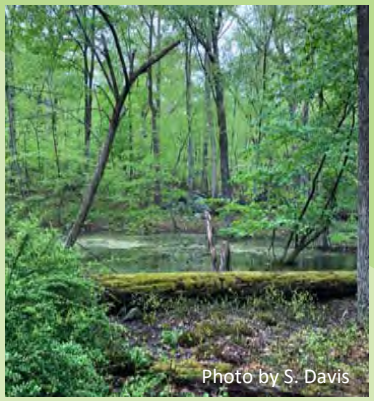


Methods

Collected at the time of sampling

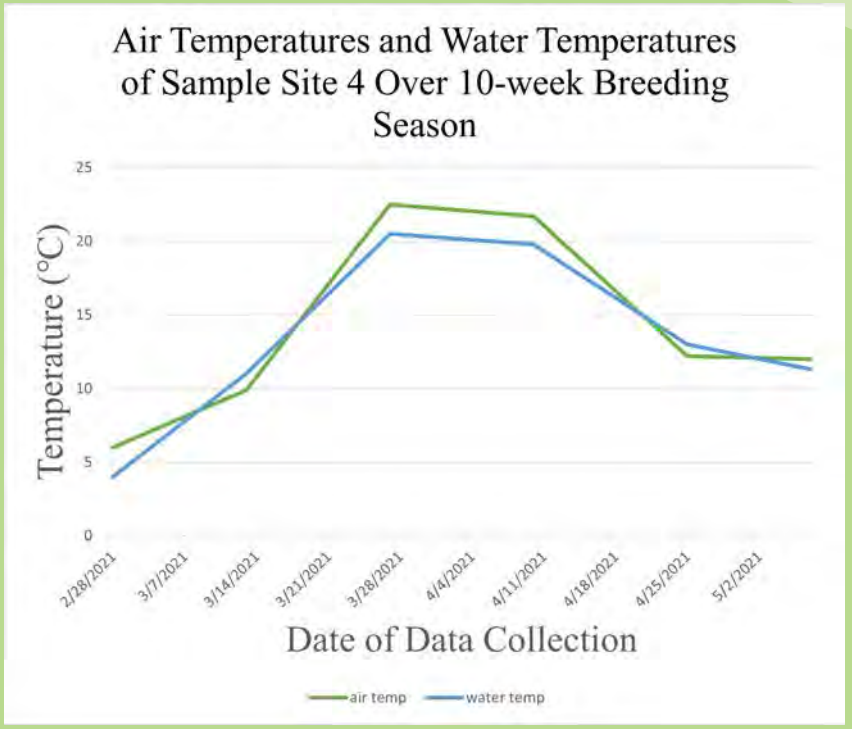
Sampled from 8 wetlands total

4 vernal pools



Results

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



Historical Data

Compare local historical data to recent temperature data.

photo link

Methods

-Compiled weekly average surface temperatures

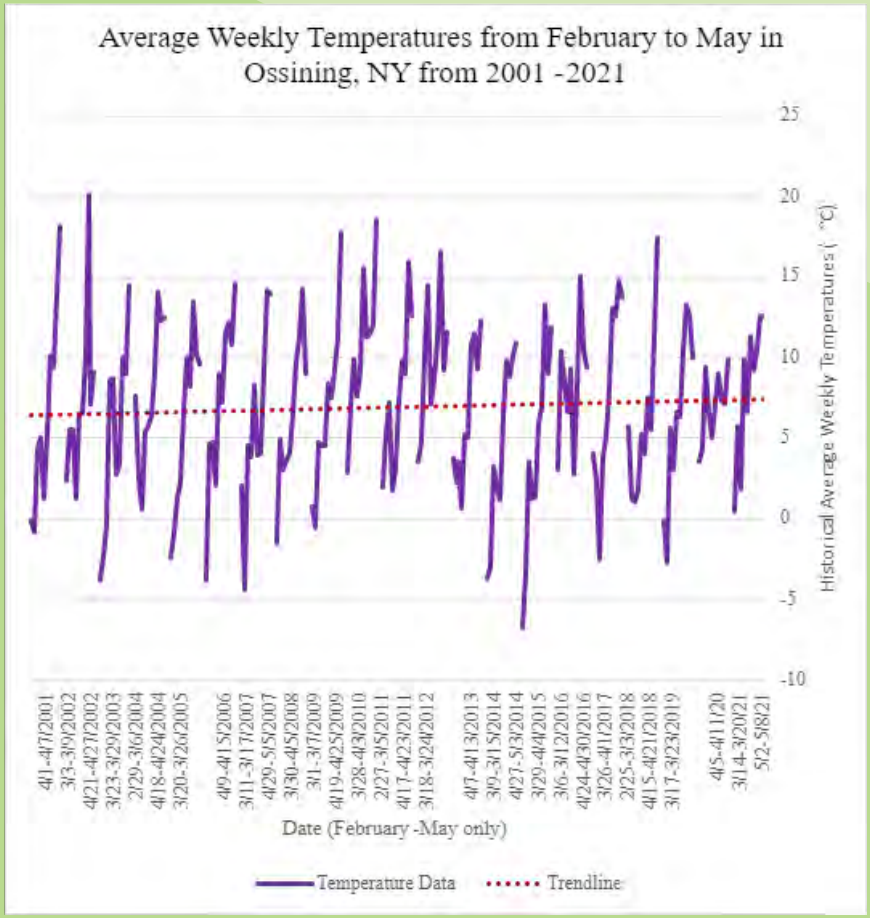
-LHV, NY, February - May 2001-2021

photo link

WEATHER UNDERGROUND

0.84°C increase over 21 years

Results



Fyfe, John C., et al. "Overestimated Global Warming over the Past 20 Years." *Nature Climate Change*, vol. 3, no. 9, 2013, pp. 767–69. *Crossref*, doi:10.1038/nclimate1972.

Methods

Finding Study Sites

In Lower Hudson valley region with vernal pools

Acquired sampling permissions and permits

Google Maps

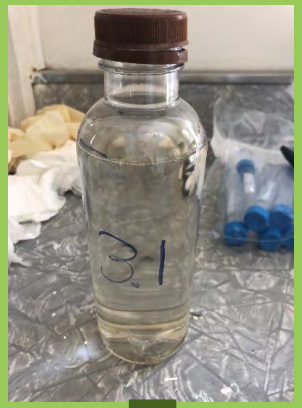
NYSDEC Environmental Resource Mapper and Google Maps

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

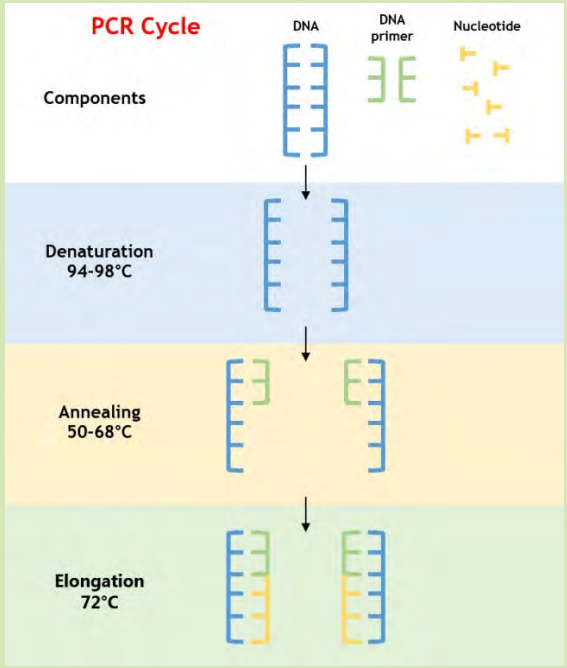
Methods

eDNA analysis

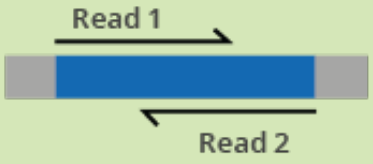
Vert. 12S mitochondrial rRNA

Riaz or MiFish primers

Amplified through PCR



Next generation sequencing



GENEWIZ

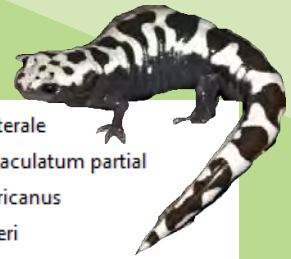
eDNA analysis

DA^2

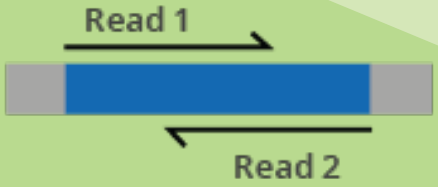
Amplicon Sequencing. **Exactly.** *Version 1.18*

BLASTed against GenBank nucleotide data

>96% identity



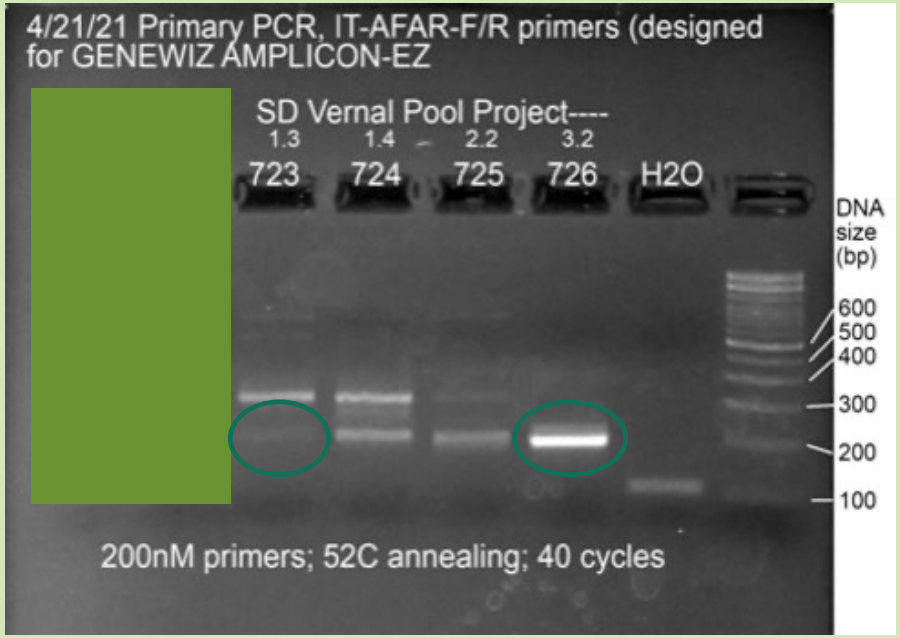
- ambystoma laterale
- ambystoma maculatum partial
- Anaxyrus americanus
- Anaxyrus fowleri
- Hemidactylium scutatum
- homo sapiens
- hyla versicolor partial
- Lithobates catesbeianus
- lithobates clamitans partial
- Lithobates palustris partial
- Lithobates pipiens
- Notophthalmus viridescens partial
- Odocoileus virginianus
- peromyscus leucopus
- Plethodon cinereus
- pseudacris crucifer partial
- rana sylvatica

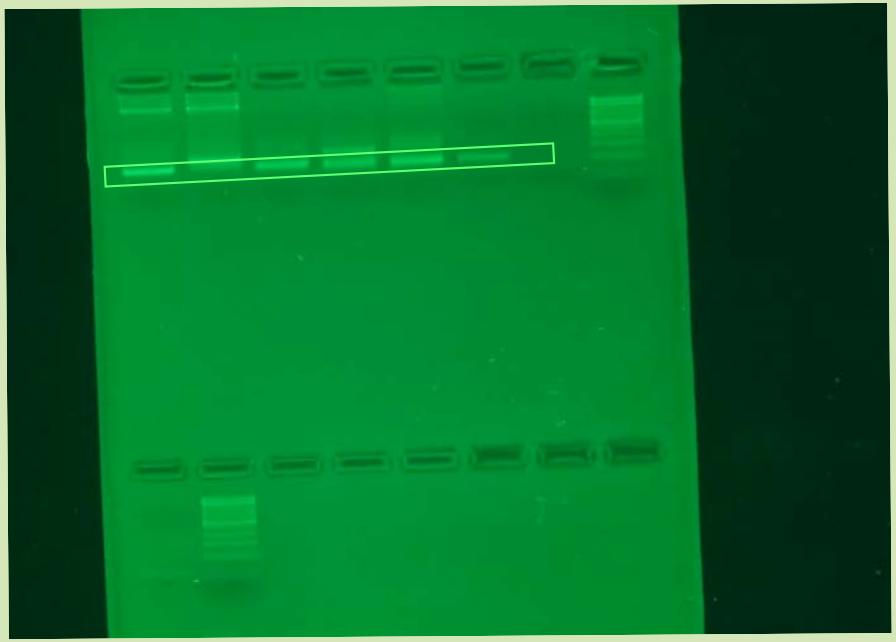


BLAST – Basic Local Alignment Search Tool

DNA Yields

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



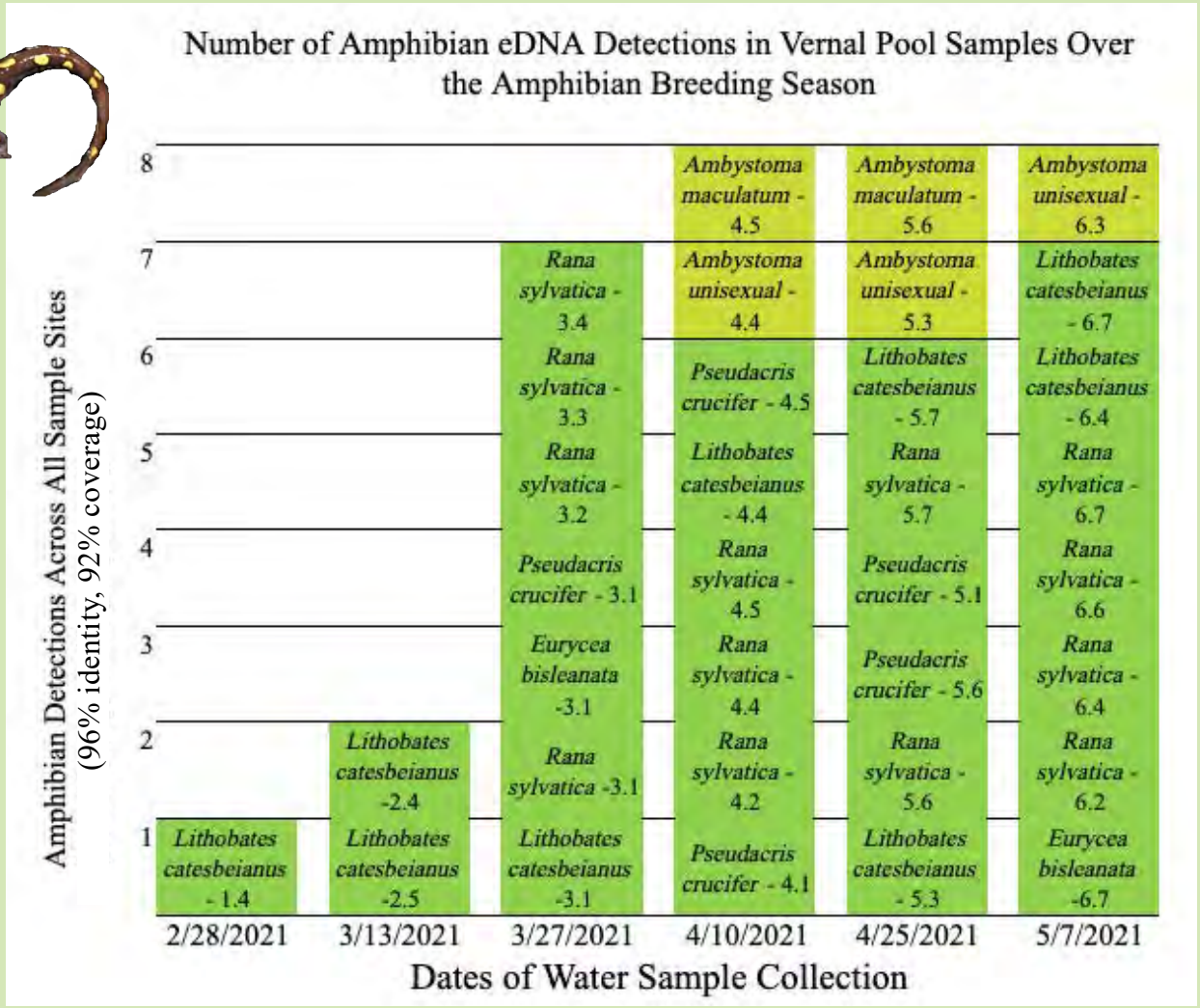


Positive eDNA Detections

eDNA detections increase as breeding season progresses

≥96% identity threshold

Conservative ≥98% identity threshold



Results

eDNA Detections

Less common

More common

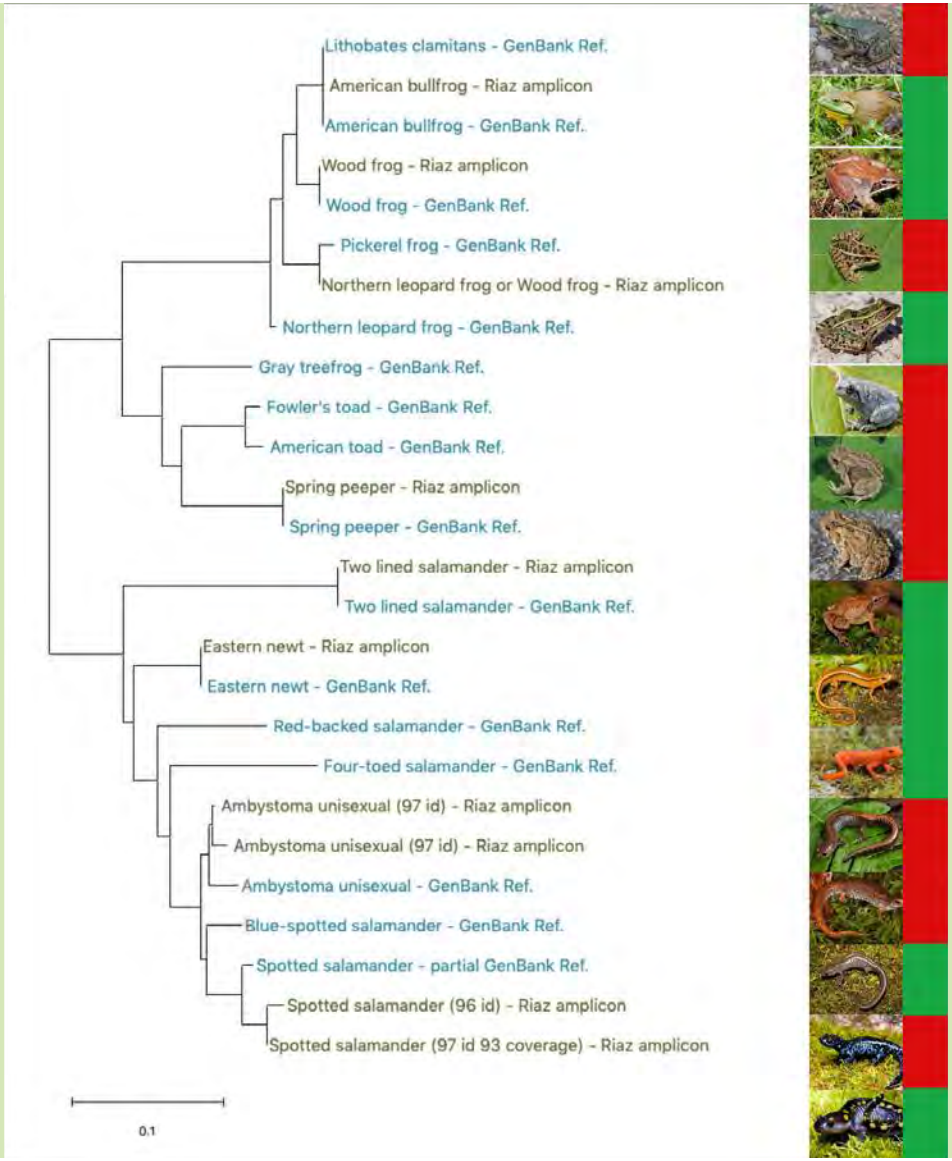
Positive detection

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

Negative detection

Results

eDNA Detections



eDNA Detections

April 25, 2021

5.0	5.1	5.3	5.6	5.7
0	0	0	0	0
0	0	36848	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	10268	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	5585	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	27
0	0	0	0	0
0	60684	0	0	0
0	0	0	0	0
0	0	0	0	0
0	35468	0	0	0
0	0	0	0	0
0	28664	0	0	0
0	0	0	0	0
0	0	0	0	0

Amphibian species

Fish species

Negative control

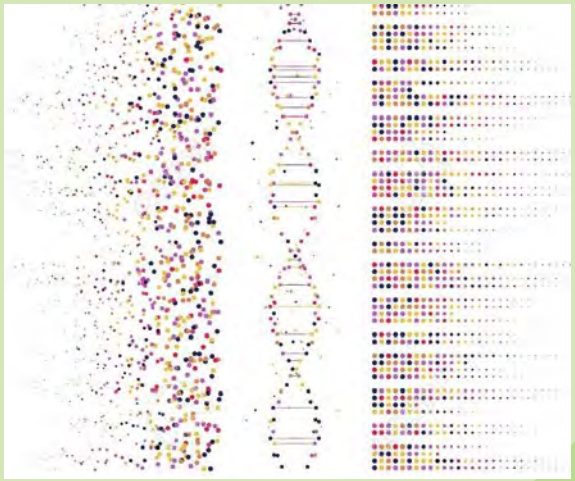
eDNA detections locality-specific

Brook

Vernal pool

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 [1]

Maine DEP

Wood frog

Jefferson salamander

Blue-spotted salamander

Significant vernal pool habitat and dependent species are protected [2]

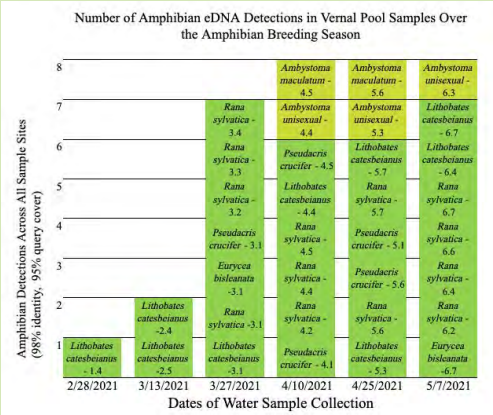
eDNA censuses vernal pools inexpensively and accurately

Contribute to vernal pool identification and protection in NY

1. New York Natural Heritage Program. 2022. Online Conservation Guide for *Vernal pool*. Available from: https://guides.nynhp.org/vernal-pool/. Accessed March 8, 2022
2. "Significant Vernal Pool Habitat, Natural Resources Protection Act, Maine Department of Environmental Protection." Maine Department of Environmental Protection, www.maine.gov/dep/land/nrpa/vernalpools. Accessed 23 Mar. 2022.

Discussion

Increase of eDNA detections over breeding season



More holistic amphibian census when amphibians are most active in the pools [1,2]

1. Souza, Lesley S. de, et al. “Environmental DNA (EDNA) Detection Probability Is Influenced by Seasonal Activity of Organisms.” *PLOS ONE*, edited by Hideyuki Doi, vol. 11, no. 10, 2016, p. e0165273. *Crossref*, doi:10.1371/journal.pone.0165273.

2. Wright, Alexander D., et al. “A Hierarchical Analysis of Habitat Area, Connectivity, and Quality on Amphibian Diversity across Spatial Scales.” *Landscape Ecology*, vol. 35, no. 2, 2020, pp. 529–44. *Crossref*, doi:10.1007/s10980-019-00963-z.

Discussion

0.84°C warming trend over 21 years

photo link

Increased threat to amphibian populations[1, 2]

photo link

2. Blaustein, Andrew R., et al. "Direct and Indirect Effects of Climate Change on Amphibian Populations." *Diversity*, vol. 2, no. 2, 2010, pp. 281–313., doi:10.3390/d2020281.
2. Brooks, Robert T. "Weather-Related Effects on Woodland Vernal Pool Hydrology and Hydroperiod." *Wetlands*, vol. 24, no. 1, 2004, pp. 104–14. *Crossref*, www.fs.fed.us/ne/newtown_square/publications/other_publishers/OCR/ne_2004brooks01.pdf.

Discussion

Hypothesis

eDNA analysis will reveal the presence of Jefferson and Blue-spotted salamanders, and other vernal pool breeding amphibians

? ?

Results

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

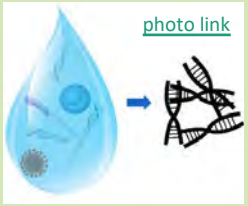
- Incomplete database

- 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

conservation planning

Increased/more effective biomonitoring

Conclusion

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.

Introduction

Review of Literature

Methods

Results

Discussion

Conclusion

Detection of Elusive Pool-Breeding Amphibians with Environmental DNA Analysis

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Photo by S. Davis

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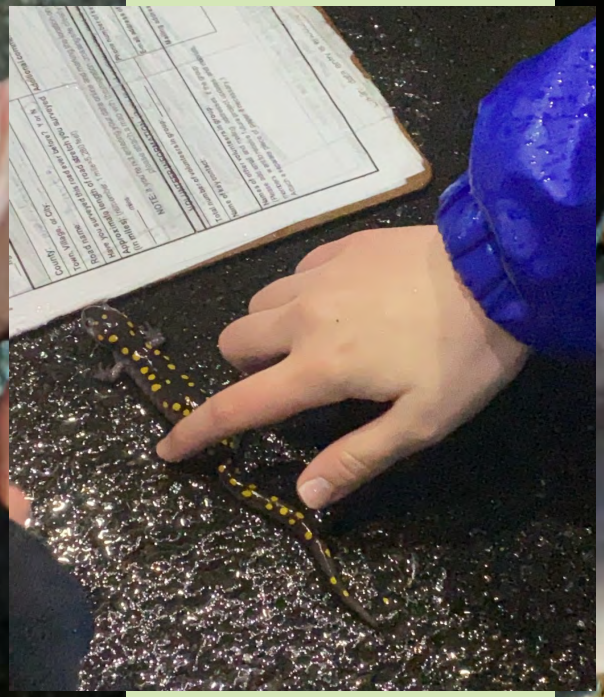


Photo by S. Davis