Validating assay for Northern leopard frogs, *Lithobates pipiens*: Ensuring accuracy and reliability

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BACKGROUND

Northern Leopard Frogs

- *Lithobates* or *Rana pipiens* are listed as "Imperiled" by the Navajo Nation[1].
- Listed as species of greatest conservation concern (SGCN Tier 1) in Arizona[2].
- *L. pipiens* require permanent water sources to reach reproductive maturity[3].
- Threats include:
  - Habitat loss
    - Climate change and grazing
  - Disease
  - Introduced species
    - Competition and predation
  - Exposure to pollutants

Environmental DNA

- eDNA is the genetic material left behind by an organism as it moves through the environment (*e.g.*, mucus, feces, tissue)[4].
- eDNA is a non-invasive method for monitoring wildlife and has worked successfully for aquatic species [4, 5].

Question

Can we use environmental DNA methods to determine Northern leopard frog distributions in Arizona and within the Navajo Nation?

METHODS

Sample Collection

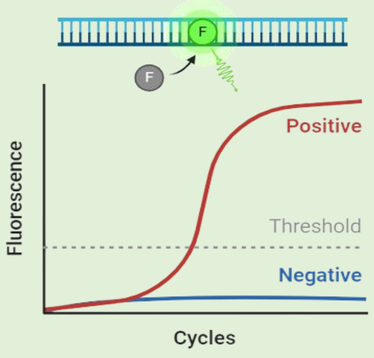
Bearizona and Stoneman Lake

Sample Filtration

Sample Preservation

DNA extraction

qPCR run



Data Analysis & Interpretation

STEPS

1. Collect 3 1L samples of water from 3 sites around each waterbody.
2. Filter collected water samples.
3. Preserve filters in silica beads.
4. Extract DNA from preserved filters.
5. Quantitative polymerase chain reaction run using extracted DNA from our samples against species-specific primers and probes provided by the Caren Helbing Lab.
6. If the species-specific probe breaks, it will cause a fluorescence, showing a positive amplification.

RESULTS

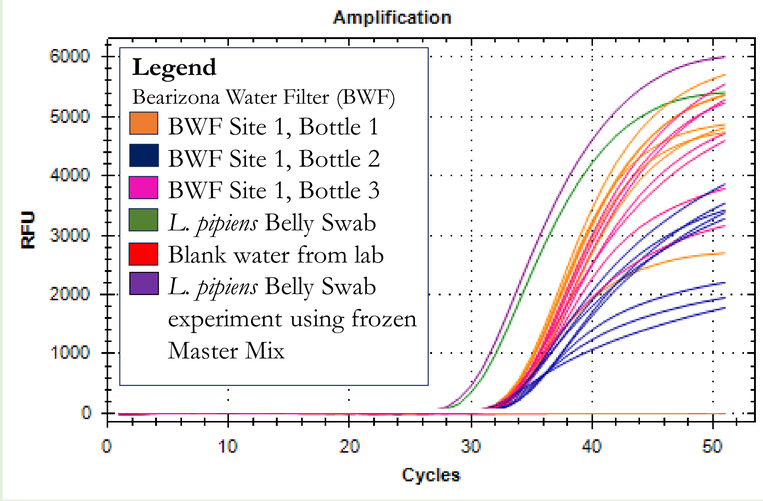


Figure 1: ***Positive Control.*** Amplification traces from a qPCR run using *Lithobates pipiens* primers and probes.

Water samples from *L. pipiens* tank enclosure and *L. pipiens* belly swabs collected from Bearizona.

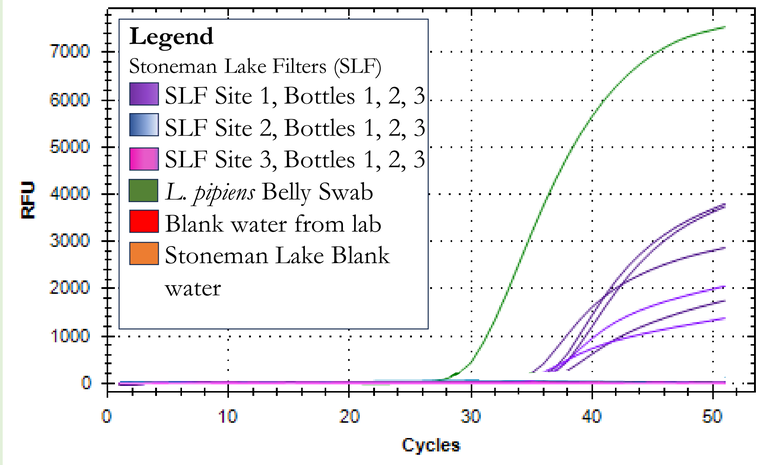


Figure 2: Amplification traces from a qPCR run using *L. pipiens* primers and probe. Collected from Stoneman Lake, AZ.

Site	Visual	eDNA Amplification
1	Yes	Yes
2	Yes	No
3	No	No

Site 1: Expected results achieved.
Site 2: *L. pipiens* visually observed but did not get a positive amplification using eDNA. Potential issues include the water body being too large or low species density.
Site 3: Poor water quality at this site. Expected results achieved.

Our successful validation of the Northern leopard frog assay using Bearizona populations and subsequent field testing at Stoneman Lake demonstrates the viability of utilizing eDNA to determine Northern leopard frog presence or absence.

NEXT STEPS

- Assay validation for more species and potential pathogens.
  - American bullfrog, crayfish, quagga mussel, chytrid, Ranavirus
- Comparing the efficacy of eDNA to more conventional wildlife monitoring methods.
  - Visual encounter surveys, audio calls, and camera traps
- Training people about the utility of this tool in the promotion of wildlife conservation and management on Indigenous lands.

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- Bearizona, Williams, AZ
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REFERENCES

1. Mikesic, D.G. 2008. *Vertebrate Animals of the Navajo Nation*. NNDFW.; **2.** Arizona Game and Fish Dept. 2023. *Rana pipiens (Northern leopard frog)*. AWCS, Awcs.azgfd.com.; **3.** US Fish and Wildlife Service. *Northern leopard frog (Rana pipiens)*. USFWS, FWS.gov.; **4.** Hobbs *et al.* 2019. *PLoS ONE*, 14(3), e0213849. **5.** Veldhoen *et al.* 2016. *PLoS ONE*, 11(11), e0164907.

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