An Unexpected Journey: Anuran decline research and the incidental elucidation of a new cryptic species endemic to the urban Northeast and Mid-Atlantic US.”

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A long time ago in a galaxy far, far away....
Enigmatic Extirpations

Can we learn more?

- Typically already occurred
- No individuals left to study
- No clear factors
- Considerable guesswork – satisfactory?
- High potential conservation value
Introduction

Leopard Frogs


Southern leopard frog
*Rana (Lithobates) sphenoecephala*

Northern leopard frog
*Rana (Lithobates) pipiens*
New York State Declines
“Staten Island’s most common species…”
_Anonymous, Proceedings of Nat. Sci. Ass. of Staten Island, 1898._

“very common on the salt marshes of [Long Island].”
_Frank Overton, Brooklyn Museum of Arts and Sciences, 1914._

“Common. Usually a salt marsh or coastal plain frog [New York City vicinity].”
_G. Kingsley Noble, AMNH, 1927._

“most abundant frog in this vicinity [Long Island].”
_Loring Turrell, The Natural History of Smithtown, 1939._

“the green frog was...never as abundant as the leopard frog”
“could have been seen almost anywhere [Long Island]”
“common in the white cedar swamp.”
_Roy Latham, Engelhardtia,1971._

“common in salt marsh areas [Long Island].”
_Sam Yeaton, TNC, 1973._

“quite common...along the Preserve’s eastern shore [Shelter Island].”
_TNC Biological Resource Inventory, 1982._
Introduction

Historical Localities & Timeline

- **1940**: Nearly ubiquitous in wetlands throughout Long Island.
- **1950**: Rural fringes in Brooklyn, Queens, Nassau. Common in Suffolk.
- **1960**: Rural fringes in Queens, Nassau. Locally common in Suffolk.
- **1970**: Rural fringes in Queens, Nassau. Locally common in Suffolk.
- **1980** - **2000**: Restricted to a few final locales in central & eastern Suffolk.

Map Source: John Cryan, NYSDEC
Biogeographic Context


Southern leopard frog
*Rana (Lithobates) sphenoecephala*

Northern leopard frog
*Rana (Lithobates) pipiens*
Background (Cryptic Species)

• What factors led to this enigmatic extirpation?
• Can *in situ* experiments provide insight?
• Can research on this decline provide a tool for investigating other declines elsewhere?
Chapter 1

Methodological Pathway

Start ➔ Egg mass collection (NJ Pinelands)

Survival Outcomes

- Tad dies
- Tad survives less growth and no metamorphosis
- Tad survives. Normal development into frogs

Raising and monitoring (several months)

Brief captive rearing

Deployed to high quality sites ~Gosner 25 (5-7mm)
Field Work
Chapter 1

Field Sites

Site 1
Site 2
Site 3
Site 4
Site 5
Site 6

No invasion site
Moderate invasion site
Heavy invasion site
Mesh enclosure, 6 tadpoles each

Experimental
Source
Initial Survival Results (Trial 1: 2007)

Date:
- 07/03/07
- 08/07
- 09/07
- 10/07
- 11/07
- 12/07

Survival Rate:
- 100%
- 90%
- 80%
- 70%
- 60%
- 50%
- 40%
- 30%
- 20%
- 10%
- 0%

Survival Percentage:
- 80.6%
- 60.0%
- 41.7%
- 30.6%
- 0.0%
Enter: Staten Island

2002-2006: Extirpation (1990s)

2007: Enclosure Experiments Begin

2007: Learned of Staten Island (SI)

2008: First breeding assemblage

2008-09: Enclosure Experiments Continue

2008-2010: “Shopped” idea to geneticists

2008: First SI observation
A new species of leopard frog (Anura: Ranidae) from the urban northeastern US

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Abstract

Past confusion about leopard frog (genus Rana) species composition in the Tri-State area of the US that includes New York (NY), New Jersey (NJ), and Connecticut (CT) has hindered conservation and management efforts, especially where populations are declining or imperiled. We use nuclear and mitochondrial genetic data to clarify the identification and distribution of leopard frog species in this region. We focus on four problematic frog populations of uncertain species affiliation in northern NJ, southeastern mainland NY, and Staten Island to test the following hypotheses: (1) they are conspecific with Rana sphenocephala or R. piperi, (2) they are hybrids between R. sphenocephala and R. piperi, or (3) they represent one or more previously undescribed cryptic taxa. Bayesian phylogenetic and cluster analyses revealed that the four unknown populations collectively form a novel genetic lineage, which represents a previously undescribed cryptic leopard frog species, Rana sp. nov. Statistical support for R. sp. nov. was strong in both the Bayesian (pp = 1.0) and maximum-likelihood (bootstrap = 99) phylogenetic analyses as well as the Structure cluster analyses. While our data support recognition of R. sp. nov. as a novel species, we recommend further study including fine-scaled sampling and ecological, behavioral, call, and morphological analyses before it is formally described.
Objectives

• Analyze mtDNA & nuDNA

• Four unknown populations:
  • Northern NJ (1)
  • Southeast NY (2)
  • Staten Island (1)

• Hypotheses:
  1. Conspecific with *R. pipiens* or *R. sphenocephala*
  2. Hybrids between *R. pipiens* and *R. sphenocephala*
  3. Neither = previously undescribed lineage
Methods

- Tissues sampled across Tri-State area (experimental & controls)
- 3-10 frogs/site
- Sequence regions:
  - Mitochondrial:
    - ND2 and 12S–16S regions (1444 bp)
  - Nuclear:
    - NTF3 (599 bp),
    - Tyr (557–585 bp),
    - Rag-1 (647–683 bp),
    - SIA (362–393 bp)
    - CXCR4 (550 bp)
- Bayesian and Maximum Likelihood Analyses for both
Results: Summary

• Both analyses = strong support for four distinct clades:
  1. *R. sphenocephala*
  2. *R. pipiens*
  3. *R. palustris*
  4. *Rana* sp. nov.

• Genetic Divergence:
  • 6.79% (*R. palustris*),
  • 11.0% (*R. sphenocephala*),
  • 12.5% (*R. pipiens*)

• Sister group: *R. palustris* (mtDNA)

• No hybridization

• Potential sympatry: CT (*R. pipiens*)
Chapter 2

Results: Phylogenies

Mitochondrial phylogeny

Nuclear phylogeny

Considerations

• Northeast/Mid-Atlantic: endemism & novel species

• Most cryptic with *R. sphenocephala* – very similar visually

• Taxonomic replacement of *R. sphenocephala* in region (e.g., NY/PA/CT)

• “Firsts”
  – NY amphibian since 1854
  – Northeast amphibian (NY + New England) since 1882
  – Anuran NA East coast since 1955
  – Anuran US/Canada since cryptic *Pseudacris fouquettiei* in 2008
Chapter 3

Taxonomy: Describing the Species

1. Bioacoustics
   - R. sp. nov
   - R. sphencephala
   - R. pipiens
   - R. palustris
   - R. sylvatica

2. Additional Genetics (holotype verification)

3. Behavior/Phenology

4. Distribution/Ecology

5. Morphology
A new species confirmed

R. sp. nov.  R. sphenoecephala  R. pipiens
Chapter 3

Secondary Diagnostics

R. sp. nov.
R. pipiens

R. sp. nov.
R. sphenocephala

Heavy Breeding Period
Scattered Breeding Activity

Philadelphia, PA
Northern Vermont

Southern LF (*R. sphenoecephala*)

Photo credit: Chris Camacho
Atlantic Coast LF (*R. kauffeldi*)
Habitats
Atlantic Coast Leopard Frog, Rana (Lithobates) kauffeldi

Chapter 3

Feinberg et al., 2014. Cryptic diversity in Metropolis: confirmation of a new leopard frog (Anura: Ranidae) from New York City and surrounding Atlantic Coast regions. PLOS One 9 (10), e0108213.
How did a large, conspicuous, acoustically distinct frog remain misidentified across a global population center with strong taxonomic infrastructure?

- Short calling season, primarily cold/rainy nights
- Call variant (chorus)
- Bioacoustic curveball: the wood frog

- *Phragmites*
- Cryptic Species
Cryptic Leopard Frog Species

*Rana* sp. nov. (NY)

*Rana* sp. nov. (NY)

*Rana* sp. nov. (NY)

*R. pipiens* (NY)

*R. sphenocephala* (NJ)

*R. sphenocephala* (NJ)
Conservation Considerations

- Uncommon endemic range (I-95 Corridor)
- Top 5 global mega-region (Florida et al. 2008)
- Expansive wetlands (clear, shallow, open-canopy)
- Industrialized landscapes (heavy impacts)
- Coastal distribution (climate change)
- Fragmented populations, clustered breeding behavior, extirpations
- Not 1, but 3 impacted species
- Reintroduction Risks
New species of frog found in New York City – first time since 1882

New Leopard Frog Found in New York City

Atlantic Coast leopard frog

Big City, Big Surprise: New York City’s Newest Species Is a Frog

A Young Scientist Makes a Remarkable Discovery in New York City
Meanwhile...
1. Were critical NYC-area populations lost or destroyed?

2. If not, what post-storm changes occurred to their size and vigor?

3. How did water chemistry, especially salinity, change among sites in the tidal storm-surge floodplain?
1. Rapid survival assessments at five focal study areas in the NYC metro region.
2. Assess size and intensity of breeding choruses, and where possible, compare to pre-storm data.
3. Measure basic water quality attributes, and compare to pre-storm data.
Chapter 4

Water Quality: Salinity

Pre-Storm sites (2006 + 2012):
- $n=14$; mean = 0.89 ppt ± 0.64 SD

Post-Storm sites (2013):
- $n=10$; mean = 2.74 ppt ± 1.56 SD

A threefold increase (207%); Significance: $t=3.55$, two-tailed $p < 0.01$
Considerations

• ACLF can survive large storms
• Total # of at-risk sites increased, but percentage decreased
  – Pre-storm: 17 (74% at-risk)
  – Post-storm: 20 (65% at-risk)
• No study area destroyed; impacts likely worse closer to Atlantic coastline.
• Sub-populations may not vanish but shift.
• Hurricane-aided expansion?
• Limitations inherent in pre-storm data
• Subset of enclosure-raised tadpoles tested for heavy metal levels
• Sibling transplant experiment
• Experimental sites + NJ source site
• Both leopard frog species included
• Wild-caught bullfrog tadpoles
## Species Comparisons

### All Tadpoles (species groups)

<table>
<thead>
<tr>
<th></th>
<th>Bullfrog</th>
<th>Leopard Frog</th>
<th>$X^2(p)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N = 11$</td>
<td>$N = 49$</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>$4.76 \pm 2.14$</td>
<td>$1.93 \pm 3.52$</td>
<td>$12.9 (&lt;0.001)$</td>
</tr>
<tr>
<td>Chromium</td>
<td>$0.25 \pm 0.21$</td>
<td>$1.44 \pm 1.39$</td>
<td>$17.3 (&lt;0.0001)$</td>
</tr>
<tr>
<td>Cadmium</td>
<td>$0.17 \pm 0.15$</td>
<td>$0.28 \pm 0.30$</td>
<td>$NS$</td>
</tr>
<tr>
<td>Mercury</td>
<td>$0.19 \pm 0.07$</td>
<td>$0.08 \pm 0.13$</td>
<td>$13.4 (&lt;0.001)$</td>
</tr>
<tr>
<td>Lead</td>
<td>$2.91 \pm 1.92$</td>
<td>$3.70 \pm 3.50$</td>
<td>$NS$</td>
</tr>
<tr>
<td>Selenium</td>
<td>$2.14 \pm 0.62$</td>
<td>$1.66 \pm 1.56$</td>
<td>$NS$</td>
</tr>
</tbody>
</table>
Conclusions

• Siblings: truly are a product of their environment
• Did not see significant differences between *R. sphenoecephala* and *R. kauffeldi*
• Combined given unbalanced sample sizes
• Adult LFs typically had lower metal levels than tadpoles
• Substantial tadpole differences between wild-caught bullfrogs vs. enclosure-reared LFs
• NJ source site (control): highest levels Pb and Cd
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