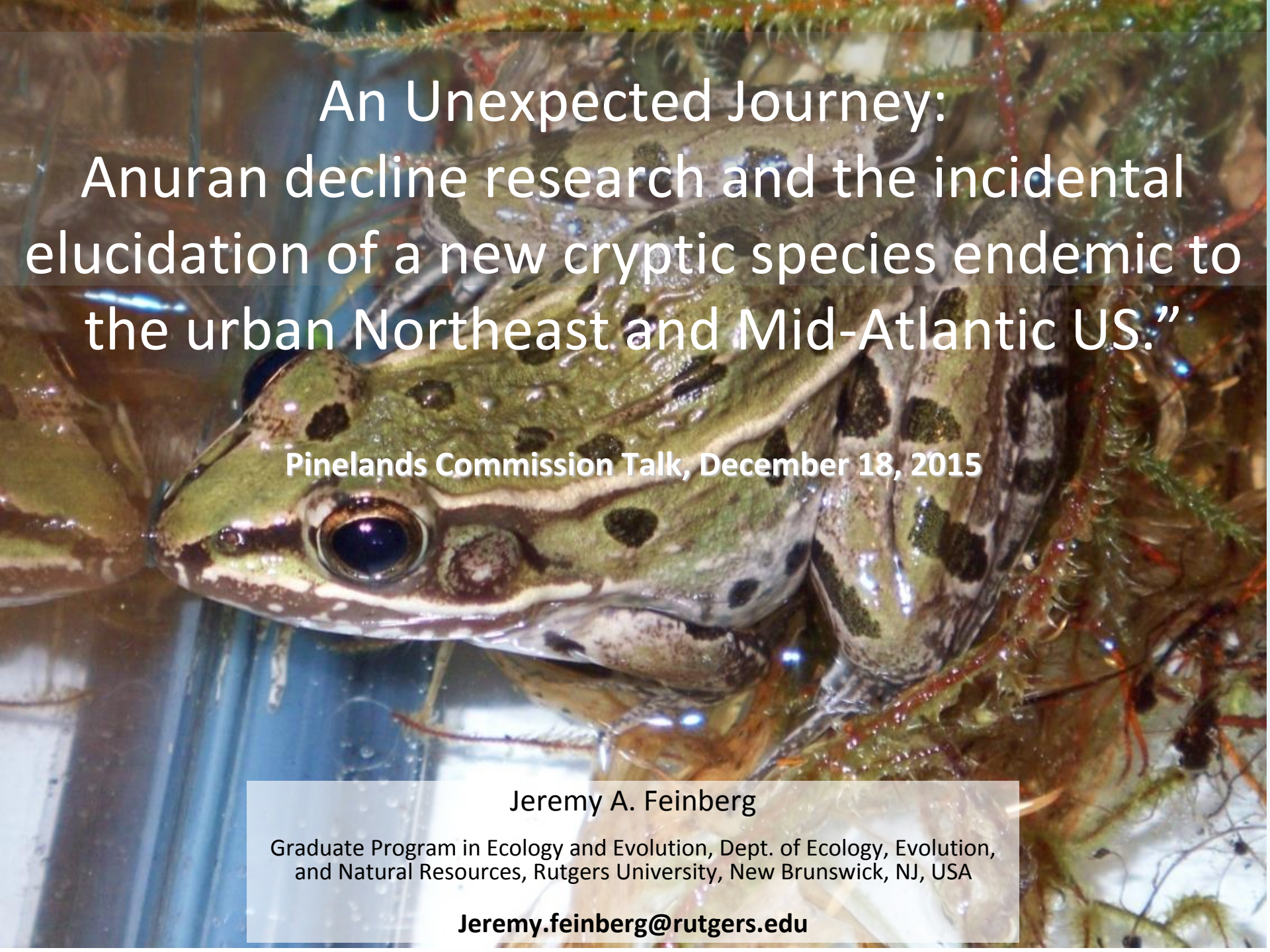


An Unexpected Journey: Anuran decline research and the incidental elucidation of a new cryptic species endemic to the urban Northeast and Mid-Atlantic US.”

Pinelands Commission Talk, December 18, 2015

Jeremy A. Feinberg

Graduate Program in Ecology and Evolution, Dept. of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ, USA

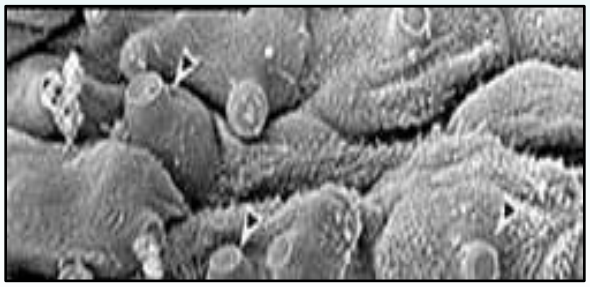
Jeremy.feinberg@rutgers.edu

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

Enigmatic Extirpations

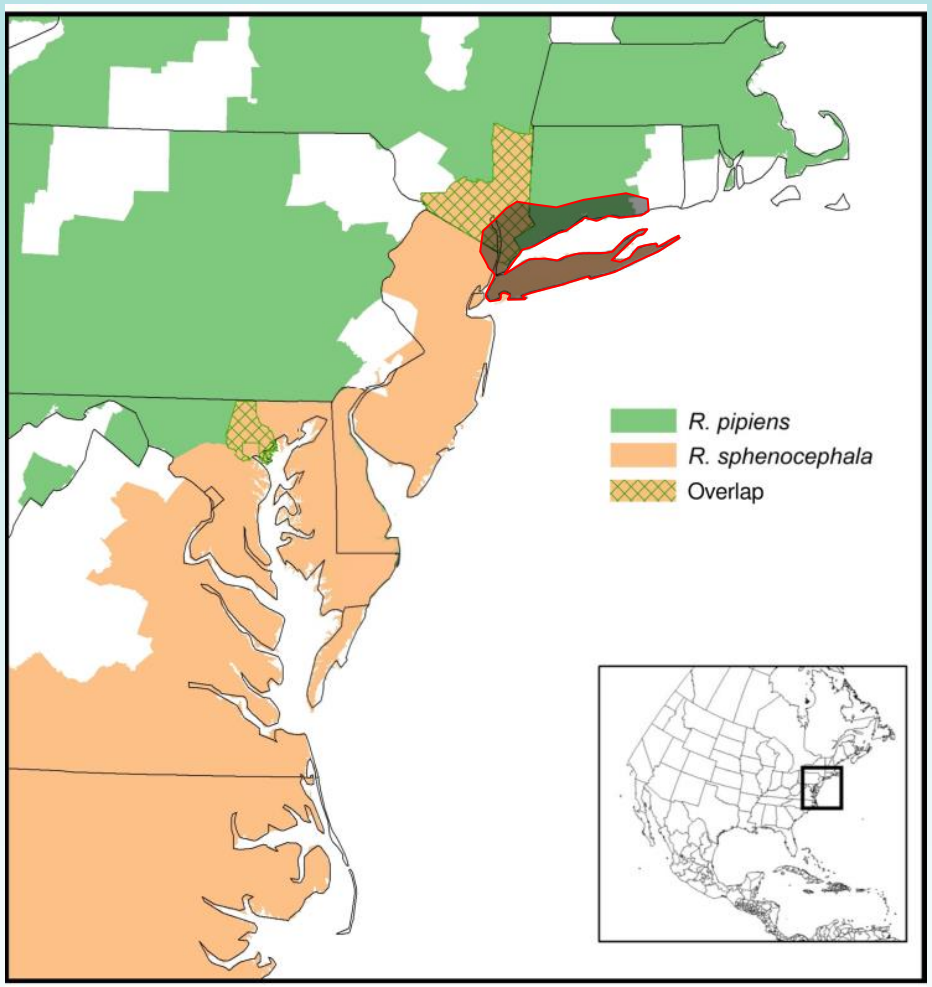
Can we learn more?

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



Leopard Frogs

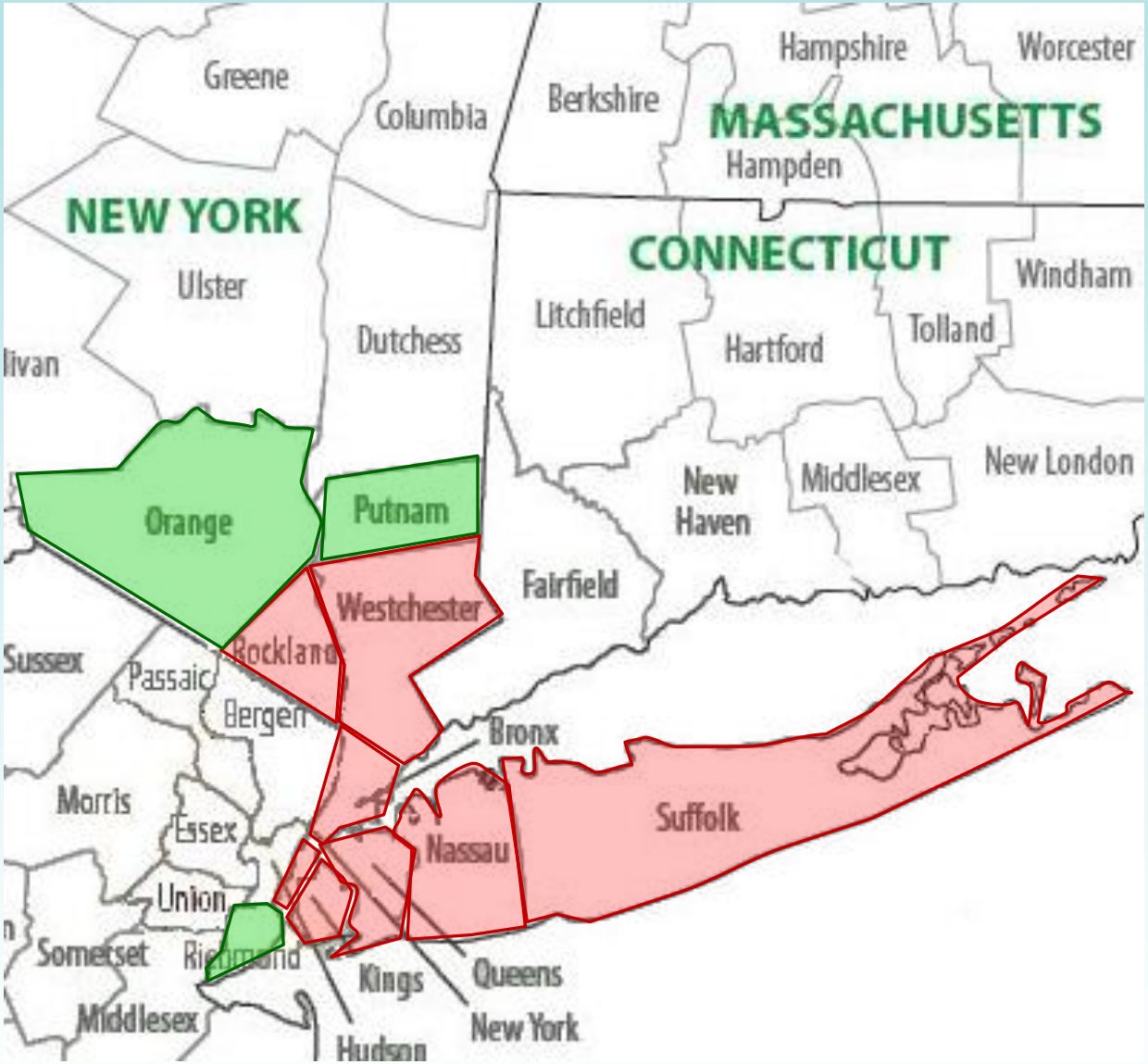
Southern leopard frog
Rana (Lithobates) sphenocephala



Map source: IUCN Red List spatial data collection (2012) (www.iucnredlist.org).

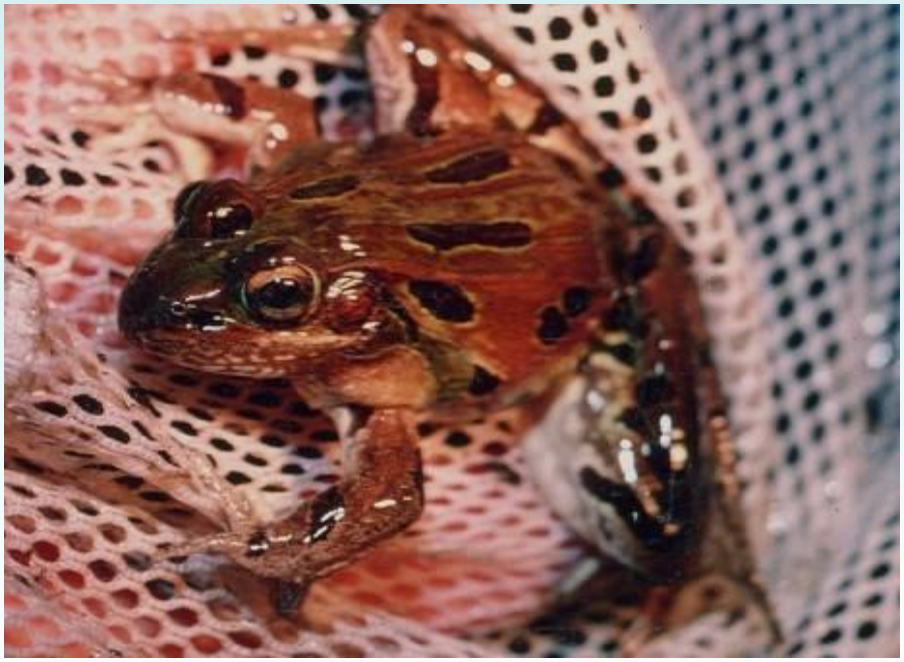
Northern leopard frog
Rana (Lithobates) pipiens

New York State Declines



Frank Overton. Fire Island. ca. 1911

Frank Overton. Fire Island. ca. 1911



Andy Sabin. Montauk. ca. 1991

Robert Villani. Montauk. ca. 1997

Historical Abundance

"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.

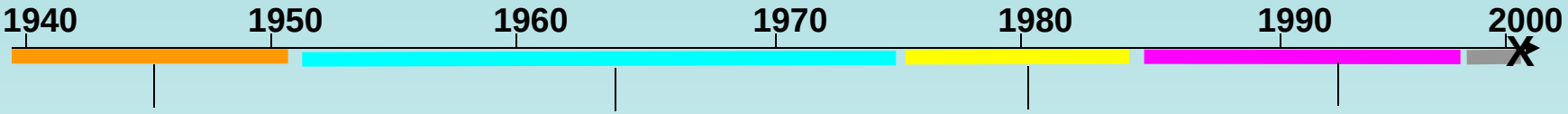
"most abundant frog in this vicinity [Long Island]."
Loring Turrell, The Natural History of Smithtown, 1939.

"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.

Historical Localities & Timeline

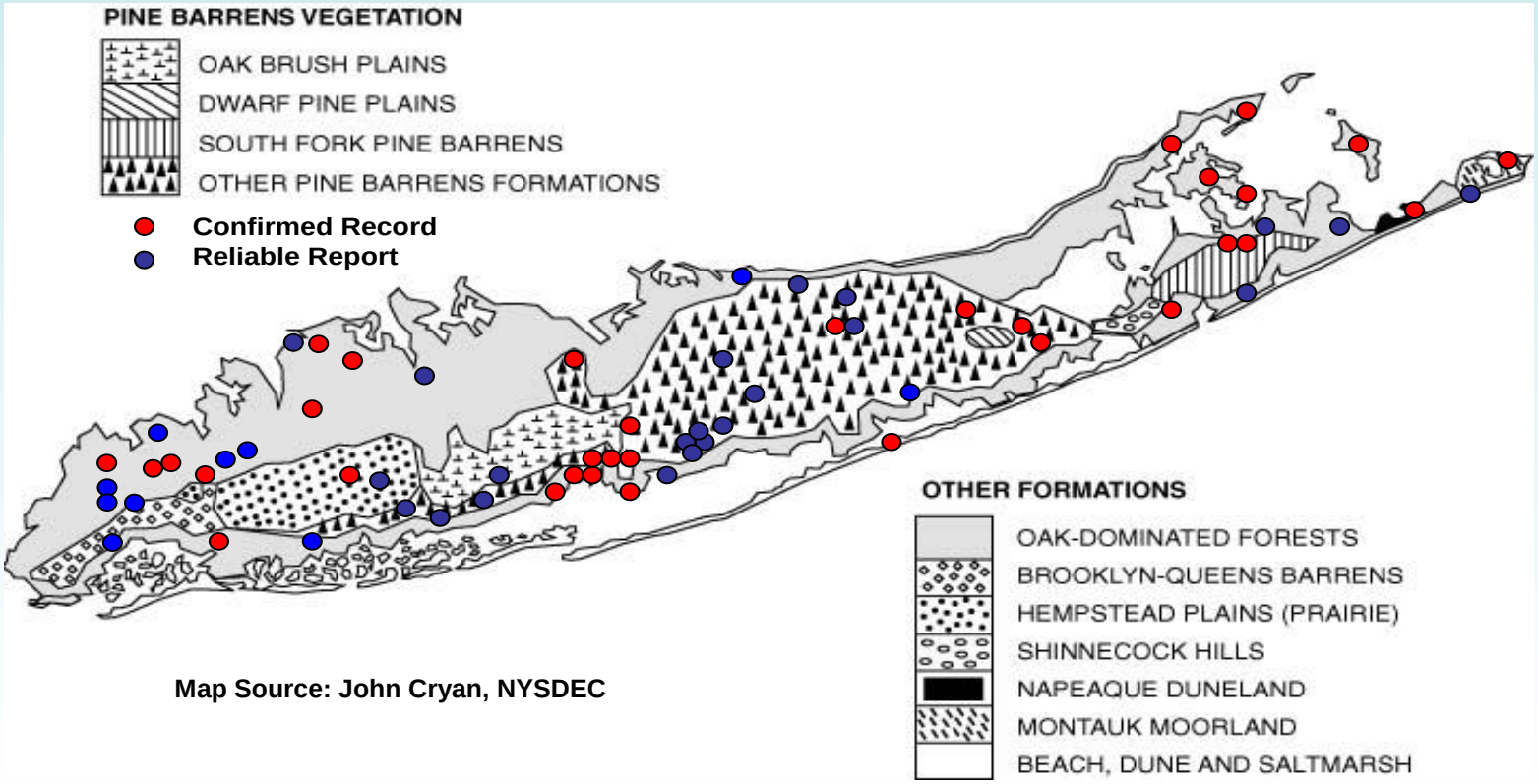


Nearly ubiquitous in wetlands throughout Long Island

Rural fringes in Brooklyn, Queens, Nassau. Common in Suffolk

Rural fringes in Queens, Nassau. Locally common in Suffolk

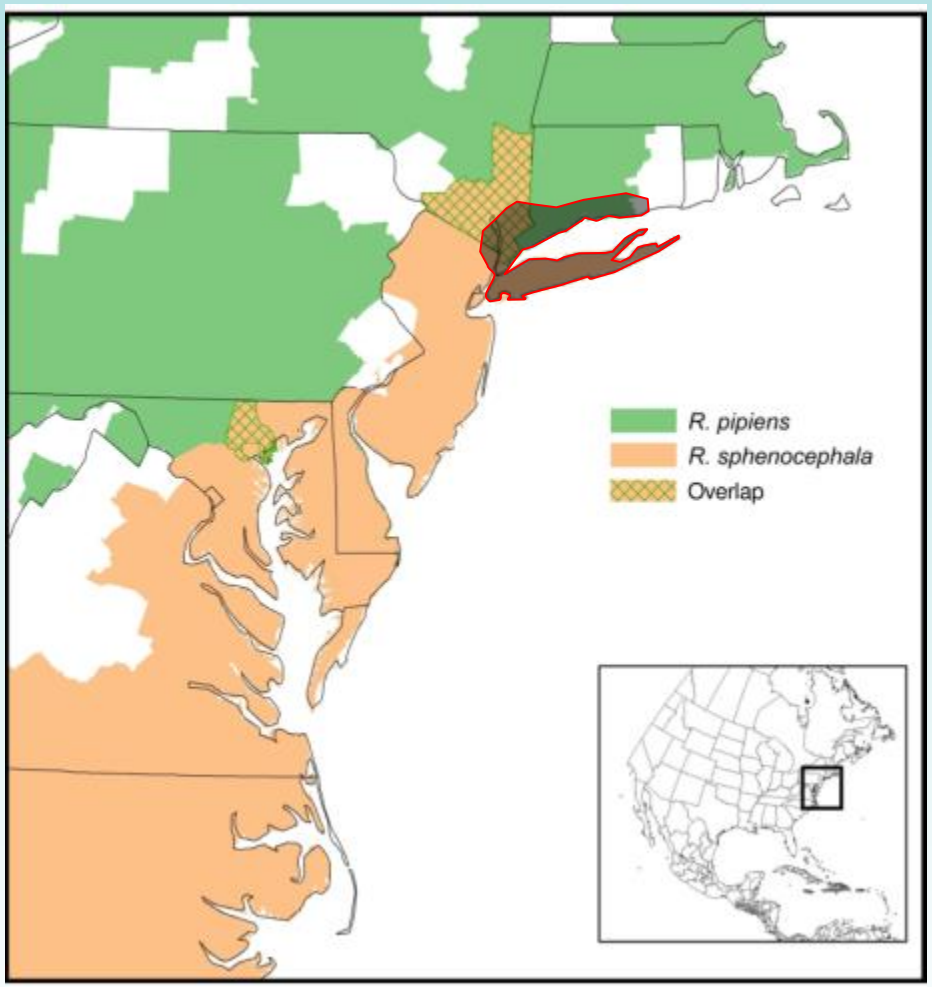
Restricted to a few final locales in central & eastern Suffolk



Map Source: John Cryan, NYSDEC

Biogeographic Context

Southern leopard frog
Rana (Lithobates) sphenocephala



Map source: IUCN Red List spatial data collection (2012) (www.iucnredlist.org).

Northern leopard frog
Rana (Lithobates) pipiens

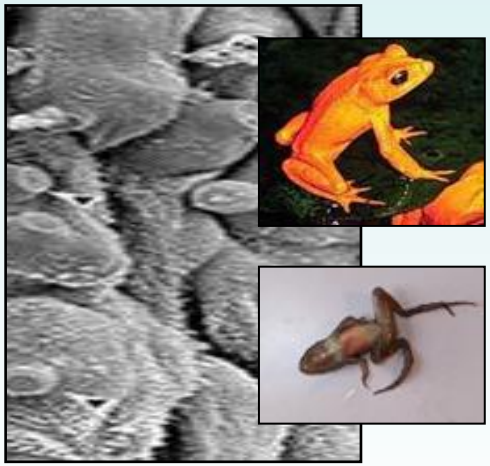
Background (Cryptic Species)

Lemmon et al. 2008. Zootaxa, 1675: 1-30

Initial Research Interests

- 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?

Disease



Contaminants*

Habitat Invasion

Methodological Pathway

Start → Egg mass collection **(NJ Pinelands)**

Brief captive rearing

Deployed to high quality sites
~Gosner 25 (5-7mm)

Raising and monitoring
(several months)

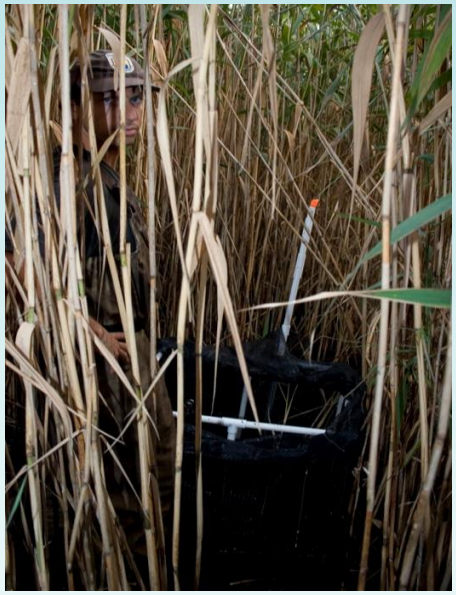
Survival Outcomes

Tad dies

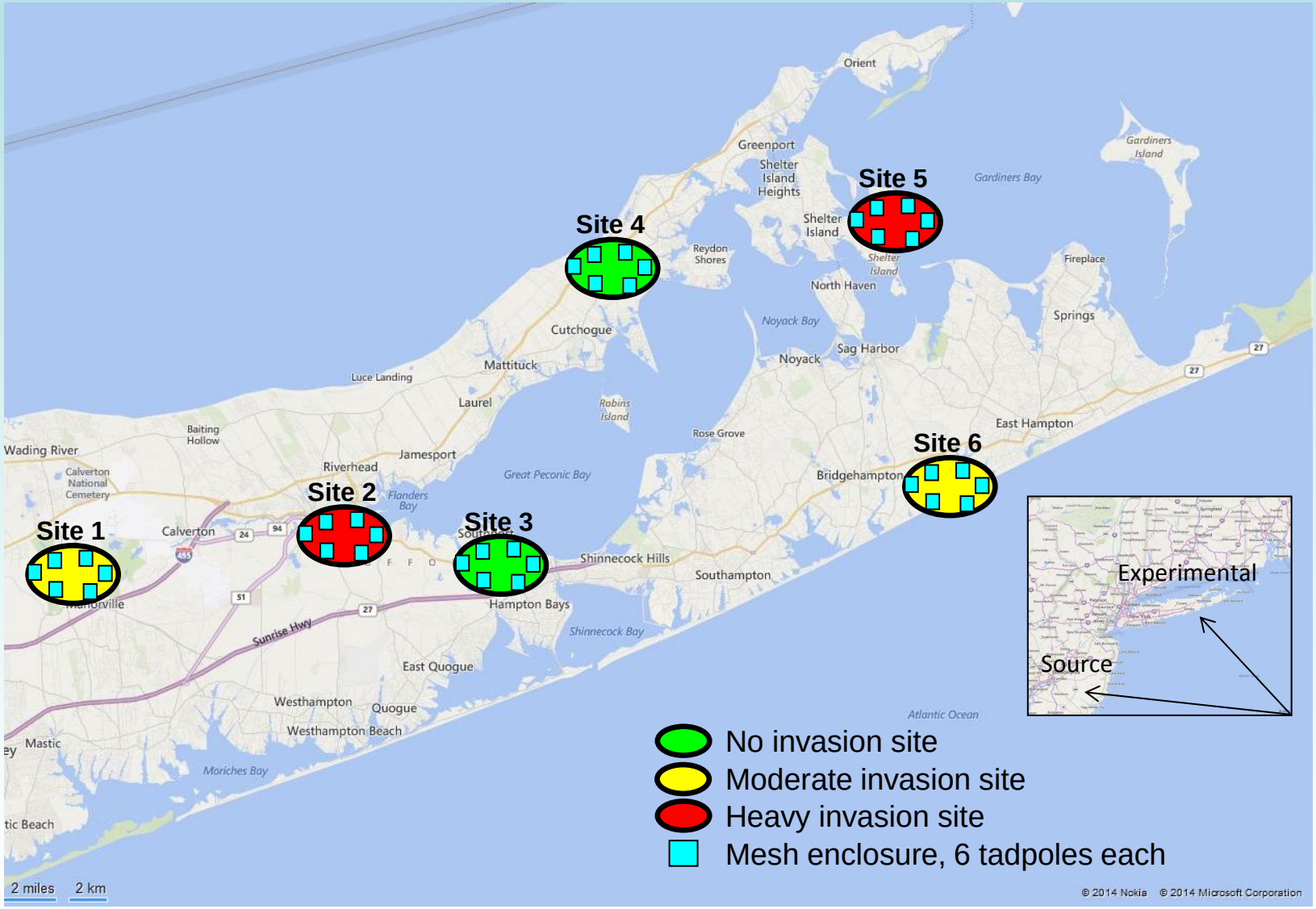
Tad survives less growth and no metamorphosis

Tad survives. Normal development into frogs

Field Work



Field Sites


Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Orient
Greenport
Shelter Island Heights
Shelter Island
Gardiners Island
Gardiners Bay
Reydon Shores
Fireplace
North Haven
Noyack Bay
Springs
Cutchogue
Noyack
Sag Harbor
Mattituck
Luce Landing
Laurel
Robins Island
East Hampton
Rose Grove
Bridgehampton
Baiting Hollow
Wading River
Jamesport
Riverhead
Calverton National Cemetery
Great Peconic Bay
Flanders Bay
Calverton
Shinnecock Hills
Southampton
Hampton Bays
Sunrise Hwy
Shinnecock Bay
East Quogue
Westhampton
Quogue
Westhampton Beach
Mastic
Moriches Bay
Atlantic Ocean
Experimental
Source
No invasion site
Moderate invasion site
Heavy invasion site
Mesh enclosure, 6 tadpoles each
2 miles
2 km
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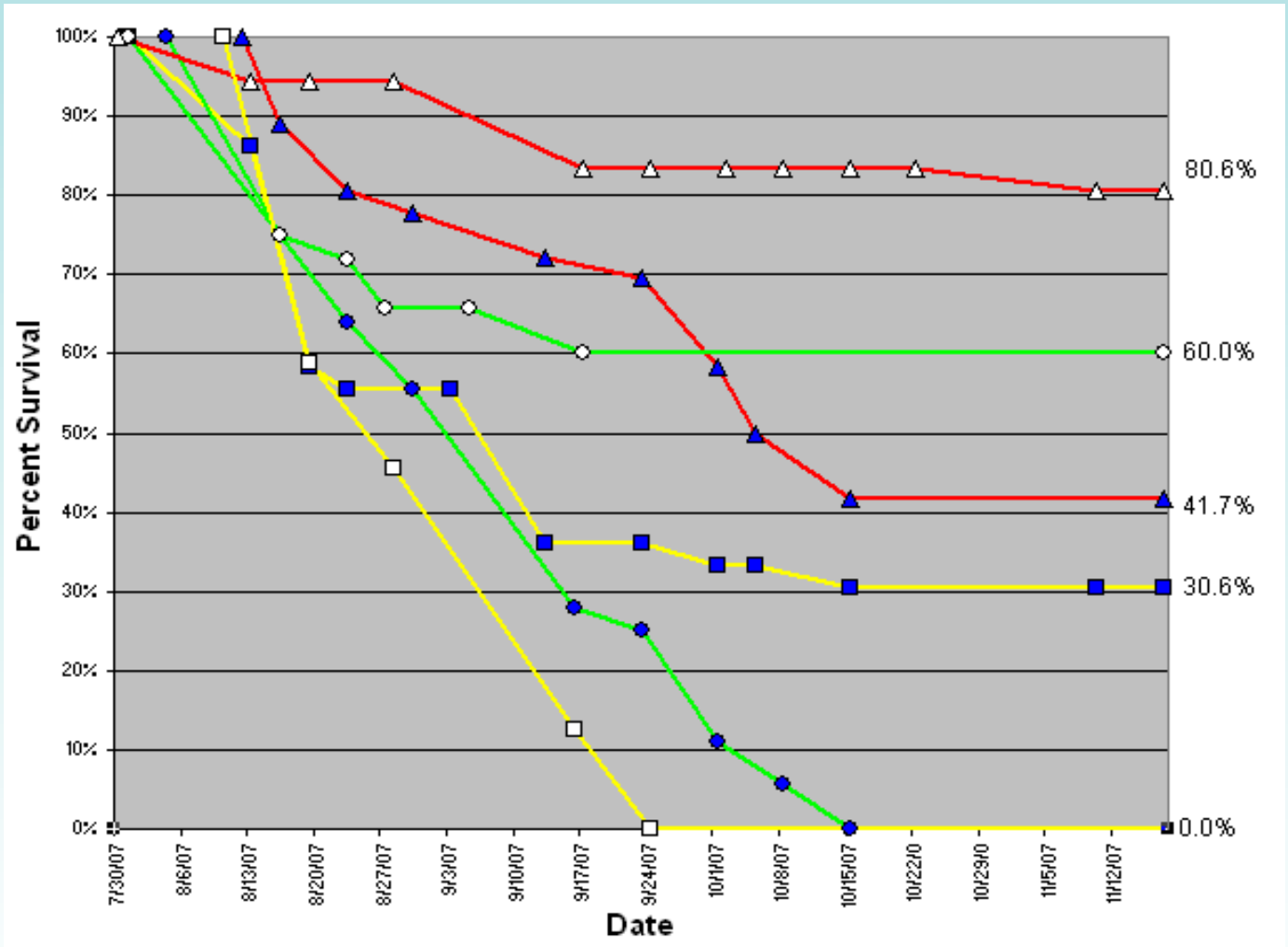
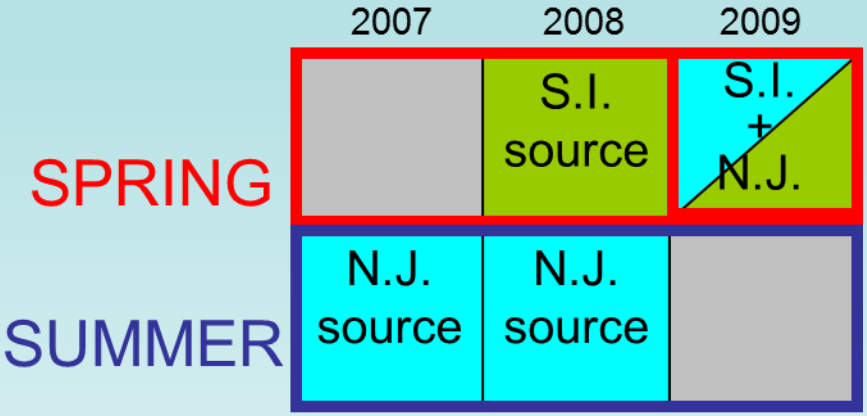
Initial Survival Results (Trial 1: 2007)



Enter: Staten Island



2002-2006: Extirpation (1990s)

2007: Learned of Staten Island (SI)

2008: First breeding assemblage

2008-2010: "Shopped" idea to geneticists

2007: Enclosure Experiments Begin

2008: First SI observation

2008-09:Enclosure Experiments Continue

Molecular Investigation

Molecular Phylogenetics and Evolution 63 (2012) 445–455

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

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

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[b] Graduate Program in Ecology & Evolution, Department of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ 08901, USA
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Amphibian decline
Species delimitation

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. pipiens*, (2) they are hybrids between *R. sphenocephala* and *R. pipiens*, 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

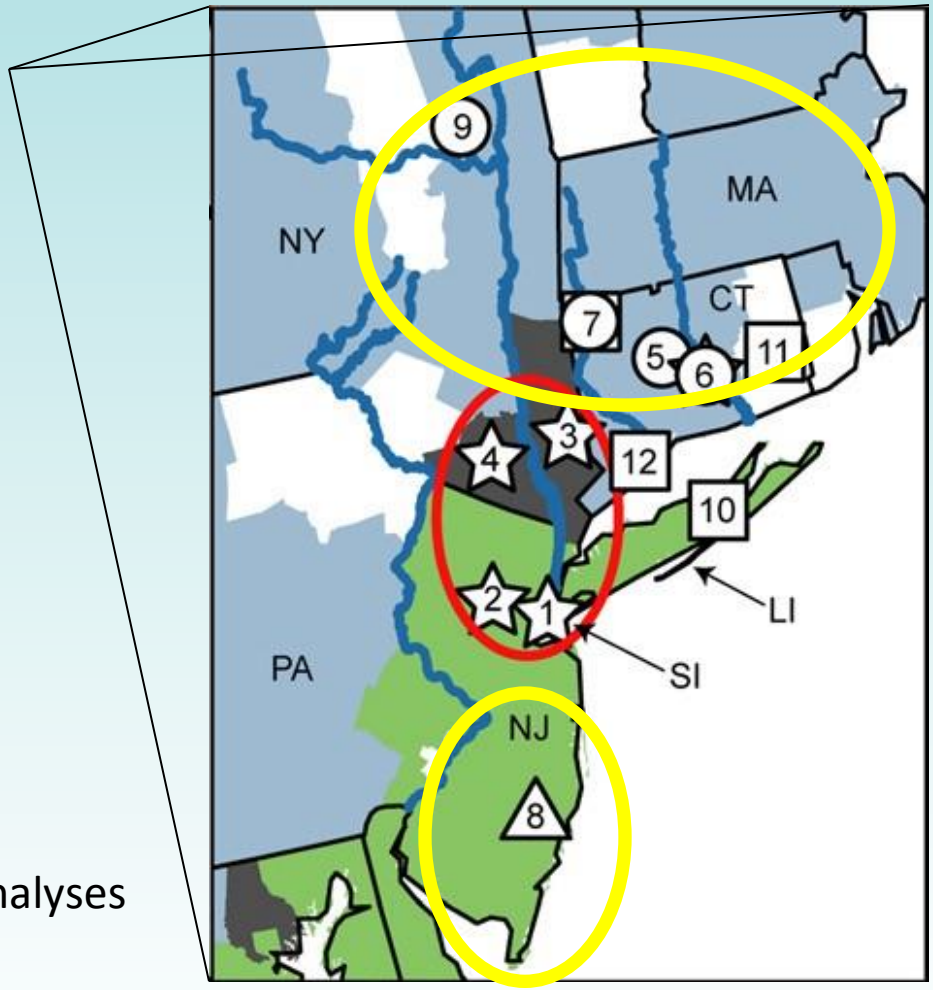
- Analyze mtDNA & nuDNA
- Four unknown populations:
  - Northern NJ (1)
  - Southeast NY (2)
  - Staten Island (1)

- 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*)

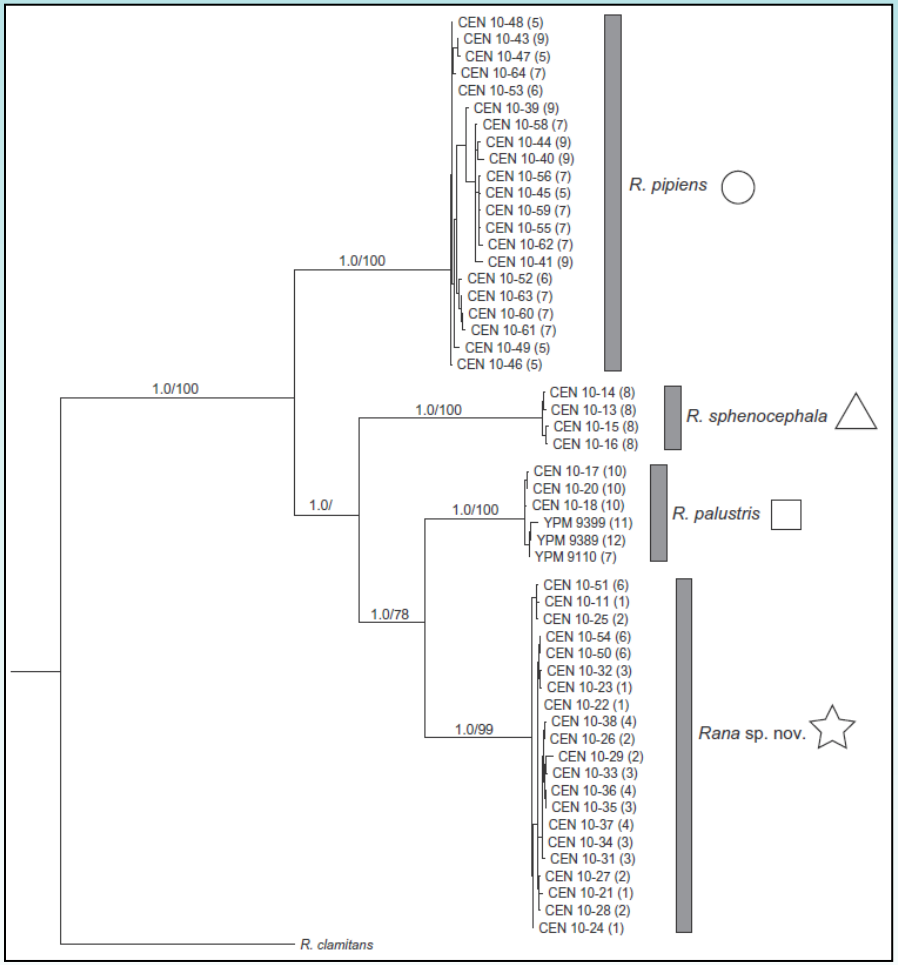
- Sister group: *R. palustris* (mtDNA)

- No hybridization

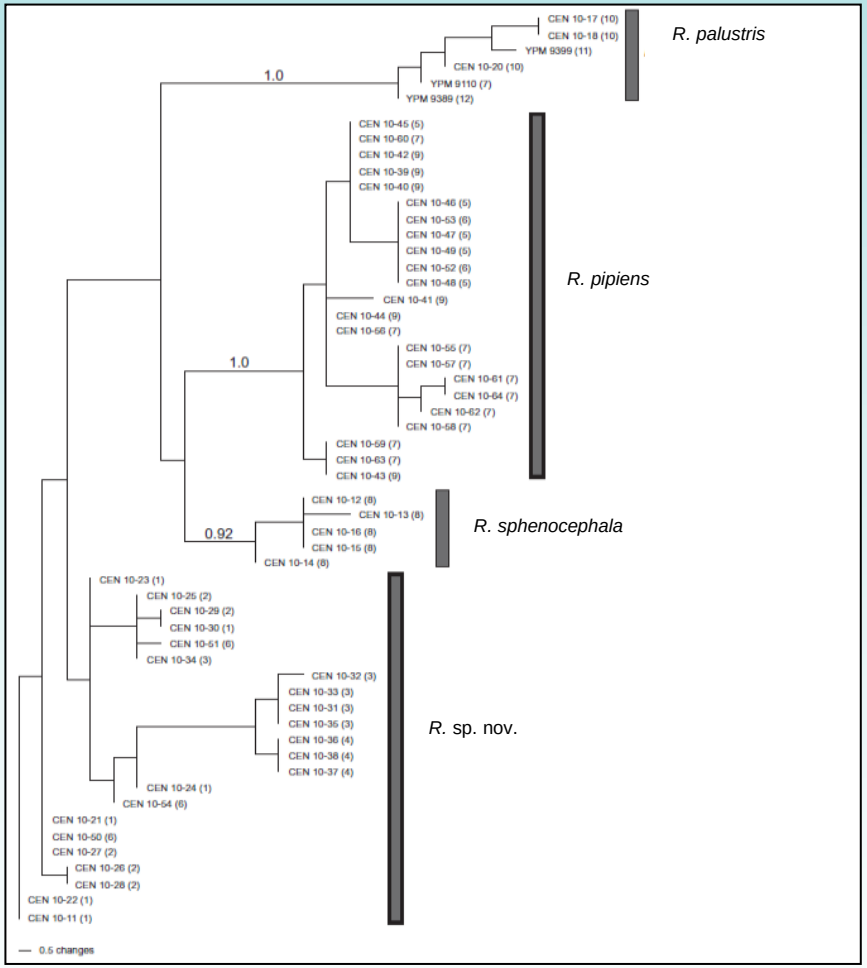
- Potential sympatry: CT (*R. pipiens*)

Results: Phylogenies

Mitochondrial phylogeny



Nuclear phylogeny



Newman et al. 2012. Molecular Phylogenetics and Evolution, 63: 445-455

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 fouquettei* in 2008

Taxonomy: Describing the Species

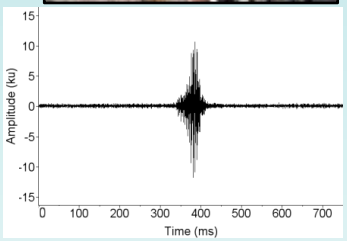
1. Bioacoustics
   - *R.* sp. nov
   - *R. sphenocephala*
   - *R. pipiens*
   - *R. palustris*
   - *R. sylvatica*
2. Additional Genetics (holotype verification)
3. Behavior/Phenology
4. Distribution/Ecology
5. Morphology

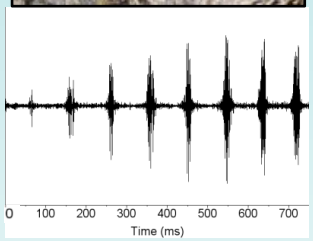
A new species confirmed

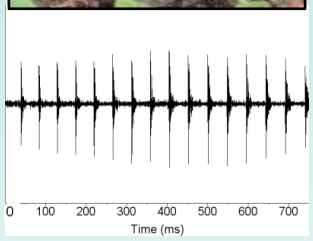
R. sp. nov.

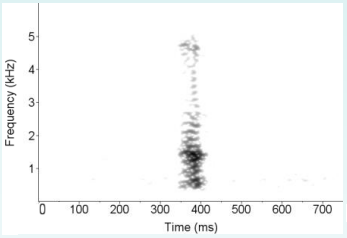
R. sphenocephala

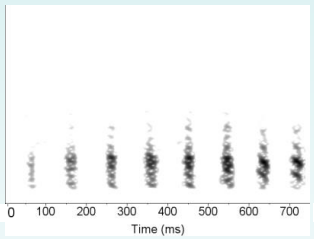
R. pipiens

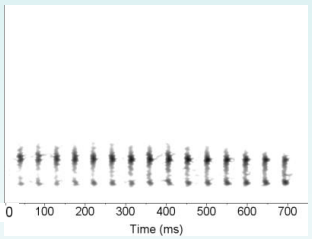


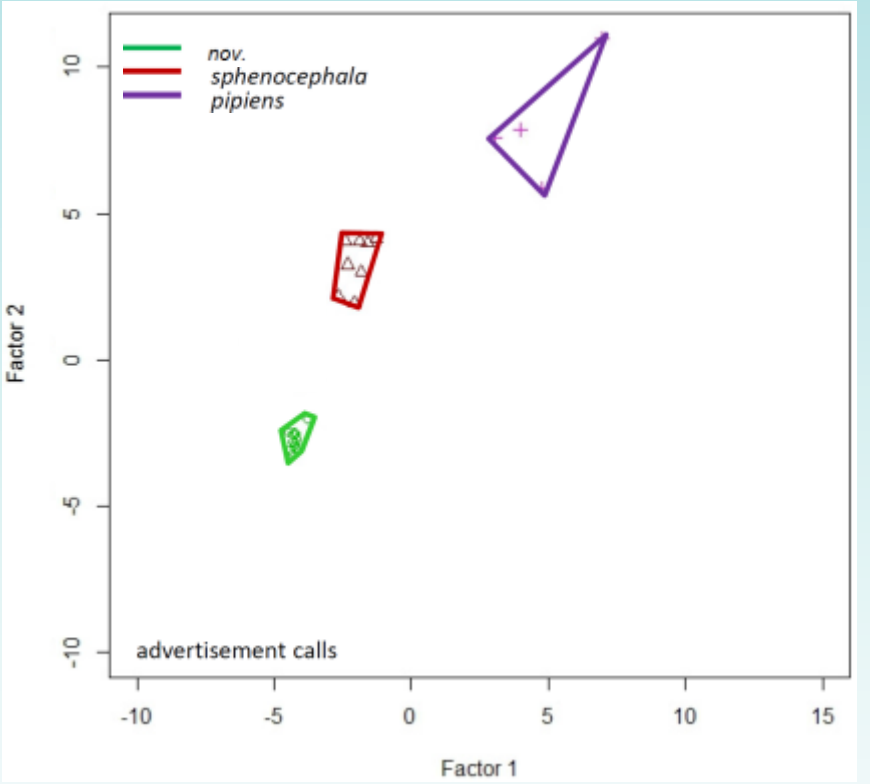




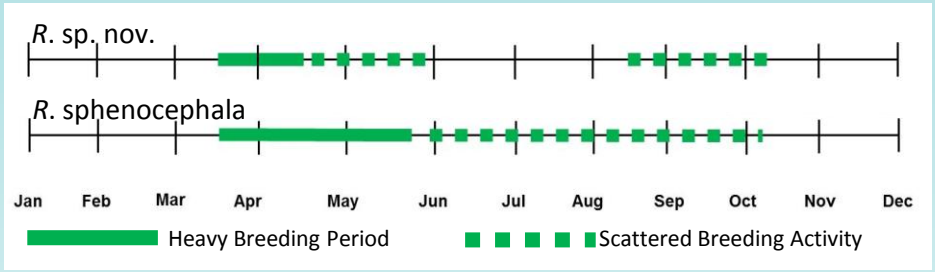








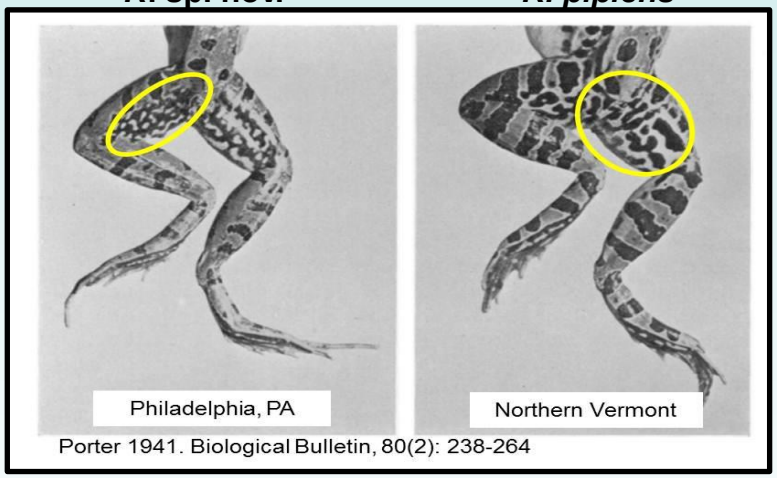
Secondary Diagnostics

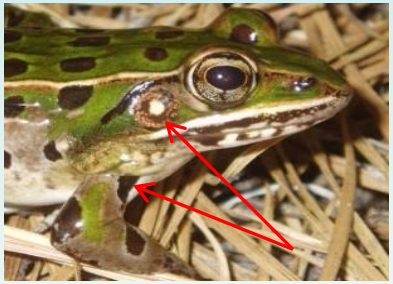


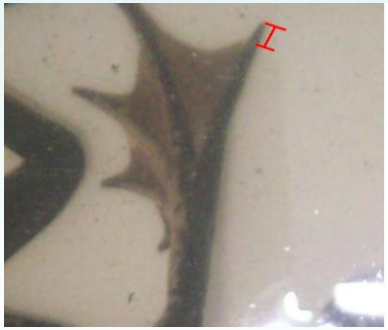
***R*. sp. nov.**

R. sphenocephala

R*. sp. nov.** ***R. pipiens







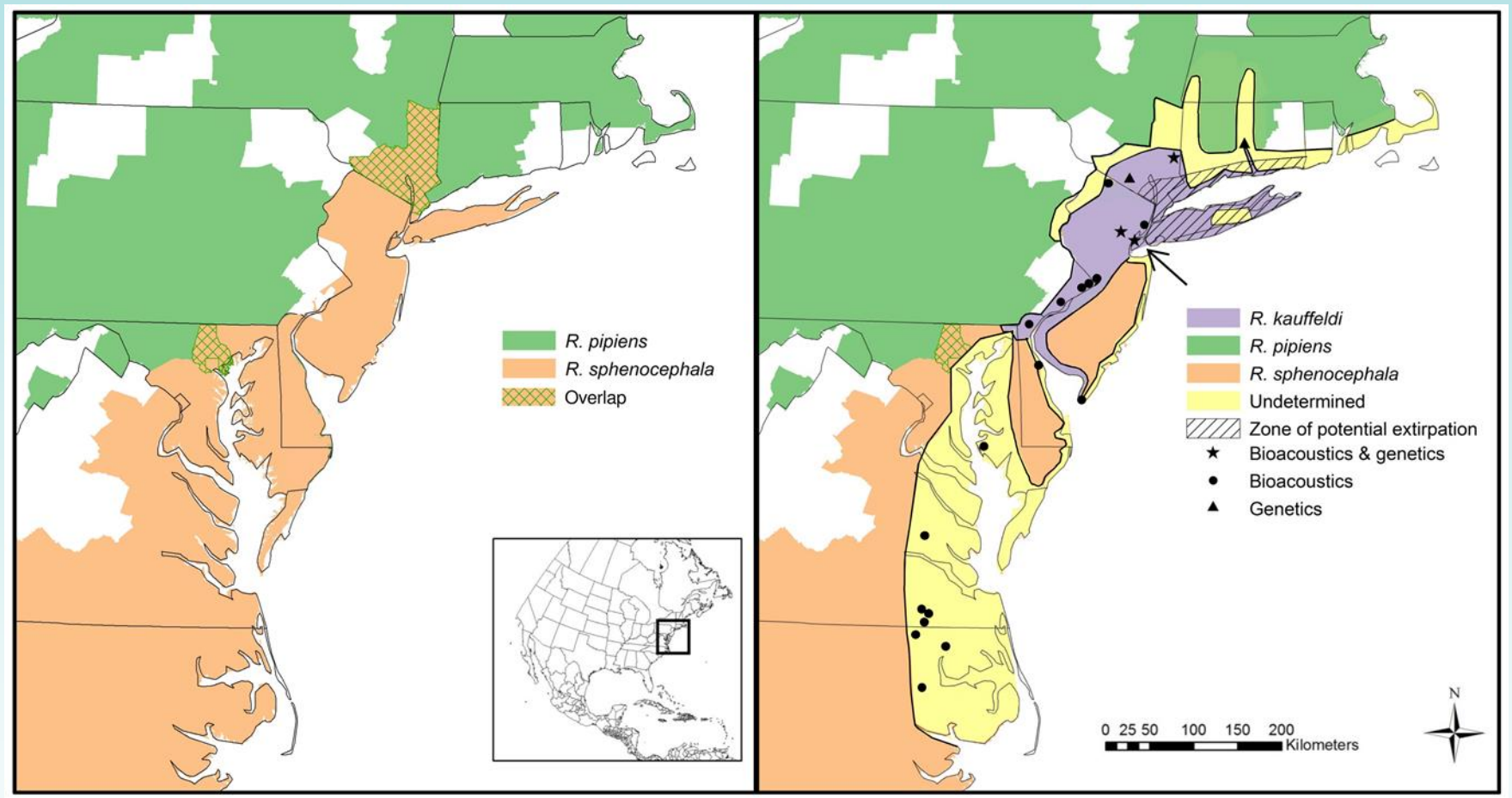
Southern LF (*R. sphenocephala*)

Photo credit: Chris Camacho

Atlantic Coast LF (*R. kauffeldi*)

Habitats

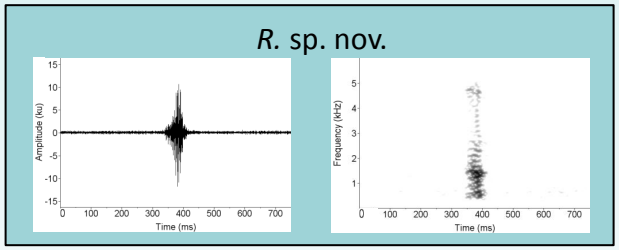
Atlantic Coast Leopard Frog, Rana (Lithobates) kauffeldi

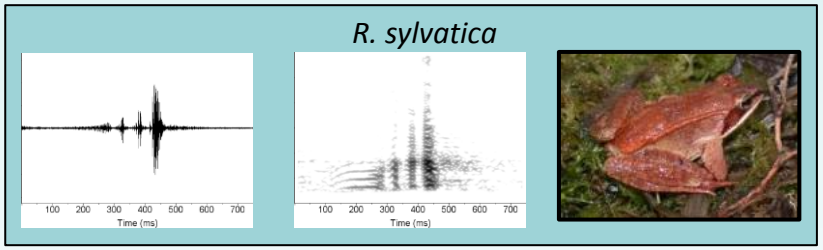


Important Questions

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



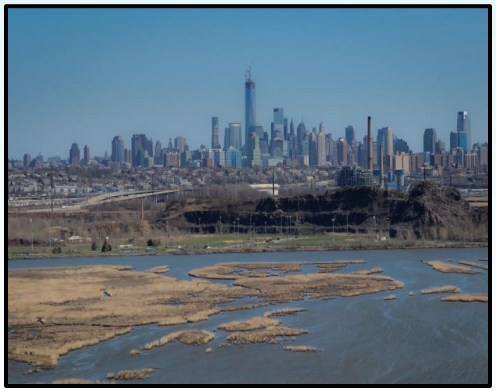


- *Phragmites*
- Cryptic Species

Cryptic Leopard Frog Species

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



The New York Times

Late Edition

NEW YORK, WEDNESDAY, MARCH 14, 2012

A New Species in New York Was Croaking in Plain Sight

BBC NEWS SCIENCE & ENVIRONMENT

29 October 2014 Last updated at 22:36 ET

Frogs' chorus leads to discovery of new species in US

By Victoria Gill
Science reporter, BBC News

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Atlantic Coast leopard frog

From Wikipedia, the free encyclopedia

The Atlantic Coast leopard frog (*Rana kauffeldi*) is a species of amphibian that is endemic to the United States. As a member of the genus *Rana*, it is classified as a true frog, with typical smooth skin and a narrow waist. Its range stretches along the northern part of Eastern Seaboard, from Connecticut to North Carolina. The species takes its common name from the speckles on its legs and back reminiscent of a leopard pattern.

It is one of several species classified as leopard frogs, distinguished as unique through its mating call, genetic differences, habitat, and morphological distinctions.

Contents [hide]

1 Etymology
2 Distribution and range
3 Characteristics
4 Breeding
5 Habitat
6 References

Etymology [edit]

The Atlantic Coast leopard frog is one of several species of leopard frogs. Its species name, *kauffeldi*, is derived from the name of herpetologist Carl Frederick Kauffeld, who in 1936 proposed that there could be a third species of leopard frog inhabiting the New York Tri-State Area. Jeremy Feinberg, who discovered the species, christened it after Kauffeld in honor of him.

Distribution and range [edit]

R. kauffeldi is found along the Northeastern Coast of the United States from central Connecticut to northeastern North Carolina. The north-south range is approximately 780 km long, and the width is about 100 km from the Atlantic shoreline inward. The range narrows as it progressed southward, mostly along the I-95 corridor. The species is thought to inhabit ten states, but the entirety of its distribution and range is not known.

The Atlantic Coast leopard frog is thought to be sympatric with both the Northern leopard frog and the Southern leopard frog

Conservation status

Not evaluated

Scientific classification

Kingdom: Animalia
Phylum: Chordata
Class: Amphibia
Order: Anura
Family: Ranidae
Genus: *Rana*
Species: *R. kauffeldi*

Binomial name

Rana kauffeldi

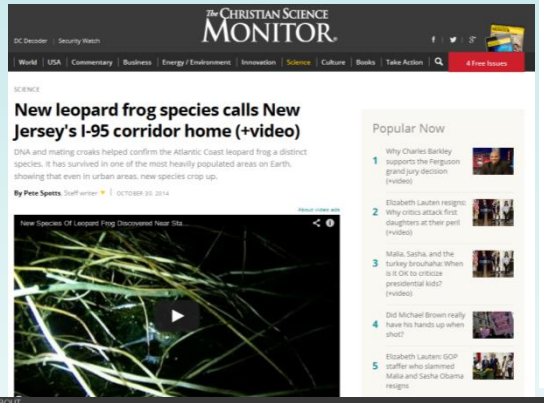
Los Angeles Times

Science Now

Discoveries from the world of science and medicine

New species of frog found in New York City -- first time since 1882

Los Angeles Times
Science / Science Now



The Christian Science Monitor

New leopard frog species calls New Jersey's I-95 corridor home (+video)

DNA and mating croaks helped confirm the Atlantic Coast leopard frog a distinct species. It has survived in one of the most heavily populated areas on Earth, showing that even in urban areas, new species crop up.

By Pete Spotts Staff writer

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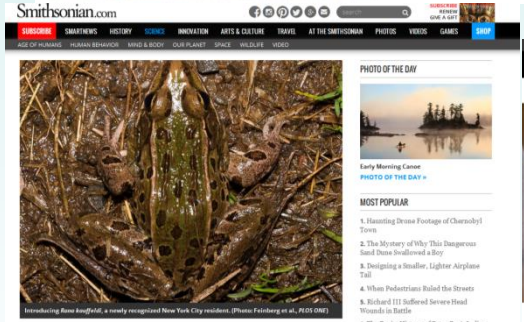

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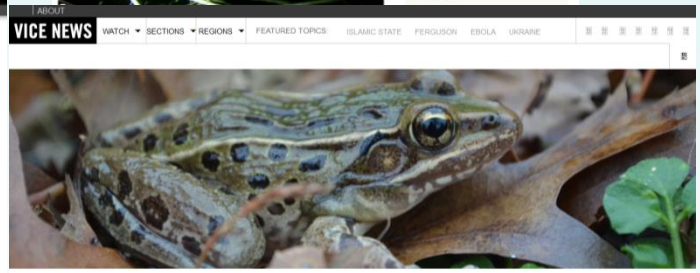
Introducing *Rana kauffeldi*, a newly recognized New York City resident. (Photo: Feinberg et al., *PLOS ONE*)

New Leopard Frog Found in New York City

The unique species, discovered near the Statue of Liberty, is the first amphibian found in the region in 150 years



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ENVIRONMENT

A Young Scientist Makes a Remarkable Discovery in New York City

By Agata Blaszczak-Bore

October 31, 2014 |

On a rainy night in 2008, Rutgers University graduate student Jeremy Feinberg was conducting

NATIONAL GEOGRAPHIC

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News

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

The amphibian stayed hidden in plain sight for decades, only to be discovered on Staten Island.

The new found leopard frog (*Rana kauffeldi*) lives in open-canopied coastal marshes and bottomland floodplains within a few miles of river mouths.

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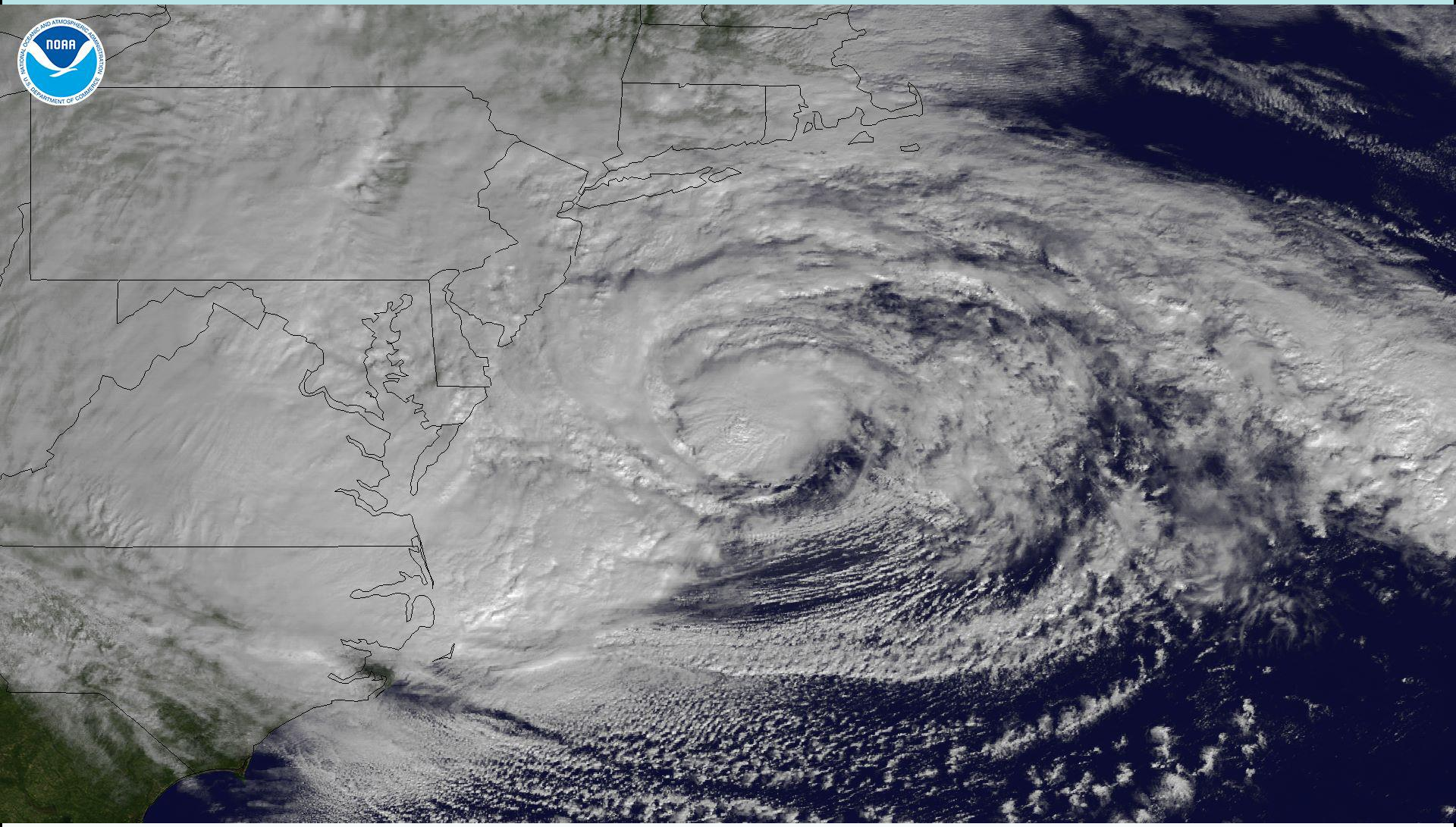


October 29, 2012

Photo Credit: NOAA/NASA

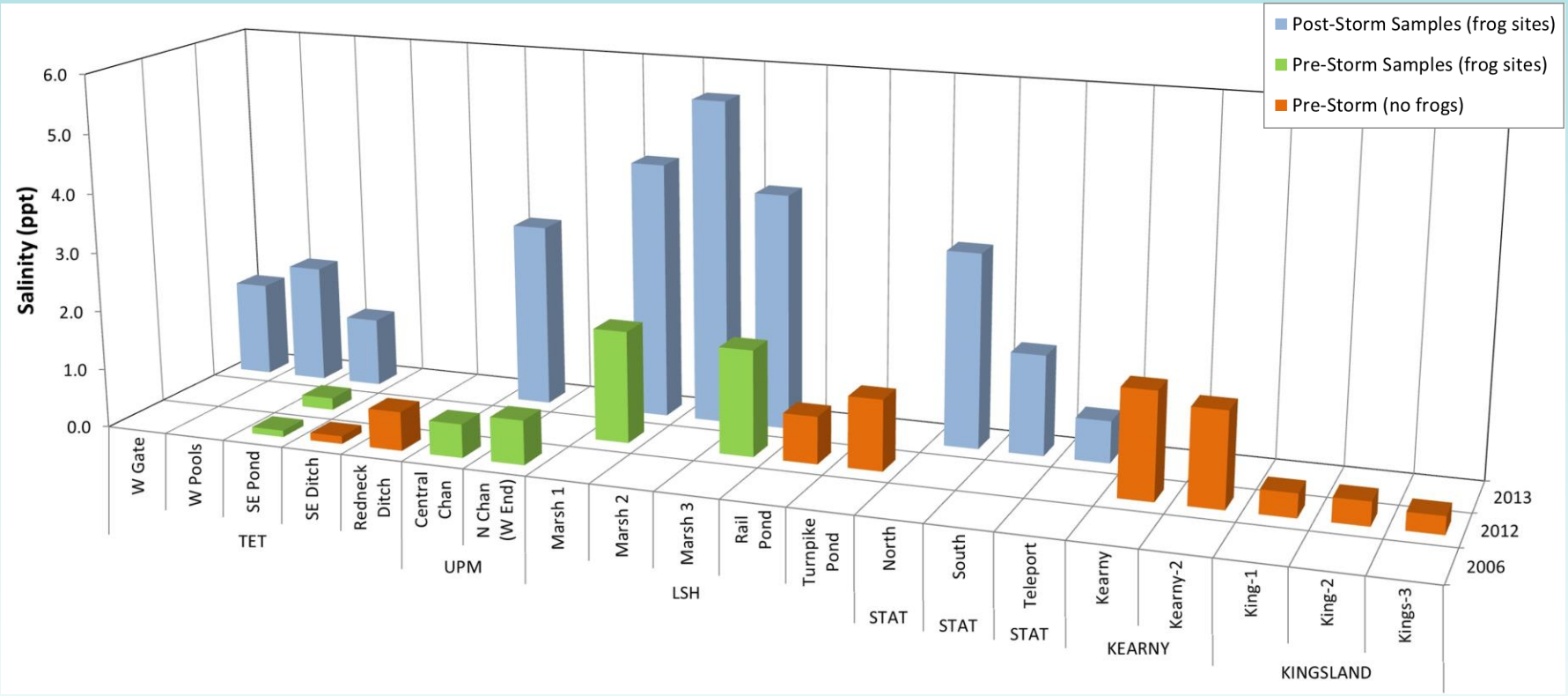
Questions

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?

Goals & Objectives

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.

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

Return to Original Project (Contaminants)

- 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)		
	Bullfrog	**Leopard Frog**	**X^2(p)**
	N = 11	N = 49	
Arsenic	4.76 ± 2.14	1.93 ± 3.52	**12.9 (<0.001)**
Chromium	0.25 ± 0.21	1.44 ± 1.39	**17.3 (<0.0001)**
Cadmium	0.17 ± 0.15	0.28 ± 0.30	**NS**
Mercury	0.19 ± 0.07	0.08 ± 0.13	**13.4 (<0.001)**
Lead	2.91 ± 1.92	3.70 ± 3.50	**NS**
Selenium	2.14 ± 0.62	1.66 ± 1.56	**NS**

Conclusions

- Siblings: truly are a product of their environment
- Did not see significant differences between *R. sphenocephala* 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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