PREVALENCE OF RANAVIRUS IN GREEN FROGS
(*Lithobates clamitans*) ACROSS INDIANA

Megan E. Winzeler[1,2], Steven J. A. Kimble[2] and Rod N. Williams[2]
[1]Savannah River Ecology Lab, University of Georgia, Aiken, SC 29802
[2]Department of Forestry & Natural Resources, Purdue University, West Lafayette, IN 47907

INTRODUCTION

Worldwide amphibian declines are due to habitat loss & fragmentation, chemical contamination of habitat, introduction of exotic species & the spread of infectious disease.

Ranavirus is a viral pathogen infecting fish, reptiles & amphibians at varying degrees of virulence. This involves complex interactions between reservoirs of the disease, direct & indirect transmission routes, anthropogenic stressors & immunity of the individual. Due to life history characteristics like habitat and a multi-stage life cycle, amphibians have an increased susceptibility to *Ranavirus*.

Indiana's landscape is highly fragmented with a long history of disturbance. The influence of anthropogenic stressors like fragmentation, pollutants and other forms of disturbance, aid in the movement of aquatic diseases across the state.

- Frog virus 3 (FV3) infects amphibian species
  - Signs of infection include emaciation & hemorrhaging (Figure 1)
  - Prevents proper osmoregulation

- Susceptibility varies among species
  - Ranid species are more susceptible due to natural history characteristics

- More virulent in larval amphibians but adults act as reservoir

- *Ranavirus* is more likely to persist in semi-permanent wetlands with a high diversity of amphibian species

Figure 1. Clinical signs of *Ranavirus*
Photos courtesy of Jason Hoverman

Figure 2. Sites sampled in Indiana
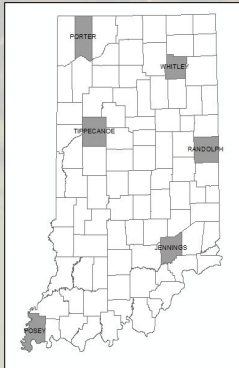
GOALS & OBJECTIVES

The goal of this project is to accurately assess the distribution and prevalence of ranavirus across the state of Indiana. This will be evaluated by:

- The collection of green frog tadpoles from six sites across Indiana (Figure 2).

- Testing for *Ranavirus* using conventional and quantitative PCR analysis.

ABSTRACT

Ranavirus is considered a **primary pathogen currently threatening amphibians**, attacking the immune system of larval amphibians and causing mass die-off events across six continents. This disease can persist in populations through sub-lethal infection of adults, spreading from one site to the next through dispersal or anthropogenic disturbance. Indiana has a highly fragmented landscape which can lead to higher degrees of anthropogenic disturbance and possibly a greater likelihood of *Ranavirus* spreading throughout the state. **We hypothesized that *Ranavirus* would be present in the state due to this fragmentation.** We sampled 6 sites (Tippecanoe, Randolph, Porter, Jennings, Posey, Whitley Counties), targeting green frog (*Lithobates clamitans*) tadpoles for collection of liver tissue. Tadpole livers (Tippecanoe: 46, Randolph: 24, Porter: 40, Jennings: 40, Posey: 3, Whitley: 16) were used to extract DNA, and conventional PCR and qPCR analysis was performed on each individual sample. Each sample was extracted and amplified under sterile conditions to prevent the cross-contamination of any one individual to another. Positive samples were identified in triplicate on agarose gels run with multiple positive and negative controls. **This study found no presence of *Ranavirus* at the six sites using conventional PCR methods.** Further research should include larger sample sizes at multiple locations at more sites should be done to determine actual prevalence of this disease in Indiana.

METHODS

Study Areas

- All sites were lentic wetlands with established green frog populations (Figure 3)
- 4 out of 6 sites near areas of high disturbance
- Surrounding habitat is disturbed by agricultural use and developed by roads

Field Sampling

- 40 minnow traps at each site
- Checked traps twice daily for 2 days
- Individuals euthanized (Figure 4) in a MS-222 & stored in ethanol
- Tadpoles identified by labial tooth rows & placed in individual 50mL conical tubes containing 70% ethanol

Genetic Sampling

- Liver samples were extracted from euthanized individuals
- Primers 4 & 5 from Mao et al. (1997) specific for a 510 bp fragment of the *Ranavirus* major capsid protein (MCP) were used in conventional PCR
- New primers were developed for qPCR analyses based on Mao et al.'s (1997) primers
- Reactions performed under a BioSafety hood with equipment & tools sterilized using ethyl alcohol & bleach solutions
- All samples were amplified 4 times, positive samples 8 times for conventional PCR

Site	# Tadpoles
Tippecanoe Co.	46
Randolph Co.	24
Porter Co.	40
Jennings Co.	40
Posey Co.	3
Whitley Co.	16

Table 1. Number of tadpoles caught at each site.

Figure 3. Minnow trap array in Whiteley Co.

Figure 4. Euthanizing tadpoles at site.

RESULTS & DISCUSSION

This study found all green frogs to be *Ranavirus* negative using conventional PCR methods.

We are still waiting on results from quantitative PCR. The assay is currently developed and based on published sequences of the *Ranavirus* FV3 MCP.

We expected there to be a high prevalence of *Ranavirus* in Indiana due to the highly fragmented landscape and history of anthropogenic disturbance. The resulting zero prevalence value may be due to the imprecise methods of conventional PCR. A more accurate representation of the prevalence of *Ranavirus* in the individuals sampled could be represented using qPCR methods, which is more sensitive and quantifies the load of the disease in the animal.

Additionally, an increased effort to cover Indiana's entire geographic range more completely with larger sample sizes at several wetlands at each site would result in a better idea of the distribution of this disease.
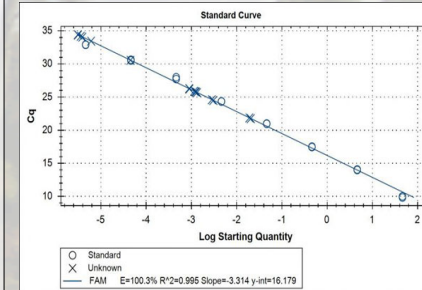

Figure 5. A standard curve based on the known *Ranavirus* standards included on qPCR plates. All unknown samples are fit to this line to determine presence of *Ranavirus*.
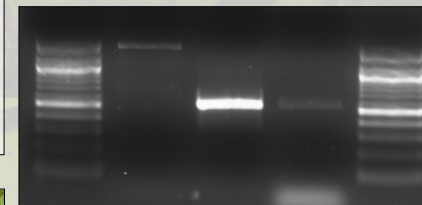

Figure 6. Gel run illustrating a negative sample (Well 2), a positive control (Well 3) & a positive sample (Well 4). Ladders are in Wells 1 & 5.

FUTURE

In the coming weeks, we will be completing the qPCR analysis in order to compare the results from qPCR and conventional PCR methods. Future research should focus on larger sample sizes covering a wider geographic range and using qPCR methods.

ACKNOWLEDGEMENTS