

Detecting the extent of mortality events from *Ranavirus* in amphibians of the Northeastern U.S.

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Project Description

Emerging infectious diseases are one of the most important factors contributing to global amphibian declines and have been implicated in local extinctions of several species. Amphibian declines due to the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), have received considerable and well-deserved attention over the last decade. However, reports of significant mortality due to outbreaks of *Ranavirus* (Family Iridoviridae) are becoming increasingly common in the U.S. with the reported number of die-offs 3-4X greater than for *Bd*. *Ranavirus* differs from *Bd* in that both amphibian and reptiles are known to be affected. Unfortunately, information on the timing, extent, and frequency of occurrence of outbreaks of *Ranavirus* remain limited, partially due to lack of surveillance and partially due to the rapid onset and mortality caused by the disease. This is especially true for amphibian larvae; in many cases, only a few days elapse between the initial signs of the disease and the disappearance of tadpoles from the environment. Thus, unless observations are directed at detecting the outbreak of the disease, it would be easy to conclude that absence of tadpoles was the result of a rapid metamorphosis, instead of mass mortality from a disease outbreak. *Ranavirus* has been confirmed in six amphibian genera found in the Northeast U.S., including the following (with the number of RCN species in that genus listed by at least one Northeastern state in parentheses): *Bufo* (3), *Hyla* (4), *Rana* (7), *Pseudacris* (5), *Ambystoma* (9), and *Notopthlamus* (1). Northeast Partners in Amphibian and Reptile Conservation's (NEPARC) considered *Ranavirus* a major threat to northeastern herpetofauna at their 2011 Annual meeting. *Ranaviruses* likely represent the greatest pathogen threat to the biodiversity of amphibians in North America. In order to begin to better understand the extent to which *Ranavirus* is impacting amphibian and reptile populations in the Northeast U.S. and to develop and test a sampling protocol that could be used throughout the region, we propose a survey of amphibian larvae at a number of wood frog (*Rana sylvatica*) breeding ponds in Maryland, Delaware, New Jersey, Pennsylvania, and Virginia. Wood frogs have the highest mortality and infection rates of northeast amphibians and their breeding ponds (primarily vernal pools) may be the main source of the disease for other affected species. Our approach involves sampling 10 ponds per state per year for two years, with samples spread over different watersheds and physiographic provinces to test the applicability of these methods to a diversity of regional conditions. Outcomes from this effort will include a standard regional *Ranavirus* sampling protocol, a relative frequency of mortality events within the 5-state sampling area

which can be extrapolated to a regional perspective, a summary of known or suspected *Ranavirus* events in the 13 northeastern states, and publications in peer-reviewed scientific journals.

Priority RCN Topic, Geographic Coverage, and Period

This project addresses Priority RCN Topic # 7- Identify and Assess Threats to NE Species of Greatest Conservation Need. *Ranavirus* is a potential direct threat to at least 29 RCN amphibian species found in the Northeast U.S., as well as an increasing number of reptile species. This project will be conducted in Maryland, Delaware, New Jersey, Pennsylvania and Virginia, however the survey protocols developed can be applied to any state in the region (and within the geographic range of the wood frog). *Ranavirus* has been detected in herpetofauna in 7 states in the Northeast (MA, ME, MD, NH, NJ, PA, VA) but likely occurs in all. The study period is for the 2013 and 2014 breeding seasons; thus the project will start in January 2013, contingent on availability of funding, and run through December 2014 (including data analysis and report writing).

Goals and Objectives

The goal of this project is to better understand the extent to which *Ranavirus* is impacting amphibian and reptile populations in the Mid-Atlantic and to develop and test a sampling protocol that could be used throughout the Northeast region. This will be accomplished by focusing on sampling at wood frog breeding ponds (vernal pools), with the order of priority as those sites at or nearest to where *Ranavirus* is known or suspected to have occurred in any amphibian or reptile species. Mortality rates are 50-99% in the larval life stage compared to low mortality rates in adults; thus larvae are the appropriate life stage to sample to increase the probability of detection of the disease. We will accomplish these goals through the following objectives:

1. In consultation with the National Wildlife Health Center, state wildlife health labs, state and federal fish and wildlife agencies, universities, and local experts identify exact locations where *Ranavirus* has been confirmed or is suspected in the five study states.
2. In consultation with state and federal fish and wildlife agencies, universities, and local experts identify exact locations of wood frog breeding ponds in the five study states, choose a subset to survey, gain permission to survey them, and secure state scientific collecting and endangered species permits to collect animals from these sites.
3. In consultation with state and federal fish and wildlife agencies, universities, and local experts identify one qualified local individual in each state to be used as a seasonal technician for the field sampling portion of this study.
4. Adaptively manage sampling protocol based on logistical challenges and input from field personnel.
5. In consultation with the National Wildlife Health Center and Montclair State University develop an efficient system for rapid shipment of animal samples to their labs for *Ranavirus* detection analysis.
6. Review pathology results from the National Wildlife Health Center and Montclair State University.
7. Prepare annual and final reports of our findings, including maps of study areas and sites of past and current *Ranavirus* outbreaks in the 5-state study region.
8. Through a questionnaire sent to states, review of scientific literature, and consultation with the National Wildlife Health Center, develop a summary of all known and suspected *Ranavirus* events in the 13 northeastern states and determine which states are actively sampling for this disease.
9. Present study findings at regional and national professional scientific meetings and on appropriate websites.
10. Publish results in peer-reviewed scientific journals, including dissemination of recommended sampling protocol to be used throughout the region.

Methodology and Approach

1. Select Study Ponds: The National Wildlife Health Center, state wildlife health labs, state and federal fish and wildlife agencies, universities, and local experts will be contacted to obtain exact locations of confirmed or expected *Ranavirus* infections in each state as well as locations of known wood frog breeding ponds. Ponds will be selected in order of highest priority from those with known or suspected disease issues, to those nearest to sites with known or suspected disease issues, to random ponds based on presence of wood frog populations. Study ponds will be ≥ 3 km apart to ensure independence. In each state, we will attempt to choose 10 study ponds in total from at least three different watersheds, and across the five states they will be picked to ensure sampling in a diversity of physiographic provinces representative of the entire region, such as the coastal plain, piedmont, valley and ridge, Blue Ridge, and Appalachian plateau. A new set of study ponds may be chosen in Year 2 depending on results from Year 1, for a potential of 100 study ponds sampled over the 2-year study.

2. Sampling at Study Ponds: Starting in February 2013, wood frog larvae at Gosner stage 27 through metamorphosis (65-130 days post-hatching) will be sampled every 2-4 days by dip-net at each of the 10 study ponds in each state. Sampling will involve 5-meter linear dip-net sweeps along the pond bottom around the pond perimeter in each of the cardinal directions (4) and two 5-meter sweeps in the central (deeper) pond area. Captured larvae will be placed in a wet bucket or tray after each sweep and sorted by species. Larvae will be visually examined for indications of ranaviral infection (reddening of their ventral skin, especially around the base of the hind limbs and the vent opening). If no symptoms are observed all larvae will be released alive back into the study pond. All boots, equipment and dip-nets will be disinfected between sites in a 10% bleach solution to ensure no disease transmission between study sites.

3. Sampling at Study Ponds where Potential Ranaviral Infection is Detected: Attempts will be made to collect ~30 larvae (live & recently dead) per potentially infected species per site. Samples will be organized, secured and labeled following National Wildlife Health Center protocol for specimen collection and all specimens will be placed in a cooler and then shipped within 24 hours to either the USGS National Wildlife Health Center (Madison, WI) or to the Department of Biology and Molecular Biology at Montclair State University (Montclair, NJ) for full pathological screening (necropsies, histology of major organs, and viral, fungal and bacterial cultures where appropriate). Infected ponds will be surveyed at the same or greater frequency as all non-infected ponds through metamorphosis to observe the length of time of the die-off and if any animals survive. Additional samples may be collected throughout the chronology of the disease event, based on consultation with the National Wildlife Health Center. All infected ponds found in Year 1 will also be surveyed in Year 2 to determine persistence of the virus at the site.



Wood frog tadpole infected with *Ranavirus*.
Montgomery County, Maryland, 2011
Photo by Scott Farnsworth



Eastern box turtle suffering from *Ranavirus* infection.
Montgomery County, Maryland, 2011
Photo by Scott Farnsworth

4. Develop a summary of all known and suspected *Ranavirus* events in the 13 Northeast states: The National Wildlife Health Center, state wildlife health labs, state and federal fish and wildlife agencies, universities, and local experts will be contacted to obtain information on confirmed or suspected *Ranavirus* infections in each state within the northeast region. A questionnaire will be developed and sent to state herpetologists to determine if any active sampling for *Ranavirus* is ongoing or has occurred in the recent past. Data requested will include: affected species, estimated mortality in numbers of individuals, lab diagnosis (if available), date of occurrence (to determine if seasonality of infections), and county of occurrence.

Products

Expected outcomes from this study include:

1. Recommendations for a standardized protocol to determine the extent of *Ranavirus*-associated die-offs that could be applied throughout the northeastern U.S.
2. A summary of states in the northeast with documented *Ranavirus* and those who are sampling for this disease.
3. Publication of one or more scientific papers in peer-reviewed journals.
4. Present findings at regional and national professional scientific meetings.
5. Put results on website for broader dissemination, such as the Global Ranavirus Consortium (<http://fwf.ag.utk.edu/mgray/ranavirus/Ranavirus.htm>).

Budget (for 2 years)

	RCN Request	Non-Federal Match	Total Cost
Personnel (MD DNR) ^{1a}	\$ 21,600.00	\$ 19,722.40	\$ 41,322.40
Fringe Benefits (MD DNR) ^{1b}	\$ 1,652.40	\$ 5,916.72	\$ 7,569.12
Contractual Service ^{2a}	\$ 48,680.00	\$ 4,120.00	\$ 52,800.00
Fringe Benefits ^{2b}	\$ 4,868.00	\$ 412.00	\$ 5,280.00
Indirect Costs ³		\$ 1,972.24	\$ 1,972.24
Supplies and Materials ⁴	\$ 2,500.00	\$ 2,200.00	\$ 4,700.00
Travel ⁵	\$ 9,600.00	\$ 4,712.00	\$ 14,312.00
In-Kind Service ⁶		\$ 49,850.00	\$ 49,850.00
Grand Total	\$88,900.40	\$88,905.36	\$ 177,805.76

^{1a} RCN Request: 1 Seasonal technician (field & data) for MD @ \$15/hour for 24 weeks (year 1) and 12 weeks (year 2).
Match: 1 classified MD DNR employee @\$33.46/hour for 15 days/yr x 2 years for field project direction and 200 hours for data analysis and report writing. Additional DNR match in classified salaries for processing grant, DVM technical assistance, and additional field assistance during outbreak events (~\$5000).

^{1b} RCN Request: @ 7.65%
Match: @30%

^{2a} RCN Request: includes 3 seasonal technicians hired by entities in PA, VA, and NJ @\$15/hour for 12 weeks X 2 years (\$43,200), plus ~57% of salary for 1 seasonal technician in DE @10\$/hour for 12 weeks x 2 years (\$5,480); estimated shipping costs for samples to labs (\$70/sample cooler X 10/yr x 2 yrs=\$1400); and estimated lab processing fees (\$4/sample for amphibian larvae at NJ lab x 200/yr for 2 years (\$1600; note: NWHC does for free with limited capacity – will go there first).
Match: ~43% of DE seasonal technician salary (\$4120).

^{2b} RCN Request: Used 10% fringe for all as is fringe with Conserve Wildlife Foundation, whom will be hiring 1 seasonal in NJ.

³ Match: @ 10% of classified MD DNR employee salary

⁴ RCN Request: Includes sampling equipment, disinfection supplies, and laboratory processing
Match: from Towson University, MD DNR and other state agencies and NGOs.

⁵ RCN Request: Mileage reimbursement for 4 seasonal technicians for 2 years @ \$0.50/mile for estimated 50 miles/day for 12 weeks (4 driving days/week). Note DE is not requesting travel funds – will use as match.
Match: \$1232 from Towson University; \$2280 from MD DNR; \$1200 from DE

⁶ Match: \$27000 pledged from PAFBC; \$6000 from DEFW; \$7200 from Towson University; \$5000 from VADGIF; \$1250 from NJDFW; \$1600 from Montclair State University; \$1800 from Conserve Wildlife Foundation

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