

# Logan Billet, PhD student

## *Ranavirus infections in vernal pools*

### Research Highlights

- Ranavirus infections can cause die-off events in tadpole populations, where > 95% of individuals die within a short period.
- I observed ranavirus-associated tadpole die-off events at 7 of the 40 ponds I surveyed in the spring/summer of 2021
- Infection prevalence tended to reach a high level ( $\geq 80\%$  of individuals infected) well before any observed mortality events (range: 2-7 weeks in advance)
- Each tadpole die-off was associated with a rapid spike in ranavirus environmental DNA (eDNA) concentrations and tadpole infection intensities
- Ponds without die-offs tended to contain no/very little detectable ranavirus eDNA and few infected tadpoles

### Research Summary

As the past several years have demonstrated, understanding the causes and consequences of epidemics is critical for human health. The same is true for wildlife conservation and management. One of the most dramatic outcomes of epidemics are mass mortality events, in which a significant portion of a population dies of disease. Mass mortality events can have profound impacts on populations and ecosystems yet are rarely studied in a systematic way because of how sporadic and difficult to detect they tend to be. Identifying and studying animal populations where we can track mass mortality events across multiple populations has the potential to greatly advance our understanding of disease-induced mass mortality events.

This past year (spring/summer 2021), I conducted surveys of a deadly amphibian pathogen, ranavirus, in tadpoles at 40 wood frog breeding ponds within the Yale-Myers Forest, surveying each pond every two weeks for three months (mid-April – Mid-July). Ranaviruses are a collection of double-stranded DNA viruses that infect ectothermic vertebrates. Ranavirus infections are responsible for die-offs of amphibians, fishes, and reptiles globally and have been implicated in a majority of amphibian mass mortality events in the United States.



A marbled salamander dying from a ranavirus infection.

### Research Summary cont.

However, due to a lack of multi-year surveys at sites with recurring epidemics, our understanding of ranavirus-induced die-off events is limited. Wood frogs are among the most susceptible species to ranavirus, and infection in natural populations often leads to catastrophic MMEs that result in  $\geq 95\%$  mortality.

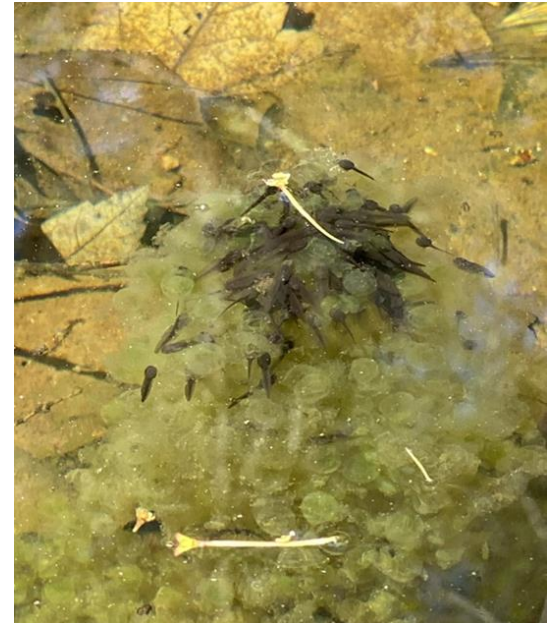
Each time I visited a pond, I collected tadpoles to measure ranavirus infection intensity (a measure of how much virus is within a tadpole) and prevalence (a measure of how many tadpoles are infected). I also collected environmental DNA (eDNA) from the water to measure community-level shedding of ranavirus into each pond. Ranavirus eDNA concentrations have been found to strongly correlate with infection intensity within wood frog tadpoles, and elevated ranavirus eDNA tends to correspond with die-offs. I also measured environmental variables such as pond temperature, oxygen levels, and tadpole densities at each visit.

The results from these initial surveys are intriguing and have provided detailed time-series data that characterize the development of ranavirus epidemics relative to ponds with no signs of disease. I observed die-off events at seven of the 40 ponds surveyed (~20% of sites). Each tadpole die-off was associated with a rapid spike in ranavirus eDNA concentrations and tadpole infection intensity, whereas infection prevalence tended to reach  $\geq 80\%$  well before any observed mortality (range: 2-7 weeks in advance). In contrast, ponds without die-offs tended to contain no/very little detectable ranavirus eDNA. I am still processing individual tadpoles from ponds without die-offs, but preliminary data suggests that these ponds have little/no ranavirus infection late in the season when prevalence is expected to be highest if ranavirus is present. It also appears that the timing of ranavirus die-offs may be shifting, occurring on average ~7 days earlier in the season in 2021 in comparison to die-off events detected at the YMF in 2013/2014, less than a decade ago. Finally, I have detected a significant difference in size at a given developmental stage at ponds experiencing die-offs versus those with no detected mortality. In other words, when controlling for stage of development, tadpoles collected from die-off ponds are larger. This could be indicative of either 1) size-selective mortality or 2) a shift in physiological rates of growth allocation at ponds with ranavirus.

While many of the observations from this work are valuable in and of themselves, this project will also act as a platform for future projects. I plan to conduct a second year of surveys in the spring and summer of 2022 that build upon this first year of work. Questions that can be answered by a second year of surveys include 1) do ranavirus die-offs happen in the same ponds every year? And 2) are there consequences of ranavirus-induced die-offs on future wood frog breeding efforts (a measure of population size)? Tracking the drivers of die-offs before declines become obvious will improve our ability to predict and respond to future outbreaks in imperiled taxa. Understanding the dynamic of lethal pathogens in model systems like wood frogs provides a powerful tool to clarify the emergence of viral epidemics in general.



Billet used this mountain bike setup to visit 40 ponds throughout the forest.



Newly hatched wood frog tadpoles in a vernal pool.