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### *Exploring the causes and consequences of ranavirus epidemics in a wood frog metapopulation experiencing recurring mass mortality events*

#### 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 and 1 of the 30 ponds I surveyed in the spring/summer of 2022.
- Some ponds may be consistently “sick” and contain ranavirus-infected tadpoles.
- Ranavirus buildup in the water column may be an important driver of die-off timing and severity.

#### Research Summary

Humans are not the only species affected by epidemics. Infectious disease outbreaks can have deadly consequences for nearly all species, from the tiniest microbes to the largest mammals. One of the most dramatic outcomes of epidemics is mass mortality events, where the majority of a population dies from disease in a short period. Mass mortality events can significantly impact populations and ecosystems yet are rarely studied systematically because of how difficult they can be to predict and detect. Because of this, we still lack answers to some of the most fundamental questions surrounding mass mortality events for many host-pathogen systems. How often do mass mortality events occur? What environmental conditions influence their severity? What are the consequences for the affected host population? The answers to these questions are important for conservation and management, so studying animal populations where we can reliably detect mass mortality events across multiple populations can greatly advance our understanding of disease-induced mass mortality events.



Preserved wood frog tadpoles collected during ranavirus field surveys.

**Research Summary cont.**

Wood frogs, a common amphibian species in New England, are among the most susceptible species to ranavirus, a deadly pathogen responsible for epidemics that often result in  $\geq 95\%$  host mortality in infected tadpole populations. However, our understanding of ranavirus-induced die-off events in tadpole populations remains limited due to a lack of survey work at sites with recurring epidemics. Over the past two years, between April and July, I have conducted surveys of ranavirus infection in wood frog tadpoles at over 30 ponds within the Yale-Myers Forest. 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 correlate with infection intensity within wood frog tadpoles strongly, and elevated ranavirus eDNA tends to correspond with die-offs. At each visit, I also measured environmental variables such as pond temperature, oxygen levels, and tadpole densities.

You can find my 2021 survey summary on YSE's Kohlberg-Donahoe webpage. In brief, I observed die-off events at eight ponds surveyed in 2021. I detected one ranavirus die-off event in 2022. Across all the ranavirus epidemics observed in 2021 and 2022, each tadpole die-off was associated with a rapid spike in ranavirus eDNA concentrations and tadpole infection intensity. In contrast, ponds without die-offs tended to contain no/very little detectable ranavirus eDNA and no/very few infected tadpoles. We are beginning to use the data collected from the 2021/2022 die-off events to piece together infection patterns and mechanisms that may be these die-offs. For example, we are finding some evidence that some ponds are consistently "sick." Although only one pond had a die-off in 2022, we detected ranavirus infections in 11 ponds where no die-off occurred. Interestingly, however, 8 of these 11 ponds did have die-offs in 2021. This finding suggests that ranavirus epidemics may be non-randomly distributed across ponds and that some ponds consistently harbor infection. Evidence also suggests that ranavirus buildup in the water column may be an important driver of die-off timing and severity. To assess the influence of pathogen buildup on the timing and severity of ranavirus die-offs, I will conduct experimental studies this year that allow or inhibit the buildup of ranavirus in the water column during an epidemic.

Amphibians are in the midst of an extinction crisis threatening over 1/3 of species globally. Ranaviruses are responsible for rapid declines in several amphibian communities, and ranavirus-induced declines are expected to increase in severity over time as environmental conditions shift with climate change. Tracking the drivers of mass mortality events 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 amphibians like wood frogs also provides a powerful tool to clarify the emergence of viral epidemics in general.



Adult wood frog found at the Yale-Myers Forest.



Tadpole carcasses resulting from a ranavirus mass mortality event at a pond in 2022.