Does Geographic Distance Between Host Population and Isolate Location Equate to Ranavirus Pathogenicity?

P. Reilly[1,2], M. Gray[1], R. Wilkes[2], J.Chaney[1], R. Hill[1], and D. Miller[1,2]
[1]Center for Wildlife Health, University of Tennessee, Knoxville, TN, USA.
[2]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.

INTRODUCTION

The occurrence of host-pathogen co-evolution has been well studied in many host-pathogen relationships (3,4);however, has been understudied in ranavirus-host systems. Storfer et al. (2007) reported complete concordance between phylogenetic trees for tiger salamanders and the ATV ranavirus, which supports a co-evolutionary history (7). If ranaviruses co-evolve with hosts and evolution is an ongoing process, it is reasonable to hypothesize that as distance between isolate and host population increases, pathogenicity of the isolate will increase. Our objective was to test for this relationship in a widely distributed anuran species. We also explored if the relationship was impacted by temperature.

METHODS

Wood frog (*Lithobates sylvaticus*) eggs are being collected from Tennessee (completed), Michigan, Manitoba Canada, and Alaska (completed). We are challenging these populations with two FV3-like ranaviruses isolated from wood frog die-off sites in Tennessee and Minnesota (5).

Experimental Design:

•Randomized block, each shelf in environmental chamber represented a block (3 controls, 6 MN isolate, 6 TN isolate).
•Chambers were set at 15 C and 25 C.
•Tadpoles were housed in 2-L tubs with 1 L of water (6).
•1 week acclimation before being inoculated with 10^3 PFU/mL of virus for 3-day exposure.

Care and Monitoring:

•Monitored morbidity and mortality for 21 days.
•Water changing and feeding with commercial fish pellets at 10% body mass every 3 days.
•Necropsy: Liver and kidney homogenate at -80°C.

UT IACUC Protocol 2074

Gross signs:

•Edema, erythema, external hemorrhaging, pale & swollen liver, and hemorrhaging of the kidneys

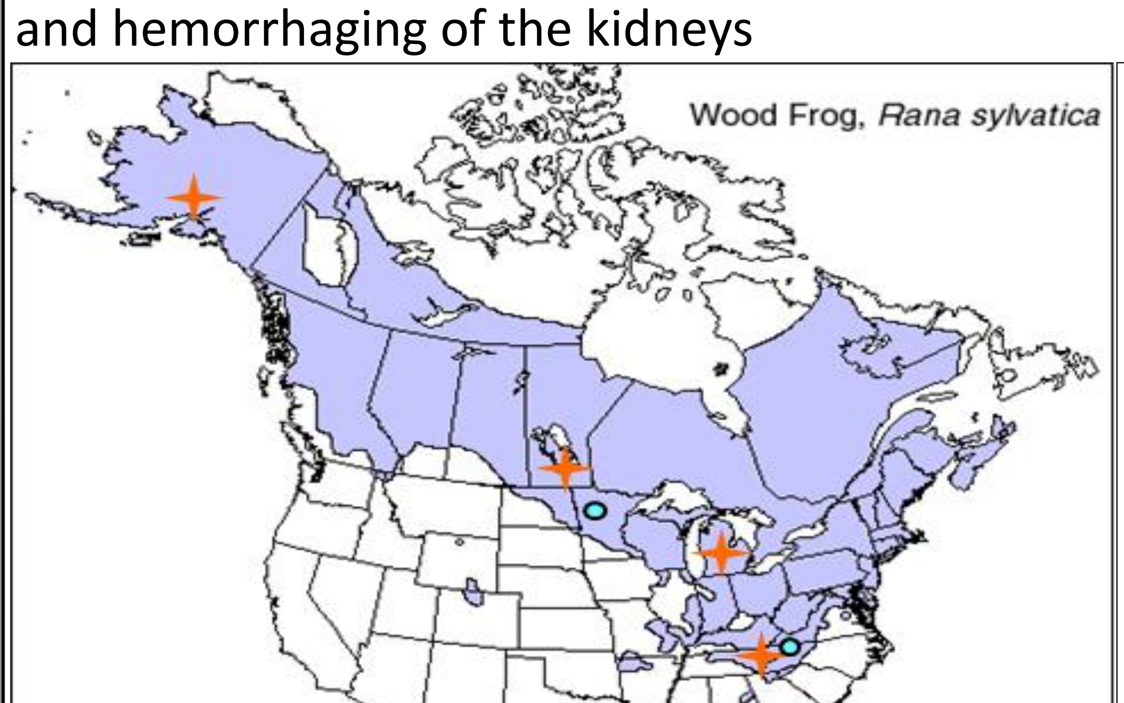
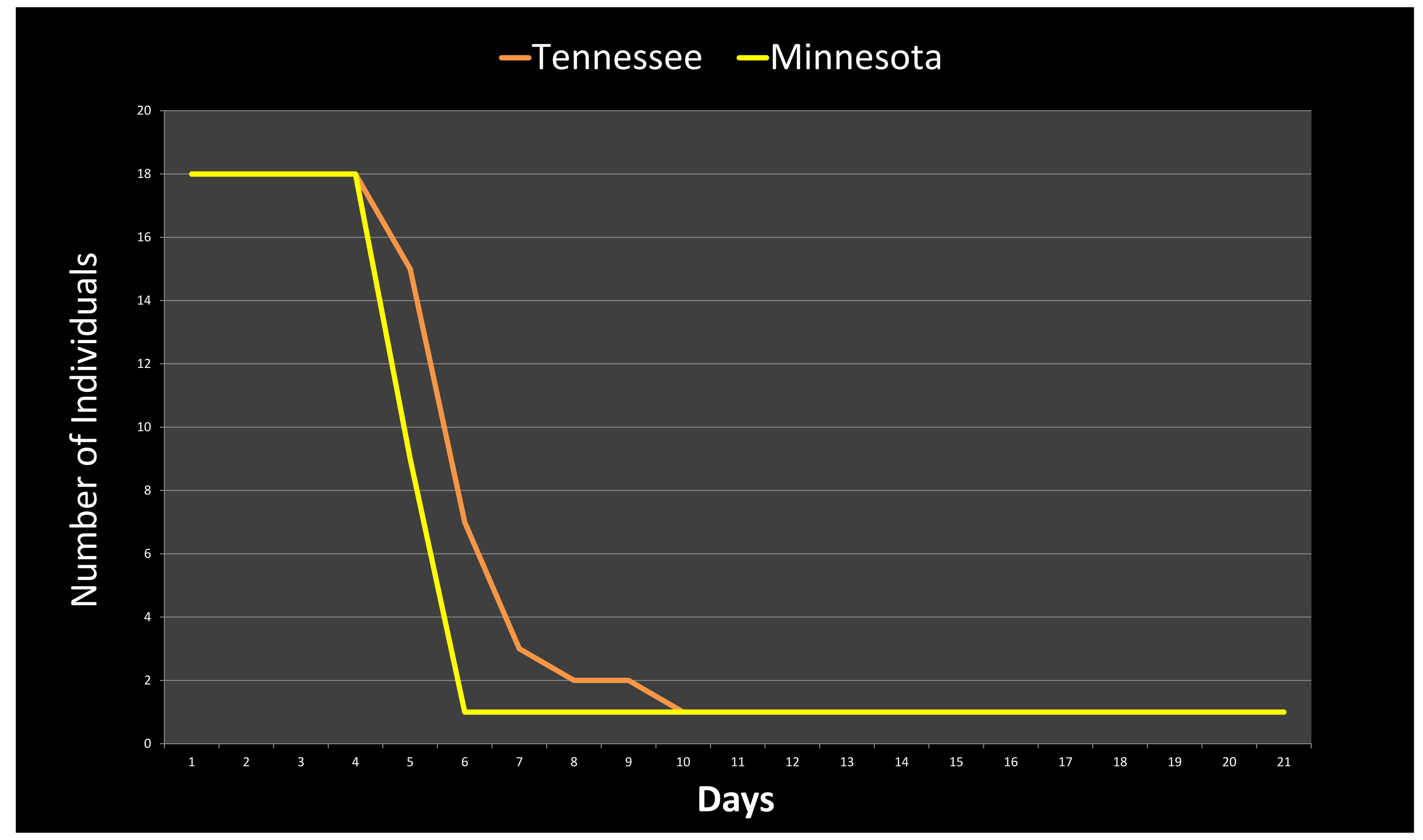


Fig 1. Map showing geographic origin of wood frog egg masses used in this study (crosses). Also shown are the geographic locations of the mortality events from which the ranavirus isolates were obtained that were used to challenge the tadpoles (circles).

https://wikispaces.psu.edu/download/attachments/131630290/Wood+Frog+map.gif?version=1&modificationDate=1367517394000

RESULTS

Survival Curve for 25°C Chamber

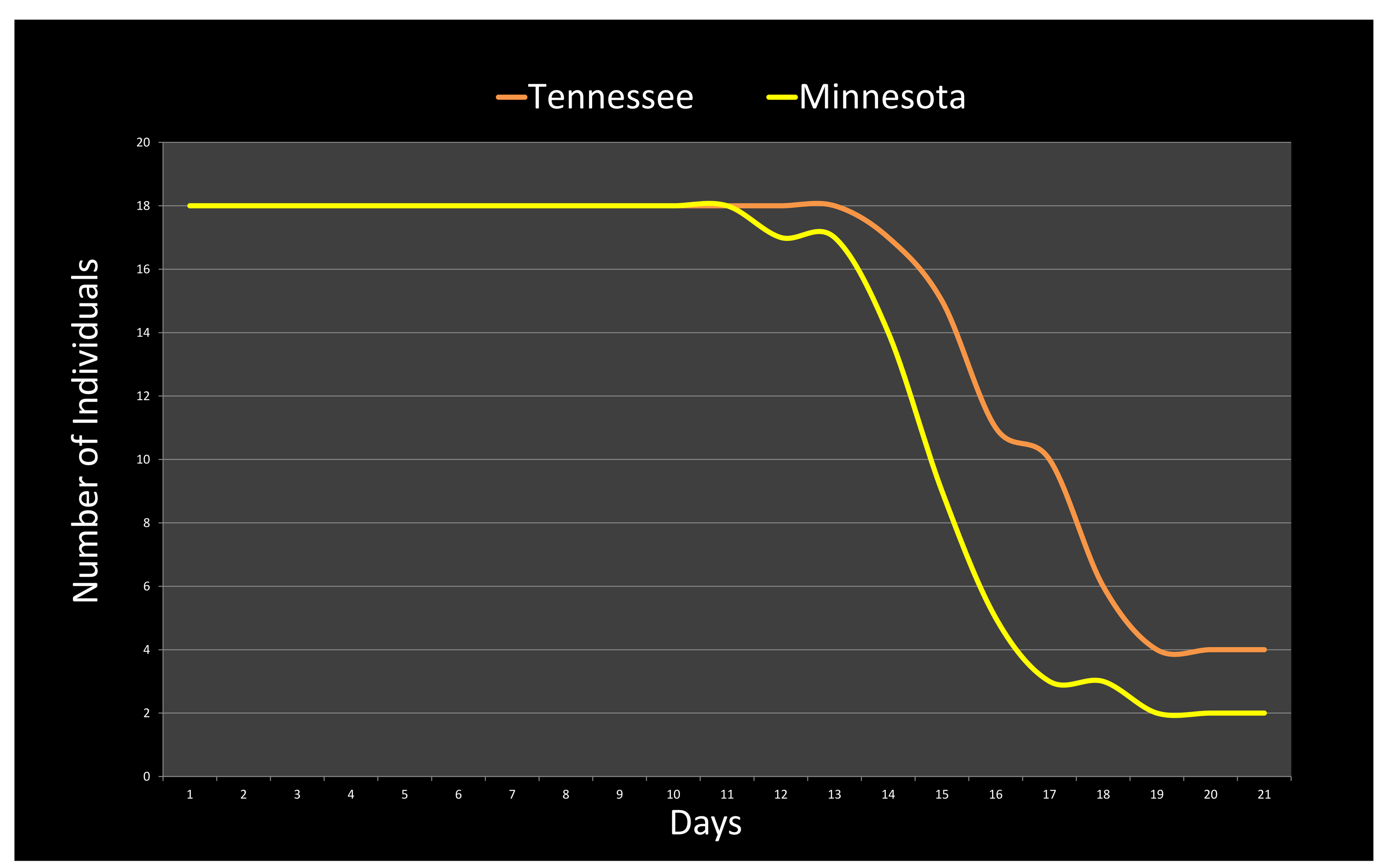


Median days to Mortality

- **Minnesota = 5.5 d**
- **Tennessee = 6 d**

Tennessee Tadpoles Died about 0.5 d Faster when Exposed to the Minnesota Isolate at 25 C

Survival Curve for 15°C Chamber



Median days to mortality

- **Minnesota =15.5 d**
- **Tennessee =18 d**

Tennessee Tadpoles Died about 2.5 d Faster when Exposed to the Minnesota Isolate at 15 C

Tadpoles in the 25 C chamber died about 10 d Faster than in the 15 C Chamber

DISCUSSION

In 2011, Hoverman et al. (2011) showed 100% mortality of wood frogs in 10 days, after being exposed to an FV3-like isolate from GA (5). These results are comparable to what we observed with the TN isolate; however, it is still slower than the MN isolate.

These preliminary results support the hypothesis of increasing pathogenicity with increasing distance from the host population.

Brenes (2013) reported a weak, positive correlation between isolate distance and pathogenicity across 16 species tested with our Tennessee isolate (2).

Our results support Bayley et al. (2013) of higher pathogenicity at warmer temperature, which may be related to an increase in viral replication or host cell division (1). Greater pathogenicity of FV3-like ranaviruses at warmer temperature may explain the occurrence of die-offs often during summer months.

Our temperature results have climate change implications.

REFERENCES

1. **Bayley, A. E., B. J. Hill, and S. W. Fiest. 2013.** Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts. Diseases of Aquatic Organisms 103:171-183.
2. **Brenes, R. 2013.** Mechanisms contributing to the emergence of ranavirus in ectothermic vertebrates communities. Dissertation, University of Tennessee, Knoxville, USA.
3. **Gagneux S. 2012.** Host-pathogen coevolution in human tuberculosis. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1590): 850-859.
4. **Gilbert SC, Plebanski M. 1998.** Association of Malaria Parasite Population Structure, HLA, and Immunological Antagonism. *Science* 279(5354): 1173-1177.
5. **Hoverman JT, Gray MJ, Haislip NA, Miller DL. 2011.** Phylogeny, Life History, and Ecology Contribute to Differences in Amphibian Susceptibility to Ranaviruses. *EcoHealth* 8:301-319.
6. **Hoverman JT, Gray MJ, Miller DL. 2010.** Anuran susceptibilities to ranaviruses: role of species identity, exposure route, and a novel virus isolate. *Diseases of Aquatic Organisms* 89:97–107.
7. **Storfer A, Alfaro ME, Ridenhour BJ, Jancovich JK, Mech SG, Parris MJ, Collins JP. 2007.** Phylogenetic Concordance Analysis Shows an Emerging Pathogen is Novel and Endemic. *Ecol. Lett.* 10: 1075–1083.

ACKNOWLEDGEMENTS

We would like to thank the following for funding and support: Merial Fellowship, UT College of Veterinary Medicine: COE Program, UT AgResearch, Dr. Wilkes, UT ETREC and JARTU (Facilities and Management).