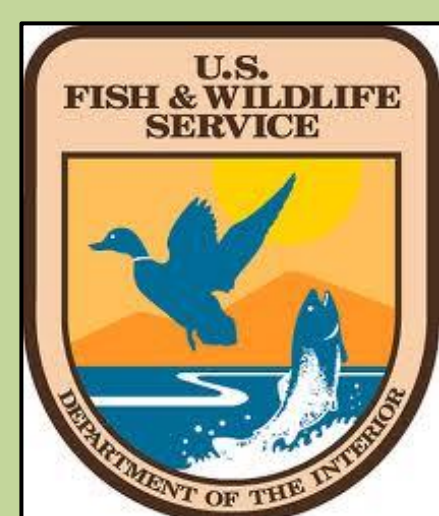# High Susceptibility of the Most Endangered Frog in North America (*Rana sevosa*) to Ranavirus

William B. Sutton[1], Matthew J. Gray[1], Rebecca H. Hardman[1], Debra L. Miller[1], and A. Kouba[2]
[1]Center for Wildlife Health, University of Tennessee, Knoxville, TN
[2]Memphis Zoo, Memphis, TN

Introduction:

- The Mississippi gopher frog (*Rana sevosa*) is a medium-sized (total length 56 – 105 mm), stout-bodied anuran native to longleaf pine (*Pinus palustris*) flatwood forests of the southeastern coastal plain.
- The historical distribution of *R. sevosa* included southwestern Alabama, Mississippi, and southeastern Louisiana, but has been reduced immensely by habitat destruction, fragmentation, and alteration of historical disturbance regimes (e.g., fire suppression).
- Periodic disturbances (e.g., wind, fire) are required to maintain open-canopy conditions in breeding habitats to speed larval metamorphosis.
- Primary conservation concerns include the continuing impacts of improper habitat management and the impacts of potentially lethal amphibian pathogens (i.e., ranavirus).
- The objective of this study was to evaluate the susceptibility of *R. sevosa* to three ranavirus exposure routes (intra-peritoneal [IP] injection, oral [OR] inoculation, and water bath [WB; transdermal]) exposure routes.
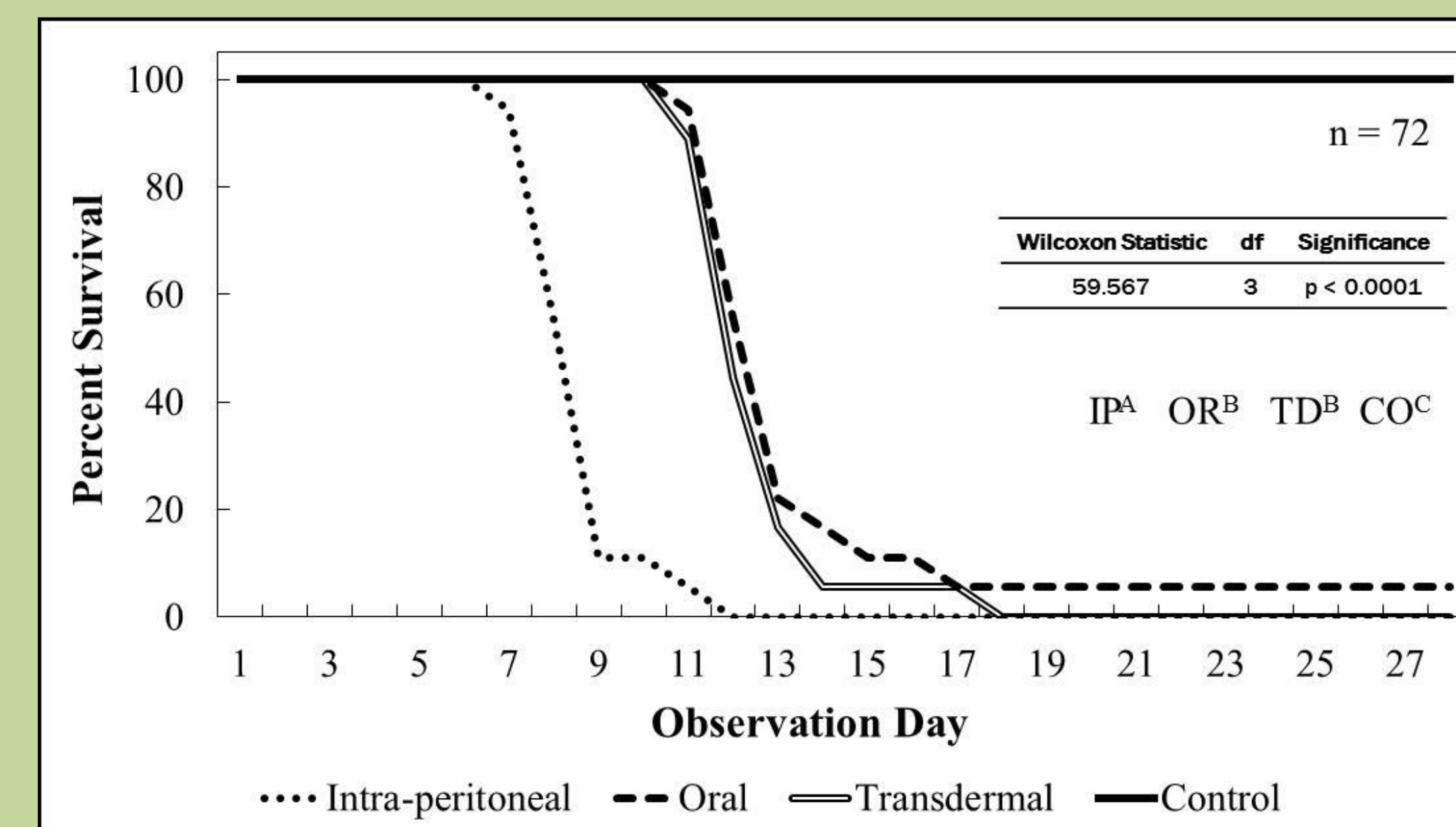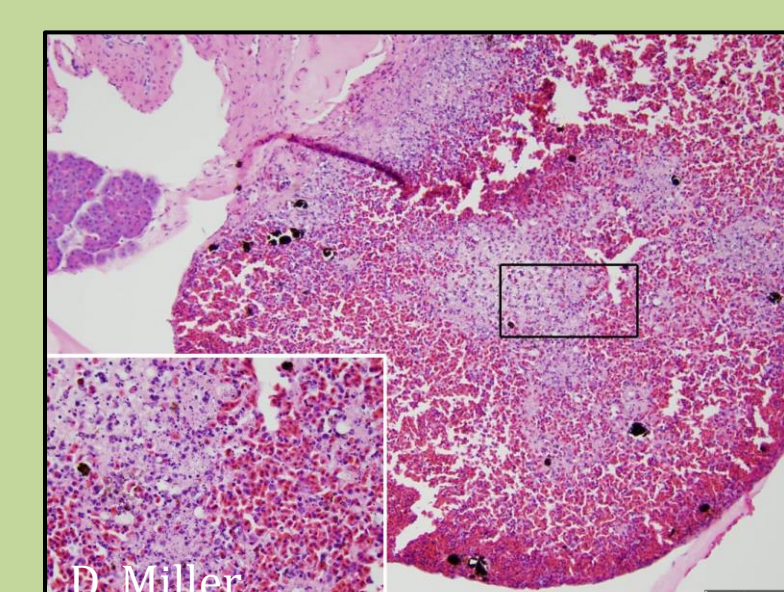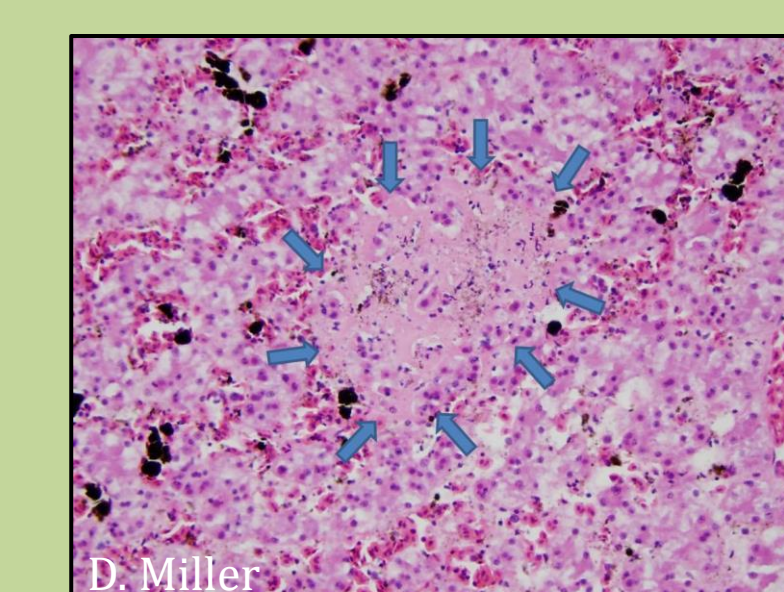
Methods:

- We obtained 74 captive-reared adult *R. sevosa* from the Omaha Zoo in Omaha, Nebraska.
- Frogs were maintained communally (7 – 8 frogs per container) in large, clear plastic Rubbermaid® containers for seven days in the Johnson Animal Research and Teaching Unit at the University of Tennessee.
- Prior to ranavirus inoculation, we sacrificed two individuals to assure that individuals were not infected prior to the experiment.
- Experimental individuals (18 per treatment) were randomly assigned to four treatments (Control, IP, OR, and WB). Ranavirus treatments (IP, OR, and WB) consisted of environmentally relevant doses (i.e., 10^3 PFUs) of an FV3-like ranavirus isolated from a morbid American bullfrog.

- We housed frogs individually in 2 L clear plastic containers and observed individuals for signs of ranavirus infection (e.g., lethargy, petechial hemorrhaging) twice daily over a 28 day experimental period.
- Every three days, we cleaned housing containers and provided each individual with three adult crickets.
- If signs of ranavirus were observed for more than 12 hours, we euthanized these individuals using benzocaine and performed necropsies to collect tissue samples from major organs and lesion sites.
- We analyzed mortality rates using Life Table Analysis in SPSS v. 21.0.

All procedures followed approved UT IACUC Protocol #2140

Figure 1: Percent survival of adult *R. sevosa* to three ranavirus exposure routes over a 28-day period
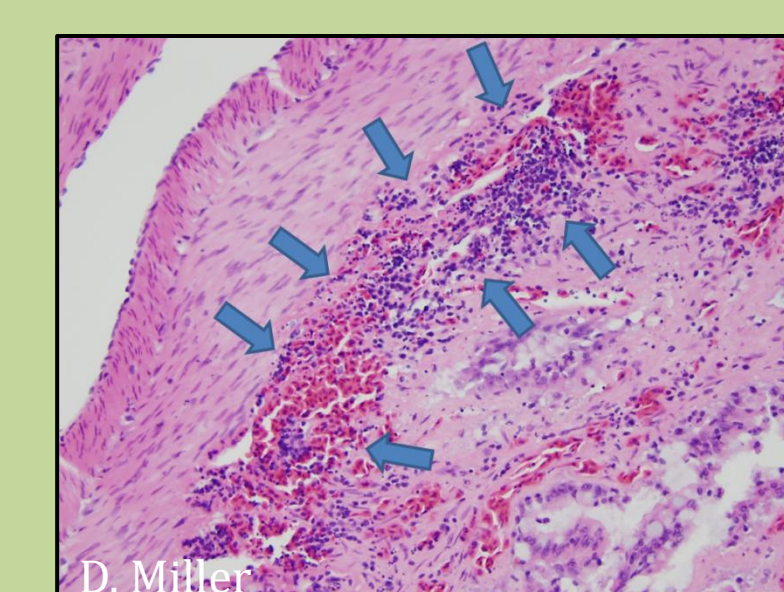

Massive Splenic Necrosis (OR)   Petechial hemorrhaging (tongue [OR])   Liver with Diffuse Degeneration (OR)   Perivascular inflammation and necrosis (WB)

Results:

- We found that survival curves differed significantly among treatments (W = 59.6; P < 0.001) with the IP route resulting in the fastest mortality (Figure 1).
- WB exposure resulted in 100% mortality in 18 days.
- One individual in the OR exposure route survived the 28-day experimental period.
- Histopathological changes included: massive splenic necrosis, hepatocellular degeneration and multifocal hepatic necrosis, and perivascular necrosis
- We observed 100% survival of control individuals.

Discussion:

- *Rana sevosa* were highly susceptible to ranavirus; therefore, this pathogen has the potential to cause die-offs in the remaining populations of *R. sevosa*.
- Using our data, Julia Earl (NIMBioS) demonstrated extinction of *R. sevosa* without a disease intervention strategy.
- Typically, adult amphibians have relatively low susceptibility to ranavirus. Isolation of the remaining *R. sevosa* may be affecting genetic diversity, which can affect susceptibility to ranavirus (Pearman and Garner 2005).
- Hoverman et al. (2011) found that species with limited geographic distribution and that exploited non-permanent breeding habitats tended to be more susceptible to ranavirus similar to *R. sevosa.*
- Future plans include testing egg, hatchling, larva, and metamorph stages.
- Researchers should focus single challenge experiments on rare species, and ensure biosecurity precautions are followed during field surveillance.