



STATE WILDLIFE GRANT PROJECT REPORT—INDIANA

Ranavirus Surveillance in Box Turtles and Aquatic Amphibians



Although box turtles can live for 100 years or more, their populations are in jeopardy from loss of habitat, disease, vehicular collisions, and illegal collection of turtles from the wild.

Current Status

First year of a two-year project

Funding Sources and Partners

State Wildlife Grant Program (T7R14)
Purdue University

Project Personnel

Principal investigators: Dr. Jason T. Hoverman, Dr. Rod N. Williams, Purdue University
Dr. Steven J.A. Kimble, postdoctoral research assistant, Purdue University

Technicians

Andrew DuBois (2014)
Chrissy Bienz (2013-2014)
Vanessa Wuertner (2013)

Background and Objectives

Ranaviruses constitute an emerging set of pathogens causing massive die-offs in reptile, amphibian and fish species worldwide, yet little is known about their distribution, prevalence, and temporal dynamics of infection.

The main goals of this project are twofold:

- 1.) to use genetic methods to assess the progress of a ranavirus outbreak in a captive population of Eastern box turtles (*Terrapene carolina*) in Indiana
- 2.) to use genetic methods to test frog tadpoles from ponds across Indiana to determine the prevalence of the virus in natural populations and its distribution across the state.

Methods

To better understand how ranavirus outbreaks affect a population, we take a blood and oral swab sample from a captive population of box turtles every two weeks during the active season (approximately April through October). Using very sensitive genetic techniques we can then tell how much, if any, virus is present in those samples. This can give us insight into the biology of the disease. For example, the disease may not thrive well in hot weather, so animals that have previously tested positive may test negative in hot summer months. This doesn't mean that the animal has been cured (it probably cannot be). It means the virus is reduced or not present in the blood or mouth. The main lesson from such a finding would be



Skin coloration on Eastern box turtles varies greatly, from dull brown to bright yellow or orange. (Photo by Steven Kimble)

that testing a wild population for ranavirus in the summer would likely give false negative results. A second insight into the disease is whether individuals can become resistant or tolerant to it. The presence of tolerant individuals would suggest that these individuals could spread the disease because by definition, they carry the virus but do not die from it. The presence of resistant individuals would suggest that some individuals are either naturally immune or can gain immunity from previous infections.

It is thought that the ranavirus present in the captive turtle population was inadvertently brought in from the wild when those individuals were removed from an interstate construction site. To understand how prevalent ranavirus may be in the wild we will be testing hundreds of green frog and bullfrog tadpoles collected from dozens of ponds around the state. These data will give us an idea about how widespread the disease may be across Indiana and thus how serious of a threat it represents to Indiana's wildlife.

Progress

In 2014, we continue to take blood and oral swab samples from the captive box turtles every two weeks. We have developed laboratory techniques to quickly process and test these samples. Unfortunately, mortality has been high in this population and about 230 individuals (out of an original population of about 320) have died or are presumed dead. The surviving 90 look healthy, but some virus is still detectable in them. We also found that mosquitoes in the area tested positive for ranavirus. This suggests a possible way disease is spread among individual turtles. This also led to a publication in a peer-reviewed journal. We will be spending the fall and winter months testing the rest of these samples. We have also received several hundred tadpoles from around the state and have begun



Oral swab samples being taken on an Eastern box turtle to test for ranavirus. (Photo by Steven Kimble)



Chrissy Bienz (Purdue University), Sarabeth Klueh-Mundy (DFW), and Andrew DuBois (Purdue University) sampling Eastern box turtles for ranavirus. The turtles are temporarily held in separate tubs to prevent cross contamination. (Photo by Steven Kimble)

to test these. Through the use of wildlife monitoring studies, this collaborative study promises to yield useful data that can help up conserve the wildlife species of Indiana.

Cost: \$189,132 for the complete 2-year project.