STATE WILDLIFE GRANT PROJECT REPORT—INDIANA
Ranavirus Surveillance in Box Turtles and Aquatic Amphibians

Chrissy Bienz (Purdue University), Sarabeth Klueh-Mundy (DFW), and Andrew DuBois (Purdue University) collect genetic samples from Eastern box turtles. Sampling takes about 10 or 15 minutes per turtle. Even with multiple sampling personnel, it still takes most of a day. (photo by Steven Kimble)

CURRENT STATUS

Final 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 and 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 Wuerthner (2013)

BACKGROUND AND OBJECTIVES

Ranaviruses are causing massive die-offs in reptile, amphibian and fish species worldwide, yet the diseases are relatively new to science and little is known about their distribution, prevalence, and patterns of infection. The main goals of this project are:

1) to use genetic methods to assess the progress of a ranavirus outbreak in a captive population of Eastern box turtles *(Terrapene carolina carolina)* in Indiana, and

2) to use genetic methods to test frog tadpoles from across Indiana to determine the prevalence of the virus in natural populations and its distribution across the state.

METHODS

To better understand how outbreaks of the emerging amphibian, fish and reptile disease ranavirus affect the

The coloration of Eastern box turtles can vary greatly, from dull brown to bright yellow or orange. Sometimes the eyes are red, sometimes brown. (photo by Steven Kimble)

health of wildlife populations, we took blood samples and both an oral and cloacal swab sample from a captive population of box turtles every two weeks during the active seasons of 2013 and 2014 (approximately April through October). We also collected more than 1,500 samples from green frogs and bullfrogs in 2013 and 2014 from 15 sites across Indiana. Using sensitive genetic techniques, we tested all of these samples to determine if ranavirus was present in them. The results gave us insight into many aspects of the biology of the disease. This insight will be useful for dealing with the disease in Indiana populations of reptiles, amphibians, and fishes.

RESULTS

We found that in the captive population of box turtles, the proportion of individuals that tested positive dropped in the summers, bottoming out in July. This doesn't mean the animals have been cured (they probably can't be), but it probably does mean that because the virus is reduced or more difficult to detect in the middle of summer, testing a wild population for ranavirus during that time period might give false negative results.

A second insight into the disease is that about a quarter of the box turtles that caught the virus continued to survive, long after most of the population succumbed. While it is good news that the virus doesn't kill every individual, it also means that these tolerant individuals might be able to spread the disease to other locations, individuals, or even other species. The presence of resistant turtles in the population also suggests that some individuals are either naturally immune to ranavirus or can gain immunity from previous infections.

From testing tadpoles, we found that ranavirus is probably already widespread in Indiana although there were no obvious signs of disease at any of the sites where they were collected. Many outbreaks are only discovered because many dead or dying amphibians are found in one location, but it appears as though at least some individuals may be able to handle the virus. Again, this means that these individuals might be able to spread the disease to other amphibian, reptile, or

In order to identify individual turtles, one method that can last for decades is filing a unique pattern of notches into the edges of the shell. The outer surface of turtle shells is made of the same material as hair and fingernail, and does not have nerves. (photo by Steven Kimble)

fish species in the area, but more research needs to be done in this area.

A third interesting result from this project is that we found that some mosquitoes also test positive for ranavirus. This does not confirm that mosquitoes could act as vectors of ranavirus, but it does mean that more research on this possibility should be pursued.

Through the use of wildlife monitoring studies such as this, we collected useful data that could help conserve the wildlife species of Indiana.

PAPERS PUBLISHED

Kimble, SJA, AK Karna, AJ Johnson, JT Hoverman and RN Williams. 2014. Mosquitoes as a potential vector of ranavirus transmission in terrestrial turtles. EcoHealth. DOI: 10.1007/s10393-014-0974-3.

COST: $189,132 FOR THE COMPLETE 2-YEAR PROJECT

Mucus oozing from the eyes and nose (milky substance in the photo) is typical of ranavirus infection in box turtles. Researchers can take samples of these fluids and test them for presence of the virus. (photo by Steven Kimble)