



# STATE WILDLIFE GRANT PROJECT REPORT—INDIANA

## Ecology and Population Genetics of Eastern Box Turtles in Indiana



*Coloration of the shell and skin of Eastern box turtles varies greatly among individuals, from dull brown to bright yellow or orange, such as on this individual. (Photo by John Maxwell, DNR.)*

### Current Status

Fifth year of a five-year project

### Funding Source

State Wildlife Grants (T07R09), Purdue University,  
DNR Nongame Fund

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### Background

Eastern box turtles are long-lived reptiles that are native to forested regions across the eastern United States. Their numbers are declining across the country, most

likely because of habitat loss, collection from the wild for sale as pets, disease and mortality on roadways. Some habitat alterations, such as timber harvests, are a common and sustainable type of habitat management, but these activities may affect forest animals in different ways. It is unclear if box turtles will be affected either positively or negatively by these types of activities.

## Objectives

The purpose of this project is to use genetic tools and radio telemetry to clarify the ecology and population organization of Eastern box turtles in Indiana for use in conservation programs. In general, we are interested in the short-term impacts of timber harvesting on Eastern box turtles' movement, home-range size and over-winter use. We are also interested in using molecular methods to assess the genetic health of Indiana's box turtle populations compared to those of others across the species' range, and to reveal cryptic aspects of their natural history.

## Methods

To understand how much turtles move during their daily activities and lives, we glued tiny radio transmitters to 50 box turtles in Morgan-Monroe and Yellowwood state forests. Many highly skilled people spent all summer and fall from 2007 to 2010 hiking in the woods, finding each turtle three times a week with radio receivers, and mapping each turtle's movements. These movements were studied in the periods before and immediately after timber harvests to help biologists understand how box turtles respond in the wild. Any changes in movement patterns in response to timber harvests might affect mating, foraging and health.

While it is important to study how turtles respond to timber harvests during summer months, it is equally important to do so during winter months, when box turtles are hibernating. Turtles were monitored during winter months using very small temperature dataloggers that were affixed to their shells. Dataloggers were also used to monitor ambient temperatures in the turtle's habitats from many locations in the forest. These dataloggers recorded temperatures every 45 minutes and provided critical information on the thermal environment in timber harvest openings versus that of uncut areas of the forest.

Spending so much time in the woods meant we saw a lot of other box turtles. From every box turtle we found we took a small blood sample for DNA analysis. In our laboratory we can get a good picture of the genetic characteristics of the box turtle populations. To accomplish this we used DNA markers that were developed for the first time in our lab specifically for use with Eastern box turtles. Just like the DNA fingerprinting used to identify humans for parentage or forensic purposes, each turtle has a unique DNA fingerprint, or genotype. More similar genotypes are found among more-related turtles, and less-related turtles have less-similar genotypes, so we can estimate how the captured turtles are related. We

can also assess other aspects of their genetic health and ask questions about natural history, topics difficult to address using non-genetic methods. Finally, to be able to compare the turtles at Morgan-Monroe and Yellowwood to those from other sites, in 2010 and 2011 we collected samples from at least 25 turtles from sites across Indiana and most of the rest of the species' range. All this may sound simple but is costly in money, skilled labor and time. While it takes all summer to find and track turtles, it takes all winter, while the turtles are over-wintering, to complete the genetic analyses, contrary to fictional forensic investigations depicted on popular television shows.

## Progress

All aspects of this project have now been completed. For the radio telemetry portion of the project we tracked 50 turtles for two years before and two years after timber harvests. Analyses of pre-harvest versus post-harvest movements suggest that Eastern box turtles tend to avoid the centers of large timber harvests but gravitate toward the edges of harvest openings during summer. During winter, when Eastern box turtles are hibernating, they choose one spot in which to burrow to escape the cold. The deeper they go, the more moderate the temperature.

Eastern box turtles in Indiana may hibernate for five to seven months of the year; that means they spend nearly half their lives underground. We found that turtles prefer to over-winter at temperatures of approximately 38°F and at an average depth of 4 inches. Although most turtles hibernated in the forest, we recorded one turtle that hibernated in a timber harvest cut.

Previously it was thought that over-wintering turtles would not use timber harvests because of the absence of a tree canopy and the lack of a leaf-litter floor mat to buffer them from extreme cold. We found that the timber harvests were colder all winter, but if turtles burrowed deeply enough, warmer temperatures could be reached and maintained. We also found that the timber harvests warmed more quickly during spring, when turtles emerge from hibernation, possibly allowing those turtles that over-wintered close to the harvest edge to emerge sooner and start feeding.

For the genetic portion of the project, we collected samples from more than 1,600 box turtles from across their geographic range. We found that there are only two genetic populations range-wide, roughly separated by the Appalachian Mountains. This was surprising because, as we learned from the study of the radio-tracked turtles at Morgan-Monroe and Yellowwood, most adult Eastern box turtles do not move around much, but keep to a small home range, and they appear to keep the same home range for years. This suggests that populations would be small and localized. However, we also have found that there are rare individuals who seem to have a different lifestyle. They do not maintain a home range but rather seem to be on some sort of journey. One individual traveled almost five miles in a nearly straight line

in one summer, a distance much greater than a normal home range. It may be that these are the individuals that make box turtle genetic populations so large because as they travel, they mate, spreading their genes across a wider geographic area. We tested the genotypes of the two individuals that appeared to be transients, and one of them was indeed from another population.

Genetic diversity is high across the range. This is good for the species; however, it doesn't mean that the box turtle is not threatened with extinction. Box turtles can live to at least 100, but we can't tell how old an individual is by looking at it. We likely sampled many old individuals who were born decades or even a century ago. Since their genes haven't changed since birth, what appears to be a genetically healthy population may be a historical artifact. As population sizes continue to decline, their genetic health will eventually decline. Box turtles are protected in Indiana (they are listed as a Species of Special Concern by the Indiana DNR) to help retain good genetic health.

We had also predicted, based on the small and consistent home ranges we observed at the Morgan-Monroe and Yellowwood site, that since there doesn't appear to be much adult dispersal (with the exception of the rare transients) that related individuals would live near each other. This would suggest that these relatives might mate with each other and that inbreeding might be high, especially in areas with significant habitat fragmentation. However, we found that neighbors are generally not related, and that inbreeding is not a problem. Some form of dispersal (perhaps pre-adult) may occur to explain this.

This project was central to the training of two graduate students and more than a dozen undergraduate students and technicians, giving them all marketable skills that can translate to good jobs and contribute to the Indiana economy. We have conveyed our findings to the public through numerous outreach presentations and publications, helping raise public awareness about the Eastern box turtle in particular and wildlife species in general. This work has resulted in at least nine peer-reviewed scientific publications. Finally, by the end of the study, we will have provided valuable information for the protection of the Eastern box turtle in Indiana and across its range in the form of management recommendations.