



## STATE WILDLIFE GRANT—INDIANA

### Genetic Assessment of Crawfish Frog Populations in Indiana



*Crawfish frogs are so named because they inhabit crawfish burrows. Here, a metamorph sits on its “doorstep.” (Photo credit: U.S. Fish & Wildlife Service and Andrew Hoffman)*

#### CURRENT STATUS

Second year of a two-year project

#### FUNDING SOURCES AND PARTNERS

State Wildlife Grant Program (T7R19)  
Purdue University  
Indiana University  
Eastern Kentucky University

#### PROJECT PERSONNEL

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#### BACKGROUND AND OBJECTIVES

Disease and habitat modification are major threats faced by amphibians, including many in Indiana. Crawfish frogs (*Lithobates areolatus*), so named because they inhabit crawfish burrows, have experienced dramatic declines in Indiana since the 1970s. They once inhabited 23 Indiana counties, mostly in the southwestern corner of the state. They have been listed as a state-endangered species since 1984. Excluding an isolated population in southeastern Indiana, crawfish frogs are now found in only eight southwestern counties.

Interest in this species in Indiana was heightened by the 2003 discovery of an isolated population, apparently thriving and outside of the historical range, at Big Oaks National Wildlife Refuge (BONWR) in Jennings, Jefferson, and Ripley counties. As the former Jefferson Proving Ground, BONWR might have been colonized by crawfish frogs by the movement of military personnel or equipment. Alternatively, the area might have been occupied by crawfish frogs moving from a



*Metamorph (juvenile) crawfish frogs pictured in a bucket trap, captured so that they can be counted and then released. The stick is to allow any mammals that fall into the trap to escape; the sponge retains moisture. Crawfish frogs are listed as state-endangered in Indiana. (Photo credit: U.S. Fish & Wildlife Service)*



*Crawfish frog metamorphs have just developed legs and left the ponds where they hatched and were tadpoles. They will continue to grow until they reach adult size. (Photo credit: U.S. Fish & Wildlife Service and Andrew Hoffman)*

previously undetected isolated population as land was cleared by settlers.

Regardless of the origin, BONWR supports one of only two known populations in Indiana that appear to be thriving. The second occupies Hillenbrand Fish & Wildlife Area in a locale that has been reclaimed from surface coal mining. Crawfish frogs were apparently among some of the earliest herpetofaunal species to recolonize this landscape. This pioneering behavior might be exploited to intentionally reestablish populations on reclaimed lands, especially those managed for wildlife. One example is Patoka National Wildlife Refuge in southwestern Indiana.

Evaluation of a species' genetic composition is a necessary first step in developing plans to establish new populations. This is useful for selecting source populations, from which individuals can be drawn to start new populations, and to preserve the remaining genetic diversity of the remnant populations. Some work has been completed to quantify the genetics of crawfish frogs in Indiana, but recent advances in techniques and technologies allow for a much broader examination of a species' genetic composition. This enables more precise estimates of genetic diversity, especially in regions of the genome that provide immunity to infection or disease. Therefore, the objectives of this study are to:

1. Improve measures of the genetic diversity in current Indiana populations of crawfish frogs, especially with respect to the genetic basis for disease resistance, and
2. Make recommendations for which source populations should be used in establishing new populations in Indiana.

## **METHODS**

In early spring, we listen for calls at sites known to have had sustained crawfish frog populations within the last decade. At those where males are heard calling, we search for adult frogs and capture them by hand or with nets or traps. A small tissue sample is collected from each captured adult, which is then immediately released. Later in spring, if needed, eggs and tadpoles are also sampled. We extract and sequence DNA from tissue samples and use these data to derive genetic diversity within each population cluster.

## **PROGRESS TO DATE**

We have collected tissue samples from about 110 individual crawfish frogs from across Indiana. We extracted DNA from these samples, sequenced them, and are processing the sequence data. We will complete the collection of the genetic data in 2018 and be able to make recommendations for the best source populations from which new populations of crawfish frogs can be established.

## **COST: \$74,584 FOR THE COMPLETE TWO-YEAR PROJECT**