



# STATE WILDLIFE GRANT PROJECT REPORT—INDIANA

## Local and Landscape Habitat Association, Population Ecology and Future Recovery of Crawfish Frogs in Indiana



*A metamorphosed crawfish frog.*

### Current Status

First year of a two-year project, after an initial five-and-a-half year project

### Funding Sources and Partners

State Wildlife Grant Program (T7R7)  
Indiana University  
Detroit Zoological Society

### Project Personnel

Dr. Michael Lannoo, Indiana University School of Medicine  
Dr. Daryl Karns, Hanover College (deceased)  
Dr. Joe Robb, U.S. Fish & Wildlife Service, Big Oaks National Wildlife Refuge  
Dr. John Whitaker, Indiana State University  
Dr. John Crawford, Lindenwood College, served on the project from Jan. 1–June 30, 2009  
Marcy Sieggreen, Detroit Zoological Society  
Rochelle Stiles, Indiana State University, graduate student  
Jonathan Swan, Indiana State University, graduate student  
Nate Engbrecht, Indiana State University, graduate student  
Jennifer Heemeyer, Indiana State University, graduate student  
Vanessa Kinney, Indiana State University, graduate student

Andrew Hoffman, Indiana State University, graduate student  
Perry Williams, U.S. Fish & Wildlife Service, Big Oaks National Wildlife Refuge  
Ben Walker, U.S. Fish & Wildlife Service, Big Oaks National Wildlife Refuge  
Todd Gerardot, Big Oaks National Wildlife Refuge, refuge intern  
Dr. Alan Pessier, San Diego Zoo, veterinarian (disease)  
Dr. Irene Macallister, U.S. Army Corps of Engineers (disease)  
Dr. Stephen Richter, Eastern Kentucky University (genetics)  
Emily Gustin, Eastern Kentucky University (genetics)  
Dr. Alisa Gallant, U.S. Geological Survey EROS Data Center (GIS)  
Dr. Robert Klaver, U.S. Geological Survey EROS Data Center (ecological modeling)  
Bill Peterman, consultant, served on the project from Jan. 1–June 30, 2009  
Kelsey Flowers, Big Oaks National Wildlife Refuge, intern  
Stephanie Bishir, Big Oaks National Wildlife Refuge, intern  
Angel Tang, Big Oaks National Wildlife Refuge, intern  
Justin Emmons, Big Oaks National Wildlife Refuge, intern  
Alex Robinson, part-time technician  
John Ryan, part-time technician



**Volunteers release crawfish frog tadpoles that were captive-reared at the Detroit Zoological Society into reptariums at Hillenbrand Fish & Wildlife Area in Greene County. (Photo by Marcy Sieggreen)**

- Shane Stephens, part-time technician
- Austin McClain, part-time technician
- B. Jagger Foster, part-time technician
- Tenia Wheat, part-time technician
- Austin May, part-time technician
- Lauren Sawyer, part-time technician
- Helen Nesius, part-time technician
- Danny Schaefer, part-time technician
- Susan Lannoo, consultant (unpaid)
- Peter Lannoo, graphics designer (unpaid)

### Background and Objectives

Crawfish frogs (*Lithobates [Rana] areolatus*) are large (adults are 3 inches or longer), heavy frogs that spend much of their adult life in crayfish burrows. In Indiana, crawfish frogs are state endangered, and their declining status across much of their range has caused broad concern about their conservation. According to Sherman Minton, crawfish frogs were locally plentiful in southwestern Indiana until about 1970. The reasons for their recent and rapid decline are the focus of this work.

Typically, crawfish frogs are associated with tallgrass prairies or other native grasslands. These habitats are increasingly being fragmented by, or converted to, row-crop agriculture. Crawfish frogs also are considered weak larval competitors. This status likely results in reduced recruitment into populations. Local and regional declines may be further enhanced by interactions with exotic species and the emergence of infectious diseases. While there is some information on general habitat use and population demographics on crawfish frogs, their fossorial nature and scarcity has made detailed investigations difficult and recovery plans ineffective.

If the ultimate goal for an endangered species is the recovery of populations, then distribution, habitat use and mechanisms of decline must be investigated. The status of the crawfish frog in Indiana presents a unique opportunity for this type of study.

### Objectives

1. Determine the status of crawfish frog populations in Indiana.
2. Develop methods to monitor the status of crawfish frog populations in Indiana.
3. Determine population parameters of crawfish frogs on public lands in an effort to delimit potential life-history bottlenecks that affect the survival of this species.
4. Define natural history features such as movement patterns (across the landscape), activity patterns (daily and seasonally) and habitat-use features (burrow location) of crawfish frogs, and identify threats to this species from current landscape attributes (roads, agricultural fields) and land-use practices (frequency of plowing, prescribed burning).
5. Determine the genetic relationships of crawfish frog populations across Indiana.
6. Define the role of disease, like chytrid fungus (*Batrachochytrium dendrobatidis*), in limiting crawfish frog populations in Indiana.
7. Determine how practical captive rearing can be for augmenting populations.
8. Run parallel studies at sites in southwest Indiana (Hillenbrand Fish & Wildlife Area-West, Dave's Pond) and southeast Indiana (Big Oaks National Wildlife Refuge).
9. Provide management recommendations to Indiana Department of Natural Resources (DNR) and U.S. Fish & Wildlife Service to maximize the likelihood that crawfish frog populations persist in Indiana.

### Methods

We have been using a wide variety of methods and techniques, including drift fences/pitfall traps, call surveys, seining, minnow trapping, radio telemetry, museum and literature searches, wildlife cameras, song meters, digital videography, pit tagging, toe clipping, microsatellite arrays, histology, PCR analyses, visual surveys, disease surveys, tissue sampling for genetic analysis, and captive rearing, as follows:



**Funnel traps used to sample for crawfish frog adults in peripheral wetlands. (Photo by Rochelle Stiles)**

- 1) Status: Literature searches, museum searches, call surveys, seining, minnow trapping, song meters
- 2) Monitoring: Occupancy modeling, song meters, minnow trapping, egg mass counts
- 3) Population parameters: Drift fences/pitfall traps, radio telemetry, pit tagging, histology
- 4) Natural history: Drift fences/pitfall traps, radio telemetry, wildlife cameras, videography
- 5) Genetics: Toe clipping, microsatellite arrays
- 6) Disease: Swabs for chytrid fungus, histology, PCR
- 7) Population augmentation: Captive rearing, diet, timing, determining rates of cannibalism and predation
- 8) Statewide comparison: Two crews, one in southwest Indiana led by Lannoo, the other at Big Oaks National Wildlife Refuge, led by Karns and Robb

### Papers in preparation/submitted

- Engbrecht, N.J., J.L. Heemeyer, C.G. Murphy, R.M. Stiles and M.J. Lannoo. Upland calling behavior in crawfish frogs (*Lithobates areolatus*) and calling triggers caused by noise pollution.
- Klemish, J.L., S.P. Aldrich, R.M. Stiles, N.J. Engbrecht, J.L. Heemeyer and M.J. Lannoo. Habitat use in a host-dependent system.
- Stiles, R.M., T.R. Halliday, N.J. Engbrecht, J.W. Swan and M.J. Lannoo. Activity patterns of a free-ranging frog.
- Terrell, V.C.K., J.C. Maerz, R.M. Stiles and M.J. Lannoo. Adult survivorship, juvenile recruitment, and juvenile fitness metrics in crawfish frogs (*Lithobates areolatus*), a cryptic, “near-threatened” species. *Journal of Wildlife Management*.

### Papers published

- Engbrecht, N.J., P.J. Williams, J.R. Robb, D.R. Karns, M.J. Lodato, T.A. Gerardot and M.J. Lannoo. 2013. Is there hope for the Hoosier Frog? An update on the status of crawfish frogs (*Lithobates areolatus*) in Indiana, with recommendations for their conservation. *Proceedings of the Indiana Academy of Science* 121:147–157.
- Engbrecht, N.J. and J.L. Heemeyer. 2010. *Lithobates areolatus circulosus* (northern crawfish frog). *Heterodon platyrhinos* (eastern hognosed snake). Predation. *Herpetological Review* 41:197.
- Engbrecht, N.J., J.L. Heemeyer and M.J. Lannoo. 2012. *Lithobates areolatus circulosus* (northern crawfish frog). *Coluber constrictor* (black racer). Thwarted predation. *Herpetological Review* 43:323–324.
- Engbrecht, N.J. and M.J. Lannoo. 2010. A review of the status and distribution of crawfish frogs (*Lithobates areolatus*) in Indiana. *Proceedings of the Indiana Academy of Sciences* 119:64–73.
- Engbrecht, N.J., S.J. Lannoo, J.O. Whitaker and M.J. Lannoo. 2011. Comparative morphometrics in ranid frogs (subgenus *Nenirana*): Are apomorphic elongation and a blunt snout responses to deep, small-bore burrow dwelling in crawfish frogs (*Lithobates areolatus*)? *Copeia* 2011:285–295.
- Engbrecht, N.J. and M.J. Lannoo. 2012. Crawfish frog behavioral differences in postburned and vegetated grasslands. *Fire Ecology* 8:63–76.
- Heemeyer, J.L., V.C. Kinney, N.J. Engbrecht and M.J. Lannoo. 2010. The biology of crawfish frogs (*Lithobates areolatus*) prevents the full use of telemetry and drift fence techniques. *Herpetological Review* 41:42–45.
- Heemeyer, J.L. and M.J. Lannoo. 2010. A new technique for capturing burrow-dwelling anurans. *Herpetological Review* 41:168–170.

- Heemeyer, J.L. and M.J. Lannoo. 2011. *Lithobates areolatus circulosus* (northern crawfish frog). *Winterkill*. *Herpetological Review* 42:261–262.
- Heemeyer, J.L. and M.J. Lannoo. 2012. Breeding migrations in crawfish frogs (*Lithobates areolatus*): Long-distance movements, burrow philopatry and mortality in a near-threatened species. *Copeia* 2012:440–450.
- Heemeyer, J.L., P.J. Williams and M.J. Lannoo. 2012. Obligate crayfish burrow use and core habitat requirements of crawfish frogs. *Journal of Wildlife Management* 76:1081–1091.
- Hoffman, A.S., J.L. Heemeyer, P.J. Williams, J.R. Robb, D.R. Karns, V.C. Kinney, N.J. Engbrecht and M.J. Lannoo. 2010. Strong site fidelity and a variety of imaging techniques reveal around-the-clock and extended activity patterns in crawfish frogs (*Lithobates areolatus*). *BioScience* 60:829–834.
- Kinney, V.C., N.J. Engbrecht, J.L. Heemeyer and M.J. Lannoo. 2010. New county records for amphibians and reptiles in southwest Indiana. *Herpetological Review* 41:387.
- Kinney, V.C., J.L. Heemeyer, A.P. Pessier and M.L. Lannoo. 2011. Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: Affirmation of Vredenburg’s “10,000 Zoospore Rule” *PLoS One* e16708. doi:10.1371/journal.pone.0016708.
- Kinney, V.C. and M.J. Lannoo. 2010. *Lithobates areolatus circulosus* (northern crawfish frog). Breeding. *Herpetological Review* 41:197–198.
- Klemish, J.L., N.J. Engbrecht and M.J. Lannoo. Positioning minnow traps to avoid accidental deaths of breeding frogs. *Herpetological Review* 44:241–242.
- Lannoo, M.J., V.C. Kinney, J.L. Heemeyer, N.J. Engbrecht, A.L. Gallant and R.W. Klaver. 2009. Mine spoil prairies expand critical habitat for endangered and threatened amphibian and reptile species. *Diversity* 1:118–132.
- Nunziata, S.O., M.J. Lannoo, J.R. Robb, D.R. Karns, S.L. Lance and S.C. Richter. 2013. Population and conservation genetics of crawfish frogs, *Lithobates areolatus*, at their northeastern range limit. *Journal of Herpetology* 47:361–368.
- Terrell, V.C.K., N.J. Engbrecht, A.P. Pessier and M.J. Lannoo. 2014. Drought reduces chytrid fungus (*Batrachochytridium dendrobatidis*) infection intensity and mortality but not prevalence in adult crawfish frogs (*Lithobates areolatus*). *Journal of Wildlife Diseases* 50:56–62.
- Terrell, V.C.K., J.L. Klemish, N.J. Engbrecht, J.A. May, P.J. Lannoo, R.M. Stiles and M.J. Lannoo. 2014. Amphibian and reptile recolonization of reclaimed coal spoil grasslands. *Journal of North American Herpetology* 2014:59–68.
- Williams, P.J., N.J. Engbrecht, J.R. Robb, V.C. K. Terrell and M.J. Lannoo. Surveying a threatened species through a narrow detection window. *Copeia* 2013:553–562.
- Williams, P.J., J.R. Robb, R.H. Kappler, T.E. Piening and D.R. Karns. 2012. Intraspecific density dependence in larval development of the crawfish frog *Lithobates areolatus*. *Herpetological Review* 43:36–38.
- Williams, P.J., J.R. Robb and D.R. Karns. 2012. Habitat selection by crawfish frogs (*Lithobates areolatus*) in a large mixed grassland/forest habitat. *Journal of Herpetology* 46:682–688.
- Williams, P.J., J.R. Robb and D.R. Karns. 2012. Occupancy modeling of breeding crawfish frogs in southeastern Indiana. *Wildlife Society Bull.* 36:350–357.



**Drift fence used to sample breeding adult and newly metamorphosed juveniles at our two primary wetlands, Nate's and Cattail ponds. (Photo by Rochelle Stiles)**

### Theses

- Engbrecht, N.J. 2010. The status of crawfish frogs (*Lithobates areolatus*) in Indiana and a tool to assess populations. M.S. Thesis, Indiana State University, Terre Haute, IN.
- Heemeyer, J.L. 2011. Breeding migrations, survivorship and obligate crayfish burrow use by adult crawfish frogs (*Lithobates areolatus*). M.S. Thesis, Indiana State University, Terre Haute, IN.
- Kinney, V.C. 2011. Adult survivorship and juvenile recruitment in populations of crawfish frogs (*Lithobates areolatus*), with additional consideration of the population sizes of associated pond breeding species. M.S. Thesis, Indiana State University, Terre Haute, IN.

### Presentations

- Lannoo, M.J. Habitats lost and habitats found. Association of Zoos and Aquariums Workshop (Keynote), Toledo Zoo, April '09.
- Lannoo, M.J. The biology of crawfish frogs. Association of Zoos and Aquariums Workshop (Keynote), April '10.
- Lannoo, M.J. The conservation biology of crawfish frogs. Iowa Lakeside Lab, June '10.
- Lannoo, M.J. The conservation biology of crawfish frogs. Hoosier Herp Society, September '10.
- Lannoo, M.J. Update on the biology of crawfish frogs. Association of Zoos and Aquariums Workshop (Keynote), April '11.
- Lannoo, M.J. The conservation biology of crawfish frogs. SE PARC February '11.
- Lannoo, M.J. Update on the conservation biology of crawfish frogs. Iowa Lakeside Lab, June '11.
- Lannoo, M.J. The biology of crawfish frogs. Canadian Association of Herpetologists' Annual Meeting (Keynote), October '11.
- Lannoo, M.J. Update on the conservation biology of crawfish frogs. Iowa Lakeside Lab, June '12.
- Lannoo, M.J. Ethics and values across changed and changing landscapes. World Congress of Herpetology (Invited), August '12.
- Lannoo, M.J. The conservation biology of crawfish frogs. University of Iowa, September '12.
- Lannoo, M.J. Can we re-introduce crawfish frogs into Iowa? Iowa State University, September '12.

- Lannoo, M.J. Recent progress on the conservation of crawfish frogs. Association of Zoos and Aquariums Workshop (Keynote), April '13.
- Lannoo, M.J. The conservation biology of crawfish frogs. University of Iowa, September '13
- Lannoo, M.J. The conservation biology of crawfish frogs. Iowa State University, September '13
- Lannoo, M.J. The conservation biology of crawfish frogs. Iowa Lakeside Laboratory, September '14
- Lannoo, M.J. The conservation biology of crawfish frogs. Oregon State University, October '14
- Lannoo, M.J. The conservation biology of crawfish frogs, University of Alaska, October '14
- Engbrecht, N.J. Status and distribution of crawfish frogs (*Lithobates areolatus*) in Indiana. Indiana Academy of Science, October '09.
- Engbrecht, N.J., V.C. Kinney and M.J. Lannoo. Using call counts to estimate anuran population sizes: an example using crawfish frogs (*Lithobates areolatus*). SE PARC, February '11.
- Engbrecht, N.J. and M.J. Lannoo. Status and conservation of crawfish frogs in Indiana. SE PARC, February '11.
- Engbrecht, N.J. Cracking the crawfish frog code: understanding and conserving one of North America's most secretive frogs. Bethel College, November '11.
- Engbrecht, N.J. The secret world of crawfish frogs: understanding and conserving one of North America's most secretive frogs. Friends of Potato Creek State Park Meeting, August '13.
- Heemeyer, J.L. Post-breeding migration and habitat selection of the crawfish frog (*Lithobates areolatus*). Indiana Academy of Science, October '09.
- Heemeyer, J.L. and M.J. Lannoo. Crawfish frog migratory behavior and survival. SE PARC, February '11.
- Hoffman, A.S., P.J. Williams, J.R. Robb and Daryl R. Karns. Activity patterns of the crawfish frog (*Lithobates [Rana] areolatus*) at crayfish burrows in Big Oaks National Wildlife Refuge, southeastern Indiana. Indiana Academy of Science, October '09
- Kinney, V.C. Breeding biology of crawfish frogs (*Lithobates areolatus*) in southwestern Indiana. Indiana Academy of Science. October '09.
- Kinney, V.C., J.L. Heemeyer, A.P. Pessier and M.L. Lannoo. Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: affirmation of Vredenburg's "10,000 zoospore rule" SE PARC February '11.
- Terrell, V.C.K., J.C. Maerz, R.M. Stiles and M.J. Lannoo. Adult survivorship, juvenile recruitment, and juvenile fitness metrics in crawfish frogs (*Lithobates areolatus*), a cryptic, "near-threatened" species. Joint Meeting of Ichthyologists and Herpetologists, July '14.
- Williams, P.J., A.S. Hoffman, J.R. Robb and D.R. Karns. Burrow selection by the crawfish frog (*Lithobates [Rana] areolatus*) in southeastern Indiana. Indiana Academy of Science, October '09.

### Progress

- We have made substantial progress in understanding the life-history and natural-history features of crawfish frogs in Indiana.
- We understand much of their historic and current

distribution, not only in Indiana but also throughout other states east of the Mississippi River. We understand when they breed and have identified a large percentage, perhaps all, of known breeding sites in Indiana. We understand survivorship in egg, larval and juvenile life-history stages, as well as in post-breeding adults. We sent water samples of breeding wetlands for analyses and have shown that neither pesticides nor metals are factors influencing survivorship.

We have successfully raised large numbers of tadpoles to metamorphosis. In 2013 and 2014, we partnered with the Detroit Zoological Society to hatch crawfish frog eggs and raise tadpoles to pre-metamorphic stages. Our results suggest that crawfish frogs can be captive-reared, but they exhibit cannibalism and are susceptible to disease in late larval stages at high densities.

In 2013, workers at Big Oaks National Wildlife Refuge continued trapping and marking efforts in five ponds; created new wetlands in suitable habitats, and relocated eggs and juveniles in an effort to populate these new areas; continued to investigate characteristics of crawfish frog breeding ponds, including the effects of raising tadpoles in ponds with cattail (*Typha spp.*) dominated substrate; and released marked tadpoles (raised at the Detroit Zoo; see above) at two sites.

We have now tracked crawfish frogs for nearly 9,000 “telemetered frog days.” From these data, we understand where adult burrows are located and have made a distinction between primary and secondary burrows. We understand activity patterns and habitat use.

We understand the pattern of infection by the chytrid fungus, which exhibits seasonal waxing and waning, and kills less than 7 percent of adults during or immediately after breeding. We now also understand how drought affects this process.

We have developed a technique for estimating crawfish frog population size based on call characteristics. There are likely fewer than 1,000 crawfish frog adults in Indiana, a figure that confirms their endangered status in the state.

We better understand the role that management techniques such as prescribed burning, cultivation, mowing and establishing food plots have on populations. Genetic analyses have been done and are published. These data show that individual breeding sites at Hillenbrand Fish & Wildlife Area are genetically distinct from those at Big Oaks National Wildlife Refuge.

We use data collected from drift fences at Nate’s Pond and Cattail Pond from 2009–2014 on adult and juvenile survivorship to calculate population trajectories. Stage-based matrix models show that Cattail Pond is a population sink, and during three of the five years of our study (2009, 2012, 2013) Nate’s Pond also was acting as a sink. In short, adult longevity does not appear to be keeping pace with larval mortality.

Our data suggest that a combination of 1 km no-plow buffer zones surrounding crawfish frog breeding wetlands in combination with captive-rearing/head-starting programs for tadpoles is sufficient to restore or establish

crawfish frog populations where upland crayfish populations are robust and healthy.

Drs. Lannoo and Robb have assembled a crawfish frog recovery plan for Indiana, and submitted it to DNR biologists in 2012. Workers within the state communicate frequently. In addition we have set up a listserv ([sevosa@listserv.eku.edu](mailto:sevosa@listserv.eku.edu)) to communicate with people working on this species group (three species: crawfish frogs, gopher frogs [*L. capito*], which have been listed for federal protection, and dusky gopher frogs [*L. sevosa*, which are federally endangered]).

**Cost: \$903,216 for the initial five-year project; \$400,317 for the additional two-year project.**



**Volunteers who released crawfish frog tadpoles, captive-reared at the Detroit Zoological Society, into reptariums at Hillenbrand Fish & Wildlife Area in Greene County. Back row: Ron Ronk, De Ronk, Danny Schaefer, Lauren Sawyer, Jonathan Swan, Kevin Pratt, Alise Vuskalns, Becky Johnson, Pat Cain. Front row: Suzie Ronk, Emma Brinson, Helen Nesius, Marcy Siegreen, Mark Vassallo, Rochelle Stiles. (Photo by Patrick Cain)**