



# STATE WILDLIFE GRANT PROJECT REPORT—INDIANA

## Local and Landscape Habitat Association, Population Ecology and Future Recovery of Crawfish Frogs (*Lithobates [Rana] Areolatus*) in Indiana



*Metamorphosed crawfish frog (Lithobates [Rana] areolatus).*

### Current Status

Fourth year of three-and-a-half year project plus one-year extension

### Funding Sources and Partners

State Wildlife Grant, Indiana University

### 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

Rochelle Stiles, Indiana State University, graduate student

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Dr. Stephen Richter, Eastern Kentucky University (genetics)  
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Dr. Alisa Gallant, U.S. Geological Survey EROS Data Center (GIS)  
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Bill Peterman, consultant, served on the project from Jan. 1–June 30, 2009  
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## 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 being assessed.

Typically, crawfish frogs are associated with tallgrass prairies or other native grasslands; however, 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 across Indiana crawfish frog populations.

6. Define the role of disease (chytrid fungus) in limiting Indiana crawfish frog populations.

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 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:

- 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, led by Karns and Robb

## Progress

### Papers in preparation:

Kinney, 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*.

Engbrecht, N.J., J.L. Heemeyer, C.G. Murphy, R.M. Stiles and M.J. Lannoo. Planes, trains, and automobiles: Upland calling behavior in crawfish frogs (*Lithobates*

*areolatus*) and triggers to calling caused by noise pollution.

Klemish, J.L., S.P. Aldrich, N.J. Engbrecht, J.L. Heemeyer and M.J. Lannoo. Habitat use in a host-dependent system.

### Papers in press:

Terrell, V.C.K., N.J. Engbrecht, A.P. Pessier and M.J. Lannoo. Drought reduces chytrid fungus (*Batrachochytridium dendrobatidis*) infection intensity and mortality but not prevalence in adult crawfish frogs (*Lithobates areolatus*). *Journal of Wildlife Diseases*.

Terrell, V.C.K., J.L. Klemish, N.J. Engbrecht, J.A. May, P.J. Lannoo, R.M. Stiles and M.J. Lannoo. Amphibian and reptile recolonization of reclaimed coal spoil grasslands. *Journal of North American Herpetology*.

### 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 hog-nosed 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.

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.

## 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.
- 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.
- 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.

## Narrative:

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 postbreeding 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, we partnered with the Detroit Zoological Society to hatch crawfish frog eggs and raise tadpoles to pre-metamorphic stages. We used two egg masses from Hillenbrand-West and one egg mass from Big Oaks. Our results suggest that crawfish frogs can be captive-reared, but they exhibit cannibalism in late larval stages at high densities. We observed a difference in the size (mass and snout vent length) of the newly metamorphosed juveniles from the two sites, suggesting that tadpole density and the local environment are key drivers of initial juvenile size in the genotypes found at Hillenbrand and Big Oaks. We also discovered



***Crawfish frog (Lithobates [Rana] areolatus) tadpoles were captive-reared in 100-gallon cattle tanks at the Detroit Zoological Society.***



***Crawfish frog (Lithobates [Rana] areolatus) tadpoles were released at Gosner Stage 39 into a mesh enclosure at Hillenbrand Fish and Wildlife Area West in Greene County.***

that concentrated release points, even when protected by fencing, attract predators, including shrews, water-snakes and wading birds.

In 2013, workers at Big Oaks 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 (*Batrachochytrium dendrobatidis*), which exhibits seasonal waxing and waning, and kills less than 7 percent of adults during or immediately after breeding; we 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.

Trying to radiotrack juveniles has been a challenge, but after a major push in 2011 we understand that juvenile dispersion mimics adult post-breeding migrations—juveniles move away from wetlands in a straight line, apparently until they intersect a suitable burrow.

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 are genetically distinct from those at Big Oaks.

We use data collected from drift fences at Nate’s Pond and Cattail Pond from 2009–2013 on adult and juvenile survivorship to calculate population trajectories. Stage-based matrix models show that Cattail Pond is a population sink, while 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. While adult inter-annual survivorship was 31.6 percent from 2009–2010 and 30.6 percent from 2010–2011, after strip-disking, survivorship was nearly halved to 15.7 percent in 2011–2012. 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: \$820,518, Extension: \$50,048**



***Conducting an experiment investigating the growth of crawfish frog (Lithobates [Rana] areolatus) tadpoles in cattail (Typha spp.) dominated substrate at Big Oaks National Wildlife Refuge.***