

2017 Arkansas State Wildlife Grant Pre-Proposal

Using a Multi-dimensional Approach to Determine the Conservation and Taxonomic statuses of Rare Crayfishes in Northern Arkansas

Project Summary: Crayfishes are one of the most threatened groups of aquatic species in the United States, yet they remain chronically understudied. We proposed to use a combination of established field and lab-based methods and expand on newly developed methods to examine the statuses of two rare Arkansas crayfish species identified in the Arkansas State Wildlife Action Plan, *Cambarus causeyi* and *C. hubbsi*. These methods will include traditional field sampling at historic and unsampled locations, habitat variable measurement and computer generated habitat modeling, species detection using environmental DNA (eDNA), and the analysis of morphological and molecular character data. Together, these methods will be used to elucidate the range, abundance, susceptibility to mineral extraction, habitat preferences, and taxonomic status of two rare Arkansas crayfishes. Information generated will assist resource managers in determining best management practices for both species. Modeling and eDNA techniques developed for this project will be applicable to other primary burrowing crayfishes found in Arkansas. The proposed research project will form the core of one University of Illinois M.S. student's thesis.

Project Leaders: Dr. Christopher A. Taylor, Senior Research Scientist, Illinois Natural History Survey, Prairie Research Institute – University of Illinois, 1816 S. Oak, Champaign, IL 61820, ctaylor@mail.inhs.uiuc.edu. 217-244-2153; Dr. Eric R. Larson, Assistant Professor, Dept. of Natural Resources and Environment Sciences, University of Illinois, Urbana, Champaign, 61801, erlarson@illinois.edu.

Project Partners: Mr. Brian Wagner, Arkansas Game and Fish Commission, 915 East Sevier St., Benton, AR 72015, bkwagner@agfc.state.ar.us.

Project Budget (35% cost-share):

SWG Grant Funds Requested:	\$109,320
Match Provided:	\$66,829
Total Project:	\$176,149

Project Statement

Need: Crayfishes are critically important members of aquatic food webs. Yet, they remain as one of the most endangered (Taylor et al. 2007) and understudied groups (Moore et al. 2013) of aquatic organisms in North America. In recognition of this, many states have increased the number of crayfish-specific priorities within their respective Wildlife Action Plans. The effective management of priority species in any location is dependent upon accurate taxonomic, biological, abundance, and distributional data.

Sitting at the junction of several ecoregions, Arkansas is home to one of the most diverse crayfish faunas found in any U.S. state. A total of 58 species in six genera are known from Arkansas waters. They occur in all habitat types including streams, swamps, large rivers, roadside ditches, and caves. Two Arkansas crayfishes have been listed as 2017 State Wildlife Grant (SWG) Funding Priorities, the Boston Mountains Crayfish (*Cambarus causeyi*) and Hubb's Crayfish (*C. hubbsi*). The most recent sampling efforts for the already narrowly endemic, and primary burrowing, Boston Mountains Crayfish (Fig. 1A) found the species at only four locations, suggesting that it has experienced a considerable range reduction (Robison et al., 2009). Threats to *C. causeyi* include gas exploration in the Fayetteville Shale region given its known habitat in mountain-side seeps. While found throughout the White River drainage of Arkansas and Missouri, the work of AGFC staff and others indicate that populations of Hubb's Crayfish outside of the Spring, Strawberry, and Eleven Point drainages are sporadic (Fig. 1B) and may represent a different taxonomic unit. Uncertainty and data deficiencies surely contribute to the priority scores for both species. Focused efforts conducted under this proposed project will resolve some of that uncertainty and inform resource managers for proper management emphasis for these two species. Methods developed and tested will also be applicable to many other crayfishes found in the state.

Purpose and Objective(s): We will use the skills and experience of the Project Leaders to design and implement a field and lab-based study that would provide information on habitat requirements, impact assessment, taxonomic status, and non-specialist sampling methods for two Arkansas crayfish species. Both of these species, the Boston Mountains Crayfish and Hubb's Crayfish, are listed as funding priorities for Arkansas's 2017 SWG program and our results would assist AFGC staff in determining what, if any, species-specific management actions are warranted.

Our objectives are as follows: 1) estimate current distribution of both *C. causeyi* and *C. hubbsi*; 2) determine taxonomic status of non-Eleven Point and Spring populations of *C. hubbsi* and describe new taxa if warranted; 3) determine if population abundances of *C. hubbsi* differ across sub-basins of the White River drainage; 4) model habitat requirements of *C. causeyi*; 5) determine the potential impact of certain gas exploration/land use activities on *C. causeyi*; 6) determine if environmental DNA (eDNA) methods performed on water samples accurately detect the presence of *C. causeyi*.

Location: Fieldwork for *C. hubbsi* will be conducted in wadable streams across the White River drainage in the Ozark Highlands and Boston Mountains ecoregions of northern Arkansas. Fieldwork for *C. causeyi* will be conducted in headwater streams in the Boston Mountains Ecoregion in Franklin, Johnson, Madison, Newton, Pope, Searcy, and Stone counties. All laboratory analyses will be conducted on the campus of the University of Illinois, Urbana.

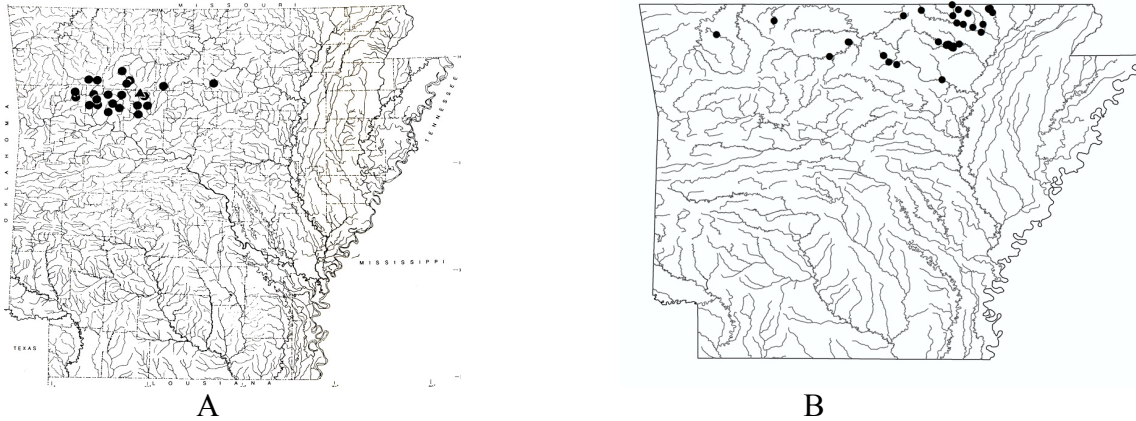


Figure 1A) current known range of *Cambarus causeyi*, denoted by black dots (from Robison and Leeds 1996); B) current known range of *Cambarus hubbsi*, denoted by black dots, data from AGFC and INHS.

Approach: Our field and lab methods will differ for each species. For *C. causeyi* we will visit and sample historical locations sampled by Robison et al. (2009) in year-one using methods developed by Rhoden et al. (2016) for burrowing crayfishes in Arkansas. These sites will encompass both positive and negative presence sites. While on-site we will also record various abiotic habitat characteristics such as canopy cover, stream flow, soil type, and vegetation present. These data will then be used to construct predictive occupancy models following Rhoden (2016). Models will be refined and tested by sampling non-visited sites selected by models in year-two. We will also develop an environmental DNA (eDNA) assay for *C. causeyi* by first developing and testing species-specific primers based on a region of the COI gene for this target species against sympatric non-target crayfishes. We will also visit a subset (10-20) of year-one sampling locations and take five 250 ml water samples for eDNA analysis. We will then filter *in situ* through 1.2 μm cellulose nitrate filters, preserve the filters in ethanol, and return these samples to the laboratory where we will extract DNA prior to amplification using quantitative PCR (qPCR). This emerging eDNA methodology may prove capable of detecting *C. causeyi* in difficult to sample habitats where conventional methods might fail to find this or other burrowing crayfish species (Ikeda et al. 2016).

For *C. hubbsi*, we will visit and sample a subset of historical locations and 20-30 new sites located in regions of the White River drainage not known to harbor the species. Sites will be sampled using traditional kick-set seining methods and relative abundance and catch per unit effort data for *C. hubbsi* will be recorded. Specimens of *C. hubbsi* at these locations will have muscle tissue samples taken and then will be preserved for examination in the laboratory where morphological counts and measurements will be recorded. Mitochondrial DNA from the 16S and COI gene regions will be sequenced from tissue samples. Phylogenetic analyses of combined DNA and morphological characters will be conducted using current Bayesian and maximum parsimony phylogenetic methods.

This project will represent the core research component of one University of Illinois (UIUC) M.S. student's thesis.

Expected Results and Deliverables:

1. Update the distribution of two rare Arkansas crayfishes, *Cambarus causeyi* and *C. hubbsi*
2. Assess the presence of unique lineages or species-level taxa within White River drainage populations of *C. hubbsi*
3. Determine if occupancy modeling will successfully predict new locations and range for an upland, primary burrowing crayfish, *C. causeyi*
4. Determine preferred habitat of and potential impacts of gas exploration/land clearing activities on an upland, primary burrowing crayfish, *C. causeyi*
5. Determine if non-invasive eDNA sampling techniques can reliably detect the presence of *C. causeyi*
6. Refine field and modeling techniques for primary burrowing crayfishes for use with other such species found in Arkansas and upland ecoregions of the United States

Other Donations:

In addition to PI time and unrecovered Facilities and Administration cost share amounts in the below Budget, the PIs will also donate \$4200 in pre-purchased molecular lab supplies. These consumable supplies will be used for mtDNA and eDNA sample preparation.

Budget:

Budget Item	Request	Match
2 yr. assistantship (UIUC)	\$49,732	
Tuition (UIUC)	\$22,009	
Staff Time (INHS/UIUC)		\$26,489
Staff Time (AGFC)		\$8,000
Supplies/contractual ser.	\$2,500	
Hourly labor (INHS/UIUC)	\$10,575	
Travel (AGFC)		\$1,800
Travel (INHS/UIUC)	\$11,500	
F&A	\$13,004	
Unrecovered F&A		\$30,540
Totals:	\$109,320	\$66,829
Project Cost:	\$176,149	

Literature Cited

- Ikeda, K., H. Doi, K. Tanaka, T. Kawai, and J.N. Negishi. 2016. Using environmental DNA to detect an endangered crayfish *Cambaroides japonicus* in streams. *Conservation Genetics Resources* 8(3):231-234.
- Moore, M. J., R. J. DiStefano, and E. R. Larson. 2013. An assessment of life-history studies for USA and Canadian crayfishes: identifying biases and knowledge gaps to improve conservation and management. *BioOne* 32(4): 1276-1287.

Rhoden, C. M. 2016. The use of right of ways by primary burrowing crayfishes in the Ouachita Mountains Ecoregion of Arkansas. M.S. Thesis, University of Illinois at Urbana-Champaign. v+151 pp.

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Robison, H. W., K. A. Crandall, and B. Wagner. 2009. Distribution, genetics, and conservation of three Arkansas crayfishes. Final Report – Project T26R-11, to Arkansas Game and Fish Commission, 7 November 2009. 101 pp.

Taylor, C. A., G. A. Schuster, J. E. Cooper, R. J. DiStefano, A. G. Eversole, P. Hamr, H. H. Hobbs III, H. W. Robison, C. E. Skelton, and R. F. Thoma. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada: the effects of 10+ years of increased awareness. *Fisheries* 32(8): 372-389.

Qualifications:

Dr. Chris Taylor is a Senior Research Biologist and Curator of Crustaceans at the Illinois Natural History Survey and an Adjunct Assistant Professor in the Department of Natural Resources and Environmental Sciences at the University of Illinois. He holds B.S. and M.S. degrees in Zoology and a Ph.D. in Natural Resources and Environmental Sciences. Chris has been conducting research on the taxonomy, conservation and ecology of eastern North American crayfishes for the past 23 years. He has published over 50 peer-reviewed papers and two books on crayfishes and has described 11 crayfish species new to science. He is co-chair of the American Fisheries Society's Endangered Species Committee's Crayfish Subcommittee.

Dr. Eric R. Larson is an assistant professor in the Department of Natural Resources and Environmental Sciences at the University of Illinois. He holds a B.S. in fishery resources from the University of Idaho, Moscow, an M.S. in biology from the University of Arkansas, Fayetteville, and a PhD in aquatic and fishery sciences from the University of Washington, Seattle. He has worked extensively on the conservation and ecology of crayfishes, as well as environmental DNA (eDNA) applications to biodiversity monitoring. He has published 42 peer-reviewed scientific papers or book chapters, including 30 on crayfishes and two recent manuscripts on eDNA.

Mr. Brian Wagner is the Nongame Aquatics Biologist with the Arkansas Game and Fish Commission. He has a Master's Degree in Fisheries from Virginia Tech, and has been involved in aquatic conservation and research with the Commission for 28 years. For the past 19 years, he has been the Commission's Nongame Aquatics Biologist. Brian coordinates the Commission's Nongame Aquatics Program and has specific oversight of nongame fish and crayfish efforts. He is a Certified Fisheries Scientist and has authored or co-authored peer-reviewed publications on sport fish, nongame fish, crayfish, reptiles, and amphibians. Brian leads the State Wildlife Grants Crayfish Taxa Team, and is also active on the Fish, Cave, and Invertebrate Taxa Teams.