

**ADRESSING DATA GAPS AND CONSERVING KARST SPECIES AND
HABITATS IN EASTERN ARKANSAS.**

Project Summary

We address the 2017 State Wildlife Grant funding karst priority “Address data gap needs for species in order to develop conservation actions” for karst species that occur in northeast Arkansas. The project will survey karst habitats (e.g. caves, springs, and seeps) to evaluate species populations, establish baseline information on groundwater quality, habitat, and abundance at existing species locations, develop environmental DNA detection assays for cave amphipod and cavefish species, and host a series of landowner-focused karst workshops to assist landowners in conserving karst habitats.

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SWG Funding Requested: \$76,500 (29.7%)

Amount and Source of Matching Funds: \$181,200 (70.3%) of non-federal match will be provided from
Arkansas Department of Environmental Quality

Total Project Costs: \$257,700

NEED: Arkansas karst terrain is underlain by limestone or dolomite, where the topography is shaped in part by sinkholes, sinking streams, closed depressions, subterranean drainage, and caves. Underground, light is absent and food limited, so many subterranean species exhibit morphological, physiological, and behavioral characteristics uniquely suited for existence in cave and groundwater habitats. These organisms, unique to our caves, springs and other subterranean habitats, are important components of species conservation planning efforts outlined in the Arkansas Wildlife Action Plan (AWAP).

The protection of karst-dependent species is problematic because their habitat has been degraded or lost due to a number of environmental perturbations. While habitat loss due to land development is observable and obvious to many people, there are other important threats such as hydrological alteration, nutrient stress, nutrient loss, sedimentation, temperature change, enrichment, and chemical pollution. Through a mix of the above and other impacts, many karst-dependent species have experienced sharp population declines. Coupled with these threats, the development of adaptive management strategies and conservation priorities is complicated for some species because determining presence/absence of populations or assessing population response to conservation actions is hampered by limited access into the subterranean environment. For example: many karst groundwater systems can only be sampled at the surface where the water resurges as seeps or springs. Another example is found in caves with narrow passages which restrict researcher access to appropriate or higher quality habitats where animals are found.

Our capacity to obtain data on species distributions and community composition via DNA has grown dramatically in recent years. We now have the capacity to collect DNA shed by an organism into its surrounding environment as a means of species detection, complimenting traditional sampling and monitoring approaches for cryptic aquatic species. These emerging technologies allow for the isolation, extraction, and analysis of DNA from easily-collected environmental samples. This DNA, termed environmental DNA or eDNA, is a powerful new tool for biodiversity monitoring. These eDNA assays provide a rapid, noninvasive, and demonstrably effective option for species monitoring, particularly for taxa that are of conservation concern, numerically rare, patchily distributed, or otherwise difficult to sample. Within the last year, the eDNA approach has shown particular utility in detection and monitoring of subterranean species, including amphipods (our research group) and salamanders.

Through a previous Arkansas State Wildlife Grant (T20-9), we assessed threats associated with karst SGCN, determining that most karst SGCN populations were threatened by human disturbance and habitat degradation (Figure 1). Many threats to these species in northwest Arkansas appear to be associated with urbanization, and the subterranean species of this area are the focus of a current Arkansas State Wildlife Grant (T63) to TNC. In contrast, threats to subterranean karst species found in northeastern Arkansas appear to be associated with rural land use practices. The 2017 AWAP Steering committee recognized that these threats may have deleterious impacts to populations of karst species found in northeast Arkansas (Table 1), and that these species were in need of additional conservation efforts. Two priority conservation actions were identified: 1.) addressing basic data gaps such as baseline abundance, habitat

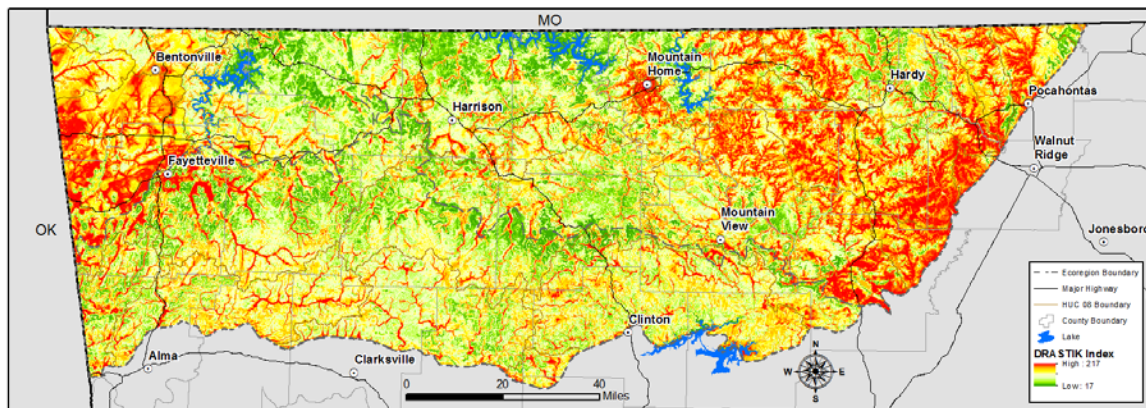


Figure 1. Groundwater vulnerability map developed as part of a previous Arkansas State Wildlife Grant (T20-9). The current project focuses on areas east of Mountain Home and Mountain View.

quality, and habitat delineation, and 2.) encouraging best management practices or BMPs through landowner outreach activities that would benefit these species. The 2017 AWAP Steering committee also recognized the need to survey and characterize spring habitats for the presence of SGCN since many of these species are suspected to be strongly associated with spring and groundwater habitats. Because most groundwater in northern Arkansas is derived from karst, the current project also contributes information on the conservation action outlined for spring and groundwater habitats in the state.

PURPOSE AND OBJECTIVES: This project will 1) address data gaps and assist landowners with implementing BMPs for karst SGCN in northeast Arkansas and 2) survey and characterize spring habitats in the Arkansas Ozarks. Project completion will take two years; proposal objectives are:

1. Address Arkansas karst SGCN data gaps through traditional cave biological inventories, habitat assessments, and water quality analysis.
2. Develop and examine the effectiveness of eDNA assays to detect and monitor groundwater biodiversity focusing on four amphipod (*Allocrangonyx hubrichti*, *Bactrurus pseudomucronatus*, *B. speleopolis*, and *Stygobromus ozarkensis*) and one cavefish species (*Typhlichthys eigenmanni*).
3. Develop and host landowner focused workshops to provide private landowners with karst conservation resources.

LOCATION OF WORK: This project will be conducted within portions of the Ozark Highlands ecoregion (northeast Arkansas), within the Ozark Highlands - Arkansas River eco-basin. The area includes all or part of the counties that occur east of Mountain Home and Mountain View (Figure 1).

APPROACH:

Objective 1. Based on information available from the ANHC Element Occurrence Records database, a list of caves in northeast Arkansas that provide habitat for karst SGCN will be developed. The list will be prioritized based on several metrics that will include number of SGCN present, availability of recent information on species abundance and habitat quality, and association with ADEQ established groundwater monitoring locations. Populations of karst SGCN at the top-ranked 10 sites will be

Table 1. Arkansas Karst SGCN. AWAP priority score is in parenthesis.

Class	Common Name	Scientific Name
Amphibians	Grotto Salamander (19)	<i>Eurycea spelaea</i>
Crayfish	Hell Creek Crayfish (80)	<i>Cambarus zophonastes</i>
Fish	Eigenmann’s Cavefish (27)	<i>Typhlichthys eigenmanni</i>
Invertebrates Other	isopod (80)	<i>Lirceus bidentatus</i>
	pseudoscorpion (65)	<i>Apochthonius titanicus</i>
	Hubricht’s Amphipod (42)	<i>Allocrangonyx hubrichti</i>
	amphipod (42)	<i>Bactrurus pseudomucronatus</i>
	isopod (42)	<i>Caecidotea dimorpha</i>
	cave obligate planarian (42)	<i>Dendrocoelopsis americana</i>
	isopod (30)	<i>Caecidotea ancyla</i>
	isopod (27)	<i>Lirceus bicuspidatus</i>
	Ozark Cave Amphipod (27)	<i>Stygobromus ozarkensis</i>
	springtail (25)	<i>Pygmarrhopalites clarus</i>
Mammals	pseudoscorpion (23)	<i>Hesperochernes occidentalis</i>
	isopod (8)	<i>Caecidotea salemensis</i>
	Ozark Big-eared Bat (80)	<i>C. townsendii ingens</i>
	Indiana Bat (46)	<i>Myotis sodalis</i>
	Gray Bat (23)	<i>Myotis grisescens</i>

assessed to establish a baseline. Water quality monitoring at these 10 sites will be conducted by collecting monthly surface grab samples at the cave mouth. Samples will be routinely analyzed for a suite of chemical parameters including: dissolved oxygen, nutrients, pH, turbidity, organics, and minerals. Every other month, samples will also be analyzed for total and dissolved metals. In addition, a minimum of 30 karst habitats (e.g. caves, springs, and seeps) will be surveyed for the presence of Arkansas karst SGCN to better understand the distributions of these species. Methodology will be species dependent (e.g. bait

stations, quadrats, timed area searches, or visual surveys). Sites will be assessed for localized impact (e.g. trash, vandalism, timing and frequency of illegal visitation).

Objective 2. A reference DNA database will be developed for the five target species and related taxa. Using molecular vouchers, a 650-bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene will be amplified using PCR and subsequently sequenced for multiple individuals of each species to complete the reference library. CO1 is a popular marker for barcoding studies of macroinvertebrates and vertebrates. Our reference database will be supplemented with additional CO1 sequences already available online for some taxa on GenBank and BOLD. The resulting reference database will be used to design species-specific primers and probes for qPCR assays. To compare the effectiveness of eDNA assays with traditional survey methods, we will collect and filter water samples from streams, pools, and other aquatic habitats at 20 cave systems. A traditional biological survey will occur following the eDNA sampling.

Objective 3. A series of Private Lands Karst Workshops will be hosted that focus on illustrating programs which landowners can use to improve wildlife habitat on private land that also benefits karst species. A minimum of two workshops will be held each year of the project. Key state and federal programs that are available through agencies such as US Fish and Wildlife Service, Arkansas Game and Fish Commission, and National Resources Conservation Service will be used as examples, and interested landowners will be provided assistance in applying for funding from these programs.

EXPECTED RESULTS AND BENEFITS: Assessing the status of SGCN populations at the 10 priority sites in northeast Arkansas will 1) assist in determining how likely conservation at individual sites is expected to benefit species and 2) generate a baseline for assessing the impact of targeted conservation actions. Water quality information will 1) allow sites to be compared to historic information, 2) establish a baseline for future comparisons, and 3) provide insight on potential stressors impacting aquatic and semi-aquatic SGCN. Inclusion of water chemistry will not only further expand ADEQ’s Ambient Groundwater Monitoring Program, but allow for early detection of any potential groundwater contamination from recent land use alteration activities. Assessing the distributions of karst species through the biological survey of additional habitats 1) will help determine the rarity of these species and 2) whether additional conservation efforts are needed to ensure their continued existence. The project will determine the use and suitability of eDNA for detection and monitoring of rare and threatened groundwater organisms, while providing the foundation for future studies to gather vital data and insights into the distributions of groundwater organisms and groundwater community assemblages, in general. Landowner workshops will facilitate the identification of landowners, tracts, and landowner interest in conservation activities which will assist with developing a prioritized list of implementation sites. The project will provide necessary information for developing priorities for habitat protection and restoration and will provide a solid foundation for implementing voluntary conservation actions, targeting funds available through other avenues, and highlighting future funding priorities for these species at these sites.

BUDGET: The total cost of this project is \$257,700.

29.7% Award – 70.3% Match	Requested SWG Funds	ADEQ Match	Total
Personnel & Fringe	\$ 28,789		\$ 28,789
Operating Expenses: Travel	\$ 3,450		\$ 3,450
Supplies	\$ 3,650		\$ 3,650
eDNA analyses, contracts	\$ 25,945		\$ 25,945
Water quality analyses		\$ 181,200	\$ 181,200
Miscellaneous expenses	\$ 250		\$ 250
Indirect Costs*	\$ 14,416		\$ 14,416
<i>Subtotal</i>	\$ 76,500	\$ 181,200	\$ 243,700
TOTAL	\$ 257,700.00		

*TNC's negotiates an indirect rate annually with USFWS. The FY2018 indirect rate is 23.22%.

ORGANIZATION AND STAFF QUALIFICATIONS: TNC, INHS, ADEQ, and USFWS have worked together on many projects to address habitat conservation, species protection, and water quality protection in the Ozark Karst Ecosystem.

Michael E. Slay is the Ozark Karst Program Director for The Nature Conservancy. Since joining The Nature Conservancy, Mike has worked closely with US Fish and Wildlife Service, US Forest Service, Arkansas Game and Fish Commission, Missouri Department of Conservation, Oklahoma Biological Survey, and Illinois Natural History Survey to conserve and protect karst species and habitats. Mike received his undergraduate degree and M.S. in Biology at the University of Arkansas, and he has authored more than 30 peer-reviewed journal articles related to the discovery and conservation of karst species.

Matthew L. Niemiller is an Associated Ecologist at INHS. He received a B.S. and M.S. in Biology at Middle Tennessee State University and a Ph.D. in Ecology & Evolutionary Biology at the University of Tennessee. He has worked closely with multiple agencies on cave and karst biology, including USFWS, USFS, The Nature Conservancy, and state agencies in Georgia, Illinois, Kentucky, Oklahoma, and Tennessee. Matthew has authored 45+ peer-reviewed journal articles and book chapters, 2 books, and 15 government reports related to subterranean biology.

Tate Wentz is the Aquatic Ecologist Coordinator for ADEQ. He has a B.S. in Fisheries and Wildlife Biology and M.S. in Biology from Arkansas Tech University. Tate has worked at ADEQ for seven years as an aquatic ecologist and researched a variety of topics from efficacy of habitat restoration to the long-term effects of major ions in wade-able streams.

Roger Miller oversees the Ambient Groundwater Monitoring Program at ADEQ. He completed a B.S. in Geology and graduate study in geochemistry and karst hydrology at the University of Arkansas, Fayetteville. He worked on mineral exploration and assessment projects in the US, Canada, and Australia, and then specialized in environmental assessment projects at various industrial sites and defense installations. He was a project manager for an engineering firm with offices in the Mid-Atlantic States, a regional hydrogeologist for the West Virginia DEP, then geology supervisor of the Arkansas Dept. of Health's Source Water Protection Program before joining ADEQ.

Tommy Inebnit is the Energy Projects Coordinator for the Arkansas Ecological Services Field Office of USFWS, and he is the T&E bat species lead, current Karst lead, and member of the Region 4 Dive Team. Tommy is a graduate of the University of Central Arkansas with a B.S. in biology and a M.S. in fish ecology. He has co-authored one scientific research publication and multiple technical documents targeting listing and recovery of threatened and endangered species in Arkansas.

Steven J. Taylor is a Senior Conservation Biologist at INHS, conducting research for the U.S. Forest Service, the National Park Service, U.S. Fish & Wildlife, and the U.S. Military, in addition to many other funding agencies. He has worked widely on karst issues including projects in Ecuador, Mexico, Belize and numerous states in the USA from California and Oregon to Virginia and Florida. Steven has authored more than 90 peer reviewed scientific publications, including articles on karst species descriptions, karst survey methodologies, and management of karst habitats.

Mark A. Davis is a Conservation Biologist and Coordinator of the Collaborative Ecological Genetics Laboratory at INHS. Mark has worked closely with USFWS, USFS, USDA, USGS, Illinois DNR, and many other agencies and organizations to protect biodiversity. Mark received Bachelor's and M.S. degrees at North Dakota State University and Colorado State University, and his Ph.D. in Natural Resources and Environmental Sciences at the University of Illinois Urbana-Champaign. He has authored more than 15 peer-reviewed journal articles and book chapters related to the conservation of fish and wildlife.