

**Alabama Cavefish**  
**(*Speoplatyrhinus poulsoni*)**

**5-Year Status Review:  
Summary and Evaluation**



Alabama Cavefish, photo by Dante Fenolio, San Antonio Zoo

**U.S. Fish and Wildlife Service**  
**Southeast Region**  
**Alabama Ecological Services Field Office**  
**Daphne, Alabama**

**February 2023**

# STATUS REVIEW

## **Alabama cavefish** **(*Speoplatyrhinus poulsoni*)**

### GENERAL INFORMATION

**Current Classification:** Endangered

**Lead Field Office:** Alabama, Ecological Services Field Office: Jennifer Grunewald, 251-424-0635.

**Reviewers:** Alabama, Ecological Services Field Office: Scott Lamont, 251-441-5857.  
Wheeler National Wildlife Refuge: Ricky Ingram, 256-353-7243.

**Lead Regional Office:** Atlanta Regional Office, Carrie Straight, (404) 679-7226.

**Date of original listing:** October 11, 1977 (42 FR 45526)

**Additional reclassification rules:** Reclassified from threatened to endangered, October 28, 1988 (53 FR 37968).

**Critical Habitat/4(d) rule/Experimental population designation/Similarity of appearance listing:** Critical habitat final rule: October 11, 1977 (42 FR 45526).

**Methodology used to complete the review:** In accordance with section 4(c)(2) of the Endangered Species Act of 1973, as amended (Act), the purpose of a status review is to assess each threatened species or endangered species to determine whether its status has changed and if it should be classified differently or removed from the Lists of Threatened and Endangered Wildlife and Plants ([50 CFR 424.11](#)). The U.S. Fish and Wildlife Service (Service) evaluated the biology, habitat, and threats of the Alabama cavefish (*Speoplatyrhinus poulsoni*) to inform this status review.

We announced initiation of this review in the Federal Register on July 14, 2021 (86 FR 37178) with a 60-day comment period. One public comment was received expressing concern for the species and the general threats of climate change and pollution to plants and animals. No specific data or information was supplied with the public comment. The primary sources of information used in this analysis were the species' recovery plan (USFWS 1990), recovery plan amendment (2019), the recent five-year review (USFWS 2017), recent studies of the species, peer-reviewed reports, agency reports, unpublished survey data and reports, and personal communication with recognized experts. This review was completed by the Service's, Alabama Ecological Services Field Office (AFO), Daphne, Alabama. All literature and documents used for this review are on file at the AFO. All recommendations resulting from this review are the result of thoroughly reviewing the best available information on the Alabama cavefish.

**FR Notice citation announcing the species is under active review:**

July 14, 2021 (86 FR 37178)

**Species' Recovery Priority Number at start of 5-year review ([48 FR 43098](#)): 4**

*Speoplatyrhinus poulsoni* is a species with a high degree of threat and a low recovery potential.

**Review History:**

Reclassification from threatened to endangered: September 28, 1988 (53 FR 37968)

Five-year Review: November 6, 1991 – No change in status was recommended

Five-year Review: August 21, 2017 – No change in status was recommended

## **REVIEW ANALYSIS**

### **Listed Entity**

#### **Taxonomy and nomenclature**

We are not aware of any changes to the taxonomy of this entity, and the Alabama cavefish is still considered a valid species by the Service.

#### **Distinct Population Segment (DPS):**

The Act defines species as including any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate wildlife. This species was not listed as a DPS, and we have no new information that would indicate the species should be listed as a DPS under the Service's 1996 DPS Policy.

### **Recovery Criteria**

#### **Recovery Plan**

Alabama cavefish (*Speoplatyrhinus poulsoni*) Recovery Plan, Second Revision. October 25, 1990.

Recovery Plan Amendment for the Alabama cavefish (*Speoplatyrhinus poulsoni*). September 27, 2019.

Recovery plans are not regulatory documents and are intended to provide guidance to the Service, States, and other partners on methods of minimizing threats to listed species and on criteria that may be used to determine when recovery is achieved. If the recovery criteria defined in the plan are still valid, meeting recovery criteria can indicate that the species no longer requires protections under the Act. However, when recommending whether a listed species should be delisted, the Service must apply the factors in section 4(a) of the Act ([84 FR 45020](#)).

The amended recovery criteria (Service 2019) establishes that the Alabama cavefish could be considered for downlisting when the following criteria are met:

Criterion 1. Existing population in Key Cave demonstrates a stable or increasing trend, evidenced by natural recruitment and multiple age classes (addresses Factor A and E).

Criterion 2. Two (2) additional populations are discovered or established that demonstrate a stable or increasing trend, evidenced by natural recruitment and multiple age classes (addresses Factor A and E).

Criterion 3. The aquifer recharge areas for these populations are thoroughly delineated, mapped, and protected from any foreseeable threats (addresses Factor A and D).

In addition to meeting downlisting criteria, the Alabama cavefish will be considered for delisting when the following criteria are met:

Criterion 4. At least two (2) additional populations demonstrate a stable or increasing trend, evidenced by natural recruitment and multiple age classes; and, for at least one of these, the aquifer recharge area is protected by a conservation mechanism (addresses Factor A, D, E).

Criterion 5. All other threats have been addressed or managed to the extent that the species will remain viable for the foreseeable future (addresses Factor A, D, E).

The Service believes these criteria are appropriate and relevant; however, none of the downlisting or delisting criteria have been met.

### **Biology and Habitat Summary**

Very little is known about the occurrence, biology, and life history of the Alabama cavefish and opportunities for surveying and sampling are limited due to the difficulty of working in its habitat and due to the fragility of the species (Boschung and Mayden 2004). Because little is known, we must determine aspects of its biology and lifecycle from knowledge of other related species. We are not aware of any additional new biology or habitat information since the most recent species review that impacts the status of the species and all the information provided in the last 5-year review remains valid (Service 2017).

The Alabama cavefish is only known from a single cave aquifer system in Lauderdale County, which is in northwest Alabama (Figure 1). The species is highly endemic with an extremely localized subterranean range (Key Cave). First discovered in 1967, survey efforts in other locations have found no additional populations (Kuhajda and Mayden 2001; Kuhajda and Fluker 2010, Service 2017). The Key Cave recharge area spans approximately 16 square miles. Of this, 1,060 acres makes up the Key Cave National Wildlife Refuge (NWR) and is managed by the Service.

Because Key Cave is a difficult and dangerous cave system to navigate, all areas of the cave system have not been explored or accessed, and the Alabama cavefish has only been located within certain pools (See Appendix A for a map of Key Cave). Surveyed pools within Key Cave are designated with a letter name A-J, along with the pool identified as Lynns Pool (Kuhajda and Fluker 2010). Pools A-D, F, G and I are likely connected, with Alabama cavefish observations restricted to Pools A-D and F.

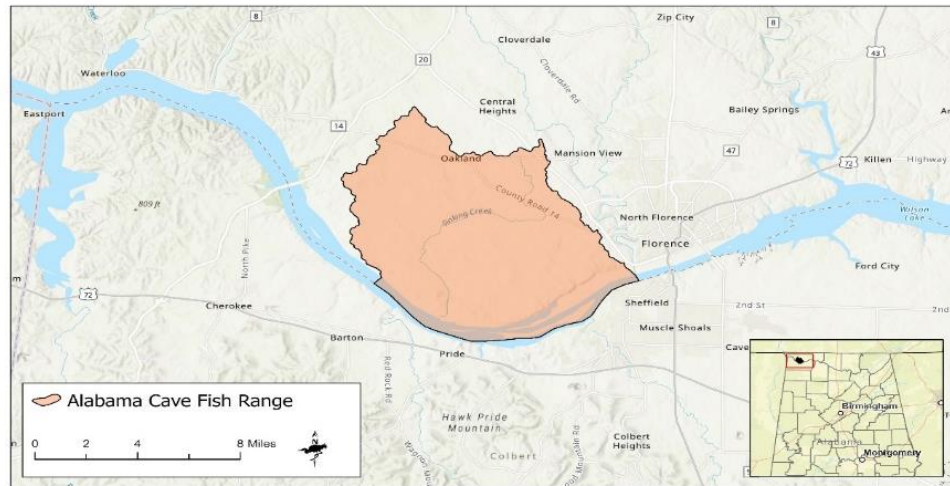


Figure 1. Current Range of the Alabama cavefish.

Researchers have recently begun to use Environmental DNA (eDNA) collection, which offers a minimally invasive and cost-effective alternative to traditional survey approaches and may allow for the Alabama cavefish to be located at new locations within the cave or at other locations with similar habitat (Niemiller, et al. 2021).

The most recent surveying for the Alabama cavefish in Key Cave occurred in November 2018 and February 2019 by Kuhajda and Niemiller, and in December 2021 by Niemiller (Tables 1-3, respectively). Refer to Appendix A for Key Cave pool locations. Survey data provided by B. Kuhajda (Kuhajda 2022, pers. comm) and M. Niemiller (Niemiller 2023, pers. comm). Based on the surveys and the extent of the cave complex and inaccessible areas the population is estimated to be less than 100 individuals (Kuhajda 2004a).

**Table 1. Surveys in 2018 for the Alabama cavefish.**

Location	# Observed	Pool	Additional Information
Key Cave	4	A	28 Nov 2018 visit
Key Cave	1	B	28 Nov 2018 visit
Key Cave	5	A	29 Nov 2018 visit
Key Cave	4	B	29 Nov 2018 visit

**Table 2. Surveys in 2019 for the Alabama cavefish.**

Location	# Observed	Pool	Additional Information
Key Cave	5	A	February 2019
Key Cave	2	C	February 2019
Key Cave	1	F	February 2019; Fin clipped for genetic study

**Table 3. Surveys in 2021 for the Alabama cavefish.**

Location	# Observed	Pool	Additional Information
Key Cave	2	A	December 2021
Key Cave	2	B	December 2021
Key Cave	3	Step-Across Pool	December 2021

### *Water Quality*

Groundwater in Key Cave is controlled by surface runoff, solubility conditions between the surrounding limestone and groundwater, and the quality of groundwater from phreatic aquifers that contribute to the cave's water supply (Ponta et. al 2018 and Ponta et al. 2021).

In addition to the fish surveys conducted by Kuhajda and Niemiller, the GSA conducted extensive water quality sampling along with well and hydrologic monitoring in 2018. The Geological Survey of Alabama (GSA) monitors water quality in Key Cave. Using loggers from November 2017 – July 2019, data showed higher precipitation events in the spring increased water levels, while drought conditions in the summer had the opposite effect. With fluctuations in water levels of approximately five meters (16ft), water quality in the cave is seasonally variable, with higher concentrations of contaminants being present during periods of low precipitation and lower contaminant levels during high precipitation events likely attributed to dilution (Ponta et al. 2021). Additionally, highly conductive soils in the area and relatively low topographic relief results in rapid water infiltration and reduced runoff rates that quickly migrate contaminants through the soil to the saturated zone (Ponta et al. 2021). Flooding in caves brings changes in water level temperature, food availability, turbidity, and water chemistry (Service 1990). Results from GSA's 2018 water quality sampling can be found in Appendix A.

### *Hydrology*

Water-level investigations in and around Key Cave indicate that the hydrology is likely controlled by groundwater originating in the soils and shallow karst terrain around the cave, direct conduits through which surface runoff enters the cave during storm events, and a phreatic zone component recharged several miles away from the cave (Ponta et. al 2018). Additionally, it has been determined that the water level in Pickwick Reservoir has a direct impact on water levels in Key Cave which demonstrates the interconnection between these water systems.

### *Other environmental factors*

Key Cave has a very stable water temperature throughout the year, ranging from 15.2 to 18.2 Celsius (C). The average temperature during GSA monitoring was around 16.0 C (Ponta et. al 2018). Temperature generally was constant (February - September), with large variations after storm events. It is unknown how these large variations in temperature might impact the species, however, it is believed that "season flooding is necessary to trigger hormonal changes within the cavefish for growth and reproduction" (Romero, 1998).

## **Threats (Five-Factor Analysis) Summary**

A detailed review of the species' threats can be found in the 2017 Alabama cavefish 5-year status review (Service 2017). The status of a species is determined from an assessment of factors specified in section 4 (a)(1) of the Act. The present or threatened destruction, modification, or curtailment of the habitat (Factor A) continues to be the primary threat to the Alabama cavefish as described in the 2017 5-year status review (Service 2017). The Key Cave aquifer and recharge area are threatened by urban and industrial growth which can lead to lowering of the water table, diminished winter flows (which provide cues to synchronize spawning), and acute and chronic water pollution (Kuhajda 2004b, 2009). The Key Cave recharge area is approximately 20 square miles in size and over 95% of it is in private ownership, which limits protections to the hydrology as described below.

**Factor A: the present or threatened destruction, modification, or curtailment of its habitat or range.**

*Urban and Industrial Development*

The Key Cave aquifer and recharge area are threatened by urban and industrial growth which can lead to lowering of the water table, diminished winter flows, and acute and chronic water pollution (Kuhajda 2004b, 2009). Reduced input of surface runoff in recharge zones could have dramatic impacts on reproduction of Amblyopsid cavefishes by disrupting the environmental cues necessary for successful reproduction which in turn could lead to greater susceptibility to extirpation (Poulson 1963; Paulson 1969; Kuhajda 2004a), or in the case of the Alabama cavefish, extinction.

Planned industrial development of the Key Cave recharge area could alter drainage and hydrological patterns within the recharge area (Service 1990) through increases in groundwater pumping, stormwater runoff, urbanization, and ancillary construction projects such as houses, small businesses, roads, utilities, and easements (Kuhajda 2004b & KPS Group 2007). The GSA has conducted extensive groundwater studies in and around Key Cave, but due to a complicated karst hydrology, a full understanding of Key Cave, its recharge area, and how water moves through this system is not known. Studies suggest however, that the water may be affected by developed land and could be vulnerable to contamination (Ponta, et al., 2021). As of 2022, the Florence area has seen increases in a diverse manufacturing base, to include the automotive and metals industries (Pruitt 2022). Other areas of growth include ports along the Tennessee River, as well as improvements to the areas of tourism, health care, and higher education. Continued growth and development in this area is likely to increase in the future and will continue to be of concern to the Alabama cavefish and its sensitive habitat requirements.

*Agricultural Impacts*

The Alabama cavefish and immediate recharge area are protected within the Key Cave NWR including approximately 242 hectares (598 ac) of farmland and old fields (Figure 2). The NWR System Improvement Act of 1997 requires that every NWR develop a Comprehensive Conservation Plan (CCP) to identify management actions for the Refuge to meet the refuge's purpose and revise it every 15 years, as needed. The CCPs allow NWR managers to take actions that improve the condition of habitats and that benefit wildlife including fish species such as the Alabama cavefish.



Figure 2. Key Cave NWR Boundary and Agricultural Zones

Agriculture can have both positive and negative effects to the Alabama cavefish. Key Cave NWR relies on cooperative farming agreements to manage the land around Key Cave. Without these agreements, the NWR would likely be overrun with invasive species, such as: Johnsongrass (*Sorghum halepense*), Chinese privet (*Ligustrum sinense*), green ash (*Fraxinus pennsylvanica*), sweetgum (*Liquidambar styraciflua*) trumpet vine (*Campsis radicans*), and bradford pear (*Pyrus calleryana*) to list a few (Hurt 2022, pers. comm). Invasive species can use more water than their native counterparts and could impact hydrology that feeds the habitat the Alabama cavefish relies on for survival (Cavaleri and Sack 2010). Although there are some concerns with the use of agricultural chemicals in the Key Cave recharge area, precautions have been put in place to minimize impacts, to include the use of buffer zones and restrictions on spraying. While long-term goals of the refuge are to convert agricultural land to native grass or oak savannah habitat, until adequate funding is available, active farming within the refuge plays an important role in conditions of water quality in Key Cave and the surrounding recharge area (Hurt 2022, pers. comm).

#### *Pesticide Use*

Future threats from pesticide use will continue to be a concern for the Alabama cavefish and its critical habitat. In 2017 the U.S. Environmental Protection Agency (EPA) began the registration review for the mosquito control pesticide, malithion. The EPA determined that the pesticide was likely to adversely affect listed species and contacted the Service for consultation. As with many other fish in the review, it was determined the Alabama cavefish would experience a high risk of mortality if exposed to this pesticide (EPA 2022: Service 2022). It was also determined that aquatic invertebrates, an important food source for the Alabama cavefish, would also suffer from a high risk of mortality if exposed to this chemical. If malathion (and other related chemicals in the future) are approved by the EPA for use, it is vitally important that the product labels and instructions be strictly adhered to. While risks exist, responsible use of the chemical and implementation of mitigation measures developed through an interagency effort by the Service, EPA, and U.S. Department of Agriculture, will minimize negative impacts to the Alabama cavefish and other aquatic species (EPA 2022: Service 2022).

#### *Gray Bat Population*

A gray bat (*Myotis grisescens*) colony in Key Cave is likely the primary source of organic matter through the deposition of guano, which is likely the foundation of the food web in the ecosystem (Service 1990). Because gray bats provide a major source of nutrients to the cave ecosystem, we can infer that a positive relationship exists between the gray bat and Alabama cavefish populations, therefore conservation of the cavefish may depend on conservation actions undertaken for the bat (Tuttle 1979). White-nose syndrome is causing increased bat mortality and population losses due to impacts on the species during hibernation and emergence (Reeder et al. 2012, Verant et al. 2014). During the last 15 years, the reduction of bat populations and guano deposits in Key Cave (Gates 2014 and 2015, pers. comm.) may have reduced the nutrient cycling in cave waters (Kuhajda 2004b), as it has in other cave systems. The loss of this important food source could have direct and indirect effects on the cavefish since diminished organic matter input adversely impacts the aquatic food base in caves (Poulson 1961).



**Factor B: overutilization for commercial, recreational, scientific, or educational purposes.**

We have no evidence that overutilization for commercial, recreational, scientific, or educational purposes are currently a threat to the species. Scientific collecting, which has not involved lethal take, is controlled by the State of Alabama.

**Factor C: disease or predation.**

While predation likely occurs by two crayfish species (*Cambarus jonesi* and *Procambarus pecki*) and the southern cavefish (*Typhlichthys subterraneus*), there is no evidence to suggest that disease and predation pose a threat to the species at-this-time.

**Factor D: the inadequacy of existing regulatory mechanisms.**

The Alabama cavefish and its habitats are afforded some protection through state laws. Code of Alabama §§ 220-2-92, makes it unlawful to take or attempt to take, capture, or kill the Alabama cavefish. The species is also afforded some protection from water quality and habitat degradation under the Clean Water Act of 1972 (33 U.S.C. 1251 et seq.) and the Alabama Water Pollution Control Act, as amended, 1975 (Code of Alabama, §§ 22-22-1 to 22-22-14). However, the 2015 Clean Water Rule defining the waters of the United States (80 FR 37054; U.S. Army Corps of Engineers 2015) excludes groundwater. Ground water allocations for Alabama is based on reasonable use, and there exists no preference for groundwater allocation in Alabama for different water uses such as domestic, agricultural, industrial, or mining at a rate of 100,000 gallons or more per day (e.g. preference for different uses are not taken into consideration for allocation: Sanjaya 2005). Because of this, public landowners may extract water on private land from the same aquifer that supplies the habitat for the species in Key Cave. Additionally, the inconsistency in implementation of Clean Water Act regulations and other best management practices, and existing regulatory mechanisms in Alabama, are still inadequate to fully protect Alabama cavefish from the ongoing threats.

**Factor E: other natural or manmade factors affecting its continued existence.**

Numerous natural and manmade factors could affect the continued existence of the Alabama cavefish. This highly endemic species with an extremely localized range makes Alabama cavefish populations vulnerable to extirpation (and possible extinction) from catastrophic events, such as toxic spills, or changes in flow regime, and changes in aquifer recharge due to pumping for public water supply or irrigation.

Although more research will need to be conducted in this area, concerns from climate change on flow rates/flood cycles could be a concern. Although temperatures have generally been unchanged since the start of the 1900s, 2016-2020 was the warmest consecutive 5-year interval on record (Runkle et al. 2022). Unlike many other states in the southeast, recent documented temperatures are below those seen in the 1920s and 1930s; however, average annual temperatures are projected to rise and exceed historical levels (Runkle et al. 2022). With predicted increases in temperature, there will likely also be associated increases in intensity of drought periods (Runkle et al. 2022). Although the magnitude is uncertain, changes in temperature and drought are both likely to impact the microclimate conditions within the cave impacting its bat fauna and is also likely to impact hydrology both of which could potentially result in changes in Alabama cavefish abundance.

## **Synthesis**

The Alabama cavefish is a small semi-transparent, pinkish-white fish which continues to be known from only a single cave system in northwest Alabama. The species is considered extremely rare with a total population estimate of less than 100 individuals. Though the Key Cave entrance and 429 ha (1060 ac) of surface property above the cave is partially protected due to its ownership by the Service, full protection is not afforded to areas on private lands, including portions of the aquifer and sink holes in the recharge area. This species exists in a fragile ecosystem and continues to be threatened by urban and industrial growth, groundwater degradation, lower groundwater levels, and diminished organic input by bats. This species' small population size also contributes to its vulnerability, putting it at risk potentially by single catastrophic events or impacts to the hydrology of the cave system. Because of these threats, the Alabama cavefish continues to meet the definition of an endangered species under the Act.

## **RECOMMENDED FUTURE ACTIVITIES**

A detailed discussion of recovery actions and criteria are presented in the Recovery Plan (Service 1990) and Alabama cavefish Recovery Plan amendment (Service 2019). During the course of this status review new and/or targeted potential recovery activities were identified and are included below.

### **Recovery Activities**

1. The species' recovery priority should be focused on establishing cooperative agreements with private landowners for the conservation of the species. The Key Cave recharge area is approximately 20 square miles in size and over 95% is in private ownership.
2. Management of Key Cave NWR habitats to improve recharge and conditions within Key Cave.
  - a. Ensure the buffer around the entrance to Key Cave is protected from harmful agricultural practices in the area.
  - b. Ensure pesticide use is minimized or not used at all near the cave entrance.

### **Monitoring and Research Activities**

1. Population monitoring should be conducted to assess the response of the Alabama cavefish to continued threats, determine the current population size, and determine other biological relationships within the Key Cave ecosystem that are currently unknown.
2. Continue water quality monitoring within Key Cave.
3. Additional studies should be conducted to determine if other populations of the Alabama cavefish exist in other locations or in other areas of Key Cave not yet explored. This could be accomplished utilizing traditional methods, eDNA monitoring, or other novel methods not yet considered. Continue support for the on-going research at a-number-of universities with faculty and graduate students conducting eDNA research on the Alabama cavefish as well as other subterranean species.

4. Additional studies of the recharge area should be conducted to get an updated understanding of current hydrological conditions and how groundwater impacts the Key Cave environment.
5. Consider gray bat monitoring to explore the relationships and benefits of a health bat population and nutrient levels within Key Cave and potential benefits to the Alabama cavefish.
6. Conduct biological studies of the entire Key Cave ecosystem.

## RESULTS / SIGNATURES

**U.S. Fish and Wildlife Service**  
**Status Review of Alabama cavefish (*Speoplatyrhinus poulsoni*)**

**Status Recommendation:**

On the basis of this review, we recommend the following status for this species. A 5-year review presents a recommendation of the species status. Any change to the status requires a separate rulemaking process that includes public review and comment, as defined in the Act.

☐ Downlist to Threatened

☐ Delist:

☐ *The species is extinct*

☐ *The species does not meet the definition of an endangered or threatened species*

☐ *The listed entity does not meet the statutory definition of a species*

☒ No change needed

**FIELD OFFICE APPROVAL:**

**Field Supervisor, Alabama Ecological Services Field Office, Fish and Wildlife Service**

Approve \_\_\_\_\_

## REFERENCES

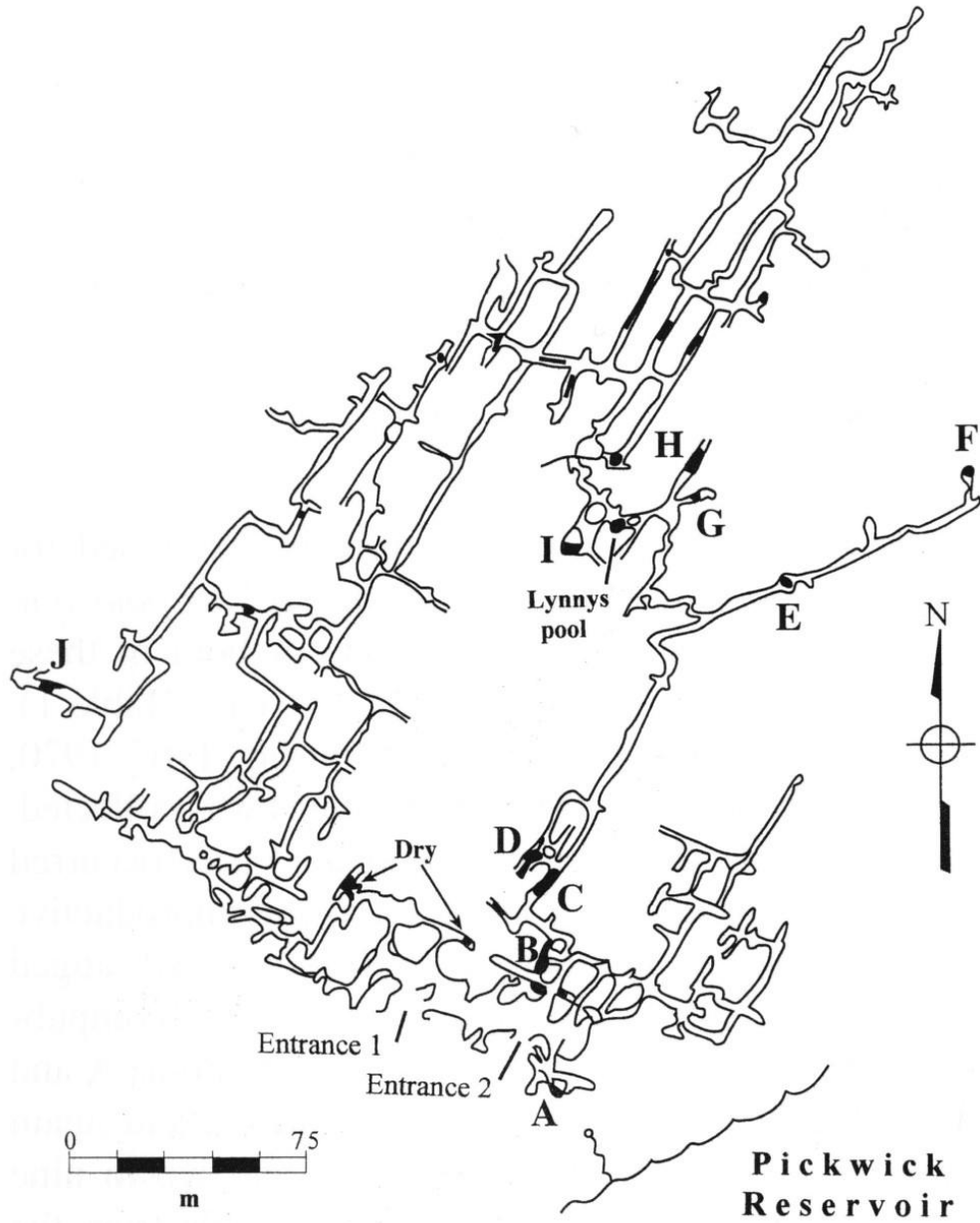
- Boyd S.H., K.D.K. Niemiller, K.E. Dooley, J. Nix, and M.L. Niemiller. 2020. Using environmental DNA methods to survey for rare groundwater fauna: Detection of an endangered endemic cave crayfish in northern Alabama. PLoS ONE 15(12): e0242741. <https://doi.org/10.1371/journal.pone.0242741>
- Boschung, H.T. and R.L. Mayden. 2004. Fishes of Alabama. Smithsonian Institution Press, Washington D.C. 736 pp.
- Cavaleri, MA. And L. Sack. 2010. Comparative water use of native and invasive plants at multiple scales: a global meta-analysis. Ecology 91: 2705-2715.
- Gates, Bill, Wheeler NWR. 2014 and 2015. Email messages concerning cave locations and bat ecology, population numbers and white nose syndrome disease in bats within local caves.
- Harris, L. D. 1984. The Fragmented Forest. University of Chicago Press. 211pp.
- Hurt, R. 2022. Personal communication. Partners Program and former Wheeler NWR employee, U.S. Fish and Wildlife Service. 2022. Telephone call regarding the history of Key Cave and past/current agricultural practices in and around the Key Cave ecosystem.
- Joshi, S. R. 2005. Comparison of Groundwater Rights in the United States: Lessons for Texas. Graduate Thesis. Texas Tech University. 17-18pp.
- KPS Group. 2007. Florence Comprehensive Plan. Recommended to the Florence Planning Commission. Feb.22. 66 pp.
- Kuhajda, B.R. 2004a. Alabama cavefish, *Speoplatyrhinus poulsoni*. Pp. 181-182. In: R.E. Mirarchi, J.T. Garner, M.F. Mettee, and P.E. O'Neil (eds.) Alabama wildlife. Volume 2. Imperiled aquatic mollusks and fishes. The University of Alabama Press, Tuscaloosa.
- Kuhajda, B.R. 2004b. The impact of the proposed Eddie Frost Commerce Park on *Speoplatyrhinus poulsoni*, the Alabama cavefish, a federally endangered species restricted to Key Cave, Lauderdale County, Alabama. Endangered Species Update 21 (2):57-65.
- Kuhajda, B.R. 2009. *Speoplatyrhinus poulsoni* -Alabama Cavefish. p. 14 In: B.R. Kuhajda, A.L. George, and J.D. Williams (eds). The Desperate Dozen: Southeastern Freshwater Fishes on the Brink. Proceedings of the Southeastern Fishes Council No. 51: 10-30.
- Kuhajda, B.R. and B.L. Flucker. 2010. Final report. Status of the Endangered Alabama Cavefish *Speoplatyrhinus poulsoni*. Univ. of AL., Tuscaloosa. 15 pp.
- Kuhajda, B.R. and R.L. Mayden. 2001. Status of the federally endangered Alabama cavefish, *Spoplatyrhinus poulsoni* (Amblyopsidae), in Key Cave and surrounding caves, Alabama. Environ. Biol. Fishes 62:215-222.

- Niemiller, M. D. Niemiller, K. Dooley, K. Giltner, and A. Guillemette. 2021. Using Environmental DNA to Detect and Monitor Cavefish and Alabama Cave Shrimp. Final Report to the Alabama Dept of Conservation and Natural Resources. 6 pp.
- Noss, R.E. and A.Y. Cooperrider. 1994. Saving Nature's Legacy. Protecting and Restoring Biodiversity. Island Press. CA. 416 pp.
- O'Neil, P. 2022. Personal communication. Biologist, USFWS consultant. Email message and telephone call discussing the organic component of bat guano in Key Cave and its effect on Alabama cavefish food sources.
- Paulson, T.L. and K.H White. 1969. The cave environment. Science 165:971-981.
- Ponta, G., S. McGregor, and S. Jones. 2018. Hydrological Assessment for Key Cave, Lauderdale County, Alabama. Open-file Report 1811.
- Ponta, G., S. McGregor, and R. Blackwood. 2021. Time Series Hydrologic Monitoring within Karst Aquifers of Key Cave and Cathedral Caverns, Alabama. 16<sup>th</sup> Sinkhole Conference.
- Poulson, T.L. 1961. Cave adaptation in amblyopsid fishes. PhD Dissertation, University of Michigan, Ann Arbor. 185 pp.
- Poulson, T.L. 1963. Cave adaptation in amblyopsid fishes. American Midland Naturalist 70:257-290.
- Pruitt, L. 2022. Spotlight on Lauderdale and Colbert: Economic Engines. Business Alabama Issue - October 2022.
- Reeder D.M, C.L. Frank, G.G. Turner, C.U. Meteyer, A. Kurta, E.R. Britzke, M.E. Vodzak, S.R. Darling, C.W. Stihler, A.C. Hicks, R. Jacob, L.E. Grieneisen, S.A. Brownlee, L.K. Muller, and D.S. Blehert. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLoS ONE 7(6):e38920.
- Romero, A. 1998. Threatened fishes of the world: *Speoplatyhinus poulsoni* Cooper & Kuehne. 1974 (Amblyopsidae). Environmental Biology of Fishes 53: 293-294.
- Runkle, J., K.E. Kunkel, L.E. Stevens, R. Frankson, and S. Rayne. 2022. Alabama State Climate Summary 2022. NOAA Technical Report NESDIS 150-AL. NOAA/NESDIS, Silver Spring, MD, 4 pp.
- Sanjaya, R.J. 2005. Comparison of Groundwater Rights in the United States: Lessons for Texas. Graduate Thesis, Texas Tech University.
- Tuttle, M.D. 1979. Status, causes of decline, and management of endangered gray bats. Journal of Wildlife Management 43(1): 1-17.

- U.S. Army Corps of Engineers. 2015. Clean Water Rule: Definition of “Waters of the United States”. Federal Register 80:37054-37124.
- U.S. Environmental Protection Agency (EPA). 2022. EPA Takes Steps to Protect Endangered and Threatened Species from Insecticide. [accessed July 19, 2022]  
<https://www.epa.gov/pesticides/epa-takes-steps-protect-endangered-and-threatened-species-insecticide#:~:text=In%20January%202017%2C%20EPA%20released,initiated%20formal%20consultation%20with%20FWS>.
- U.S. Fish and Wildlife Service (Service). 1977. Final Threatened Status and Critical Habitat for Five Species of Southeastern Fishes. Federal Register 42: 45526-45530.
- U.S. Fish and Wildlife Service (Service). 1988. Endangered and Threatened Wildlife and Plants; Reclassification of the Alabama Cavefish from Threatened to Endangered. Federal Register 53: 37968-37970.
- U.S. Fish and Wildlife Service (Service). 1990. Alabama Cavefish (*Speoplatyrhinus poulsoni*) Recovery Plan. Second Revision. Southeast Region, Atlanta, Georgia. 22 pp.
- U.S. Fish and Wildlife Service (Service). 2017. Alabama Cavefish (*Speoplatyrhinus poulsoni*) 5-Year Review. Southeast Region, Jackson, Mississippi. 26 pp.
- U.S. Fish and Wildlife Service (Service). 2019. Alabama Cavefish (*Speoplatyrhinus poulsoni*) Recovery Plan Amendment. Southeast Region, Atlanta, Georgia. 5 pp.
- U.S. Fish and Wildlife Service (Service). 2022. Biological and Conference Opinion on the Registration of Malathion Pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act. Appendix K-A6 – Fishes Integration and Synthesis Summaries-CONUS-Animals. U.S. Fish and Wildlife Service, Ecological Services Program, Headquarters. 542 pp.  
<https://fws.gov/media/biological-and-conference-opinion-registration-malathion>
- Verant, M.L., C.U. Meteyer, J.R. Speakman, P.M. Cryan, J. M. Lorch and D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. BMC Physiology 14: 10. <http://www.biomedcentral.com/1472-6793/14/10>.

## APPENDIX A. SUPPORTING DOCUMENTATION

Key Cave Pool Map. Surveyed pools are those labelled as A-J along with Lunnys Pool. Alabama cavefish observations restricted to Pools A-D and F.





GSA Water Quality Monitoring (2018) Results (Ponta et al. 2018)

Analyte	GSA Method	Chemical/Physical Method	Parent Method
Aluminum	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200.7 rev. 4.4
Ammonia as Nitrogen	2350.4	Colorimetric, automated segmented-flow, salicylate-hypochlorite)	USGS 1-2522-85
Arsenic	2200.9	Stabilized temperature platform graphite-furnace atomic absorption spectrometry	EPA 200.9 rev. 2.2
Barium	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Beryllium	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Biochemical Oxygen Demand, 5-day	2405.0	Electrometric, 5-day	SM 5210 B
Cadmium	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200.7 rev. 4.4
Calcium	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Carbon, Total Organic	2415.1	Catalytic combustion	EPA 415.1
Chloride	2300.1A	Ion chromatography	EPA 300.1 rev. 1.0
Chromium	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200.7 rev. 4.4
Conductance, Specific (Conductivity)	field		
Copper	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Iron	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200.7 rev. 4.4
Lead	2200.9	Stabilized temperature platform graphite-furnace atomic absorption spectrometry	EPA 200.9 rev. 2.2
Manganese	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Mercury	2245.7	Cold-vapor atomic fluorescence spectrometry	EPA 245.7
Nitrate as Nitrogen	2300.1A	Ion chromatography	EPA 300.1 rev. 1.0
Nitrite as Nitrogen	2300.1A	Ion chromatography	EPA 300.1 rev. 1.0
Oxygen, Dissolved	field		
pH	field		
Phosphorus, Total	2200. 7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4
Selenium	2200.9	Stabilized temperature platform graphite-furnace atomic absorption spectrometry	EPA 200.9 rev. 2.2
Solids, Total Suspended	2160.3	Gravimetric, dried at 103-105°C	USGS 1-3765-85
Solids, Total Dissolved	2160.2	Gravimetric, dried at 180°C	USGS 1-1750-85
Temperature	field		
Thallium	2200.9	Stabilized temperature platform graphite-furnace atomic absorption spectrometry	EPA 200.9 rev. 2.2
Turbidity	2180.0	Nephelometric	EPA 180.1 rev. 2.0
Zinc	2200.7	Inductively coupled plasma-atomic emission spectrometry	EPA 200. 7 rev. 4.4