Ozark cavefish
(*Amblyopsis rosae* Eigenmann 1898)

Tommy Inebnit, USFWS

5-Year Review:
Summary and Evaluation

U.S. Fish and Wildlife Service
Southeast Region
Arkansas Ecological Services Field Office
Conway, Arkansas

5-YEAR REVIEW
Ozark Cavefish (*Troglichthys rosae*)

I. GENERAL INFORMATION

A. Methodology used to complete the review

We announced initiation of this review and requested information from the public in a published *Federal Register* notice with a 60-day comment period on June 30, 2017 (82 FR 29916). During the public comment period, we did not receive any additional new information about Ozark Cavefish. The Service's lead recovery biologist in the Arkansas Ecological Services Field Office (AES) completed this review. Additional information used in this report was gathered from peer reviewed literature, published and unpublished data provided by species experts, and from Service files.

We circulated a draft of this 5-year review to five persons for peer review. Comments and suggestions regarding the review were received from Brian Wagner (Arkansas Game and Fish Commission; AGFC), Bryan Rupar (Arkansas Natural Heritage Commission; ANHC), and Jacob Westhoff (Missouri Department of Conservation; MDC). We did not contract any part of this review to an outside party. Recommendations are a result of thoroughly reviewing the best available information on Ozark Cavefish (*Amblyopsis rosae)* (see section II C 1 b for more detail).

Special thanks to private landowners, developers, and communities who with their input, support, and cooperative spirit have made Ozark Cavefish recovery efforts successful. To respect private and other landowners' wishes, thereby, not encouraging search of and entry into cavefish locations; specific cave locations will not be discussed.

B. Reviewers

Lead Region: Southeast Region: Kelly Bibb, (404) 679-7132

Lead Field Office: AES – Tommy Inebnit, (501) 513-4483

Cooperating Field Offices: Oklahoma Ecological Services Field Office – Brian Fuller, (918) 382-4514.

Ozark Plateau National Wildlife Refuge – Richard Stark, (918) 382-4520.

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Cooperating Regional Offices – Southwest Region: Brady McGee, (505) 248-6657; Midwest Region: Laura Ragan, (612) 713-5157

C. Background

1. **Federal Register Notice initiating this review**: June 30, 2017. (82 FR 29916)

   Species Status: Stable. Graening et al. 2010 reported over a 50% decrease in occupied/active sites observed from 1990 to 2010. The number of occupied/active sites remained stable from 2010 to present. However, sufficient documentation does not exist at this time to indicate whether the decrease in occupied/active sites is indicative of large-scale population declines, site-specific declines at the extant localities, or other unknown causes. Of the current occupied/active sites, we have no evidence since the last 5-year review (Service 2011) to indicate additional population declines.

2. **Listing History**:
   Original Listing
   FR notice: 49 FR 43965
   Date listed: November 1, 1984
   Entity listed: Species
   Classification: Threatened

3. **Associated rulemakings:** None.

4. **Review History:**
   5-Year Reviews
   U.S. Fish and Wildlife Service. 2011. Ozark Cavefish 5-year review: summary and evaluation. Conway, Arkansas. 28 pp.

   In this review, a range-wide estimate of countable cavefish using the most recent population monitoring numbers resulted in 213 individuals, although not indicative of actual population total. Overall threats to the species are stable or increasing at the majority of active sites. Threats include human entry, agriculture, and urbanization/development. We discussed lack of groundwater regulatory mechanisms and associated monitoring in the threats analysis. No change in the fish's listing classification was appropriate.

   U.S. Fish and Wildlife Service. November 6, 1991 (56 FR 56882) 5-year review of listed species.

   In this review, we evaluated the status of many species simultaneously with no in-depth assessment of the five factors or threats as they pertain to the individual species. The notice stated that the Service was seeking new or additional information reflecting the necessity of a change in the status of the species under review. The notice indicated that if significant data were available warranting a change in a species' classification, the Service

would propose a rule to modify the species' status. We determined no change in the fish's listing classification was appropriate.

Status Reviews

Westhoff, J.T. and D.C. Novinger. 2016. FY16 Ozark Cavefish Monitoring. Unpublished Report. Missouri Department of Conservation.

Graening, G.O., D.B.Fenolio, M. L. Niemiller; A.V. Brown, and J.B. Beard. 2010. The 30-year recovery effort for the Ozark cavefish (*Amblyopsis rosae*): Analysis of current distribution, population trends, and conservation status of this threatened species. Environ. Biol. Fish., Vol. 87, pp. 55-88. DOI 10.1007/s10641-009-9568-2.

Graening, G.O. and A.V. Brown. 1999. Cavefish population status and environmental quality in Cave Springs Cave, Arkansas. Arkansas Water Resources Center. Publication No. 276. 38 pp.

Romero, A. 1998. Threatened fishes of the world: *Amblyopsis rosae* (Eigenmann, 1898) (Amblyopsidae). Environmental Biology of Fishes 52:434.

Brown, Arthur V. and Todd, C.S. Status review of the threatened Ozark cavefish (*Amblyopsis rosae*). Proceedings, Arkansas Academy of Science. 1987; 41:99-100

Willis, L.D. 1984. Distribution and habitat requirements of the Ozark cavefish, *Amblyopsis rosae*. M.S. Thesis, University of Arkansas, Fayetteville, AR. 35pp.

Jones, Stephen R. and Rimbach, Don. 1983. (ABS) Notes on the status of *Amblyopsis rosae* in southwestern Missouri and water quality data on its habitat. National Speleological Society Bulletin 45: Insert

Brown, A.V., K.B. Brown, L.D. Willis, D.C. Jackson, P.P. Brussock. 1982. Distribution and abundance of the Ozark cavefish *Amblyopsis rosae* (Eigenmann) in Missouri. Final Report submitted to Missouri Department of Conservation. 20pp.

5. **Species Recovery Priority Number at start of review (48 FR 43098)**: 5c. The degree of threat to Ozark Cavefish caves and recharge zones is high as urbanization and development continue to increase, lands are converted for agricultural purposes, and caving as a recreational activity increases. Recovery potential is low because of the increase in and limited ability to reduce existing threats from urbanization and difficulty implementing conservation actions. Furthermore, recovery of this species

remains problematic until there is a better understanding of its biology and groundwater habitat. We previously added "C" to the new recovery priority number as this species is in conflict with construction, development, and other forms of economic activity.

6. **Recovery Plan:**
Name of Plan: A Recovery Plan for the Ozark Cavefish (*Amblyopsis rosae*).
Date Issued: December 17, 1986
Revised: November 19, 1989

II. REVIEW ANALYSIS

A. Application of the 1996 Distinct Population Segment (DPS) policy:

1. Is the species under review listed as a DPS? No.

2. Is there relevant new information that would lead you to consider listing this species as a DPS in accordance with the 1996 policy? No.

B. Recovery Plan and Criteria

1. Does the species have a final, approved recovery plan containing objective measurable criteria? Yes

2. Adequacy of recovery criteria

a. Do the recovery criteria reflect the best available and most up-to-date information on the biology of the species and its habitat? No. Recovery criteria for delisting in the original recovery plan were based on the best available science at the time of listing and may not represent the current status. Ozark Cavefish biology and life history are poorly understood with little data available to suggest life span, spawning season, number of eggs, egg survival, mouth brooding or not, population genetics, and various other aspects of its ecology.

b. Are all of the five listing factors that are relevant to the species addressed in the recovery criteria? No.
Information from Missouri has revealed Ozark Cavefish populations in springs and wells. Recovery criteria do not specifically address protection of springs and wells in groundwater systems that may have no other surface access, albeit we address protection of recharge areas in the criteria.

3. List the recovery criteria as they appear in the recovery plan, and discuss how each criterion has or has not been met, citing information.

Recovery criteria identified to delist Ozark Cavefish include:

1) Eight caves (Cave Springs and Logan in Arkansas; Twin and Engelbrecht in Oklahoma; Ben Lassiter, Kellhofers, Sarcoxie, and Turnback Creek in Missouri) and their recharge areas are protected.

Ozark Cavefish are restricted to the Springfield plateau geologic province of the Ozark ecoregion, with current populations spanning eight counties in Arkansas, Missouri, and Oklahoma, but there are historical and rumored accounts acknowledging further distribution in 52 caves in 14 counties (Service 1989).

Our partners prioritized these eight caves protection efforts through community outreach, data sharing, and on-the-ground conservation measures. Formal or estimated recharge zones exist for all recovery caves. Implementation of conservation actions within these recharge zones is ongoing and has been generally successful. Much work remains within recharge zones, such as developing conservation agreements, land use practice guidance, and encouraging adoption of conservation measures as part of county and community land use plans. Despite efforts to protect these caves from human disturbance through the installation of cave gates or fences, human disturbance at seven of these sites (all but Kellhofer Cave) continues to be a concern due to periodic vandalism (J. Westhoff, MDC, pers. comm., 2018; M. Slay, TNC, pers. comm., 2019).

2) The [Ozark] cavefish population in each of these caves remains stable or increasing as evidenced by observation of no less than 100 per survey visit in Cave Springs and no less than 20 per survey visit in each of the other caves over at least a 10 year period.

Population goals have been met in Logan (i.e., at least 20 individuals per survey; exception of the last attempt in 2017 which resulted in a partial survey) and Cave Springs caves (i.e., at least 100 individuals per survey; exception of the 2002 survey described as incomplete due to turbidity) over a ten year period (Table 1). Other caves generally have less than five individuals per survey visit with 0-2 individuals common. Therefore, 75% (6) of the recovery sites are currently unable to meet this criterion based on 10 – 20 years of survey data.

Table 1. Survey data depicting number of cavefish observed at Recovery Caves from 2000 to 2017.

Survey Date	Cave Springs Cave	Logan Cave	Englebrecht Cave	Twin Cave	Turnback Cave	Ben Lassiter Cave	Kellhofer Cave	Sarcoxie Cave
2000	164	36		1				
2001				4				
2002	60	48				0		
2003		46	0					4
2004	155	45	0			15		
2005			0			2		
2006	123	43	0				12	1
2007					0			0
2008			0					
2009	145	49			0			2
2010							6	
2011						2		
2012	132	23	0					
2013								
2014					0			
2015	162	28						
2016				0		0		8
2017	173	9						

3) At least three additional cavefish populations are confirmed and protected in Greene County, Missouri (to restore historic distribution), with a minimum sighting threshold of five cavefish each per survey visit.

In 2010, MDC identified a new population (Johnson Cave) in Greene County with eight individuals observed. Johnson Cave (8 cavefish observed) and Johnson Well (9 cavefish observed) were the only two sites with more than 5 cavefish during the last survey. No sites in this county have met the goal of five cavefish per visit.

The 1989 Ozark Cavefish Recovery Plan (USFWS 1989) includes the following tasks. Each recovery task is discussed below. New information is available for Tasks 1.1, 2.1, 2.3, 2.4, and 3.1 since the previous five-year review.

Task 1.1 Determine recharge area for recovery caves

All recovery caves have recharge zones either formally delineated using standard dye tracing methods or predicted using aerial photographs, soils/topographic/geology maps, mapped photolineaments (geologic fractures), hydrography, and cave maps where available. Additionally, Missouri Department of Conservation (MDC) completed recharge delineations for Fitzpatrick Cave, Johnson Cave, Buddy Well, and Jenkins Creek in 2012. In Arkansas, the Service and AGFC redelineated Cave Springs Cave (Beeman and Aley 2015) and Civil War Cave (Aley and Aley 2014) since the last review. Also, a new discovery at Charlie Craig State Fish Hatchery in Centerton led to a new delineation of the Centerton Spring and Fish Hatchery Spring in 2014.

Task 1.2 Determine the extent of continuous habitat in all recovery caves

Of the original recovery caves, Englebrecht Cave in Oklahoma is the only unmapped cave. Delineated recharge zones only account for areas of surface influence and does not account for the entire groundwater basin where the species may exist. Further assessment of this should occur across the range.

Task 2.1 Obtain conservation agreements with private landowners

The U.S. Fish and Wildlife Service (Service) and the Arkansas Natural Heritage Commission, respectively purchased Logan and Cave Springs Caves since listing, and specifically manage for cavefish. Two of four Missouri recovery caves are on private land with no conservation agreements in place. Of the other two Missouri recovery caves, a regional land trust organization owns one and MDC manages the other. One of the Oklahoma caves is private with no management agreement, while The Nature Conservancy owns the other. Five of eight recovery caves have management (conservation agreement or similar protections) in place for conservation of cavefish. Although not a recovery cave, Tom Wasson Cave (or Mud Cave), along Flint Creek in Benton County, Arkansas was acquired by Northwest Arkansas Land Trust in 2018 through land donation, and the property is being managed to benefit Ozark cavefish.

Task 2.2 Develop and install gates/fences or other methods of limiting access to public and privately owned caves that will not interfere with bats using the caves

Other than Kellhofer Cave in Missouri, installation of cave gates or fences protects all recovery sites to the best of our ability from human disturbance. Gating Kellhofer Cave entrance is due to its small entrance, which typically requires displacing gravel for entry (pers. comm. with Jacob Westhoff, MDC). Vandalism and unauthorized entry at recovery caves continues to be problematic with several undocumented incidents.

Task 2.3 Develop and implement habitat protection strategies for all recovery caves

Specific habitat protection strategies have been developed by the U.S. Fish and Wildlife Service and their partners for recovery caves and their recharge zones, including development of best management practices (BMP's) for the Cave Springs Cave recharge zone. Logan and Cave Springs caves are in public ownership with entrances and immediately adjacent lands managed for Ozark Cavefish. This does not include the recharge zone, although at Cave Springs Cave, three municipalities (Rogers (Rogers, AR 2018), Springdale (Springdale,

AR 2018), and Lowell (Lowell, AR 2018)) have implemented ordinances that include karst BMP measures for new development activities within the recharge area. Missouri has developed general BMPs for Ozark Cavefish (https://mdc.mo.gov/sites/default/files/downloads/Ozark%20Cavefish.pdf). Twin Cave in Oklahoma has a site conservation plan in place developed by The Nature Conservancy. This plan includes goals such as monitoring cavefish populations, maintaining and restoring water quality in the cave stream, and protecting lands and water in the recharge area (TNC 1996).

Task 2.4 Investigate the feasibility of introducing bats into uncolonized recovery caves.

Willis and Brown 1985 hypothesized that bat guano, particularly gray bat (*Myotis grisescens*), may be necessary or at least ideal for cavefish presence in a cave. Although gray bats and cavefish often occur in the same caves, studies suggest cavefish can benefit either directly or indirectly from the nutritive value of guano (Poulson 1960, Poulson 1963, and Fenolio et al. 2006). Graening et al. (2010) assert the majority of known Ozark Cavefish sites do not have appreciable bat guano deposits or even suitable habitat for bats (e.g., wells in Missouri or karst "windows" like a few sites in Arkansas). Furthermore, guano piles are statistically more likely to be absent at a cavefish site. There also is a lack of substantial evidence that cavefishes (Ozark and Southern cavefish) require this trophic input (Graening et al. 2010).

Task 2.5 Coordinate with State and private agencies to make spelunkers aware of the harm caving can inflict upon cavefish

Despite efforts with interested parties to preclude spelunkers from recovery caves with gates and fences, vandalism and unauthorized access at these sites is periodically a problem. Caves with gates or fences are signed indicating closure. Closure of caves for any reason receives scrutiny from the caving community.

Task 3.1 Monitor water quality in recovery caves annually

Annual water quality monitoring has not occurred at recovery caves since listing, but periodic sampling is ongoing. Work is underway in Arkansas to examine water quality (ongoing) in multiple biologically significant caves in northern Arkansas including Logan and Cave Springs Cave (pers. comm. Mike Slay, TNC). Novinger et al. 2012 assessed water quality in the Tri-State Mining District of Jasper and Newton Counties, Missouri and described the relationship between mining related contamination and the distribution of Ozark Cavefish. Poor water quality within mining-impacted areas reduces distribution and abundance of Ozark Cavefish and other stygobionts (obligate

subterranean aquatic organisms). Oklahoma sites need water quality monitoring.

Task 3.2 Monitor cavefish populations in known locations
Population monitoring of Ozark Cavefish is ongoing by all three states involved in Ozark Cavefish conservation and recovery. Monitoring at two to three-year intervals occurs at the majority of Ozark Cavefish sites with human access.

Task 3.3 Survey historic[al] and potential Ozark Cavefish sites
Monitoring of additional active cavefish sites is ongoing and conducted when possible across the range. MDC is evaluating a list of unconfirmed and potential Ozark Cavefish sites. Novinger et al. (2012) discovered four new sites in Missouri. Service biologists in Oklahoma have made a recent (May 2018) cavefish observation at a historical site. Further genetic analysis should confirm species. All states are pursuing leads when possible. Additionally, current research from Oklahoma State University (OSU) is aimed at developing and evaluating an environmental DNA (eDNA) protocol to detect the presence of Ozark Cavefish as well as cave crayfishes, then compare the efficiency of eDNA to traditional visual surveys.

C. Updated Information and Current Species Status

1. Biology and Habitat

a. Spatial distribution, abundance and population trends
Ozark Cavefish distribution is restricted to the Springfield plateau geologic province of Arkansas, Missouri, and Oklahoma. The Springfield plateau encompasses approximately 21,000 km^2 and drains the White, Neosho, and Osage rivers. Ozark Cavefish historically occurred at approximately 52 sites (Brown and Todd, 1987). Graening et al. (2010) reported 83 confirmed sites, plus at least 37 unconfirmed sites. There are an estimated 41 active Ozark Cavefish caves and wells in Arkansas, Missouri, and Oklahoma. Active sites are those with a positive observation since 1990, and positive observation includes either visual observation or (when it becomes available) a positive eDNA signal. As evidence that cavefish are rare (or difficult to detect), many of the "confirmed" sites reported by Graening et al. (2010) have no positive observation in the last 10 years. The 41 active sites are distributed throughout 10 counties including Benton County in Arkansas; Greene, Jasper, Lawrence, Newton, Christian, Barry, and Stone Counties in Missouri; and Delaware and Ottawa Counties in Oklahoma.

Arkansas has 8 caves (pers. comm. with Mike Slay, TNC), Missouri has 25 caves and wells (pers. comm. with Rick Horton, MDC), and Oklahoma has 8 caves (pers. comm. with Richard Stark, FWS) (Table 2).

The current range-wide estimate is 235 individuals compared to 213 individuals reported in the 2011 status review. The small increase in total numbers is encouraging, but an overall increase masks count variability between sampling periods at individual sites. For instance, there were an additional 11 cavefish observations at Cave Springs in 2017 compared to 2015. During the same sampling period, the number of cavefish observed at Logan Cave dropped by 19 individuals, most likely because of equipment failure during the 2017 survey (pers. comm. with Mike Slay, TNC). While cavefish are easily observable at Cave Springs and Logan caves, accessible cavefish habitat at most other caves is small. Limited accessible habitat coupled with low detectability of individuals and inconsistent periodic surveys means that the most "recent" count total may not be the best estimate for the species. Trend analysis that incorporates longer datasets of cavefish counts may be a more useful way to characterize population fluctuations (Graening et al 2010). Since the last status review, new surveys occurred at most cavefish sites a few times. Additional surveys are necessary prior to reassessing population trends (Table 2). Of populations that are undetermined and/or unoccupied, infrequency of survey and site accessibility issues may be contributing factors.

b. Demographic characteristics

Willis and Brown (1985) found a strong correlation between caves with plankton and/or benthos populations and presence of Ozark Cavefish. This study also documented the presence of gray bat maternity colonies at six of the 13 cavefish sites. Graening and Brown (2000a) similarly documented presence of gray bat maternity sites and suggested no correlation between presence of cavefish and presence of bat colonies. Specific breeding habits of Ozark Cavefish are unknown, including the number of eggs produced and whether they mouth brood or not. While reproductive season is not documented, Boyd (1997) located 10 mm,

Table 2. Observations from 41 current active Ozark Cavefish sites. Recovery caves are in bold.

Cave Name	1990-1999 (most recent observation listed)	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	last survey	last observed
ARKANSAS																						
Cave Springs		164	n	60		155		123			145			132			162		173		2017	2017
Civil War		**1**					**1**	**1**											**1**		2006	2017
Hewlitt Well	2		**0**																		2007	1992
Logan		36		48	46	45		43			49			23			28		9		2017	2007
Rootville		**1**	**1**						**0**												2007	2001
Tom Allan 1		**9**						**0**													2006	2000
Tom Allan 2																					2006	2000
Wassons Mud/James Ditto		**0**		**2**					**0**												2007	2002
MISSOURI																						
Ben Lassiter				0		15	2						2					0			2016	2011
Billies Creek aka Predator Cave			1				0					0				0					2014	2001
Buddy Well												5			0	0		2			2016	2016
Capps Creek Well 1									2		0				1			6			2016	2016
Capps Creek Well 2								1	0		0		2		0	0		1			2016	2016
Cave Spring	1						0						0								2011	1995
Fantastic Caverns	1							0													2006	1996
Fitzpatrick Cave											1										2009	2009
Harrison aka Faye Valley									3			3				8					2014	2014
Hayes Spring Cave									1							0					2014	2007
Hearrell Spring								1	0		1				0		0				2015	2009
Jackson Cave								1	0		0					0					2014	2006
Johnson Cave												8									2010	2010
Johnson's Well									1		6		3		0	6	9				2015	2015
Kellhofer's Cave								12			n	6									2010	2010
Kenney Cave									2			1									2010	2010
Kenney Well												3	0								2011	2010
Moore Cave	3																				1992	1992
Poor Well										1	0		1		0			0			2016	2011
Sarcoxie					4			1	0		2							8			2016	2016
Sperandio Well													1		0						2013	2011
Turnback Creek	1								0		0					0					2014	1997
Viebrock Well									1				2			0					2014	2011
Walbridge Spring													1		0			1			2016	2016
Jenkins Creek Cave aka Wright Cave										1	0	0									2010	2008
OKLAHOMA																						
Englebrecht	present				0	0	0	0	n	0				0							2005	1990
Jail					2				0	1									1		2018	2017
Longs			12				1		7							1					2014	2014
Mcgee			2						0	3						3					2014	2014
Mitchell			0						0											1	2018	2018
Star						0	0	0												1	2018	2018
Twin		1	4															0			2006	2001
Cave Springs Ranch						2	1														2005	2005

presumed young of the year, cavefish in Logan Cave during July, and Kampwerth (pers. obs. 2005) observed similar sized young of the year in January in Cave Springs Cave. Efforts have been made during counts at Cave Springs Cave and Logan Cave to assess size class over the past decade (see Figures 1 and 2). During efforts to assess size class over the past decade, surveyors recorded each individual observed as a small (>1 inch), medium (1 – 2 inches), or large (<2 inches) sized fish (Figures 1 and 2). These data were collected via observer estimates, not from physical collection and measuring of fish.

Figure 1. Size class observations at Cave Springs Cave from 2004 to 2017.

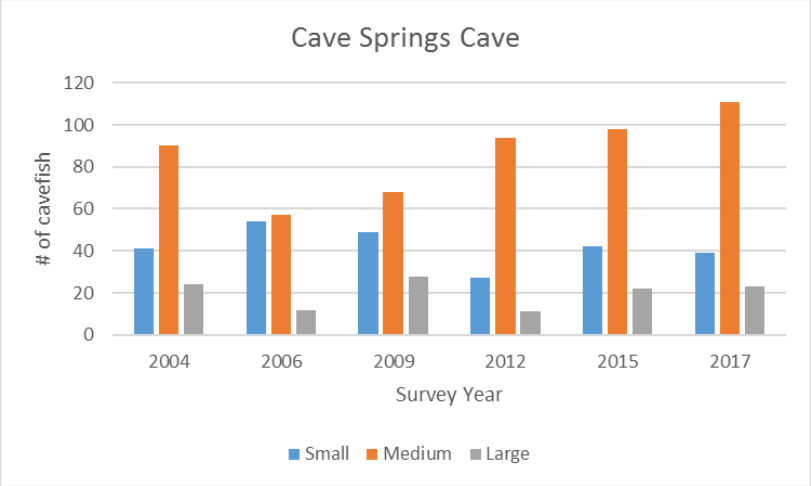
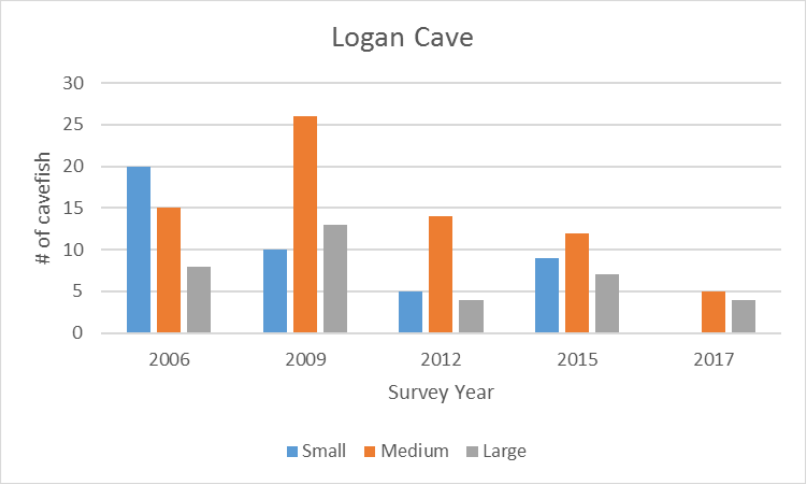


Figure 2. Size class observations at Logan Cave from 2006 to 2017.



Bergstrom (1997) conducted genetic analysis of six populations and suggests that based on intraspecific divergence Ozark Cavefish is a four subspecies complex. This suggests that each site is a deme with small isolated populations and a degree of cave or watershed endemism, which may constitute an Evolutionarily Significant Unit (Noltie and Wicks 2001). Neimiller (pers. comm., 2018) found a common haplotype between Logan and Cave Springs Cave genetic samples suggesting contemporary gene flow between populations. Logan and Cave Springs Caves are divided by Osage Creek and approximately 16 km (25.6 miles). Additional work should be conducted to determine population genetics and rangewide abundance. Niemiller (2011) suggested resurrection of the genus *Troglichthys* for the Ozark Cavefish based on eye histology, phylogenetic analyses, and rhodopsin evolution. The scientific community currently does not accept the reclassification of the nomenclature and *Amblyopsis* remains the senior synonym.

c. Habitat

Cavefish occur in groundwater habitats (the Springfield Plateau Aquifer) within Boone and Burlington Formation limestones, in cave streams with chert rubble substrate, wells and sinkholes, and occasionally in the soil phreatic zone (Poulson, 1960, 1963; USFWS, 1986). Woods and Inger (1957) suggest cavefish dispersal occurs through phreatic cave passages. Noltie and Wicks (2001) suggests that due to shale geologic confining units, Ozark Cavefish occur in near surface and epikarst habitats.

2. Five Factor Analysis (threats)

a. Present or threatened destruction, modification, or curtailment of its habitat or range:

Overall, threats appear to be stable or increasing at 41 active sites throughout the range. Threats at caves/wells and within recharge zones include human entry (discussed under Factor E), agriculture, and urbanization/development.

To respect private landowners wishes and not encourage search of and entry into cavefish locations, locations will not be discussed in great detail. We will discuss Logan and Cave Springs Caves as examples inferring similar threats and issues across the range.

Agriculture

In Benton County, Arkansas, 47.1% of land is in pasture and 28.9% is in cropland (Census of Agriculture 2012). Valuable canopy cover for ground temperature regulation and soil moisture retention is lost due to forest conversion to pasture. Chemicals and fertilizers applied to pastures rapidly infiltrate during precipitation events into groundwater systems. Graening and Brown (2000) found metals bioaccummulated in surface crayfish removed from Cave Springs Cave. They attribute these metal concentrations to land application of poultry litter in the recharge zone. Graening further suggests the decline in amphipods and an increase in isopods may be due to an increase in nutrient loads. Cave Springs Cave and Logan Cave occur in a Nutrient Surplus Area (NSA) in Northwest Arkansas. An NSA is an area designated by the Arkansas General Assembly as having such high concentrations of one or more nutrients that continued unrestricted application of the nutrient could negatively affect soil fertility and waters of the state. The Ozarks are a leading producer of poultry in the United States (United States Department of Agriculture 2018). In Arkansas, most poultry litter land application is on pasture or forage land near production (Miller and Tharp 1994).

Aley et al. 2015 suggests that there are only a few operating confined animal feeding operations compared to early 1990's and no sewage sludge application to pasturelands in the Cave Springs Cave Recharge Area. This is primarily due to the conversion of pasturelands to suburban development. In 2010, the Northwest Arkansas Conservation Authority opened a $55 million regional wastewater treatment facility four miles southwest of Cave Springs Cave in its recharge area. With the current proposed expansions, this facility will be able to treat 80 million gallons per day (mgd) for a population of approximately one million people.

In 1968, 59 percent of the Logan Cave recharge zone was forest, with a decrease to 43 percent by 1987 (Aley and Aley 1987). Harvest of mature forest continues to decrease important surface cover and is likely due to conversion to pasture land, home development, and some Confined Animal Feeding Operation (CAFO) developments. This 11 square mile recharge zone had approximately 50 hog and poultry facilities during the 1980s (Aley and Aley 1987). Updated data are not currently available on the number of CAFOs in this specific area. However, desktop assessment via satellite

imagery suggests reduced pasture or forest conversion within the past decade compared to the 1980's. Although, as animal production sites generally occur on well-drained slopes, potentially high levels of biological and chemical contaminants are rapidly transported, which can influence Logan Cave waters. Metals and other contaminants pass through poultry/livestock and can reach groundwater through land application of wastes.

Urbanization/development

Of the 41 active sites, urbanization/development is a leading threat in recharge zones in northwest Arkansas and parts of southwest Missouri. As development increases, areas that allow natural infiltration and percolation are lost or significantly diminished. As impervious surfaces increase, stormwater directed to engineered or natural outlets no longer finds natural groundwater flow paths. Outfalls often lead to adjacent losing streams whereby stormwater is ultimately transported to groundwater. Stormwater runoff contains numerous contaminants including automotive fluids, brake dust, roof tar, pesticides, and herbicides. Stormwater runoff leads to acute pulses of contaminated waters underground, of which some contaminants remain in the system for years. A substantial amount of groundwater contamination occurs from inadequate or un-maintained sewage disposal systems. Increased groundwater withdrawals for home, community, and agricultural use, depletes groundwater and limits available habitat (https://www.epa.gov/nutrientpollution/sources-and-solutions-stormwater).

Based on aerial photography, in 1990 approximately 2,729 acres or 28 percent of the 15 square mile Cave Springs Cave recharge zone was developed, while in 2006 that number was 6,751 acres or 70 percent, a 147 percent increase in developed acres. The population in Benton County increased 57.3 percent between 1990 and 2000. In April 2000, Benton County had a population of 153,406, and in July 2006 a population of 176,756, or a 15 percent increase (one of the highest in Arkansas). In April 2010, Benton County had a population of 221,339, and in July 2017 a population of 266,300, or a 20% increase. According to the U.S. Census Bureau, the Fayetteville-Springdale-Rogers metro area in Benton and Washington counties combined has seen a population increase of 9.1% from 2013 to 2017, from 492,739 to 537,463. It is likely that this metro area of Northwest Arkansas will join the Top 100 metropolitan statistical areas by the end of 2019.

Benton County is the only county in Arkansas with Ozark Cavefish.

Development increases at two of the active cavefish sites in suburban northwest Arkansas are likely similar to that of Cave Springs Cave, while more rural Benton County sites, such as Logan Cave, appear to be stable.

b. Over-utilization for commercial, recreational, scientific, or educational purposes:
There is no evidence to suggest that over utilization is a threat. While this was a threat in the past; site protection, scientific collection permit regulation and public outreach has nearly eliminated this threat.

c. Disease or predation:
Ozark Cavefish have few natural predators. Cave use by mammals such as raccoons and invasion of cave streams/springs by surface fishes like sunfish and minnows do occur. However, predation by these animals, although possible, is not considered a substantive threat at this time. We have no evidence of disease in Ozark Cavefish. Cavefish biologists clean equipment between caves to reduce potential transfer of disease or parasites from one cave to another. Since the discovery of white-nose syndrome in the U.S., including Arkansas, the Service has developed decontamination procedures for cleaning of cave gear. The most recent decontamination procedures can be found at https://www.fws.gov/arkansas-es/docs/National%20WNS%20Decon%20Protocol_04.12.2016.pdf.

d. Inadequacy of existing regulatory mechanisms:
Regulatory mechanisms (e.g. Clean Water Act Section 106) are in place to protect water quality and habitat for Ozark Cavefish. Arkansas Department of Environmental Quality (ADEQ) groundwater staff craft guidelines to address both point and nonpoint sources of pollution. ADEQ is supporting groundwater protection strategies through coordination of permit review and comment by the Service prior to issuance. However, existing regulatory mechanisms regarding the protection of groundwater resources are limited compared to surface waters. ADEQ conducts groundwater quality monitoring throughout the state, but cavefish sites are not included in their scheduled sampling.

Arkansas legislation requires land application of poultry litter under an approved nutrient management plan. The plan relies on soil and vegetative communities present, and recommends distances from waterways that litter application. As enforcement is limited and water quality in caves and wells shows increases in nutrients and metals, it appears adherence to or success of these plans are limited at this time.

Missouri Department of Natural Resources (MDNR) relies on criteria to protect beneficial uses, coordinates with the Service prior to permit issuance, and successfully implemented procedures for Missouri's antidegradation rule in 2016 (Code of State Regulations, Division 20, Chapter 7.031(3) (i.e., 10 CSR 20-7.031(3)) and federal antidegradation policy at Title 40 Code of Federal Regulations (CFR) Section (§)131.12). The MDNR is required by 40 CFR §131.12(a) to develop and adopt a statewide antidegradation policy and to identify procedures for implementing that policy. Implementation generally includes: identifying the antidegradation review levels (i.e., the "tiers") that apply to a surface water; determining existing water quality (EWQ); assessing and determining appropriate extent of water quality degradation; identifying and assessing less-degrading or non-degrading alternatives; determining the importance of economic or social development to justify degradation of waters; and establishing intergovernmental coordination and public participation processes.

The Oklahoma Department of Environmental Quality (ODEQ) is responsible for issuing discharge permits for industries and municipalities that dispose of treated wastewater. ODEQ also is responsible for storm water discharge associated with construction and industrial sites. These permits are required for any storm water discharge associated with construction activities that would result in land disturbance equal to or greater than one acre. Prior to the last review, the Oklahoma Ecological Services Field Office completed consultation with the ODEQ on issuance of construction storm water permits in the state. Further coordination with the Service will take place for any proposed activities within a watershed that contains an Ozark Cavefish cave. This coordination will facilitate the development and implementation of appropriate BMPs to avoid and minimize adverse effects on cavefish.

Agencies are requiring stormwater management plans under Environmental Protection Agency (EPA) MS4 phase 2

regulations whereby development activities greater than two acres in size have to develop a stormwater management plan. The EPA has regulations and standards outlining water quality conditions for groundwater based on human health standards. Regulations and management guidance necessary to protect groundwater from nonpoint source pollution are not available. Water quality threats are typically non-point source derived and difficult to regulate. In general, regulations are not specific enough to protect, they contain no guidance on how to protect, and enforcement is understaffed.

The Ozark Cavefish is listed as endangered by the State of Missouri (Rule 3CSR10-4.111 of the Wildlife Code of Missouri) and certain state statutes are applicable that would not otherwise apply to unlisted species. MDC (1999) developed an Action Plan, similar to a recovery plan, for Ozark Cavefish, which identifies objectives and strategies. MDC plans to revise the plan periodically.

In 2015, Northwest Arkansas Regional Planning Commission initiated a Cave Springs Karst Study. This study resulted in the development of the "Chapter 10. Cave Springs Direct Recharge Area Water Quality Protection Requirements" (Cave Springs Karst Study 2015). These requirements, if enacted across the species range, promote recovery by minimizing adverse effects to water quality, habitat, and hydrology, associated with rapid urbanization in the region. The cities of Rogers, Lowell, and Springdale have all adopted these, or a similar version of, these requirements into their city drainage criteria manuals. However, the city of Cave Springs, which contains the majority of the "High" and "Extremely High Vulnerability" areas, has yet to adopt these water quality protections. The Service is currently working with the city of Cave Springs on moving forward with adopting these conservation measures.

e. Other natural or man-made factors affecting its continued existence:

Human entry

Sixteen cavefish sites are gated or fenced to reduce human disturbance. Entry is the primary threat at one site (Logan Cave) in Arkansas, which harbors the second largest population. Use at ungated caves is occurring based on evidence such as new paint, footprints, rafts, and writing found during biannual monitoring surveys. As interest in recreational

caving continues to increase, caves supporting cavefish are likely to receive additional unauthorized entry.

Human entry causes increased turbidity decreasing cavefish sensory ability, increases the potential for direct mortality due to trampling of individuals, and can interrupt feeding and breeding behaviors.

Contaminant spills/accidents

Transportation and pipeline routes can cause sediment and other contaminants to enter the groundwater system. Leaks and spills along roadways do occur and threaten groundwater. Prior to the last review, a spill of 60,000 gallons of gasoline in Benton County Arkansas immediately went underground. Arkansas DEQ conducted well and spring water quality sampling, finding no evidence of the fuels' groundwater dispersal. Spill residue may resurface during significant precipitation events flushing it from karst conduits. In 2017, over 15,000 gallons of light crude oil spilled from the Ozark Pipeline at the Lawrence Pump Station in Lawrence County, Missouri. There was no report of effects on Ozark Cavefish. Information pertaining to this spill is available at https://www.ozarksfirst.com/news/pipeline-leaks-more-than15000-gallons-of-crude-oil-in-lawrence-co/641318061.

Other Threats

Recent unpublished water quality studies at springs, wells, and streams in Arkansas, Oklahoma, and Missouri found numerous contaminants at low but detectable levels. Brown et al. (1998) found mean total coliform counts of 500 MPN (most probable number)/100ml at base flow, and 20,000 MPN/100mL during storm events at Cave Springs Cave. Graening and Brown (2003) consistently found high levels of fecal coliform, excess nutrients, and metals in water, sediment, and tissue samples at Cave Springs Cave. They further identified beryllium, copper, selenium, and zinc at levels exceeding Arkansas Maximum Contaminant Levels (MCL) for chronic and acute toxicity to aquatic life.

A U.S. Geological Survey (USGS) study in 2004 found 42 pharmaceuticals and other organic wastewater constituents in selected northern Arkansas stream sites (Galloway et al. 2004). Most streams in northern Arkansas are losing streams that contribute to groundwater. Contaminants identified include antibiotics, antioxidants, detergent metabolites, disinfectants, fire retardants, fragrance/flavor compounds, insect

repellant/pesticides, non-prescription drugs, polycyclic aromatic hydrocarbons, plasticizers, solvents, and steroids. Threats from these contaminants as suggested by the USGS include abnormal physiological processes and reproductive impairment, increased incidences of cancer, and the potential increased toxicity and carcinogenic activity of the chemicals and mixtures of the constituents.

Bidwell et al. 2010 found a range of organic wastewater compounds in Ozark Cavefish waters in Arkansas and Oklahoma during a study conducted in 2006 using polar organic chemical integrative samplers and semi-permeable membrane devices. Probable sources of contaminants include wastewater treatment facility discharges, septic systems, land application of livestock litter and biosolids, agricultural chemicals, homeowner application of chemicals, and other unknown sources. Although levels were generally low, pharmaceutical and wastewater constituents present a concern until we understand possible effects on cave fauna.

Additionally, multiple reports provide baseline, water quality data and/or summaries for Ozark Cavefish sites (Slay and Snell 2005, Slay 2011, Aley et al. 2015, Aley and Slay 2015, and Beeman and Aley 2015). This information may be useful when assessing future water quality in these systems.

In 2018, the Missouri American Water Company initiated conversations with landowners regarding a proposed 1,500-acre water supply reservoir in Newton County, Missouri. This reservoir would occur in an area southeast of Joplin and would affect either Cedar Creek or Baynham Branch, both occurring in the Shoal Creek watershed. Although the proposed footprint of either option does not inundate any known locations for Ozark Cavefish, both sites are in karst areas very near (< 6 km from) existing (Kenny Well/Cave and Buddy Well) and other potential Ozark Cavefish sites.

3. Conservation Measures

There is new relevant information regarding implementation of conservation measures that benefit the Ozark Cavefish. Arkansas, Missouri, and Oklahoma completed various conservation measures necessary for cave and recharge zone protection.

Logan Cave National Wildlife Refuge completed a Comprehensive Conservation Plan in 2008 (Service 2008) and a Habitat

Management Plan in 2013 (Service 2013). Goals and objectives of these plans account for the needs of subterranean fauna including Ozark Cavefish. In 2008, an effort focused on cave radio work delineating the extent of cave passage on adjacent private lands. These data informed decision making for a hazmat storage site close to identified cave passages. Efforts focused on landowner outreach within the recharge zone. Prior to the last review, the Service met with the Benton County road department about road improvements, discussed nutrient management and litter application with poultry growers, established a working relationship with adjacent landowners to the Refuge, funded the installation of a permanent water quality monitoring station, installed a new cave fence and gate, and continue population monitoring efforts. Currently, the Service continues to work with partners to accomplish population monitoring, water quality monitoring, eDNA research, sediment reduction projects by the county on upaved roads as well as two box culvert installations proposed for 2019. The Nature Conservancy with funding from the Service, installed upgrades to problematic septic tanks, and studied habitat quality and pollution effects. As this area is prime development real estate, diligent involvement, primarily by the Service, Arkansas Game and Fish Commission, Arkansas Natural Heritage Commission, and The Nature Conservancy, in the county planning process is paramount to continued conservation within this recharge zone.

The Cave Springs Cave system has had numerous conservation measures conducted successfully since listing. These include acquisition of the cave entrance and 15 acres by The Nature Conservancy with transfer to the Arkansas Natural Heritage Commission and a recent donation of 42 acres through AES negotiation with developers that included lands immediately over cave passages and along the primary losing stream that contributes water to the cave stream. The donation earned three developers the U.S. Fish and Wildlife Service Regional and National Directors Conservation Awards. In 2005, a new easement granted to the Arkansas Natural Heritage Commission for a bat friendly fence at the cave entrance. They completed the project and land acquisition in 2005. They also restored land above the known cave passages by planting trees in an attempt to convert agricultural fields back to forest. In addition, they conducted groundwater dye trace studies to determine the potential surface groundwater area of influence, with data used to establish the corridor for an interstate highway, which completely avoided the recharge zone. In 2012, the Illinois River Watershed Partnership (IRWP) acquired a downstream lake and land (totaling 30 acres) adjacent to Cave Springs Cave, which

provides improved access control to the cave and protection of bat foraging habitat.

In 2004, a large partnership was established with numerous agencies, communities, private landowners, engineering firms, attorneys, and academia coming together to develop a mechanism for guidance to conserve groundwater while supporting community growth. One outcome was the development of a document entitled "Community Growth Best Management Practices for Conservation of the Cave Springs Cave Recharge Zone" (Service 2005). This document outlines recommendations for development and construction activities within the delineated recharge zone, including establishing conservation zones, stormwater management recommendations, general construction BMP's, and guidance for water and sewer line installation. In 2014, multiple local interests created the Cave Springs Area Karst Resource Conservation Initiative. This Initiative has three objectives:

- Seek out, consolidate and analyze existing water quality, species population, and development data in and around the study area, defined as the Cave Springs Cave Recharge Area, and determine trends and needs for additional data.

- Work with the scientific community to determine appropriate actions necessary to ensure adequate protection of local karst recharge zones that support threatened and endangered species, and build on previous efforts for karst conservation.

- The third objective was to work with local, county and state officials/administrators and other relevant stakeholders to determine the best mechanism to ensure conservation actions are effective in the appropriate areas.

This initiative lead, in part, to creation of a Drainage Criteria Manual entitled "Chapter 10: Cave Springs District Recharge Area Water Quality Protection Requirements". Although the residents of the city of Cave Springs own the majority of the recharge area for Cave Springs Cave, they are the only one out of four cities/communities that have yet to adopt the "Chapter 10 Requirements" as a part of their city ordinance.

Through a lengthy informal consultation, we evaluated a large chlorinated community water line for threats and applied conservation measures to reduce potential risk. In 2007, a dye study conducted by the city of Rogers wastewater treatment

facility to determine if contaminants found during recent water quality work at the cave originated from the plant. Results of this study showed no connectivity to Cave Springs Cave.

Landowner relations at other Ozark Cavefish caves and within their respective recharge zones are beneficial. Ownership changes frequently due to land prices and development activity. As development and community growth activities expand in recharge zones, the Service reviews them in coordination with the ADEQ and/or communities. It is important to implement conservation measures, including conservation zone establishment and stormwater management techniques,

Additional conservation efforts in Arkansas:

- development of BMP's for the Ozark National Forest Plan (Karst).
- development of Forest Industry karst BMP's fact sheet with Arkansas Forestry Commission.
- conducting educational programs ranging from elementary through college level, holding public meetings with Audubon Society, Ozark Society, cave clubs, boy scouts, church groups, statewide attorneys meeting, engineering organizations, and real estate organizations.
- leading cave trips for groups discussed above.
- establishing a relationship with Arkansas Highway and Transportation Department whereby recharge zones are considered in development activities.
- inclusion of recharge areas as a component of developing acquisition priorities for the Northwest Arkansas Open Space Plan.

Conservation efforts in Oklahoma include:

- cave gate and fence installation and monitoring.
- survey and monitoring of cavefish populations.
- delineation of recharge zones.
- land management in coordination with landowners, TNC, and the Ozark Plateau National Wildlife Refuge.
- invasive species control within recharge zones.
- coordination with the city of Tulsa and Land Legacy to develop riparian and groundwater protection projects.
- water quality studies including pharmaceutical and wastewater compounds.
- purchase of caves by TNC.
- coordination with the Army Corp of Engineers, the Federal Energy Regulatory Commission, and Grand River Dam Authority on reservoir effects and cave gate maintenance.

- coordination with local caving organizations to build and monitor cave gates, and map caves to determine land use activities over known cave passages.
- coordination with the Cherokee Nation on survey efforts and management of caves and recharge zones.

Conservation efforts in Missouri include:

- continuation of the Ozark Cavefish working group and an Ozark cavefish action plan.
- a Shoal Creek Restoration Group comprised of representatives from many agencies and organizations in Missouri has formed to consider management of water resources and biota in the Shoal Creek watershed.
- consistent monitoring of existing sites.
- continue searching for new cavefish locations.
- highway realignment based on cavefish recharge zone studies.
- install and maintain cave gates and fences.
- install riparian and livestock exclusion fences.
- protective well capping where cavefish are found.
- site specific water quality monitoring.
- site acquisition and easements.
- recharge delineation and mapping for all 25 active locations in Missouri.
- cave mapping.
- hazard identification and assessment.
- create and distribute educational articles.
- develop outreach materials including coffee mugs, magnets, stickers, and placemats.
- public outreach including fair booths, youth programs, and local watershed committee involvement.
- support for eDNA research to be developed for surveys and monitoring.
- hold public meetings for specific caves and recharge zones.
- apply for and receive grants targeting cavefish conservation actions.
- receive a landowner incentive program (LIP) grant for Ozark Cavefish biologist.
- grant obtained for easements and long term protection of sensitive areas.
- numerous landowner contacts via mailings, phone, and in person.
- installation of selected BMPs to protect groundwater quality and karst habitats within known recharge areas of Ozark Cavefish sites.

- development of the Ozark Cavefish National Wildlife Refuge Habitat Management Plan (Service 2015).

Conservation activities are ongoing and widely applied across the range of Ozark Cavefish.

D. Synthesis

At the time of listing, there were eight populations identified for protective measures. Of those sites, only two have met population goals, with the exception of the recent Logan Cave count, although hindered by effort, none met the goal of protecting all lands within the recharge zone. Surveyors consistently see Ozark Cavefish at 16 of 41 known sites. However, they have not seen cavefish at the majority of active sites in ten or more years. The top four sites based on the most recent (2016-2017) monitoring counts, include Cave Springs Cave (173), Logan Cave (9), Johnson Well (9), and Sarcoxie Cave (8), with Cave Springs and Logan caves alone representing approximately 78% of countable Ozark Cavefish across all known sites. The other 12 occupied sites are represented by counts of 1-2 individuals typically, although higher counts have occurred (e.g., Capps Well at 6 in 2016).

While it is impossible to determine accurate historical distribution and site locations, recognized historical and rumored sites, the Service and its partners evaluate caves when landowner permission is available. These efforts led to the discovery of seven new sites in Missouri since 2000 and a recent observation at a historical site in Oklahoma (May 2018). Diligent effort focused on locating new sites as well as protecting existing populations and their recharge zones is necessary. As population growth (urbanization) influences groundwater quality and quantity, resource agencies need to focus Ozark Cavefish conservation efforts at threats associated with urban and agriculture development.

Urbanization and development are the primary threat within 17 recharge zones, it is critical to establish cooperative partnerships with city and county officials and others with responsibility for planning and development to ensure conservation practice implementation. Attendance and active participation at city and county planning board meetings are required for successful conservation measures. Once interested parties identify conservation practices, it is necessary to encourage and support adoption into city and county planning ordinances. For long-term protective measures to be effective, city and county ordinances have to specify conservation measures necessary for protection of groundwater through appropriate land management recommendations. One measure might be a required increase in green space to include conservation zones around losing streams, springs, sinkholes, and cave entrances, which are

the most sensitive features in a recharge zone. Ball fields, trails, other community amenities, and stormwater management features can occur in this green space. The Service and other agencies must cooperate so that notification of and response to permitted activities are coordinated. Establishing common ground between community growth and necessary conservation requires cooperative partnerships between private landowners, communities, interested parties, and agencies.

Unmanaged stormwater runoff poses a significant groundwater contamination threat without site-specific management practices. As established developments and agricultural activities have limited ability for modification, communities and their partners need to continue development and implementation of guidelines for post-construction stormwater management. New development projects must manage their site's stormwater runoff to reduce threats to groundwater. This benefits cavefish conservation, the communities that drink groundwater, and private landowners who use it for home, farm, and ranch purposes.

There is a need for water quality monitoring and studies targeted on effects of pharmaceuticals and other contaminants. Pharmaceuticals and other contaminants are present at some cavefish sites. It is unclear what effects these have on reproduction, recruitment, and survival of cavefish. An agreement between the Pharmaceutical Research and Manufacturers of America, the Service, and the American Pharmacists Association should help protect the nation's fish and aquatic resources from improper disposal of medication. The campaign informs people on safe disposal of medicines and highlights the environmental threat (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2866706/). Additional studies should focus on methods for wastewater treatment facilities to treat phamaceuticals.

Since private landowners are probable owners of unknown Ozark Cavefish sites, public outreach, information sharing, and relationship building must continue in order to locate unknown sites and monitor historical sites. Additional Ozark Cavefish populations likely will occur on private lands. Contacts should occur in person without fear of additional regulatory burdens. Many private landowners are cautious of agencies and therefore do not acknowledge sites, but much of this concern can be overcome with agreements, and honest open communication. When private landowners recognize that cavefish are not a liability but an asset in the conservation of groundwater, they often want to assist with conservation efforts. Private landowners are conscientious and concerned with issues surrounding groundwater. Greater effort focused on contacting, establishing trust, and building relationships with private landowners.

Additional outreach and information sharing should focus on spelunkers and organized caving clubs. There are approximately 426 members of the National Speleological Society in Arkansas, Missouri, Oklahoma, and many independent spelunkers. These groups and individuals visit thousands of sites each year and have knowledge of rumored, historical, and other potential cavefish sites. Given site endemicity of cave species, including microbiological communities, the caving community is encouraged to clean equipment between caves in order to reduce potential cave-to-cave transfer of unknown species and disease. Once introduced into other cave systems, the effect, although initially unapparent, could pose a significant threat to cave species including cavefish. A recent threat to bats called "white nose syndrome" is considered transmittable by cavers and decontamination procedures have been developed and distributed.

As knowledge of cavefish life history is negligible, the scientific community and our partners need to consider methods to study cavefish without harming individuals, populations, or habitat. An understanding of how and when cavefish spawn, whether they mouth brood or not, and general population ecology can aid in conservation and recovery efforts. Developing methods for propagation and establishing a conservation population should occur while population numbers are generally stable.

Ozark Cavefish is still threatened by potential risks to water quality and quantity associated with urbanization and other land use development activities, a lack of knowledge regarding the species life history and ecology, potential indirect effects to the species life history and ecology posed by "white nose syndrome" effects to bat populations, trespass and vandalism, and trampling either by inadvertent researchers or spelunkers. Based on this status review, we believe Ozark Cavefish still meets the definition of threatened as defined by the ESA.

III. RESULTS

A. Recommended Classification:

The status of the Ozark Cavefish should remain unchanged.

IV. RECOMMENDATIONS FOR FUTURE ACTIONS

1. As the majority of community and agricultural development activities have no federal nexus, it is imperative that the Service and its partners continue to establish and/or foster cooperative relationships with city councils, planning boards, quorum courts, county commissioners, tribes, and others involved in the economic development of communities and their growth.

2. Establish trust and relationships with private landowners as the likelihood of future discovery exists mostly on private lands.

3. Revise the recovery plan to reflect current knowledge, refine reclassification criteria, re-define delisting criteria, and accurately address the five factors.

4. Determine life history characteristics of Ozark Cavefish.

5. Determine the importance of gene flow between individual populations.

6. Research use of mucous samples for genetic analysis.

7. Establish methodology for the propagation of Ozark Cavefish.

8. Conduct tissue analysis of non-sensitive species, and sediments in caves for contaminants and metals.

9. Continue and expand water quality monitoring, including pharmaceuticals and other contaminants.

10. Determine occurrence and accurate status of sites with cavefish records greater than 10 years old.

11. Continue monitoring of the groundwater basin within the Springfield plateau for potentiometric surface and water quality.

12. Evaluate alternative incentives for cooperating private landowners, developers, and communities.

13. Investigate and implement security measures at caves. These may include pressure plates, cameras, sensors, data loggers, and cave stewards.

14. Ascertain methods for Ozark Cavefish population enumeration throughout the Springfield Plateau by sampling groundwater portals (i.e.: wells, springs, etc.) for cavefish. Development of a model may prove beneficial in this effort.

15. Investigate alternatives to land application of litter within recharge zones and educate landowners on appropriate setbacks from sensitive karst features and appropriate timing of land application when applying nutrients to soil.

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U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW of Ozark cavefish (*Amblyopsis rosae*)

Current Classification: Threatened

Recommendation resulting from the 5-Year Review:

_x_ No change is needed

Review Conducted By: Tommy Inebnit, AES

FIELD OFFICE APPROVAL:

Lead Field Supervisor, Fish and Wildlife Service

Approve: MELVIN TOBIN Digitally signed by MELVIN TOBIN Date: 2019.06.25 14:13:10 -05'00' Date: _____

REGIONAL OFFICE APPROVAL:

Cooperating Assistant Regional Director, Fish and Wildlife Service, Southwest Region

X Concur ____ Do Not Concur

Signature: Kevin Hall for Jonna Polk Cooperating Field Supervisor, Southwest Region Date: 8/9/2019

Cooperating Assistant Regional Director - Ecological Services, Fish and Wildlife Service, Midwest Region

X Concur ____ Do Not Concur

Signature: Leonard Nemeth Date: 8/8/2019

***APPENDIX A: Summary of peer review for the 5-year review of* Ozark Cavefish (*Amblyopsis rosae*)**

Reviewers:
Dr. Jacob Westhoff, Resource Scientist-Aquatic Systems
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Brian Wagner, Nongame Aquatics Biologist
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Bryan Rupar, Chief of Land Acquisition and Stewardship
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A. Peer Review Method: A draft copy of this 5 year review was sent to the above knowledgeable individuals for their review and comment. These biologists were selected based on their current active involvement with Ozark cavefish conservation efforts and/or knowledge with this fish.

B. **Peer Review Charge:** Reviewers were charged with providing a review of the document including any other comments and/or additions appropriate to include. We did not ask peer reviewers to evaluate our status recommendation.

C. **Summary of Peer Review Comments:** Reviewers responded verbally and/or by email. They did provide some additional references and recommendations that were incorporated into the 5-year review as appropriate. A brief summary of some significant comments are provided below.

D. Response to Peer Review: Most recommendations from the reviewers were included in the document. Any recommendation not included is listed below with a justification. Recommendations primarily consisted of updated survey data, cave site information, and recent conservation efforts.

Bryan Rupar had no comments.

Dr. Jacob Westhoff provided minor editorial changes. Dr. Westhoff noted they use *Troglichthys* in Missouri rather than *Amblyopsis* for the genus of Ozark Cavefish.

Our Response: Niemiller (2011) suggested resurrection of the genus *Troglichthys* for the Ozark Cavefish based on eye histology, phylogenetic analyses, and rhodopsin evolution. The reclassification of the nomenclature currently is not accepted within the scientific community and *Amblyopsis* remains the senior synonym. Once accepted, an official

change in 50 CFR 17.11 would require the Service to publish a notice in the federal register for public comment.

Dr. Jacob Westhoff suggested adding the most recent 2016 cavefish monitoring report in the "Status Reivews" section.

Our Response: The Service added the *FY16 Ozark Cavefish Monitoring* report to the "Status Review" section and the literature cited.

Dr. Jacob Westhoff provided corrections to survey data listed in Table 1.

Our Response: The Service applied correct data to Table 1.

Dr. Jacob Westhoff suggested adding a recent proposal to create a water supply reservoir in the Shoal Creek watershed within the Ozark cavefish range to "Other Threats".

Our Response: The Service added the suggested information.

Dr. Jacob Westhoff suggested adding Shoal Creek Restoration Group to Missouri conservation efforts.

Our Response: The Service added the suggested information.

Brian Wagner provided minor editorial changes. Mr. Wagner also noted an error in the interpretation of Willis and Brown (1985) regarding the correlation between cavefish presence and gray bat colonies.

Our Response: The Service corrected the description to "Willis and Brown (1985) found a strong correlation between caves with plankton and/or benthos populations and presence of Ozark Cavefish. This study also documented the presence of gray bat maternity colonies at six of the 13 cavefish sites. Graening and Brown (2000a) similarly documented presence of gray bat maternity sites and suggested no correlation between presence of cavefish and presence of bat colonies."