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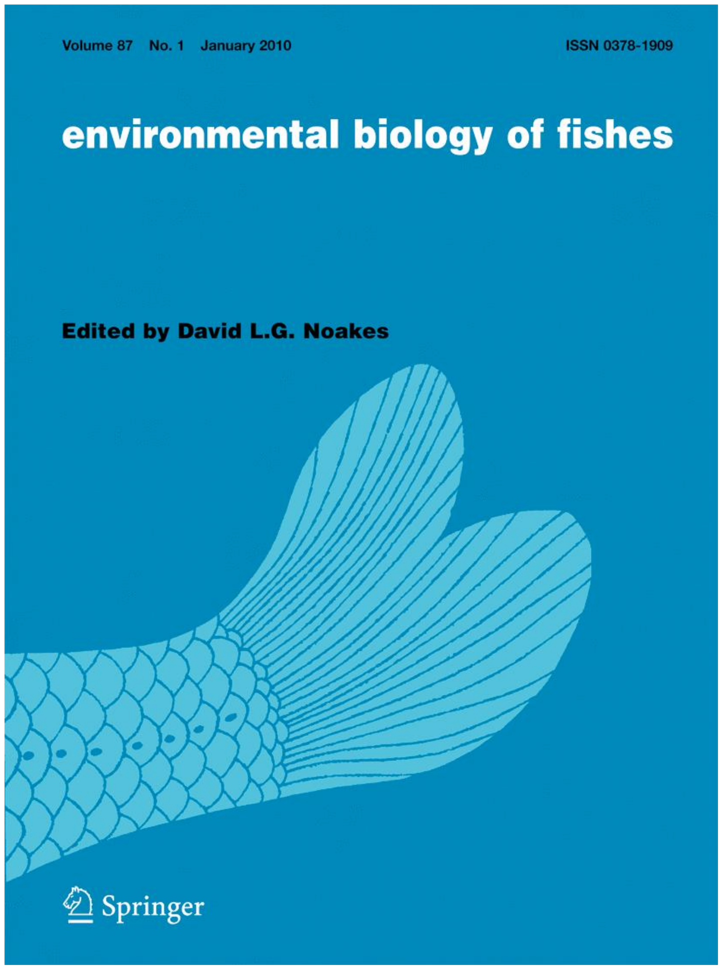

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The 30-year recovery effort for the Ozark cavefish (*Amblyopsis rosae*): Analysis of current distribution, population trends, and conservation status of this threatened species

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Abstract Here we review the thirty year recovery effort and conservation status of the Ozark cavefish, *Amblyopsis rosae*. We summarized the historic and current range of the species, and report county range extensions for both *A. rosae* and its confamilial *Typhlichthys subterraneus*. Ozark cavefish survey data spanning almost a century were analyzed for temporal trends using the Mann-Kendall Test/Sen's Slope Estimator Method. Results were inconclusive because variance was high and the majority of data sets were not sufficiently large to detect a trend. However, the two largest populations (Cave Springs Cave and Logan Cave, Benton Co., Arkansas) have stabilizing or increasing survey counts. While the number of active cavefish sites has decreased over 50% since 1990, the number of surveyed individuals has not. Reasons for endangerment were reanalyzed since federal listing; the primary threat has shifted from overcollection to habitat degradation. We analyzed the progress of recovery task implementation, and we critically evaluated the basis of delisting criteria. Recovery Task 1, the hydrogeologic delineation of subterranean habitats, is almost complete. Recovery Task 2 prescribes protection and management for Recovery Caves, and important progress has been made. Recovery Task 3 involves the development and implementation of monitoring programs in Recovery Caves. Several important studies have been performed, and indicate that many cavefish populations are experiencing chronic, low-level exposure to a suite of anthropogenic contaminants. Delisting conditions are largely unattainable as currently worded. We suggest that recovery criteria be amended such that habitat protection goals are attainable, that the list of Recovery Caves can be periodically updated, and that the recovery

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population goal is increased and distributed between more sites.

Keywords *Amblyopsis rosae* · Amblyopsidae · Endangered species · Groundwater pollution · Ozark cavefish · Population dynamics · Recovery plan · Trend analysis

Introduction

The thirty-year effort to bring the Ozark cavefish, *Amblyopsis rosae sensu lato* (Eigenmann 1898), back from the brink of extinction exemplifies the challenges facing conservation biologists who manage rare species with life history traits that are largely incompatible with human enterprises. This cavefish, and stygobionts (animals restricted/adapted to subterranean aquatic habitats) in general, are particularly difficult to manage because their habitat is inaccessible to scientific study and because subterranean ecosystems are vulnerable to a plethora of anthropogenic stressors that are related to human dependence upon, and exploitation of, groundwater (Elliott 2000; Proudlove 2006). Stygobiotic fishes are a particularly imperiled guild with approximately 61% of the 104 known taxa on the International Union for Conservation of Nature (IUCN) Red List (Proudlove 2006; IUCN 2007). Prominent on this list is the Ozark cavefish (Fig. 1), which has ironically served as both a "lab rat" for the testing of evolutionary theories and a flagship species for conservation actions designed specifically for subterranean environments. We evaluated the efficacy of the federal recovery plan written by the United States Fish and Wildlife Service (USFWS) for this species (USFWS 1989), with the hope that lessons learned here can be applied to other endangered subterranean species.

Fig. 1 Photograph of the Ozark cavefish in a cave stream in Delaware County, Oklahoma, by D. Fenolio

Enigmatic species such as cavefish are difficult to census or even to define in discrete population units. Here we establish a survey protocol and a population definition based upon habitat, as well as suggest critical habitat to be legally assigned. Historical population baselines are lacking for most vertebrate species, which impedes the creation of accurate restoration targets or management plans (McClenachan 2009); the judicial use of anecdotal information sources is often the only way to establish historical estimates of abundance (Pauly 1995). We analyzed ten years of new population survey data, which included both protocol survey counts as well as anecdotal counts by hydrologists and speleologists, in order to establish population baselines and a method for detecting population trends. Nonparametric rank correlation methods, used for decades in other disciplines such as hydrology (Hipel and McLeod 1994; USEPA 2006), are now preferred by biologists to test for a trend in census time series; such ranking procedures are more appropriate when exact estimates of population size are not known, when data gaps are present, when normality assumptions are not met, etc. (Thompson et al. 1998).

Historic distribution

The Ozark cavefish was apparently once fairly abundant and widespread in the phreatic habitats throughout the entirety of the Springfield Plateau, which encompasses northwestern Arkansas, northeastern Oklahoma, and southwestern Missouri (Fig. 2). Early publications on *Amblyopsis* (=*Typhlichthys, Troglichthys*) *rosae* indicated it was ubiquitous under southwestern Missouri wherever there was (human) surface access to underground waters (termed karst windows). USFWS (2003) described this cavefish's folklore status: "*When early settlers drew water from their wells, it was common to find Ozark cavefish swimming in their buckets. Believing this was a good luck charm, as well as a sign that the water was safe to drink, they called the fish 'spring keepers' or 'well keepers'*." In addition to the type locality—Sarcoxie Cave—R. Hoppin reported cavefish from at least 12 wells near Sarcoxie, Missouri (Garman 1889). Early diving explorations of Ozark springs also documented numerous cavefish sightings. For example, 13 sites were

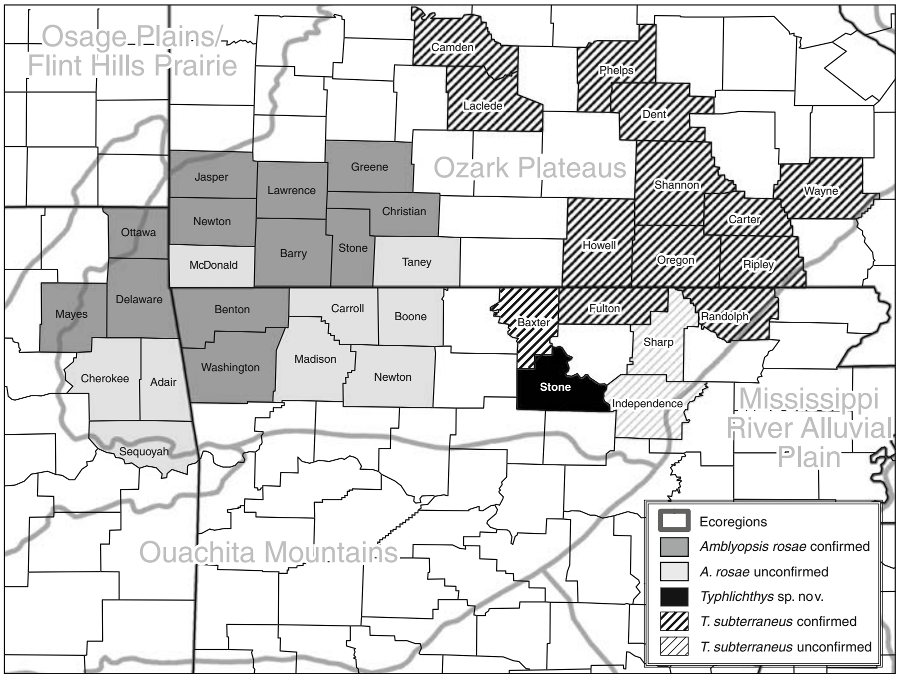


Fig. 2 County range map of *Amblyopsis rosae* and proximal confamilials in the tri-state region of Arkansas, Oklahoma, and Missouri, central USA. These cavefishes are confined to the Ozark Plateaus Ecoregion (ecoregions delineated by gray lines)

reported in Greene County, Missouri, by cave diver D. Rimbach during SCUBA surveys from 1966–1968 (Jones and Taber 1985). Heavy construction equipment used in major earthmoving projects has triggered ceiling collapse of shallow conduits (dolines) and facilitated the discovery of new cavefish occurrences. For instance, Ozark cavefish were uncovered during the construction of an Arkansas Game and Fish Commission (AGFC) fish nursery pond and during forest clearing on private property at Monte Né (both on Beaver Reservoir, Benton Co., Arkansas), and the construction of an earthen flood control dam on Whitewater Creek, Delaware Co., Oklahoma (Tafanelli and Russell 1972; Brown and Willis 1984).

By the late 1960s however, scientists began to document the increased exploitation of groundwater resources and the resulting degradation of stygobiont habitat. Especially prominent was the loss of karst windows. For example, during his unsuccessful search for historic locations of stygobiotic crayfish (*Cambarus* spp.) in wells of Jasper Co., Missouri, Marquart (1979) explains, "*Electric pumps have made open wells obsolete. Many of these locations have probably been sealed or possibly filled; none have been located*". Whereas the hand-dug wells of the 19th century functioned as karst windows, modern (post mid-20th century) wells, with their casings, caps, and shock chlorination, are designed specifically to exclude surface access to the phreatic zone.

In 1976, A. Brown and his research team (L. Willis and other students) initiated a project to update the distribution and status of the stygobiotic fishes and crayfishes of the Ozark Plateaus ecoregion. Through literature reviews, interviews, and museum searches, Brown et al. (1982) compiled a list of at least 52 Ozark cavefish sites, confirmed and unconfirmed, in the following tri-state region: Arkansas–Benton Co.; Missouri–Barry, Christian, Greene, Jasper, Lawrence, McDonald, Newton, Stone, Taney, and Webster Cos.; and Oklahoma–Delaware, Mayes, and Ottawa Cos. We add Washington Co. to this county checklist of the species' historic range because at least five sites were reported by reputable scientists (Buchanan 1974; Cloutman and Olmsted 1976; Aley and Aley 1979). Figure 2 delineates this historic range. Documented occurrences of Ozark cavefish are conspicuously absent from McDonald Co., although it is presumed to be part of the historic range (Brown et al. 1982). Noltie and Wicks (2001) reasoned that suitable habitat is lacking in this county because of its geologic setting; however, there are at least eight caves in McDonald Co. harboring stygobionts (including two sites for the Bristly Cave Crayfish, *Cambarus setosus*; Graening et al. 2006a); hence, at least some of this county contains suitable habitat. Ozark cavefish are rumored to occur at numerous localities at the periphery of its range in the tri-state area: Arkansas–Boone, Carroll, and Madison Cos.; and Oklahoma–Cherokee and Sequoyah Cos. (Fig. 2).

Brown et al. (1982) also surveyed 89 sites in ten counties in Missouri for Ozark cavefish in 1979, but found just 11 individuals at three caves in Jasper Co. and a single individual in Lawrence Co. The authors implicated over-collection and habitat destruction for this apparent range reduction; Brown petitioned the USFWS to designate this fish an endangered species in 1982, and two years later USFWS listed the species "threatened" under the Endangered Species Act of 1973, as amended. Survey efforts expanded to over 300 caves and springs in the tri-state area for cavefish in the 1980s (Brown and Willis 1984; Willis 1984; Willis and Brown 1985; Brown and Todd 1987). These surveys confirmed Ozark cavefish populations in only 16 caves: Arkansas—seven caves in Benton Co.; Missouri—three in Jasper Co., and one each in Greene, Lawrence, and Newton Cos.; and Oklahoma—three in Delaware Co. Willis and Brown (1985) concluded that its status was still "seriously threatened".

A resurvey of localities in Arkansas performed by Brown (1991) resulted in the confirmation of nine of 21 probable sites in Benton County. Renewed efforts during the 1990s in Missouri by MDC expanded the number of Missouri localities to 20 in the six county area–Barry, Greene, Jasper, Lawrence, Newton, and Stone (Pflieger 1997; Noltie and Wicks 2001). No new sites in Oklahoma were discovered during this period. Graening and Brown (2000b) reinitiated intensive survey efforts in 1999 in Arkansas and Oklahoma; the most interesting result was the confirmation of caver reports of new cavefish populations in Stone Co., Arkansas, which are being described as a new species of *Typhlichthys* by Graening et al. (in review). Renewed survey efforts in the last decade by K. Lister, D. Figg, W. Elliott, and J. Beard have discovered new Ozark cavefish populations in Missouri (Table 1). Recently (February 2009), a live amblyopsid was discovered by fisherman S. Teems near the shore in Norfork Lake—the first record of the fish in Baxter County, Arkansas (S. Todd, AGFC, unpub. data). Molecular phylogenetic analyses determined the specimen to be *T. subterraneus sensu lato* (M. Niemiller, unpub. data).

Historic abundance

Although historically widespread in the tri-state area, the Ozark cavefish has never been observed in large numbers. At the time of publication, Pflieger (1997) reported that Ozark cavefish were known from 12 sites in Missouri, but that a maximum of eight individuals were seen in any single survey. The Ozark cavefish has one of the lowest reported population densities of any stygobiotic fish, ranging from 0.005 to 0.15 individuals per square meter (see review by Trajano 2001). Published population size estimates for amblyopsids span two orders of magnitude, but 150 individuals is a common estimate. Poulson (1963) estimated an average population size of 76 for Ozark cavefish and 41 for its relative, the Southern cavefish (*Typhlichthys subterraneus*), based upon field observations. Later, Poulson (1985) estimated that typical *A. rosae* populations have 100 to 200 individuals. Willis and Brown (1985) estimated that an average deme of Ozark cavefish contains 150 individuals based upon field observations. Poulson (1960, p. 65–66) estimated the Cave Springs Cave population in Benton Co., Arkansas: "*the entire population probably numbered 150 before extensive collections were made (Tulane 10719, 16723, 11602, and 16561)*". Poulson (1960, 1963) reported a visual survey count range in the 1960s of 39–97.

In Logan Cave (also in Benton Co.), snorkel surveys consistently detected 10–30 cavefish (Brown and Todd 1987; Brown 1991), but later mark-recapture studies by Means (1993) and Brown (1996) estimated this entire population to be three times the detected size. Pflieger (1997) gave a "conservative" population estimate of 90 individuals of *T. subterraneus* in The Gulf, Wayne County, Missouri; Tryon (1971) estimated this same population at about 70 individuals.

Methods

Visual population surveys were performed by the method established by Poulson (1960), refined by Brown et al. (1982), and adopted by USFWS (1989) as follows: using bright dive lights in the water, two to three surveyors move side by side slowly and quietly upstream and tally cavefish as they are sighted. Snorkeling gear was necessary in deep water,

Table 1 Summary of all known locations and their reported occurrences of Ozark cavefish, with columns indicating: date of observation, survey, or collection; whether or not a complete visual survey of the habitat was performed; the number observed, counted, or collected; the number collected; and whether or not the site sustains a large bat population capable of depositing appreciable quantities of guano into the aquatic habitat

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
ARKANSAS						
Benton County						
AGFC Nursery Pond (Historic site)	20-Jan-1987	yes	1		no	J. Meinecke (A. Brown, unpub. data)
	early 1987	yes	14			R. Fourt (AGFC) unpub. data
	18-Jun-1987	yes	5	2		KU-21800[a]; Brown & Todd 1987
	14-Nov-1987	yes	0			S. Todd, unpub. data
	1990–1991	yes	0			Brown 1991
	13-Oct-1999	yes	?			Plugged from soil subsidence (Graening and Brown 2000a, b)
Bear Hollow Cave (Historic site)	circa 1984	no	1		no	1 amblyopsid scale found (Willis and Brown 1985)
	22-Oct-2001	yes	0			This study
	8-Feb-2006	yes	0			M. Slay, unpub. data
Cave Springs Cave (Recovery Cave)	before 1955	n/a	150		yes	Poulson 1963
	2-Sep-1955	no	31	30		TU-10719; USFWS 1989
	18-Nov-1955	no	27	27		TU-11602; USFWS 1989
	4-Oct-1957	no	55	55		TU-16561; USFWS 1989
	12-Oct-1957	no	16	16		TU-16723; USFWS 1989
	Jul-1958	no	50			Poulson 1960
	Aug-1959	no	6	6		Count & collection by Poulson (USFWS 1989)
	25-Oct-1959	no	16	16		TU-22675; USFWS 1989
	Aug-1960	yes	93	10		Count & collection by Poulson (USFWS 1989)
	Oct-1967	no	6	6		Count & collection by Poulson (USFWS 1989)
	Aug-1968	no	3	3		Count & collection by Poulson (USFWS 1989)
	Aug-1969	yes	78	5		Count & collection by Poulson (USFWS 1989)
	3-Mar-1983	yes	97			USFWS 1989
	26-Mar-1984	yes	100			USFWS 1989
	4-Mar-1986	yes	122			Brown and Todd 1987
	16-Nov-1990	yes	139			Brown 1991
	1995	yes	153			A. Brown, unpub. data
	25-Jan-1998	yes	106			Brown et al. 1998
	16-Dec-1998	yes	166			Graening et al. 2001
	11-Oct-1999	no	82			Graening et al. 2001
	16-Feb-2000	yes	102			Graening et al. 2001
	30-Nov-2000	yes	164			Graening et al. 2001

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	6-Mar-2002	no	60			This study
	7-Apr-2004	yes	155			M. Slay, unpub. data
	7-Feb-2006	yes	123			M. Slay, unpub. data
Civil War Cave (Current site)	late 1930s	no	30	30	no	Aley and Aley 1979; TU?
	1979	no	1			Aley and Aley 1979
	1980–1983	yes	4			Max. count during this period; Brown 1991
	14-Jan-1984	no	1			L. Willis, unpub. data
	24-Mar-1984	yes	2			A. Brown, unpub. data
	8-Oct-1986	yes	5			Brown 1991
	1990–1991	yes	2			Max. counted during this period; Brown 1991
	23-Nov-1999	yes	1			Graening and Brown 2000b
	29-Oct-2000	yes	1			Graening et al. 2001
	20-Sep-2005	no	1			Gillip 2007
	3-Nov-2006	no	1			Gillip 2007
Hewlitt's Spring Hole (Current site)	1979	yes	2		no	Max. count during this period; T. Aley, unpub. data
	26-Jun-1992	yes	2			Aley 1992
	18-Feb-01	yes	0			This study
James-Ditto (Wasson's Mud) Cave (Current site)	29-Jan-1987	yes	2		no	Brown and Todd 1987
	19-May-1987	no	1			A. Brown, unpub. data
	12-Nov-1990	yes	0			Brown 1991
	27-Aug-1999	yes	3			Graening and Brown 2000b
	6-Dec-2000	yes	0			Graening et al. 2001
	5-Jul-2002	yes	2			This study
Logan Cave (Recovery Cave)	1979	no	20		yes	Max. count during this year; Aley & Aley 1979
	1980–1983	yes	12			Max. count during this period; Brown and Todd 1987
	16-Jan-1986	no	25			A. Brown, unpub. data
	20-Feb-1986	yes	32			Brown and Todd 1987
	25-Feb-1987	yes	23			A. Brown, unpub. data
	7-Nov-1990	yes	14			Brown 1991
	10-Mar-1991	yes	18	4		Brown 1991; 4 collected by Means (1993)
	1992	yes	30			Max. count during this year; Means & Johnson 1995
	1993	yes	23			Max. count during this year; Means 1993
	1994	yes	29			Max. count during this year; Brown and Johnson 2001

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	1995	yes	22			Max. count during this year; Brown and Johnson 2001
	31-Dec-1999	yes	31			Graening et al. 2001
	21-Nov-2000	yes	36			Graening et al. 2001
	13-Feb-2002	yes	48			This study
	22-Jan-2003	yes	46			This study
	24-Mar-2004	yes	45			B. Wagner, unpub. data
	6-Feb-2006	yes	43			M. Slay, unpub. data
Monte Né Sinkhole (Historic site)	1-Oct-1990	yes	4		no	Brown 1991
	1991	yes	0			bulldozed shut by landowner (A. Brown, unpub. data)
Mule Hole Sink (Historic site)	4-Mar-1983	yes	4		no	Willis and Brown 1985
	21-Nov-1983	yes	0			A. Brown, unpub. data
	17-Jan-1985	yes	0			Brown and Todd 1987
	11-Jun-1986	yes	0			Brown and Todd 1987
	2-Feb-2001	yes	0			Plugged from soil subsidence (Graening et al. 2001)
Rootville Cave (Current site)	17-Jan-1986	yes	1		no	Brown and Todd 1987
	1990–1991	yes	0			Brown 1991
	1-Sep-1999	yes	0			Graening and Brown 2000b
	19-Apr-2000	yes	1			Graening and Brown 2000b
	10-Mar-2001	yes	1			Graening et al. 2001
	19-Aug-2001	yes	1			This study
Tom Allen's Cave # 1 & 2 (Current site)	12-Nov-1990	yes	4		no	Brown 1991
	2000	yes	9			This study
Washington Co.						
Brush Creek (Historic site)	before 1976	no	present		no	Cloutman and Olmsted 1976
Cave Spring (Historic Site)	1968	no	present		?	Aley and Aley 1979
Ozark Spring (Johnson's Fish Farm) (Historic site)	before 1984	no	present		no	Reported by T. Aley (Willis 1984)
Split Cave (Historic site)	before 1970	no	present		no	Aley and Aley 1979
MISSOURI						
Barry Co.						
Cave near Cassville (Historic site)	circa 1957	?	present		?	Woods and Inger 1957
Hankin's Well (Historic site)	Oct-1940	?	1	1	no	MO Natural Heritage Database; UMMZ-150420
	30-Nov-1995	yes	0			"No water seen", K. Lister, MO Natural Heritage Database
Johnson's Well (Historic site)	1930s	?	1	1	no	collection by landowner (Brown et al. 1982)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	circa 1991	yes	0			Brown et al. 1982
Moore Cave (Current site)	Jun-1938	?	1	1	?	collection reported (Mohr 1950)
	7-Apr-1992	yes	3			MO Natural Heritage Database
Christian Co.						
Atkinson Spring Cave #1 (Historic site)	before 1981	?	1		?	Reported by T. Aley (USFWS 1989)
	13-Aug-1981	yes	0			Brown et al. 1982
Baker Spring Cave (Historic site)	before 1982	no	present		?	Reported by D. Rimbach (Brown et al. 1982)
Fitzpatrick Cave (Current site)	13-June-2009	no	1		?	J. Beard, unpub. data
Swan Cave (Historic site)	before 1982	no	present		no	Reported by T. Aley (Brown et al. 1982)
Virgin Cave (Ed Smith Cave) (Historic site)	before 1982	no	present		no	Reported by T. Aley (Brown et al. 1982)
	13-May-1982	yes	0			Brown et al. 1982
Wilson Cave (Historic site)	before 1982	?	present		?	Reported by T. Aley (Brown et al. 1982)
Greene Co.						
Fantastic Caverns (Current site)	1968	no	present	1	no	D. Rimbach reported "several", at least one collected (Jones and Taber 1985)
	20-Feb-1982	yes	2			Willis 1984
	23-Feb-1987	yes	0			Brown and Todd 1987
	1989	?	1			Sigheted by D. Rimbach (MO Natural Heritage Database)
	15-Aug-1989	no	0			J. Beard, unpub. data
	Nov-1991	yes	0			MO Natural Heritage Database
	31-Jan-1994	?	1			Sighted by employee (MO Natural Heritage Database)
	14-Mar-1996	yes	0			MO Natural Heritage Database
Jackson Cave (Current site)	Oct-1992	yes	6		?	Survey by K. Lister (MO Natural Heritage Database)
	1993–1995	yes	0			Survey by K. Lister (MO Natural Heritage Database)
	Feb-1996	yes	0			Survey by K. Lister (MO Natural Heritage Database)
	17-Jul-1996	yes	1			Survey by K. Lister (MO Natural Heritage Database)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	29-Sep-1999	yes	1			Survey by K. Lister (MO Natural Heritage Database)
Minch Cave (Historic site)	before 1982	?	present	1	?	Collection reported (Brown et al. 1982)
Moore's Spring & Cave (Historic site)	26-Jun-1938	?	1	1	no	Woods and Inger 1957; UMMZ-156671
	15-Jun-1958	?	3			Poulson 1960
	1967	no	present			Reported by D. Rimbach (Brown et al. 1982)
	1980–1982	yes	0			Brown et al. 1982
Pfaff Cave (Historic site)	12-Aug-1971	?	1	1	?	Collected by W. Pflieger (Jones and Taber 1985)
	circa 1984	yes	0			Willis and Brown 1985
	1990	?	0			MO Natural Heritage Database
	Nov-1991	?	0			MO Natural Heritage Database
Raney Creek Cave (Historic site)	Mar-1958	?	1	1	?	Willis & Brown 1985; UMC 5063 (or 5693)
Road Cave Pit (Historic site)	before 1982	?	present		?	Reported by D. Rimbach (Brown et al. 1982)
Sam William's Spring (Historic site)	15-Jun-1952	?	1	1	?	Willis and Brown 1985; TU-7036
	1995	yes	0			MO Natural Heritage Database
	3-Dec-1996	yes	0			MO Natural Heritage Database
Sammon's Well (Historic site)	Aug-1968	?	1	1	no	collection by Poulson (Brown et al. 1982)
	circa 1982	?	0			Brown et al. 1982
Well on private land (Current site)	2006	no	1		no	MO Natural Heritage Database
Well on private land # 2 (Current site)	2007	no	1		no	MO Natural Heritage Database
Jasper Co.						
Adam's Well (Historic site)	circa 1889	?	1	1	no	Collection by R. Hoppin (Garman 1889)
Armstrong's Well (Historic site)	circa 1889	?	2	2	no	Collection by R. Hoppin (Garman 1889)
Cave Spring Cave (Current site)	25-Jan-1957	no	present		?	O. Hawksley reported "many seen" (Marquart 1979)
	9-Jul-1981	yes	0			Brown et al. 1982
	1989	yes	0			MO Natural Heritage Database
	1991	yes	0			Survey by K. Lister (MO Natural Heritage Database)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	1995	yes	1			Survey by K. Lister (MO Natural Heritage Database)
Coolbrook Spring Cave (Historic site)	before 1979	no	present		?	Marquart (1979) reported "Amblyopsid fish"
	circa 1981	yes	0			Brown et al. 1982
Kellhaufer's Cave (Recovery Cave)	9-Jul-1981	yes	4	?	?	Previous collections by landowner? (Brown et al. 1982)
	1986	?	4			Reported by landowner (MO Natural Heritage Database)
	14-Nov-1989	yes	6			Survey by D. Figg (MO Natural Heritage Database)
	1992	?	0			MO Natural Heritage Database
	Sep-1994	yes	5			K. Lister reported "5 fish seen over 4 visits" (MO Natural Heritage Database)
Sarcoxie (Downer's, Day's) Cave (Recovery Cave)	circa 1888	no	6	1	no	R. Hoppin reported "half a dozen or more", 1 collected (Garman 1889)
	1898	no	5	5		Romero and Conner 2007; BMNH 1898.30.31.19-23
	1935	no	2	2		Collection by B. Marshall (Romero and Conner 2007)
	1962	no	2	2		Collection by Taber (MO Natural Heritage Database); Pittsburg State Univ., Kansas, collection
	circa 1979	no	present			Marquart 1979
	9-Jul-1981	yes	3			Brown et al. 1982
	4-Feb-1984	yes	3			L. Willis, unpub. data
	21-Nov-1996	yes	1			Survey by K. Lister (MO Natural Heritage Database)
	1997	no	1			J. Beard, unpub. data
	29-Jan-2000	no	3			J. Beard, unpub. data
	10-Feb-2001	no	2			J. Beard, unpub. data
	8-Feb-2002	yes	5			This study
	13-Apr-2002	yes	5			J. Beard, unpub. data
	12-Apr-2003	no	2			J. Beard, unpub. data
	18-Nov-2004	yes	3			Survey by M. Slay et al. (MO Natural Heritage Database)
Wilson's Cave (Historic site)	circa 1889	no	9	9	?	Collections by R. Hoppin (Garman 1889); MCZ-27585, 27587

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	5-May-1940	?	5	5		Poulson 1960; UMMZ?
	3-Oct-1951	?	1	1		Poulson 1960; CNHM?
	5-May-1958	?	6			Poulson 1960
	16-Jan-1982	yes	4			Willis 1984
	4-Feb-1984	yes	2			L. Willis, unpub. data
	Mar-1984	yes	2–3			MO Natural Heritage Database
	Oct-1984	?	1			MO Natural Heritage Database
	1987	?	1			MO Natural Heritage Database
	14-Nov-1989	yes	5			Survey by D. Figg (MO Natural Heritage Database)
	10-Aug-1991	?	0			Vandike 1992b
	1992–1995	yes	0			MO Natural Heritage Database
	3-Dec-1996	yes	0			MO Natural Heritage Database
Unnamed well in Sarcoxie (Historic site)	circa 1889	no	3	3	no	Romero and Conner 2007; MCZ 27586
10 Unnamed wells (Historic sites)	circa 1909	no	present		no	Eigenmann 1909
Lawrence Co.						
Billies Creek Cave #1 (Current site)	1995	yes	1		?	Survey by K. Lister (MO Natural Heritage Database)
	1996	?	0			4 separate surveys (MO Natural Heritage Database)
	24-Nov-1999	yes	0			MO Natural Heritage Database
	4-Mar-2002	yes	0			MO Natural Heritage Database
Faye Valley Cave (Current site)	6-Sep-1996	?	0		?	MO Natural Heritage Database
	14-Oct-1996	?	2			T. Aley sighted (MO Natural Heritage Database)
	21-Jan-1998	yes	2			Survey by W. Elliott (MO Natural Heritage Database)
	24-Nov-1999	yes	0			MO Natural Heritage Database
	19-Apr-2000	no	2			J. Beard, unpub. data
	4-Mar-2002	yes	2			Survey by D. Novinger (MO Natural Heritage Database)
	7-Mar-2009	no	2			J. Beard, unpub. data
Johnson Spring Well (Current site)	before 1957	?	1		no	3 surveys by Woods & Inger (MO Natural Heritage Database)
	11-Oct-1994	?	0			MO Natural Heritage Database
	18-May-2006	yes	13			MDC protocol survey (Aley and Aley 2007)
	2007	no	4			Max. counted in 7 trips (Aley and Aley 2007)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
Turnback Cave (Current site)	29-Jul-1981	yes	1		yes	Brown et al. 1982
	13-Dec-1983	yes	1			A. Brown, unpub. data
	1992	?	present			MO Natural Heritage Database
	1995	yes	0			MO Natural Heritage Database
	28-Feb-1996	yes	0			MO Natural Heritage Database
	Nov-1996	yes	1			Crede and Skinner counted 1 (MO Natural Heritage Database)
	9-Nov-1997	yes	0			MO Natural Heritage Database
	1999	yes	0			MO Natural Heritage Database
	5-Jun-2000	yes	0			MO Natural Heritage Database
	4-Mar-2002	yes	0			Survey by D. Novinger, MO Natural Heritage Database
Newton Co.						
Ben Lassiter Cave (Recovery Cave)	Aug-1924	?	8	8	yes	USFWS 1989; UMMZ-64947
	Aug-1930	?	30–40			Poulson 1960
	Sep-1940	?	4	4		USFWS 1989; UMMZ-151466
	Jul-1958	no	6	3		Collection by Poulson (USFWS 1989)
	22-Aug-1958	?	11			Poulson 1960
	Aug-1959	yes	11	5		Collection by Poulson (USFWS 1989)
	Aug-1960	yes	7	5		Collection by Poulson (USFWS 1989)
	Oct-1967	yes	14	7		Collection by Poulson (USFWS 1989)
	Aug-1968	yes	5	3		Collection by Poulson (USFWS 1989)
	Aug-1969	yes	15	5		Collection by Poulson (USFWS 1989)
	1980–1983	yes	4			Max. count during this period (Willis and Brown 1985)
	16-Mar-1983	no	3			USFWS 1989
	Sep-1983	yes	6			Willis and Brown 1985
	16-Sep-1983	?	3			L. Willis, unpub. data
	Feb-1984	no	0			USFWS 1989
	26-Jun-1987	yes	2			Brown and Todd 1987
	19-Aug-1991	?	3			Vandike 1992b
	1992	?	present			MO Natural Heritage Database
	1994	yes	4			Survey by K. Lister (MO Natural Heritage Database)
	30-Mar-1995	yes	26			Survey by K. Lister (MO Natural Heritage Database)
	18-Dec-1996	yes	0			Survey by K. Lister (MO Natural Heritage Database)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	23-Aug-1999	yes	2			Survey by K. Lister (MO Natural Heritage Database)
	19-Oct-1999	yes	5			Survey by Skinner et al. (MO Natural Heritage Database)
	5-Mar-2002	yes	0			Survey by D. Novinger (MO Natural Heritage Database)
Blinzer's Well Cave (Historic site)	before 1982	?	present		?	Reported by D. Rimbach (Brown et al. 1982)
	circa 1982	yes	0			Brown et al. 1982
Capps Creek Wells (Historic site)	5-Mar-2002	?	1		no	Reported by D. Novinger (MO Natural Heritage Database)
	19-Nov-2004	yes	2			Survey by W. Elliott & M. Slay (MO Natural Heritage Database)
Hearrell Spring (Current site)	10-Jan-1990	?	8		no	Survey by Hines (MO Natural Heritage Database)
	Apr, Jun-1992	?	1			Surveys by Ziehmer, D. Noltie (MO Natural Heritage Database)
	1993–1996	yes	1–5			count range during this period (MO Natural Heritage Database)
	25-Apr-1996	yes	10			Survey by Hendrix (MO Natural Heritage Database)
	5-Mar-2002	?	0			MO Natural Heritage Database
Cave on private land (Current site)	2007	?	2	?	?	MO Natural Heritage Database
Spring in Newtonia	circa 1909	no	present		?	Eigenmann 1909
Whispering Springs (Historic site)	before 1982	no	present	?	?	Supposed collection (Brown et al. 1982); UMMZ?
	18-Jan-82	yes	0			Brown et al. 1982
Stone						
Cave (Elsey) Spring (Current site)	1956	no	present		?	MO Natural Heritage Database
	1957	no	present			Woods and Inger 1957
	4-Oct-1958	no	1	1		Poulson 1960
	1989	yes	0			MO Natural Heritage Database
	23-May-1995	yes	1			Survey by K. Lister (MO Natural Heritage Database)
	Aug-1999	yes	0			Survey by K. Lister (MO Natural Heritage Database)

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
Galena Spring (Historic site)	before 1982	?	present		no	Reported by D. Rimbach (Brown et al. 1982)
	circa 1982	yes	0			Brown et al. 1982
Gentry Cave (Historic site)	before 1982	?	present		?	Reported by D. Rimbach (Brown et al. 1982)
	12-Aug-1981	yes	0			Brown et al. 1982
Hayes Spring Cave (Current site)	1-Nov-1989	yes	4		?	Survey by K. Lister (MO Natural Heritage Database)
	8-Dec-1994	yes	1			Survey by K. Lister (MO Natural Heritage Database)
Indian Creek Caverns (Historic site)	before 1982	?	present		?	Reported by T. Aley (Brown et al. 1982)
	12-May-82	yes	0			Brown et al. 1982
Reed's Spring (Historic site)	before 1982	?	present		no	Reported by D. Rimbach (Brown et al. 1982)
	circa 1982	yes	0			Brown et al. 1982
Unnamed cave (Historic site)	before 1982	?	present		?	Reported by D. Rimbach (Brown et al. 1982)
	circa 1982	yes	0			Brown et al. 1982
OKLAHOMA						
Delaware Co.						
January-Stansbury Cave (Historic site)	1960s	?	present		no	Reports by Looney Family and D. Russell (USFWS, unpub. data)
	2000–2006	yes	0			This study
Engelbrecht Cave (Current site)	1980–1983	yes	1		no	Max. counted during this period; Brown and Todd 1987
	8-Jul-1983	yes	2			W. Puckette, unpub. data
	1985–1987	yes	0			Brown and Todd 1987
	1987	?	1			USFWS, unpub. data
	Jan-1990	yes	present			Mehlhop-Cifelli 1990
	8-Dec-2003	yes	0			This study
	15-Dec-2004	yes	0			This study
	8-Aug-2005	yes	0			This study
	2006	yes	0			This study
	29-Sep-2008	yes	0			This study
Wilkerson Swim Hole Cave (Inglebrook Spring) (Historic site)	2-Aug-83	yes	0		no	Willis and Brown 1985
	1985	yes	1			USFWS, unpub. data
	8-Aug-2005	yes	0			This study
Jail Cave (Current site)	24-Sep-1967	?	1	1	no	Looney 1969; OSUS-7106
	circa 1971	no	present			Black 1971
	25-Dec-1978	yes	2			L. Willis, unpub. data
	Mar-1983	yes	1			L. Willis, unpub. data

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	19-Nov-1983	yes	3			Brown and Willis 1984
	22-Nov-1983	yes	2			L. Willis, unpub. data
	1984	yes	3			OK Natural Heritage Database
	26-Jan-1984	yes	1			Willis and Brown 1985
	19-Apr-1986	?	3			Puckette 1986
	10-Jul-1986	yes	3			S. Todd, unpub. data
	6-Nov-1989	?	1			Sighted by T. Aley (OK Natural Heritage Database)
	1990	?	1			Sighted by T. Aley (OK Natural Heritage Database)
	1991	?	2			Sighted by T. Aley (OK Natural Heritage Database)
	1991	?	1			Aley and Aley 1991
	6-Dec-2003	yes	2			This study
	21-Feb-2007	yes	0			This study
	2-Oct-2008	yes	1			This study
Long's Cave (Current site)	1990	no	6		no	B. & B. Howard, unpub. data
	27-Sep-1991	yes	1			Vaughn & Certain 1992b
	1999	yes	19			B. & B. Howard, unpub. data
	18-Mar-2001	no	12			Bergey et al. 2003
	31-Aug-2001	yes	12			Graening et al. 2006b
	26-Jul-2005	no	1			This study
	5-Dec-2007	yes	7			Survey by R. Stark, S. Wallace, S. Hensley (USFWS, unpub. data)
McGee Cave (Current site)	1990	yes	6		no	B. & B. Howard, unpub. data
	27-Sep-2001	yes	2			Vaughn and Certain 1992
	18-Mar-2001	yes	0			Bergey et al. 2003
	31-Aug-2001	yes	1			Graening et al. 2006b
	21-Feb-2007	yes	0			This study
	2-Oct-2008	yes	3			This study
Mitchell's Caves (Historic site)	circa 1970	?	1	1	no	Collection by J. Black (Mehlhop-Cifelli 1990)
	1980–1983	yes	0			Brown and Todd 1987
	circa 1983	yes	2			Looney 1984
	1985–1987	yes	0			Brown and Todd 1987
	23-Oct-1989	yes	0			OK Natural Heritage Database
	26-Oct-1991	yes	0			OK Natural Heritage Database
	30-Nov-2001	yes	0			This study
	21-Feb-2007	yes	0			This study

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
Star Cave (Current site)	circa 1971	no	present		no	Black 1971
	1-Sep-1971	?	5	5		OSUS-7271
	4-Apr-1992	?	1			Sighted by W. Puckette (OK Natural Heritage Database)
	3-May-2004	yes	0			This study
	1-Aug-2005	yes	0			This study
	Jun, Aug-2006	yes	0			Surveys by R. Stark, S. Hensley (USFWS, unpub. data)
Twin Cave (Recovery Cave)	29-Nov-1970	yes	22		yes	Black 1971
	3-Oct-1971	?	6			Puckette 1986
	7-Nov-1971	?	1	1		OSUS-23868
	1972	?	4			Puckette 1986
	7-Mar-1981	?	4			Puckette 1986
	13-Aug-1982	?	1			Puckette 1986
	22-Nov-1983	yes	3			Brown and Todd 1987
	1980–1983	yes	5			Max. count during this period (Willis and Brown 1985)
	26-Jan-1984	yes	2			L. Willis, unpub. data
	1985–1987	yes	3			Max. count during this period (Brown and Todd 1987)
	30-Apr-1986	?	2			Puckette 1986
	7-Feb-1987	?	3			W. Puckette, unpub. data
	1988	?	1			OK Natural Heritage Database
	26-Oct-1990	?	4			Survey by N. Jones, B. Hamilton (OK Natural Heritage Database)
	12-Feb-1991	?	3			W. Puckette, unpub. data
	7-Jun-1991	no	2			Aley and Aley 1991
	1-Feb-2000	no	1			This study
	5-Mar-2001	yes	4			This study
Whitewater Creek Flood Control Dam (Historic site)	3-Oct-1971	yes	20	12	no	Tafanelli and Russell 1972; OAM-7271
	circa 1984	yes	0			Site no longer exists (Willis 1984)
Mayes Co.						
Site near Disney (Historic site)	circa 1972	?	1	1	?	Reported by H. Robison (Tafanelli and Russell 1972)
Ottawa Co.						
Cave Springs Ranch Cave (Current site)	24-Apr-1954	?	1	1	no	Collection by T. Denesha (Hall 1956); KU-3210
	1966	?	1	1		Collection by R. Nolan (Mayden and Cross 1983); KU-14007

Table 1 (continued)

Site name	Survey date	Complete survey?	No. reported	No. collected	Has bat pop. ?	Data source; museum catalog number given where known[a]
	1967	no	present			Branson 1967
	1969	?	1			Looney 1969
	5-Aug-1969	?	2	1		Tafanelli and Russell 1972; OSUS- 7105
	17-Mar-1983	yes	0			L. Willis, unpub. data
	14-Dec-2004	yes	0			This study
	31-Dec-2004	yes	2			Survey by S. Wallace et al. (USFWS, unpub. data)
	1-Aug-2005	no	1			Survey by R. Stark, S. Hensley (USFWS, unpub. data)

[a] Museum abbreviations: Tulane Museum of Natural History, Louisiana (TU); University of Michigan Museum of Zoology, Ann Arbor (UMMZ); Kansas University, Lawrence (KU); British Museum of Natural History, London (BMNH); Harvard University Museum of Comparative Zoology (MCZ); Oklahoma State University Museum of Natural History (OSUS)

but even in shallow water snorkeling improves detection of fish (Brown and Todd 1987; Trajano 2001). Standardization reduces variation in population indices (Greenwood 1996), and these surveys were standardized by using the same method as those previous and included at least one of the surveyors used in a previous survey. Although some scientists such as Means and Johnson (1995) discount the visual survey method as too inaccurate, this technique is the primary method used worldwide to determine population sizes of cavefish (Trajano 2001). Underwater and bankside observations of fish are a common survey method and can approach the accuracy of more inclusive techniques, such as electrofishing or rotenone collections (Hankin and Reeves 1988; Perrow et al. 1996). However, the visual survey method is biased towards the recording of conspicuous individuals and may overlook inconspicuous ones (Perrow et al. 1996). Schubert et al. (1993) and Brown (1996) predict that underestimation will be common in visual population estimates due to the tendency of Ozark cavefish to hide in gravel interstices. During surveys, cavefish were visually categorized into broad size classes as a surrogate for age classes: 1) young of year to juvenile–less than 2.5 cm total length; 2) juvenile to adult–between 2.5 and 5 cm 3) adult–greater than 5 cm. The location of each fish sighted was recorded on the cave map on a waterproof tablet.

The bulk of survey data derives from 25 years of direct observation by the authors and colleagues. We compiled all other known survey and locale data, primarily from the following sources: field reports by L. Willis and S. Todd; the Natural Heritage Program databases maintained by the Arkansas Natural Heritage Commission, the Oklahoma Biological Survey (University of Oklahoma at Norman), and the Missouri Department of Conservation (MDC); W. Elliott's Missouri Cave Life Database (MDC); museum collections identified by Poulson (1960) and Romero and Conner (2007); field reports by the Arkansas Game and Fish Commission's Non-game Program, Tulsa Regional Oklahoma Grotto (National Speleological Society), the USFWS Oklahoma Ecological Services Office, and The Nature Conservancy (TNC) Oklahoma Field Office.

Incomplete survey counts, casual numeric observations, and collection events were used in lieu of complete surveys where the observation was made by a qualified professional familiar with the differences between stygobiotic fishes and epigean fishes and larval salamanders. This included SCUBA divers (e.g., D. Rimbach, S. Wallace), speleological societies (e.g. J. Beard of the Missouri Cave and Karst Conservancy), and hydrologists (e.g., T. Aley, V. Brahana) that have been trained by biospeleologists (such as the authors).

Because of the inaccessibility of subterranean habitats, population censuses for this fish are impossible. However, the authors and colleagues have consistently employed the visual observation method described previously; the result is a data set of population indices spanning three decades that can be analyzed for increasing or decreasing trends. Anectodal data were used cautiously, and trend analyses were run with and without these data to determine the significance of inclusion of this data type. Summary statistics of cavefish counts were performed using SPSS statistical software (version 15.0, SPSS, Inc.). Consistent with bat survey data analyses (e.g. Ellison et al. 2003; Graening et al. in review), a time series of counts required at least four annual counts (but not necessarily spanning consecutive years). Where a count was reported as a range, the mean of the lower and upper bound was used. Note that none of the counts included estimates of sampling-based variation, and most lacked replicate counting (both are suggested for future counts, where possible). The MAKESENS software created by Salmi et al. (2002) was used to perform the nonparametric Mann-Kendall Test (Kendall 1938; Mann 1945) and Sen's Slope Estimator Method (Sen 1968a,b), which test for a monotonic trend in the time series and the magnitude of the trend, respectively, at significance level α=0.05. For time series with less than 10 observations, the S statistic was calculated, and for larger time series (n =/> 10), a normal approximation to the Mann-Kendall procedure is used with the Z statistic. The Mann-Kendall Test involves computing the Z (or S) statistic, which is the difference between the number of pairwise differences that are positive and the number negative (USEPA 2006). If Z is a large positive value and the probability value is less than 0.05, then there is evidence of an increasing trend in the data, and vice versa. The null hypothesis or baseline condition is that there is no temporal trend in the time series (USEPA 2006). Following Ellison et al. (2003), the coefficient of variation (CV, ratio of a parameter's standard deviation estimate to its mean, expressed as a percent) was used to further evaluate potential trends because it is a dimensionless measure of precision and can compare data sets of unequal variance (Thompson et al. 1998). Where a time series did not have a significant upward or downward trend, we followed Ellison et al. (2003)'s use of an arbitrary CV cutoff point of 50% to interpret its meaning. Where the CV was less than 50%, the count was inferred to stabilize around a mean value; where a time series had a larger CV, we inferred that the counts were too variable to interpret any trend (until a later date when the time series dataset was large enough to have a reduced CV). Note that trend analyses results were similar using linear regression of time series (α=0.05).

Geographic information system software (ArcGIS 9.3, Environmental Systems Research Institute, Inc.) was used to explore potential zoogeographical patterns. Each site was also assigned to a watershed (scale of 1:250 000) using the US Geological Survey hydrography dataset, National Map Program.

Results

Current distribution

Our data compilation produced a total of 83 confirmed sites, plus at least 37 unconfirmed sites (Table 1). We assigned most of these confirmed localities as historic where no sightings of Ozark cavefish have occurred within the past twenty years. Ozark cavefish have current occurrences at 32 sites in the tri-state area.

In agreement with past studies (Poulson 1963; Noltie and Wicks 2001), the Ozark cavefish still appears to be distributed within the confines of the Springfield Plateau physiographic province. Suitable habitat is specifically defined as the phreatic zones of karstified carbonate bedrocks of the Mississippian Period (the Boone, Keokuk, Burlington, and Pierson Formations), collectively termed the Springfield Plateau Aquifer (or Boone-St. Joe aquifer; Fig. 3). Within this region, suitable habitat is absent only where the carbonate bedrocks are too thin to sustain permanent groundwater or are lacking entirely and underlying shales exposed, such as in much of McDonald Co., Missouri (see review by Noltie and Wicks 2001).

Beginning with Swofford (1982), phylogenetic studies involving Ozark cavefish have revealed considerable genetic divergence across its range. Bergstrom et al. (1995) and Bergstrom (1997) concluded that subspecific separation was warranted, partitioned by watershed: 1) Illinois River drainage in northwestern Arkansas, 2) White River drainage in southwestern Missouri, 3) Neosho River drainage in southwestern Missouri, and 4) Neosho River drainage in northeastern Oklahoma. Recent molecular studies by Niemiller and Fitzpatrick (2008) and T. Near et al. (unpub. data) cast doubt even on the interspecific relationships of this species with other amblyopsids.

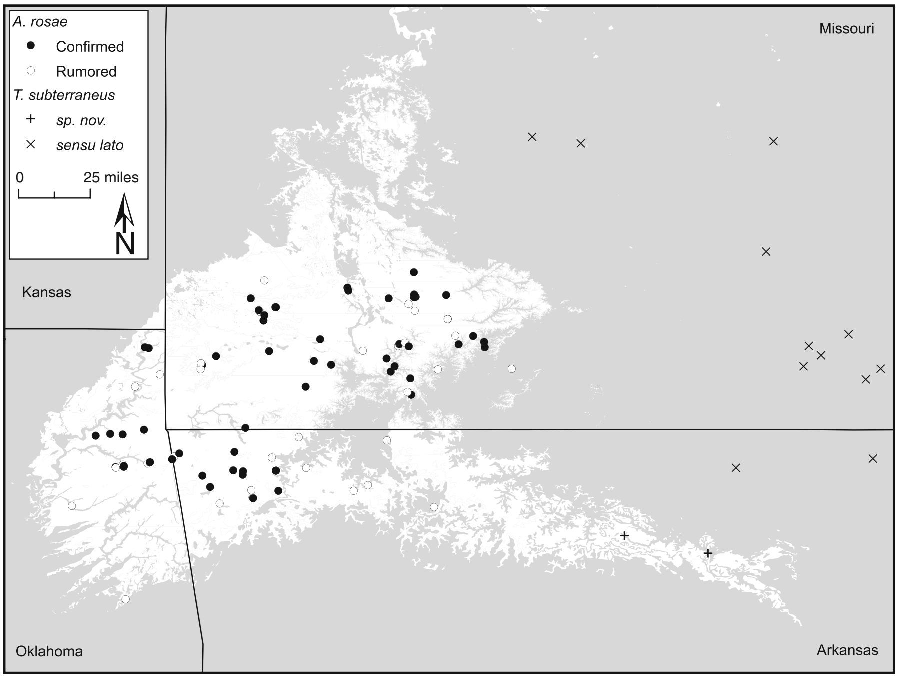


Fig. 3 Distribution of confirmed (black circle) and unconfirmed *A. rosae sensu lato* sites (white circle) and confirmed *Typhlichthys* spp. sites ("+" is *T.* sp. nov. and "x" is *T. subterraneus sensu lato*) in relation to the surface expression of the Mississippian Period carbonate bedrocks of the Springfield Plateau in the tri-state region. This geographic analysis confirms previously published conclusions that *A. rosae* is confined to this physiographic province

Current abundance

Our compilation of all known data sources resulted in a total of 419 occurrence records for Ozark cavefish (Table 1). The summation of the most recent survey in every current site is only 222 individuals. To estimate the potential for this species to achieve its maximum population size in its known habitat, we summed the highest recorded count in each site, ignoring any biases resulting from differences in survey method, season, or calendar date. The summation of each of these maxima is 515 individuals in a total of 82 localities—a crude indication of this fish's population size potential in its visible habitat.

We also compared historic maximum counts to recent maximum counts at each site using the year 1990 as the breakpoint, which was the year that intensive survey efforts by Brown and colleagues ended. Where cavefish were reported at a site without a numeric count, a count of one cavefish was assigned to that site. Before 1990 there were a maximum of 402 cavefish counted in 70 sites; after 1990 there were a maximum of 353 cavefish counted in 34 sites. Only 12 sites had higher survey counts after 1990, but the discovery of new, and relatively large, populations after 1990 has offset this apparent decline (Fig. 4). There is no significant difference between tallies of maximum counts in surveys before and after 1990, according to a paired Student *t*-test (two-tailed, $n=83$, $t=0.826$, $P=0.411$).

Population trend and structure in Recovery Caves

Trend analyses were conducted on survey data from populations of Ozark cavefish where a sufficiently long time series existed. The number of cavefish surveyed in Cave Springs Cave (Benton Co., AR) is significantly increasing since T. Poulson began the survey effort in 1960 (Fig. 5). Protocol surveys ranged from 93 to 166, with relatively small variance ($\bar{x}=123$, SD=29.7, CV=24%). The Mann-Kendall Test indicated a significant upward trend ($n=13$, $Z=2.62$, $P=0.007$), while Sen's Slope Estimator Method indicated a rate of increase of approximately two cavefish per year ($Q=1.62$, $B=14.83$). This linear increase is significant even if anecdotal sources are included—Poulson (1960)'s estimate of 150 cavefish prior to intensive collections in the mid-1960s and his post-collection estimate of 50 cavefish in 1958 (Mann-Kendall Test/Sen's Method, $n=15$, $Z=2.47$, $P=0.012$, $Q=1.41$, $B=29.00$). This linear increase is not significant if only our surveys (1983–2006) are used (Mann-Kendall Test/Sen's Method, $n=11$, $\bar{x}=130$, $Z=1.71$, $P=0.07$, $Q=2.13$, $B=-23.50$). Because count variance is low and the CV is less than 50% (SD=26.7, CV=21%), we infer that the count is

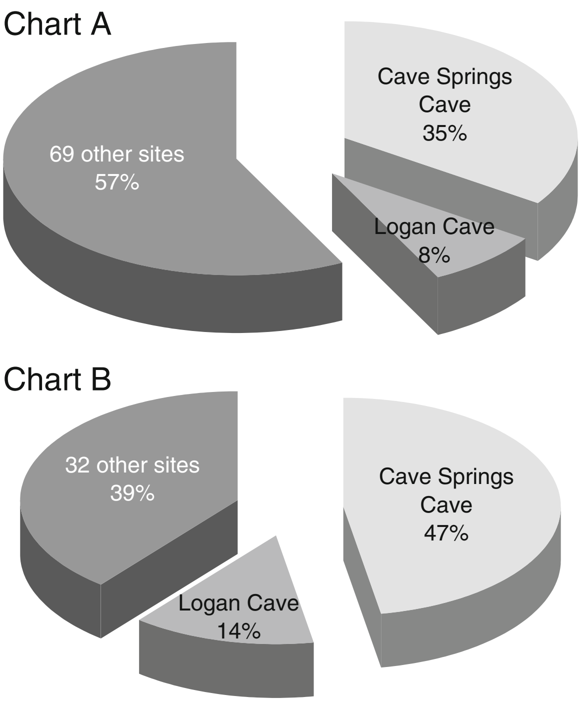


Fig. 4 Pie charts showing the relative proportion of surveyed Ozark cavefish in the two largest caves compared to all other sites, where chart A is the historical (pre-1990) maximum counts in each of 71 historically active sites, with 403 total cavefish counted, including 139 in Cave Springs Cave and 32 in Logan Cave; and chart B is the more recent maximum counts in 34 post-1990 active sites, with 353 total cavefish counted, including 166 in Cave Springs Cave and 48 in Logan Cave. There is no statistical difference in these cavefish tallies before and after 1990

stabilizing around the mean value of 130 fish (i.e., the count is not increasing nor decreasing temporally).

The population structure of Ozark cavefish was described as skewed toward older (and thus larger) individuals by Poulson (1960), who stated, "*The first year class is small or lacking, even in the most favorable habitats (Cave Springs Cave).*" This is typical for a *K*-selected life history strategy (MacArthur and Wilson 1967). However, we document a shift in population structure that is now skewed towards younger (measured as smaller) individuals, and we attribute this shift to the cessation of historic harvest of breeding adults from this locality (Fig. 6).

The number of cavefish surveyed in Logan Cave (Benton Co., AR) is significantly increasing since protocol surveys were started in 1983 by Willis and Brown (1985): Mann-Kendall Test/Sen's Method, $n=11$, $\bar{x}=32$, SD=13.26, CV=42%, $Z=2.34$, $P=0.016$, $Q=1.44$, $B=-78.56$ (Fig. 7). If we include anecdotal data back to 1979 (Aley and Aley 1979; Means and Johnson 1995; Brown 1996), the result is the same—a slow rate of increase in counts of approximately one cavefish per year: Mann-Kendall Test/Sen's Method, $n=16$, CV=39%, $Z=3.06$, $P=0.002$, $Q=1.27$, $B=-65.45$.

The population structure (Fig. 8) in Logan Cave appears normally distributed, and few collections have been made at this site; however, no historical population structure data exists for this site. Note that Means and Johnson (1995) and Brown (1996) tagged at least 124 apparently unique adult cavefish in Logan Cave, and their Jolly-Seber population estimate was 93 individuals (Brown 1996).

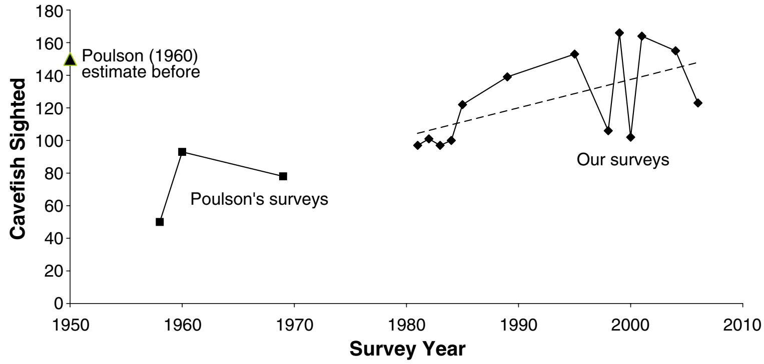


Fig. 5 Population surveys of Ozark cavefish in Cave Springs Cave, Benton Co., Arkansas. Surveys before federal listing were performed by Poulson (1960, 1963) who documented a population decline attributed to specimen collection, and more recent surveys performed by the authors who documented a significant increasing trend, or at least a stabilizing trend, in this population index

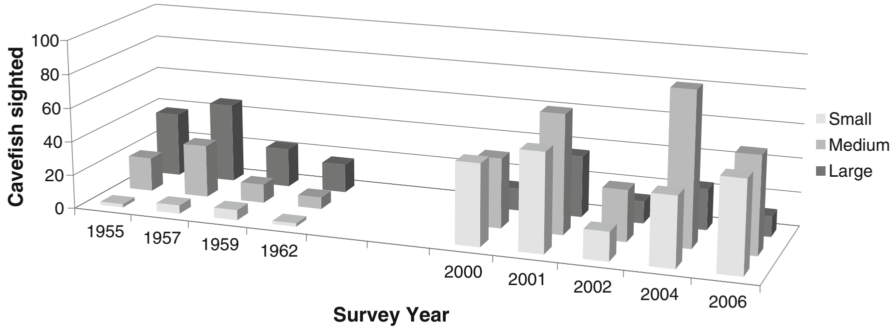


Fig. 6 Population structure of Ozark Cavefish in Cave Springs Cave, Benton Co., Arkansas, before and after federal listing (Poulson 1963; this study), with size classes of small (<2.5 cm), medium, and large (>5 cm). Population structure and size have apparently changed over time: the most likely reasons are the cessation of collection of adults and the successful recruitment of young-of-year

Fig. 7 Summary of all known visual population surveys of Ozark cavefish in Logan Cave, Benton Co., Arkansas (Brown and Todd 1987; Means and Johnson 1995; Brown and Johnson 2001; this study); statistical analyses indicates significantly increasing trend in survey counts

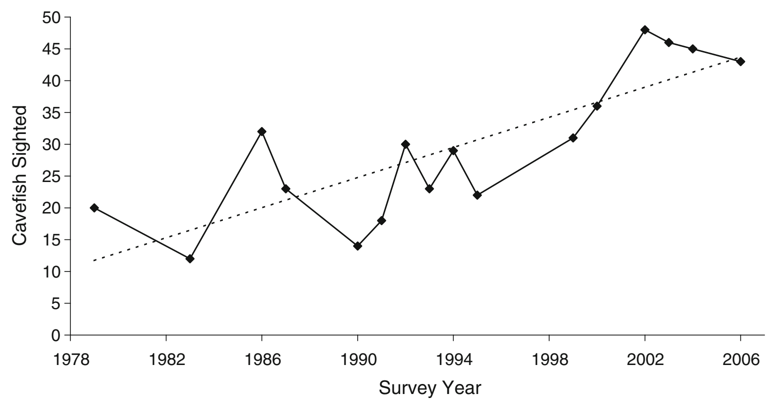
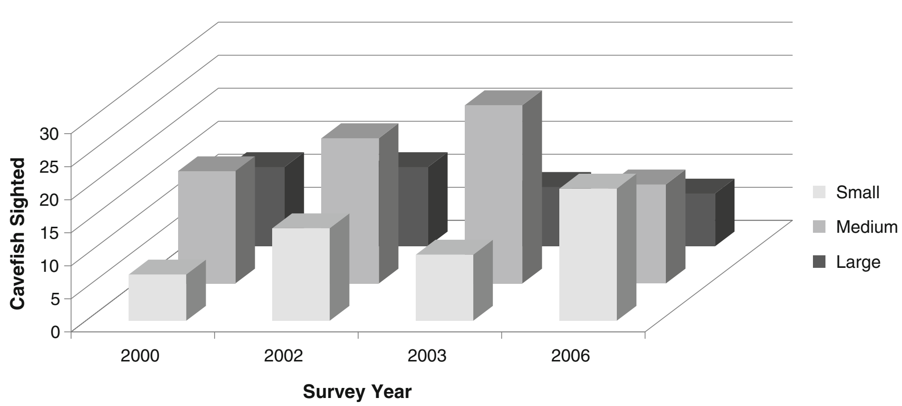


Fig. 8 Population structure of Ozark cavefish in Logan Cave, Benton Co., Arkansas (this study)



Trend analyses of the protocol surveys from 1959 to 2002 of Ben Lassiter Cave (Newton Co., MO) could not detect a significant trend: Mann-Kendall Test/Sen's Method, $n=11$, $Z=-1.33$, $P>0.1$, $Q=-0.16$, $B=15.95$ (Fig. 9). This is probably due to high variance in counts ($\bar{x}=9$, SD=7.43, CV=86%); the inclusion of collection data and anectodal counts (e.g. Vandike 1992b) that span 1924–2002 do not improve the trend or lower the variance: Mann-Kendall Test/Sen's Method, $n=16$, $\bar{x}=9$, SD=9.30, CV=99%, $Z=-1.54$, $P<0.1$, $Q=-0.08$, $B=10.11$.

Trend analysis of counts in Sarcoxie Cave (Jasper Co., MO) indicated a small but persistent population, which has not achieved its former survey size since scientific collection (Fig. 10). Using only recovery period surveys (1981–2004), no trend was detected: Mann-Kendall Test/Sen's Method, $n=5$, $\bar{x}=3$, SD=1.41, CV=47%, $S=0.28$, $P>0.1$, $Q=0.056$, $B=-2.00$. Even if numbers of collected specimens are treated as survey counts and anecdotal counts by J. Beard are used, no strong trend emergences: Mann-Kendall Test/Sen's Method, $n=12$, $Z=-0.07$, $P>0.1$, $Q=0.00$, $B=2.50$.

The population of Long's Cave (Delaware Co., OK) has not been surveyed for a long enough period of time (1990–2007) to detect a trend, and counts have ranged from one to 19 cavefish: Mann-Kendall Test/Sen's Method, $n=5$, $\bar{x}=9.0$, SD=6.82, CV=76%, $S=2$, $P>0.1$).

No trend was detected in protocol surveys from 1983 to 2001 for the small population at Twin Cave (Delaware Co., OK): Mann-Kendall Test/Sen's Method, $n=4$, $\bar{x}=4$, SD=1.3, CV=37%, $S=0$, $P>0.1$, $Q=0.008$, $B=2.91$ (Fig. 11). If anecdotal counts from 1970 are included (Black 1971; Puckette 1986; OK Natural Heritage Database) there is still no trend and variance is increased: Mann-Kendall Test, $n=13$, $\bar{x}=5$, SD=5.5, CV=118%, $Z=-1.88$, $P=0.06$.

Trend analysis of the few protocol surveys that have been performed at Jail Cave (Delaware Co., OK) revealed no trend and high variance: Mann-Kendall Test/Sen's Method, $n=4$, $\bar{x}=2$, SD=1.29, CV=86%, $P=0.17$, S=−4.0, $Q=-0.10$, $B=9.74$). The inclusion of anecdotal data since 1967 (Looney 1969; Black 1971; Puckette 1986; OK Natural Heritage database) did not create a significant trend, but did lower the variance: Mann-Kendall Test/Sen's Method, $n=11$, $\bar{x}=2$, SD=1.01, CV=59%, $Z=-1.15$, $P=0.17$, $Q=-0.03$, $B=3.93$.

Discussion

Evaluation of historic and current threats

Globally, major threats to stygobiotic fishes have been grouped into five categories: habitat degradation, hydrological manipulations, environmental pollution, overexploitation, and impacts of introduced aquatic animals (Proudlove 2006). More specifically, USFWS (1989) lists the factors for decline of Ozark cavefish as overcollection, habitat destruction, disturbance by cavers, and "lack of reproduction". In the following discussion, we analyze these factors in light of our current understanding of the species and 30 years of conservation efforts.

Scientific and amateur collection during the late 19th and early 20th centuries has severely impacted the stygobiotic amblyopsids (Poulson 1960; Culver 1986; Romero 1998a, b; Elliott 2000). The primary reason that USFWS listed Ozark cavefish as federally threatened was overcollection, and critical habitat was not designated for fear of advertising the location of remaining habitat to collectors (USFWS 1984a, b). At least 313 Ozark cavefish are catalogued in museum collections (Table 1), a number that exceeds any

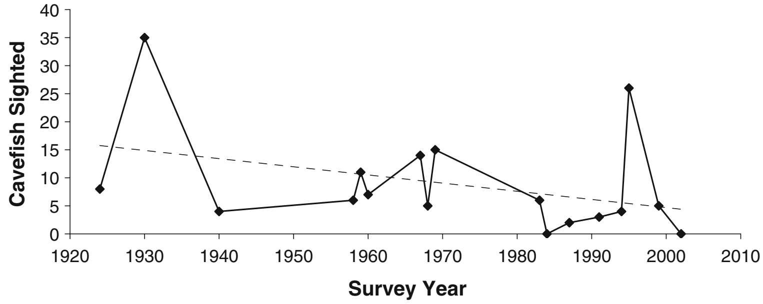


Fig. 9 All known visual surveys of Ozark cavefish in Ben Lassiter Cave, Newton Co., Missouri (Willis and Brown 1985; USFWS 1989; Missouri Natural Heritage Database data, this study). Statistical analyses revealed no trend in the surveys

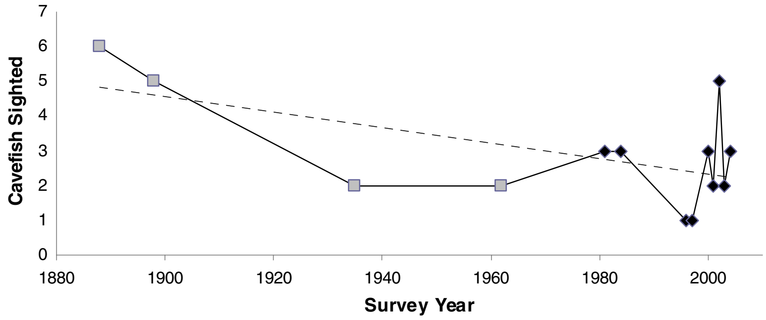


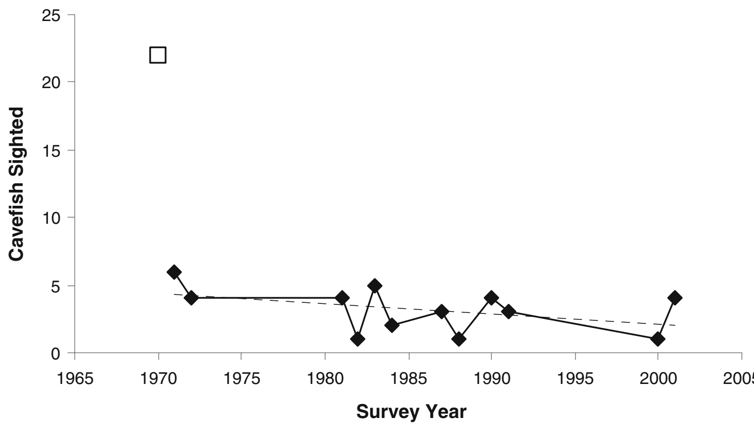
Fig. 10 All known collection events (gray squares) and visual surveys (black diamonds) of Ozark cavefish in Sarcoxie Cave, Jasper Co., Missouri (Garman 1889; Marquart 1979; Brown et al. 1982; Missouri Natural Heritage Database data; this study). Statistical analyses revealed no trend in the surveys regardless of whether collection events are equated to survey events

published total population estimate for the species. We cannot estimate the additional numbers that were taken by other biologists, cave tourists, or aquarium enthusiasts.

In the most critical of all habitats—Cave Springs Cave—at least 174 cavefish have been collected (Table 1). Poulson (1963) estimated the historic population in Cave Springs Cave to be about 150 before R. Suttkus and colleagues collected 144 cavefish in the 1950s for the Tulane Museum of Natural History (Belle Chasse, Louisiana). Poulson (1960) counted only 72 in 1960, and it has taken this population 40 years to recover to its previous abundance (Graening and Brown 2000a; this study). In the second largest historic population—Ben Lassiter Cave—at least 40 individuals were taken from the 1920s to 1950s. Yet the maximum historic count in Ben Lassiter Cave was only 26, and the most recent survey in 2002 detected only five individuals. Thirty cavefish were collected in Civil War Cave (Benton Co., Arkansas) in one collection event in the 1930s and deposited at Tulane University (Aley and Aley 1979), but surveys since then have never reported more than two individuals. At the type locality (Sarcoxie Cave), at least ten specimens have been collected, yet surveys since 1960s have detected no more than five cavefish. At least 14 cavefish were collected from Wilson's Cave (Jasper Co., Missouri) in the first half of the 20th century (Garman 1889; Poulson 1960); since then, surveyed numbers have declined and no cavefish have been detected in the last 20 years (Willis 1984; Vandike 1992b; this study). Since specimen collection, Ozark cavefish have never been detected in subsequent surveys at these sites: Missouri–Hankin's Well in Barry Co.; Moore's Spring Cave, Pfaff's Cave, Raney Creek Cave, Sam William's Spring, and Sammon's Well in Greene Co.; Adam's Well and Armstrong's Well in Jasper Co.; and in Oklahoma–Mitchell's Cave in Delaware Co. and an unnamed cave in Ottawa Co.

An important, but often understated, factor in amblyopsid habitat degradation is reservoir impound-

Fig. 11 All known visual surveys of Ozark cavefish in Twin Cave, Delaware Co., Oklahoma (Black 1971; Puckette 1986; Brown and Todd 1987; Aley and Aley 1991; Puckette, unpub. data; TNC unpub. data; this study). Statistical analyses revealed no trend in the surveys (with or without the historic count of 22 in 1970)



ment of karstified river drainages. Erection of dams on the Green River in Kentucky raised the base water level in Mammoth Cave, potentially impacting amblyopsids by disrupting normal sediment flux and organic matter inputs (reviewed by Proudlove 2001). Pickwick Reservoir has degraded Southern cavefish habitat at Key Springs Cave in Alabama (Kuhajda and Mayden 2001). The major impoundments on the White River of Arkansas and Missouri (Beaver, Table Rock, and Bull Shoals Reservoirs) have inundated some of the most extensive cave and karst systems in the heart of the Ozark cavefish range (Bretz 1956; D. Taylor, Arkansas Association for Cave Studies, pers. comm. 2002). Furthermore, Looney (1972) reports that some of the best caving areas within the Oklahoman range of the Ozark cavefish (i.e. Spavinaw Valley) were flooded by the creation of Lake Eucha.

Another habitat degradation factor is geomorphic instability in these epikarst terrains. Soil subsidence has clogged karst windows such as Mule Hole Sink, the Arkansas Game and Fish Commission Nursery Pond, and perhaps the Oklahoma Whitewater Creek flood control structure (Graening et al. 2001). Karst windows are also lost by human intervention; a landowner's fear of incurring endangered species liability motivated him to bulldoze his Monte Né sinkhole site shut (at Beaver Reservoir, Arkansas); Moore's Cave (Greene Co., Missouri) was sealed shut "for reasons of safety", and a parking lot is now on top of this site (Marquart 1979; Brown et al. 1982). While it is unknown what effect inundation or soil subsidence has upon cavefish habitat, such habitat alterations make it impossible to monitor these cavefish populations.

Although few studies have examined the effects of groundwater pollution on cavefish populations, several researchers implicate this threat in population declines (Keith and Poulson 1981; Crunkilton 1985; Tercafs 1992; Lewis 1996). Brown et al. (1998) attributed a 30% population decline in Ozark cavefish at Cave Springs Cave to increased levels of inorganic and organic compounds. Many studies express concern with the number of pollution point sources within Ozark cavefish groundwater recharge zones. Aley and Aley (1999) considered confined animal feeding operations (CAFOs) to be the greatest threat to water quality for the recharge basins of Long's Cave, McGee Cave, and Engelbrecht Cave. Highway runoff could threaten Twin Cave (Aley and Aley 1991, 1999). Septic system leachate and CAFOs threaten the water quality of Cave Springs Cave and Logan Cave in Benton Co., Arkansas (Aley 1978; Graening and Brown 2003). Aley et al. (2008) ranked 46% of the recharge zone areas of 24 Ozark cavefish sites as high or extremely high vulnerability, and inventoried over 200 CAFOs in these zones. Aley et al. (2008) also report petroleum spills at two airports located within cavefish recharge zones, and numerous pollution point sources exist in other recharge zones such as major interstate roadways, chemical pipelines, salvage yards, municipal landfills, and wastewater treatment plants.

Other limiting factors include caver trampling, expulsion by floods and other natural mortality factors. Trampling of cavefish is a very real threat during cave visitation, even for scientific purposes. Ozark cavefish cannot easily be avoided unless they are spotted in clear water, but they often forage and seek refuge in gravel interstices; narrow passageways require cavers to walk in streams, and visibility is quickly obscured by disturbance of clay sediment. One cavefish was inadvertently trampled during the annual population survey at Cave Springs Cave in 2000 (Graening and Brown 2000a, b). Additional accounts of trampling have been reported for other stygobiotic species (Weingartner 1977; Lewis 1991; Graening et al. 2006c).

We agree with Poulson (1960), who concluded that floods expelling fish out of subterranean habitats is another important, but poorly documented, mortality factor. Smith (1980) reported major flood events washing Southern cavefish from caves. An Ozark cavefish was apparently collected outside of a cave circa 1889: the museum catalogue (MCZ-27587) note states, "*From brook outside of Wilson's Cave near Sarcoxie; 50 feet from entrance to cave*" (Romero and Conner 2007). Graening observed three live Ozark cavefish in the trout runs downstream of the Cave Springs Cave resurgence after a major storm event in 1999 (Graening and Brown 1999). Another live Ozark cavefish was observed in the pond downstream of Logan Cave, again after major flow events (Graening and Brown 2000b). These fish were carefully returned to their subterranean streams, but we cannot predict how many other expelled cavefish have perished in epigean environments.

Other threats include cannibalism of young-of-year (Poulson 1960) and predation by epigean fish, especially sport fish (e.g., rainbow trout, *Oncorhynchus mykiss*, which are introduced into commercial caves to enhance the tourist experience) and cave-inhabiting sculpin (*Cottus carolinae*). Poulson (1960) believed that disease or parasitism in amblyopsids is negligible because of the dearth of pathological records for cavefish. "Lack of reproduction" is listed vaguely as a limiting factor by USFWS (1989), in reference to their low reproductive potential and its other *K*-selected life history traits in an energy poor environment. It is impossible to assess this limiting factor, which was surmised to be coupled to resource availability (Poulson 1960; Brown et al. 1994), and subsequent research has failed to find a link between seasonal energy inputs and metabolic activity in Ozark cavefish (e.g., Adams and Johnson 2001). Furthermore, lack of recruitment in an Ozark cavefish population has never actually been documented. In this study we demonstrated evidence to the contrary.

Evaluation of habitat delineation and protection efforts

Here we analyze the progress to date of implementation of the recovery tasks identified in the USFWS (1989) Recovery Plan. Recovery Task 1 involves the physical delineation of each subterranean habitat and discrete entry points for surface contaminants, and this task is almost complete; Table 2 summarizes all hydrologic studies performed to date. Hydrogeologic studies in karst terrain traditionally focus upon establishing connections by dye trace from discrete inputs, such as dolines (sinkholes) and losing streams, to resurgence points, such as springs or cave streams (Aley et al. 2008). Surficial photolineaments and fault and fracture traces are also considered, as well as bedrock geologic contacts. Other hydrogeologic data, such as drilling logs and groundwater potentiometric surfaces, help generate an understanding of karst flowpaths; surface river basin boundaries may poorly describe groundwater basin boundaries because of interbasin transfer, flow reversal, etc. The resulting study delineates a groundwater recharge zone in which a given cavefish population resides; this zone, or basin, describes the total habitat for that population. The network of conduits within this zone must, of course, be phreatic and large enough for a fish to enter (Noltie and Wicks 2001). The known recharge zones with viable Ozark cavefish populations should be federally designated as critical habit units. Once the zone is delineated, point and non-point pollution sources should be inventoried and vulnerability mapping performed (reviewed by Aley et al. 2008).

Recovery Task 2 prescribes protection and management for these critical habitats, which are less formally designated as "Recovery Caves" by USFWS (1989); Recovery Caves, as defined, include their recharge zones, and are a novel type of Endangered Species Act management unit (typically defined as a population grouping based on restricted demographic interchange; Taylor and Dizon 1999). Table 2 identifies the primary recharge zone management units and the major land acquisitions and other conservation activities.

Evaluation of other management actions prescribed by the Recovery Plan

Public outreach efforts include MDC's program in Missouri (Canaday and Vitello 1996), and the USFWS' Arkansas Ecological Services Office's Karst Resources Support Team, created in 2004. This office also teamed with TNC to develop an Endangered Species Act Section 10 safe harbor program called "Cave Harbor" to encourage compatible land use on private lands for cave-dependent species, including Ozark cavefish.

Guano, particularly that of the gray bat (*Myotis grisescens*), has been hypothesized to be necessary or ideal for cavefish presence (Willis and Brown 1985) because gray bats and amblyopsids frequently cohabitate caves and because guano has been reported to be a food source for cavefish, either directly or indirectly via secondary crustacean production (Poulson 1960, 1963). Recent studies have determined the nutritive value of gray bat guano and that other syntopic groundwater inhabitants also feed directly on it (Fenolio et al. 2006). In Shelta Cave (Madison Co., Alabama), Southern cavefish congregate where bat guano accumulates in the water (Poulson 1960); we too have noticed higher densities of Ozark cavefish near gray bat deposits, especially in Cave Springs Cave. Yet the vast majority of known Ozark cavefish sites do not have appreciable bat guano deposits or even suitable habitat for bats, especially wells in Missouri or other karst windows such as Mule Hole Sink, James Ditto Cave, and the AGFC Nursery Pond sinkhole in Arkansas. Furthermore, guano piles are

Table 2 Summary of the habitat size, associated scientific citations, and conservation status of the management units (caves and hydrologically connected springs and their combined recharge zones) currently occupied by Ozark cavefish

Management unit	Recharge zone Size (hectare)	Hydrogeologic studies	Ownership / management and conservation status
Arkansas			
Benton County			
Cave Springs Cave / Reed Spring Complex	3,800 primary; 5,000 total	Aley 1978; Williams 1991; Aley and Moss 2001	ANHC's Cave Springs Natural Area (23 hectares); remainder privately owned. Closed to public; ANHC and USFWS implementing site conservation plan.
Logan Cave / Lower Palmer Spring / Chaney Springs Complex	3,000	Aley and Aley 1987	USFWS Logan Cave National Wildlife Refuge (50 hectares); remainder privately owned. Closed to public; Conservation Plan (USFWS 2008) being implemented.
Hewlitt's Spring Hole / Rone Spring / Stillhouse Spring	1,300	Aley 1992	Private ownership.
Civil War Cave / McKisic Creek	470 to 1,000	Gillip 2007	Private ownership. Informal management agreement with TNC allows monitoring.
Rootville Cave / Spavinaw Creek	unknown	Brahana and Phelan 2002	Private ownership. Informal management agreement with TNC allows monitoring.
Bear Hollow Cave	900	Aley and Aley 1998a	TNC's Bear Hollow Preserve (3 hectares); remainder privately owned. Closed to public; TNC and USFWS implementing conservation plan.
Beaver Reservoir Nursery Pond Sinkhole	unknown		AGFC owns. Closed to public.
James-Ditto (Wasson's Mud) Cave	unknown		Private ownership. Informal management agreement with TNC allows monitoring.
Tom Allen's Cave System	unknown		Private ownership. Informal management agreement with TNC allows monitoring.
Missouri			
Barry County			
Moore Hollow Spring and Cave	200	Aley and Aley 2005	Private ownership.
Greene County			
Fantastic Caverns / Big Williams Spring	4,000	Aley and Thomson 2002, Aley and Moss 2004	Fantastic Caverns, Inc.; remainder in private ownership. Cave managed for commercial tours; aquatic habitat closed to public; informal management agreement with MDC allows monitoring.
Jackson Cave	600 in primary; 1,800 total	Aley and Aley 1997	Private ownership.
Viebrock Well / Trogdon Spring	150 primary; 2,500 total	Aley and Aley 2008	Private ownership.
Jasper County			
Kellhofer Cave	2,100	Aley and Moss 2002	Private ownership.

Table 2 (continued)

Management unit	Recharge zone Size (hectare)	Hydrogeologic studies	Ownership / management and conservation status
Wilson's Cave	30	Vandike 1989, Aley and Moss 2002	Private ownership.
Sarcoxie Cave	400	Aley and Moss 2002	Ozark Regional Land Trust's Sarcoxie Cave and Spring Preserve (1.2 hectares); remainder privately owned. Closed to public; MDC monitors water quality; MCKC assists in management.
Lawrence County			
Billies Creek Cave / Predator Cave / Faye Valley Cave / Spring Complex	3,100	Aley and Aley 1997	Private ownership.
Johnson Spring Well / Marbut Spring	300 primary; 800 total	Aley and Aley 2007	Private ownership.
Turnback Cave	10,400	Aley and Aley 2005	USFWS's Ozark Cavefish National Wildlife Refuge / Neosho National Fish Hatchery (17 hectares); remainder privately owned. Closed to public.
Newton County			
Ben Lassiter Cave	1,200	Vandike 1992a, Aley and Moss 2002	Private ownership.
Capps Creek Wells 1 and 2	100 primary; 300 total	Aley and Aley 2005	MDC's Capps Creek Conservation Area (293 hectares); remainder privately owned.
Hearrell Spring / South Big, Carter, and Highway 71A Springs / Sallee Spring and Park Spring Branches	1,500	Aley and Aley 1997, 1998b	Portion of recharge zone owned by USFWS and managed as the Ozark Cavefish National Wildlife Refuge and Neosho National Fish Hatchery; remainder privately owned. Closed to public.
Stone County			
Elsey Cave Springs	200	Aley and Aley 2005	Private ownership.
Hayes Spring and Cave	3,700	Aley and Aley 1997	MDC's Hayes Spring Conservation Area (42 hectares); remainder privately owned. Closed to public.
Oklahoma			
Delaware County			
Engelbrecht Cave / Inglebook (Wilkerson's) Spring	6,200	Aley and Aley 1990	Private ownership. Closed to public. Informal management agreement with USFWS allows monitoring.
Jail Cave	400	Aley and Aley 1990, 1991	Private ownership. Informal management agreement with USFWS allows monitoring.
Long's Cave / McGee Cave / Parchcorn and Cherokee Springs	4,400	Aley and Aley 1990, 1991, 1999	TNC's Eucha Nature Preserve (60 hectares) ; the remainder is privately owned
Mitchell's Cave System	unknown		Private. Uncooperative landowner
Star Cave / Muskrat and Drowning Creeks / Shelf Rock, Pond, Property Line, Collapsed Blocks, and Kelly Springs	100 primary; 1,000's in total	Aley 2005	Private ownership. Informal management agreement with USFWS allows monitoring.

Table 2 (continued)

Management unit	Recharge zone Size (hectare)	Hydrogeologic studies	Ownership / management and conservation status
Twin Cave	600	Aley and Aley 1990, 1991	TNC's Twin Caves Preserve (10 hectares); remainder privately owned. Closed to public.
Ottawa County			
Cave Springs Ranch Cave	in progress		Sky Ranch (non-profit) owns cave; remainder in private ownership. Informal management agreement with USFWS allows monitoring.

statistically more likely to be absent in a cavefish site, and the majority of sites do not even contain appreciable guano piles: of 57 sites where bat resources were known, only 5 sites had colonial bat guano piles, Pearson Chi-square test, df=1, X=38.75, P>0.001. In an extensive trophic study of Cave Springs Cave, Graening and Brown (2003) determined that guano was not a major contributor to the detrital resource base for isopods (*Caecidotea*), which were the primary diet of Ozark cavefish. We reject this recovery task of translocation of gray bats not only for the welfare of the endangered gray bat, which also has resisted previous translocation efforts, but because there is no substantial evidence that cavefishes require this trophic input.

Evaluation of monitoring efforts

Recovery Task 3 involves the development and implementation of monitoring programs for habitat quality in recovery caves, population surveys in known locations, and searches for additional populations. Although water quality monitoring has not occurred at the frequency dictated by the Recovery Plan (per annum), some important studies have been performed in the last two decades. Results are equivocal. The numerous recharge zone delineation studies performed by T. Aley (Ozark Underground Laboratory) have identified potential pollution sources in many Ozark cavefish habitats. For example, Aley (2005) determined that much of the water discharged from the City of Jay (Oklahoma) sewage treatment plant subsequently flows through Star Cave. No pesticide or herbicide contamination was detected in a study of Wilson's Cave and Ben Lassiter Cave (Vandike 1992b). Intensive environmental quality sampling from 1997 to 2001 in Cave Springs Cave indicated that this habitat was consistently contaminated with fecal coliform bacteria, excess nutrients, and dissolved metals (Graening and Brown 2003). Toxic metals were also detected in cave sediments and the tissues of cave isopods and one Ozark cavefish (Graening and Brown 2003). A study of six cavefish caves (Bear Hollow, Cave Springs, Civil War, January-Stansbury, Logan, and Rootville caves) by Graening (2005) concluded that these sites had detectable contamination of water, sediments, and animal tissue by nutrients, toxic metals, and coliform bacteria, originating probably from septic systems and land application of CAFO wastes. Adornato (2005) performed a contaminant study of several caves, including January-Stansbury Cave, and reported low concentrations of organic and inorganic contamination (pesticides) in the study sites, but concluded that there was not yet significant contamination of January-Stansbury Cave, even though it was downstream of land applications of CAFO wastewater treatment plant effluents. Bidwell et al. (2008) performed an investigation into the potential occurrence of pharmaceuticals and other wastewater compounds in six Ozark cavefish caves (Cave Springs, January-Stansbury, Logan, Long's, Star, and Twin caves); low concentrations of organic wastewater compounds and other organic compounds were detected in the cave water of these caves. These studies indicate that many cavefish habitats are experiencing chronic, low-level exposure to a suite of anthropogenic contaminants; however, the effect of this exposure upon the species is uncertain.

Population surveying at recovery sites and historic sites has not been performed at the frequency dictated by the Recovery Plan (every three years and every five years, respectively), but intensive survey efforts have been performed in the last three decades. The survey effort began with A. Brown, L. Willis, and S. Todd's original cavefish distribution studies in the 1980s, which established the historic range of the species. Intensive survey efforts in the 1990s to update the status of the Ozark cavefish in Missouri were conducted by researchers at MDC and University of Missouri at Columbia, and their survey efforts continue. From 1998 to 2006, the authors have performed surveys for additional cavefish sites and regularly surveyed known cavefish sites in Arkansas and Oklahoma (Brown et al. 1998; Graening and Brown 1999, 2000a, b, 2001). The USFWS now coordinates monitoring efforts rangewide with cooperating agencies, including Arkansas Game and Fish Commission, Arkansas Natural Heritage Commission, Missouri Department of Conservation, Oklahoma Department of Conservation, and The Nature Conservancy.

Evaluation of conservation status and delisting criteria

The Recovery Plan defines, without explanation, eight "Recovery Caves": Cave Springs Cave and Logan Cave in Arkansas; Ben Lassiter Cave, Kellhaufer's Cave, Sarcoxie Cave, and Turnback Creek Cave in Missouri; and Twin Cave and Engelbrecht Cave in Oklahoma. The Recovery Plan then identifies two delisting criteria: (1) the recovery caves and their recharge zones are protected; and (2) the 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 Cave and no less than 20 per survey visit in each of the other seven recovery caves over at least a ten year period. As currently worded, the species may never be delisted because the delisting conditions are largely unattainable.

Although all of the Recovery Caves have had their entrances gated and have had their recharge zones delineated, protection of these recharge zones from all anthropogenic stressors, and resulting habitat degradation, is virtually impossible. Aley et al. (2008) concluded that "*many of the habitat sites cannot be effectively defended against land use activities or accidents that could seriously damage or destroy some of the populations*." For example, the Cave Springs Cave recharge zone, as currently defined by Aley and Moss (2001), comprises about 5000 hectares of land area, and extends into the metropolitan center of Springdale with a population of ca. 60 000 people. How will land use restrictions, or even voluntary use of water quality best management practices, be implemented or enforced in such a large suburban area? Because groundwater travel rates in the Cave Springs area often exceed 1 km·day^{-1} (Aley 1978), and because numerous sinkholes and fracture traces are present that allow surface water to rapidly infiltrate the aquifer (Graening and Brown 2001), there is very little time for hazardous material response teams to intercept an accidental release of a toxic substance into the recharge zone. Furthermore, the Cave Springs Cave recharge zone is situated in a region with a high density of CAFOs and residential septic systems, all of which dispose of organic wastes directly to the soil subsurface (Graening and Brown 2003; Bidwell et al. 2008). We suggest that this recovery criterion be amended to consist of the successful implementation of groundwater protection programs at each recovery cave's recharge zone, rather than the elusive goal of complete protection of the recharge zone. These programs should be at least as comprehensive and binding as municipal source water protection programs administered by the U.S. Environmental Protection Agency and regulated by the Safe Drinking Water Act and Clean Water Act.

The second delisting criterion presupposes that all the designated recovery caves support large cavefish populations. Yet, surveys over the last century in Turnback Creek Cave, Sarcoxie Cave, Kellhaufer's Cave, and Engelbrecht Cave have never even approached ten individuals. Ben Lassiter Cave and Twin Cave have had historic reports of more than 20 cavefish, but surveys in the last two decades have averaged less than five. Consistently detecting at least 20 cavefish in these sites for a decade seems to be an unreasonable and unattainable goal. The second delisting criterion has been achieved in only two of the eight recovery caves; more than 100 have been consistently observed in Cave Springs Cave and more than 20 in Logan Cave in the last decade. This delisting criterion also implies that a consistent survey of 240 cavefish (100 in Cave Springs Cave, 20 each in seven other recovery caves) is a sufficiently large population to be deemed safe from threat of extinction, and constitutes a stable total population size. Yet this is less than half of the textbook "50–500" rule; Franklin (1980) calculated that an effective population size of at least 500 individuals are needed to be safe from inbreeding depression, and that an effective size of less than 50 individuals is in danger of extinction (e.g., from deleterious mutation accumulation). Lynch et al. (1995) conclude that populations as large as one thousand are still susceptible to "mutational meltdown."

We suggest that adaptive management be employed and that "Recovery Caves" be defined as an updatable list of at least 20 sites that have had a consistent sighting of at least two cavefish in the last ten years, with the list updated at least every five years. Using this definition, recovery caves would currently consist of: Cave Springs Cave, Logan Cave, and Tom Allen's Cave complex in Arkansas: Ben Lassiter Cave, Kellhaufer's Cave, Sarcoxie Cave, and Hearrell Spring in Missouri; and Twin Cave and Long's / McGee Cave complex in Oklahoma. Furthermore, we suggest that the total population size recovery goal be increased to at least 500 surveyed cavefish (ideally, breeding adults). Critical habitat should be legally designated as the recharge zones of recovery caves, especially Cave Springs Cave and Logan Cave.

Conclusions

The survey count trend analyses for Ozark cavefish at recovery sites was largely inconclusive because variance was high and the majority of data sets were not sufficiently large to detect a trend. However, the two largest populations (Cave Springs Cave and Logan Cave) have stabilizing or increasing survey counts. While the number of active cavefish sites has decreased over 50% since 1990, the number of total surveyed individuals has not significantly changed; this is another indication that persisting populations may be increasing. Important progress has been made towards protection of the recovery caves and their recharge zones. Further, state and federal agencies are actively monitoring cavefish habitat and encouraging landowners to implement best management practices. Nevertheless, the continuing expansion of human enterprises into these rural recharge zones leaves little hope for ever delisting this species. We recommend changes to the criteria defining successful recovery. The list of "recovery caves" should be re-evaluated on a regular basis, and should reflect the viability of each cavefish subpopulation and the environmental quality and defensibility of its habitat. Additionally, the total population size recovery goal (the sum of surveyed individuals across all sites in a given year) should be increased from an arbitrary value of 240 individuals to a calculated minimum viable population size.

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