Status and Life History of the Amblyopsid Cavefishes in Kentucky

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Introduction

The Appalachians and Interior Plateau support the highest aquatic subterranean biodiversity within the continental U.S. (Culver et al. 2003). However, over 95% of subterranean species in North America are vulnerable or imperiled (Culver et al. 2000) because of restricted geographic distributions (Culver et al. 2000, 2003) and a number of threats, such as groundwater pollution and habitat degradation (Elliott 2000; Danielopol et al. 2003; Boulton 2005). Unfortunately, the distribution and status of many species is incomplete or lacking entirely, making conservation and management decisions difficult. Here we investigate the distribution, ecology, conservation status, and threats to three cave-associated fish species in the family Amblyopsidae in Kentucky (Fig 1): the Northern Cavefish (*Amblyopsis spelaea*), Spring Cavefish (*Forbesichthys agassizii*) and Southern Cavefish (*Typhlichthys subterraneus*). Despite large distributions in central Kentucky, little is known regarding life history of these species, particularly of the obligate cave-dwelling *A. spelaea* and *T. subterraneus*. Pursuant with Kentucky's priority research and survey needs, the objectives of this study were to (1) conduct baseline surveys and status assessments of each amblyopsid species to determine their distribution and conservation status in the state, (2) obtain cavefish biology information, such as habitat requirements, ecology, and demography for each species, and (3) identify potential threats to existing and significant populations of each species and develop recommendations for

Cavefish / Dante Fenolio

Figure 1: *Four species of amblyopsid cavefishes occur in Kentucky: the Spring Cavefish (Forbesichthys papilliferus) (top left), Northern Cavefish (Amblyopsis spelaea) (top right), Southern Cavefish (Typhlichthys subterraneus) (bottom left), and Kentucky Cavefish (bottom right).*

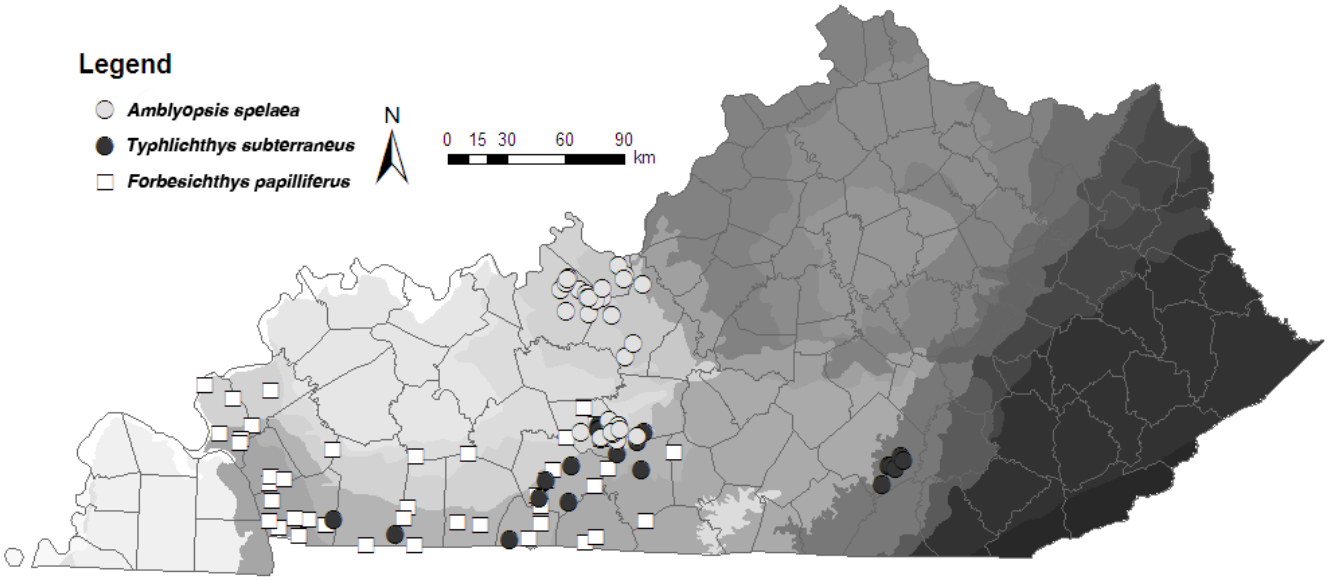

Figure 2: *The distributions of amblyopsid cavefishes in Kentucky are confined to cave- and karst- bearing regions. Ecoregions are colored.*

status evaluations and monitoring.

Methods

Field Surveys

We searched for Northern Cavefish, Southern Cavefish, and Spring Cavefish from May 2007 through September 2011 in caves, springs, and spring-fed streams throughout the Interior Plateau and along the Cumberland Plateau of Kentucky, including several historic localities. We conducted surveys during all months of the year, but concentrated during periods of favorable conditions in subterranean streams (i.e., shallow, clear water with little flow) or during spring when water levels were higher and Spring Cavefish can be found in surface habitats. Surveys for cave species (i.e., Northern Cavefish and Southern Cavefish) were temporarily discontinued in 2009 and 2010 because of concerns of the spread of White Nose Syndrome affecting cave-roosting bats.

To locate cavefish, we donned wetsuits and slowly walked along, waded through, or crawled in the cave stream channel and thoroughly scanned the streambed with the beams of our headlamps. We also carefully lifted flat rocks, small cobble, and detritus under which smaller individuals might seek refuge. Lifted rocks were returned to their original positions to minimize habitat disturbance. A similar approach was taken in surface springs, streams, and ponds while surveying for Spring Cavefish. We used large dipnets to search through aquatic vegetation and detritus where Spring Cavefish might seek refuge during the day. We also searched beneath rocks, logs, and other potential cover objects. A tally of each individual found was kept, and a concerted effort was made to capture, with small bait nets, each cavefish encountered.

Captured fish were placed in clear plastic bags until standard length (SL) was measured to the nearest mm using a small metric rule or digital calipers. Other data were gathered from each captured fish if possible, including sex, condition (e.g., injuries, growths, or presence of parasites), habitat (aquatic: stream pool, stream riffle, rimstone pool; terrestrial: mud bank, bank-cut, crevice), substrate (mud, sand, cobble, gravel, bedrock, organic debris, artificial), cover type (rock, log, crevice, organic debris), and other aspects of life history (diet, behavior, community associates). Additionally, we excised a small tissue sample from the right pectoral fin or caudal fin of one or more cavefish captured at each locality (up to 15 at a given locality) for subsequent genetic analyses.

Results and Discussion

Spring Cavefish Distribution

Within Kentucky, Spring Cavefish have been reported from at least 48 localities in 17 counties, including at least seven records from caves (Fig. 2). We did not observe Spring Cavefish during any cave surveys; however, the species has been reported from a few caves in the Western Pennyroyal Karst. Spring Cavefish occur in four ecoregions in Kentucky. This distribution extends through much of the

southern Interior Plateau in the central part of the state, including the Western Highland Rim, Eastern Highland Rim, Crawford-Mammoth Cave Uplands and Western Pennyroyal Karst from the Mammoth Cave region in Edmonson and Hart counties south to the Tennessee border and west to Trigg, Lyon, and Livingston counties along the Cumberland River. At least six records exist within the Caseyville Hills of the Interior River Valleys and Hills. The highest density of Spring Cavefish localities occurs in the Land Between the Lake area in Lyon and Trigg counties, as well as south of the Bowling Green area in Warren County. Included in the distribution of the Spring Cavefish in Kentucky are nine HUC8 watersheds, including the Cumberland, Green, Lower Ohio, and Lower Tennessee basins. Spring Cavefish have the largest geographic extent of all amblyopsids in Kentucky with an extent of occurrence (EOO) of 14,786.2 km^2, and an area of occupancy (AOO) of 720.0 km^2 (based on 4 x 4 km grid cells). We discovered one new locality in Todd County in the Red River watershed.

Southern Cavefish Distribution

Within Kentucky, Southern Cavefish have been reported from at least 29 localities, including 27 caves, one spring, and one well in eight counties (Fig. 2). The highest density of Southern Cavefish localities occurs in Edmonson County. Southern Cavefish occur in four ecoregions in Kentucky. This distribution extends through much of the southern Interior Plateau in the central part of the state, including the Crawford-Mammoth Cave Uplands and Western Pennyroyal Karst from the Mammoth Cave region in Edmonson and Hart counties south to the Tennessee border and west to Trigg County. Southern Cavefish in this region have an EOO of 4,547.9 km^2 and an AOO of 320.0 km^2. A disjunct cluster of populations occurs in Plateau Escarpment ecoregion of the Southwestern Appalachians. Included in the distribution of the Southern Cavefish in Kentucky are five HUC8 watersheds. We documented several new populations in Pulaski County in the Upper Cumberland watershed. Prior to this study, *Typhlichthys* were confirmed from only Sloans Valley Cave (Cooper and Beiter 1972), which is partially inundated by Lake Cumberland. With the assistance of the Greater Cincinnati Grotto, we discovered new populations in three nearby cave systems with unconfirmed reports from two additional cave systems. All localities occur within the Plateau Escarpment of the Southwestern Appalachians and are isolated from other populations in both Kentucky and Tennessee. This set of populations only has an EOO of 38.3 km^2 and an AOO of 80.0 km^2.

Northern Cavefish Distribution

Within Kentucky, Northern Cavefish have been reported from at least 39 localities, including 38 caves and one spring in five counties (Fig. 2). Northern Cavefish occur in three ecoregions in the state: the Crawford-Mammoth Cave Uplands and Mitchell Plain of the Interior Plateau and the Caseyville Hills of the Interior River Valley and Hills (two localities). The highest density of Southern Cavefish localities occurs in the Sinking Creek valley in Breckinridge County and in the Mammoth Cave region in Edmonson County. Included in the distribution in Kentucky are three HUC8 watersheds: the Rough and Upper Green watersheds of the Green River Basin and the Blue-Sinking Watershed of the Lower Ohio River Basin. Northern Cavefish have an EOO of 2700.6 km^2 and an AOO of 432.0 km^2 in the state.

Relative abundance, population size and trends

Few studies have attempted to quantify population sizes and relative abundance of amblyopsids, and most of these studies have focused on caves that are known to contain relatively large populations. Other studies for which the most reliable estimates of abundance have been obtained have focused on the species of conservation concern. Additional demographic studies, including long-term censuses, are needed for both surface and subterranean populations.

Historically, Spring Cavefish has been considered rare to uncommon throughout much of its range. In Kentucky, this species has been widely reported but most localities yield fewer than ten fish during a single survey (Fig. 3). To our knowledge, Spring Cavefish have only been observed in excess of 25 fish at two localities: a ditch off of Morton Road in Todd County and Rich Pond in Warren County. Most surveys yield just a few fish; however, this likely is an artifact of habitats sampled, as many ichthyological surveys focus on streams and other larger bodies of water rather than spring runs and springs. Moreover, most springs are located on private property and consequently have been poorly sampled. Because Spring Cavefish return and persist in spring heads and underground waters when their surface habitats dry in late summer and autumn, the best chance of detecting this species occurs when water levels are high in late winter and early spring.

We discovered a new, significant population of Spring Cavefish in a spring-fed ditch off of Morton Road in Todd County (Fig. 4). This stream has been channelized for irrigation and averages ca. 2 m wide. It is full of aquatic vegetation, which provides ample cover for the species. During our first visit on 31 Mar 2010, we captured 77 fish in the 30 m stretch upstream of the road crossing and we estimated a population density of 12,833 fish per hectare at this locality. However, the number of fish dramatically decreased in subsequent weeks as water levels began to decrease and fish presumably moved upstream. By mid-June in both 2010 and 2011,

we were unable to capture a single fish at this site. A similar phenomenon was observed at Rich Pond in Warren County. We surveyed a 50 m section of stream upstream of the road crossing on several occasions throughout the year. The stream at Rich Pond issues from a series of small springs then flows for a few hundred meters through an agricultural field before issuing into a large depression in an agricultural field. In the spring during high water levels, the water from the stream issues into this depression forming a large pond (up to 340 acres in size). However, as the season progresses, water levels drop and flow is usually reduced to a small stream that eventually goes completely dry by July or August. During our surveys, we observed as few as zero and as many of 203 Spring Cavefish in this 50 m section amidst aquatic vegetation. We estimated a population density up to 27,067 fish per hectare in the spring but dropping to 0 fish per hectare in the autumn when fish move underground and the stream dries.

Sixty-three percent of reported Southern Cavefish localities yield fewer than ten fish during a single survey. Only Hawkins River in Mammoth Cave, Hidden River Cave in Hart County, and L & N Railroad Cave in Barren County have historically produced 25+ fish during a single survey (Fig. 3). Although Southern Cavefish have been found in many portions of the Mammoth Cave system, the vast majority of cavefish observed are from the Proctor Cave section of the system, and more specifically Hawkins River. Pearson and Boston (1995) observed up to 104 *Typhlichthys* during several surveys in 1993 and 1994. We visited the Logsdon River section and observed 19 cavefish in 2010 even though water levels were slightly elevated from recent rainfall. Pearson and Boston (1995) observed up to 45 cavefish during several surveys of L & N Railroad Cave in 1993 and 1994. We visited the cave on four occasions and observed between 8, 15, 22, and 27 cavefish, respectively, in a ca. 300 m section of the stream. This population is unusual in that cavefish are found in the cave stream with considerable flow, often underneath rocks in the middle of the channel or under undercut ledges around bends and meanders. During two surveys, water levels were elevated with low visibility and we observed few cavefish. We estimated a population density of 450 cavefish per hectare in this section of stream. We discovered a new significant population of *Typhlichthys* in Pulaski County at Drowned Rat Cave. We searched ca. 400 m of stream passage on four occasions and observed 31, 17, 24, and 14 cavefish, including presumably young-of-the-year fish. We estimate a population density of 258 cavefish per hectare in this section of stream.

Like Southern Cavefish, most Northern Cavefish localities yield few cavefish, as ten or fewer cavefish have been observed from 64% of localities in Kentucky (Fig. 3). The largest populations exist in Breckinridge County, including Webster's Cave, Penitentiary Cave, Amblyopsis Cave, and Under the Road Cave where over 100 individuals have been observed during a single survey. This area and the Mammoth Cave system have been identified as population centers for *Amblyopsis* in Kentucky (Pearson and Boston 1995). Our surveys focused primarily on the northern population center in Breckinridge County where we observed significant numbers in several caves, including Under the Road Cave, which may have experienced a population decline (Pearson and Boston 1995). Webster's Cave

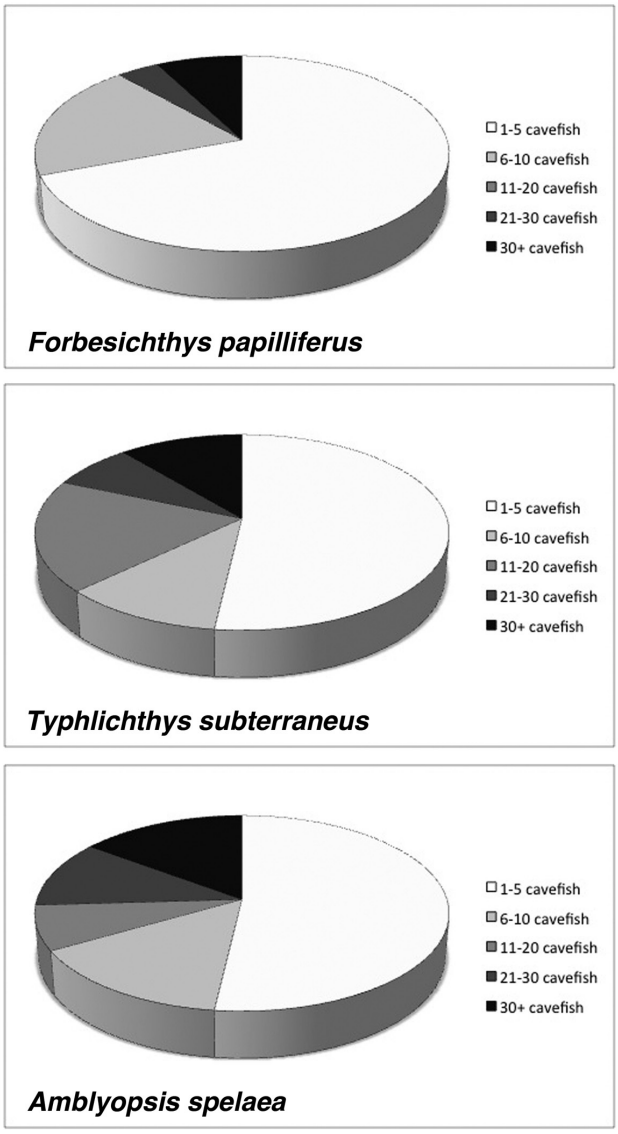

Figure 3: *Proportion of Spring Cavefish (top), Southern Cavefish (middle), and Northern Cavefish (bottom) localities in Kentucky categorized by the maximum number of cavefish observed during a single survey. Most localities yield few individuals.*

in Breckinridge County also supports a large population of *Amblyopsis*. Louis (1999) estimated a population size of 211 ± 37 individuals in a 2530 m section of stream passage at Webster's Cave using mark-recapture with visual implant elastomers. However, this estimate likely is conservative given that Pearson and Boston (1995) observed 162 individuals during a single survey and estimated a population size of 456 cavefish. We observed as many as 51 individuals during our surveys of the first 1200 m of this passage. Based on Pearson and Boston's (1995) data, we estimated a population density of 64 cavefish per hectare in the surveyed portion of Webster's Cave, but it should be noted that cavefish have been observed all throughout the cave system, including areas not subject to survey (Chris Anderson, personal communication).

Although these results might be a reflection of true abundance, the distribution and abundance of the cave-dwelling amblyopsids likely is greater than currently realized. Localities for which Southern Cavefish and Northern Cavefish have been reported represent but a fraction of total available habitat accessible to cavefish. This was clearly illustrated during a fertilizer pipeline break within the recharge zone of Meramec Spring that resulted in the death of at least 1,000 Southern Cavefish and likely many more. This unfortunate kill is informative because the drainage basin had no records for the species previously. The problem with inferring population densities from such fish kills is that we do not know the volume or extent of habitat impacted. Most observations of Southern Cavefish and Northern Cavefish are restricted to caves near the surface and there is some controversy as to whether even the best cavefish caves are sources or sinks (Niemiller and Poulson 2010). Habitats where few or no cavefish are observed likely represent population sinks and not sources. Wells and short stream segments encountered in an otherwise dry cave may not be representative of the habitat that most cavefish inhabit. Cavefish can disperse through and occupy submerged passages inaccessible to humans but these habitats are probably neither usual for the fish nor optimal. These habitats likely act as corridors for dispersal. Given their longevity, low metabolic rates, and foraging efficiency, cavefish likely can move relatively long distances but data are lacking to support this hypothesis.

Determining the actual population sizes of amblyopsid cavefishes is extremely challenging because of the difficulty and inaccessibility of the habitats that each species inhabits. Only a fraction of the actual census population likely is sampled during a given survey; however, even estimating that fraction sampled is not trivial. Here we apply an order of magnitude scaling factor for estimating population size, but recognize that actual population sizes could be lesser or greater than our coarse estimates. We estimate a minimal population size of over 12,000 individuals for Spring Cavefish, 3,200 individuals for Southern Cavefish, 500 individuals for new lineage of *Typhlichthys* in Pulaski County and 14,900 individuals for Northern Cavefish in Kentucky.

Trends refer to directional change over the short-term (within three generations) and long-term (within 100 years) in population size, EOO, AOO, or number of occurrences. There is no current evidence to suggest that there have been substantial changes in any of these factors over the short-term or long-term for amblyopsid cavefishes in Kentucky, although these factors should be reassessed every 5–10 years. The population of *Typhlichthys* at Sloans Valley Cave has not been confirmed since the late 1960s, but cavers have reported seeing white, blind fish in the same pools where Cooper and Beiter (1972) collected cavefish over the past two decades. If this population was extirpated, a significant reduction in EOO and AOO would occur for this lineage. Northern Cavefish may have experienced a population decline after excessive collections in the late 1800s (Niemiller and Poulson 2010) but there is no evidence to suggest that current population densities are any less than those in the mid 1800s when the species was first discovered in the Mammoth Cave system.

Management Recommendations

Several conservation measures have been proposed or implemented for populations of cave amblyopsids in Kentucky. Fencing or gating of cave entrances have been proposed or implemented to reduce and control human visitation to sensitive cave ecosystems, such as the many entrances to the Mammoth Cave system in Edmonson County, Thornhill Cave in Breckinridge County and Parker Cave in Barren County. Special bat gates are needed to allow entry and exit by bats but stop human entry. Bat Conservation International and The National Speleological Society have been leaders in the improvement and installation of such gates on an increasing number of bat caves. At other caves, such as Wells Cave in Pulaski County, signs have been posted to help reduce illegal visitation. Protection of cave surface and subsurface watersheds is probably the most important intervention for cavefish localities. Watershed protection has included establishing preserves as well as institution of best land management practices around sinkholes and sinking creeks, including reforestation. Indeed, a number of cave systems receive some protection by occurring on state or federally owned land or are owned or leased by conversation agencies. In other cases, water tracing has identified the source of pollutants and so allowed legal action that remedied the situation. Hidden River Cave in Hart County, Kentucky is one example. We suggest that demographic source caves deserve complete protection of their watersheds, such as Northern Cavefish local-

ities located in Sinking Creek. Only a few caves have the vast majority of all Northern Cavefish ever censused. Attention to protecting these caves should be a top priority for the near future. Likewise, source populations of Spring Cavefish, such as Rich Pond, should be identified and protected. To this end, several management policies should be implemented in the immediate recharge basins of significant cavefish populations to protect the health and integrity of source populations: (1) alter land use practices and implement runoff control measures to reduce the input of sediments and runoff into cave systems, (2) reduce or eliminate the use of toxic pesticides and herbicides known to negatively impact the fragile subterranean ecosystem, (3) identify and protect critical input points (sinkholes and sinking streams) into cave systems, and (4) limit access to areas within cave systems that support large cavefish subpopulations.

In light of the current state of knowledge regarding amblyopsid populations in Kentucky, we offer the following recommendations for future research and conservation management:

Spring Cavefish

1. Identify and survey springs located on private property located within the suspected distribution of the species to discover additonal significant populations.

2. Work to protect the Rich Pond population through purchase of the spring and surrounding area, implementing habitat protection strategies, or by obtaining a conservation agreement with the private landowner.

3. Additional population genetic analyses and long-term mark-recaptured are warranted to determine connectivity of populations and dispersal ability of the species in the Western Pennyroyal Karst. Although dispersal ability in amblyopsids is generally thought to be low, major flood events, such as the event during May 2010, may be important for long distance dispersal in this species.

4. Establish a yearly census at the two most significant localities (Morton Road in Todd County and Rich Pond in Warren County) during April or May to monitor population and demographic trends over time.

5. Delineate the recharge zone and conduct annual monitoring water quality at Rich Pond.

Southern Cavefish

1. Delineate the recharge zones of known localities of the undescribed species in Pulaski County, particularly the Coral Cave system and Hail Cave system.

2. Additional surveys are needed to document additional sites for the


Newly found cavefish habitat / Matthew Niemiller

Figure 4: *This spring-fed ditch off of Morton Road in Todd County contains a newly discovered population of Spring Cavefish (F. papilliferus).*

undescribed species in Pulaski and determine if the distribution extends to the southwest along the escarpment of the Cumberland Plateau in Wayne County.

3. Determine the point source of groundwater contamination at Friendship Cave in Warren County and initiate a chemical cleanup of the cave if possible.

4. Implement a public awareness program to inform landowners and others of the harmful impacts of dumping into sinkholes on groundwater and life it contains.

5. Remove the dilapidated pump house and other debris at the entrance of L & N Railroad Cave in Barren County to improve terrestrial and aquatic habitat in the cave.

Northern Cavefish

1. Surveys are needed of cave systems that occur between the main centers of distribution for *Amblyopsis spealea* in parts of Grayson, Hardin and Hart counties to determine if the two main population centers in Kentucky are continuous or isolated by the Hart County Ridge. Additionally, future genetic work should focus on determining with relationships of southern populations of *Amblyopsis* in the Mammoth Cave area with those to the north in the the Sinking Creek area of Breckinridge County. This latter recommendation is currently underway.

2. Because the populations in Sinking Creek in Breckinridge County represent the most significant population center of the species, efforts should be made to protect these populations through landowner agreements, the purchase of cave entrances and surrounding land within recharge zones, and measures to reduce development and construction activities in the area.

3. Implement a public awareness program to inform landowners and others of the harmful impacts of dumping into sinkholes on groundwater and life it contains.

4. Conduct *in situ* studies to determine if Rainbow Trout and Banded Sculpin successfully prey on subterranean fauna, including Northe Cavefish, in subterranean habitats and determine their influence on subterranean faunal abundance and behavior.

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