

**U.S. FISH AND WILDLIFE SERVICE
SPECIES ASSESSMENT
AND LISTING PRIORITY ASSIGNMENT FORM
March 14, 2023**

SCIENTIFIC NAME: *Amblyopsis spelaea*

COMMON NAME: Northern cavefish

LEAD REGION: 4

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DATE INFORMATION CURRENT AS OF: 8/21/2023

STATUS/ACTION

☒ Species petitioned for listing which we have determined does not warrant listing (does not meet the definition of a threatened or endangered species)

Petition Information:

☒ Petitioned; Date petition received: April 20, 2010

90-day “substantial” finding: September 27, 2011 (76 FR 59835)

PREVIOUS FEDERAL ACTIONS

On April 20, 2010, we received a petition from the Center for Biological Diversity, Alabama Rivers Alliance, Clinch Coalition, Dogwood Alliance, Gulf Restoration Network, Tennessee Forests Council, and West Virginia Highlands Conservancy to list 404 aquatic, riparian, and wetland species, including the northern cavefish (*Amblyopsis spelaea*), as endangered or threatened species under the Act. On September 27, 2011, we published a 90-day finding (76 FR 59836) that the petition contained substantial information indicating listing may be warranted for the species. This document constitutes our 12-month finding on the April 20, 2010, petition to list the northern cavefish under the Act.

TAXONOMY

FISH, Amblyopsidae

ANALYTICAL FRAMEWORK

To assess northern cavefish viability, we conducted a species status assessment (SSA) using the three conservation biology principles of resiliency, redundancy, and representation (Shaffer and Stein 2000, pp. 306–311). Briefly, resiliency supports the ability of the species to withstand environmental and demographic stochasticity (for example, wet or dry, warm or cold years, variation in demographic rates), redundancy supports the ability of the species to withstand catastrophic events (for example, droughts, large pollution events), and representation supports the ability of the species to adapt to both near-term and long-term changes in its physical and biological environment (for example, climate change, disease). A species with a high degree of resiliency, representation, and redundancy is better able to adapt to novel changes and to tolerate environmental stochasticity and catastrophes. In general, species viability will increase with increases in resiliency, redundancy, and representation (Smith et al. 2018, p. 306). Using these principles, we identified the species' ecological requirements for survival and reproduction at the individual, population, and species levels, and described the beneficial and risk factors influencing the species' viability.

We use the SSA framework to assemble the best scientific and commercial data available for this species. The SSA framework consists of three sequential stages. During the first stage, we evaluate the species' needs. The next stage involves an assessment of the historical and current condition of the species' demographics and habitat characteristics, including an explanation of how the species arrived at its current condition (i.e., how threats and conservation actions have influenced the species). The final stage of the SSA framework involves assessing the species' plausible range of future responses to positive and negative environmental and anthropogenic influences. The SSA framework uses the best available information to characterize viability as the ability of a species to sustain populations in the wild over time and is used to inform our regulatory decision.

The SSA report does not represent a decision by the Service on whether the northern cavefish should be listed as an endangered or threatened species under the Act. However, it does provide the scientific basis that informs our regulatory decisions, which involve the further application of standards within the Act and its implementing regulations and policies. The Species Status Assessment Report for the Northern Cavefish (*Amblyopsis spelaea*) – April 2022, Version 1.0 (SSA Report) is a summary of the information we have assembled and reviewed and incorporates the best scientific and commercial data available for this species. Excerpts of the SSA Report are provided in the sections below. For more detailed information, please refer to the SSA Report (Service 2022, entire).

BIOLOGICAL INFORMATION

Native to central Kentucky, the northern cavefish is a small, cave-dwelling fish found only in subterranean drainages. It is characterized by its rudimentary eyes, lack of skin pigment, a large, flat head, and a tubular, non-streamlined body (Figure 1). The standard length (tip of nose to end

of last vertebra) of adult northern cavefish typically ranges from approximately 60 to 80 mm (2.4 to 3.1 inches). The maximum known age for northern cavefish is 10 years, but the lifespan may be 20 - 40 years with an age of 30 - 40 years more likely achieved in caves with consistent food availability (Poulson *et al.* 2001, p. 358; Niemiller and Poulson 2010, p. 221; Adams p. 305, 316). The species has four life stages: egg, protolarva, juvenile, and adult. Eggs and protolarvae are held in the female's gill chamber until reaching the juvenile stage, when they swim freely, apart from the mother. Age at reproductive maturity (adulthood) is around 6 years.



Figure 1. Photo of northern cavefish (Credit: National Park Service Digital Image Archives)

The range of the northern cavefish is subterranean streams in Meade, Breckinridge, Hardin, Hart, and Edmonson counties, Kentucky, south of the Ohio River (Figure 2). In Kentucky, this area is characterized as a karst ecosystem with underground drainage systems comprised of sinkholes and caves. Because northern cavefish inhabit underground stream networks that cannot be completely mapped or surveyed, the species likely occurs at sites that are inaccessible, and the true distribution and number of populations within the range of the northern cavefish is unknown.

The closely related Hoosier cavefish, which is restricted to Indiana north of the Ohio River, formerly was recognized as northern cavefish but is now known to be a distinct taxon based on morphological and genetic differences (Chakrabarty et al. 2014, entire). Although they are different species with separate ranges, northern cavefish and Hoosier cavefish have very similar life history traits. The two taxa are the only two members of the genus *Amblyopsis*. Nearly all natural history data for the northern cavefish are from two sources which either group Hoosier cavefish with northern cavefish or pertain to Hoosier cavefish entirely (Eigenmann 1909, Poulson 1963; summarized in Poulson 2001, Niemiller & Poulson 2010, pp. 220 -249 and Adams 2020, pp. 304-310). Thus, the current state of knowledge concerning life history and resource needs of northern cavefish is from studies on the genus *Amblyopsis*, which include both species in the genus in some cases and in others, includes only Hoosier cavefish.

The life history of the northern cavefish entails a naturally low population growth rate and is characterized by long-lived adults that produce a few large eggs resulting in occasional strong year classes (Poulson 2001, p. 350, 355). Adult northern cavefish mortality is low as they are top predators, but food is often limited for growth and reproduction (Niemiller and Poulson 2001, p. 221). Females may only spawn only three to five times in their lifetime and about five percent of females in a population are estimated to breed each year (Niemiller and Poulson 2001, p. 226). Northern cavefish have low metabolic rates and can survive more than a year without feeding (Eigenmann 1909, p. 81; Niemiller and Poulson 2010, p. 228, 236-237).

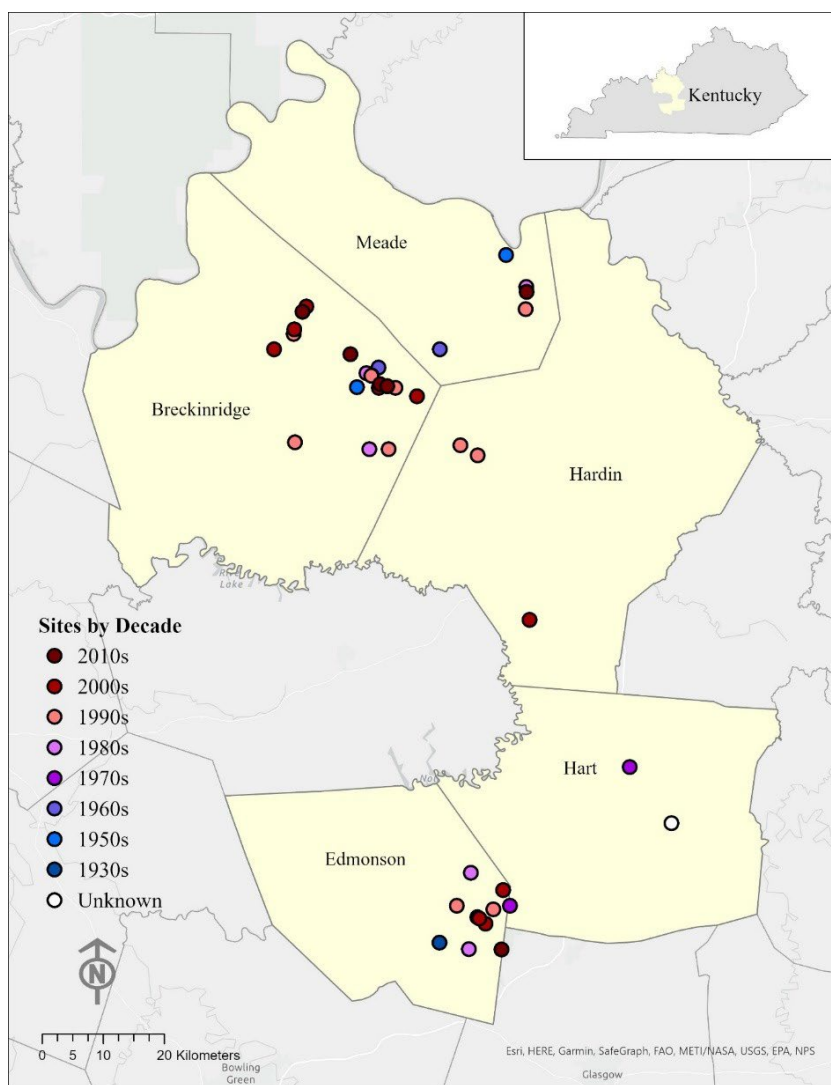


Figure 2. Range of the northern cavefish (yellow areas). Records since 2000 are considered current occurrences in the SSA.

Individuals of all northern cavefish life stages need generally cool water temperatures, sufficient dissolved oxygen, low salinity, and flowing water. The species needs slow flowing pools or

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shoals, a food supply of invertebrates (may occasionally consume other northern cavefish), and substrates composed of fine particles. Northern cavefish are most numerous in flood refuges (slackwater areas, breakdown and other rock piles), as well as deep pools and shoals with ledges, and near or under overhangs (Niemiller and Fitzpatrick 2013 p. 26). A common feature of these habitats is the accumulation of organic matter washed in from the surface along with mud, silt, sand, and cobble substrates (Poulson 1963, p. 264; Poulson 2001, p. 359; Niemiller and Fitzpatrick 2013 p. 26). Floods are important for juveniles and adults as they provide detritus and food resources. Food in the northern cavefish's ecosystem comes from two main sources, sinking streams and cave inhabiting animals. Sinking streams carry various plant materials as well as bacteria, fungi, and zooplankton into caves. Cave animals deposit eggs, feces, and often their bodies in caves. Bacteria and fungi decompose these materials, supplying food for detritus-feeding animals, which are in turn eaten by predators like the northern cavefish (Barr 1967, p. 476).

At the population level, floods are important for reproduction (renewing generations) and maintaining connectivity and gene flow. Northern cavefish may be reproductively active whenever detrital renewal occurs. The reproductive periods are noted as February through April, but also March through November (Eigenmann 1909 p. 93, 206; Adams p. 305). The associated rise in water level likely triggers breeding, but the amount of detrital renewal exhibits spatiotemporal variation; successful reproduction is infrequent and linked to significant flooding events (Poulson 2001, p. 348). During flood events, northern cavefish likely move from one location to another either intentionally or passively via bedrock conduits that connect caves (McCandless 2005, pp. 54-55).

Major rivers are thought to be natural dispersal barriers for northern cavefish. Fluvial (rivers and streams) barriers may not be permeable to larger subterranean aquatic fauna (e.g., crayfishes, cavefishes), as they cannot travel through small solution or alluvial channels that likely are filled with sediment and glacial outwash in larger rivers like the Ohio River (Niemiller et al. 2013, p. 1021). Therefore, the Green River and its northern tributary, Nolin River, potentially act as barriers to northern cavefish dispersal. In addition, it is likely that the Rough Creek Fault Zone (RCFZ) is a dispersal barrier for northern cavefish (Figure 3), as it significantly disrupts the continuity of the limestone containing the caves the species occupies. There are likely at least two northern cavefish groups separated by the Rough Creek RCFZ: one south of the ridge centered in the Mammoth Cave region in Edmonson County and the other north of the ridge centered in the Sinking Creek Valley in Breckinridge County (Niemiller et al. 2013a, p. 1013). We treated the two areas as species' representation units in our SSA, given the very limited connectivity between them.

It is possible that most caves occupied by northern cavefish are hydrologically linked and therefore represent only a few large population centers. However, some research suggests that these population centers exhibit a metapopulation structure (Pearson and Boston 1995, p. 88; McCandless 2005, pp. 170, 54-56), whereby a single large population (metapopulation) is

composed of subpopulations with varying levels of spatiotemporal connectivity. Genetic and survey data indicate that northern cavefish observed in individual caves are part of a larger metapopulation where some sites serve as sources comprised of many individuals and good habitat while others likely represent sinks that require the immigration of these individuals to maintain a viable population in less suitable habitat (Niemiller 2021, pers. comm.). Based on expert elicitation (Kuhajda, Pers. Comm. 2021) that relied on information on genetics, geology, subterranean hydrology, and distance between northern cavefish sites, a total of six metapopulations were delineated for the northern cavefish. Three metapopulations each are in the northern and southern representation units, which are separated by the RCFZ (Figure 4). Two of the six metapopulations have not been surveyed recently (since 2000) to determine whether they are still extant. Two purported populations (sites 7 and 8, in Figure 4) are from single anecdotal observations of fish that could not be verified as northern cavefish and were not included in our analysis of the status of the species.

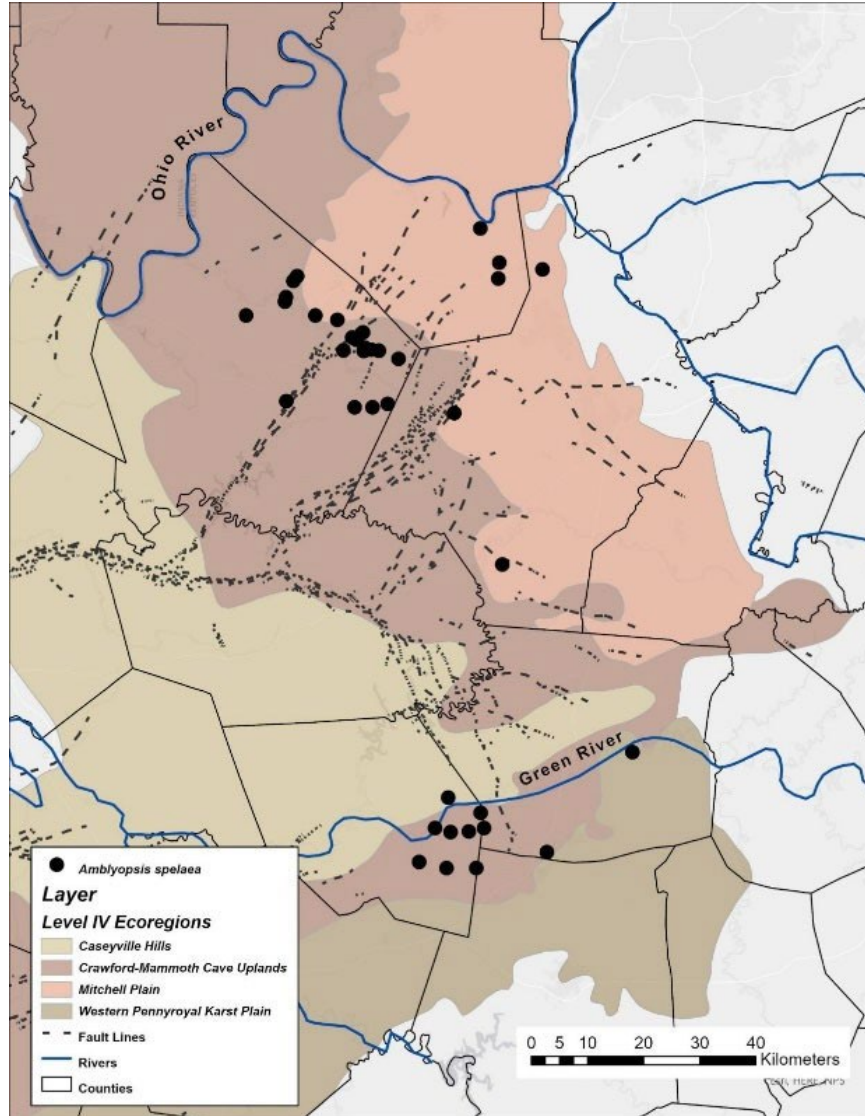


Figure 3. Northern cavefish sites and the level IV ecoregions they occupy. Fault lines are included, with the main east-west fault series referred to as the Rough Creek Fault Zone (general Rough Creek area outlined in grey; Johnson and Shwalb 2021, p.85). Imagery provided by the Tennessee Aquarium Conservation Institute

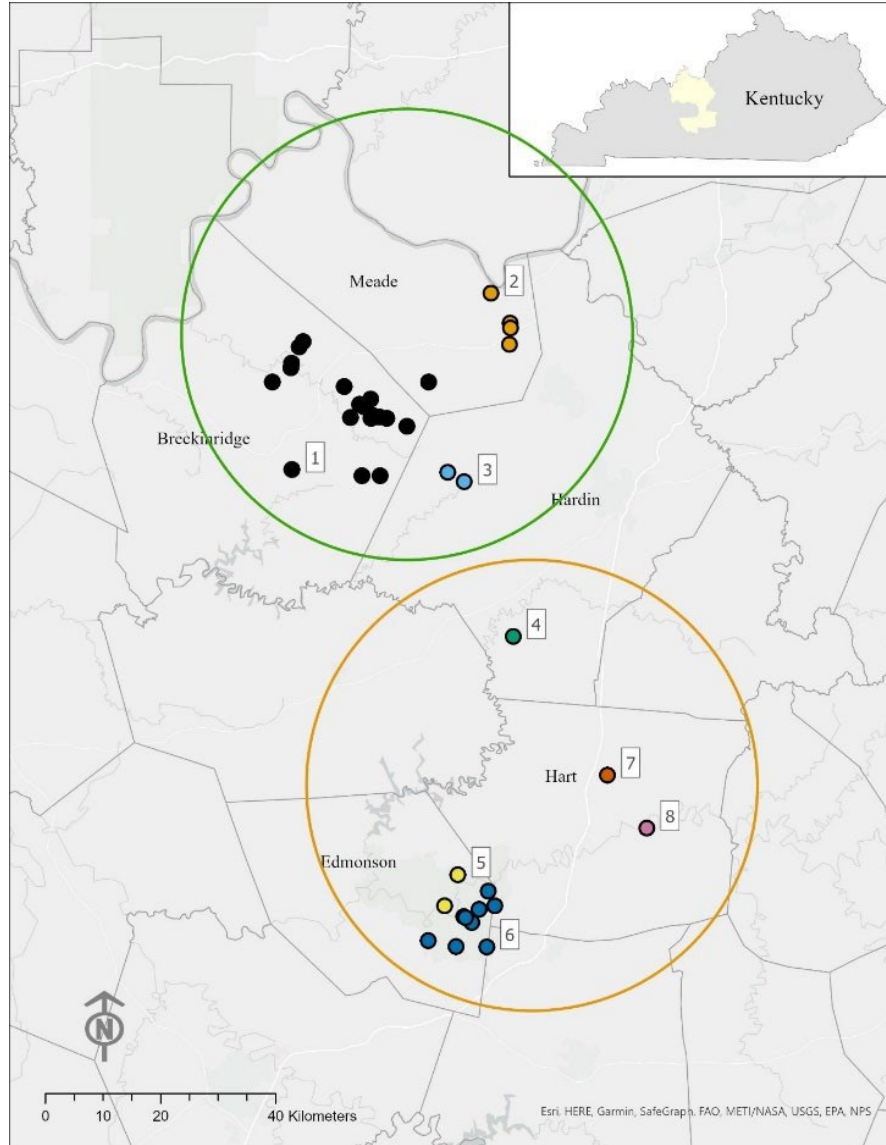


Figure 4. Population delineation for the northern cavefish. Six historical populations (grouped by color and numbers 1-6); with four of the six verified extant at this time. Two unsubstantiated sites (7 and 8) were not considered to influence or contribute to the status of the northern cavefish. Representative units included (north unit in green; south unit in orange).

For additional information on the species description, taxonomy, habitat/life history, historical and current range/distribution please refer to pp. [14-31] of the SSA report. For additional information on population and species needs, please refer to pp. [49-76] of the SSA report.

FACTORS INFLUENCING THE STATUS

The Act directs us to determine whether any species is an endangered species or a threatened
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species because of any factors (or threats) affecting its continued existence (i.e., whether it meets the definition of a threatened species or an endangered species). We use the term “threat” to refer in general to actions or conditions that are known to or are reasonably likely to negatively affect individuals of a species. The term “threat” includes actions or conditions that have a direct impact on individuals, as well as those that affect individuals through alteration of their habitat or required resources. The term “threat” may encompass—either together or separately—the source of the action or condition, or the action or condition itself.

However, the mere identification of any threat(s) does not necessarily mean that the species meets the statutory definition of an “endangered species” or a “threatened species.” In determining whether a species meets either definition, we must evaluate all identified threats by considering the expected response by the species, and the effects of the threats—in light of those actions and conditions that will ameliorate the threats—on an individual, population, and species level. We evaluate each threat and its expected effects on the species, then analyze the cumulative effect of all of the threats on the species as a whole. We also consider the cumulative effect of the threats in light of those actions and conditions that will have positive effects on the species—such as any existing regulatory mechanisms or conservation efforts. The Secretary determines whether the species meets the definition of an “endangered species” or a “threatened species” only after conducting this cumulative analysis and describing the expected effect on the species now and (if evaluating whether a species is a threatened species) in the foreseeable future.

Threats

A detailed discussion of northern cavefish threats is provided in Chapter 3 of our SSA report (Service 2022, pp. 30-48). An overarching threat to northern cavefish populations is pollution of the karst system (underground drainage system with sinkholes and caves) through water contamination (Adams et al. 2020, p. 318). Contaminants enter water bodies through both point and nonpoint sources, including spills, industrial sources, mining and quarrying, municipal effluents, and runoff from agricultural and developed areas. Threats that contribute to water contamination include expansion of land uses such as agriculture, urban development, transportation, mining, clear cuts, and logging infrastructure (especially roads), and impoundments (Pearson and Boston 1995, pp. 85-89; Proudlove 2001, pp. 201-203). Poor land use practices may destroy or degrade northern cavefish habitat through alteration of surface drainage that feeds karst systems.

Municipal, industrial, and other water contamination associated with development may pose a threat to the northern cavefish’s habitat. Contamination may be acute (such as a spill that occurs in a single moment) or chronic, over a longer period. In a Hart County cave, a southern cavefish (*Typhlichthys subterraneus*) population was severely impacted by groundwater pollution from domestic sewage, industrial waste, and chemical spills in the 1980s (Niemiller and Fitzpatrick 2013, p. 42). Northern cavefish occur in Hart County, although are not known from the same cave where the pollution affecting the southern cavefish occurred. Additionally, oil and gas

development is a potential threat to northern cavefish via releases of contaminants to groundwater. For example, in Barren County, just south of the Mammoth Cave population of northern cavefish, a southern cavefish site was impacted by suspected illegal injection of waste brine from nearby oil and gas operations (Niemiller and Fitzpatrick 2013, pp. 56-57).

Agriculture and forest loss can contribute to groundwater pollution if not properly managed, including use of pesticides, fertilizers, animal waste, and underground storage tanks. For example, a fertilizer pipeline break within the recharge zone of a spring in Missouri resulted in the death of at least 1,000 southern cavefish (Niemiller and Fitzpatrick 2013, p. 44). Pesticides (those applied to crops) have been implicated as the source of “broken back syndrome” in a cave inhabited by the Hoosier cavefish in Indiana (Keith and Paulson 1981, entire). Two fish with the syndrome were tagged and recaptured after two months and were found to still be alive. A population census of the affected cave revealed a slight decline in reproduction, but it was not statistically significant (Keith and Paulson, 1981 p. 47-48). Untreated fecal material adds excess organic material to the water which decays and depletes the water of oxygen, which may kill fish. High fecal coliform counts at Rimstone Cave in Breckinridge County, which is occupied by northern cavefish, was attributed to the large number of surface ponds used for watering livestock and the scattered homes with septic systems in the recharge area (Niemiller and Fitzpatrick 2013, pp. 55-56). High levels of nitrogen and phosphorus inputs have been recorded from caves near Mammoth Cave, which is occupied by northern cavefish, and are associated with sewage from urbanized areas, runoff from confined animal feeding operations on pasture lands, and fertilizer runoff from croplands after precipitation events (Pearson and Boston 1994, p. 22). Intentional sinkhole dumping of waste is a problem in the vicinity of the northern cavefish site, Under the Road Cave, in Breckinridge County (Niemiller and Fitzpatrick 2013, p. 56).

Impoundments have been identified as a potential threat to cavefish populations, however we lack data on how northern cavefish individuals and populations may respond to changes in the water table. Previous dam construction on the Green River in Kentucky resulted in increased water levels in Mammoth Cave, increasing deposition of silt and sediment, and reducing the input of particulate organic matter, a major food component in the aquatic cave system (Proudlove 2001, p. 202). The construction of the Green River Lock and Dam 6 (GRLD6) below Mammoth Cave has resulted in increasing flood levels over time, and the Styx and Echo River areas in the Mammoth Cave system experienced subsequent declines in cave biota (Elliott 2000, p. 672).

In our SSA, we considered the potential impact of four threats and determined they likely have limited influence on northern cavefish relative to the influence of the primary threats to the species (i.e., land uses that contribute pollutants to the karst system): (1) climate change, (2) declines in cave-hibernating bats due to the fungal disease, white-nose syndrome, (3) collection of northern cavefish, and (4) human traffic in caves.

Regarding climate change, fluctuations in stream discharge due to drought conditions have the potential to lead to fluctuations in groundwater temperature, although species experts have noted that fluctuations of temperature outside of the range of tolerance for northern cavefish does not seem to be a threat to the species currently (Kuhajda, Pers. Comm., 2021). At the opposite extreme, flash flooding associated with heavy precipitation events can impact populations through degradation and modification of habitat and displacement, injury, or death of individuals. At the center of the northern cavefish's range, climate models project temperatures to be warmer and wetter. These predicted increases in precipitation and subsequent flooding events can have positive and negative effects on the northern cavefish, but the impacts to individuals and populations are essentially unpredictable (Poulson 2001, p. 348). Southern cavefish, which partly overlap the range of northern cavefish, were assessed as "presumed stable" to the impacts of climate change (Glick et al. 2015, p. 99). Due to the uncertainty in effects (positive and negative) and particularly the uncertainty in the response of northern cavefish to climate change, we found climate change will likely have both positive and negative influences on the northern cavefish. Although the net effect to the species due to climate change cannot be predicted, climate change is likely to have a small influence on the species relative to the potential influence of land uses that contribute pollutants to the karst system.

The contribution of bats, particularly summer bat residents (primarily gray bats (*Myotis grisescens*) in Kentucky) to the *Amblyopsis* food chain is uncertain. Top predators in caves, such as salamanders (*Eurycea spelaea*), have been documented feeding directly on bat guano (Fenolio et al. 2006, entire), and three southern cavefish (*Typhlichthys subterraneus*) in a Tennessee cave inhabited by gray bats had guano visible in their guts that may have been ingested incidentally (Niemiller and Fitzpatrick 2013, p. 48). The tendency for northern cavefish to occur deeper in caves and in smaller passages not typically occupied by bats may mean the decomposition of plant material is more important than guano in these areas. In addition, gray bats exhibit low prevalence of the fungus causing white-nose syndrome and have not experienced dramatic population declines due to the disease (Powers et al. 2016, p. 133; Jackson et al. 2022, p. 2). As such, we did not find declines in some North American cave-roosting bats to be having a species-level effect on the northern cavefish.

Capturing and keeping individual northern cavefish has been noted as a threat for the species (Niemiller and Fitzpatrick 2013, p. 57). Some researchers have speculated that collection of fish in the Echo River and River Styx sections of Mammoth Cave led to dramatic declines of populations during the 1800s (Poulson 1968; Elliott 2000), although it appears that this threat was minimal in other populations (Niemiller and Fitzpatrick 2013, p. 57). Cavefishes are not legally sold in the pet trade, and they are not useful for food or bait given the small population sizes and density of most species (Adams et al. 2020, p. 320). Although northern cavefish may have been collected in the 1800s, the best available information does not indicate that these

activities are currently happening. Based on the above evidence, overcollection for commercial or scientific purposes is not a current threat to the northern cavefish.

Increased human traffic in cave systems has been noted as a potential threat to northern cavefish (Niemiller and Fitzpatrick 2013, p. 57). Commercial exploitation of caves can degrade and potentially destroy cavefish habitat through increased human traffic, leading to direct disturbance of individuals, as well as increased nutrient inputs and light levels (Pearson and Boston 1995, pp. 87-88; Niemiller and Fitzpatrick 2013, p. 57; Adams et al. 2020, p. 318, 320). There is evidence of indirect impacts due to commercial cave tours for three populations of southern cavefish, and cave tours at Mammoth Cave have the potential to impact northern cavefish, although there is much uncertainty surrounding the exact impacts and long-term effects of cave tour operations (Niemiller and Fitzpatrick 2013, p. 57; Adams et al. 2020, p. 320). Among the more than 2,500 caves in Kentucky (Keith 1988, p. 77), there are two inhabited by northern cavefish where commercial tours are offered (Adams et al. 2020, p. 320). It is unlikely the majority of cave system where northern cavefish occur have been affected by human disturbance and there have been no reported observations of northern cavefish being directly harmed by human traffic.

Conservation Measures and Existing Regulatory Mechanisms

Some conservation actions that benefit the northern cavefish have been implemented (e.g., removal of impoundments on Green River). Other activities are underway (e.g., dye tracing to delineate drainage basins and potential sources of contamination). Delineating drainage basins and potential sources of contamination, as well as subsequent protection of surface and subsurface drainage basins, is probably the most important conservation measure to protect the species (Niemiller and Poulson 2010). For example, the Kentucky Geological Survey has mapped sinkholes throughout the state, as well as karst valleys that are mostly confined to the Western Pennyroyal physiographic region, which includes the range of the northern cavefish.

Protection and conservation of the northern cavefish also results from the Kentucky Cave Protection Law (KRS 433.877), which makes it unlawful to remove, kill, harm, or otherwise disturb any naturally occurring organism found within any cave, except for safety or health reasons, and it provides for scientific collecting permits. Additionally, the northern cavefish and its habitats are afforded some protection from water quality and habitat degradation under the Clean Water Act of 1977 (33 U.S.C. 1251 et seq.), Kentucky's Agriculture Water Quality Act of 1994 (KRS 224.71-140), Kentucky's Groundwater Protection Plan (KRS 224; 401 KAR 5:037), Kentucky's Forest Conservation Act of 1998 (KRS secs. 149.330–355); Kentucky's Agriculture Water Quality Act of 1994 (KRS secs. 224.71–140); and additional Kentucky laws and regulations regarding natural resources and environmental protection (KRS secs. 146.200–360; KRS sec. 224; 401 KAR secs. 5:026, 5:031). However, current laws may not completely protect the northern cavefish and its habitats from nonpoint-source pollution. Local ordinances in Meade, Hart, Hardin, and Edmonson Counties also provide for protection of sinkholes, which reduces the risk of degradation of cavefish habitat.

The northern cavefish receives incidental protection under the Endangered Species Act of 1973 (Act), as amended (16 U.S.C. 1531 et seq.) due to habitat overlap with the federally endangered Kentucky cave shrimp (*Palaemonias ganteri*). However, only a small part of northern cavefish range, exclusively in Edmundson and Hart Counties, is shared with the Kentucky cave shrimp.

The northern cavefish likely benefits from implementation of federal management plans where it occurs on land owned by the National Park Service and Department of Defense. The Mammoth Cave National Park Cave and Karst Management Plan (2019) provides a consistent framework for managing the cave and karst resources in the park and the broader Mammoth Cave Area International Biosphere Reserve. The Fort Knox Integrated Natural Resources Management Plan (INRMP, 2018) guides the implementation of the natural resources program for the U.S. Army Garrison, Fort Knox, Kentucky. The program, through the INRMP and its Endangered Species Management Plans helps to ensure the conservation of Fort Knox's natural resources while maintaining/emphasizing compliance with related environmental laws and regulations. Measures in the INRMP and management plans that afford protection to the northern cavefish include that no stocking of trout (a potential predator) is to occur in the stream draining the cave inhabited by the northern cavefish, as well as maintaining a fenced entry to that cave; a requirement for consulting with the Service and State agencies on pest management activities; and requirements for erosion and sediment control for land disturbing projects.

Cumulative Effects

We note that, by using the SSA framework to guide our analysis of the scientific information documented in the SSA report, we have analyzed the cumulative effects of identified threats and conservation actions on the species. To assess the current and future condition of the species, we evaluate the effects of all the relevant factors that may be influencing the species, including threats and conservation efforts. Because the SSA framework considers not just the presence of the factors, but to what degree they collectively influence risk to the entire species, our assessment integrates the cumulative effects of the factors and replaces a standalone cumulative-effects analysis.

ANALYSIS

Our review of the threats to northern cavefish needs revealed several influences that pose risks to the viability of the species. These risks are largely related to groundwater contamination, which can be linked to habitat changes from anthropogenic land uses. Therefore, we first evaluated the 3Rs under current land use patterns to assess the species' current condition. Next, we assessed the species' future condition, using land use change projections and socioeconomic pathways from climate change models.

CURRENT CONDITION

There have been limited quantitative studies to determine the rangewide population status and

trend of northern cavefish to date; therefore, it was not possible in the current condition analysis to directly assess population resiliency using estimates of population size. Although population size estimates are lacking, for many sites where there are recent (since 2000) and historical survey data showing persistence over time, we can infer that there is some resiliency but the level of resiliency (e.g., low or high) is not possible to measure. Previous mark-recapture censuses of northern cavefish provide insight regarding densities of fish that would be expected in a high-resiliency or healthy population. In habitats of different complexities and numbers of hiding places, these surveys provided estimates of fish density ranging from 1.5 to 2.5 times the number of fish censused per square meter (Niemiller and Poulson 2010, p. 229). Using a multiplier factor of 1.9 at the Penitentiary Cave site, Kentucky, where, as reported in 1995, 519 northern cavefish were censused in an area of 3702 square meters, yielded an estimate of 2643 fish per hectare. Penitentiary Cave is the largest known subpopulation of northern cavefish and the fish density value there, estimated in 1995, likely represents a high resiliency population. The level of resiliency also may be indicated by the number of subpopulations that comprise a metapopulation (within metapopulation redundancy). However, there is substantial uncertainty in estimating the true number of subpopulations, as many areas in proximity to the metapopulations cannot be surveyed. Known subpopulations represent a minimum number, on which our analysis is based. Changes in representation and redundancy for the species were inferred based on changes from the historical distribution.

Attributes used to assess current population (metapopulation) condition are shown in Table 1 and described as follows:

- Verified Extant refers to populations observed since 2000, given northern cavefish likely live 20 years or longer (the oldest “extant” record is from 2002). Although Populations 5 and 3 are not verified as extant, the sites where they occurred have not been surveyed since 2000 and their persistence or extirpation at these sites cannot be confirmed.
- A Substantiated Site is considered a subpopulation in a metapopulation (Populations 1 through 6 were considered metapopulations in our analysis).
- Greater than 20 Fish Post 2000 is the maximum number observed in any survey after year 2000. We note that 20 individuals observed would be lower than a mark-recapture census estimate and, while the spatial area where the observations occurred is not reported or readily available to estimate fish density, the true number of fish at a site given 20 or more observed is likely in the hundreds, or possibly more (discussed above in CURRENT CONDITION).
- Maximum Number Ever Recorded is the maximum number of fish observed in any reported survey, regardless of date of observation.
- Recruitment is a measure of new cohorts or generations being added to a population and

is indicated by observations multiple size classes or the presence of small individuals that are likely juveniles.

- Distance is the straight-line length between nearest substantiated sites and is a measure of connectivity.
- Modeled Inherent Groundwater Vulnerability ratings are from the COP model (Vias et al. 2006, entire), in which C refers to concentration of flow, O refers to overlying soil layers, and P refers to precipitation. As indicated by the model output, inherent vulnerability of the ground water for surface watersheds associated with each population is ranked as Very High due to the high number of sinkholes throughout the species' range.
- Percent Area Potential Contaminant Source is the percentage of land use types (e.g., developed, cropland, pasture, etc.) that are potential sources of groundwater contamination. Where there are two surface watersheds overlying the subterranean drainage occupied by the population, two percentages, one for each surface watershed, are shown.
- Percent Protected Lands values are from the 2019 Protected Areas Database, the official national inventory of U.S. terrestrial and marine protected areas, and includes the best available aggregation of federal land areas provided directly by managing agencies. Where there are two surface watersheds overlying the subterranean drainage occupied by the population, two percentages, one for each surface watershed, are shown.

Table 1. Indicators of northern cavefish population resiliency (described further in text above).

Population	Verified Extant?	Substantiated Sites	>20 Fish Post 2000	Maximum Ever Recorded	Recruitment	Distance (km)	Modeled Inherent Groundwater Vulnerability	% Area Potential Contaminant Source	% Protected lands
1	Yes	16	Yes;	515 in 1994	Yes	13	Very High	47% & 54%	0.01% & 0%
6	Yes	6	Yes;	75 in 1994	Yes	3	Very High	59% & 32%	9.6% & 64%
2	Yes	4	No;	~20 from 1975 - 85	Yes	13	Very High	53%	19%
4	Yes	1	No;	2 in 2008	No	22	Very High	67%	1%
5	No	2	No;	8 in 1987	n/a	3	Very High	59% & 32%	9.6% & 64%
3	No	1	No	2 in 1994	n/a	11	Very	49%	0.1%

							High		
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At this time, four of six historical populations of northern cavefish are verified extant. Two populations (3 and 5) have not been verified as extant or extirpated because they have not been surveyed in over 25 years. Of the known extant populations, two (Populations 1 and 2) are in the north representation unit and two (Populations 4 and 6) are in the south representation unit. Risk of water quality contamination is high for all populations due to the large number of sinkholes distributed across the range of the northern cavefish, although there are no documented water contamination events that have affected the species to date. Furthermore, the dispersion of the populations confers redundancy to the species from a catastrophic event, as all populations are unlikely to be affected simultaneously because the groundwater connections between them are limited or lacking. The Sinking Creek area (Population 1) in the north and the Mammoth Cave system (Population 6) in the south have the highest number of fish observed and the highest number of subpopulations (16 and 6 subpopulations, respectively) and are likely the most resilient.

Within the six surface watersheds in the range of the northern cavefish, the largest protected areas are the federally owned Mammoth Cave National Park (43935 ha), where Population 6 occurs, and Fort Knox US Army Installation (9507 ha), where Population 2 occurs. Together, these Federal lands constitute over 90 percent of protected areas within the range of the species.

FUTURE CONDITION

Northern cavefish resiliency, representation, and redundancy were projected into the future using approximately 30 and 50-year time horizons (to years 2050 and 2070, respectively). These time horizons were chosen to correspond to the range of available urbanization and land use change model forecasts. The 2050 timeframe provides a near-term projection with relatively reliable forecasts, while the 2070 timeframe represents a potential longer-term trajectory for the species, but with greater uncertainty. Furthermore, approximately 30 and 50 years represent timeframes for the species to respond to potential changes on the landscape and is estimated to correspond to approximately three to four and five to six generations, respectively.

The primary future driver of species condition, anthropogenic land use, was assessed in two future scenarios developed to project northern cavefish future condition at the 2050- and 2070-time steps (Table 2). The future scenarios reflect differing land use change projections incorporating climate change and socioeconomic pathways through use of the USGS Forecasting Scenarios of land use (FORE-SCE) model (Sohl et al. 2018, data release). To forecast viability using these projections, we assessed the future condition in the context of risk to ground water quality (a habitat-based analysis). Because there is uncertainty as to the level and extent of future urbanization and land use change, we used the two scenarios to capture low and high rates of change. These future scenarios provide a range of plausible predictions of threats to viability on the species. The two FORE-SCE projection storylines in our analysis include the A2 storyline (is

similar, but not identical to RCP 8.5 and a higher emissions scenario, particularly late century) and B1 (is similar, but not identical to RCP 4.5 and a lower emissions scenario throughout the timeline to 2100) (Nakicenovic et al. 2000, entire; Sohl et al. 2014, entire). Under scenario A, the area of new urban lands is low due to a focus on environmentally friendly lifestyles and “smart” urban growth (Sohl et al. 2014, p. 1021), and urbanization is concentrated in urban centers. The environmentally focused scenario A showed agricultural land declines, while forested areas did not show these same declines (Sohl et al. 2014, p. 1022). Under scenario B, agricultural lands (cropland and hay/pasture) increased and forested area declined. Scenario B also had the highest rate of urban increase, but the increase was not necessarily concentrated in urban centers; where agricultural land (cropland or hay/pasture) extent decreased through time, it was typically converted to urban landcover (Sohl et al. 2014, p. 1022). Regardless of scenario or time step, projected land cover changes were small. As such, in the foreseeable future, the threat level experienced by northern cavefish is not expected to improve or worsen much relative to current conditions, and overall, resiliency, redundancy, and representation are unlikely to change.

Table 2. Percentage of total land cover composed of incompatible land cover types (i.e., development, mining, and agriculture) within watersheds occupied by delineated northern cavefish populations for current (2020) and future (2050 and 2070) timesteps, under two scenarios (FORE-SCE SRES B1 (Scenario A in SSA) and A2 (Scenario B in SSA)). Site 8 based on unverified report of species and is not considered to contain a valid population (table from SSA report, p. 80)

Watersheds (& Populations)	Scenario A			Scenario B		
	B1 2020	B1 2050	B1 2070	A2 2020	A2 2050	A2 2070
Sinking Creek (1)	40%	37%	37%	46%	47%	51%
North Folk-Rough River (1)	49%	43%	42%	56%	54%	57%
Otter Creek-Ohio River (2)	48%	47%	48%	53%	55%	61%
Clifty Creek-Rough River (3)	46%	46%	47%	50%	53%	61%
Middle Nolin River (4)	62%	65%	66%	69%	73%	65%
Beaverdam Creek-Green River (5 & 6)	28%	27%	28%	30%	30%	33%
Ugly Creek-Green River (5, 6, & 8)	56%	56%	57%	58%	60%	65%

FINDING

Regulatory Framework

Section 4 of the Act (16 U.S.C. 1533) and its implementing regulations (50 CFR part 424) set forth the procedures for determining whether a species is an “endangered species” or a “threatened species.” The Act defines an endangered species as a species that is “in danger of extinction throughout all or a significant portion of its range,” and a threatened species as a

species that is “likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.” The Act requires that we determine whether any species is an “endangered species” or a “threatened species” because of any one or a combination of the following factors:

- (A) The present or threatened destruction, modification, or curtailment of its habitat or range;
- (B) Overutilization for commercial, recreational, scientific, or educational purposes;
- (C) Disease or predation;
- (D) The inadequacy of existing regulatory mechanisms; or
- (E) Other natural or manmade factors affecting its continued existence.

These factors represent broad categories of natural or human-caused actions or conditions that could have an effect on a species’ continued existence. In evaluating these actions and conditions, we look for those that may have a negative effect on individuals of the species, as well as other actions or conditions that may ameliorate any negative effects or may have positive effects.

The Act does not define the term “foreseeable future, which appears in the statutory definition of “threatened species.” Our implementing regulations at 50 CFR 424.11(d), as revised in 2019, set forth a framework for evaluating the foreseeable future on a case-by-case basis. The term “foreseeable future” extends only so far into the future as we can reasonably determine that both the future threats and the species’ responses to those threats are likely. In other words, the foreseeable future is the period of time in which we can make reliable predictions. “Reliable” does not mean “certain”; it means sufficient to provide a reasonable degree of confidence in the prediction. Thus, a prediction is reliable if it is reasonable to depend on it when making decisions.

It is not always possible or necessary to define the foreseeable future as a particular number of years. Analysis of the foreseeable future uses the best scientific and commercial data available and should consider the timeframes applicable to the relevant threats and to the species’ likely responses to those threats in view of its life-history characteristics. Data that are typically relevant to assessing the species’ biological response include species-specific factors such as lifespan, reproductive rates or productivity, certain behaviors, and other demographic factors.

Status Assessment

Status Throughout All of Its Range

After evaluating threats to the species and assessing the cumulative effect of the threats under the section 4(a)(1) factors, we assessed the current status of the northern cavefish throughout its range. Historically, there were at least six metapopulations (single population with subpopulations at different sites and some connectivity between sites) of northern cavefish. Two of those populations have not been surveyed since the 1990s and cannot be confirmed to be

extant or extirpated. Based on occurrence records since 2000 (assuming the species has a life span of 20 - 40 years), the other four northern cavefish metapopulations are known to remain extant in two representation units. The representation units are separated by the Rough Creek Fault Zone, which is likely a barrier to cavefish dispersal. Population resiliency was not directly assessed; however, the number of individuals encountered during surveys of most sites is 20 or less; but some sites (subpopulations) have documented hundreds of northern cavefish (Pearson and Boston 1995, p. 49; McCandless 2003, p. 20; Pearson and Jones pers. comm. in McCandless 2005, pp. 180-190; Niemiller and Fitzpatrick 2013, pp. 42-44).

Northern cavefish may be negatively impacted by groundwater contamination via runoff or intentional disposal of wastes in sinkholes, which are predominant landscape feature in the species' range. While there is risk of a spill or surface release of contaminants to groundwater, there have been no documented cases of northern cavefish being harmed by such an event. In addition, it is unlikely contamination events would affect all populations, as the two representation units are separated by a fault zone barrier. Further, there is redundancy of subpopulations within three of the four known, extant metapopulations (at least one metapopulation in each representation unit has multiple subpopulations). Because there has been little to no change in the range and the available information does not indicate a loss of any populations of northern cavefish and there are multiple subpopulations distributed across a wide area, which buffers the impacts of adverse events, the current risk of extinction is low. Thus, after assessing the best available information, we conclude that the northern cavefish is not in danger of extinction throughout all of its range.

Therefore, we proceed with determining whether the northern cavefish is likely to become endangered within the foreseeable future throughout all of its range. Our future conditions analysis for the northern cavefish used projections of land uses and climate to assess potential ground water contamination. to 30- and 50-year time horizons. It is reasonable to rely on these time horizons because they correspond to the range of available urbanization and land use change model forecasts. Furthermore, approximately 30 and 50 years represent time frames for the species to respond to potential changes on the landscape and is estimated to correspond to approximately 3-4 and 5-6 generations, respectively. Two scenarios were projected, one under which human population growth economic development is slow, and another under which development is more rapid. Climate in the species' range is expected to be warmer and wetter but is unlikely to be a major threat to the species at the time horizons considered in our analysis. Likewise, under both scenarios and time horizons, the portion of developed land is expected to change very little. Given the projected small changes in threats and land use to 2070, we expect the northern cavefish will maintain species' redundancy and representation similar to current levels. In addition, the best scientific information indicates population condition has not substantially changed over time and is not expected to change in the foreseeable future given the projected small changes in land uses and threats. After assessing the best available information, we conclude that the northern cavefish is not likely to become an endangered species within the foreseeable future throughout all of its range.

Status Throughout a Significant Portion of Its Range

Under the Act and our implementing regulations, a species may warrant listing if it is in danger of extinction or likely to become so in the foreseeable future throughout all or a significant portion of its range. Having determined that the northern cavefish is not in danger of extinction or likely to become so in the foreseeable future throughout all of its range, we now consider whether it may be in danger of extinction or likely to become so in the foreseeable future in a significant portion of its range—that is, whether there is any portion of the species’ range for which it is true that both (1) the portion is significant; and (2) the species is in danger of extinction now or likely to become so in the foreseeable future in that portion. Depending on the case, it might be more efficient for us to address the “significance” question or the “status” question first. We can choose to address either question first. Regardless of which question we address first, if we reach a negative answer with respect to the first question that we address, we do not need to evaluate the other question for that portion of the species’ range.

In undertaking this analysis for the northern cavefish, we chose to address the status question first. First, we evaluated the range of the northern cavefish to determine if the species is in danger of extinction in any portion of its range. The range of a species can theoretically be divided into portions in an infinite number of ways. We focused our analysis on portions of the species’ range that may meet the definition of an endangered species or a threatened species. For northern cavefish, we considered whether the threats or their effects on the species are greater in any biologically meaningful portion of the species’ range than in other portions such that the species is in danger of extinction now or likely to become so in the foreseeable future in that portion. We examined the following threats: Groundwater contamination, agriculture, logging, mining, municipal and industrial development, surface water impoundments, and climate change, including cumulative effects.

We evaluated both the northern and southern representation units to assess the potential for either area to have a different status because the units are likely isolated. Groundwater contamination is the primary, potential threat for the northern cavefish due to the inherent vulnerability of the karst ecosystem the species inhabits. Based on the best available science, this threat is not concentrated, nor is the species response more pronounced in a specific portion of the range such that the species’ status may be different in that portion of its range. Further, both units exhibit sufficient resiliency such that neither portion is likely to have a different status. In addition, there is redundancy of subpopulations within at least three of the four known, extant metapopulations (one in each representation unit). Given the current species’ redundancy with extant subpopulations within each representation unit of northern cavefish, we found, at the scale of the two units, no portion of the range where the species is currently in danger of extinction.

Considering even smaller portions of the range in our evaluation of status, with each of the four areas occupied by the metapopulations as a portion, Population 4 may be in danger of extinction

because surveys have found just two individuals at an individual site and has no known subpopulations. Populations 2, 6, and 1, with 4, 6, and 16 subpopulations, respectively, likely have sufficient redundancy of subpopulations to withstand stochastic disturbances and are, therefore, not currently in danger of extinction. Even if Population 4 is truly small and isolated, as very limited survey effort suggests, there is nothing unique or important to the life history processes of the northern cavefish afforded by the habitat Population 4 occupies. The size of the subterranean areas occupied by the four metapopulations is not known. However, based on individuals observed and number of known subpopulations within each of the four metapopulations, it can be inferred that Population 4 likely occupies the smallest area. Even if we assumed all four areas are equal in size, Population 4 would contain 25 percent of the species range, and we cannot determine with any certainty that are only 4 populations. Given the small area likely occupied by Population 4 and the lack of unique important habitat features in the area it occupies, we find that the area where Population 4 occurs does not constitute a significant portion of the species range.

We then considered whether there was any portion of the range that may have a different status from the remaining portions in the foreseeable future. When evaluating projections in land use under the two future scenarios, the species' representation and redundancy is expected to remain similar to current levels. Accordingly, we found that, in regard to the two representation units the northern cavefish is not likely to become an endangered species within the foreseeable future and does not have a different status than the remainder of the species' range. As previously noted, when each of the four populations is considered as a portion of the range, Population 4 may currently be in danger of extinction but the area it occupies does not currently constitute a significant portion of the range.

Therefore, we find that the species is not in danger of extinction now or likely to become so in the foreseeable future in any significant portion of its range. This does not conflict with the courts' holdings in *Desert Survivors v. Department of the Interior*, 321 F. Supp. 3d 1011, 1070-74 (N.D. Cal. 2018), and *Center for Biological Diversity v. Jewell*, 248 F. Supp. 3d 946, 959 (D. Ariz. 2017) because, in reaching this conclusion, we did not apply the aspects of the Final Policy on Interpretation of the Phrase "Significant Portion of Its Range" in the Endangered Species Act's Definitions of "Endangered Species" and "Threatened Species" (79 FR 37578; July 1, 2014), including the definition of "significant" that those court decisions held to be invalid.

Date: 11/15/2023



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