REPTILES & AMPHIBIANS

Survey of Southern Missouri Plethodontidae in Ozark Caves

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Abstract.—Plethodontidae is a large family of salamanders with a distribution ranging from eastern North America to northern South America. Three genera and nine species occur in Missouri, most of which are typically found in caves for at least part of their lives. These species have been recorded to emerge from hibernation in early April and throughout May. This study shares the species abundance and diversity in ten Missouri Ozark caves: seven near St. Louis and three near Springfield. Each cave was surveyed at least once between November 2021 and September 2022 using visual encounter surveys. Observational data were recorded without handling any individuals to minimize disturbance. The three caves near St. Louis had few to no salamanders present in early April, despite optimal weather conditions, but tended to have larger populations throughout the summer. Two caves near Springfield held at least a dozen individuals of multiple species in mid-April. Few individuals were recorded in the most southwestern cave sampled, potentially due to pollution or recent flooding. Our findings could aid others studying plethodontid salamanders to determine the prime conditions for emergence from hibernation, optimal habitats for different species in each cave zone, and potential biological or chemical impacts that could affect salamander abundance in caves.

Karst topography is characterized by geological features that include cave systems, sinkholes, and springs, and occurs in regions containing soluble bedrock that has been dissolved over time by carbonic acid present in groundwater (Dreybrodt 1988). The presence of limestone or dolomite bedrock and the historical consistency of precipitation and groundwater circulation have created ideal conditions for karst development in the Ozarks. Thousands of caves have been identified and this area is among the most significant karst regions in the United States (Kemmerly and Siska 2008). The Ozarks karst region is significant at the national level for its biodiversity and endemic taxa (Culver et al. 2000). Conservation of this karst region is necessary for the protection of endemic species, the relative sensitivity of this type of topography, and the high degree of connectivity between areas that this type of geology supports. Pollutants can move and spread rapidly through local karst groundwater systems (Bauffaut and Benson 2009). Biodiversity surveys of subterranean systems are an important tool for natural resource management, land use planning, and assessing ecosystem changes (e.g., Zigler et al. 2020).

Amphibian populations are useful indicators of environmental quality because of their sensitivity to ecological changes, important role within food webs, and ease of encounter and visual field identification compared to other taxa (Hopkins 2007). The permeability of amphibian skin used for gas exchange, makes these organisms particularly vulnerable to environmental contaminants (Rohr and Palmer 2005). Amphibians also serve as important links between trophic levels and between terrestrial and aquatic environments (Regester et al. 2008). They play important roles in nutrient cycling and community composition in caves (Bradley 2018). Additionally, amphibians are relatively easily collected and confined in artificial environments, lending themselves well to study in comparison to other vertebrates (Hopkins 2007). The above traits are especially important in subterranean habitats, in which environmental changes due to factors such as surface pollution or climate change can be difficult to measure. Together these traits illustrate the importance of performing salamander diversity surveys and maintaining historical records of species presence, location, and behavior.

Two important factors to consider when surveying cave environments for amphibians are the degree to which the species are adapted to subterranean life and how common they are in the region. Many organisms found in caves are adapted to permanently inhabit this environment, whereas others may use the cave for only part of their lives. Troglobionts are animals that live in caves permanently and have features adapted for cave living; troglophiles live in caves but also can be found on the surface and are not cave-adapted; and trogloxenes are occasionally reported from caves but require the surface environment for a significant part of their life cycle (Elliott 2007).

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Having current, updated abundance data for managers to effectively conserve lands is important. An effective, low-cost tool for this with amphibians is conducting regional observation-based abundance surveys.

The most recent cave faunal inventory in the greater Ozark region (Graening et al. 2006) focused on the Buffalo National River region in four counties of northern Arkansas. The second-most abundant vertebrate reported was an amphibian, the Cave Salamander (*Eurycea lucifuga*), which was found in 53 of 143 sites inventoried. The same study also found Dark-sided Salamanders (*Eurycea melanopleura*) at 17 sites, Grotto Salamanders (*Eurycea spelaea*) in 25 caves, and Western Slimy Salamanders (*Plethodon albagula*) in 37 caves. A slightly earlier inventory in 64 caves in Pulaski County, Missouri, at a U.S. Army installation found 11 Cave Salamanders in seven caves, five Grotto Salamanders in three caves, two Ozark Zig-zag Salamanders (*Plethodon angusticlavius*) in one cave, and 22 Western Slimy Salamanders in 12 caves (Taylor et al. 2003).

Based on these two previous inventories, salamanders expected to be documented in our survey of southern Missouri should be lungless salamanders in the family Plethodontidae. Plethodontids are the largest and most diverse group of salamanders and occupy a variety of regions and moist habitats including caves, forests, springs, streams, and seeps. In the Ozarks, plethodontids are the only salamanders likely to be observed in caves, although other groups such as the mole salamanders (Ambystomatidae) spend much of their adult lives underground in burrows and could be considered to occupy shallow subterranean habitat (Culver and Pipan 2014; Burkhart 2018). The six taxa we expected to encounter in our survey were Cave Salamanders, Long-tailed Salamanders, Dark-sided Salamanders, Grotto Salamanders, Western Slimy Salamanders, and Southern Red-backed Salamanders (*Plethodon serratus*).

The Cave Salamander (*Eurycea lucifuga*) is bright orange to yellow with dispersed dark spots along its body (Briggler and Johnson 2021). *Eurycea lucifuga* is considered secure (G5/S5) in Missouri (NatureServe 2023) and is classified as a troglophile (Elliott 2007; Ringia and Lips 2007), although the lifestyle of this species might differ depending on location and season across the United States. Facultative cave-dwelling salamanders are more likely to be found in caves in higher numbers during late spring and summer. A likely explanation for this behavioral pattern is that salamanders use caves at least partially to avoid hot, dry epigean conditions (Camp et al. 2014). The amount of moisture present on the walls and floor of a cave has a direct effect on the presence of *E. lucifuga*. This species tends to be found close to the entrance of caves during moist seasons when the entrance is consistently damp. When the epigean environment and the mouth of the cave are drier, these salamanders retreat farther into the cave twilight and dark zones, where humidity is likely to be higher (Hutchison 1958). As is characteristic of salamanders in general, the reproductive cycle of *E. lucifuga* is intrinsically linked to water. This species has demonstrated a preference for slow-flowing streams, and usually lays eggs in cave streams between June and November, when water levels are low (Ringia and Lips 2007). They deposit their eggs on underwater rocks, suggesting that one reason for their preference for slow-moving water is the protection of hatchlings from swift water (Hutchison 1958).

The Long-tailed Salamander (*Eurycea longicauda*) and the Dark-sided Salamander (*E. melanopleura*), until recently considered to be subspecifically related (Raffaëlli 2022), are yellow, yellow-orange, or green-yellow, with longitudinal dark brown or black marks and spots along bodies and tails; these salamanders also are characterized by large eyes, slender bodies, and stout limbs (Briggler and Johnson 2021). The distribution of *E. longicauda* includes the Ozarks karst region of Arkansas, Kansas, Missouri, and Oklahoma, as well as the eastern U.S. karst regions, where it has been recently reported from cave surveys in Georgia and Tennessee (Niemiller et al. 2016; Slay et al. 2016; Zigler et al. 2020), whereas *E. melanopleura* is found in the Ozark karst region and adjacent western Illinois (Frost 2023). The species (*sensu lato*; i.e., both "subspecies") is considered secure (G5/S5) in Missouri (NatureServe 2023). Adults are terrestrial and live in riparian wooded areas of streams and ponds, whereas larvae are entirely aquatic (Briggler and Johnson 2021). We consider these species to be troglophiles as both can be found in caves for parts of their life cycles. These salamanders have been recorded from shale banks, springs, spring runs, riparian zones, flood plains, caves, and mines (Anderson and Martino 1966). They are most active at dusk on humid or rainy nights and are generalist invertebrate predators (Briggler and Johnson 2021). Breeding takes place from late fall to early spring. Females lay 60 to 100 eggs on the undersides of rocks in cold streams and springs, typically in caves, and hatching takes place 4–10 weeks later depending on local ambient temperatures (Briggler and Johnson 2021). The Dark-sided Salamander might hybridize with the Cave Salamander (Slay et al. 2016).

The Grotto Salamander (*Eurycea spelaea*) is a slender, flat-headed species, partially or completely blind with small eyes, and may exhibit a range of colors from white to pink due to the lack of pigmentation (Briggler and Johnson 2021). The species is considered a troglobiont and is endemic to wet caves of the Ozark Highlands in Missouri, where it requires total darkness and springs or running water (Briggler and Johnson 2021). Adults mate in the late spring or early summer, likely due to the influx of prey items at this time of year; eggs are laid 1–4 months after mating, and larvae tend to be found in streams both inside and/or outside of caves (Briggler and

Johnson 2021). Larvae commonly feed on small invertebrates from bat guano piles and tend to have the largest populations in caves with bat colonies (Slay et al. 2016; Briggler and Johnson 2021). The species is considered imperiled (G4/S2) in the Ozarks region, and regions with deteriorated groundwater habitats are considered a threat to the species (Slay et al. 2016; NatureServe 2023).

The Western Slimy Salamander (*Plethodon albagula*) is all-black with randomly distributed white spots all over its body (Briggler and Johnson 2021). Populations are known from throughout the Ozarks karst region in southern Missouri, northern and western Arkansas, and also from the Balcones Escarpment area of south-central Texas (Frost 2023). *Plethodon albagula* is considered secure (G5/S5) in Missouri, Texas, and Arkansas, but its conservation status is unknown in Oklahoma (NatureServe 2023). The species occasionally occurs in caves and is more commonly found under rocks or logs in damp ravines and moist wooded hillsides (Johnson 1977). Eggs are laid in damp protected locations underground, and females remain with their eggs until hatching in late summer and early fall; the larval stage is not aquatic, and larvae reach maturity within three years (Garrett and Barker 1987). The species is most active at night or after heavy rains, and its diet primarily consists of small arthropods and annelids (Briggler and Johnson 2021). The species is poorly vagile, with home ranges of individuals ranging from 3–26 m and dispersal ranges within 300 m (Mahoney 2001).

The Southern Red-backed Salamander (*Plethodon serratus*) is black with a thick red middorsal stripe (Briggler and Johnson 2021). The species is distributed in Missouri, Oklahoma, Arkansas, Tennessee, North Carolina, Texas, Louisiana, Alabama, and Georgia; within the Ozarks karst region the species has been reported from northern and southeastern parts of the region (Slay et al. 2016; Briggler and Johnson 2021). Throughout its range it is considered secure (G5/S5) (NatureServe 2023). In the drier summer months, the species seeks shallow subterranean habitat, burrowing underground or in springs and seepages, which can complicate detection of the species. It is known to occur in the twilight zone of caves but more typically is found in woodland areas under rocks, moss, and deadwood (Herbeck and Semlitsch 2000). We consider the species a troglophile. Southern Red-backed Salamanders breed from December to March, lay their eggs from May to July, eggs hatch between September and October, and larvae mature 24–36 months after hatching (Herbeck and Semlitsch 2000).

Methods

In the field, surveyors were equipped appropriately for both the safety of the surveyors and protection of cave life and the fragile physical cave environment with synthetic quick-drying clothing, boots, gloves, helmets, and at least three sources of light per surveyor. Field equipment was cleaned prior to entering caves following established protocols to prevent the spread of White-Nose Syndrome, a fungal disease that invaded cave environments in the mid-2000s and devastated bat populations across the United States (Puechmaille et al. 2011; WNS Decontamination Team 2012). Permission was obtained from landowners or property managers prior to entering each cave.

Observational data were recorded from ten Missouri caves in the Ozarks karst region (Fig. 1): two caves in Christian County (Breakdown Cave, Fitzpatrick Cave); two caves in Crawford County (Cathedral Cave, Onondaga Cave); four caves in Franklin County (Fisher Cave, Hamilton Cave, Indian Cave, Sheep Cave); one cave in Greene County (Junction Cave); and one cave in Washington County (Green's Cave). Dimensions of the cave entrances were measured, and coordinates were taken with either a handheld GPS unit or a mobile phone, although we do not provide coordinates to comply with U.S. cave conservation practices (only approximate locations of caves sampled are indicated in Fig. 1). Temperature and humidity were measured using a FisherScientific digital thermohygrometer at the drip line of the cave entrance, the point where a salamander was first encountered, and at the start of the dark zone. General notes on the outside condition of the cave were taken in addition to notes on the abundance and diversity of each amphibian encountered. Photographs were taken with a mobile phone for each salamander that could not be immediately identified in the field. On-site identification was aided by a field identification guide for Ozark cave life (Slay et al. 2016) and off-site identification was done by comparing photographic vouchers with descriptions and photographs in that guide and in Briggler and Johnson (2021). The map (Fig. 1) was created

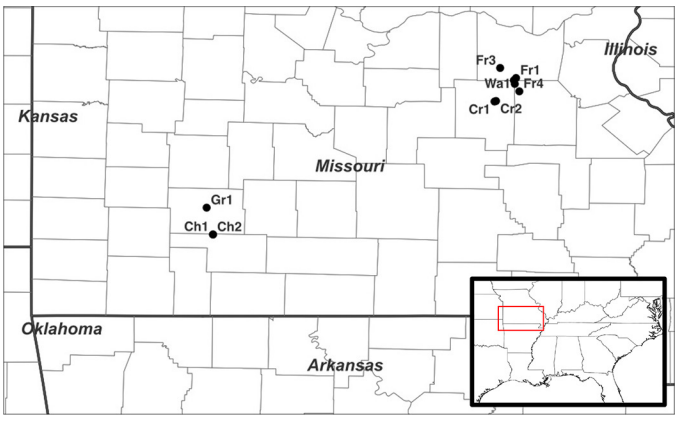

Figure 1. Map of southern Missouri showing the approximate location of Ozark caves sampled during this study (inset: southeastern USA). Christian County: Ch1 = Breakdown Cave, Ch2 = Fitzpatrick Cave; Crawford County: Cr1 = Cathedral Cave, Cr2 = Onondaga Cave; Franklin County: Fr1 = Fisher Cave, Fr2 = Hamilton Cave, Fr3 = Indian Cave, Fr4 = Sheep Cave; Greene County: Gr1 = Junction Cave; Washington County: Wa1 = Green's Cave.

with QGIS v. 3.26 "Buenos Aires," using geographic data layers downloaded from GADM v.1 (GADM 2022).

Christian County.—Breakdown Cave was surveyed twice, on 9 April 2022 by a team of seven and on 10 October 2022 by a team of four. It is privately owned and located ~13 km south of Springfield and near the James River. The entrance (Fig. 2G) is small (~3 m wide by 0.5 m high) and leads to a crawling passage that is gated; in both April and October the entrance exterior was heavily vegetated. Little light penetrates beyond the gate. The cave is horizontal with two levels and no pits; following the entrance zone crawling passage, passage dimensions in the transition and dark zones are variable but never exceed 30 m, with some standing passage in wide rooms and several small side passages that fan out and reconnect with the main passage. Permanent pools and a slow-moving stream passage with water of subterranean origin are farther into the cave. The nearby James River occasionally floods part of the passage, as evidenced by debris washed into the cave at ceiling height. Surveys were conducted in the late morning in April and in the early evening in October.

Fitzpatrick Cave was surveyed on 9 April 2022 by a team of six and on 10 October 2022 by a team of two. The cave is privately owned, gated, and does not have a stream or standing pools of water. Although evidence of human activity exists, the cave is relatively well-preserved. The entrance (Fig. 2B) measures approximately 3.5 m wide and 1 m tall and is on a north-facing slope with dense vegetation that does not allow for much sunlight penetration; as a result, the environment around the cave is cool, damp, and covered by mosses. The multiple entrances of these caves are situated on the same slope and are separated by a few hundred meters; however, no subterranean connection has been discovered that connects passages of the two systems. The entrance and transition zones inside Fitzpatrick Cave were dry and contained little life.

Crawford County.—Cathedral Cave is a public show cave in Onondaga Cave State Park. Its primary entrance is closed off by a door, with an 'airlock chamber' through which all visitors must pass before the interior cave door is opened. The door to Cathedral Cave is visible after a ten-minute hike on a rocky park trail. The entrance is ~2 m tall and 1 m wide. We followed a concrete path that extends throughout the cave. The cave was surveyed on 4 September 2022 in the early afternoon by a group of three. Permission to access and survey Cathedral Cave was granted by the head naturalist of the state park.

Onondaga Cave is another popular public show cave in Onondaga Cave State Park. The main entrance is an unnatural entrance which is accessed through a door in the visitor center building. The entrance passage is approximately 1.8 m tall and 1 m wide. We followed the concrete tour path until we reached Missouri Caverns, which is a non-toured portion of Onondaga Cave. At the end of the Missouri Caverns

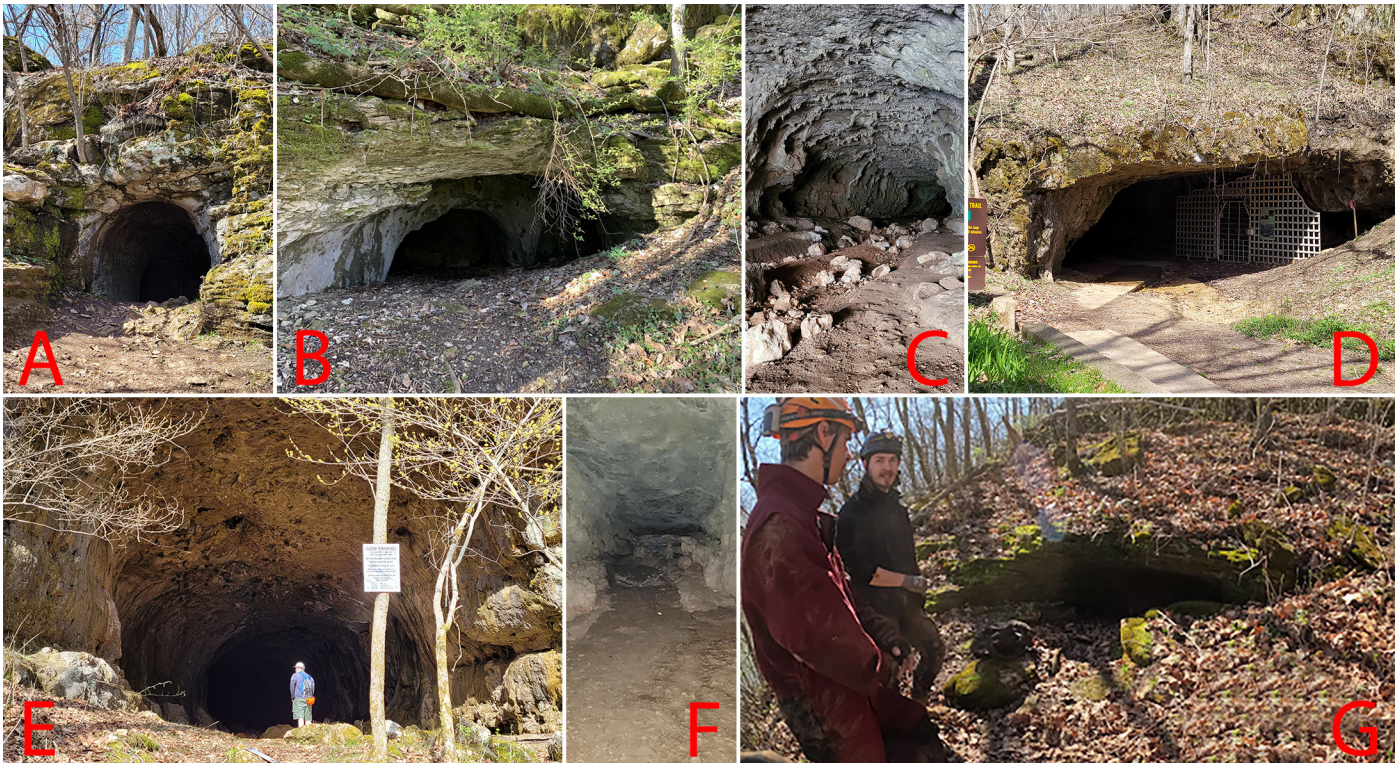

Figure 2. Entrances of Ozark caves surveyed during this study: Junction Cave in Greene County (A); one of three entrances to Fitzpatrick Cave in Christian County (B); interior of Sheep Cave (C); Fisher Cave in Franklin County (D); Sheep Cave in Franklin County, with a surveyor for size reference and a posted sign that discourages the public from entering during the winter due to White-Nose Syndrome (E); interior of Indian Cave in Franklin County (F); Breakdown Cave in Christian County (G). Photographs taken by Samantha Grove (A, C, D, E, F) and Clayton Austin (B, G).

passage, there are stairs that lead to a locked door which used to open to the surface. Onondaga Cave was surveyed on 3 September 2022 in the late afternoon by two people. Permission was given to enter Onondaga Cave by the head naturalist of the state park.

Franklin County.—Fisher Cave is a large public show cave in Meramec State Park. The approximately 4-m tall and 15-m wide entrance (Fig. 2D) faces a parking lot and is gated. The entirety of the cave is approximately 3.5 km long, but only the first 100 m were surveyed. The cave widens and narrows at various points with a slow moving stream through the entrance and twilight zones. Fisher Cave was surveyed four times throughout the spring and summer months on 3 April, 22 June, 8 July, and 15 July 2022. Fisher Cave surveys were conducted by 2–3 persons during various times of day and surface conditions. We followed the concrete path to observe salamanders. Permission to enter all caves located in Meramec State Park was granted by the head naturalist of the park.

Hamilton Cave is located in Meramec State Park. It was surveyed once on 11 September 2022 by four people. This cave is hidden in the woods in Hamilton Hollow, accessible only by hiking an extremely overgrown path. The cave entrance is approximately 7.5 m tall and 4 m wide with a large gate prohibiting entry approximately 6 m inside the drip line. In the past we found many plethodontid salamander larvae in a cave stream flowing out of the entrance. The survey was conducted at dusk by looking in the cave stream, under rocks, and along walls outside the gate. The gate was not opened for this survey.

Indian Cave also is in Meramec State Park. It was surveyed twice by a team of two on 3 April and 22 June 2022. It is publicly accessible and about 400 m from a public hiking trail with a large wooden staircase leading to its entrance. The entrance measures approximately 4 m high and 30 m wide. A hallway measuring about 1.75 m high by less than 1 m wide leads into a small room measuring approximately 2 m tall and 2 m wide. Inside the cave (Fig. 2F), the floor is composed of clay, no stream is present, and noticeable breakdown and rimstone dams prevent further exploration. Indian Cave was surveyed in the early morning of both days.

Sheep Cave, also in Meramec State Park, was surveyed twice by a team of 2–3 on 3 April and 22 June 2022. This is a publicly accessible cave on a hillside near a creek, picnic area, and playground, with a fast-flowing stream inside. The entrance (Fig. 2E) is approximately 6 m tall and 4 m wide and extends approximately 240 m. The entrance was covered with lichens, mosses, and algae signifying recent and abundant water flow. The cave stream flows through the middle of the cave, so both sides of the stream were accessible for observing salamanders. On the 3 April visit, at 120 m, about halfway through the cave passage, rimstone dams and high water were encountered and the survey stopped at this point. Data collection on the 22 June survey stopped at the same rimstone dam. Both surveys were conducted in the early morning.

Greene County.—Junction Cave is in Ritter Springs Park, a municipal park managed by the City of Springfield. The cave is located ca. 10 m up the east side of a hill near the Little Sac River, does not contain a cave stream but does have humid areas resulting from penetration of water. We encountered no standing pools on any visit. The cave is not gated and is publicly accessible. Evidence of frequent human activity included broken glass on the cave floor, graffiti on the walls and ceiling, and charred wood from a fire. The entrance (Fig. 2A) is about 2 m in diameter and the cave passage stays consistently near that height. Surveys were conducted in the afternoon and late evening.

Washington County.—Green's Cave is in Meramec State Park. It was surveyed once by a team of three on 5 September 2022. The single entrance is located on a bluff adjacent to the Meramec River, several meters above the water line. The entrance is 7 m by 3 m, and the cave functions as a spring with groundwater flowing out the entrance. The cave is not gated; however, park signage discourages unsanctioned visitation. Most of this horizontal cave is stream passage. The survey began in the early afternoon during a light rain shower.

Results

We observed six species of plethodontid salamanders (n = 252) in ten caves (Table 1). Two individuals could not be positively identified. We also noted other vertebrate and invertebrate life in each cave. Salamander diversity (including one salamandrid) across all caves and counties is in Table 2.

Christian County.—On 9 April 2022 in Breakdown Cave we recorded 16 individuals (two *Eurycea lucifuga* and 14 *Eurycea* larvae) from a small pool of standing water in a side passage of the dark zone. Few other organisms were evident. On 10 October 2022 we recorded four *Plethodon albagula* from the same pool in the dark zone. Additionally, we observed a single Pickerel Frog (*Lithobates palustris*) in that pool. Environmental measurements taken on 10 October were 25 °C and 41% relative humidity (RH) in the entrance zone, 23 °C and 45% RH in the transition zone near the gate, and 23 °C and 56% RH in the dark zone at the pool where amphibians were found. The Shannon diversity index was 1.13 (Table 2).

On 9 April in Fitzpatrick Cave we recorded one *Eurycea longicauda* in a wall crevice and 12 *E. lucifuga* on the walls, in wall crevices, and underneath rocks in the dark zone. Other fauna noted in the transition and dark zones included bats (*Perimyotis subflavus*), cave crickets (*Ceuthophilus* sp.), spiders, and mosquitoes. Environmental measurements were 5 °C and 69% RH in the entrance zone and 14 °C and 68% RH in the dark zone. On 10 October we recorded one *Plethodon albagula* under a rock about 10 m from the entrance in the transi-

Table 1. Presence of plethodontid salamanders in sampled Ozark caves. Cave codes are listed in Fig. 1.

Species	Ch1	Ch2	Cr1	Cr2	Fr1	Fr2	Fr3	Fr4	Gr1	Wa1
Eurycea longicauda	—	X	—	—	X	—	—	—	—	—
Eurycea lucifuga	X	X	X	X	X	X	X	X	X	X
Eurycea melanopleura	—	—	—	—	X	X	—	—	X	—
Eurycea spelaea	—	—	X	—	—	—	—	—	—	—
Plethodon albagula	—	X	—	X	X	—	—	—	X	—
Plethodon serratus	—	—	—	—	X	X	—	X	—	—
Species A	—	—	—	—	—	X	—	—	—	—
Species B	X	—	—	—	X	X	—	—	X	—

Table 2. Shannon diversity indices (H) for counties and caves sampled for salamanders. Cave codes are listed in Fig. 1.

County	H (county)	Cave	H (cave)
Christian	0.46	Ch1	1.13
		Ch2	0.54
Crawford	0.55	Cr1	0.36
		Cr2	0.53
Franklin	1.26	Fr1	0.88
		Fr2	0.83
		Fr3	0
		Fr4	0.45
Greene	0.76	Gr1	0.76
Washington	0	Wa1	0
All counties	1.34	—	—

tion zone and two *E. lucifuga* and two *P. albagula* in the dark zone. Environmental measurements were 25 °C and 41% RH in the entrance zone and 26 °C and 41% RH in the transition zone. The Shannon diversity index was 0.54 for Fitzpatrick Cave and 0.46 for Christian County (Table 2).

Crawford County.—In Cathedral Cave we found one *Eurycea lucifuga* inside the airlock where humidity was high (20 °C and 70% RH) and another on the stairs immediately after entering the cave after the airlock room. At the end of the tour route, we found two small *E. spelaea* in a small pool of water. Two more *E. spelaea* were found in the cave stream near the "Cathedral Bell" flowstone formation. Temperature and humidity varied near the bell (18–20 °C, 59–63% RH). We found one additional *E. lucifuga* in a pool a few hundred meters from the entrance on our way back. The Shannon diversity index was 0.36 (Table 2).

No salamanders were found near the door or in the passage regularly used for tours in Onondaga Cave. Surveys were conducted in the "Missouri Caverns" section of the cave that is not regularly used by tours. At the end of "Missouri Caverns," humidity is high where steps and a door lead to the outside. Two *Eurycea lucifuga* and one *Plethodon albagula* were on the door of the cave and five *E. lucifuga* were on the walls and stairs near the artificial entrance. One additional *P. albagula* was approximately 3 m into the cave. We also observed one Ring-necked Snake (*Diadophus punctatus*), in the "Missouri Caverns" section. Environmental measurements were 23 °C and 43% RH at entrance, 24 °C and 69% RH 3 m into the cave where the first salamander was noted past the artificial entrance zone. The Shannon diversity index was 0.53 for Onondaga Cave and 0.55 for Crawford County (Table 2).

Franklin County.—In Fisher Cave we recorded two individuals of two species (one subadult *Eurycea lucifuga* and one adult plethodontid not identified to species) in the dark zone during the April 2022 survey. Other vertebrates noted included two species of hibernating bats (*Perimyotis subflavus* and *Myotis* sp.) and remains of a dead mammal (likely *Didelphis virginiana*). Environmental measurements taken from the dark zone were 15 °C and 47–49% RH. In June 2022 we recorded five individuals of three species, three *E. lucifuga* (Fig. 3B), one *E. longicauda*, and one *E. melanopleura* (Fig. 3C), on walls in the transition zone and 16 *E. lucifuga* in the dark zone. Environmental measurements were 21 °C in the transition zone and 13–19 °C in the dark zone (RH was not recorded). On 8 July 2022 we recorded 25 individuals of four species: three *E. lucifuga* and two *E. melanopleura* in the entrance zone; ten *E. lucifuga* and one *Plethodon serratus* in the transition zone; and seven *E. lucifuga*, one *E. longicauda,* and one *E. melanopleura* in the dark zone. Environmental measurements were 22 °C and 92–100% RH in the entrance zone, 20 °C and 90% RH in the transition zone, and 14–15 °C and 98% RH in the dark zone. On 15 July 2022 we recorded 24 individuals of four species: three *E. melanopleura*, one *E. lucifuga*, three *P. albagula*, and one *Eurycea* larva that was not identified to species in the entrance zone; three *E. lucifuga*, one *E. longicauda*, and one *P. albagula* on walls in

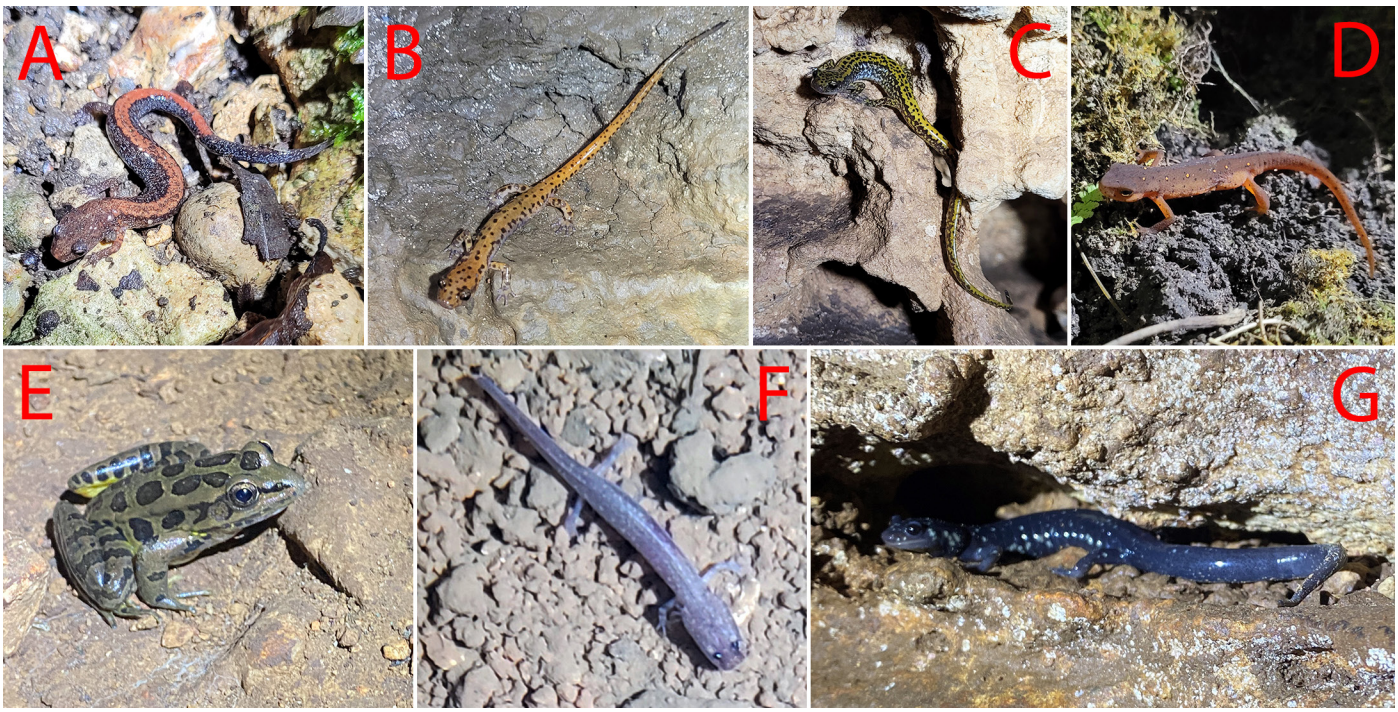

Figure 3. Representative amphibians encountered during surveys of Ozark caves in Missouri: Southern Red-backed Salamander (*Plethodon serratus*) in Hamilton Cave (A); Cave Salamander (*Eurycea lucifuga*) in Fisher Cave (B); Dark-sided Salamander (*Eurycea melanopleura*) in Fisher Cave (C); Central Newt (*Notophthalmus viridescens*) eft in Hamilton Cave (D); Pickerel Frog (*Lithobates palustris*) from Junction Cave (E); Grotto Salamander (*Eurycea spelaea*) from a cave in Shannon County (F), taken in October 2022; Western Slimy Salamander (*Plethodon albagula*) in Junction Cave (G). Photographs by Samantha Grove (A, B, C, D), Charles D.R. Stephen (E, G), and Gabrielle Campbell (F).

the transition zone; and 11 *E. lucifuga* on the ground, walls, and in the cave stream of the dark zone. Environmental measurements were 18 °C and 90% RH in the entrance zone, 15 °C and 94% RH in the transition zone, and 12 °C and 100% RH in the dark zone. The Shannon diversity index was 0.88 (Table 2).

In Hamilton Cave we observed 58 individuals: 40 *Plethodon serratus* (Fig. 3A), eight *Eurycea lucifuga*, six *E. melanopleura*, three *Eurycea* sp. larvae, and one eft of the Central Newt (*Notophthalmus viridescens*) (Fig. 3D). Salamanders were on walls, in the flowing cave stream, and under warm rocks inside and outside of the dripline. The temperature around the cave outside the dripline was 21–24 °C and RH was 29–61%; 21–22 °C and 46–56% RH in the entrance zone; and 22–23 ºC and 47–55% RH in the transition zone. Other wildlife seen included hundreds of bats (*Myotis* sp.) flying out of the cave at dusk. The Shannon diversity index was 0.83 (Table 2).

In April 2022 in Indian Cave we encountered no salamanders. No environmental measurements were taken but humidity in the cave was high. We also noted dozens of flying insects, pieces of trash, and graffiti. In June 2022 we recorded only one *Eurycea lucifuga* in the entrance zone, a second in the transition zone, and a third in the dark zone. Environmental measurements were not taken. The Shannon diversity index was 0 (Table 2).

In April 2022 in Sheep Cave we encountered no salamanders. No environmental measurements were taken. In June 2022 we detected three species of salamanders, three *Eurycea lucifuga* and one *Plethodon serratus* in the entrance zone and one *E. lucifuga* in the transition zone. Additionally, we encountered one Pickerel Frog (*Lithobates palustris*). No environmental measurements were taken. The Shannon diversity index was 0.45 for Sheep Cave and 1.26 for Franklin County (Table 2).

Greene County.—On 20 November 2021 in Junction Cave a team of ten persons observed 28 amphibians during two afternoon hours: 16 *Eurycea lucifuga*, ten *Plethodon albagula*, and two *Lithobates palustris*. On 5 April 2022 a team of three recorded 11 salamanders on the left wall and floor and seven salamanders on the right wall and ceiling in the late evening, when temperature was 24 °C and RH was 40%. Five of the salamanders were *P. albagula* (Fig. 3G), 12 were *E. lucifuga*, and one remained unidentified. Two *P. albagula* were juveniles, all others were adults. Nine salamanders were in the transition zone, seven in the entrance zone, and one in the dark zone. On 29 September 2022 CDRS surveyed the total extent of the cave for 0.5 h. Entrance conditions were 21 °C and 32% RH. He encountered 16 salamanders and 10 frogs, one *E. melanopleura*, eight *E. lucifuga*, seven *P. albagula*, and ten *Lithobates palustris* (Fig. 3E). One bat (*Perimyotis subflavus*) was on the ceiling in the dark zone and

did not present external signs of White-Nose Syndrome. The diversity index for this cave and county was 0.76 (Table 2).

Washington County.—In Green's Cave we found only two adult *Eurycea lucifuga* on walls, one in the transition zone (25 °C and 50% RH) and one in the dark zone (25 °C and 55% RH). Evidence of human disturbance was apparent by the small pieces of plastic trash littered through the cave. Three bats (*Myotis* sp.) were huddled together on the ceiling. The Shannon diversity index for this cave and county was 0 (Table 2).

Discussion

Across the ten caves surveyed, diversity was generally low and abundance varied widely, from no individuals in several caves to 58 individuals in a single survey of Hamilton Cave. The most plethodontids were recorded in Hamilton, Fisher, and Junction Caves. Possible reasons for the variation in abundances include varying levels of direct and indirect human disturbance, seasonality and environmental differences, and local geography. Differences in active seasons, weather conditions, and phenology could explain why we saw more of certain species than others.

Examples of direct human disturbance observed in our surveys include vandalism, such as damage or removal of speleothems and graffiti on walls and ceilings. Also, debris on the floors of caves included broken glass, aluminum cans, and other litter. Less intentional anthropogenic damage to caves from skin contact with limestone can introduce microbes, fungi, dirt, and oils that could ultimately impact the development of speleothems and potentially affect cave life (Bunting and Balks 2001).

Additional potential threats to cave life from indirect human disturbance comes from pollutants dissolved in water that infiltrate through the karst landscape into caves. These can include fertilizers, pesticides, road runoff, sewage, and industrial waste. Salamanders rely on water sources to reproduce, so pollutants in the watershed surrounding the caves can negatively impact the life cycles of cave-dwelling species. While adult salamanders have permeable skin used for gas exchange that make them vulnerable to water quality changes, the most at-risk are larval salamanders that must remain in flowing water, have highly sensitive gills, and need clean water to support a steady food supply (Forson and Storfer 2006). Notably, the three caves with the lowest diversity of species were the most easily accessible by the public. However, this is not the sole determining factor for salamander populations, as Junction Cave also is publicly accessible and had a relatively high abundance of troglophilic species.

Weather, seasonality, and time of day likely affected our data, although temperatures recorded inside caves generally reflect annual average temperature in the region. Nevertheless, all caves ideally would have been sampled on the same day or at least season and time of day.

Excessive rain can flood caves and introduce organic material. Two caves we surveyed (Breakdown and Fitzpatrick Caves) have entrances within the floodplain of the James River and are impacted by floods as evidenced by flotsam found in the caves. This section of the river is affected by upstream agricultural pollution from adjacent croplands in Christian County. Many organisms, including salamanders, are sensitive to these chemicals; therefore, we suspect pollutants affected the abundance and diversity of species observed in these caves. Prior to our visits to these caves, the James River had flooded. Our results serve to demonstrate how quickly flooding from polluted surface waters can affect subterranean life. Fitzpatrick Cave was noticeably humid and wet in the dark zone. For example, we saw minimal to no life in the area of the dark zone where a pool with a subsurface connection to the James River regularly floods. Although Breakdown Cave had high humidity and pools when not flooded, it was nearly devoid of life during our survey. Flooding of these pools could also have negative effects on cave life by changing the typical flow to which the organisms are adapted.

Time of year almost certainly influenced our data. The most frequently encountered species, *Eurycea lucifuga* and *Plethodon serratus*, were more abundant during summer months than in springtime. Salamanders usually emerge from hibernation in early spring and can be active throughout the summer. This was most evident in Fisher Cave, where only two individuals were found in early spring and the average number of individuals found during summer surveys was 18 salamanders. This pattern was not evident in every cave, likely due to other variables, including time of day and conditions in and around the caves.

For example, during early spring surveys, some salamanders might not have emerged from hibernation, and more abundant rainfall and temperature variation outside the cave might affect not only salamander activity but access to some cave passages. Ideally, future cave surveys should be conducted on a single day or serially over a period of a few months to a year in order to accommodate seasonal variations.

Our findings are relevant for management in Missouri for the six species we observed. Because some plethodontids use caves to overwinter but are more frequently seen in early spring and summer, our admittedly preliminary data could be used to identify conditions for emergence of salamanders from hibernation. Some of our other observations could lead to additional studies that might identify optimal habitats for different species in each cave zone, especially if certain species are present only in specific zones. Also, the variations in abundance that we observed, many likely attributable to human impact, could inspire research focusing on potential biological or chemical factors that affect cave life. Regardless of motivation, more studies must be conducted across many types of caves over multiple years to evaluate the variables

affecting plethodontid salamanders in subterranean environments.

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