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Via Email and Certified Mail, Return Receipt Requested

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RE: Notice of Violations of the Endangered Species Act Regarding Determination that Endangered Species Act Protection of the Berry Cave Salamander Is Not Warranted

Dear Secretary Haaland:

This letter serves as a 60-day notice of intent to sue the U.S. Fish and Wildlife Service ("Service") from the Southern Environmental Law Center on behalf of the Center for Biological Diversity ("Center") for violations of the Endangered Species Act ("ESA"),[1] relating to the Service's October 7, 2019, decision to deny listing protections to the Berry Cave salamander (*Gyrinophilus gulolineatus*) under the ESA.[2] The Center for Biological Diversity is a national, non-profit conservation organization supported by more than 1.7 million members and online activists. The Center is dedicated to securing a future for all species, great and small, hovering on the brink of extinction.

The Berry Cave salamander ("salamander") is a cave-obligate aquatic amphibian that occupies a very small range in eastern Tennessee. The species exists in extremely low numbers across nine or fewer caves and is imperiled by habitat loss and degradation, particularly from water quality declines and changes to stream flow associated with increasing urbanization and development in the surrounding area. The salamander's low abundance and limited range cause the species to be at an even greater risk of extirpation due to the effects of climate change, including increased droughts, and stochastic events such as severe storms.

Despite the ongoing threats facing the species and its documented decline, on October 7, 2019, the Service determined that ESA protections were "not warranted" for the salamander.[3] This determination arbitrarily and capriciously departed from the Service's

[1] 16 U.S.C. §§ 1531–1544.
[2] *See* 16 U.S.C. § 1540(g)(1)(C).
[3] 84 Fed. Reg. 53,338 (Oct. 7, 2019).

prior decision that the species is threatened or endangered, ignored substantial scientific evidence before the agency showing that the salamander faces extinction, and relied on hypothetical and unspecified future conservation measures, all in violation of the ESA and Administrative Procedure Act.

I. BACKGROUND – THE BERRY CAVE SALAMANDER

The salamander exists only in the subterranean waters of nine or fewer caves in eastern Tennessee.[4] As a cave-obligate species, the salamander cannot survive outside of these cave systems. Based on mark-recapture studies, home ranges for salamanders within the caves are also thought to be small, as individuals exhibit high site fidelity.[5] While little is known about the salamander's life history, it is thought to have a lifespan of 20 years or more.[6]

The salamander's diet consists of invertebrates, a food source dependent on the amount of detritus—nutrient-rich, organic material from vegetation on the surface—present in the watershed.[7] The salamander is found in water depths up to four meters and is typically observed resting on the bottom of pools or under rocks, logs, and other organic cover material.[8] The species is thought to require rock habitat of high quality and quantity to escape predators and to use as substrate for egg deposition.[9] Crucial to its survival is the availability of high-quality water; all life stages rely on sufficient water flow, and the species is very sensitive to pollutants.[10]

The salamander exists in extremely low numbers and is declining. While the species has historically been reported from twelve different caves or sites, the Service believes it to currently exist in only nine caves, and recent (2018) population surveys have only been able to confirm its continued presence in four of those.[11] In the majority of caves where it persists, its surveyed numbers have declined over at least the last ten to fifteen years, and in some cases the last thirty years, depending on when the cave was first surveyed.[12]

Ongoing threats to the salamander include chemical toxicants, sediment, fecal coliform bacteria, reduced detrital input, historic quarry operations, urbanization, collection, hybridization with spring salamanders, disease, and climate change.[13] These threats are compounded by the fact that the salamander exists only in very small population sizes, making it particularly vulnerable to environmental and demographic stochasticity.

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[4] U.S. Fish and Wildlife Service, Species Status Assessment for the Berry Cave Salamander (*Gyrinophilus gulolineatus*), iv (2019) ("SSA").
[5] SSA at 11.
[6] SSA at iv.
[7] SSA at 9.
[8] *Id.*
[9] *Id.*
[10] *See, e.g.*, SSA at 9, 12 (Table 2-1), 23.
[11] SSA at 16 (Table 2-2), 52–54 (Appendix A). Surveys of all eleven historically known caves, except for Christian Cave, in 2018 only found salamanders in Meads Quarry Cave, Mudflats Cave, Berry Cave, and the Lost Puddle.
[12] *See, e.g.*, SSA at 16 (Table 2-2), 52–54 (Appendix A).
[13] SSA at v, 17–23.

The precise suite of threats facing the salamander vary depending on the cave it inhabits. For instance, salamanders in Meads Quarry Cave face not only the threats of urbanization, fecal coliform bacteria, and climate change, but are also harmed by toxic waste leachate that remains in the cave from historic quarry operations.[14] The number of salamanders in Meads Quarry Cave—one of only two relative "strongholds" for the species—has declined significantly, and individuals with burn-like lesions resulting from the leachate have been observed.[15] This cave also faces threats from urban encroachment and resulting increases in sediment deposition, as well as "moderate to high" human visitation that can result in the crushing or collection of salamanders.[16] The other "stronghold," Berry Cave, similarly has its own unique stressors, as it is the only cave in which salamanders have been found with nodules of suspected parasitic origin.[17]

And while the Service believes that the salamander exists in low numbers in all the caves, some populations are threatened more severely by low abundance and lack of demographic complexity (e.g. variety in observed age classes). For instance, in the Meads River and Fifth Entrance Caves, only one salamander was observed during surveys in 2007, and zero salamanders were observed in 2018.[18] Aycock Spring and Christian Caves also likely have very low, if any, abundance, as only one salamander has been observed in each—more than 15 years ago.[19] Small Cave similarly has only one recorded salamander on record.[20] Mudflats Cave, where only two salamanders were observed in 2018 surveys, is also threatened by low abundance and is additionally harmed by water quality issues from adjacent urban development.[21] Additionally, Berry Cave salamanders may be threatened by hybridization with spring salamanders in Mudflats Cave, Meads Quarry Cave, Meads River Cave, and Small Cave, where the two species are known to coexist.[22]

II. STATUTORY FRAMEWORK

Congress passed the ESA to conserve endangered and threatened species and the ecosystems upon which they depend.[23] The Supreme Court's review of the ESA's "language, history, and structure" convinced the Court "beyond a doubt" that "Congress intended endangered species to be afforded the highest of priorities."[24]

A species is "endangered" if it "is in danger of extinction throughout all or a significant portion of its range."[25] A species is "threatened" if it "is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range."[26]

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[14] SSA at 17.
[15] *Id.*; *see* also SSA at 16 (Table 2-2), 27, 52–54 (Appendix A).
[16] SSA at 19, 27–28.
[17] SSA at 29.
[18] SSA at 16 (Table 2-2).
[19] *Id.*
[20] *Id.*
[21] SSA at 28 (Table 4-2), 30.
[22] SSA at 6, 20–21, 55–57 (Appendix B).
[23] 16 U.S.C. § 1531(b).
[24] *Tennessee Valley Authority v. Hill*, 437 U.S. 153, 174 (1978).
[25] 16 U.S.C. § 1532(6).
[26] *Id.* § 1532(20).

A "species" "includes any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature."[27]

Any person may petition the Service to list a species under the ESA.[28] Within ninety days of receiving a listing petition, the Service "shall make a finding as to whether the petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted."[29] "If such a petition is found to present such information, [the Service] shall promptly commence a review of the status of the species concerned[,]"[30] and within twelve months of receiving the petition, shall make and promptly publish a finding as to whether the proposed action is either "warranted," "not warranted," or "warranted, but . . . precluded by [other] pending [listing] proposals . . . ."[31] A negative twelve-month finding is subject to judicial review under the ESA.[32]

No matter how imperiled a species might be, it does not receive any protection under the ESA unless it is officially listed under Section 4 of the Act as either threatened or endangered.[33] In determining whether a species is threatened or endangered, the Service must consider five statutory listing criteria:

> (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; and
> (E) other natural or manmade factors affecting its continued existence.[34]

If a species meets the definition of threatened or endangered because it is imperiled by any one or a combination of these five factors, the Service must list the species.[35] The Service must base all listing determinations "solely on the basis of the best scientific and commercial data available."[36]

The lawfulness of the Service's conduct in making listing determinations is typically reviewed under the Administrative Procedure Act ("APA").[37] The APA governs the procedural requirements for federal agency decision-making and directs a reviewing court to "hold unlawful and set aside agency action, findings, and conclusions" that are "arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law," "without observance of the procedure required by law," or "in excess of statutory

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[27] *Id.* § 1532(16).
[28] *Id.* § 1533(b)(3).
[29] *Id.* § 1533(b)(3)(A).
[30] *Id.*
[31] *Id.* § 1533(b)(3)(B).
[32] *Id.* § 1533 (b)(3)(C)(ii).
[33] *Id.* § 1533.
[34] *Id.* § 1533(a)(1).
[35] *Id.* § 1533(1).
[36] *Id.* § 1533(b)(1)(A).
[37] 5 U.S.C. §§ 551 *et seq.*

jurisdiction, authority, or limitations, or short of statutory right."[38] An agency action is arbitrary and capricious under the APA where "the agency has relied on factors which Congress has not intended it to consider, entirely failed to consider an important aspect of the problem, offered an explanation for its decision that runs counter to the evidence before the agency, or is so implausible that it could not be ascribed to a difference in view or the product of agency expertise."[39]

III. THE SERVICE'S LISTING DECISION FOR THE BERRY CAVE SALAMANDER

Citizens and conservationists have long tried to secure ESA listing protections for the rare endemic Berry Cave salamander. And for nearly a decade, the Service had consistently assured the public of its plans to list the salamander, as it agreed the species was threatened or endangered and required legal protections. In 2019, however, the Service abruptly announced in a batched Federal Register notice that it no longer believed the salamander merited ESA listing.[40]

A. Listing History

On January 22, 2003, Dr. John Nolt, a University of Tennessee professor and Knoxville area resident, petitioned the Service to list the salamander as an endangered or threatened species. After the Service failed to act on the listing petition for more than seven years, the Center for Biological Diversity sued the Service on February 17, 2010, for its unlawful delay in issuing a 90-day finding on the petition.[41] On March 18, 2010, the Service published a 90-day finding in the Federal Register concluding that Dr. Nolt's petition presented substantial information indicating that listing the salamander may be warranted.[42] On March 22, 2011, the Service published a finding that listing the salamander under the ESA was warranted but precluded by higher priority species.[43]

The 2011 finding noted that two additional populations had been discovered since the 2003 listing petition—in Aycock Springs and Christian Caves—but still found that the present or threatened destruction, modification, or curtailment of the species' habitat or range (Listing Factor A) presented a "significant threat of moderate magnitude" due to increasing development, urbanization, and associated water quality impacts.[44] The 2011 decision also specifically found that the salamander was threatened or endangered under Listing Factor D (inadequacy of existing regulatory mechanisms) because habitat degradation and water quality declines were ongoing despite protections afforded by state and federal laws.[45] Additionally, the 2011 finding concluded that the salamander was threatened or endangered under Listing Factor E (other natural or manmade factors) because of the risk of hybridization between Berry Cave salamanders and spring salamanders, especially in Meads Quarry Cave, and discussed how the species is predicted to be particularly

[38] 5 U.S.C. § 706(2).
[39] *Motor Vehicle Mfrs. Ass'n of U.S., Inc. v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 43 (1983).
[40] 84 Fed. Reg. 53,335 (Oct. 7, 2019).
[41] Complaint, *Center for Biological Diversity v. Salazar*, 10-cv-00230 (D.D.C. Feb. 17, 2010).
[42] 75 Fed. Reg. 13,068 (March 18, 2010).
[43] 76 Fed. Reg. 15,919 (March 22, 2011).
[44] 76 Fed. Reg. at 15,923.
[45] 76 Fed. Reg. at 15,924 ("[W]e find the inadequacy of existing regulatory mechanisms to be a significant threat of high magnitude.").

vulnerable to the adverse impacts of climate change due to its limited range, limited dispersal ability, and dependence on subterranean aquatic environments in a region where drought has consistently been increasing over the last several decades and is expected to continue to increase, which will impact stream flow volumes and organic input into cave systems.[46]

For the next several years, the Service annually reaffirmed the salamander's status as a candidate species that warranted listing as threatened or endangered, including after discovering the salamander's presence in an additional cave in 2012.[47] Then, on October 7, 2019, the Service changed course and found that the salamander did not warrant listing, removing it from the candidate list.[48] The Service published the "not warranted" decision for the salamander as part of a batched Federal Register notice containing "not warranted" decisions for twelve species, half of which came from the Service's Southeast Region.[49] At that time, the Southeast Region was pursuing what it called a "wildly important goal" of downlisting, delisting, or precluding from listing an arbitrary quota of at least 30 species each year.[50]

B. The Species Status Assessment

Accompanying its 2019 listing decision, the Service published a "species status assessment" ("SSA") for the salamander evaluating what the Service refers to as the "3Rs"—resiliency (ability to withstand stochastic events), representation (ecological diversity across the species' range and ability to adapt to changing conditions), and redundancy (ability to withstand catastrophic events).

The Service divided the known salamander populations, which are distributed across nine caves, into six Analysis Units ("AUs"), two of which contained multiple caves. While the caves in AU1 (Meads Quarry, Meads River, Fifth Entrance), are generally considered to be part of the same system and are managed by Ijams Nature Center as part of Knoxville's

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[46] 76 Fed. Reg. at 15,925. The Service further explained that "[b]ecause the available evidence would suggest that the Berry Cave salamander exists in relatively low population densities and distribution is confined to subterranean waters within the Tennessee River and Clinch River watersheds, the species cannot readily tolerate losses of populations *or even many individuals*." *Id.* (emphasis added) (internal citations omitted).

[47] *See* 77 Fed. Reg. 69,994, 70,020 (Nov. 21, 2012) (noting that a new population was discovered at the Lost Puddle Cave in May 2012 but still finding that the species faces imminent threats and warrants listing); 78 Fed. Reg. 70,104, 70,125 (Nov. 22, 2013) (noting that salamanders were discovered in the Lost Puddle Cave in May 2012 and still retaining the same listing priority); 79 Fed. Reg. 72,450, 72,467–68 (Dec. 5, 2014) (maintaining listing priority); 80 Fed. Reg. 80,584, 80,597 (Dec. 24, 2015) (maintaining listing priority); 81 Fed. Reg. 87,246, 87,257 (Dec. 2, 2016) (maintaining listing priority).

[48] 84 Fed. Reg. 53,338 (Oct. 7, 2019).

[49] *Id.*

[50] *See, e.g.*, Clare Fieseler, *Tiny Flowers, Big Secrets: Why the Feds Want to Strip Protections from This Rare Plant*, Post and Courier (Mar. 23, 2023), https://www.postandcourier.com/environment/tiny-flowers-big-secrets-why-the-feds-want-to-strip-protectionsfrom-this-rare-plant/article_c060a0d4-c27b-11ed-ae0b-2f870242b096.html; Jimmy Tobias, *Fish and Wildlife is 'Conserving' Imperiled Animals by Denying Them Protection*, Pacific Standard (May 1, 2019), https://psmag.com/environment/fish-and-wildlife-is-conserving-nearly-extinct-animals-bydenying-them-protection.

Urban Wilderness, the caves in AU2 (Aycock Spring and Christian) are generally thought to be separate systems. The Service provided no evidence of connectivity between them and noted that there is no evidence of intercave dispersal of the salamander.[51]

The SSA first assessed the salamander's current condition and then made predictions about the species' future viability at each AU. The Service considered six elements that it asserts influence survival and reproduction of the species: abundance, population/demographic complexity, water quality (including toxicants, fecal coliforms, and sediment), availability of rock habitat, detrital load, and human visitation.[52] The Service evaluated the current resiliency of each AU in light of the aforementioned elements and the threats it deemed specific to each cave. The SSA determined the current resiliency of only AU3 (Berry Cave) to be "high;" the resiliency of AU1 (Meads Quarry, Meads River, Fifth Entrance) and AU5 (Lost Puddle) to be "moderate;" and the resiliency of AU2 (Aycock Spring and Christian Caves), AU4 (Mudflats Cave), and AU6 (Small Cave) to be "moderate to low."[53] After evaluating each AU's resiliency, the Service concluded that the species' redundancy as a whole is currently "moderate to low" and its representation is "not high" due to the species' "low overall adaptive potential."[54]

The Service next modeled the salamander's future viability under three different scenarios. Each scenario varied only in the level of conservation effort applied and used the same modeling results for urbanization and climate change.

Under all scenarios, a "significant level of increase in development is anticipated" adjacent to at least five of the six AUs.[55] This is expected to result in additional habitat degradation and higher levels of water contamination.[56] Likewise, under all scenarios, climate change is expected to cause increases in average and extreme temperatures, leading to lower dissolved oxygen levels and increased pathogen risks; increases in drought that will likely result in reduced groundwater flow, adversely impacting Berry Cave salamander habitat and potentially leading to population declines; and extreme precipitation events that will result in increased streambank erosion and sediment deposition, also adversely impacting water quality and flow.[57]

Under Scenario 1, however, the Service assumed that limited and unspecified conservation measures in the form of forested riparian habitat maintenance and a "low level of improvement" over current existing levels of conservation would mitigate these impacts from urbanization and climate change.[58] Under this scenario, the Service predicted that

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[51] SSA at 24 ("[M]ovement of Berry Cave salamanders from one cave to another within these units has not been documented . . . ."), 29 ("Although possible, a sub-surface hydrological connection between [Aycock Spring and Christian Caves] has not been documented.").
[52] SSA at 24.
[53] SSA at 28 (Table 4-2).
[54] SSA at 32.
[55] SSA at 33.
[56] SSA at 34.
[57] *Id.*
[58] SSA at 35, 38.

overall redundancy would be “moderate to low” through year 2080 and representation would remain “low.”[59] Resiliency for each AU would remain unchanged from the present.[60]

Under Scenario 2, conservation measures would be implemented to a “greater extent” than in Scenario 1.[61] The Service assumed conservation measures such as livestock fencing and/or installation of waste-containment structures, expansion of forested riparian zones, and removal of quarry waste materials will occur.[62] According to the Service, these measures would assist in mitigating negative effects of climate change.[63] Redundancy would remain “moderate to low” through 2080, and overall representation would remain “low.”[64] Thanks to these theoretical conservation measures, the two currently “moderate” resiliency AUs (AU 1 and AU5) would improve to a “high to moderate” status, and AU2 would improve from “moderate to low” to “moderate” resiliency.[65]

Under Scenario 3, conservation measures would remain limited, and no improvements in conservation would be assumed as in the other two scenarios.[66] The species would again exhibit “moderate to low” redundancy, and representation would remain “low.”[67] Berry Cave (AU3), the most stable population today, would decline to only “moderate” resiliency under this scenario, and three AUs (AU2, AU4, AU6)—half of all salamander populations—would face potential extirpation.[68]

The Service then predicted the likelihood of these three scenarios occurring over two different time periods: 11 years (to represent the species’ estimated generation time or the average difference in age between parent and offspring) and 61 years (to represent two to three lifespans). At 11 years, without pointing to any evidence for planned or site-specific conservation improvements, the Service predicted that Scenario 1 was very likely to occur, Scenario 2 was likely, and Scenario 3 was unlikely.[69] The Service then concluded that all three scenarios are “as likely as not” to occur at the 61-year timeframe, but noted that “because there is potential for implementation of conservation actions, our confidence in scenario 3 transpiring . . . is less than for scenario 2.”[70]

Despite its blind confidence in theoretical improvements in conservation efforts, the Service acknowledged that, to date, conservation work in the watersheds occupied by the salamander “has been limited.”[71] And rather than pointing to specific future conservation measures that would significantly change and improve current water quality management

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[59] SSA at 39.
[60] *Compare* SSA at 39 (Table 5-1) *with* SSA at 28 (Table 4-2).
[61] SSA at 40.
[62] *Id.*
[63] *Id.*
[64] SSA at 41.
[65] SSA at 40–41.
[66] The Service’s future scenarios also create confusion around the Service’s ultimate conclusions because the agency does not clearly identify whether it intends Scenario 3 to represent a baseline, or “status quo,” scenario or something else.
[67] SSA at 43.
[68] *Id.*
[69] SSA at 46.
[70] SSA at 46–47.
[71] SSA at 34.

regimes at the state or federal level, the Service merely contemplated vague potential conservation measures such as expansion of forested buffer zones along streams and "attentiveness in applying best management practices," which "could improve water quality."[72] The SSA also stated that prudent livestock management and removal or containment of waste leachate from the Meads Quarry Cave could benefit the species,[73] as could precautionary measures to minimize the spread of potential pathogens.[74] The SSA did not point to plans for any of these possible future conservation efforts.

C. The Listing Priority Assignment Form

The Service also completed a Species Assessment and Listing Priority Assignment Form ("Decision Form") to supplement its October 7, 2019, batched Federal Register notice, which had devoted only about 350 words to the Service's "not warranted" decision for the salamander. The Decision Form document summarized the contents of the SSA and concluded that the salamander is not threatened or endangered in all or a significant portion of its range.

In its summary of threats section, the Decision Form outlined several threats facing the salamander and explained that "any factor that impacts the [salamander's] physical habitat [or] water quality of the streams it inhabits will likely have a deleterious effect upon the species."[75] Like the SSA, the Decision Form did not identify any planned or proposed conservation efforts. Yet it doubled down on the SSA's assertions that "conservation efforts are likely to counteract some sources of stress to [salamander populations],"[76] and that "even if urbanization were to overcome conservation efforts, the species would be expected to persist as a result of the inherent adaptability that it has demonstrated to date."[77]

In its finding, the Service stated that the salamander does not warrant listing as an endangered species because its resiliency is "sufficient at each [AU] that the stressors are acting at the individual level and not raising to the population level." [78] This, it asserted, is demonstrated by the salamander's continued existence.[79]

Using a 50-year timeframe as its horizon to evaluate whether the salamander is likely to become endangered within the "foreseeable future," the Service concluded that, "the stressors acting on the Berry Cave salamander are not projected to substantially reduce the overall resiliency, redundancy, or representation of the species in the near term or within the next 50 years"[80] The Service then published the "not warranted" finding in its October 7, 2019, Federal Register notice.

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[72] SSA at 34.
[73] SSA at 23, 34.
[74] SSA at 34.
[75] U.S. Fish and Wildlife Service, Species Assessment and Listing Priority Assignment Form: Berry Cave Salamander (*Gyrinophilus gulolineatus*) 19 (May 22, 2019) ("SAF").
[76] SAF at 20.
[77] SAF at 19.
[78] SAF at 21–22.
[79] *Id.*
[80] SAF at 22.

IV. LEGAL VIOLATIONS

The Service's finding that the salamander does not warrant listing under the ESA relied on severely flawed, conclusory analyses of the species' current status and future viability, and arbitrarily reversed the agency's own prior decisions. After determining that the salamander merited listing as a threatened or endangered species in 2011, the Service repeatedly affirmed that decision until its abrupt reversal in 2019. Neither the Decision Form nor the Federal Register notice explained why threats the Service identified in 2011 under Listing Factors A, D, and E have sufficiently been abated such that the salamander no longer merits listing, nor did they sufficiently examine other threats to the species that may have arisen or increased during that time. Instead, the Decision Form merely gestured at a vague "better understanding" of the salamander that the agency claimed it possessed in 2019.[81] As discussed in more detail below, this alleged "better understanding" is unsubstantiated, as the agency repeatedly ignored the newest, best available scientific evidence to reach its conclusions about the species' likely future viability.[82]

The Service's not warranted finding violated the requirements of Section 4 of the ESA in several ways,[83] including by ignoring the best available science, discounting threats to the species, and unlawfully assuming positive outcomes in the face of uncertainty, as described in greater detail below.

A. The determination improperly relied on theoretical future conservation actions to reach its future viability estimates.

The Service violated the ESA in its analysis of the species' future viability by assuming that unplanned conservation measures sufficient to mitigate threats to the survival of the species were likely to occur. Of the three possible future scenarios that the Service modeled in its SSA, two assumed improvements over current levels of conservation measures for the species: Scenario 1 assumed that current conservation measures will be improved, and Scenario 2 additionally assumed that new measures will be implemented.[84] The Service then assumed that these unspecified new or improved conservation measures would provide a panacea for the wide array of threats facing the salamander. For instance, the Service assumed in Scenarios 1 and 2 that due to future hypothetical conservation measures over the next 61 years, the salamander "will respond to drought and flooding conditions in a manner that results in continuing viability."[85] Elsewhere, the SSA contradicts that assumption, acknowledging that the agency is "not certain of the Berry Cave salamander's potential response to conservation measures . . . ."[86] By contrast, under Scenario 3, which did not include new or improved conservation measures, the Service acknowledged

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[81] SAF at 21.

[82] Additionally, the same scientists who provided updated population surveys and other data to the Service subsequently published a paper based on that data explicitly rebuking the Service's "not warranted" finding for the salamander. *See* Matthew L. Niemiller et al., *Distribution, Ecology, Life History, and Conservation Status of the Berry Cave Salamander* (Gyrinophilus gulolineatus), 16(3) Herpetological Conservation and Biology 686–703 (2021), provided with accompanying *Supplemental Information* as Attachment 1.

[83] 16 U.S.C. § 1533.

[84] SSA at 35, 38, 40.

[85] SSA at 35.

[86] SSA at 45.

that "climate change would exacerbate the effects of the other stressors" and "could potentially result in extirpation of half of the salamander populations."[87]

The Service then assumed, without explanation or evidence, that Scenarios 1 and 2 were each more likely to occur than Scenario 3.[88] The Service incorporated this assumption of likelihood into the summary of threats section of the Decision Form, where, without providing any support and without identifying a single proposed or planned conservation action, the Service stated that "[c]onservation measures are likely to counteract some sources of stress to Berry Cave salamander populations, as predicted under our future scenarios."[89] The Service made no attempt to reconcile this assertion with its 2011 finding that existing conservation measures and regulatory mechanisms, including state and federal water quality regulations, are not sufficient to mitigate threats to the species.[90]

The Service thus relied on these purely hypothetical conservation measures to make its not-warranted determination. In its finding, the Service referred back to the SSA's projections and stated that "the stressors acting on the salamander are not projected to substantially reduce the overall resiliency, redundancy, or representation of the species."[91] This effectively ignored the SSA's warning that under Scenario 3, there would be "significantly greater impacts than predicted in the other two scenarios" and *half* of remaining salamander populations could be extirpated in 61 years.[92] The Service acted arbitrarily and capriciously and violated the ESA and Service policies by relying on hypothetical, unplanned conservation measures to justify its not-warranted finding, because "future and uncertain actions cannot justify a negative listing decision" and an agency may not assume the best-case scenario in light of uncertainty.[93]

B. The Service improperly ignored the best available science on abundance and relied on faulty assumptions about the species' persistence.

To reach its unjustified not warranted finding, the Service painted an unreasonably rosy picture of both the current population health and future viability of the Berry Cave salamander. The Service violated the ESA by ignoring current numeric survey data that it possessed, which largely indicated declines in population numbers, and assuming that healthy populations existed by default wherever population data was limited.

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[87] SSA at 46–47.
[88] SAF at 19.
[89] SAF at 20.
[90] Likewise, the Service failed to analyze the efficacy of *existing* regulatory mechanisms here.
[91] SAF at 23.
[92] SSA at 46–47.
[93] *Sw. Ctr. for Biological Diversity v. Norton*, Civil Action No. 98-934 (RMU/JMF), 2002 U.S. Dist. LEXIS 13661, at *27 (D.D.C. July 29, 2002); *see also Ctr. for Biological Diversity v. U.S. Fish & Wildlife Serv.*, No. 21-CV-5706 (LJL), 2023 WL 5747882, at *12–14 (S.D.N.Y. Sept. 5, 2023) (finding that the Service arbitrarily and capriciously considered improper factors and ignored the best available science when it relied on not-yet-implemented conservation efforts to justify its decision not to list the eastern hellbender); *Ctr. for Biological Diversity v. Haaland*, 562 F. Supp. 3d 68, 85 (D. Ariz. 2021) ("Future actions are not relevant to the determination of whether a species should be listed"); *Greater Yellowstone Coal., Inc. v. Servheen*, 665 F.3d 1015, 1034 (9th Cir. 2011) ("The Service's reliance on voluntary action is contrary to law").

For instance, without acknowledging the apparent > 60% decline in the number of salamanders observed in Meads Quarry Cave in the last ten years,[94] the Service focused on the salamander's continued persistence in the cave as evidence of the population's alleged health.[95] Even worse, the Service went on to irrationally assert that the current persistence of salamanders in Meads Quarry Cave shows that the toxic leachate in the cave "is only impacting individuals that come into direct contact with it and not the population as a whole."[96]

For caves with lower reported abundances than Meads Quarry Cave (sometimes with only one observed salamander), the Service similarly relied on persistence to claim current population resiliency. While recognizing that recent survey results indicated likely declines in several populations, the Service asserted that the populations in several caves were already so low that "trends in abundance at those sites [were] difficult to discern."[97] Rather than making best efforts to discern trends from the best available data, or otherwise grappling with the low numbers observed, the Service instead assumed, without adequate justification, that sufficiently robust numbers of salamanders are likely present deeper within these cave systems. [98]

The Service likewise assumed, without scientific or legal support, that the salamander also continues to persist at healthy population levels even in caves where *no* salamanders were observed in 2018, caves that have not been surveyed for over a decade, and caves where only one observed salamander has ever been reported. For example, Aycock Spring and Christian Caves (AU1) were previously surveyed only once in 2005, and only a single salamander was found at each site.[99] Since then, significant residential development has occurred in the area immediately surrounding the caves, and "[n]ew houses are [now] located adjacent to Christian Cave, increasing the potential for introduction of toxicants into the cave system."[100] When the SSA was published, only 38% of the habitat within a half mile of the caves was forested (the lowest amount of forest of any AU),[101] raising concerns about high levels of sediment transport and runoff of lawncare chemicals and other toxicants into the caves.[102] In 2018, surveyors were unable to find any salamanders in

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[94] *See, e.g.*, SSA at 16 (Table 2-2), 52–54 (Appendix A).
[95] SAF at 22.
[96] SAF at 22, 25. This logic conflicts with the Service's recognition in the SSA that "salamander populations that already exhibit lower densities due to predation, human collection, or other means can be especially sensitive to [sediment load, toxicants, and climate change], and multiple simultaneous or chronic stressors could result in negative, synergistic effects on the viability of the [species]." SSA at 22. Put simply, when a population is very small, as Berry Cave salamander populations are believed to be, then *any* direct mortality or stress to individuals can impair reproductive success and recruitment and cause population level effects.
[97] SAF at 8.
[98] SAF at 9 ("[I]t is thought the species uses areas further into the cave system where surveys cannot be conducted; survey results more than likely only represent a subset of the entire population due to the lack of human accessibility.")
[99] SSA at 16 (Table 2-2), 29.
[100] SSA at 17, 19.
[101] SSA at 19.
[102] SSA at 40, 42.

Aycock Spring Cave and were unable to access Christian Cave.[103] Despite this, the Service still assumed that a "moderate to low" resiliency salamander population persists in the AU but provided no rational explanation as to why a lack of evidence of a viable population should support this conclusion.[104]

The Service reached identical conclusions on the viability of Small Cave (AU6) despite Small Cave having only been surveyed once—in 2014—with only one salamander observed and despite the fact that residential development, originating within 0.3 miles of the cave entrance, is expected to continue steadily through 2080.[105]

The Service cannot reasonably ignore numeric population data demonstrating observed declines in favor of less informative assessments of persistence. Furthermore, the Service cannot reasonably conclude that the threats facing the salamander are not having population-level effects simply because the species continues to exist. And finally, the Service cannot reasonably conclude that healthy populations continue to exist even in areas of severe habitat degradation where recent surveys have found no salamanders, or where no recent survey data is available. In doing so, the Service repeatedly ignored the best available science.

C. The Service unreasonably discounted future threats to the species.

The Service further compounded its faulty conclusions about the salamander's current and future resiliency levels by additionally ignoring the best available science and substituting unsubstantiated claims dismissing future threats to the species. Throughout the SSA, the Service recounted the future compounding threats of urbanization and consequent water quality degradation, as well as climate change, but irrationally discounted those and other threats when assessing the salamander's extinction risk.

The agency failed to adequately address the clear threat of urbanization, for instance by failing to meaningfully evaluate the impacts of development and grazing on detritus and sediment input and by failing to explain how the *doubling* of development in large portions of the species' range will result in only "*somewhat limited* effects on water quality through the year 2080."[106]

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[103] The SSA and SAF are internally inconsistent in their reporting on Aycock Spring Cave population surveys. While the SSA states at page 29 that the cave was last surveyed for Berry Cave salamanders in 2005, the data presented in Table 2-2 (p. 16) acknowledge that Aycock Spring Cave was again visited in 2018 with no salamanders found. The same table as reproduced in the SAF at page 9, however, lacks the 2018 data for Aycock Spring Cave. As reported to the Service, surveyors visited Aycock Spring Cave on July 10, 2018, but observed no salamanders.
[104] To the extent that the Service grouped the two caves together into one AU for the purpose of bolstering its viability assumptions, this was also improper.
[105] SSA at 41. The Service's analysis is also arbitrary here because it treats a distance of 0.3 miles as being so small as to group Christian and Aycock Spring Caves together as one AU with a potential hydrological connection, SSA at 29, but so large as to claim that residential development occurring within 0.3 miles of the mouth of Small Cave (AU 6) will have little to no water quality impacts and will not impact the salamander population there, SSA at 33–34.
[106] SSA at 37 (emphasis added).

Similarly, the Service failed to acknowledge or analyze available numeric measures of fecal coliform bacteria levels despite identifying fecal coliform bacteria as a threat to the species and noting that livestock and septic systems impact the watersheds of the cave systems. Instead, the Service discounted threats from water quality as only "moderate" in both Meads Quarry Cave, which is already threatened by toxic leachate, and Berry Cave, where salamanders have repeatedly been found with nodules of suspected parasitic origin, without providing an adequate rationale for this choice and without citing any measures of water quality.[107] Likewise, the Service erred by effectively dismissing the presence of these nodules on salamanders in Berry Cave and failing to analyze their significance under Listing Factor C.

The Service also irrationally claimed that the salamander's "inherent adaptability," as evidenced by its persistence today, will help it overcome the impacts of climate change,[108] a threat that elsewhere the Service anticipated could result in the extirpation of half of remaining salamander populations absent improvements in conservation measures.[109] Similarly, the Service did not, and cannot, reconcile its assertions of the salamander's "inherent adaptability" with evidence of the salamander's presumed extirpation from caves it formerly inhabited. For example, the Service did not engage in any meaningful way with the extirpation of the salamander from Blythe Ferry Cave, at the southwest end of its range, where the cave is now considered too dry to support a salamander population.[110] Likewise, the Service failed to reconcile its assertions of adaptability with the suspected extirpation of Berry Cave salamanders from Cruze Cave (a highly disturbed site where the species may have been outcompeted by spring salamanders).[111]

The Service cannot rationally point to the salamander's current persistence across a handful of caves as a reason why the species will adapt to the significant cumulative threats it faces in the future. The Service improperly discounted the threats facing the salamander in violation of the ESA and its "institutionalized caution" mandate.[112] These assumptions necessarily tainted the Service's analysis of whether the salamander is threatened or endangered in all or a "significant portion of its range," because the Service could not accurately assess whether threat levels in a particular area render the salamander threatened or endangered in part of its range when starting from such an inaccurate representation of those population and threat levels.

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[107] SSA at 29.
[108] SSA at 47.
[109] SSA at 46–47.
[110] SSA at 31. The Service also failed to analyze the loss of Blythe Ferry Cave (at the southwest corner of the salamander's range), the lack of evidence to support any continuous presence of salamanders near the 1953 roadside ditch sighting in Athens, TN (the southeast corner of its range), and the fact that only one salamander has ever been observed at Small Cave. Together, these losses point towards potential range contraction across Meigs and McMinn Counties, which comprise the southern half of the species' mapped range, that the Service should have analyzed both under Factor A and its significant portion of the range analysis.
[111] *See* SSA at 14 (citing Niemiller et al. 2018, p. 23, for documented evidence of hybridization between Berry Cave salamanders and spring salamanders at Cruze Cave); SSA at 21 (recognizing that while "the reason for current absence of the Berry Cave salamander is not entirely clear," spring salamanders "may simply have an advantage over the Berry Cave salamander in the ability to compete for food resources, resulting in higher population densities").
[112] *See Tenn. Valley Auth. v. Hill*, 437 U.S. 153, 184 (1978).

V. CONCLUSION

In sum, the best available science paints a grim picture of the salamander's current status and future viability due to its very low abundance, myriad ongoing and unmitigated threats, significant downward population trends, and multiple suspected extirpations. Only by repeatedly assuming the best-case scenario when faced with uncertainty and ignoring the best available science did the Service find that the salamander is not threatened or endangered. For these and other reasons, the Service's finding is arbitrary and capricious, contrary to the best available science, and in violation of the ESA.

If the Service does not remedy these violations, the Center for Biological Diversity and Southern Environmental Law Center intend to pursue legal action. If you believe any of the foregoing to be in error, have any questions, or wish to discuss this matter, please do not hesitate to contact us.

Sincerely,

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ATTACHMENT 1

Niemiller et al. 2021 and Supplement Information

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Distribution, Ecology, Life History, and Conservation Status of the Berry Cave Salamander (*Gyrinophilus gulolineatus*)

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Abstract.—**Berry Cave Salamanders (*Gyrinophilus gulolineatus*) are neotenic, stygobitic salamanders endemic to the Appalachian Valley and Ridge of eastern Tennessee, USA. We conducted surveys for *G. gulolineatus* from 2017–2019 to assess the status, locate new populations, and address knowledge gaps related to life history and population ecology required for conservation assessment. We confirmed the presence of *G. gulolineatus* at four of 11 historical sites, but we did not observe it at any additional caves. At the three known cave sites with greatest abundance, visual counts per survey ranged 0–19 salamanders in 2017–2019. There was no apparent trend in abundance at Berry Cave. Visual counts declined 65% since the mid-2000s at Meads Quarry Cave and 80% since the early 1980s at Mudflats Cave. Mark-recapture studies in 160-m of cave stream at Berry Cave in 2017–2018 and 900-m of cave stream at Meads Quarry Cave in 2008 yielded population size estimates that ranged from 34–78 and 15–65 individuals, respectively. We identified 13 existing or potential threats to populations. Habitat degradation and groundwater contamination represent the most evident threats to long-term viability. Based on our conservation assessments, we recommend a rank of Endangered under Red List criteria of the International Union for Conservation of Nature and Critically Imperiled-Imperiled (G1G2) under NatureServe criteria. In opposition to the recent U.S. Fish and Wildlife Service decision, we advocate that, at a minimum, *G. gulolineatus* remain a Candidate Species, and we offer recommendations for research, conservation, and management of this rare salamander.**

Key Words.—Appalachian Valley and Ridge; demography; groundwater; home range; karst; population size; subterranean; threat assessment

Introduction

Salamanders and fishes are the only two vertebrate groups with species restricted to subterranean aquatic habitats, such as cave streams and groundwater aquifers (Gorički et al. 2012, 2019; Soares and Niemiller 2013, 2020). Among salamanders, 14 species in two families are considered troglobionts, i.e., obligate cave-dwellers, with most diversity (13 species) in three genera in the family Plethodontidae (Gorički et al. 2012, 2019; Phillips et al. 2017). In the Interior Low Plateau and Appalachians karst regions of the eastern U.S., three species of the genus *Gyrinophilus* are considered stygobionts, aquatic, obligate-subterranean organisms: Tennessee Cave Salamanders (*G. palleucus*), Berry Cave Salamanders (*G. gulolineatus*), and West Virginia Spring Salamanders (*G. subterraneus*; Gorički et al. 2012, 2019). Both *G. palleucus* and *G. gulolineatus* are neotenic, i.e., they attain sexual maturity without metamorphosing and retain larval characteristics (Miller and Niemiller 2008; Gorički et al. 2012, 2019). The latter can attain a snout-vent length > 145 mm and is, therefore, one of the largest species of plethodontid salamanders (Gladstone et al. 2018).

Gyrinophilus gulolineatus has been assessed as Endangered [B1ab(iii)+B2ab(iii)] on the Red List of the International Union for Conservation of Nature (IUCN) because of its limited extent of occurrence, severe fragmentation of populations, and continuing decline in the extent and quality of habitat (Hammerson 2004). Likewise, the species has been assessed as Critically Imperiled (G1Q) by NatureServe (https://

Figure 1. An adult Berry Cave Salamander (*Gyrinophilus gulolineatus*) from the type locality in Roane County, Tennessee, USA. (Photographed by Matthew L. Niemiller).

explorer.natureserve.org/). *Gyrinophilus gulolineatus* was petitioned for federal listing as Endangered under the U.S. Endangered Species Act (ESA) in January 2003 by the U.S. Fish and Wildlife Service (USFWS; 2010). At that time, this species was known from eight sites in Tennessee, including one surface record from a roadside ditch in McMinn County in 1953 (Brandon 1965) and seven caves that occur predominantly in the metropolitan area of Knoxville. The entire known range is within the Upper Tennessee River and Clinch River watersheds of Knox, McMinn, Meigs, and Roane counties, within the Appalachians karst region and Appalachian Valley and Ridge (AVR) physiographic province of eastern Tennessee (Niemiller and Miller 2010; Table 1). Based on morphology and genetics, the salamanders at one of the sites in Knox County were later determined to be related Spring Salamanders (*G. porphyriticus*; Miller and Niemiller 2008; Niemiller et al. 2008). In 2010, a 90-day petition finding was published by the U.S. Fish and Wildlife Service (USFWS 2010), which ruled that information available at the time did warrant federal listing. A subsequent 12-mo status review (USFWS 2011) concluded that, although listing was warranted, it was precluded by higher priority actions. Concurrently, *G. gulolineatus* was included on the list of Candidate Species, and the USWFS indicated that a proposed rule to list the species would be developed. Since it was first petitioned for federal listing in 2003, *G. gulolineatus* have been discovered at four additional caves (Miller and Niemiller 2008; Niemiller and Miller 2010; Niemiller et al. 2008, 2010, 2016b), which increased the total number of known sites to 11, which includes eight distinct cave systems and a record from the roadside surface ditch (Table 1).

Although *G. gulolineatus* has been known to science for more than 50 y and received recent research prioritization, we still know relatively little about its distribution, ecology, life history, and threats potentially impacting populations. Most populations appear small (Miller and Niemiller 2008), but this is based on past visual censuses. Because of their proximity to metropolitan Knoxville, some populations may be in decline because of threats to habitat caused by groundwater contamination and sedimentation associated with urban development, past mining operations including direct habitat loss and leaching of crushed lime into cave systems, flooding following dam construction, and possible hybridization with *G. porphyriticus* in one cave system (Beachy 2005; Niemiller and Miller 2011; USFWS 2016a).

To assist the USFWS with a Species Status Assessment (SSA; USFWS 2016b) used to determine to list *G. gulolineatus* under the U.S. Endangered Species Act, we conducted new surveys for the species

Table 1. Historical sites of Berry Cave Salamanders (*Gyrinophilus gulolineatus*) in eastern Tennessee, USA. For caves, additional details are reported, including the overall passage length, geological formation, and whether the cave has been mapped. For Meads River Cave, only a partial map exists. The last survey year is included, as well as the maximum number of salamanders observed during a visual census during any survey trip. Refer to Supplemental Information Table S1 for a summary of all observation data for *G. gulolineatus*. The abbreviation NA = not applicable.

Site	County	Length (m)	Geologic Formation	Mapped	Last surveyed	Maximum observed
Aycock Spring Cave (TKN172)	Knox	90	Newala Formation	No	2018	1
Christian Cave (TKN49)	Knox	415	Newala Formation	Yes	2005	1
Fifth Entrance Cave (TKN167)	Knox	54	Holston Marble	No	2018	1
Meads Quarry Cave (TKN28)	Knox	1830	Holston Marble	No	2019	24
Meads River Cave (TKN151)	Knox	305	Holston Marble	No	2018	1
Mudflats Cave (TKN9)	Knox	101	Lenoir Limestone	Yes	2018	6
The Lost Puddle (TKN145)	Knox	156	Maynardville Limestone	Yes	2018	4
Blythe Ferry Cave (TME1)	Meigs	311	Knox Group	Yes	2018	1
Ditch along Oostanaula Creek S of Athens	McMinn	NA	NA	NA	1953	3
Small Cave (TMM5)	McMinn	90	Newala Formation	No	2014	1
Berry Cave (TRN3)	Roane	365	Mascot Dolomite	No	2019	19

in 2017–2019. Our aims were to (1) assess the status of the species and extant populations in eastern Tennessee; (2) survey for new populations within its suspected distribution; (3) address knowledge gaps related to life history and population ecology that are required for accurate conservation assessment; (4) identify priority populations and habitats for immediate conservation and management efforts; and (5) use these data to update IUCN Red List and NatureServe conservation ranks through new conservation assessments. The USFWS published a rule for *G. gulolineatus* (USFWS 2019b) in October 2019. This rule followed a review of the best available scientific information, which included data presented herein, in a Species Status Assessment (SSA; USFWS 2019a). The SSA is an analytical approach to support an in-depth review of the biology and threats to a species, an evaluation of biological status, and an assessment of the resources and conditions needed to maintain long-term viability (USFWS 2016b). An SSA relies on what is called the three Rs under a range of future scenarios: (1) Resiliency describes the ability of a species to persist in the face of random disturbance events through demographic processes at the population or metapopulation level; (2) Redundancy describes the ability of the species to withstand catastrophic events through the occurrence of multiple resilient populations; and (3) Representation describes the capacity of the species to adapt to changing conditions through the existence of ecologically relevant variance (i.e., genetic, life historical, habitat). Ultimately, the USFWS concluded that *G. gulolineatus* will persist in the foreseeable future, which precluded listing as Threatened or Endangered under the ESA. *Gyrinophilus gulolineatus* remains listed as Threatened at the state level in Tennessee. Thus, in addition to the goals stated above, we discuss the challenges associated with conservation assessments of cave-obligate organisms and how they might affect inferences under the SSA framework.

Materials and Methods

Study area.—We visited and surveyed the biota of 88 caves within the AVR physiographic province of eastern Tennessee, USA (Fig. 2; Supplemental Information Tables S1 and S2), to assess presence of *G. gulolineatus.* We selected non-historical sites based on location within or near the suspected range of *G. gulolineatus*, accessibility and presence of aquatic habitat, from a list of caves maintained by the Tennessee Cave Survey (TCS), an organization affiliated with the National Speleological Society that, among other responsibilities, maintains a database on caves in Tennessee. We attempted to revisit all 11 historical sites but could not arrange permission to access two caves and could not identify a cave associated with the surface record near Athens, Tennessee (Table 1). To protect the species and sensitive cave resources, we do not list exact geocoordinates for sampled caves herein; however, cave location data can be requested from the TCS or the corresponding author.

Cave surveys and data collection.—We conducted new surveys from October 2017 to July 2019. We also included in our analyses data from surveys conducted in the AVR of eastern Tennessee from 2007 to 2019

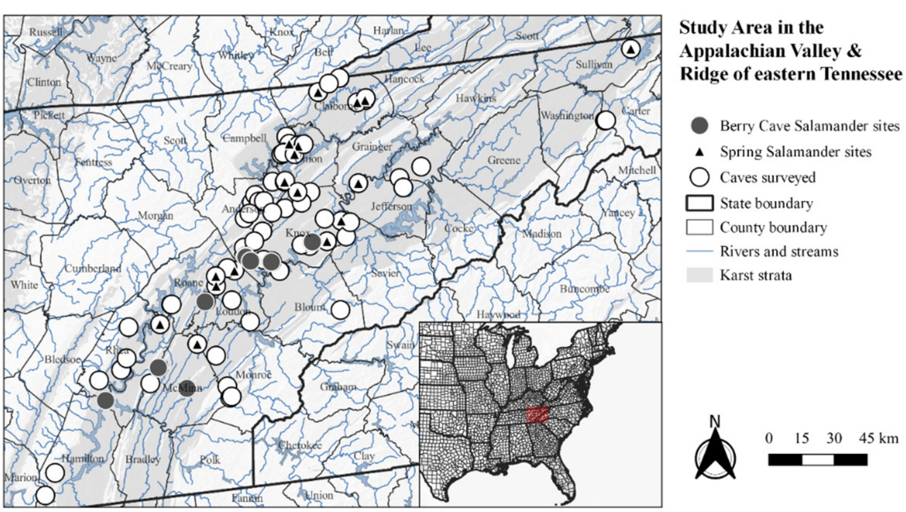

Figure 2. Distribution of the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and locations of 98 caves surveyed between 2004–2019 in the Appalachian Valley and Ridge of eastern Tennessee, USA. Karst carbonate rock are depicted in gray (U.S. Karst Map; Weary and Doctor 2014). Dark circles represent caves with occurrence records of Berry Cave Salamanders, and caves with occurrence records of Spring Salamanders are noted with a black triangle. Caves surveyed but with no Berry Cave or Spring salamanders are shown as open circles.

in association with other projects in the region (e.g., Niemiller et al. 2016b; Gladstone et al. 2019). All cave surveys to locate salamanders were conducted by many of the same personnel who employed the same level of effort and approaches for surveying aquatic habitats; specifically searching all human-accessible streams, pools, rimstone pools, and phreatic waters with headlamps and handheld dive lights, carefully lifting rocks and other debris, and hand-sifting small cobble and detritus. At least two surveyors were present for each survey and survey duration was recorded. We made a concerted effort to capture each salamander encountered with handheld bait nets and made note of any individuals that escaped capture. We placed captured salamanders into a clear plastic bag or other small container until we found a suitable site to process the salamander, which usually took < 5 min. In addition to recording the general position where they were observed (e.g., underneath a submerged rock, in an open pool, etc.), we weighed and measured each salamander. We used spring scales (Pesola AG, Schindellegi, Switzerland) to weigh salamanders to the nearest 0.2 g, and metric calipers to measure total length (TL) and snout–vent length (SVL; from the tip of the snout to the posterior margin of the vent) to the nearest 0.5 mm. Furthermore, we noted any physical abnormalities, such as tail damage, tail regeneration, missing limbs, presence of parasites, or lesions. Because sex is difficult to determine in species of *Gyrinophilus* without examination of cloacal anatomy, we identified sex only of females when developing ova were visible through the abdominal wall. Based on dissections, Simmons (1975) found that males and females were sexually mature at 70 mm SVL; therefore, we classified each salamander we captured as either a juvenile (< 70 mm SVL) or an adult (≥ 70 mm SVL).

Water quality measurements.—We used standard methods (U.S. Geological Survey [USGS] 2015) to examine water quality at two locations in Berry Cave (June 2018) and at two locations upstream and downstream of a large white speleothem (i.e., structure formed from mineral deposits) that occurs at 335 m upstream of the main entrance at Meads Quarry Cave (January 2008 and June 2018). This speleothem formed below large piles of lime on the surface that originated from past quarrying operations. In 2018, we used 0.2-mm polyvinylidene fluoride Millipore filters to obtain water samples for laboratory analyses of alkalinity and major anion and cation (which were acidified to pH 2.0 with trace metal grade nitric acid) concentrations to evaluate contamination indicators from ratios of ion concentrations (e.g., Wakida and Lerner 2005; Panno et al. 2006). We determined total and fecal bacterial coliform (e.g., *Escherichia coli* and other intestinal Enterobacteriaceae) colony forming units (CFU) per mL from the water using RIDA® COUNT (R-Biopharm AG, Darmstadt, Germany) test kits, according to manufacturer instructions and previous modifications (Mulec et al. 2012). We interpreted coliform results to indicate potential fecal contamination while acknowledging that pathogenicity and health risk cannot be determined unless other tests are performed.

Mark-recapture studies.—We conducted mark-recapture studies at Meads Quarry Cave in Knox County over 10 surveys from January 2008 to September 2008 and at Berry Cave in Roane County over 13 surveys from October 2017 to December 2018 to estimate population sizes of salamanders at both caves, and home range and movement at Meads Quarry Cave. These two sites were chosen based on highest abundance during past surveys. We supplemented visual encounter surveys at Meads Quarry Cave from January 2008 to June 2008 using unbaited minnow traps set every 40 m along a stream transect beginning from about 800 m from the downstream entrance and ending about 640 m upstream of the main upstream entrance. At these sites, we marked captured salamanders by injecting a 1.2 × 2.7 mm visible implant (VI) alpha tag (Northwest Marine Technology Inc., Shaw Island, Washington, USA) into the dermis of the tail. This approach has been applied in population studies of Grotto Salamanders, *Eurycea spelaea* (Fenolio et al. 2014a), and *G. palleucus* (Huntsman et al. 2011; Niemiller et al. 2016a). Because of the size of the VI alpha tag injection needle and potential for harm to the animal, we did not mark salamanders < 40 mm SVL. After marking, we allowed salamanders to recover for about 5–15 min, then released each at its point of capture. Migration of VI alpha tags has been reported in other amphibians (Heard et al. 2008; Kaiser et al. 2009), and we have experienced low levels of local tag migration, and in some cases, inversion of tags in *G. gulolineatus* (e.g., Niemiller et al. 2016a). Because they were injected just underneath the translucent epidermis of the tail, we could discern the color and alphanumeric code of most tags. To maximize retention, we briefly restrained salamanders in plastic bags during marking, and placed tags away from entry wounds to minimize their expulsion (Osbourn et al. 2011; Niemiller et al. 2016a). We suspended the 2008 capture-mark-recapture study at Meads Quarry Cave because of cave closure associated with concerns regarding potential spread of White-nose Syndrome or its causative fungus (*Pseudogymnoascus destructans*) in bats. We suspended the most recent capture-mark-recapture study at Berry Cave because of record high-levels of precipitation that occurred from January to March 2019 in the region.

***Estimating population size, detectability, and survival rates.*—**We investigated whether abundance (i.e., direct visual counts) changed over time at Berry, Mudflats, and Meads Quarry caves. We used count data from our surveys in addition to data from Ron Caldwell and John Copeland (unpubl. report) and Miller and Niemiller (2008). We used Generalized Linear Models (GLM) with the census counts as the response variable and survey date (as days since 1 January 1983 before the first survey in the dataset) as the explanatory variable. Because count data often exhibit a Poisson or negative binomial distribution and also can be zero-inflated (Lindén and Mantyniemi 2011), we explored the best fit of several different distributions for each cave, including zero-inflated and non-zero-inflated Poisson, negative binomial, and negative binomial with NB2 parameterization [variance = $\mu(1 + \mu /k)$], using the glmmTMB (Brooks et al. 2017) package in the R statistical computing environment (v.4.0; R Core Team 2020). We developed zero-inflated models using a single zero-inflation parameter; but we also developed hurdle models that first modeled the binary likelihood that a 0 value is observed, and we modeled the non-zero observations using a truncated Poisson or negative binomial model. We determined the best fitting models using Second Order Akiake Information Criterion (AICc) using the bblme package in R (Bolker et al. 2017). The best fitting model was used to estimate the overall trend for each cave.

We used the package RCapture (Baillargeon and Rivest 2007) in R to estimate population size, capture probabilities, and assess general trends in apparent survival over time by fitting a Jolly-Seber Open Population Model following the Loglinear approach of Cormack (1985, 1989) based on the mark-recapture data from the two populations studied: Meads Quarry Cave in 2008 and Berry Cave in 2017–2018. RCapture uses Poisson regressions fitted using the glm function and then transforms loglinear parameters into demographic parameters, which include population size, capture probability at each sampling occasion, and survival. An open population model is most appropriate for these datasets for several reasons (Niemiller et al. 2016a), including that birth and death likely contribute to a lack of closure, immigration and emigration by adults and larvae likely occur, and salamanders can live in habitats inaccessible to humans (Miller and Niemiller 2008; Gorički et al. 2019). Because there were several surveys with a low number of captures, we reduced the capture history matrix for the Berry Cave dataset from 13 capture occasions to four periods by pooling surveys in 3-mo intervals. We evaluated two models, one that allows capture probabilities to vary between periods and another that holds capture probabilities equal across periods. We assessed model fit by examining plots of Pearson residuals versus number of captures and selected the best model using AICc in the bblme package. We report relative abundances as the mean ± one standard deviation (SD) and capture probabilities and population estimates as mean ± one standard error (SE).

***Estimating movements and home range.*—**We examined potential factors affecting movement of *G. gulolineatus* at Meads Quarry Cave in 2007–2008. We measured distance along the cave stream for each capture and later used these points to calculate linear distance moved, directionality of movement between capture occasions (upstream versus downstream), and total distance moved for all salamanders with at least two captures. During exploratory analysis, we used Mixed-effects Models (with a random intercept term for individual) via the lme4 package in R (Bates et al. 2015) to model movement metrics as functions of size (SVL), stage class (adult or juvenile), time between captures, number of recaptures, and stream flow direction. We also applied GLM and visualized distributions according to each of these factors, alone and in combination. No patterns were evident in these exploratory analyses; thus, we present only visualizations and basic descriptive statistics herein.

To investigate site fidelity and homing behavior along the cave stream at Meads Quarry Cave, we quantified variance in the directionality of individual and population level movements. We calculated movement vectors as distance and direction (upstream versus downstream) moved between captures. We either nested movement by individual or treated them as independent observations in two separate analyses. Movement vector or individual means were then resampled 10,000 times for each measure, and the distributions were compared to a null value of 0 (no directional bias) to determine whether there was directionality. Greater overlap in individual movements (vector means that approached 0, which indicates bidirectionality) provided a measure of homing behavior. We used estimates based on pooled values to assess any individual-independent effects on movement up or downstream.

***Conservation assessment.*—**We employed NatureServe and IUCN Red List conservation assessment protocols to evaluate the conservation status of *G. gulolineatus*. The system of NatureServe to assess conservation status uses 10 primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent, area of occupancy, number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short- and long-term trends in population size, extent of occurrence, area of occupancy, number of occurrences, and viability or ecological integrity of occurrences. Finally, threat factors include threat impact and intrinsic vulnerability to threats. Other information can be used, and we included information on the number of protected and managed occurrences. We calculated NatureServe conservation status assessments using default points and weights with the NatureServe Rank Calculator worksheet available in Excel (Faber-Langendoen et al. 2009).

To determine the appropriate Red List classification for each species, we compiled all available information with reference to each of five criteria. A species may be classified as Critically Endangered (CR), Endangered (EN), or Vulnerable (VU) on the IUCN Red List if it meets specific conditions under any one of these five criteria (IUCN 2012): (1) past, present, or projected reduction in population size over three generations; (2) small geographic range in combination with fragmentation, population decline or fluctuations; (3) small population size in combination with decline or fluctuations; (4) very small population or restricted distribution; and (5) a quantitative analysis of extinction risk. Species should be assessed against all criteria, when possible, to confirm that the highest possible threat classification is obtained (IUCN 2001).

We calculated two measures of geographic range size for IUCN Red List and NatureServe conservation assessments, EOO (Extent of Occurrence; also referred to as range extent) and AOO (Area of Occupancy; area within EOO that a species actually occupies; IUCN 2012), in the web-based program GeoCAT (Bachman et al. 2011; http://geocat.kew.org). EOO was calculated as a minimum convex hull. We used a grid size of 2 km (4 km^2) to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010). We determined changes in EOO, AOO, number of occurrences, relative abundance, and quality of habitat over short- and long-term timescales when such data were available. Long-term trends are considered from the year of first discovery of a species to the present day, whereas short-term trends are considered over the last 10 y (Faber-Langendoen et al. 2009; IUCN 2010).

We determined whether occurrences were located on state or federal protected areas or private easements (e.g., state parks, natural areas, national parks, state and national forests, and non-governmental organization-protected lands). Protected areas were obtained from the USGS Protected Areas Database (PAD-US) version 1.3 (shapefiles available at http://gapanalysis.usgs.gov/padus/). To assist with identification of current and potential threats, we used the IUCN Threats Classification Scheme (v3.2; http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme). Additionally, we examined land cover from the 2016 release of the National Land Cover Database (NLCD; Homer et al. 2020) for a 2.5 km buffer (19.6 km^2 area) around each occurrence in ArcGIS Pro 2.6.0. We collapsed land use into six categories: Water, Developed, Forest, Grass/Scrub, Pasture, and Crops. We also calculated percentage increase in urban development from 2001–2016 within these same regions using the 2001 and 2016 release of the NLCD. We considered total loss and gain of naturally vegetated areas owing to impervious surface and pasture/crops, which can affect karst hydrology (Price 2011; Hamel et al. 2013) and subsurface water quality (Bonneau et al. 2017), within the respective surface catchment area of each site, as identified via the High Resolution release of the National Hydrography Dataset (https://www.usgs.gov/core-science-systems/ngp/national-hydrography/access-national-hydrography-products).

Uncertainty in values of assessment criteria is an important consideration when assessing conservation status, as uncertainty can strongly influence the assessment of extinction risk (Akcakaya et al. 2000; IUCN 2001; Gillespie et al. 2011). NatureServe accounts for uncertainty by allowing a range of ranks to show the degree of uncertainty in a conservation status when available information does not permit a single status rank (Master et al. 2009). The IUCN Red List assessment also deals with uncertainty by allowing a plausible range of values to be employed to evaluate criteria (IUCN 2001, 2010; Mace et al. 2008). We adopted a moderate dispute tolerance considering the most likely plausible range of values for a criterion and excluding extreme or very unlikely values (Faber-Langendoen et al. 2009; IUCN 2010). We set risk tolerance and dispute tolerance to 0.5 (risk neutral) for all assessments.

Results

Surveys.—In 2017–2019, we visited eight of the 10 historical cave sites (six of eight cave systems) over 35 cave surveys (Table 1). We confirmed species presence at four caves: Berry, The Lost Puddle, Meads Quarry, and Mudflats (Table 1; Supplemental Information Table S1). We did not observe *G. gulolineatus* at Aycock Spring, Blythe Ferry, and Meads River caves. We searched for *G. gulolineatus* in 35 non-historical cave sites in 11 counties during 43 cave surveys in 2017–2019, and 88 sites in 19 counties over 124 AVR cave surveys from 2007 to 2017 (Fig. 2; Supplemental Information Table S2). We did not observe *G. gulolineatus* at any of these additional locations.

Relative abundances.—Direct observations of *G. gulolineatus* were highly variable among surveys at individual sites (Fig. 3; Supplemental Information Table S1). At Berry Cave, we observed 0–19 salamanders

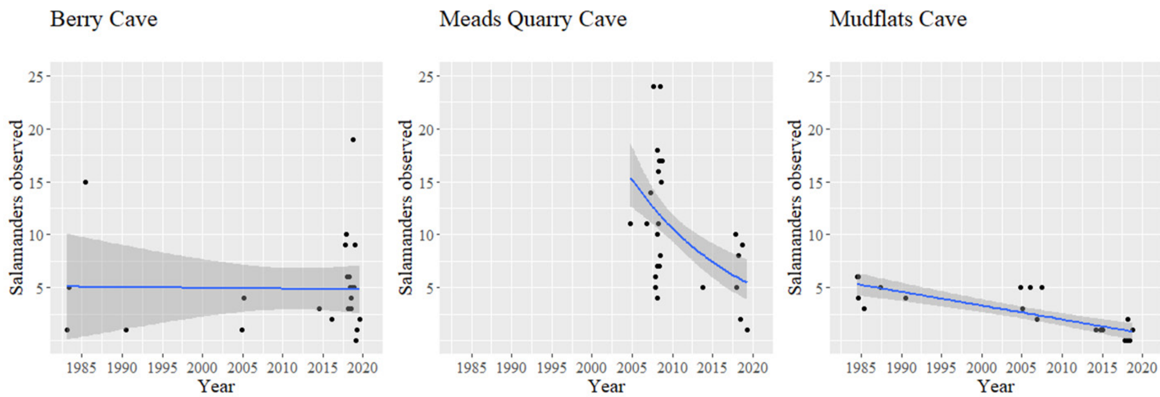

Figure 3. Trends in relative abundance (direct visual counts) of the Berry Cave Salamander (*G. gulolineatus*) at Berry Cave, Mudflats Cave, and Meads Quarry Cave, Tennessee, USA, based on data from Caldwell and Copeland (1992), Miller and Niemiller (2008), and the current study. Blue line is the best fit regression (see Results), and shaded gray is ± one standard error around the trend line.

over 17 surveys in 2017–2019, with a mean ± 1 standard deviation of 5.3 ± 4.6 salamanders observed per survey. The best fitting models (negative binomial and negative binomial with ND2 parameterization; AICc = 139.2; Supplemental Information Table S3) showed no trend in abundance from the early 1980s to the late 2010s (Fig. 3). At Meads Quarry Cave, we observed 0–10 salamanders over six surveys in 2017–2019 (5.8 ± 3.8 salamanders per survey), which was lower than visual counts (range 4–24 salamanders; mean 12.6 ± 6.6 salamanders per survey) over 15 surveys in 2007–2008, and suggested a 65% decline in abundance from the mid-2000s to the late 2010s (Fig. 3) based on the best fitting models (negative binomial and negative binomial with ND2 parameterization; AICc = 157.3 and 157.5, respectively; Supplemental Information Table S3). At Mudflats Cave, we observed only three salamanders over seven surveys in 2017–2018 (0.4 ± 0.8 salamanders per survey): two salamanders on 16 March 2018 and one salamander on 22 September 2018. These observations represented an 80% decline in abundance from the early 1980s to the late 2010s (Fig. 3) based on the best fitting model (zero-inflated hurdle Poisson and Poisson; AICc = 73.8 and 75.4, respectively; Supplemental Information Table S3). At The Lost Puddle, we observed six salamanders over two surveys (3.0 ± 1.4 salamanders per survey): four salamanders on 23 March 2018 and two salamanders on 10 July 2018.

Population size, detectability, and survival rates.—Between 31 January 2008 and 10 September 2008 in 902 m of cave stream at Meads Quarry Cave, we captured and marked 63 unique individuals > 40 mm SVL over 10 cave surveys. We recaptured 28 salamanders at least once, including one salamander that we recaptured on six occasions. An open model with equal capture probabilities among surveys was a better fit (deviance = 148.1, df = 1012, AICc = 254.1) compared to a model with unequal capture probabilities (deviance = 136.5, df = 1002, AICc = 262.5). Capture probability was estimated at 27.3 ± 3.9% among surveys under the best model. Individual survival probabilities for each 3-mo period estimated under the best model were generally high (63.1–100.0%) throughout the study period. Estimates of population size for individual surveys ranged from 14.6 ± 6.6 (31 January 2008) to 64.8 ± 9.5 (4 June 2008) salamanders, with an overall population size during the study period (January to September 2008) of 98.5 ± 11.7 individuals.

In about 160 m of cave stream at Berry Cave between 30 October 2017 and 8 December 2018, we captured and marked 51 unique individuals > 40 mm SVL over 13 cave surveys. We recaptured 14 salamanders at least once, with one salamander captured on four occasions. An open model with equal capture probabilities among surveys was a better fit model (deviance = 5.69, df = 8, AICc = 50.41) compared to a model with unequal capture probabilities (deviance = 4.92, df = 6, AICc = 53.64). Capture probability was estimated at 30.8 ± 10.8% among surveys under the best model. Individual survival probabilities for each 3-mo period estimated under the best model were variable (35.4–100.0%) throughout the study period. Estimates of population size ranged from 34.2 ± 13.7 (February to April 2018) to 77.8 ± 25.4 (September to December 2018) salamanders among the four periods with an overall population size during the study period (October 2017 to December 2018) of 113.1 ± 30.0 individuals.

Observations on growth rate.—We recaptured a salamander in November 2017 at Meads Quarry Cave that was first captured and marked in April 2008. At initial capture, this individual measured 75 mm SVL. In November 2017, this same salamander measured 80.5 mm SVL, growing only 5.5 mm SVL in 9.5 y. In contrast, some juvenile salamanders at Berry Cave exhibited faster growth rates. For example, a 43.5 mm SVL individual grew 5.0 mm SVL in just 33 d and another salamander that measured 42.5 mm SVL at initial capture, grew 12 mm SVL by the time it was recaptured 155 d later.

Home range and movement.—We obtained at least three captures (maximum = 7, mean = 3.9) for 27 individual salamanders to estimate movement metrics at Meads Quarry Cave in 2008. Mean distance moved between recaptures was 16.8 m ± 5.0 (SE) and mean estimated activity range size during 2008 was 26 m ± 6.8. We found no evidence of directionality of movements at the individual (P = 0.44) or population level (P = 0.20). Moreover, all salamanders with at least three captures either did not move between capture occasions or exhibited overlap with prior movements, which suggests the existence of core activity ranges or territories. The largest salamanders captured at Meads Quarry Cave exhibited the lowest degree of spatial overlap with other individuals (Fig. 4). In addition, only one salamander crossed a potential barrier to dispersal in Meads Quarry Cave: a 1.5-m tall flowstone cascade located 336 m upstream of the main entrance (Fig. 4). This salamander was captured on three occasions on 30 March, 30 April, and 4 June 2008 in the same location at 325 m before traveling upstream past the flowstone cascade where it was recaptured at 340 m on 27 June 2008 then back downstream where it was recaptured at 330 m on 9 September 2008.

Extent of occurrence and area of occupancy.—*Gyrinophilus gulolineatus* is known from 11 sites (10 caves and one surface record from a roadside ditch in McMinn County), with an EOO estimated at 1,873 km^2 and AOO estimated at 36 km^2. New sites have been found in recent years that increased EOO and AOO, but it is highly unlikely that range size has expanded or decreased significantly since the species discovery in the 1950s.

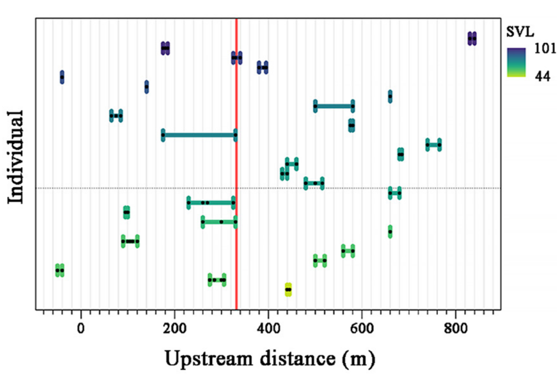

Figure 4. Capture locations (distance in meters along cave stream transect from main entrance) for individual Berry Cave Salamanders (*Gyrinophilus* gulolineatus) captured at least twice at Meads Quarry Cave, Tennessee, USA, in 2008. Points are recapture occasions, bars are minimum and maximum distance moved, and colors represent relative body size (SVL in mm) on a continuous scale. Individuals below the horizontal dotted line are considered sexually immature (< 70 mm SVL). The red vertical line marks the location of the white formation where a significant amount of alkaline lime leaches into the cave system.

Threats.—We identified 13 threats that may impact populations at present or in the near future (Supplemental Information Tables S4 and S5). Several of these threats (e.g., urbanization, groundwater contamination from runoff, septic tanks and spills, past quarry operations, and possible hybridization or competition with Spring Salamanders, *Gyrinophilus porphyriticus*), have been implicated or have the potential to cause population declines and threaten the long-term persistence of the species. Using NLCD data, total loss of natural area from 2001 to 2016 within all 2.5-km buffers around each *G. gulolineatus* site was about 5.97 km^2 (range: 3.6–11.5% for the 11 sites; Fig. 5). Within the respective catchment at each site, total percentage of area converted from naturally vegetated to either impervious surface or agricultural use was 9.8% ± 0.6 (SE) and ranged from 3.4–16.3% per catchment. Approximately 2.1 m^2 ± 0.2 of naturally vegetated area were lost to every 1 m^2 gained (i.e., converted to and from developed or agricultural, respectively) from 2001 to 2016. Additional undocumented development has occurred since 2016, particularly near Meads Quarry Cave. Populations at Mudflats Cave, Christian Cave, and Aycock Spring Cave are potentially impacted by road construction and residential housing developments nearby.

On occasion in Meads Quarry Cave, dying metamorphosed *G. gulolineatus* and several live metamorphosed salamanders and larvae with burn-like lesions were found near and in the pool downstream of the white speleothem demarking leakage of surface lime deposits. One dead *G. porphyriticus* was found in 2018. From 2008 and 2018 surveys, the pH of the cave stream more than 5 m downstream of the speleothem consistently ranged from 7.75 to 8.40, but pH was caustic (pH 10.0 to 12.7) in the pool immediately downstream of the speleothem. Upstream of the speleothem, pH was 7.40 (Fig. 6). Oxidative reductive potential (ORP) in the pool was quite low, reaching -320 mV, compared to higher levels (from -25 to -80 mV) upstream and downstream of the speleothem (Fig. 6). In 2018, more detailed water quality parameters at Mead's Quarry Cave revealed high nitrate, at 3.3 mg/L, and Cl/Br and Na/K ratios. The coliform counts ranged from 86,000 to 99,000 CFU per 100 mL. At Berry Cave, contamination indicators, especially the Na/K ratio, indicated sewage contributions, which was corroborated by coliform counts at 96,000 to 150,000 CFU per 100 mL.

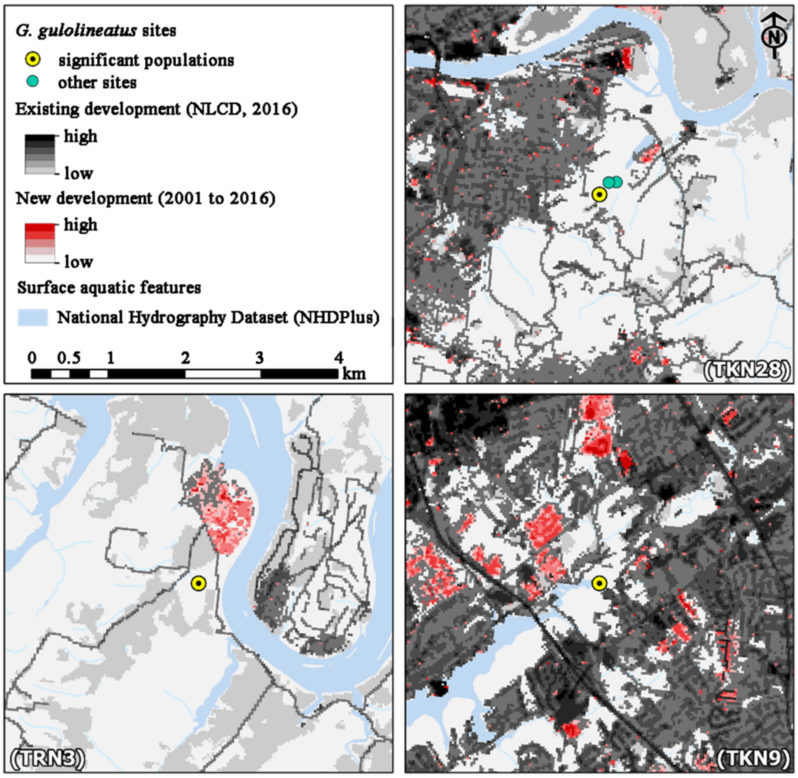

Figure 5. Examples of land cover change (i.e., percentage increase in development) from 2001–2016 at Meads Quarry Cave (upper left), Mudflats Cave (lower right), and Berry Cave (lower left), Tennessee, USA. Greyscale is existing development, and red is new development since 2001. The darkest shading for both indicates impervious surface, and the lightest shading indicates agricultural or lawn where runoff and infiltration of surface contaminants remain a threat.

Current conservation measures.—We compiled a list of existing and recommended conservation and management actions (Supplemental Information Table S4). The Berry Cave landowners entered into a conservation agreement with USFWS, Tennessee Wildlife Resources Agency, and The Nature Conservancy to protect and manage the cave in 2003. Much of the Meads Quarry Cave system, including the entrances to Meads Quarry, Fifth Entrance, and Meads River caves, occurs in the Knoxville Urban Wilderness that is managed by Ijams Nature Center. All entrances are gated, with restricted public access. Blythe Ferry

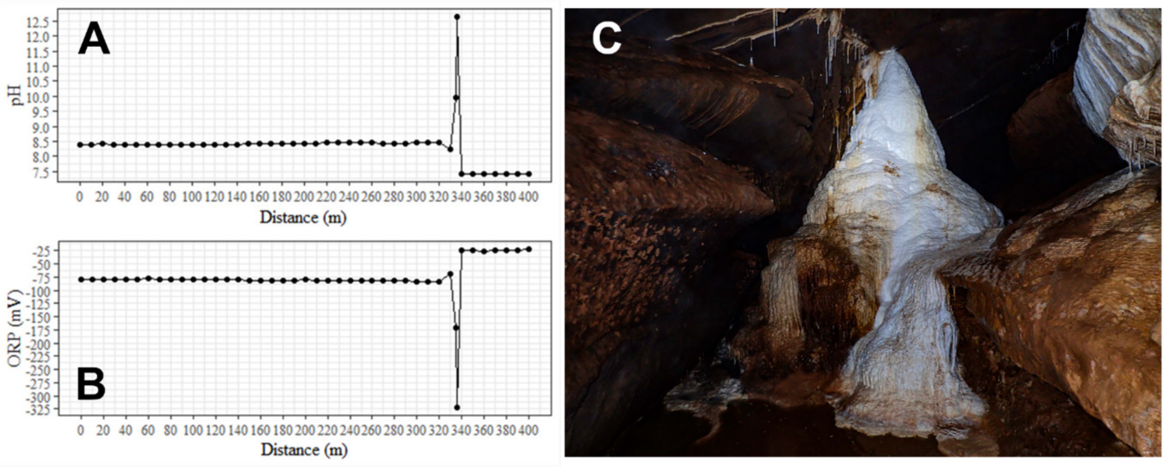

Figure 6. Cave stream transect at Meads Quarry Cave, Tennessee, USA, for (A) pH and (B) oxidation reduction potential (ORP), as measured January 2008. (C) The white speleothem marks where lime deposits from past quarry operations leach into the Meads Quarry Cave system at 336 m upstream of the main entrance. (Photographed by Matthew L. Niemiller).

Cave in Meigs County is owned by the Tennessee Valley Authority. A fence has been constructed around the entrance to restrict access; however, the fence has been breached on occasion. All other cave entrances are privately owned.

Discussion

Abundance, population size, and trends.—Our results corroborate previous suggestions that most populations of *G. gulolineatus* are small (Simmons 1975; Petranka 1998; Beachy 2005; Miller and Niemiller 2008), and repeated salamander observations in the same general area of a cave stream likely represent the same animals. Estimating population sizes of stygobiont salamanders is difficult because of challenges associated with surveys of subterranean habitats. Consequently, size and stability of *G. gulolineatus* populations are often based on relative abundance, and low recapture probabilities suggest that most salamanders are undetected during any given survey (e.g., Miller and Niemiller 2008; Ron Caldwell and John Copeland, unpubl. report). Salamanders clearly exploit smaller passages inaccessible to human exploration, however, which can lead to larger aquatic environments with potential to support a population. Based on our abundance estimates, the two most significant populations, Berry Cave in Roane County and the Meads Quarry Cave system in Knox County, contained up to 19 and 24 salamanders, respectively, during a single survey (Miller and Niemiller 2008; this study). The capture-mark-recapture study by Simmons (1975) estimated *G. gulolineatus* population sizes of 24.7 and 32.0 salamanders at Berry and Mudflats caves, respectively. Our capture-mark-recapture studies (of individuals > 40 mm SVL) at Berry Cave from 2017–2018 and at Meads Quarry Cave in 2008 suggest minimum population sizes of > 95 salamanders at each cave, which offers a better outlook for population persistence. These population size estimates are comparable to population estimates of the related Tennessee Cave Salamander (*G. palleucus*): 95% confidence intervals span 31–302 salamanders, depending on the cave (Huntsman et al 2011, Niemiller et al 2016a).

Qualitative assessments of population trends are critical to assessing threat classification. Some authors suggest that some *G. gulolineatus* populations are in decline (e.g., Berry Cave and Mudflats Cave; Petranka 1998; Beachy 2005; Ron Caldwell and John Copeland, unpubl. report) or possibly extirpated (e.g., Mudflats Cave; USFWS 2010). Our recent surveys in 2017–2019 highlight the high variation in visual count data, and when we include data from reported surveys from the 1980s to the late 2010s, the Berry Cave population appears to be stable over the past three or more decades and Mudflats and Meads Quarry cave populations show a signature of decline over the past 35+ and 10+ y, respectively. We caution, however, against the inference that these populations are on the brink of extirpation. One consideration is how local weather directly or indirectly affect environmental conditions and the presence and detection of salamanders. For the most part, we conducted surveys during optimal environmental conditions for humans (i.e., low water level, flow, and turbidity); however, conditions varied. For example, water levels at Mudflats Cave may fluctuate 2.5 m or more annually in relation to precipitation and water levels of nearby Ft. Loudon Lake, and we have visited the cave when high water levels prevented survey and during periods of drought when water could only be found in the footprints left behind from past surveys.

Our observations of small population size, site fidelity, and low vagility of *G. gulolineatus* (Simmons 1975; our data from Meads Quarry Cave) might be general characteristics of adult stygobitic salamanders (Huntsman et al. 2011; Fenolio et al. 2014a; Niemiller et al. 2016a; Balázs et al. 2020); however, population sizes on the order of 100–150 individuals (as estimated for Meade Quarry and Berry Caves) are well below minimum population sizes estimated for long-term population viability (e.g., Frankel and Soule 1981; Lochran et al. 2007; Flather et al. 2011). Therefore, even in the absence of external threats, avoidance of extinction for *G. gulolineatus* depends on the frequency of dispersal between populations and the potential existence of viable source populations that are undiscovered or inaccessible. These questions about population structure might be answerable with a large population genetics study.

Threats.—Threats to *G. gulolineatus* populations include habitat degradation and contamination associated with urbanization, which likely pose the greatest and most urgent threats, particularly those near Knoxville (Meads Quarry Cave system, Mudflats Cave, Aycock Spring Cave, and Christian Cave), as well as alternations to surface stream flow, cave visitation, and hybridization. Historical impoundments on the Clinch and Tennessee rivers, such as the construction of Melton Hill Lake in the 1960s and Ft. Loudon Lake in the 1940s, have potentially impacted local populations by altering stream flow dynamics and surface to groundwater connectivity. Flooded cave passages may have also allowed predatory surface fishes, such as catfishes (*Ictalurus* spp.) and sunfishes (*Lepomis* spp.), which have been observed at low densities in the Berry Cave stream (Niemiller et al. 2016b), access to previously inaccessible *G. gulolineatus* habitat.

Urbanization can also lead to contamination, although the sources, scope, and potential severity of habitat degradation vary among populations. For example, Mudflats Cave has been receiving excess sediment from the nearby Gettysvue housing development and development within the Ten Mile Creek watershed in west Knoxville (USFWS 2011). Shortly after salamanders were discovered in Christian and Aycock Spring caves, construction of the Covered Bridge residential development in Hardin Valley began within the immediate vicinity. The population at Meads Quarry Cave continues to be threatened, despite being protected in the Knoxville Urban Wilderness, a 688-ha collection of parcels in south Knoxville (Zefferman et al. 2018), also having avoided being impacted from a proposed but unmaterialized James White Parkway extension (USFWS 2010).

Our data indicate that past quarry operations and associated lime deposits continue to affect water quality and probably contribute to unhealthy salamanders. Leakage of septic tanks, which is a pervasive problem in urbanized karst terrain, can be a source of elevated ion concentrations, like nitrate and chloride (Wakida and Lerner 2005), as well as high fecal coliform counts. Elevated bacterial loads in surface water can lead to reduced oxygen concentrations (Ya Zheng et al., unpubl. report). Decreased dissolved oxygen has become a major concern for stygobitic Barton Springs Salamanders (*E. sosorum*) and Austin Blind Salamanders (*E. waterlooensis*) in Texas, USA (USFWS 2016c); however, a paucity of information about critical levels of sediment, ion, and bacterial contaminants for particular amphibian species and conditions limits application to viability assessments (Egea-Serrano et al. 2012; USFWS 2016c).

Because entrances to most caves with populations of *G. gulolineatus* do not occur on public lands, access to the caves and the salamander populations is entirely controlled by private landowners. Several caves are gated (Meads Quarry Cave system, Blythe Ferry Cave, and Christian Cave), and a conservation agreement among landowners, the USFWS, Tennessee Wildlife Resources Agency, and The Nature Conservancy exists at Berry Cave. Although there is potential risk of over-collection by unscrupulous hobbyists, we believe this threat is quite low given the difficulty in accessing and surveying caves and catching *G. gulolineatus.* There may be greater impacts associated with recreation at some caves. Cave visitation may increase the risk for accidental injury, death, and loss of oviposition sites under rocks and other cover objects, but we have not observed oviposition sites in primary cave passages, and most salamanders appear to avoid footfall as the pulse waves created by people moving in water stimulates an escape response. Overall, data are lacking to substantiate hypotheses about direct impacts owing to cave visitation.

The range of *G. porphyriticus* overlaps completely with that of *G. gulolineatus*, and the two species are syntopic at Mudflats Cave, Small Cave, and the Meads Quarry Cave system (Simmons 1975; Miller and Niemiller 2008; Niemiller et al. 2016b). Although *G. porphyriticus* can occur at high densities in caves in the AVR (Osbourn 2005; Miller and Niemiller 2008) where larvae may live in cave streams for several years before undergoing metamorphosis (Culver 1975), *G. gulolineatus* outnumber *G. porphyriticus* at sites where they co-occur. In general, *G. porphyriticus* occurs at higher densities closer to entrances of cave systems with in-flowing streams compared to sections of cave streams that have been flowing underground for several hundred meters. Areas in dark zones where *G. porphyriticus* and *G. gulolineatus* may interact likely serve as sink habitats for *G. porphyriticus.* Loss and degradation of surface habitat might facilitate greater use of subterranean habitats by *G. porphyriticus* and contribute to increased levels of competition or hybridization.

Molecular evidence indicates that low levels of interbreeding have occurred relatively recently between *G. gulolineatus* and *G. porphyriticus* at Meads Quarry Cave (Niemiller et al. 2008, 2009) and perhaps at Cruze Cave (USFWS 2011). Hybridization could influence the long-term viability of *G. gulolineatus* populations and lead either to extinction if hybrids experience low fitness (Rhymer and Simberloff 1996) or to so called genomic extinction if genetically pure *G. gulolineatus* are replaced by individuals of mixed ancestry. The philosophical and ecological ramifications of the latter are not well-understood, but hybridization can be a threat, particularly if human activities affect the probability of interbreeding or the ecological viability of hybrids (e.g., Fitzpatrick and Shaffer 2007; Fitzpatrick et al. 2010). Regardless, we do not believe that contemporary hybridization currently is a major threat to *G. gulolineatus.* Even if the level of gene flow between *G. gulolineatus* and *G. porphyriticus i*s low (e.g., Niemiller et al. 2008, 2009), it is unknown whether this is primarily a function of low contact rates or intrinsic isolating mechanisms. In addition, we do not know the probability of interbreeding when the two species do co-occur.

Conservation status.—*Gyrinophilus gulolineatus* is considered extant at nine distinct caves that represent seven cave systems (USFWS 2019a). We confirmed their presence at five of these cave systems in the last 10 y. We were unable to acquire authorization to resurvey Christian Cave. We surveyed Blythe Ferry Cave in January 2018 but found little significant aquatic habitat except for a few shallow epikarst-fed drip pools, and this site is not considered to represent an extant population (USFWS 2019a). The single occurrence from this cave is based on a specimen collected by bat biologist Merlin Tuttle during a bat survey in July 1975 (specimen USNM 319407) from a small, shallow pool (about 3 cm deep and about 25 cm diameter) near the main bat roosting area (Liz Burton Hamrick, pers. comm.). This observation, in addition to the three specimens collected from a roadside ditch near Athens in McMinn County (Johnson 1958; Brandon 1965), further suggest that *G. gulolineatus* is more widely distributed than previously thought but occurs in groundwater largely inaccessible to humans.

Gyrinophilus gulolineatus was last assessed as Endangered B1ab(iii) + 2ab(iii) in 2004 under IUCN Red List criteria because of an EOO < 5,000 km^2, a severely fragmented distribution, and evidence of continuing decline in the extent and quality of habitat (Hammerson 2004). Based on our conservation assessment, we recommend no change to this conservation rank. Similarly, *G. gulolineatus* was last assessed as Critically Imperiled (G1Q) in 2004 (last reviewed in 2019) under NatureServe criteria because of a small range extent (250–5000 km^2), few occurrences, very few occurrences with good viability, evidence of a short-term population decline (< 30% to relatively stable), and medium to very high overall threat impact (https://explorer.natureserve.org/). Similarly, we recommend a NatureServe conservation rank of G1G2 (Critically Imperiled to Imperiled), given uncertainty in the number of occurrences with good viability, evidence for a short-term population decline (< 30% to relatively stable), and impacts of threats (medium to very high threat impact). *Gyrinophilus gulolineatus* remains listed as Threatened by the state of Tennessee, and no populations are expected to occur outside of the state. The determination by the USFWS not to list *G. gulolineatus* was based largely on newly discovered populations since the last 12-mo finding (USFWS 2011).

Recommendations.—We recommend that *G. gulolineatus* continue to be considered for listing under the ESA based on available information on threats to populations and our conservation assessments; however, more information is needed to clarify demographic and life-history parameters of even the most studied populations (Berry, Meads Quarry, and Mudflats caves). Such data are critical to predict population viability and resiliency under future scenarios. Together with a paucity of information on diet, diseases, parasites, tolerance to low oxygen conditions, poor water quality, and habitat degradation, and other aspects of life history, predictions from even the most sophisticated analyses can hold little to no value for decision makers (Coulson et al. 2001).

We have made a concerted effort in recent years to bioinventory cave systems in the AVR (Niemiller et al. 2016b; Zigler et al. 2020; this study). Despite very few new occurrences, we remain optimistic that additional populations will be discovered. Although surveys have been conducted in many larger caves near historical *G. gulolineatus* sites, dozens of smaller caves (< 150 m in length) have not been surveyed biologically, particularly in the southern AVR. Moreover, at least 15 caves with streams or other hypogean waters with potential to support *G. gulolineatus* exist north of Melton Hill Lake in portions of Anderson and Roane counties in Tennessee. These caves occur within 2–3 km from Aycock Spring Cave with direct hydrologic connection via the Clinch River system, which was impounded to create Melton Hill Lake in the 1960s. The caves might benefit from highly restricted access as part of the Oak Ridge Environmental Research Park of the U.S. Department of Energy but are subject to various contaminants associated with past U.S. Department of Energy activities (Carter et al. 2019).

Additional studies are needed to determine the sources, nature, and extent of threats to populations, and mitigate these threats whenever possible. Groundwater recharge zones and flow patterns should be delineated for all populations, such as through dye tracing programs, and water quality should be regularly assessed at Berry, Meads Quarry, Mudflats, and The Lost Puddle caves, among others, to monitor environmental changes and contaminant sources. Vulnerability mapping should be conducted to estimate the risk and impacts of potential contamination sources to assist in land management decisions and species protection. For instance, Ijams Nature Center staff are now consulting with geologists regarding possible measures to remove surface lime deposits and reduce leaching into the Meads Quarry Cave system (Ben Nanny, pers. comm.).

Protection of the cave surface and subsurface drainage basins is probably the most important intervention for many populations of *G. gulolineatus*. Minimally, this should include application of best land management practices (e.g., stormwater mitigation and erosion control) and more stringent associated regulations around sinkholes and sinking creeks. Permits are currently required by the Tennessee Department of Environment and Conservation for major impacts to sinkholes, but the regulations apply under rather specific scenarios (e.g., solid waste treatment and injection wells; https://www.tn.gov/environment/program-areas/solid-waste/sw-regulations.html). Private landowners are rarely educated on state environmental regulations, and there is little incentive to follow existing regulations even when they are known, as the state lacks the ability to monitor most private sites.

Finally, we strongly advocate for the immediate development of captive breeding programs (CBPs) for *G. gulolineatus*. The establishment of CBPs has become a popular conservation tool for many herpetofaunal groups (Griffiths and Pavajeau 2008; Browne et al. 2011), including groundwater salamanders (Fenolio et al. 2014b). For extremely limited populations of a species, CBPs provide a preemptive safeguard against species loss but ideally should be developed before collection (and necessary experimental rearing and breeding) of individuals itself poses additional risk to viability in the wild. Importantly, CBPs should be researched and implemented only by those accredited institutions that possess the infrastructure and professional networks required to support tasks ranging from long-term breeding to monitoring the success of reintroduction efforts (Heinrichs et al. 2019). This ensures that CBPs have the capacity to adapt protocols under controlled conditions and can extend success when complications arise (e.g., Williams and Hoffman 2009).

Conclusions.**—**We still understand relatively little about the biology, life history, and ecology of *G. gulolineatus.* Shortfalls in our knowledge are commonplace for most subterranean fauna given the inherent difficulties associated with studying and monitoring organisms living underground (Mammola et al. 2019). Consequently, nearly all subterranean taxa that are evaluated under the SSA framework will suffer from the same or similar deficiencies to inform the 3 Rs. Filling in these knowledge gaps will best inform viability and guide decisions under the ESA and inform resiliency, redundancy, and representation, as used in the SSA framework of the USFWS (and described by Shaffer and Stein 2000). In the case of *G. gulolineatus*, we recommend that the species remain a Candidate Species at minimum due to documented and potential threats, low apparent abundance and number of occurrences, and both uncertainty and lack of data for many aspects of the ecology and life history. Most of what we know about *G. gulolineatus* supports only the broad conclusions that the species is geographically restricted to aquatic subterranean environments of eastern Tennessee, exploits areas that may not be readily accessible or surveyable by humans, especially during important life-history events (e.g., egg deposition), and that individuals appear to exhibit high site fidelity within the survey durations considered herein. We know almost nothing about where, when, and over what distance dispersal might take place within and between cave systems, the extent that movements are restricted to aquatic subterranean systems, and whether dispersal is active, passive, or both across life stages. Owing to impoundment of major rivers and habitat loss over the past 50+ y, it is possible that most or all inhabited cave systems are isolated. Such recent isolation events would be difficult to quantify when one considers that dispersal, long-term movement distance, and generation times in *G. gulolineatus* are unknown.

Even if one considers each cave with at least one *G. gulolineatus* observation to be a population, all populations would be restricted to the AVR within eastern Tennessee, with little opportunity for dispersal between segmented karst and watershed units (Niemiller et al. 2018). Sites that do have potential for gene flow occur within the rapidly developing Knoxville metropolitan area, and unknown aspects of life history, particularly the length of larval period, life span, and fecundity, and timing of responses to stressors by *G. gulolineatus*, are clearly needed to understand resiliency under future scenarios in the context of impacts from urbanization. Noninvasive sampling methods (e.g., Fenolio et al. 2017) and innovative methods of detection, such as environmental DNA (Gorički et al. 2017; Vörös et al. 2018; Niemiller et al. 2018; DiStefano et al. 2020; Boyd et al. 2020), from groundwater systems inaccessible to human surveyors may be used to assess representation and redundancy. Until these data can be collected, existing viability models might hold little weight to predict population outcomes under future scenarios. Moreover, although small population size and potential isolation would not indicate a positive long-term outlook for *G. gulolineatus*, it remains possible that this stygobitic species might benefit from directed conservation strategies, such as CBPs (Valbuena-Ureña et al. 2017).

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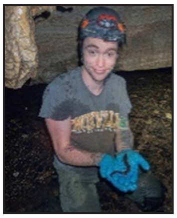

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SUPPLEMENTAL INFORMATION

THE DISTRIBUTION, ECOLOGY, LIFE HISTORY, AND CONSERVATION STATUS OF THE BERRY CAVE SALAMANDER (*GYRINOPHILUS GULOLINEATUS*)

MATTHEW L. NIEMILLER, EVIN T. CARTER, NICHOLAS S. GLADSTONE, K. DENISE KENDALL NIEMILLER, LINDSEY E. HAYTER, ANNETTE S. ENGEL, BRIAN T. MILLER, AND BENJAMIN M. FITZPATRICK

The following material is provided by the authors and was not subjected to editing by *Herpetological Conservation and Biology*.

TABLE S1. Summary of surveys for Berry Cave Salamanders (*Gyrinophilus gulolineatus*) at historical sites in eastern Tennessee, USA between 2004 and 2019, including the current study.

County	Cave	TCS no.	Date	Salamanders observed
Knox	Aycock Spring Cave	KN172	9/17/2005	1
Knox	Aycock Spring Cave	KN172	7/10/2018	0
Knox	Christian Cave	KN49	9/17/2005	1
Knox	Fifth Entrance Cave	KN167	10/23/2004	0
Knox	Fifth Entrance Cave	KN167	11/8/2007	1
Knox	Fifth Entrance Cave	KN167	7/14/2018	0
Knox	Meads Quarry Cave	KN28	10/23/2004	11
Knox	Meads Quarry Cave	KN28	11/4/2006	11
Knox	Meads Quarry Cave	KN28	4/22/2007	14
Knox	Meads Quarry Cave	KN28	9/9/2007	24
Knox	Meads Quarry Cave	KN28	11/8/2007	5
Knox	Meads Quarry Cave	KN28	11/24/2007	6
Knox	Meads Quarry Cave	KN28	1/24/2008	7
Knox	Meads Quarry Cave	KN28	1/31/2008	18
Knox	Meads Quarry Cave	KN28	3/1/2008	10
Knox	Meads Quarry Cave	KN28	3/6/2008	4
Knox	Meads Quarry Cave	KN28	3/30/2008	16
Knox	Meads Quarry Cave	KN28	4/10/2008	11
Knox	Meads Quarry Cave	KN28	4/30/2008	17
Knox	Meads Quarry Cave	KN28	5/15/2008	7

Knox	Meads Quarry Cave	KN28	6/4/2008	24
Knox	Meads Quarry Cave	KN28	6/27/2008	8
Knox	Meads Quarry Cave	KN28	7/30/2008	15
Knox	Meads Quarry Cave	KN28	9/10/2008	17
Knox	Meads Quarry Cave	KN28	10/5/2013	5
Knox	Meads Quarry Cave	KN28	11/22/2017	10
Knox	Meads Quarry Cave	KN28	1/13/2018	5
Knox	Meads Quarry Cave	KN28	3/10/2018	8
Knox	Meads Quarry Cave	KN28	6/17/2018	2
Knox	Meads Quarry Cave	KN28	9/23/2018	9
Knox	Meads Quarry Cave	KN28	4/5/2019	1
Knox	Meads River Cave	KN151	10/23/2004	0
Knox	Meads River Cave	KN151	4/22/2007	0
Knox	Meads River Cave	KN151	11/8/2007	0
Knox	Meads River Cave	KN151	11/24/2007	1
Knox	Meads River Cave	KN151	12/2/2007	0
Knox	Meads River Cave	KN151	9/10/2008	0
Knox	Meads River Cave	KN151	2/17/2018	0
Knox	Meads River Cave	KN151	7/14/2018	0
Knox	Mudflats Cave	KN9	11/20/2004	5
Knox	Mudflats Cave	KN9	1/6/2005	3
Knox	Mudflats Cave	KN9	12/30/2005	5
Knox	Mudflats Cave	KN9	11/12/2006	2

Knox	Mudflats Cave	KN9	6/7/2007	5
Knox	Mudflats Cave	KN9	4/5/2014	1
Knox	Mudflats Cave	KN9	10/20/2014	1
Knox	Mudflats Cave	KN9	1/8/2015	1
Knox	Mudflats Cave	KN9	10/29/2017	0
Knox	Mudflats Cave	KN9	11/25/2017	0
Knox	Mudflats Cave	KN9	2/27/2018	0
Knox	Mudflats Cave	KN9	3/16/2018	2
Knox	Mudflats Cave	KN9	5/10/2018	0
Knox	Mudflats Cave	KN9	6/18/2018	0
Knox	Mudflats Cave	KN9	9/22/2018	1
Knox	The Lost Puddle	KN145	5/8/2012	3
Knox	The Lost Puddle	KN145	3/23/2018	4
Knox	The Lost Puddle	KN145	7/13/2018	2
McMinn	Small Cave	MM5	5/10/2014	1
Meigs	Blythe Ferry Cave	ME1	1/26/2018	0
Roane	Berry Cave	RN3	12/17/2004	1
Roane	Berry Cave	RN3	3/5/2005	4
Roane	Berry Cave	RN3	6/28/2014	3
Roane	Berry Cave	RN3	2/14/2016	2
Roane	Berry Cave	RN3	10/30/2017	9
Roane	Berry Cave	RN3	12/4/2017	10
Roane	Berry Cave	RN3	1/6/2018	6

Roane	Berry Cave	RN3	2/17/2018	3
Roane	Berry Cave	RN3	3/16/2018	3
Roane	Berry Cave	RN3	4/13/2018	6
Roane	Berry Cave	RN3	5/10/2018	5
Roane	Berry Cave	RN3	6/18/2018	3
Roane	Berry Cave	RN3	7/20/2018	4
Roane	Berry Cave	RN3	8/12/2018	5
Roane	Berry Cave	RN3	9/15/2018	19
Roane	Berry Cave	RN3	10/21/2018	5
Roane	Berry Cave	RN3	12/8/2018	9
Roane	Berry Cave	RN3	1/19/2019	0
Roane	Berry Cave	RN3	2/17/2019	0
Roane	Berry Cave	RN3	4/6/2019	1
Roane	Berry Cave	RN3	7/20/2019	2

TABLE S2. Summary of caves surveyed during the current study (2017–2019) and additional surveys associated with other projects (e.g., Niemiller et al. 2016b, 2017) between 2004 and 2017 in the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee, USA, including survey dates and Tennessee Cave Survey (TCS) number. Berry Cave Salamanders (*Gyrinophilus gulolineatus*) were not observed at these sites. Sites where related Spring Salamanders (*G. porphyriticus*, Gpor) were observed are indicated.

County	Cave	TCS no.	Date	Gpor
Anderson	Blowing Springs Cave	AN1	2016: 3 Jun 2018: 13 Apr	
Anderson	Offut Cave	AN12	2018: 18 May	
Anderson	Weaver Cave	AN22	2016: 22 Mar	
Anderson	Springhill Saltpeter Cave	AN3	2017: 28 Oct	
Anderson	Martin Cave	AN31	2016: 21 Feb	
Anderson	Rieders Lost Creek Cave	AN36	2016: 30 May	
Anderson	Wallace Cave	AN37	2015: 25 Oct	
Anderson	Rainy Knob Cave	AN42	2019: 10 May	
Anderson	Demarcus Cave	AN5	2018: 26 Jun	Y
Anderson	Robert Smith Cave	AN6	2018: 26 Jun	
Anderson	Carters Pit	AN8	2015: 19 Dec	
Blount	Tuckaleechee Caverns	BA11	2014: 20 Mar	
Campbell	Panther Cave No. 1	CM8	2015: 23 Mar 2018: 19 Jul	
Campbell	Panther Cave No. 2	CM9	2018: 19 Jul	

Carter	Carter Saltpeter Cave	CR1	2014: 14 May	
Carter	Rockhouse Cave	CR3	2014: 14 May	
Claiborne	Obie Mill Cave	CB14	2019: 16 Mar	
Claiborne	Powell Mountain Cave	CB15	2019: 16 Mar	Y
Claiborne	Station Creek Cave	CB17	2019: 6 Jun	
Claiborne	Sour Kraut Cave	CB46	2015: 1 Jun	
Claiborne	Buis Saltpeter Cave	CB48	2015: 1 Jun	Y
Claiborne	Tom Balls Cave	CB51	2019: 6 Jun	
Claiborne	Kings Saltpeter Cave	CB52	2015: 30 May	Y
Claiborne	Coonsies Creek Cave	CB57	2016: 23 Mar	
Claiborne	Tiprell Spider Cave	CB78	2019: 6 Jun	
Claiborne	Fools Cave	CB90	2016: 23 Mar	
Grainger	Indian Cave	GA4	2014: 22 Feb; 29 Jun	Y
Hamblen	Soard Cave	HB3	2015: 29 Dec	
Hamblen	Miller Cave	HB5	2015: 29 Dec	
Hamilton	Pan Gap Cave	HM11	2019: 10 Jun	
Hamilton	Read Spring Cave	THM47	2019: 25 May	
Jefferson	Silo Pit Cave	JF71	2015: 3 Aug	
Jefferson	Tater Cave	JF8	2015: 3 Aug	
Knox	Campbell Cave	KN1	2014: 23 Dec	
Knox	Pedigo Cave	KN103	2018: 14 Jul; 26 Jul; 15 Dec 2019: 27 Jan	

Knox	Pedigo Cave No. 2	KN108	2018: 14 Jul	
Knox	Out and In Cave No. 1	KN111	2019: 13 Jan	
Knox	Brents Cave	KN112	2012: 8 May 2018: 23 Mar	
Knox	Heiskell Pit	KN12	2015: 19 Dec	
Knox	Burnett Cave	KN125	2008: 21 May	Y
Knox	Chriscroft Cave	KN127	2014: 20 Oct	
Knox	Carter Cave	KN14	2008: 21 May	Y
Knox	Ebenezer Rising Cave	KN150	2004: 20 Nov 2018: 22 Sep	
Knox	Watercress Cave	KN153	2019: 13 Jan	
Knox	Keller Bend Cave	KN16	2013: 16 May	
Knox	Steamboat Crawl	KN173	2007: 5 Apr	
Knox	Blowing Hole Cave	KN19	2013: 16 May 2015: 14 Nov	
Knox	Cherokee Caverns	KN22	2014: 5 Apr	
Knox	Cruze Cave	KN24	2004: 31 Oct 2005: 6 Jan; 6 Mar; 31 Dec 2006: 18 Jul; 10 Sep; 19 Nov 2008: 19 May; 7 Jul 2013: 13 May; 15 Jun	Y

			2014: 10 Apr; 11 May; 19 Jun; 14 Aug; 13 Oct 2018: 3 Jul	
Knox	Cherokee Bluff Cave	KN4	2015: 7 Mar	
Knox	Conner Creek Cave	KN50	2018: 10 Jul	
Knox	Kirkpatrick Cave	KN62	2014: 9 Feb; 6 Jul 2019: 25 Jun	Y
Knox	Wilke Waller Cave	KN80	2019: 10 Jul	
Knox	Thumping Cave	KN82	2019: 25 Jun	
Knox	Unreported Cave	KN90	2014: 5 Apr	
Loudon	Blankenship Cave	LN1	2014: 25 Jan	
Loudon	Benjos Cave	LN11	2014: 30 Aug	
Loudon	Ghost Cave	LN3	2014: 30 Aug	Y
Loudon	Melton Hill Spring Cave	LN4	2018: 6 Oct	
McMinn	McCorkle Cave	MM10	2018: 6 Jul	
McMinn	Too Small Cave	MM6	2014: 10 May	
Meigs	Sensabaugh Cave	ME3	2014: 31 Aug	Y
Monroe	The Lost Sea	MO1	2014: 9 Sep	
Monroe	Gay Cave	MO3	2013: 16 Nov	
Monroe	Morgan Cave	MO5	2013: 26 Oct	
Monroe	Nobletts Cave	MO6	2014: 26 Nov	Y
Monroe	Lick Creek Cave	MO8	2013: 16 Nov	

Monroe	Alans Hideway Cave	MO9	2013: 16 Nov	
Rhea	Dayton Quarry Cave	RH1	2017: 14 Jul	
Rhea	Grassy Creek Cave	RH2	2014: 22 Dec	
Rhea	Starve Rock Cave	RH7	2016: 26 Mar	
Rhea	Clear Creek Cave	RH8	2016: 26 Mar	
Rhea	Piney River Cave	RH9	2016: 26 Mar	
Roane	Big Cave	RN13	2005: 5 Mar	
Roane	Chimney Cave	RN14	2005: 5 Mar	
Roane	Marble Bluff Cave	RN19	2018: 27 Feb	
Roane	Cave Creek Cave	RN5	2007: 7 Jun 2014: 28 Jun 2018: 3 May; 3 Jun; 3 Jul; 15 Dec 2019: 3 Feb	Y
Roane	Eblen Cave	RN6	2005: 30 Dec 2013: 15 May 2019: 3 Feb; 24 Mar	Y
Sevier	Two County Cave	SV36	2014: 5 Jul	
Sullivan	Bristol Caverns	SL1	2017: 17 Oct	Y
Union	Big Cave	UN10	2015: 22 Mar	Y
Union	Rogers Hollow Cave	UN23	2015: 22 Mar	
Union	Mossy Spring Cave	UN25	2015: 22 Mar	

Union	Big Coon Caverns	UN30	2018: 19 Jul		
Union	Little Coon Cave	UN36	2018: 19 Jul		
Union	Ellison Hollow Cave	UN46	2015: 22 Mar		
Union	Oaks Cave	UN5	2015: 23 Mar		Y
Union	Wright Cave	UN9	2015: 21 Mar		

TABLE S3. Summary of average parameter estimates and AICc for best model distributions comparing abundance over time (salamanders observed ~ days) at Berry, Mudflats, and Meads Quarry Cave. Days represents number of days since 01 January 1983 (before first survey in dataset). Conditional (c.m.) and zero-inflation (zi.m) model parameters of hurdle models are included. Models in bold indicate top fitting distributions (i.e., ΔAICc < 2). Significance: *** - p < 0.001; ** p < 0.01; * p < 0.05.

Model distribution	Berry Cave	Mudflats Cave	Meads Quarry Cave
Gaussian	intercept: 5.09*	intercept: 5.53***	intercept: 28.1***
	days: -2.0e-5	days: -3.57e-4***	days: -0.0018**
	df: 3	df: 3	df: 3
	AICc: 153.2	AICc: 76.4	AICc: 161.9
Poisson	intercept: 1.63***	**intercept: 1.80*****	intercept: 4.29***
	days: -4.04e-6	**days: -1.29e-4*****	days: -1.95e-4***
	df: 2	**df: 2**	df: 2
	AICc: 172.1	**AICc: 75.4**	AICc: 171.9
Zero-inflated Poisson	intercept: 1.60***	intercept: 1.80***	intercept: 4.29***
	days: 6.54e-6	days: -1.29e-4***	days: -1.95e-4***
	zi.m. intercept: -2.50**	zi.m. intercept: -21.03	zi.m. intercept: -21.78
	df: 3	df: 3	df: 3
	AICc: 168.0	AICc: 78.1	AICc: 174.5

Zero-inflated hurdle Poisson	intercept: 1.59*** days: 7.46e-6 zi.m. intercept: 106.8 zi.m. days: 0.008 df: 4 AICc: 166.4	**intercept: 1.72** **days: -9.7e-5** **zi.m. intercept: -35.69** **zi.m. days: 0.0028** **df: 4** **AICc: 73.8**	intercept: 4.29 days: -1.96e-4 zi.m. intercept: -0.437 zi.m. days: -0.0023 df: 4 AICc: 177.3
Negative binomial	**intercept: 1.52***** **days: 5.77e-6e-4***** **k: 2.83** **df: 3** **AICc: 139.2**	intercept: 1.81*** days: -1.30e-4*** k: 0.09 df: 3 AICc: 78.1	**intercept: 4.26***** **days: -1.92e-4**** **k: 1.75** **df: 3** **AICc: 157.3**
Zero-inflated negative binomial	intercept: 1.52 days: 5.77e-6 k: 2.83 zi.m. intercept: -20.1 df: 4 AICc: 142.1	intercept: 1.80 days: -1.29e-4 k: 0.09 zi.m. intercept: -20.9 df: 4 AICc: 81.2	intercept: 4.29 days: -1.92e-4 k: 1.75 zi.m. intercept: -21.8 df: 4 AICc: 160.3
Zero-inflated hurdle negative binomial	**intercept: 0.760** **days: 6.85e-5** **zi.m. intercept: -106.8** **zi.m. days: 0.008**	intercept: 1.72 days: -9.74e-5 zi.m. intercept: -35.7 zi.m. days: 0.003 k: 2.43e-6	intercept: 4.39 days: -2.06e-4 zi.m. intercept: 0.003 zi.m. days: -0.001 k: 1.87

	k: 3.44 **df: 5** **AICc: 139.8**	df: 5 AICc: na	df: 5 AICc: 162.8
Negative binomial with NB2 parameterization	**intercept: 1.63***** **days: -3.80e-6** **k: 1.72** **df: 3** **AICc: 139.2**	intercept: 1.80*** days: -1.29e-4*** k: 6.56e7 df: 3 AICc: 78.1	**intercept: 4.35***** **days: -2.02e-4***** **k: 6.16** **df: 3** **AICc: 157.5**
Zero-inflated negative binomial with NB2 parameterization	intercept: 1.63*** days: -3.80e-6 zi.m. intercept: -20.4 k: 1.72 df: 4 AICc: 142.1	intercept: 1.80 days: -1.29e-4 zi.m. intercept: -16.5 k: 3.22e7 df: 4 AICc: na	intercept: 4.35 days: -2.02e-4 zi.m. intercept: -22.1 k: 6.16 df: 4 AICc: 160.3
Zero-inflated hurdle negative binomial with NB2 parameterization	**intercept: 1.44**** **days: 7.89e-6** **zi.m. intercept: -106.8** **zi.m. days: 0.008** **k: 1.38** **df: 5** **AICc: 140.6**	intercept: 1.72*** days: -9.74e-5 zi.m. intercept: -35.7 zi.m. days: 0.003 k: 3.91e6 df: 5 AICc: na	intercept: 4.39*** days: -2.07e-4 zi.m. intercept: -0.362 zi.m. days: -0.0024 k: 5.89 df: 5 AICc: 163.2

TABLE S4. Threats, existing and recommended conservation and management actions for Berry Cave Salamander (*Gyrinophilus gulolineatus*) sites in east Tennessee, USA.

Location	Last observed	Last surveyed	Threats/impacts	Contribution to assess species viability	Severity (expert opinion)	Actions in place	Specific recommended actions
Aycock Spring Cave (TKN172)	2005	2018	• Habitat degradation and contamination associated with urbanization (residential)	Low	High to medium	None	• Water quality monitoring • Delineate recharge basin
Christian Cave (TKN49)	2005	2005	• Habitat degradation and contamination associated with urbanization (residential)	Low	High to medium	Gated	• Water quality monitoring • Delineate recharge basin
Fifth Entrance Cave (TKN167) Meads Quarry Cave (TKN28) Meads River Cave (TKN151)	2007 2019 2007	2018 2019 2018	• Habitat degradation and contamination associated with urbanization (residential and commercial) • Habitat loss and degradation and changes in hydrology associated with past mining operations • Possible competition/hybridization with *G. porphyriticus* • Human visitation	Very high	Very high to high	Gated Managed by Ijams Nature Center	• Remove lime deposits in recharge zone • Water quality monitoring • Increased regulation of cave visitation • Increase natural buffers around infiltration and recharge zone • Assess levels and risk of hybridization with *G. porphyriticus*

Mudflats Cave (TKN9)	2019	2018	• Habitat degradation and contamination associated with urbanization (residential & commercial) • Habitat loss/degradation and changes in hydrology associated with impoundments • Possible competition/hybridization with *G. porphyriticus* • Human visitation	High	Very high to high	None	• Water quality monitoring • Delineate recharge basin • Assess levels and risk of hybridization with *G. porphyriticus*
The Lost Puddle (TKN145)	2018	2018	• Habitat degradation and contamination associated with urbanization (residential)	High	Medium to low	None	• Water quality monitoring
Oostanaula Creek south of Athens	1953	1953	• Unknown	Very low	Na	None	• Determine aquatic/karst connectivity
Small Cave (TMM5)	2014	2014	• Habitat degradation and contamination associated with urbanization (residential) • Possible competition/hybridization with *G. porphyriticus* • Human visitation	Low	Medium to low	None	• Water quality monitoring • Delineate recharge basin
Blythe Ferry Cave (TME1)	1975	2018	• Habitat loss/degradation and changes in hydrology associated with impoundments • Human visitation	Very low	High	Gated Owned and managed by TVA	• Water quality monitoring • Increased regulation of human visitation • Delineate recharge basin
Berry Cave (TRN3)	2019	2019	• Habitat degradation and contamination associated with	Very high	High to medium	Conservation easement	• Water quality monitoring • Increase natural buffers around infiltration and recharge zone

	urbanization (residential) and agriculture (pasture/cattle)
General recommended actions for all sites	• Map hydrologic and karst connectivity • Delineate surface recharge zones • Identify and mitigate contaminant sources • Limit cave visitation without compromising facultative cave fauna • Develop captive breeding programs (accredited) • Monitor human-inaccessible habitats • Leverage noninvasive survey methods (e.g., eDNA)

TABLE S5. Potential threats facing Berry Cave Salamanders (*Gyrinophilus gulolineatus*). Threat impacts are negligible (N), low (L), medium (M), high (H), and very high (VH) based on the scope, severity, and known timing of each threat.

Threat	Threat impact
Residential & commercial development	H
Housing & urban areas	H
Commercial & industrial areas	L
Tourism & recreation areas	L
Agriculture & aquaculture	L
Mining & quarrying	L
Transportation & service corridors	L
Roads & railroads	L
Biological resource use	L
Hunting & collecting animals	L
Human intrusions & disturbance	L
Recreational activities	L
Natural system modifications	M
Dams & water management/use	M
Invasive & other problematic species, genes, & diseases	L
Introduced genetic material	L
Contamination and pollution	M-H
Domestic & urban wastewater (i.e., sewage)	M-H
Agricultural & forestry effluents	L
Climate change & severe weather	L?
Droughts	L?

Storms & flooding	L?