Herpetological Conservation and Biology 16(3):686–703.
Submitted: 5 March 2021; Accepted: 28 October 2021; Published: 16 December 2021.

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

Matthew L. Niemiller[1,8], Evin T. Carter[2], Nicholas S. Gladstone[3], K. Denise Kendall Niemiller[1], Lindsey E. Hayter[4], Annette S. Engel[5], Brian T. Miller[6], and Benjamin M. Fitzpatrick[7]

[1]Department of Biological Sciences, 301 Sparkman Drive Northwest, University of Alabama in Huntsville, Alabama 35899, USA
[2]Environmental Sciences Division, 1 Bethel Valley Road, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA
[3]School of Fisheries, Aquaculture and Aquatic Sciences, 203 Swingle Hall, Auburn University, Auburn, Alabama 36849, USA
[4]Natural Resources Management Program, 1 Bethel Valley Road, Oak Ridge National Laboratory (RSI Services), Oak Ridge, Tennessee 37830, USA
[5]Department of Earth and Planetary Sciences, 602 Strong Hall, University of Tennessee, Knoxville, Tennessee 37996, USA
[6]Department of Biology, 1672 Greenland Drive, Middle Tennessee State University, Murfreesboro, Tennessee 37132, USA
[7]Department of Ecology and Evolutionary Biology, 569 Dabney Hall, University of Tennessee, Knoxville, Tennessee 37996, USA
[8]Corresponding author, e-mail: mn0039@uah.edu

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

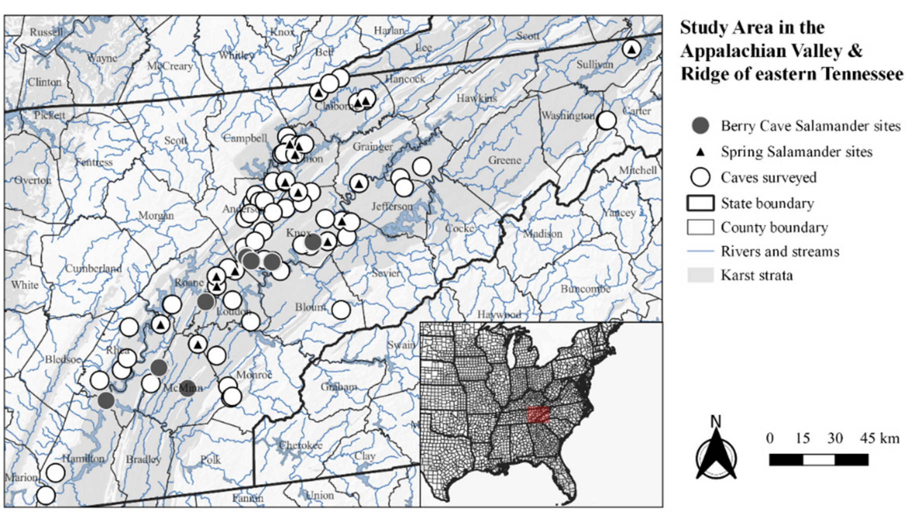

Figure 2. Distribution of the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and locations of 98 caves surveyed between 2004–2019 in the Appalachian Valley and Ridge of eastern Tennessee, USA. Karst carbonate rock are depicted in gray (U.S. Karst Map; Weary and Doctor 2014). Dark circles represent 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

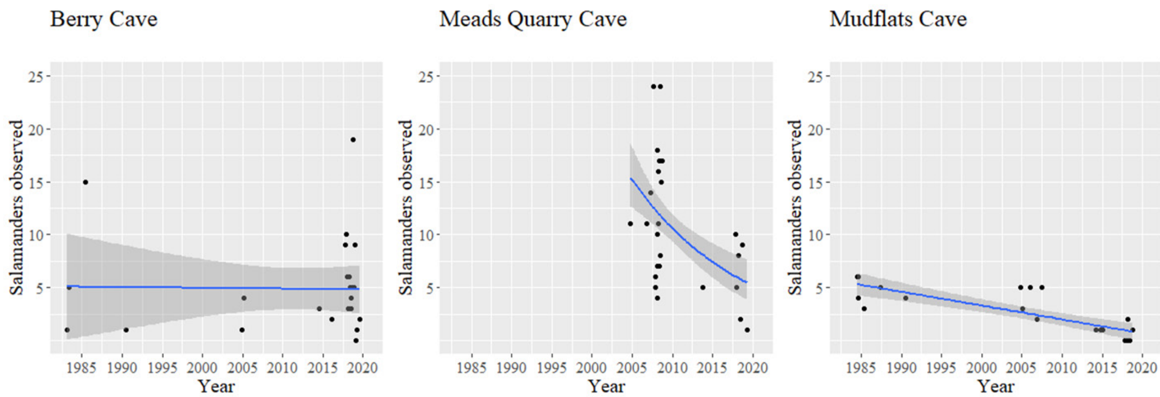

Figure 3. Trends in relative abundance (direct visual counts) of the Berry Cave Salamander (*G. gulolineatus*) at Berry Cave, Mudflats Cave, and Meads Quarry Cave, Tennessee, USA, based on data from Caldwell and Copeland (1992), Miller and Niemiller (2008), and the current study. Blue line is the best 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.

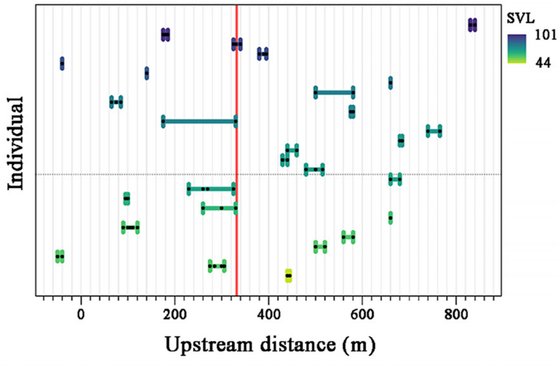

FIGURE 4. Capture locations (distance in meters along cave stream transect from main entrance) for individual Berry Cave Salamanders (*Gyrinophilus* gulolineatus) captured at least twice at Meads Quarry Cave, Tennessee, USA, in 2008. Points are recapture occasions, bars are minimum and maximum distance 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.

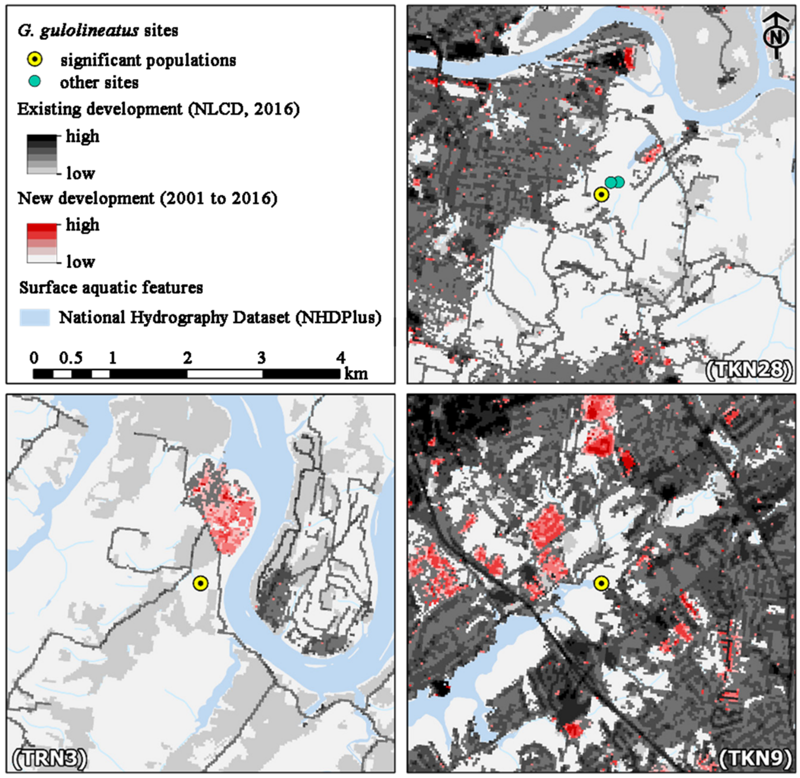

Figure 5. Examples of land cover change (i.e., percentage increase in development) from 2001–2016 at Meads Quarry Cave (upper left), Mudflats Cave (lower right), and Berry Cave (lower left), Tennessee, USA. Greyscale is existing development, and red is new development since 2001. The darkest shading 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

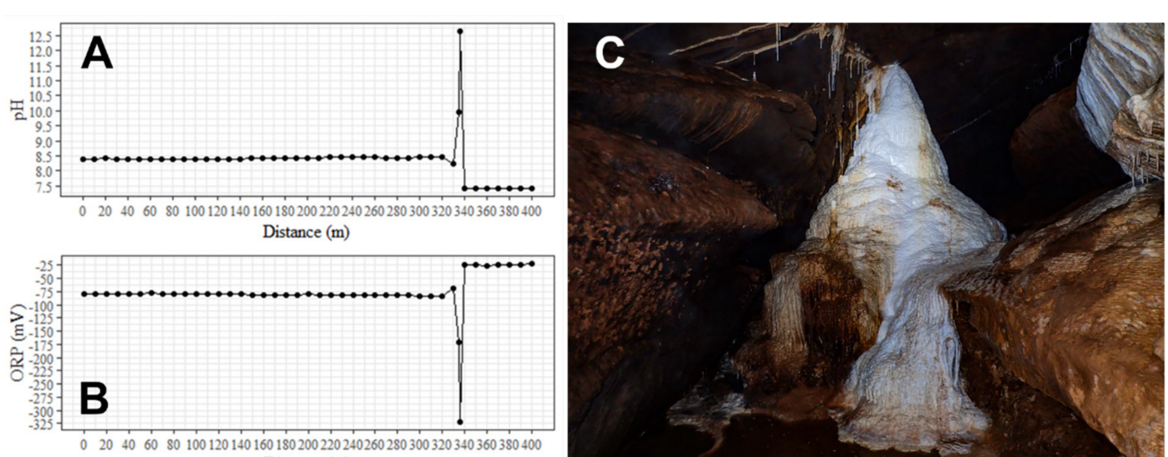

Figure 6. Cave stream transect at Meads Quarry Cave, Tennessee, USA, for (A) pH and (B) oxidation reduction potential (ORP), as measured January 2008. (C) The white speleothem marks where lime deposits from past quarry operations leach into the Meads Quarry Cave system at 336 m upstream of the main entrance.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

Acknowledgments.—Funding for this project was provided by the U.S. Fish and Wildlife Service (grant no. F17AC00939), Tennessee Wildlife Resources Agency (TWRA; contract nos. ED-04-01467-00 and ED-06-02149-00), Cave Conservancy Foundation (contract no. CCF #A14-0574), Department of Biology at Middle Tennessee State University, departments of Ecology & Evolutionary Biology and Earth and Planetary Sciences as well as the Donald H. and Florence Jones Endowment at the University of Tennessee-Knoxville, and the SSAR Dean Metter Memorial Award. All research was conducted under a TWRA scientific collection permit (nos. 1385, 1450, 1585, and 1605) and following approved Institutional Animal Care and Use Committee protocols at the University of Alabama in Huntsville (protocol no. 2017.R005) and Middle Tennessee State University (protocol no. 04-006). We thank Christopher L. Barber (Tennessee Valley Authority; TVA), Tim Burkhardt, Jance Carter, Jeremy Clothier (Ijams Nature Center), Bryan Eads, Jack Gress (Ijams Nature Center), Daniel R. Istvanko (TWRA), Liz Burton Hamrick (TVA), Ryan Hunt, Sarah W. Keenan, Christina Kendall, Ben Nanny (Ijams Nature Center), Chris Ogle (TWRA), David Pelren (USFWS), Evelyn Pieper, Todd Pierson, M.J. Ravesi, Jesse Troxley (TVA), Lauren Van Fleet (Ijams Nature Center), and Jacob Wessels for assistance in the field or sharing data during recent surveys. Audrey Paterson, Abigail Harmon, and Holly Nichols assisted with the water quality and coliform count analyses. We especially thank the Healy family, Jackson family, Tom Coe, Ijams Nature Center (especially Ben Nanny), Tennessee Valley Authority, and other landowners for allowing and arranging access to caves on their properties.

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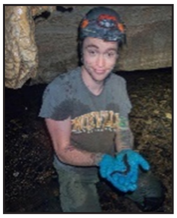
Supplemental Information: http://www.herpconbio.org/Volume_16/Issue_3/Niemiller_etal_2021_Suppl.

Matthew L. Niemiller is an Assistant Professor in the Department of Biological Sciences at the University of Alabama in Huntsville, USA, since 2017. He has a B.S. and M.S. in Biology from Middle Tennessee State University (Murfreesboro, Tennessee, USA) and a Ph.D. in Ecology & Evolutionary Biology from the University of Tennessee (Knoxville, USA). His research for the past 18 y has focused on the ecology, evolution, and conservation of cave life, with an emphasis on salamanders and cavefishes. Matthew also works with herpetofauna that do not live underground. (Photographed by Alfred Crabtree).

Evin T. Carter is the Wildlife Ecology Principal Investigator in the Biodiversity and Ecosystem Health Group of Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. He has a B.S. in Biology from Indiana University Southeast, New Albany, USA, a M.S. in Biology from Purdue University, Fort Wayne, Indiana, USA, and a Ph.D. in Ecology and Evolutionary Biology from the University of Tennessee, Knoxville, USA. Evin merges field-based studies and quantitative methods to address problems in the conservation and evolutionary ecology of salamanders and snakes. He most enjoys the field-based components that lead to and result from these efforts. (Photographed by Dante Fenolio).



Nicholas S. Gladstone is a Ph.D. student at Auburn University, Auburn, Alabama, USA. He has a B.S. in Environmental Studies and a M.S. in Geology from the University of Tennessee, Knoxville, USA. His research interests revolve around documenting biodiversity and studying the evolutionary processes that promote diversification. Nicholas applies molecular and morphological data to investigate the systematics, phylogeography, population genetics, and comparative biology of invertebrate animals. He is also passionate about studies of natural history and conservation of stygobitic animals. (Photographed by Matthew L. Niemiller).

K. Denise Kendall Niemiller is a Lecturer in the Department of Biological Sciences at the University of Alabama in Huntsville, USA, since 2017. She has an A.A. from Pellissippi State Technical Community College (Knoxville, Tennessee, USA) as well as a B.S. in Biological Sciences and Ph.D. in Ecology & Evolutionary Biology from the University of Tennessee (Knoxville, USA). Denise also holds a secondary education professional teaching certificate with an emphasis in Biology. Her research for the past 12 y has focused on science education, ecology, evolution, and conservation. (Photographed by Matthew L. Niemiller).

Lindsey E. Hayter is a Natural Resources Technician employed by the Natural Resources Management Program of Oak Ridge National Laboratory as a subcontractor via RSI Services Inc. (Oak Ridge, Tennessee, USA). She has a B.S. in Wildlife Biology from Ball State University, Muncie, Indiana, USA, and has completed coursework for a M.S. in Biology at Purdue University Fort Wayne, Indiana, USA, where her thesis tests artificial road crossing designs for imperiled herpetofauna. An avid and professional naturalist, her focal areas are public education and outreach related to herpetofauna and aiding fellow researchers in conservation projects. (Photographed by Evin T. Carter).

Annette Summers Engel is a Professor in the Department of Earth and Planetary Sciences at the University of Tennessee-Knoxville, USA. She has a B.A. in Geology from Wittenberg University (Springfield, Ohio, USA), an M.S. in Geology and M.S. in Biology from the University of Cincinnati, Ohio, USA, and a Ph.D. in Geology from the University of Texas at Austin, USA. Her interdisciplinary research focuses on understanding how life becomes distributed within the landscape through time. Among her diverse projects during her career, she focused on uncovering microbial life in cave and karst systems, the genetic underpinnings of chemosymbiotic associations, and characterizing the effects of anthropogenic activities on biogeochemical processes and biodiversity in ecosystems like caves and coastal wetlands. (Photographed by Annette S. Engel).

Brian T. Miller is a Professor of Biology at Middle Tennessee State University, Murfreesboro, Tennessee, USA, where he teaches Comparative Vertebrate Anatomy, Vertebrate Zoology, Herpetology, Scanning and Transmitting Electron Microscopy, and freshman biology courses. He received his B.S. and M.A. from the University of Missouri, Columbia, USA, and his Ph.D. from Washington State University, Pullman, USA. His research for the past four decades has focused on the natural history, morphology, and conservation of amphibians and reptiles, especially that of salamanders. (Photographed by Dante Fenolio).

Benjamin M. Fitzpatrick is a Professor of Ecology and Evolutionary Biology at the University of Tennessee, Knoxville, USA. His research includes conservation biology and population genetics, especially of salamanders. (Photographed by Angeline Fitzpatrick).