
THE DISTRIBUTION AND CONSERVATION STATUS OF THE GREEN SALAMANDER (*ANEIDES AENEUS*) IN TENNESSEE, USA

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Abstract.—Green Salamanders (*Aneides aeneus*) are endemic to the eastern U.S. with a patchy distribution that spans the southern Appalachian Mountains and Cumberland Plateau from Mississippi and Alabama northward into Pennsylvania and Maryland. Green Salamanders are believed to be a specialist of mature hardwood cove forests. Alterations in this habitat from anthropogenic disturbance may be driving population declines that have led to the species being a candidate for listing under the U.S. Endangered Species Act. In Tennessee, Green Salamanders are considered a Species of Conservation Concern; however, their ecology, life history, and conservation status are poorly understood. We conducted the most comprehensive assessment of the distribution and status of the species in Tennessee to date. Specifically, we sought to (1) identify priority populations, (2) address knowledge gaps in its ecology and life history, and (3) complete a comprehensive threat assessment of the species. We conducted surveys of sites with known occurrences and at other sites with suitable habitat, determined geographic extent, and identified existing and future stressors. We documented Green Salamanders at 47 sites during field surveys conducted in 2017–2019, including 30 new sites along the escarpments of the Cumberland Plateau, Cumberland Mountains, Appalachian Valley and Ridge, and northern Eastern Highland Rim. Green Salamanders are now known from 160 localities in 30 Tennessee counties. Existing or potential threats include forest loss and fragmentation from development and logging, construction of water impoundments, over-collection for the pet trade, emergent diseases, and climate change. We offer several recommendations for future research, conservation, and management of the species in Tennessee.

Key Words.—Appalachian Valley and Ridge; Cumberland Plateau; Plethodontidae; species; threat assessment

INTRODUCTION

The Green Salamander, *Aneides aeneus* (Fig. 1), is one of two species of the North American genus *Aneides* endemic to eastern USA. The other species, the Hickory Nut Gorge Green Salamander (*Aneides caryaensis*) was described recently from the Hickory Nut Gorge area in Rutherford County, North Carolina (Patton et al. 2019). Green Salamanders have a wide but scattered distribution across much of the southern Appalachians and Cumberland Plateau from northeastern Mississippi and northern Alabama northward into southwestern

Pennsylvania and western Maryland (Petranka 1998; Powell et al. 2016). These lungless salamanders are considered habitat specialists of moist crevices in rock outcrops, woody cover, and arboreal habitats in mature hardwood cove forests (Gordon 1952; Cupp 1991; Patton et al. 2019), which can render populations difficult to monitor while increasing their vulnerability to habitat loss, fragmentation, and other stressors (Patton et al. 2019). The Green Salamander has been assessed as Near Threatened on the International Union for Conservation of Nature (IUCN) Red List because of evidence of declining populations in



FIGURE 1. (A) Green Salamander (*Aneides aeneus*) at Savage Gulf State Natural Area, Grundy County, Tennessee, USA, 19 June 2018. (B) Searching for salamanders in a sandstone rock outcrop at Bear Hollow Mountain Wildlife Management Area, Franklin County, Tennessee, in 2018. (C) Green Salamander guarding her hatchlings in a rock crevice in the Bays Mountain region of Hawkins County, Tennessee, 17 July 2016. (D) Loose bark habitat where a Green Salamander was found on a fallen tree on a dry, south facing slope in Sullivan County, Tennessee. (A and B photographed by Matthew L. Niemiller; C and D photographed by Tristan M. Clark).

portions of its range (Hammerson 2004). Likewise, the species has been assessed as Vulnerable (G3) by NatureServe (NatureServe. 2021. NatureServe Explorer [web application]. NatureServe, Arlington, Virginia. Available <https://explorer.natureserve.org/>. [Accessed 10 July 2021]) and is a species of conservation concern in every state throughout its distribution. The salamander was petitioned for federal listing as endangered under the U.S. Endangered Species Act in July 2012 (Center for Biological Diversity. 2012. Petition to list 53 amphibians and reptiles in the United States as threatened or endangered species under the Endangered Species Act. Center for Biological Diversity. Available from <https://ecos.fws.gov/docs/petitions/92210/662.pdf>. [Accessed 10 July 2021]). The U.S. Fish and Wildlife Service (USFWS) ruled in 2015 that the petitioned action may be warranted and initiated a status review (USFWS 2015). A 90-d finding conducted by the USFWS determined that listing may be warranted, and the species is currently under a full status review (USFWS 2019. Conserving South Carolina's At-Risk species: species facing threats to their survival. USFWS. Available <https://www.fws.gov/southeast/pdf/fact-sheet/green-salamander.pdf>. [Accessed 5 December 2019]).

In Tennessee, Green Salamanders have been found primarily in association with mesic forests with humid, well-shaded sandstone bluffs and rock outcrops with damp, deep crevices (Gentry 1955; Snyder 1991); however, individuals also have been found in association with limestone (Snyder 1991) and beneath loose bark

on living and dead trees in mesic upland hardwood forests (Gentry 1955; Redmond and Scott 1996; Miller and Reynolds 2011). Most of the > 40 known occurrences are located along the Cumberland Plateau, Cumberland Mountains, and northern Eastern Highland Rim, but disjunct populations have been documented in the Central Basin and on Short Mountain in central Tennessee and the Appalachian Ridge and Valley, Bays Mountains, Clinch Mountain, and the Great Smoky Mountains in eastern Tennessee (Redmond and Scott 1996; Miller and Reynolds 2011; Thames et al. 2014; Argo and Argo 2018; Atlas of Amphibians in Tennessee: <https://www.apsubiology.org/tnamphibiansatlas/title.htm>).

The ecology and life history of populations of Green Salamanders in Tennessee are poorly understood (Miller and Reynolds 2011), despite the type locality occurring near the mouth of Nickajack Cave in Marion County, Tennessee (Cope and Packard 1881). Most of our knowledge on reproductive ecology are based on studies of populations in other states. Furthermore, data on abundance are lacking for most sites in Tennessee; consequently, determining whether individual populations are stable, increasing, or decreasing is extremely difficult.

The lack of information on trends and potential threats to populations from anthropogenic disturbances illustrates the critical need for an updated assessment of population health and conservation status of Green Salamanders in Tennessee. Such an assessment is

paramount given recent evidence that more than one genetically distinct lineage (and management unit) may exist in Tennessee (Patton et al. 2019). Accordingly, the overall aim of this study was to assess the status of the species and of extant populations in central and eastern Tennessee to identify priority populations and habitats for immediate conservation and management efforts. In addition, we sought to identify and then contribute data to aspects of ecology and life history that are required for accurate conservation assessment but for which little if any information is currently available. We conducted surveys for Green Salamanders at sites with known occurrence (= historical sites) and at other localities in regions with suitable habitat that lie within the potential range of the species based on a recent distribution model (Thames et al. 2021). We also determined geographic extent and identified existing and future stressors that potentially threaten the species in Tennessee.

MATERIALS AND METHODS

Study area.—We conducted field surveys for Green Salamanders at historical and potential sites within forest habitats along escarpments of the Cumberland Plateau, Cumberland Mountains, and Eastern Highland Rim physiographic regions, as well as mountain ranges in the Appalachian Valley and Ridge physiographic region in eastern Tennessee, USA. Potential sites were selected non-randomly based on a maximum entropy distribution model for the species in Tennessee (Thames et al. 2021) and accessibility; consequently, surveys were conducted primarily on public lands. In addition, we conducted opportunistic surveys at and around entrances and within twilight zones of caves and rock shelters associated with other cave-related research projects by the authors. Surveys occurred in the following counties in Tennessee: Anderson, Campbell, Cannon, Claiborne, Coffee, Cumberland, DeKalb, Fentress, Franklin, Grundy, Hamilton, Hawkins, Knox, Loudon, Lincoln, Marion, Meigs, Overton, Putnam, Rhea, Roane, Sullivan, Union, Van Buren, Warren, White, and Wilson.

Field surveys and data.—Our surveys for Green Salamanders consisted of time-constrained searches of vertical and horizontal crevices in rock outcrops that we illuminated with LED headlamps and hand-held flashlights (Fig. 1). These surveys were conducted primarily from April to early June after salamanders had dispersed from their hibernacula (a post-hibernation period). We conducted additional surveys from July to September (the breeding and incubation period), and from October to December (the pre-hibernation period). We conducted diurnal surveys from morning to early afternoon, usually on days when weather conditions might stimulate activity of salamanders (e.g., overcast

or rainy days or days with high humidity). We also conducted surveys, however, when weather conditions would be considered suboptimal, such as on warm, sunny, and dry days. Furthermore, we searched trees and fallen logs in adjacent forest habitat within 3 m of rock outcrops by visually inspecting bark up to 6 m above ground, under sloughed bark, and within knotholes and hollow cavities in trees with the aid of headlamps and handheld flashlights. Our surveys were conducted on average by three researchers (range 1 to 16). We recorded time of day, vertical position, horizontal position, and substrate for each salamander found.

Compiling additional occurrence records.—In addition to field surveys, we searched for records of occurrence for Green Salamanders (e.g., observations from specific sites) from Tennessee in the scientific literature, unpublished reports, biodiversity databases, and museum accession records as of June 2020. Literature sources included peer-reviewed journals, books, proceedings, theses and dissertations, and government reports. Searches of literature sources included keyword queries of ISI Web of Science, Google Scholar, and Zoological Record. We also queried the VertNet database (<http://www.vertnet.org>), iNaturalist (<http://www.inaturalist.org>), HerpMapper (<http://www.herpMapper.org>), Global Biodiversity Information Facility (GBIF; <http://www.gbif.org>), Tennessee Natural Heritage (TNH) Database, and the Tennessee Wildlife Resources Agency (TWRA) State Wildlife Action Plan (SWAP) Database. We made a concerted effort to georeference all occurrences compiled from these sources.

Conservation assessment.—We used the NatureServe conservation assessment protocol to determine the conservation rank for populations in Tennessee. This approach assessed the conservation status using 10 primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors included extent of occurrence (EOO), area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors included both short-term and long-term trends in population size, EOO, AOO, number of occurrences, and viability or ecological integrity of occurrences. Finally, threat factors included threat impact and intrinsic vulnerability to threats. We calculated the NatureServe conservation status assessment using default points and weights with the NatureServe Rank Calculator worksheet available in Excel (FaberLangendoen et al. 2009).

We calculated two different measures of size of the geographic range for populations in Tennessee,

EOO and AOO, in the web-based program GeoCAT (Bachman et al. 2011; available at <http://geocat.kew.org>). We calculated EOO as a minimum convex hull, and we used a grid size of 2 km (4 km²) to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010). When data were available, we used changes in EOO, AOO, number of occurrences, and quality of habitat to determine trends in population dynamics over short- and long-term timescales. We defined long-term trends as those occurring over 30 y or more, and short-term trends as those occurring over the last 10 y (2011 to present). We assumed that environmental specificity was narrow (specialist or community with key requirements common), which is consistent with past NatureServe assessments for the species.

We determined whether occurrences occurred on state- or federally protected areas and private easements of NGO-protected lands (e.g., state parks, natural areas, national parks, state and natural forests, etc.) or private lands without conservation easements. We used the USGS Protected Areas Database (PAD-US) version 1.3 (available at <http://gapanalysis.usgs.gov/padus/>) and state sources (TWRA and TDEC) to determine if lands with records of occurrence were protected. To help 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>) following Salafsky et al. (2008).

Uncertainty in values of assessment criteria is an important consideration when assessing conservation status, as uncertainty can strongly influence the assessment of extinction risk (Akçakaya et al. 2000; 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). 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).

RESULTS

Field surveys.—In 2017–2019, we found Green Salamanders during 68 of 122 surveys at 47 of 84 historical and potential sites in Tennessee (Appendix Table). We found Green Salamanders at 17 historical sites and 30 new sites, which were primarily along the escarpments of the Cumberland Plateau and in the Cumberland Mountains. We also located populations at previously unknown localities in the Eastern Highland Rim and Appalachian Valley and Ridge physiographic provinces in middle and eastern Tennessee.

Abundance.—We counted 329 Green Salamanders during 122 surveys (range, 0–31 salamanders per survey; Appendix Table), with a mean of 4.4 ± 5.2 (standard deviation) salamanders/survey. Our search effort averaged 5.2 ± 6.2 person-hours per survey and ranged from 0.33 to 40.0 person-hours. Over all surveys, salamanders observed per person-hour of search effort was 0.52, and our salamanders observed per per-hour of search effort was 0.70 and ranged 0.0 to 8.0. We observed more than 10 salamanders during a single survey at six sites (Appendix Table): Signal Mountain in Hamilton County, Bear Hollow Mountain Wildlife Management Area (WMA)-Cedar Ridge area in Franklin County, No Business Branch and Stinking Creek Road near Pioneer in Campbell County, Ozone Falls State Natural Area (SNA) in Cumberland County, and Short Mountain in Cannon County. We found more than five salamanders during a survey at several additional sites (Appendix Table). We found Green Salamanders during almost every month of the year (the exception was January; Fig. 2). Salamanders were most common during the post-hibernation and pre-hibernation periods in late February into March and September into November, respectively.

Habitat.—We found Green Salamanders most frequently in deep, moist, horizontal crevices on rock outcrops and large boulders ($n = 282$); however, we also observed some salamanders ($n = 23$) in vertically oriented crevices. We found individuals on sandstone and limestone outcroppings and on large boulders, typically in well-shaded, forested gorges and ravines

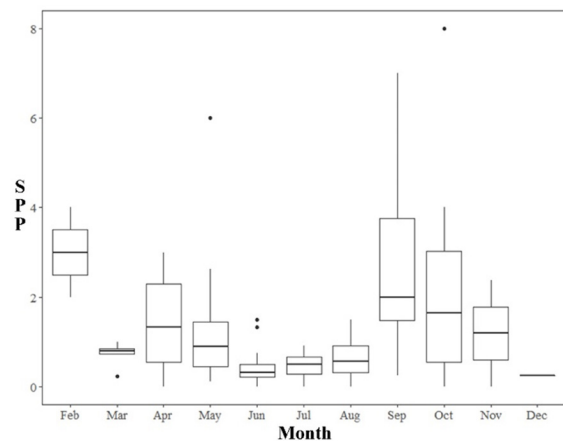


FIGURE 2. Green Salamander (*Aneides aeneus*) abundance (SPP = salamanders per person-hour effort) by month of year based on 75 surveys of new and historical sites in Tennessee, USA. Salamanders were not observed in January during the study. The upper and lower limits of the box represent the first and third quartiles, with the horizontal line representing the median. Whiskers extend to the minimum and maximum values, and points represent outliers.

associated with the escarpments of the Cumberland Plateau and Cumberland Mountains, including the Bays and Clinch Mountains, but also along the escarpment of the Eastern Highland Rim.

Three Green Salamander observations did not occur on rock outcrops. We found an adult on the end of a fallen log about 0.9 m off the forest floor within 3 m of a rock outcrop at Bear Hollow Mountain WMA–Cedar Ridge area 10 July 2018. Also, we found a juvenile climbing up a Chestnut Oak (*Quercus montana*) about 2.5 m from a sandstone outcrop at 1149 at Franklin-Marion State Forest in Marion County 16 July 2018. The salamander was 0.8 m above the forest floor at the time of the observation. A juvenile was found 0.3 m above the ground on a Mountain Laurel (*Kalmia latifolia*) immediately adjacent to a sandstone outcrop (< 0.2 m away) at Signal Mountain in Hamilton County 20 March 2019. Additionally, we received an anecdotal observation of an adult found 25 September 2019 under bark on a log just outside the entrance of Tumbling Rock Cave in Jackson County, Alabama (Kayla Wilson and Joe Lamb, pers. obs.).

We found Green Salamanders either just inside or at the entrance of five caves: Panther Cave no. 1 in Campbell County; York Cave, Mountain Eye, and Zarathustras caves in Fentress County; and Blowing Spring Cave in Anderson County. We also received anecdotal reports of Green Salamanders at Lost Creek Cave in White County (Kristen Bobo, pers. comm.) and Hubbard's Cave in Warren County (Cory Holliday, pers. comm.). Historical cave records in Tennessee included a cave at Norris Dam State Park in Campbell County, Ingram Cave in Clay County, near Nickajack Cave in Marion County, Hazzard Cave in Pickett County, a small cave

at Oak Ridge National Laboratory in Roane County, and Big Bone Cave in Van Buren County (museum records and Tennessee Natural Heritage database).

Occurrences and distribution.—Along with locations recorded from field surveys in the current study, we compiled a list of 160 sites in 30 counties that could be georeferenced in Tennessee from the primary literature, state databases (TNH and TWRA), museum accessions, iNaturalist, and other sources. Based on these records, Green Salamanders are known primarily from higher elevations along the escarpments of the Cumberland Plateau and northern Eastern Highland Rim and ridges in the Appalachian Valley and Ridge (Fig. 3). Several populations are known along the western escarpment of the Cumberland Plateau from Franklin and Grundy counties northward to White, Putnam, Fentress, and Pickett counties. Along the eastern escarpment of Cumberland Plateau and Walden Ridge, populations are known from the Chattanooga area in Hamilton County and eastern Marion County northward through Rhea and Cumberland counties. The species also is known from Lookout Mountain in Hamilton County as well as from escarpments along the Sequatchie Valley in Marion and Sequatchie counties. Populations have been documented from the Cumberland Mountains of Cumberland, Morgan, Scott, Campbell, and Claiborne counties. Scattered populations are known from the northern Eastern Highland Rim, particularly in Overton, Putnam, DeKalb, and Cannon counties, with several populations known from rock exposures associated with the transition from the Eastern Highland Rim into the Central Basin. Additional disjunct populations have been reported from the Blue Ridge Mountains and the Central Basin.

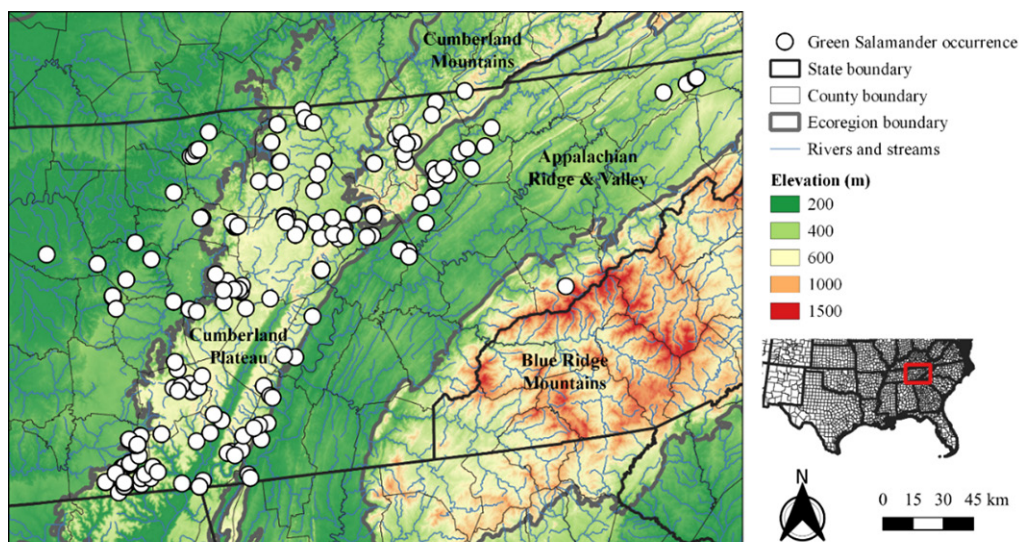


FIGURE 3. Distribution of Green Salamanders (*Aneides aeneus*) in Tennessee, USA, showing 160 occurrences (white dots) compiled from literature, museum, state databases, iNaturalist, and the current study.

Nesting ecology.—We found five nests during the current study, including a female with eggs 2 August 2018 at Bear Hollow Mountain WMA below the horse trail parking in Franklin County, and two nests with attending females 10 September 2018 at Bear Hollow Mountain WMA—Cedar Ridge area in Franklin County. Furthermore, we found a female with her hatchlings at Stinking Creek Rd near Pioneer in Campbell County 18 September 2018 and at Short Mountain in Cannon County 25 October 2018. In addition, observation no. 31413649 by user cem9235 of a female with eggs at Pickett State Park in Pickett County found 24 August 2019 was uploaded to iNaturalist. Finally, TMC found a female with eggs at Bays Mountain Park in Hawkins County 17 July 2016. The same female was observed guarding hatchlings during a return to the site 21 September 2016.

Conservation status and threat assessment.—We estimated EOO and AOO at 4,856 km² and 220

TABLE 1. List of possible threats facing southern and northern lineages of the Green Salamander (*Aneides aeneus*) in Tennessee, USA, following the classification proposed by Salafsky et al. (2008). Threat impacts are negligible (N), low (L), medium (M), high (H), and very high (VH).

Threat	Southern	Northern
1. Residential & commercial development	L	L
1.1 Housing & urban areas	L	L
1.2 Commercial & industrial areas	N	N
1.3 Tourism & recreation areas	M	M
2. Agriculture & aquaculture	L	L
2.2 Wood & pulp plantations	L	L
2.3 Livestock farming & ranching	L	L
3. Energy production & mining	L	L
3.2 Mining & quarrying	L	M
4. Transportation & service corridors	M	M
4.1 Roads & railroads	M	M
4.2 Utility & service lines	M	M
5. Biological resource use	M	M
5.1 Hunting & collecting terrestrial animals	L	L
5.3 Logging & wood harvesting	H	H
6. Human intrusions & disturbance	L	L
6.1 Recreational activities	L	L
7. Natural system modifications	L	L
7.2 Dams & water management/use	L	L
8. Invasive & other problematic species & genes	LM	LM
8.1 Invasive non-native/alien species/diseases	LM	LM
8.2 Problematic native species/diseases	LM	LM
11. Climate change & severe weather	M	M
11.1 Habitat shifting & alteration	M	M
11.2 Droughts	M	M
11.3 Temperature extremes	L	L

km² in Tennessee, respectively, with 160 documented occurrences. New sites found in recent years have increased EOO and AOO, but we doubt that range size changed significantly during the last 30+ y. We identified 18 threats (nine threat categories) that are or may impact populations at present or in the near future (Table 1). We assessed the overall threat impact as medium in Tennessee. At least 97 occurrences documented from state or federal lands are afforded some protection, including Bear Hollow Mountain WMA, Franklin-Marion State Forest (SF), Prentice Cooper SF, Prentice Cooper WMA, Mr. and Mrs. Henry Lee Carter SNA, Hawkins Cove SNA, Natural Bridge SNA, South Cumberland State Park (SP), Savage Gulf SNA, Alvin C. York SP, Bays Mountain Park and SNA, Big Bone Cave SNA, Big Ridge SP, Big South Fork National River and Recreation Area, Bridgestone-Firestone WMA, Catoosa WMA, Centennial Wilderness WMA, Cove Creek WMA, Chuck Swan WMA and SF, Edgar Evins SP, Fall Creek Falls SP and WMA, Frozen Head SP and SNA, Hubbard’s Cave SNA, Lost Creek SNA, Norris Dam SP, North Cumberland WMA, Oak Ridge National Laboratory, Ozone Falls SNA, Pea Ridge WMA, Pickett SP and SF, Pickett WMA, Rock Island SP, and Standing Stone SP and SF. Based on our NatureServe conservation assessment, we evaluated the Green Salamander as S3S4 (Vulnerable—Apparently Secure) in Tennessee.

DISCUSSION

Distribution, habitat, and abundance.—Redmond and Scott (1996) report the Green Salamander from 38 sites in Tennessee. As of July 2021, 44 sites have been documented in the Atlas of Amphibians in Tennessee (<https://www.apsubiology.org/tnamphibiansatlas/title.htm>), reflecting six recent literature records (Miller and Reynolds 2011; Daniels et al. 2012; Thames et al. 2014; Argo and Argo 2018). Our documentation of 160 sites within 30 counties, although predominately from escarpments of the Cumberland Plateau, Cumberland Mountains, Appalachian Valley and Ridge, and northern Eastern Highland Rim, indicates that our understanding of the distribution of Green Salamanders in Tennessee is improving as more people become aware of the species, and surveys no longer restrict searches to sandstone outcroppings at historical sites. For example, populations also exist in disjunct regions in the Blue Ridge Mountains and Central Basin. The enigmatic occurrence of Green Salamanders in the Blue Ridge Mountains of Tennessee is based on one specimen collected in 1929 from under a log along a trail in the Cherokee Orchard area on the north-facing slope at the base of Mount LeConte (Weller 1931; King 1939; Dodd 2004). A species distribution model for Green Salamanders in Tennessee suggests that

suitable habitat is available in the Blue Ridge Mountains from Cocke County southward into Polk County in the state (Thames et al. 2021). Despite repeated surveys in this area and in the Great Smoky Mountains National Park in general for salamanders and the presence of suitable habitat, another individual has never been found, and the species is no longer thought to occur in Great Smoky Mountains National Park (Redmond and Scott 1996; Dodd 2003, 2004). Populations have declined dramatically in the nearby disjunct populations of the Blue Ridge Escarpment in North Carolina, South Carolina, and Georgia (Corser 2001). If populations did exist in the Blue Ridge Mountains of Tennessee, then dramatic declines possibly occurred here as well.

McKinney and Snyder (1973) reported Green Salamanders from a sinkhole at Cedars of Lebanon State Park in the Central Basin of Wilson County. During a study of the shrew community at Cedars of Lebanon State Park and adjacent Cedars of Lebanon State Forest (Relford 1999), several amphibians and reptiles, especially salamanders, were unintentionally captured in pitfall traps. Upon examination of the bycatch years later, a juvenile Green Salamander was discovered, representing just the second record from Cedars of Lebanon State Park (Niemiller et al. 2011). These occurrences are 90-km west of the major population cluster on the Cumberland Plateau and possibly represent a relic population (Jordan 1986). Recent surveys at Cedars of Lebanon State Park and Forest during the current study and by Niemiller et al. (2011) have failed to locate any additional individuals. The current status of this population is unknown.

Several areas in Tennessee appear suitable for the Green Salamander (Thames et al. 2021) but lack records of occurrences. Notably, Putnam and Overton counties along the western escarpment of the Cumberland Plateau, Bledsoe County on both the eastern and western sides of the Sequatchie Valley, and the border of Hancock and Hawkins counties in the Ridge and Valley physiographic region have large areas of high suitability with few to no occurrences. Most of these areas are privately owned and likely have never been surveyed systematically. We suspect that Green Salamander populations occur in these geographic areas.

Abundance data for populations of Green Salamander in Tennessee are limited, but data are available for two populations. Seven salamanders were reported from No Business Branch in Campbell County in October 1977 (TWRA SWAP Database), and 11 salamanders were observed in March 2010 (unpubl. data). Relative abundance during the current study (seven to 18 salamanders) at this site suggests that this population has remained stable. Green Salamanders have been reported periodically from the Ozone Falls area since 1940 (accessions in Royal Ontario Museum and

Louisiana State University Museum of Natural History collections), but data suggest Green Salamanders occur at low density. During a survey in September 2009, however, 37 salamanders were observed (unpubl. data), and we observed as many as 17 salamanders during our surveys. Additional historical sites with observations from the 1930s to 1950s, such as Stinking Creek Rd area near Pioneer in Campbell County, Natural Bridge SNA in Franklin County, and the Domain on the campus of the University of the South in Franklin County, remain extant. Detection of Green Salamanders is influenced by time of year, time of day, observer, and weather conditions (e.g., temperature, cloud cover), among other factors (John 2017; Smith et al. 2017; Newman et al. 2018; Williams et al. 2020), which may account for variation in abundance among surveys at Ozone Falls SNA and other sites. Green Salamanders are often difficult to detect during field surveys. Future studies on Tennessee populations should prioritize examining factors that influence microhabitat preferences, detection, and abundance at the site level.

Nesting ecology and activity.—The nesting ecology of Green Salamanders in Tennessee has not been well-studied or documented. Wyatt (2010) observed two nests in July 2008 and two nests in July 2009 at Myatt Creek at Catoosa WMA in Cumberland County. Cantrell (2012) observed a nest for two consecutive years at a rock outcrop at Myatt Creek at Catoosa WMA in August 2010 and 2011. A nest was observed at this same rock outcrop by Wyatt (2010), but it is unknown if the same or a different female produced these nests. These studies and our observations support a nesting season from July through September, which is consistent with the reported summer nesting season in other parts of the range (e.g., Woods 1969; Cupp 1991; Canterbury and Pauley 1994).

Our observations that Green Salamanders are most active and, consequently, more likely to be found, during the post-hibernation period in May and the pre-hibernation period from September to October is consistent with past studies. For example, Green Salamanders presumably become less active and tend to move deeper into crevices periods of high temperatures and dry conditions (Pauley and Watson 2005), which typically occurs during July and August in the lower elevations of West Virginia (Thomas Pauley, unpubl. report). In higher elevations of northern West Virginia, Green Salamanders are most active in June, with little activity from the first week in July to the following May (Waldron 2000). In Kentucky, the highest densities of salamanders are observed prior to hibernation from late October into mid-December (Cupp 1991) before salamanders retreat deep into crevices. Furthermore, in North Carolina, salamanders that are not present during

summer reappear in October just prior to hibernation (Gordon 1952).

Threats and conservation assessment.—Any change in land use from a natural to a disturbed state can potentially negatively impact Green Salamander populations. Threats that result in habitat loss, fragmentation, or degradation likely have the largest negative impacts on populations, particularly logging, construction of open corridors associated with roads, railroads, and powerlines, and past and current mining operations along the Cumberland Plateau and Cumberland Mountains. These threats, however, vary in impact among physiographic regions and populations.

Presumed rarity of Green Salamanders across its range may be linked to the loss of the American Chestnut (*Castanea dentata*) and Old-growth Forests, in general (Wilson 2003). Spaces under bark of large snags and logs, presumably important microhabitats for the species, are now relatively scarce in secondary- and third-growth forests compared to Old-growth Forests. Consequently, Green Salamanders are now largely restricted to crevices in rock outcrops and large boulders (Gordon 1952; Corser 1991, 2001; Petranka 1998; Wilson 2003). Timber harvest and clearing adjacent to rock outcrops can increase both airflow and insolation, leading to increased temperatures and decreased moisture in crevices (Pauley and Watson 2005), which may cause local extirpations. To counter the potential decrease in suitable habitat with deforestation, several authorities recommend maintenance of forest buffers of at least 100 m around rock outcrops (Petranka 1998; Waldron and Humphries 2005; Miloski 2010).

Other significant threats to populations in Tennessee include water impoundments, over-collection associated with the pet trade, amphibian diseases, and climate change. Impoundments on the Tennessee River, such as Wheeler, Guntersville, Nickajack, and Chickamauga reservoirs, have likely isolated southern populations on opposite sides of the river; however, genetic analyses of populations on opposing sides of reservoirs have yet been conducted to test the veracity of these hypotheses. The impacts on populations in Tennessee from over-collection by amateur amphibian enthusiasts for the pet trade are unknown, but this threat may exist (Mitchell et al. 1999; Corser 2001). Climate change has been implicated in the decline of Green Salamander populations in the southern Appalachians (Corser 2001; Staudt et al. 2013). Potential impacts of climate change on Tennessee populations are unknown; however, disjunct (Central Basin and Blue Ridge) and peripheral populations of both northern and southern lineages may be at elevated risk.

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), and *Ranavirus*

infections have been documented in populations of Green Salamanders in North Carolina, South Carolina, and Virginia. Chytridiomycosis has been documented only on a captive Green Salamander (Bauer et al. 2018), however, and otherwise only detected subclinically in wild populations at varying prevalence: 7.7% in North Carolina (Moffitt et al. 2015), 2.4% in South Carolina (Newman et al. 2019), and 15% in southwestern Virginia (Blackburn et al. 2015). *Ranavirus* is another potential pathogen of Green Salamanders and has only been reported at subclinical levels in a few individuals in southwestern Virginia (Blackburn et al. 2015). Seasonally balanced sampling of Green Salamanders, including thorough physical examination for signs of disease, will be needed to help guide mitigation of potential infectious disease threats in the state.

The Green Salamander is currently assessed as Vulnerable-Apparently Secure (S3S4) in Tennessee under NatureServe criteria (NatureServe *op. cit.*). Based on our conservation assessment, we recommend no change to the NatureServe conservation rank. The Green Salamander is considered rare and tracked by the state of Tennessee but is not state listed (Tennessee Department of Environmental & Conservation Rare Species Data Viewer; https://dataviewers.dec.tn.gov/pls/enf_reports/f?p=9014:3).

Recommendations.—Given results of our study and available (or lack thereof) information on Tennessee populations, we offer several recommendations for future study, conservation strategies, and management of Green Salamanders. First, additional research is needed to estimate important demographic and life-history variables of populations in Tennessee. The ecological study of the species in Tennessee is in its infancy. Some studies examining habitat preferences have been conducted recently (e.g., Armstrong 2010; Wyatt 2010; Cantrell 2012), but information on population size, detectability/occupancy, age-based survival, growth, lifespan, and reproduction ecology are critically lacking. These data are essential to predict quantitatively the future status of populations (i.e., Population Viability Analysis). In addition, limited information exists on diet, diseases, parasites, and other life-history traits of Tennessee populations. Acquiring such data is challenging because of the reclusive nature of the species and that only a fraction of available crevices on rock outcrops (up to 2–3 m above ground level) are typically accessible to researchers during field surveys (Smith et al. 2017). Many salamanders cannot be captured easily for capture-mark-recapture studies without great risk of harm; however, the use of photo identification approaches to accurately identify individual salamanders (e.g., Gamble et al. 2008; Bendik et al. 2013) based on unique color patterns

may facilitate such studies (e.g., unique patterning on the head or body of Green Salamanders photographed while in crevices). Moreover, we recommend that a long-term, standardized population monitoring program be initiated, which includes several priority sites throughout the range of the species identified in this study. Although we have generated important baseline data for several sites, regular monitoring is necessary to detect population trends and assess impacts of stochastic events (e.g., Smith et al. 2019). Most occurrences are known from state and federal land; however, as of 2005, approximately 92% of land in Tennessee is privately held (Tennessee Wildlife Resources Agency 2005) and there is high potential to discover new occurrences in the state.

Most occurrences during the current study were associated with shaded, rock outcrops and large boulders in mature mixed hardwood or hardwood-pine forests. Species distribution modelling suggests that forest canopy cover has a strong positive correlation with Green Salamander habitat suitability (Thames et al. 2021), providing further evidence that management efforts in Tennessee should focus on protecting forests surrounding rock outcrops (Wyatt 2010). Loss of forest habitat around rock outcrops leads to increased insolation and loss of moisture within crevices and is associated with negative impacts and extirpation of local populations (Petranka 1998; Pauley and Watson 2005). Forests regulate temperature and humidity regimes around the rock outcrops and trees near outcrops may be additionally important for foraging during rain events (Smith et al. 2017). In Tennessee, these features are particularly important along the margins of the Cumberland Plateau, the Cumberland Mountains, and northern Ridge and Valley physiographic regions where the landscape is most suitable for the species. Forest buffers maintained around important rock outcrops may help protect populations of Green Salamanders (e.g., Petranka 1998; Waldron and Humphries 2005; Miloski 2010); however, research is needed to determine the appropriate buffer size that balances both protection and connectivity of local (sub)populations with land use needs.

Green Salamanders use woody and arboreal habitats (Waldron and Humphries 2005; Smith et al. 2017; this study), but more research is needed to determine the importance of woody and arboreal habitat in the southern portion of the range of the species. Likewise, observations around the entrances and within the twilight zones of caves in Tennessee and Alabama (unpubl. data) have increased in recent years. We observed Green Salamanders using caves during both winter and summer seasons; however, we do not yet fully understand why and to what extent this species uses caves.

Diseases can impact amphibian population health without notable die offs. The detection of *Bd* and ventral skin lesions in some Green Salamander populations in states surrounding Tennessee warrants continued, regular range-wide monitoring for the foreseeable future. We recommend targeting a few representative sites across the range of the species for regular pathogen sampling across multiple seasons. We further recommend ancillary diagnostics in individuals with lesions or displaying abnormal behaviors as this will greatly aid in determining the effects of the presence of these pathogens if encountered. Green Salamanders are likely experiencing multiple environmental stressors from climate change and habitat alteration that may influence disease dynamics and impacts of endemic pathogens. Intensive and targeted sampling will be required to fully understand the dynamics of *Bd* and other unknown infectious and noninfectious agents on local populations. Finally, Patton et al. (2019) found phylogenetic and phylogenomic support for a polytypic Green Salamander species complex composed of *A. caryanensis* and *A. aeneus* with three cryptic, largely allopatric lineages, two of which occur in Tennessee. These two lineages may overlap along the Cumberland Plateau and additional genetic analyses with more intensive sampling are needed to determine if these lineages should be recognized and managed as evolutionary significant units (ESUs; sensu Moritz 1994) or treated as a single entity for conservation purposes.

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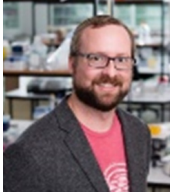


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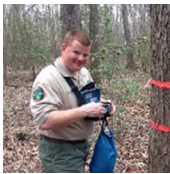


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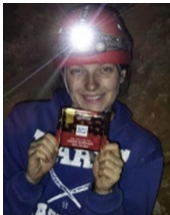
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APPENDIX TABLE. Date, location, and search effort of surveys conducted for Green Salamanders (*Aneides aeneus*) in Tennessee, USA, between October 2017 and November 2019. Historical populations or localities are indicated with an asterisk and recently discovered localities are in bold. Search effort includes number of researchers, total search time (in hours), search effort in person-hours, and number of salamanders observed per person hour (SPP). Abbreviations are WMA = Wildlife Management Area and SNA = State Natural Area. Exact locality data are intentionally withheld but are available from the authors and Tennessee Wildlife Resources Agency.

Date	County	Locality	No. observed	Personnel	Total time	Person-hours	SPP
9 October 2017	Franklin	Bear Hollow Mtn WMA - Stubblefield Hollow*	1	1	0.33	0.33	3.03
10/9/2017	Franklin	Bear Hollow Mtn WMA - Stubblefield Hollow*	4	1	0.5	0.50	8.00
29 November 2017	Franklin	Bear Hollow Mtn WMA - Keith Cove	1	1	0.42	0.42	2.38
26 January 2018	Meigs	entrance to Blythe Ferry Cave	0	2	0.25	0.50	0.00
23 March 2018	Knox	entrance to Brents Cave	0	2	0.25	0.50	0.00
23 March 2018	Knox	entrance to The Lost Puddle	0	2	0.25	0.50	0.00
24 March 2018	Campbell	No Business Branch*	9	16	2.5	40.00	0.23
4 April 2018	Franklin	Bear Hollow Mtn WMA - Keith Cove	1	1	0.4	0.40	2.50
13 April 2018	Anderson	entrance to Blowing Springs Cave*	0	3	0.25	0.75	0.00
27 April 2018	Franklin	Natural Bridge SNA*	1	2	1.25	2.50	0.40
28 April 2018	Grundy	Savage Gulf SNA - Stone Door area	3	2	1.5	3.00	1.00
2 May 2018	Franklin	Bear Hollow Mtn WMA - Williams Cove	1	1	0.38	0.38	2.63
3 May 2018	Franklin	University of the South Domain*	3	6	2.75	16.50	0.18
8 May 2018	Franklin	Bear Hollow Mtn WMA - Stubblefield Hollow*	7	3	4.5	13.50	0.52
11 May 2018	Cumberland	Ozone Falls SNA*	17	4	2.75	11.00	1.55
14 May 2018	Sullivan	Bays Mountain Park*	2	1	1.5	1.50	1.33
16 May 2018	Franklin	Bear Hollow Mtn WMA - Williams Cove	0	2	1	2.00	0.00
16 May 2018	Franklin	Bear Hollow Mtn WMA - Wolf Cove	2	2	2.78	5.56	0.36
16 May 2018	White	Bridgestone-Firestone WMA*	9	1	4	4.00	2.25
22 May 2018	Franklin	Bear Hollow Mtn WMA - Pumplog Hollow	9	2	4.92	9.84	0.91
22 May 2018	Rhea	Piney Falls SNA	0	3	3.5	10.50	0.00
23 May 2018	Franklin	Bear Hollow Mtn WMA - Williams Cove	3	2	1.68	3.36	0.89
29 May 2018	Franklin	entrance to Keith Cave	0	4	0.25	1.00	0.00
31 May 2018	Fentress	off Manson Rd near Sandy	1	1	4	4.00	0.25
31 May 2018	Franklin	Bear Hollow Mtn WMA - Pumplog Hollow	4	2	3	6.00	0.67
4 June 2018	Franklin	Bear Hollow Mtn WMA, Little Coon Creek	5	6	3.58	21.48	0.23
5 June 2018	White	Bridgestone-Firestone WMA*	1	1	4	4.00	0.25
6 June 2018	Franklin	Bear Hollow Mtn WMA - Flat Top	0	2	2.17	4.34	0.00
6 June 2018	Grundy	Savage Gulf SNA, Collins West area	4	3	4.33	12.99	0.31
10 June 2018	Hawkins	Bays Mountain Park*	3	1	2	2.00	1.50
19 June 2018	Grundy	Savage Gulf SNA - Greeter Falls area	2	4	2.25	9.00	0.22
21 June 2018	Cumberland	Catoosa WMA	2	1	4	4.00	0.50
21 June 2018	Cumberland	Catoosa WMA	3	1	4	4.00	0.75
26 June 2018	Rhea	Stinging Fork Falls SNA	0	2	3	6.00	0.00

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APPENDIX TABLE (CONTINUED). Date, location, and search effort of surveys conducted for Green Salamanders (*Aneides aeneus*) in Tennessee, USA, between October 2017 and November 2019. Historical populations or localities are indicated with an asterisk and recently discovered localities are in bold. Search effort includes number of researchers, total search time (in hours), search effort in person-hours, and number of salamanders observed per person hour (SPP). Abbreviations are WMA = Wildlife Management Area and SNA = State Natural Area. Exact locality data are intentionally withheld but are available from the authors and Tennessee Wildlife Resources Agency.

Date	County	Locality	No. observed	Personnel	Total time	Person-hours	SPP
3 July 2018	Franklin	Bear Hollow Mtn WMA - Boundary Rd	6	3	3.67	11.01	0.54
7 July 2018	Hawkins	Bays Mountain Park*	0	2	1.75	3.50	0.00
7 July 2018	Sullivan	Bays Mountain Park*	2	2	1.25	2.50	0.80
9 July 2018	Franklin	Bear Hollow Mtn WMA - Boundary Rd	3	4	3.72	14.88	0.20
10 July 2018	Franklin	Bear Hollow Mtn WMA - Cedar Ridge	13	4	3.58	14.32	0.91
16 July 2018	Marion	Franklin-Marion State Forest	6	5	3.28	16.40	0.37
19 July 2018	Campbell	rock outcrop near Panther Cave no. 1	1	4	0.5	2.00	0.50
19 July 2018	Union	entrance to Big Coon Caverns	0	4	0.25	1.00	0.00
19 July 2018	Union	entrance to Little Coon Cave	0	4	0.25	1.00	0.00
26 July 2018	Knox	entrance to Pedigo Cave	0	2	0.25	0.50	0.00
2 August 2018	Franklin	Bear Hollow Mtn WMA – near horse trail parking	1	1	2	2.00	0.50
30 August 2018	Franklin	Bear Hollow Mtn WMA – Cave Springs Rd	0	1	1.5	1.50	0.00
31 August 2018	Van Buren	Fall Creek Falls State Park - Wheeler Farm	1	1	4	4.00	0.25
10 September 2018	Franklin	Bear Hollow Mtn WMA - Cedar Ridge	5	1	2.5	2.50	2.00
13 September 2018	Warren	entrance to Hazel Ward Cave	0	4	0.25	1.00	0.00
13 September 2018	White	Bridgestone-Firestone WMA	1	1	4	4.00	0.25
17 September 2018	Franklin	Bear Hollow Mtn WMA - Barking Frog Pond area	4	1	2.75	2.75	1.45
20 September 2018	Lincoln	entrance to Kelso Saltpeter Cave	0	4	0.25	1.00	0.00
22 September 2018	Knox	entrance to Mudflats Cave	0	2	0.25	0.50	0.00
23 September 2018	Knox	entrance to Meads Quarry Cave	0	4	0.25	1.00	0.00
6 October 2018	Cumberland	Ozone Falls SNA*	16	2	2.75	5.50	2.91
6 October 2018	Loudon	entrance to Melton Hill Spring Cave	0	2	0.33	0.66	0.00
20 October 2018	DeKalb	Edgar Evins State Park*	0	2	1.75	3.50	0.00
20 October 2018	Hawkins	Bays Mountain Park*	8	1	2	2.00	4.00
20 October 2018	Warren	Rock Island State Park*	0	2	2.5	5.00	0.00
24 October 2018	White	Bridgestone-Firestone WMA	3	1	4	4.00	0.75
25 October 2018	Cannon	Short Mountain*	31	5	3.75	18.75	1.65
12 December 2018	Fentress	Alvin C York State Park	1	1	4	4.00	0.25
2 February 2019	Fentress	East Eye entrance of Mountain Eye Cave	2	2	0.25	0.50	4.00
2 February 2019	Fentress	entrance to Zarathustras Cave	1	2	0.25	0.50	2.00
13 February 2019	Jackson	Cordell Hull WMA	0	1	4	4.00	0.00
9 March 2019	Cumberland	Ozone Falls SNA*	4	2	2.5	5.00	0.80
18 March 2019	Campbell	Titus Hollow Rd near Royal Blue*	2	1	2	2.00	1.00
18 March 2019	DeKalb	Pea Ridge WMA	0	1	4.5	4.50	0.00

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Date	County	Locality	No. observed	Personnel	Total time	Person-hours	SPP
20 March 2019	Hamilton	Signal Mountain - Rainbow Trail*	11	4	3.25	13.00	0.85
22 March 2019	Jackson	Cordell Hull WMA	0	1	4	4.00	0.00
25 March 2019	White	Bridgestone-Firestone WMA	0	1	4.5	4.50	0.00
26 March 2019	White	Bridgestone-Firestone WMA	0	1	4.5	4.50	0.00
27 March 2019	Jackson	Washmorgan Hollow	0	1	4	4.00	0.00
28 March 2019	Campbell	No Business Branch*	11	5	3	15.00	0.73
28 March 2019	Overton	Alpine Mountain WMA	0	1	4.5	4.50	0.00
2 April 2019	Campbell	Stinking Creek Rd near Pioneer*	5	3	1	3.00	1.67
6 April 2019	Hawkins	Bays Mountain Park*	6	1	2	2.00	3.00
25 April 2019	Cumberland	Crab Orchard Property	0	1	4	4.00	0.00
26 April 2019	Cumberland	Crab Orchard Property	0	1	4	4.00	0.00
7 May 2019	Cumberland	Crab Orchard Property	0	1	4	4.00	0.00
8 May 2019	Jackson	Washmorgan Hollow	0	1	4	4.00	0.00
9 May 2019	Cumberland	Catoosa WMA	3	1	4	4.00	0.75
10 May 2019	Wilson	Cedars of Lebanon State Forest	0	9	3.5	31.50	0.00
16 May 2019	Campbell	Cross Hollow near Royal Blue	1	3	0.33	0.99	1.01
16 May 2019	Campbell	No Business Branch*	18	4	0.75	3.00	6.00
17 May 2019	Franklin	Bear Hollow Mtn WMA - Little Coon Creek	3	10	2.5	25.00	0.12
28 May 2019	Grundy	entrance to Big Mouth Cave	0	6	0.25	1.50	0.00
10 June 2019	Warren	Hubbards Cave SNA*	3	3	2.33	6.99	0.43
13 June 2019	Cannon	entrance to Frog Hole	0	3	0.25	0.75	0.00
14 June 2019	Overton	entrance to Armour Cave	0	4	0.25	1.00	0.00
14 June 2019	Overton	entrance to Water Supply caves	0	4	0.5	2.00	0.00
15 June 20189	Putnam	entrance to Kuykendall Cave	0	3	0.25	0.75	0.00
15 June 2019	Putnam	entrance to Stamps Cave	0	3	0.25	0.75	0.00
16 June 2019	Sullivan	Bays Mountain Park*	0	3	1.75	5.25	0.00
18 June 2019	White	Bridgestone-Firestone WMA	1	1	4.5	4.50	0.22
20 June 2019	Cumberland	Catoosa WMA	0	1	4	4.00	0.00
20 June 2019	Warren	Rock Island State Park*	1	3	2.25	6.75	0.15
21 June 2019	Cumberland	Ozone Falls SNA*	1	2	3	6.00	0.17
24 June 2019	White	Bridgestone-Firestone WMA	2	1	4.5	4.50	0.44
24 June 2019	White	Bridgestone-Firestone WMA	6	1	4.5	4.50	1.33
27 June 2019	Marion	Prentice Cooper WMA	2	1	4	4.00	0.50
20 July 2019	Roane	entrance to Berry Cave	0	3	0.25	0.75	0.00
25 July 2019	Cumberland	Catoosa WMA	0	1	4	4.00	0.00

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Date	County	Locality	No. observed	Personnel	Total time	Person-hours	SPP
31 July 2019	White	Chestnut Mountain	0	1	4	4.00	0.00
2 August 2019	Hamilton	Cumberland Trail - Rock Creek Gorge	0	2	5.33	10.66	0.00
2 August 2019	White	Chestnut Mountain	4	1	4	4.00	1.00
4 August 2019	Campbell	No Business Branch*	7	4	2.75	11.00	0.64
8 August 2019	White	Chestnut Mountain	6	1	4	4.00	1.50
8 August 2019	Wilson	Cedars of Lebanon State Forest	0	3	2.5	7.50	0.00
8 August 2019	Wilson	Cedars of Lebanon State Park - Jackson Cave*	0	3	1.25	3.75	0.00
8 September 2019	Sullivan	Bays Mountain Park*	2	1	0.5	0.50	4.00
16 September 2019	Union	Chuck Swan State Forest (SF) - Clear Creek Road	0	1	1	1.00	0.00
16 September 2019	Union	Chuck Swan SF - outside of Mossy Springs Cave	0	1	1	1.00	0.00
16 September 2019	Union	Chuck Swan SF - outside of Oaks Cave	0	1	1	1.00	0.00
17 September 2019	Campbell	Cove Creek WMA - Cumberland Mountain Trail	3	2	1	2.00	1.50
17 September 2019	Campbell	Cove Creek WMA*	7	2	1	2.00	3.50
18 September 2019	Campbell	North Cumberland WMA-Cell Tower Rd	0	2	1	2.00	0.00
18 September 2019	Campbell	Stinking Creek Rd near Pioneer*	14	2	1	2.00	7.00
12 October 2019	Cumberland	Ozone Falls SNA*	3	2	2.75	5.50	0.55
2 November 2019	Grundy	South Cumberland State Park - Fiery Gizzard Trail	0	3	3.25	9.75	0.00
16 November 2019	Grundy	Savage Gulf SNA, Collins West area	0	3	4.25	12.75	0.00
24 November 2019	Coffee	Davidson Branch	0	3	1.75	5.25	0.00