A SURVEY OF THE CAVE-ASSOCIATED HERPETOFAUNA OF THE EASTERN UNITED STATES WITH AN EMPHASIS ON SALAMANDERS

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Only four salamander species are considered obligate inhabitants of caves (troglobites) east of the Mississippi River in the United States. However, many other amphibians and reptiles use cave habitats to varying degrees for aspects of their life histories, such as reproduction, refuge, and hibernation. From 2004 to 2008, we surveyed 172 caves for amphibians and reptiles in Alabama, Georgia, Indiana, Kentucky, Tennessee, Virginia, and West Virginia using standard visual survey techniques in both aquatic and adjacent terrestrial habitats. Our survey documented 4542 occurrences of 29 species of amphibians (4081 of 18 salamanders and 461 of 11 anurans) and 11 occurrences of six reptile species. The troglophilic Cave Salamander (*Eurycea lucifuga*) represented nearly a third of all salamander occurrences and the Pickerel Frog (*Rana palustris*) represented over one half of all anuran occurrences. Three troglobitic species accounted for 1016 salamander occurrences from 34 caves: the Berry Cave Salamander (*Gyrinophilus gulolineatus*), Tennessee Cave Salamander (*G. palleucus*), and the West Virginia Spring Salamander (*G. subterraneus*). Although the occurrence of many amphibian and all reptile species in this survey can be categorized as accidental, we observed evidence of reproduction (courtship, nests, or young larvae) for several salamanders including *Eurycea cirrigera*, *E. longicauda*, *E. lucifuga*, *G. gulolineatus*, *G. palleucus*, *G. porphyriticus*, *G. subterraneus*, *Plethodon dorsalis*, and *Pseudotriton ruber*. Furthermore, recent studies have shown that several amphibian species lacking obvious adaptations to subterranean habitats are nonetheless reliant on caves to complete some aspect of their life histories. Thus, our data adds to a growing body of evidence indicating that caves are critical habitats that should be protected for proper amphibian conservation and management.

1. Introduction

The Interior Low Plateau (ILP) and Appalachian Valley (AV) karst regions represent two of the most diverse cave regions in North America. Accordingly, these areas and caves in general have received increased emphasis in conservation efforts because of their high levels of endemism and unique fauna and ecosystems (ELLIOTT, 2000). Salamanders are a conspicuous component of this unique fauna. Three troglobitic salamander species that all belong to the plethodontid genus *Gyrinophilus* occur in the ILP and AV: *G. gulolineatus* (Berry Cave Salamander), *G. palleucus* (Tennessee Cave Salamander), and *G. subterraneus* (West Virginia Spring Salamander). Because of their complete dependence on subterranean habitats, these species have received considerable attention from biologists seeking to understand the ecology and evolution of cave habitation (BESHARSE AND HOLSINGER, 1977; BRUCE, 1979; MILLER AND NIEMILLER, 2008; NIEMILLER et al., 2008).

However, cave and karst habitats are important not only to troglobitic species, but also to several non-troglobitic amphibian species. Many species of this latter group use caves on a temporary or semi-permanent basis for aspects of their life histories, including reproduction, refuge from harsh surface conditions (e.g., extreme temperatures or drought), and foraging (WEBER, 2000; BRIGGLER AND PRATHER, 2006; CAMP AND JENSEN, 2007; NIEMILLER AND MILLER, 2007; MILLER et al., 2008). Considerable research conducted on non-obligate, cave-associated salamanders in the ILP and AV karst regions (HUTCHINSON, 1958; CAMP AND JENSEN, 2007; NIEMILLER AND MILLER, 2007; MILLER et al., 2008), have emphasized species' distributions, generation of species lists, and obtaining life-history data; however, most of these studies centered on surveys of just a few species or of relatively few caves. Consequently, limited data are available on the use of cave habitats by amphibian species not typically classified as cave-associated, particularly from Tennessee and northern Alabama. Therefore, we present herein the results of four years of surveys for amphibians and reptiles from 172 caves throughout the karst regions of north Alabama, northwest Georgia, south-central Indiana, southeast Kentucky, Tennessee, southwest Virginia, and southeast West Virginia. Our study represents one of the most comprehensive surveys of cave-associated amphibians and reptiles in terms of number of caves visited and surveyed, as well as the total number of species and individuals encountered.

2. Materials and Methods

2.1 Study sites.

From June 2004 through December 2008, we conducted 301 surveys of 172 caves throughout the ILP and AV in seven states: north Alabama (14 surveys of 14 caves), northwest Georgia (8 surveys of 7 caves), south-central Indiana (3 surveys of 3 caves), southeast Kentucky (8 surveys of 8 caves), Tennessee (262 surveys of 131 caves), southwest Virginia (5 surveys of 5 caves), and southeast West Virginia (5 surveys of 4 caves). A detailed list of the caves surveyed, including coordinates, can be obtained from the authors. Surveys were conducted during all months of the year with emphasis on periods of favorable stream conditions (i.e., shallow, clear water with low flow): December–February (54 surveys of 44 caves), March–May (70 surveys of 56 caves), June–August (108 surveys of 73 caves), and September–November (69 surveys of 46 caves). Most caves searched contained flowing streams or numerous, isolated pools.

2.2 Survey methods.

To locate aquatic amphibians, we donned wetsuits and slowly walked along, waded through, or crawled in the cave stream and thoroughly scanned the streambed and adjacent stream edge for amphibians. We carefully lifted rocks, cobble, and detritus under which amphibians might seek refuge both in the water and stream edge. Lifted rocks and other cover objects were returned to their original positions to minimize habitat disturbance. We searched crevices in the cave wall and wall proper for terrestrial amphibians and reptiles, particularly near a cave entrance and associated twilight zone. In addition, we searched underneath rocks and other debris for herpetofauna. The developmental stage (egg, larva, juvenile, or adult) of each individual observed was recorded and a count was kept of each species encountered.

2.3 Ecological classification.

We classified species/populations of cave-associated amphibians and reptiles following BARR (1968) into four categories: troglobite (TB), troglophile (TP), trogloxene (TX), and accidental (AC). Note that here we define accidental as an occurrence that is incidental to the larger cave habitat; herpetofaunal observations in this category tend to occur at the cave mouth or in the twilight zone, which is analogous to non-cave habitats such as talus and bluff shelters. Considerable debate continues over the utility of ecological classification of cave-associated organisms and we refer the reader to SKET (2008) and FENOLIO et al. (this volume) for a review and discussion of this subject and in relation to amphibians, respectively. Historically, the range-wide tendencies of a species indicate the proper cave-associated ecological classification (see SKET, 2008), rather than the attributes of a local population. We agree with POLY AND BOUCHER's (1996) assessment that unique populations of epigean species capable of subterranean existence or those that utilize subterranean habitats for some aspect of their life history should not be ignored and are of "great importance concerning evolution of cave-dwelling organisms."

3. Results

3.1 Species encountered.

We conducted 301 surveys of 172 caves throughout the ILP and AV in seven states. We recorded 4542 observations of 29 species amphibians and six species of reptile from 151 caves (269 surveys) from 2004–2008. Eighteen species of salamander accounted for 4081 observations from 144 caves (Table 1) including three troglobitic species (*G. gulolineatus*, *G. palleucus*, and *G. subterraneus*). The troglophilic *Eurycea lucifuga* (Cave Salamander) represented nearly a third of all salamander observations (n=1267) and was observed in 73% of caves. *Gyrinophilus porphyriticus* (Spring Salamander) and *Plethodon glutinosus* (Northern Slimy Salamander) were the second and third most frequently encountered salamanders. Eleven species of anurans accounted for 461 observations from 73 caves (Table 2), but eight of these species were classified as accidental. The three most commonly encountered species were not classified as accidental. These three ranids (American Bullfrog, *Rana catesbeiana*; Green Frog, *R. clamitans*; and Pickerel Frog, *R. palustris*) represented 97% of all anuran observations, with *R. palustris* the most frequently encountered species (227 observations from 42 caves). Six reptiles (four snakes, one lizard, and one turtle species) accounted for 11 observations from nine caves (Table 3). All reptiles encountered were categorized as accidental. The *Terrapene carolina* (Eastern Box Turtle) was the most frequently observed species (five observations from four caves).

3.2 Life stages encountered and subterranean reproduction.

Adult was the predominant life stage encountered for the majority of salamanders (Table 1), including two paedomorphic taxa (*G. gulolineatus* and *G. palleucus*); however, larvae of a number of species were also observed, including *Desmognathus conanti* (Spotted Dusky Salamander), *E. cirrigera* (Southern Two-lined Salamander),

Species	Ecological Classification[a]	States (No. of Counties)	No. Caves	No. Surveys	No. Observed	Max. Observed	Life Stages Observed[b]
Family Plethodontidae							
Desmognathus abditus	AC	TN(1)	1	1	1	1	A
Desmognathus conanti	TX/AC	AL(1), GA(1), TN(10)	14	14	37	17	L, A
Desmognathus fuscus	TX/AC	KY(1), VA(1)	2	2	2	1	A
Desmognathus ochrophaeus	AC	WV(1)	2	2	2	1	A
Eurycea cirrigera	TX/AC	GA(1), TN(8), WV(2)	12	18	83	31	E, H, L, J, A
Eurycea longicauda	TP/TX	AL(1), GA(2), KY(1), TN(8), WV(2)	22	32	109	15	L, A
Eurcyea lucifuga	TP	AL(5), GA(2), IN(2), KY (1), TN (29), VA(2), WV(1)	125	209	1267	99	E, H, L, J, A
Gyrinophilus gulolineatus	TB	TN(2)	6	28	250	24	H, J, A
Gyrinophilus palleucus necturoides	TB	TN(7)	14	43	459	34	H, J, A
Gyrinophilus palleucus palleucus	TB	AL(1), GA(1)[c], TN(2)	11	17	278	41	J, A
Gyrinophilus porphyriticus	TP	AL(1), GA(2), TN(9), VA(2), WV(2)	28	41	674	84	E, H, L, J, A
Gyrinophilus subterraneus	TB	WV(1)	1	2	45	29	L, A
Hemidactylium scutatum	AC	TN(1)	1	1	1	1	A
Plethodon dorsalis	TX	AL(1), GA(1), TN(12)	21	22	76	31	E, J, A
Plethodon glutinosus	TP/TX	AL(2), GA(2), KY(1), TN(15)	41	48	539	102	J, A
Plethodon petraeus	TX	GA(1)	1	2	6	4	A
Pseudotriton montanus	AC	KY(1)	1	1	1	1	J
Pseudotriton ruber	TP/TX	AL(1), GA(1), TN(8), VA(1)	27	40	249	33	E, H, L, J, A
Family Salamandridae							
Notophthalmus viridescens	AC	TN(2)	2	2	2	1	J
Overall: 18 species		AL(7), IN(2), GA(2), KY(1), TN(31), VA(2), WV(2)	144	261	4081	112	

[a] According to Barr (1968): TB – troglobite, TP – troglophile, TX – trogloxene, AC – accidental

[b] Life Stages: E - eggs, H - hatchling, L - larva, J - juvenile (sexually immature metamorph), A - adult

[c] The Georgia record has not been determined to subspecies

Table 1: Abundance, life stages, and ecological classification of salamander species observed during the present study from 2004–2008.

Species	Ecological Classification[a]	States (No. of Counties)	No. Caves	No. Surveys	No. Observed	Max. Observed	Life Stages Observed[b]
Family Bufonidae							
Bufo americanus	AC	TN(2)	3	3	3	1	A
Bufo fowleri	AC	TN(1)	1	1	1	1	A
Family Hylidae							
Acris crepitans	AC	TN(2)	2	2	2	1	A
Hyla chrysoscelis	AC	TN(1)	1	1	1	1	A
Pseudacris crucifer	AC	TN(2)	2	2	2	1	A
Pseudacris feriarum	AC	TN(1)	1	1	1	1	A
Family Microhylidae							
Gastrophryne carolinensis	AC	TN(1)	1	1	1	1	A
Family Ranidae							
Rana catesbeiana	TX	AL(3), GA(1), IN(1), KY(1), TN(15), VA(2), WV(1)	33	47	66	4	L, J, A
Rana clamitans	TX	AL(1), GA(1), KY(1), TN(14), VA(2), WV(1)	33	60	156	11	J, A
Rana palustris	TX	AL(2), GA(2), IN(1), KY(1), TN(18)	42	68	227	21	J, A
Rana sphenocephala	AC	TN(1)	1	1	1	1	A
Overall: 11 species		AL(7), IN(2), GA(2), KY(1), TN(31), VA(2), WV(2)	73	128	461	31	

[a] According to Barr (1968): TB – troglobite, TP – troglophile, TX – trogloxene, AC – accidental

[b] Life Stages: L - tadpole, J - juvenile (sexually immature metamorph), A - adult

Table 2: Abundance, life stages, and ecological classification of anuran species observed during the present study from 2004–2008.

Species	Ecological Classification[a]	States (No. of Counties)	No. Caves	No. Surveys	No. Observed	Max. Observed	Life Stages Observed[b]
Family Colubridae							
Pantherophis spiloides	AC	TN(1)	1	1	1	1	A
Family Crotalidae							
Crotalus horridus	AC	TN(1)	1	1	1	1	A
Family Dipsadidae							
Diadophis punctatus	AC	GA(1), TN(1)	2	2	2	1	A
Family Emydidae							
Terrapene carolina carolina	AC	TN(3)	4	4	5	2	J, A
Family Natricidae							
Nerodia sipedon	AC	TN(1)	1	1	1	1	A
Family Scincidae							
Scincella lateralis	AC	TN(1)	1	1	1	1	A
Overall: 6 species		GA(1), TN(7)	9	10	11	2	

[a] According to Barr (1968): TB – troglobite, TP – troglophile, TX – trogloxene, AC – accidental

[b] Life Stages: J - juvenile, A - adult

Table 3: Abundance, life stages, and ecological classification of reptile species observed during the present study from 2004–2008.

E. longicauda (Long-tailed Salamander), *E. lucifuga*, *G. porphyriticus*, *G. subterraneus*, and *Pseudotriton ruber* (Red Salamander). We observed evidence of reproduction (courtship, oviposition, nests, or recently hatched young) for several plethodontids including the *E. cirrigera*, *E. longicauda*, *E. lucifuga*, *G. gulolineatus*, *G. palleucus*, *G. porphyriticus*, *Plethodon dorsalis* (Zigzag Salamander), and *P. ruber*. Detailed descriptions of these observations for many species have been reported elsewhere (see Discussion). Only juveniles or adults were observed for ten of the eleven anuran species (Table 2); however, two tadpoles of *R. catesbeiana* were found in the cave stream of Cow Cave (TWR286), Warren Co., Tennessee, and were, presumably, washed in from a cattle pond located upstream of the main cave. Adults accounted for nearly all reptile observations (Table 3), the only exception was a hatchling *T. carolina* found in Big Mouth Cave (TGD2), Grundy Co., Tennessee. This turtle was nearly dead when found and apparently washed into the cave after a significant rainfall event a few days earlier.

4. Discussion

Many species of non-troglobitic amphibians and reptiles have been reported from subterranean habitats, including caves, and most species we observed have been reported previously from caves. Individuals from any species may accidentally enter a cave (e.g. by falling into a pit, wandering into an entrance by following a stream, or being washed in by an inflowing stream). However, only those individuals with the necessary morphological, physiological, or behavioral adaptations may persist and exploit subterranean habitats for extended periods of time. Throughout the ILP and AV, several amphibian species use caves to varying degrees for aspects of their life histories, including reproduction, foraging, and shelter from harsh surface conditions.

Evidence of reproduction in subterranean habitats was noted for several plethodontid salamander species during this study. Although hatchlings were observed for many species, we observed nests, many with attending females, for several salamanders including *E. cirrigera*, *E. lucifuga*, *G. porphyriticus*, *P. dorsalis*, and *P. ruber*. Nests of *E. lucifuga* and *G. porphyriticus* in caves are not surprising; however, nests of the other three species are noteworthy. Although *E. cirrigera* are occasionally reported from caves (e.g., HIMES et al., 2004 and OSBOURN, 2005), the use of caves for reproduction in this species was first reported for a cave-breeding population in middle Tennessee during the course of this study (NIEMILLER AND MILLER, 2007). Nests of *P. dorsalis* in the literature are few (reviewed in NIEMILLER AND MILLER, 2008) and all are from subterranean habitats. Some authors have claimed that *P. dorsalis* is not a cave-dwelling species (DODD et al., 2001); however, subterranean nesting and observed aggregations during summer months (NIEMILLER AND MILLER, 2008) suggests this species is more reliant on subterranean habitats than previously perceived. Likewise, *P. ruber* has occasionally been reported from caves in the ILP and AV (BRODE, 1958; GREEN AND BRANT, 1966; BUHLMANN, 2001; OSBOURN, 2005). We previously reported on nests of this species from Tennessee (MILLER AND NIEMILLER, 2005) and Georgia (NIEMILLER et al., 2006), in addition to nesting location, oviposition behavior, and nest defense by attending females (MILLER et al., 2008). Given recent evidence, we argue that at least some populations of this species are adapted and live the majority of their lives in caves and should, therefore, be classified as troglophiles. As more comprehensive life histories are conducted, we expect that many other non-troglobitic amphibians will be shown to use subterranean habitats for reproduction, which will further obscure the discrete categories of troglophile and trogloxene.

Several salamanders utilize caves in a seasonal fashion (BRIGGLER AND PRATHER, 2006; CAMP AND JENSEN, 2007) and our results (not shown) also support seasonality of cave use for several species. Although *E. lucifuga* was found year-round, *P. glutinosus* was predominately found during the summer months in the twilight zones of several caves during this study, likely in response to warm and dry surface conditions. Many plethodontids forage and move through the leaf litter of the forest floor. However, surface activities become nearly impossible during periods of extreme heat, cold, or drought. Consequently, the cool and moist environment found in caves and other subterranean haunts provides sanctuary into which herpetofauna can retreat when the surface is inhospitable (BRIGGLER AND PRATHER, 2003; CAMP AND JENSEN, 2007). The anuran *R. palustris* exploits caves for similar reasons. Although our surveys reinforce the observations of others that *R. palustris* has a high affinity for caves (BARR, 1953; BRODE, 1958; GREEN AND BRANT, 1966; CLIBURN AND MIDDLETON, 1983; OSBOURN, 2005), recent work by FENOLIO et al. (2005) suggest that this species is not a significant subterranean predator and caves are being used as refugia only. Similar to most anurans, reptiles are rarely encountered in caves in the ILP and AV (CLIBURN AND MIDDLETON, 1983; OSBOURN, 2005; this study). Most reptile records represent incidental entry into subterranean habitats; however some species, such as *T.*

carolina, may seek refuge in caves during hot, dry periods.

Our study represents one of the most comprehensive surveys of cave-associated herpetofauna, and our data suggest that several non-troglobitic amphibian species are reliant upon caves and subterranean habitats. Many taxa use caves on a temporary or semi-permanent basis for shelter, foraging, and reproduction. Indeed, the use of caves by amphibians is a more complex behavior than previous work has indicated (CAMP AND JENSEN, 2007). Our study adds to a growing body of evidence indicating that in addition to the surrounding forest, caves are critical habitats for many species, and, therefore, should be protected for proper amphibian conservation and management.

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