

2010

Conservation Status and Habitat Use of the West Virginia Spring Salamander (*Gyrinophilus Subterraneus*) and Spring Salamander (*G. Porphyriticus*) in General Davis Cave, Greenbrier Co., West Virginia

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Original Publication Citation

Niemiller, M. L., Osbourn, M. S., Fenolio, D. B., Pauley, T. K., Miller, B. T., & Holsinger, J. R. (2010). Conservation status and habitat use of the West Virginia Spring Salamander (*Gyrinophilus subterraneus*) and Spring Salamander (*G. Porphyriticus*) in General Davis Cave, Greenbrier Co., West Virginia. *Herpetological Conservation and Biology*, 5(1), 32-43.

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CONSERVATION STATUS AND HABITAT USE OF THE WEST VIRGINIA SPRING SALAMANDER (*GYRINOPHILUS SUBTERRANEUS*) AND SPRING SALAMANDER (*G. PORPHYRITICUS*) IN GENERAL DAVIS CAVE, GREENBRIER CO., WEST VIRGINIA

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Abstract.—The West Virginia Spring Salamander (*Gyrinophilus subterraneus*) is one of four obligate cave-dwelling species of plethodontid salamanders found east of the Mississippi River in the United States. This species is endemic to a single cave system; General Davis Cave, in Greenbrier Co., West Virginia, where it is syntopic with the closely-related Spring Salamander (*G. porphyriticus*). Accordingly, the West Virginia Spring Salamander is a species of critical conservation concern. Because of its conservation status and lack of data regarding the ecology and life history, particularly about population trends, we present data on relative abundance of and habitat use by the West Virginia Spring Salamander during a 33-year period from 1975–2008. Specifically we address: (1) stability of the population during the last 33 years; (2) variation in habitat use by life stage and between species (Spring Salamanders and West Virginia Spring Salamanders); (3) plausibility of neoteny in the West Virginia Spring Salamander; and (4) the conservation status of the West Virginia Spring Salamander. We recorded 324 observations of *Gyrinophilus* salamanders, of which 192 were West Virginia Spring Salamanders, within the study area during 17 surveys. While both larval and metamorphosed West Virginia Spring Salamanders were encountered, only metamorphosed Spring Salamanders were observed. West Virginia Spring Salamander larvae were encountered in pools more often than in riffle habitat. Spring Salamanders were encountered more often in terrestrial habitats versus aquatic habitats. West Virginia Spring Salamanders reach relatively large size before metamorphosing, with some individuals becoming sexual mature as larvae. It remains unknown whether any of these individuals reproduce, however. Although the populations of both species appear to be stable over the past 33 years and not in immediate danger of extinction, the West Virginia Spring Salamander is still of critical conservation concern because of its extremely restricted distribution and current threats to the cave system it resides in.

Key Words.—cave-dwelling; *Gyrinophilus*; neoteny; Spring Salamander; subterranean; troglodyte; West Virginia

INTRODUCTION

Because of their unique habitat, life histories, and generally restricted distributions, most species of obligate cave-dwelling salamander species are of conservation and management concern. Included in this group are cave-dwelling species of *Gyrinophilus* (family Plethodontidae) endemic to the Interior Low Plateau and Appalachian Valley and Ridge of the eastern United States, the Tennessee Cave Salamander complex and the West Virginia Spring Salamander (*Gyrinophilus subterraneus*). The Tennessee Cave Salamander complex includes the Berry Cave Salamander (*G. gulolineatus*) endemic to the Appalachian Valley and Ridge in east Tennessee, and two subspecies of the Tennessee Cave

Salamander, the Pale Salamander (*G. palleucus palleucus*) and the Big Mouth Cave Salamander (*G. p. necturoides*) associated with caves in the Central Basin, Highland Rim, and Cumberland Plateau of Alabama, Georgia, and Tennessee (Miller and Niemiller 2008). In contrast to the Tennessee Cave Salamander complex, the West Virginia Spring Salamander presumably is not neotenic and readily undergoes metamorphosis, albeit at an exceptionally large size up to 95 mm snout-vent length (SVL; Besharse and Holsinger 1977). Interestingly, the largest larvae we examined are sexually mature. These circumstances suggest the potential for neoteny, but it remains unknown whether such individuals actually reproduce as larvae.



FIGURE 1. Whole body images of Spring Salamanders (*Gyrinophilus porphyriticus*) and West Virginia Spring Salamanders (*G. subterraneus*) for comparative purposes: (A) larval Spring Salamander from Rehoboth Church Cave, Monroe County, West Virginia (W. VA.), USA, (B) larval West Virginia Spring Salamander from General Davis Cave, Greenbrier County, W. VA., (C) late stage larval West Virginia Spring Salamander from General Davis Cave, Greenbrier County, W. VA., (D) metamorphosed West Virginia Spring Salamander from General Davis Cave, Greenbrier County, W. VA., (E) metamorphosed Spring Salamander from General Davis Cave, Greenbrier County, W. VA. (Photographed by Danté Fenolio)

As described by Besharse and Holsinger (1977), the West Virginia Spring Salamander (Fig. 1) is endemic to General Davis Cave in the Appalachian Valley and Ridge karst region of Greenbrier County in West Virginia, where it is syntopic with the closely-related Spring Salamander (*G. porphyriticus*). Little is known about the ecology and life history of the West Virginia Spring Salamander; what information is available has been summarized by Petranka (1998) and Beachy (2005). Although not in immediate danger of extinction, the West Virginia Spring Salamander is of critical conservation concern because of a restricted distribution and anthropogenic threats to the cave system and associated watershed where it is found. The Nature Conservancy owns an easement on the General Davis

Cave system and title to the main entrance; however, the principal upstream source of the cave stream and the entire watershed remain unprotected. Consequently, NatureServe lists the West Virginia Spring Salamander as “Critically Imperiled” (NatureServe 2009. NatureServe Explorer; an online encyclopedia of life. Version 7.1. Available from <http://www.natureserve.org/explorer> [Accessed 9 August 2009]), whereas IUCN lists the West Virginia Spring Salamander as “Endangered” because of a putative population size of less than 250 individuals (IUCN 2008. 2008 IUCN Red List of Threatened Species. Available from <http://www.Iucnredlist.org> [Accessed 31 March 2009]). Furthermore, this species is included on the West Virginia list of rare, threatened, and endangered species; however, the United States Fish and Wildlife Service currently does not designate special protection to the West Virginia Spring Salamander.

Because of the conservation status and lack of data regarding the ecology and life history of the West Virginia Spring Salamander, particularly population trends, we present data herein on relative abundance and habitat use during a 33-year period from 1975–2008. Specifically we address: (1) dynamics of the population during the last 33 years; (2) variation in habitat use by life stage (larva and metamorphs) and between species (Spring Salamanders and West Virginia Spring Salamanders); (3) plausibility of neoteny in West Virginia Spring Salamander; and (4) the conservation status of the West Virginia Spring Salamander. Past authors have disputed the validity of the West Virginia Spring Salamander as a distinct species (Blaney and Blaney 1978), claiming the salamanders in General Davis Cave represent an extreme variant of a phenotypically plastic species (i.e., *G. porphyriticus*). In light of data collected during our study, we also comment on the taxonomic status of the West Virginia Spring Salamander.

MATERIALS AND METHODS

Description of study site.—General Davis Cave is located in the Greenbrier River watershed in Greenbrier Co., West Virginia, within the Appalachian Valley and Ridge physiographic province. The cave is developed in the Greenbrier Series of the Union Limestone with the main entrance at an elevation of 503 m. The stream passage is intersected approximately 200 m from the entrance. Downstream the cave stream flows through a 1.2 m high and 3.0 m wide passage for 70 m before the stream sumps and the passage is blocked by clay fill. The stream can be followed upstream for approximately 890 m until the ceiling lowers to within 0.5 m at the “Hurricane Siphon.” During periods of low stream flow the siphon can be passed through to access 2.5 km of additional cave passage that eventually ends in a



FIGURE 2. Deposits of allochthonous coarse particulate organic matter up to a meter thick found along the stream in General Davis Cave, Greenbrier County, West Virginia, USA. This habitat contains an abundance of earthworm castings and salamanders were usually found in the vicinity of patches of coarse particulate organic matter. (Photographed by Michael S. Osbourn)

terminal siphon. Our surveys focused on the first 290 m of stream passage, which is dominated by mud banks and deposits of coarse particulate organic matter (Fig. 2). The substrate of the cave stream consists predominately of small cobble and gravel with intermittent mud or bedrock.

Species determination.—West Virginia Spring Salamanders and Spring Salamanders coexist syntopically in General Davis Cave. Although closely related, a suite of morphological features can readily distinguish larvae and metamorphic individuals of these two species (Fig. 1). In particular, larvae of West Virginia Spring Salamanders have smaller eyes, wider heads, more premaxillary and prevomerine teeth, and are larger and more robust relative to similar-sized Spring Salamanders. Furthermore, West Virginia Spring Salamander larvae are paler than, and have darker reticulation usually with two to three irregular rows of pale yellow spots running the length of the body that are lacking in Spring Salamander larvae (Besharse and Holsinger 1977; Petranka 1998). West Virginia Spring Salamander larvae undergo metamorphosis at a larger size (> 95 mm SVL) than larval Spring Salamanders from local populations (55–70 mm SVL; Besharse and Holsinger 1977). Metamorphosed (adult) Spring Salamanders and West Virginia Spring Salamanders also can be easily distinguished from each other (Fig. 1), as the former are gaunter in appearance than the latter. Furthermore, metamorphosed West Virginia Spring Salamanders have reduced eyes, an indistinct *canthus rostralis*, and retain the reticulate patterning found in larvae. In addition, the premaxilla is undivided in metamorphosed West Virginia Spring Salamanders (a

trait shared with metamorphosed Tennessee Cave Salamanders and Berry Cave Salamanders), but is divided in Spring Salamanders.

Survey techniques.—We searched ca. 290 m of linear cave stream and adjacent stream bank habitat for terrestrial and aquatic salamanders on 17 occasions from 30 May 1975 to 8 October 2008. To locate salamanders, we slowly walked along, waded through, or crawled in the cave stream and thoroughly scanned the streambed and adjacent stream bank with our headlamps. We carefully overturned rocks and logs and searched through cobble and detritus within stream pools and riffles and adjacent terrestrial habitats. Overturned objects were returned to their original positions to minimize habitat disturbance. Although the same observers were not present on every survey (Table 1), we feel that the same survey strategy was employed during all surveys for which abundance data were obtained and presented herein. Moreover, environmental conditions (e.g., water level, clarity, and flow) were similar across all surveys included in the current dataset. We did not include abundance data from several other surveys where water levels were elevated, flow increased, and clarity reduced because of recent precipitation.

Data collection.—Upon capture, individuals were classified according to species (*G. porphyriticus* or *G. subterraneus*) and life stage (larva or metamorph). During surveys on 21 August 2002, 9 October 2003, 10 August 2007, and 8 October 2008, we measured to the nearest mm for SVL and total length (TL) with small metric rulers to the nearest mm, and weighed each salamander (in 2007 and 2008) to the nearest 0.5 g with a Pesola® spring scale (Pesola AG, Baar, Switzerland). Although we were unable to determine the sex of most salamanders, ova were visible in several metamorphosed female salamanders. For each salamander captured, we also recorded the distance from the downstream sump, habitat type (terrestrial: mud bank, organic mat, or bedrock; aquatic: pool, run, or riffle), substrate, water depth, and position (under cover object or uncovered). Finally, we excised the tail tip from several salamanders for subsequent genetic analyses. Each salamander was returned to its capture location immediately after processing.

Data analysis.—All statistical analyses were performed in the program *R 2.4.1* (R Development Core Team 2006). We used the nonparametric Mann-Kendall test implemented in the *Kendall* package in *R* to examine trends in abundance from 1975–2008 for the four salamander groups: (1) all *Gyrinophilus* pooled; (2) metamorphosed Spring Salamanders; (3) metamorphosed West Virginia Spring Salamanders; and

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TABLE 1. *Gyrinophilus* abundance data from 17 surveys of ca. 290 m cave stream in General Davis Cave, Greenbrier Co., West Virginia, USA. Observers Present indicates which authors were present for a given survey.

Date	All <i>Gyrinophilus</i>	Spring Salamander	West Virginia Spring Salamander		Observers Present
			Metamorphosed	Larvae	
30 May 1975	14	7	6	1	JRH
26 Sep 1976	9	1	1	7	JRH
28 Sep 1979	27	15	10	2	JRH
24 Jul 1982	11	6	2	3	JRH
02 Jul 1983	23	11	4	8	JRH
21 Sep 1984	21	9	3	9	JRH
26 Sep 1986	13	6	1	6	JRH
07 Oct 1988	21	7	1	13	JRH
28 Sep 1990	12	5	1	6	JRH
01 Oct 1993	11	6	0	5	JRH
29 Sep 1995	12	7	0	5	JRH
02 Oct 1998	17	9	2	6	JRH
28 Sep 2001	8	6	0	2	JRH
21 Aug 2002	38	10	3	25	MSO
09 Oct 2003	22	7	3	12	MSO
10 Aug 2007	40	11	1	28	MLN, MSO, DBF, BTM
08 Oct 2008	25	9	1	15	MLN

(4) West Virginia Spring Salamander larvae. This test is appropriate for time series data when assumptions required for regression analyses cannot be met. This test can only determine if the data are increasing or decreasing and cannot account for the magnitude of change (Thompson et al. 1998). We examined SVL-mass variation between metamorphosed Spring Salamanders and larval West Virginia Spring Salamanders using analysis of covariance (ANCOVA) to test for different slopes with SVL as the covariate. Pearson correlation was used to examine the relationship between water depth and salamander body size for larval West Virginia Spring Salamanders. Binomial probability tests were used to determine whether metamorphosed Spring Salamanders and West Virginia Spring Salamanders were observed more often in terrestrial habitats than aquatic habitats. Likewise, we conducted binomial probability tests to determine if metamorphosed Spring Salamanders were observed more often on mud banks rather than organic matter and bedrock combined, and if larval West Virginia Spring Salamanders were observed more often in pool compared to riffle habitats. For all tests, $\alpha = 0.05$.

RESULTS

We recorded 324 observations of *Gyrinophilus* salamanders (192 identified as West Virginia Spring Salamanders) within the study area during 17 surveys conducted from 1975–2008 (Table 1, Fig. 3). Of the West Virginia Spring Salamanders observed, only 39

(20.3%) were metamorphosed individuals; whereas all salamanders identified as Spring Salamanders were metamorphosed (i.e., no larval Spring Salamanders were found). Several female Spring Salamanders and a single West Virginia Spring Salamander were gravid.

Population size and trend.—We observed on average 19.1 *Gyrinophilus* salamanders per survey (range 8–40) in the study area with an average of 7.8 Spring Salamanders (range 1–15) and 11.3 West Virginia Spring Salamanders (range 5–29) observed. Most salamanders identified as West Virginia Spring Salamanders were larvae (mean = 9.0, range 1–28); few metamorphosed (adult) West Virginia Spring Salamanders were found (mean = 2.3, range 0–10). There were no significant trends in salamanders observed from 1975–2008 ($n = 17$) for metamorphosed Spring Salamanders, metamorphosed West Virginia Spring Salamanders, or all *Gyrinophilus* pooled; however, a significant increasing trend was detected for West Virginia Spring Salamander larvae (Table 2, Fig. 3).

Demography and morphometrics.—We measured SVL for 102 *Gyrinophilus* salamanders (35 metamorphosed Spring Salamanders, eight metamorphosed West Virginia Spring Salamanders, and 59 West Virginia Spring Salamander larvae) during 2002, 2003, 2007, and 2008 surveys (Fig. 4). Mean SVL of metamorphosed Spring Salamanders, metamorphosed

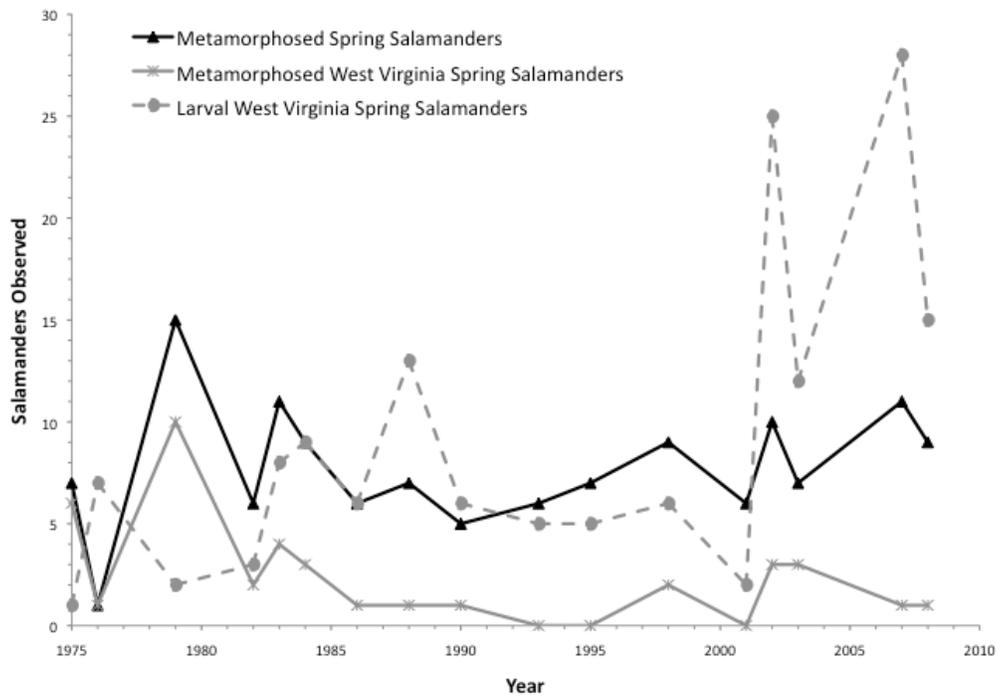


FIGURE 3. Abundance of *Gyrinophilus* salamanders from 1975–2008 from surveys of ca. 290 m of cave stream in General Davis Cave, West Virginia, USA.

TABLE 2. Results of Mann-Kendall tests to determine trends in abundance at General Davis Cave, Greenbrier Co., West Virginia, USA, from 1975–2008.

Group	<i>n</i>	<i>Z</i>	τ	<i>P</i>
All <i>Gyrinophilus</i>	17	1.12	0.201	0.265
Metamorphosed Spring Salamanders	17	0.92	0.172	0.356
Metamorphosed West Virginia Spring Salamanders	17	-1.70	-0.321	0.089
Larval West Virginia Spring Salamanders	17	2.19	0.397	0.028

West Virginia Spring Salamanders, and West Virginia Spring Salamander larvae was 109.7 mm (range 92–25), 101.4 mm (range 87–113), and 86.3 mm (range 48–117), respectively. We measured mass of 50 *Gyrinophilus* salamanders (18 metamorphosed Spring Salamanders, two metamorphosed West Virginia Spring Salamanders, and 30 West Virginia Spring Salamander larvae) during the 2007 and 2008 surveys. Mean mass of metamorphosed Spring Salamanders, metamorphosed West Virginia Spring Salamanders, and West Virginia Spring Salamander larvae was 20.4 g (range 13.0–30.0), 17.8 g (range 7.0–28.5), and 13.2 g (range 2.5–40.0), respectively. West Virginia Spring Salamander larvae were heavier than metamorphosed Spring Salamanders

(Fig. 5). However, slopes of linear regression lines were not significantly different between these two groups ($F = 0.10$; $df = 1,44$; $P = 0.7556$). West Virginia Spring Salamander larvae were also larger than metamorphosed West Virginia Spring Salamanders, but the sample size was only two (Fig. 5). We observed and measured 11 gravid female Spring Salamanders from 2002–2008. Mean SVL and mass for gravid females was 110.9 mm (range 87–125) and 21.1 g (range 7.0–30.0), respectively.

Habitat use.—The mean water depth where larval ($n = 49$) and metamorphosed West Virginia Spring Salamanders ($n = 2$) were captured was 7.5 cm (range = 1–20.5) and 5.5 cm (range = 5.5–5.5), respectively. Only one metamorphosed Spring Salamander was found in water, at a water depth of 6 cm. Larval West Virginia Spring Salamander SVL was not correlated with water depth ($r = 0.136$; $P = 0.36$). Terrestrial and aquatic observations for metamorphosed Spring Salamanders and metamorphosed and larval West Virginia Spring Salamanders are presented in figures 6 and 7, respectively. Metamorphosed Spring Salamanders (34 out of 35; $P < 0.001$) were encountered more often in terrestrial habitats versus aquatic habitats, whereas metamorphosed West Virginia Spring Salamanders were not encountered more frequently in either habitat type (6 out of 8 occurrences in terrestrial habitats; $P = 0.29$).

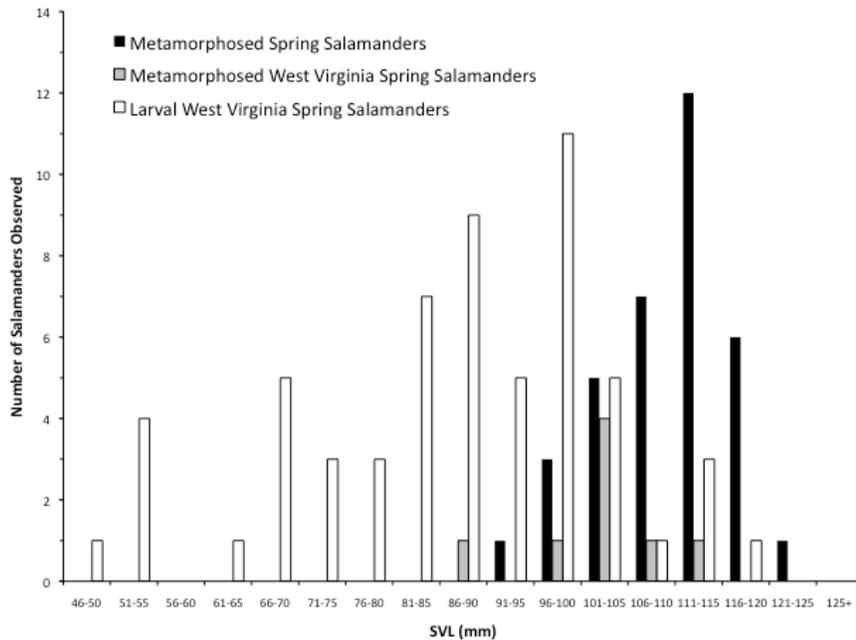


FIGURE 4. Body size histogram of snout-vent length (SVL) of metamorphosed Spring Salamanders (*Gyrinophilus porphyriticus*) and metamorphosed and larval West Virginia Spring Salamanders (*G. subterraneus*) in General Davis Cave, West Virginia, USA.

Among terrestrial habitats, metamorphosed Spring Salamanders were observed more often on mud banks than organic matter and bedrock combined (28 out of 34; $P < 0.001$). West Virginia Spring Salamander larvae were encountered in pools more often than in riffle habitat (44 out of 58; $P < 0.001$). One larva was found

crawling on land between pool habitats.

DISCUSSION

Demography, population size, and trend.—Little information is available on relative abundance or

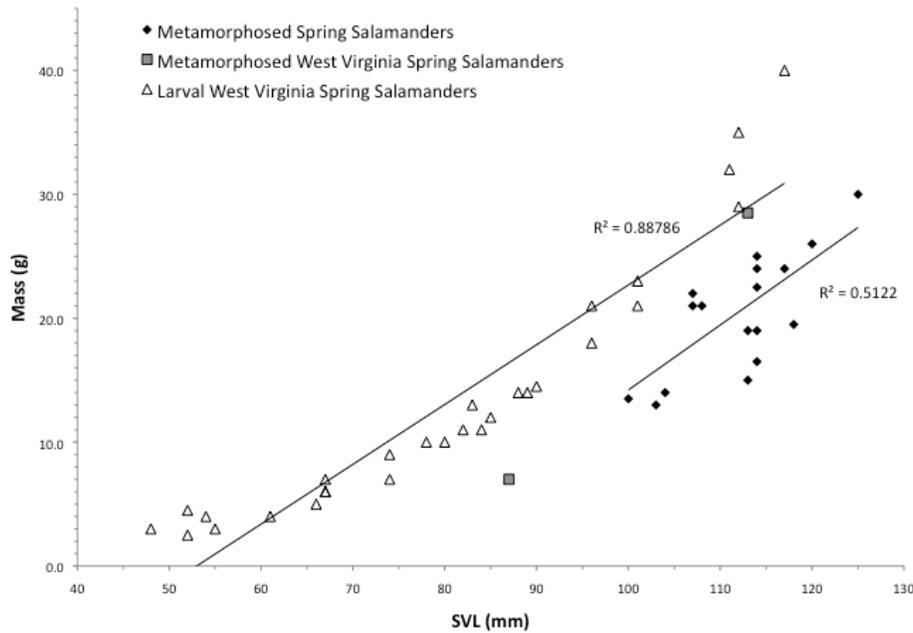


FIGURE 5. Relationship between snout-vent length (SVL) in mm and mass in grams of *Gyrinophilus* salamanders in General Davis Cave, West Virginia, USA. Linear regression trend lines are for Spring Salamander metamorphs and West Virginia Spring Salamander larvae.

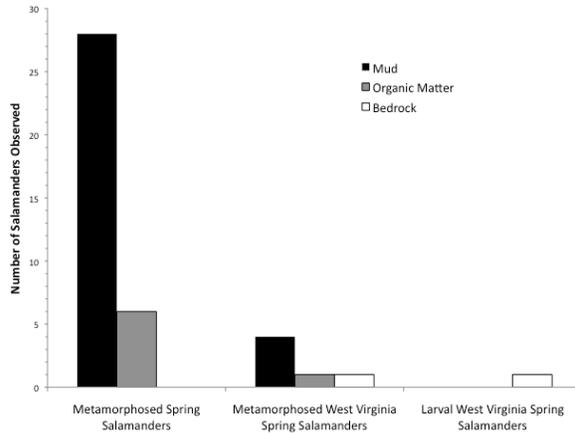


FIGURE 6. Terrestrial observations of *Gyrinophilus* salamanders on mud, organic matter, and bedrock from surveys from 2002–2008 at General Davis Cave, West Virginia, USA within the study area.

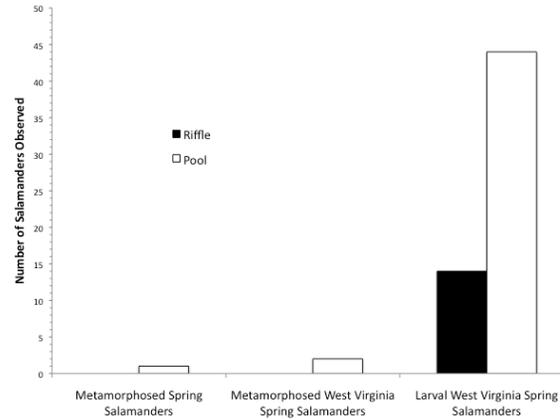


FIGURE 7. Aquatic observations of *Gyrinophilus* salamanders in riffles and pools from surveys from 2002–2008 at General Davis Cave, West Virginia, USA within the study area.

population trends for cave-dwelling *Gyrinophilus* salamanders, largely because of the inherent difficulties in surveying cave-dwelling populations. Our 33-year dataset represents one of the longest studies on relative abundance of a cave-dwelling salamander population. Although mark-recapture studies provide a more accurate estimate of population size, the sensitivity of the salamander population in General Davis Cave prevented us from using mark-recapture techniques. Miller and Niemiller (2008) demonstrated that census data could be used to estimate the relative abundance of several populations of Tennessee Cave Salamanders and Berry Cave Salamanders in Tennessee and Alabama. Although there are inherent flaws with this approach, the same salamander survey techniques were used in the same area at approximately the same time of year over the course of the present study. Because of the similarity of methods employed by different observers, we assume that our success at observing salamanders corresponds to the relative abundance of salamanders inhabiting the study area.

We failed to detect trends in abundance for metamorphosed Spring Salamanders, metamorphosed West Virginia Spring Salamanders, and for all *Gyrinophilus* salamanders pooled. Larval West Virginia Spring Salamander observations did appear to increase in recent surveys, although we recognize that could be the result of observer bias or improved environmental conditions (e.g., water level, flow, and clarity). The same observers were not present on every survey; however, we feel that the same survey strategy was employed during all 17 surveys for which abundance data were obtained and included in the current dataset. Increased observations of larvae during recent surveys likely do not reflect improved sampling techniques. Moreover, environmental conditions were similar across

all surveys; indeed, we did not include abundance data from several other surveys where water levels were elevated and clarity was reduced because of recent precipitation. Our study suggests that the West Virginia Spring Salamander population is stable. The presence of smaller larvae in earlier surveys (J.R. Holsinger, unpublished data) and the presence of both gravid metamorphosed Spring Salamanders and West Virginia Spring Salamanders indicate that reproduction is ongoing. Of concern, however, is the absence of young West Virginia Spring Salamander larvae (< 40 mm SVL) during the final four surveys (2002–2008) and our failure to detect any Spring Salamander larvae in the General Davis Cave stream. Although potentially an indication of failed reproduction, the absence of Spring Salamander larvae and smaller West Virginia Spring Salamander larvae observations are more likely the result of: (1) size-based detection bias; (2) size-based habitat segregation; or (3) low survivorship of eggs and larvae. Smaller larvae are more difficult to detect than larger larvae and other studies on cave-dwelling *Gyrinophilus* species also showed a bias towards larger larvae during surveys (Miller and Niemiller 2005. The Tennessee cave salamander complex: distribution, demography, and phylogenetics. Unpublished report. Tennessee Wildlife Resources Agency, Nashville, Tennessee, USA; Miller and Niemiller 2008). Smaller larvae might be down in the interstitial spaces of the streambed or in other habitats away from the main cave stream inaccessible to humans. Seeps, drip pools, and rimstone pools may provide refuge from potential predators, such as crayfish, and larger conspecifics. Data on diet are lacking for West Virginia Spring Salamanders, but other *Gyrinophilus* species are generalists and known to cannibalize smaller conspecifics (Lazell and Brandon 1962; Simmons 1975;

Matthew Niemiller and Brian Miller, unpubl. data). Any larvae in our study area too large to retreat into interstitial spaces of the streambed, yet still smaller than the large West Virginia Spring Salamander larvae could be vulnerable to predation.

The pressure from conspecific predation and competition could result in gravid females selecting isolated habitats away from the main cave stream for oviposition. Tennessee Cave Salamanders appear to oviposit in areas away from the main cave stream, as suggested by the absence of gravid females or nests in the main cave stream during late autumn and early winter (Matthew Niemiller and Brian Miller, unpubl. data). Spring Salamander hatchlings have been observed in a seep puddle 30 m from the main stream passage in another West Virginia cave (Michael Osbourn, unpubl. data). West Virginia Spring Salamander hatchlings and small larvae might also live in such secluded areas until they grow to sufficient size to escape predation. More studies are needed on larval survival and detectability of smaller size classes before conclusions about population health can be made.

Habitat use.—Our observations that larval West Virginia Spring Salamanders frequent shallow pools and avoid cover objects, such as rocks or logs are surprising, as this open habitat seems to make individuals particularly susceptible to predation. This behavior contrasts with Tennessee Cave Salamanders, which are most frequently found underneath submerged rocks, logs, and other debris (Miller and Niemiller 2005, *op. cit.*; Matthew Niemiller and Brian Miller, unpubl. data). Although we did not quantify available cover within the cave stream, the number and density of cover objects did not appear appreciably different than many other caves surveyed by the authors in the Valley and Ridge physiographic province. Our observations suggest there might be a behavioral basis for the lack of cover use by larval West Virginia Spring Salamanders, possibly due to a lower susceptibility to predation for larger individuals.

We encountered metamorphosed Spring Salamanders most often on the large slopping mud banks that flank the cave stream. This habitat contained an abundance of earthworm castings and salamanders were usually found in the vicinity of patches of coarse particulate organic matter. In some areas near the intersection of the stream and entrance passages, mud and organic matter was piled in layers over a meter deep (Fig. 2). Allochthonous coarse particulate organic matter in the form of fallen leaves supply most of the energy inputs to headwater streams in eastern deciduous forests (Wallace et al. 1995), and in caves with inflowing surface streams these inputs are also crucial for supplying nutrients to terrestrial habitats (Culver 1982). While surveying cave-dwelling Spring Salamander populations throughout

Greenbrier and Monroe Counties in West Virginia, Osbourn (2005) observed higher densities of salamanders congregated in areas where streams sump or become constricted. During seasonal flood events these areas become swirling eddies, accumulating organic debris. Leaves, small sticks, logs, and silt settle and form thick-layered deposits. These areas of concentrated nutrients contain abundant terrestrial invertebrate communities, which is likely the reason for the higher metamorphosed Spring Salamander observations there, as compared with other terrestrial or stream habitats.

Most salamanders observed were large and robust, indicating successful foraging in complete darkness. Although metamorphosed Spring Salamanders might feed underground, the lack of observations of Spring Salamanders in the stream within General Davis Cave suggests greater terrestrial than aquatic foraging. In total darkness, metamorphosed salamanders must rely on non-visual senses, such as touch and olfaction, to locate and capture prey in terrestrial habitats. Terrestrial cave habitats are generally thought to harbor less biomass than aquatic habitats (Hüppop 2000; Poulson and Lavoie 2000), and might have led to the evolution of neoteny in troglobitic salamanders (Bruce 1979). However, there is an abundance of invertebrates, particularly annelids, within the mud banks and organic debris along the cave stream in General Davis Cave, which might support a large and healthy population of metamorphosed *Gyrinophilus*. Clearly, studies are needed that examine trophic relationships of terrestrial cave-dwelling salamander populations and foraging success of cave-dwelling species, such as Spring Salamanders, in total darkness.

Neoteny.—Neoteny, or attaining sexual maturity via delayed metamorphosis, is a common phenomenon in cave-dwelling salamanders that appears to have evolved after subterranean colonization (Ryan and Bruce 2000). Most obligate, cave-dwelling species of *Eurycea*, the Tennessee Cave Salamander, and the Berry Cave Salamander are neotenic, although the latter two species can be induced to undergo metamorphosis in the laboratory (Dent and Kirby-Smith 1963; Brandon 1971). West Virginia Spring Salamanders appear intermediate between metamorphosing Spring Salamanders and neotenic Tennessee Cave Salamanders. Our observations and that of Besharse and Holsinger (1977) demonstrate that West Virginia Spring Salamanders metamorphose at a relatively large size, from 87 to as much as 117 mm SVL. Metamorphosed West Virginia Spring Salamanders observed in this study measured 87–113 mm SVL with larvae up to 117 mm SVL. Although we could not determine if the largest larvae were sexually mature, Besharse and Holsinger (1977) reported both sexually mature male (pigmented testes) and female (enlarged and convoluted oviducts) larviform

individuals. Three other large larvae that have been dissected had undeveloped gonads, however (Michael Osbourn and Thomas Pauley, unpubl. data). Bruce (1979) argued that neoteny in *Gyrinophilus* was an adaptation to insufficient food resources in terrestrial cave habitats, which would compensate for a metabolically demanding metamorphosis and subsequent niche shift, from an aquatic to a terrestrial existence. At first glance, the gaunt to nearly emaciated appearance of metamorphosed West Virginia Spring Salamanders, relative to the large and robust appearance of larvae might support Bruce's (1979) insufficient terrestrial resources hypothesis. The presence of large, robust, and apparently healthy metamorphosed Spring Salamanders, however, contradicts such an inference unless terrestrial conditions were significantly harsher than today throughout much of the evolutionary history of the West Virginia Spring Salamander. Unfortunately, the collection and dissection of additional specimens are needed to elucidate whether the West Virginia Spring Salamander is truly neotenic.

Taxonomic status.—Blaney and Blaney (1978) questioned the taxonomic validity of the West Virginia Spring Salamander shortly after its description by Besharse and Holsinger (1977). It has been argued that the Spring Salamander is highly polymorphic with regard to eye size, pigmentation, and neoteny (Blaney and Blaney 1978) and is phenotypically plastic (J.H. Howard et al., unpubl. data), although this latter point has yet to be demonstrated. Blaney and Blaney (1978) argued that larval Spring Salamanders exhibit considerable variation in pigmentation from darker individuals in surface populations to pale individuals in cave populations. Likewise, the authors claimed that eyes range from normal to reduced and nonfunctional. Accordingly, Blaney and Blaney (1978) argued that the West Virginia Spring Salamander population is a transitional cave form with varying levels of neoteny and represents just one of several possible phenotypes of the Spring Salamander, as speciation between the two species is incomplete.

The key argument for recognition of the West Virginia Spring Salamander as a distinct species is the co-occurrence of two distinct forms in General Davis Cave; that is, are there one or two diagnosable forms? Morphological evidence suggests that both larval and metamorphosed West Virginia Spring Salamanders are distinct from local Spring Salamander populations including individuals from General Davis Cave (Besharse and Holsinger 1977; Osbourn 2005). Limited genetic work also suggest West Virginia Spring Salamanders are distinct from Spring Salamanders, as *G. subterraneus* possessed six allozyme alleles not shared with *G. porphyriticus* individuals examined (J.H. Howard et al., unpubl. data). However, their results are

inconclusive because of small sample sizes. Unfortunately, until thorough genetic analyses are conducted on the *Gyrinophilus* inhabiting General Davis Cave, and larvae are successfully reared through metamorphosis, the taxonomic status of the West Virginia Spring Salamander cannot be supported or refuted.

Conservation status.—The decline of surface amphibian populations worldwide has received considerable attention in recent years and several factors have been implicated in declines, including habitat destruction and degradation, pollution, disease, and overcollection (Blaustein et al. 1997; Alford and Richards 1999; Semlitsch 2003). Concern has also been expressed for subterranean salamanders, as many species, such as the West Virginia Spring Salamander, are particularly susceptible to decline because of small, restricted distributions and small population sizes (Chippindale and Price 2005; Miller and Niemiller 2008). Although the population appears to be stable over the past 33 years and not in immediate danger of extinction, the West Virginia Spring Salamander is still of critical conservation concern because of its extremely restricted distribution (known only from a single cave system) and current threats to the cave system it resides in. Accordingly, the West Virginia Spring Salamander is listed as a species of concern by IUCN (2008. *op. cit.*), NatureServe (2009. *op. cit.*), and by the state of West Virginia, but the species is not afforded special protection by the United States Fish and Wildlife Service. In 2001 the Karst Waters Institute named the Greenbrier Valley where General Davis Cave is located as one of the top ten most endangered karst areas in the world (Tronvig and Belson 2001). Major impacts on the valley's caves include siltation, agricultural runoff, water contamination, and development. The Nature Conservancy owns an easement on the General Davis Cave system and title to the main entrance; however, the principal source of the cave stream in General Davis Cave (Sinks of the Run Cave) and the entire watershed remain unprotected. Furthermore, the landowner has proposed logging within the recharge basin of the cave system, which could jeopardize the integrity of the cave stream. Indeed, the integrity of the entire aquatic ecosystem in General Davis Cave is dependent upon the main surface feeder stream on Muddy Creek Mountain upstream of the cave system. Any significant changes in land use above the area where this stream enters Sinks of the Run Cave and ultimately feeds the main cave stream in General Davis Cave will impact the aquatic ecosystem and likely affect this unique salamander population. Protection of the land area upstream and surrounding the General Davis Cave system is just as critical, if not more so, than the protection of the cave interior itself. General Davis Cave is one of the most biologically significant

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caves in West Virginia and, in addition to the West Virginia Spring Salamander, harbors several endemic or rare species of invertebrates, and serves as a hibernaculum of the federally listed endangered Indiana Bat (*Myotis sodalis*) (West Virginia Department of Natural Resources, Natural Heritage Program, unpublished data). Although efforts have been made to purchase rights to some of the surrounding surface area, significant parts of the surface drainage area above General Davis Cave remain unprotected. It is clear what we must protect in order to prevent degradation of this and other biologically significant subterranean ecosystems. Regardless of the ultimate taxonomic designation of the West Virginia Spring Salamander, the unique population at General Davis Cave is deserving of every necessary protection to insure its persistence.

Acknowledgments.—Funding for this project was provided by the Department of Ecology and Evolutionary Biology at the University of Tennessee, the Department of Biology at Middle Tennessee State University, the Marshall University Graduate College Summer Research Grant (MSO), the American Museum of Natural History Theodore Roosevelt Memorial Fund (MLN), the American Society of Ichthyologists and Herpetologists Helen T. and F.M. Gaige Fund (MLN), the Cave Research Foundation Cave and Karst Grant-in-Aid (MLN), the West Virginia Association for Cave Studies (MSO), and the West Virginia Wildlife Diversity Program (MSO). We thank the Nature Conservancy in West Virginia for access into General Davis Cave. We are grateful to Casey Bartkus, Amy Cimarolli, Joe Greathouse, Stesha Pasachnik, Jennifer Wykle, Mark Watson, Bill Sutton, Zac Loughman, Lisa Smith, and William Balfour for assistance with fieldwork. We also thank Ray Semlitsch and R. Graham Reynolds for providing helpful comments on an earlier version of the manuscript. Fieldwork was conducted under authorization of the West Virginia Division of Natural Resources Nongame and Natural Heritage Program (permits issued to MLN, MSO, TKP, and JRH) and in accordance with animal care and use protocols (UTK IACUC # 1589-0507 to MLN).

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