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## SUBTERRANEAN REPRODUCTION OF THE SOUTHERN TWO-LINED SALAMANDER (*EURYCEA CIRRIGERA*) FROM SHORT MOUNTAIN, TENNESSEE

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**Abstract.**—The Southern Two-lined Salamander, *Eurycea cirrigera*, typically inhabits the margins of small, rocky streams, springs, and seeps in forested areas. The species is found only occasionally in subterranean habitats and, consequently, is considered a cave visitor (accidental or troglaxene). However, we discovered egg clutches in the deep cave zone of a subterranean stream during January and February 2005, indicating that some individuals are adapted to reproduce in caves. Eggs were attached singly to form monolayer masses on the undersurfaces of submerged rocks; females were found attending three of nine clutches. We determined the total number of eggs/clutch, mass surface area, egg and embryo size, and stage of development using digital images of each clutch. Embryo length correlated positively with developmental stage. Duration of embryonic period ranged from 35-42 days; consequently, eggs were laid from early to mid-January and larvae hatched from late February to early March. Adult males and females migrated into the cave to breed during late autumn. Males exited the cave after mating; whereas, females brooded their eggs and exited the cave only after eggs hatched. Larvae drifted downstream and out of the cave following heavy winter and early spring rains. Reproduction of this population is relatively early compared to epigeal populations and may be associated with the stable aquatic cave environment.

**Key Words.**—Cumberland Plateau; *Eurycea cirrigera*; Middle Tennessee; Southern Two-lined Salamander; Subterranean Reproduction

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### INTRODUCTION

*Eurycea* is the most speciose genus of spelrepine salamanders (Family Plethodontidae: Subfamily Spelerpinae) and has disparate centers of diversity in the Appalachian Highlands, Interior Highlands, and the Edwards Plateau of central Texas (Petranka 1998; Chippindale et al. 2000; Bonett and Chippindale 2004). Several species of *Eurycea* are associated with subterranean environments and many of them have developed cave-associated morphologies (Petranka 1998; Chippindale 2000; Chippindale et al. 2000; Hillis et al. 2001). Most of these species are aquatic paedomorphs that inhabit caves and springs along the Edwards Plateau of central Texas (Chippindale 2000; Chippindale et al. 2000; Hillis et al. 2001; Wiens et al. 2003). Several of the paedomorphic species (e.g., *E. tridentifera*, *E. troglodytes*, *E. latitans*, *E. rathbuni*, and *E. robusta*) are stygobites (i.e., obligate residents of subterranean waters); whereas, others (e.g., *E. neotenes*) are stygophiles (i.e., able to complete their life cycle in either hypogean or epigeal waters). Some paedomorphic species on the Edwards Plateau (e.g., *E. nana* and *E. pterophila*) are not reliant on subterranean waters, but may occasionally enter and spend a part of their life cycle in this environment. These latter species are troglaxenes or accidental cavernicoles.

Fewer cave-associated species of *Eurycea* are known from the Appalachian and Interior Highlands. The paedomorphic *Eurycea wallacei* (= *Haideotriton wallacei*) is the only true stygobite, although *E. lucifuga* and *E. longicauda* are troglaphiles. Additionally, the Grotto Salamander, *E. spelaea*, has an unusual life history that includes a cavernicolous phase. Larval *E. spelaea* have well-developed, fully functional, eyes and live in small springs outside of caves; whereas, in association with metamorphosis, the eyes become non-functional and transformed individuals migrate into subterranean habitats. Despite its unusual life history, *Eurycea spelaea* is generally classified as a troglabite (Weber 2000).

Although members of the wide-ranging Northern Two-Lined Salamander species complex (*E. bislineata*, *E. cirrigera*, *E. wilderae*, *E. aquatica*, and *E. junaluska*) occasionally occur in caves, species within this complex lack the morphological and behavioral characteristics associated with subterranean life, and are not generally associated with subterranean habitats. The absence of cave-associated populations of members of this complex is particularly puzzling because of the abundance of caves within the complex's range. For instance, the Southern Two-lined Salamander, *E. cirrigera*, has a broad distribution throughout much of the karst regions of the Interior Low Plateau and Appalachian Highlands of the



**FIGURE 1.** Chronology of development of Southern Two-lined Salamander (*Eurycea cirrigera*) clutch no. 1 from a privately-owned cave on Short Mountain, Cannon Co., Tennessee. Eggs at (A) Harrison stage 11 on 26 January 2005, (B) Harrison stage 31 on 09 February 2005, (C) Harrison stage 42 on 26 February 2005, and (D) a hatchling without yolk collected on 26 February 2005.

southeastern United States northward into Illinois, Indiana, and southern Ohio (Pauley and Watson 2005). The species is common throughout its range and typically inhabits the margins of small, rocky streams and seeps in forested areas, but is common also in and around springs and spring runs (Mount 1975; Petranka 1998). Oviposition typically occurs in flowing water where eggs are attached singly in a monolayer to the undersurfaces of submerged rocks or logs that are embedded in the stream bottom (Baumann and Huels 1982; Martof et al. 1980; Mount 1975; Petranka 1998). Although Southern Two-lined Salamanders occasionally are reported from subterranean environments (West Virginia, Carey 1973; Pauley 1993; Osbourn 2005; Mississippi, Carey 1982; Himes et al. 2004), neither the significance of their subterranean occurrence, nor their use of cave systems for reproduction has been discussed. Here, we describe several reproductive parameters (timing of migration by adults, timing of oviposition, incubation period, clutch size, and size at hatching) of a population of the Southern Two-lined Salamander in middle Tennessee that uses a subterranean stream for reproduction.

#### MATERIALS AND METHODS

**Study Area.**—The population we studied inhabited a small stream that flowed from a privately-owned cave on the southwest slope of Short Mountain, near the head of Mountain Creek in Cannon County, Tennessee. Short Mountain is an outlier of the Cumberland Plateau and is located on the western margin of the Eastern Highland Rim. The cave developed in St. Louis Limestone at an elevation of 384 m. The cave passage averaged 1 m high and 2–3 m wide, and a shallow (< 0.25 m) out-flowing stream emerged from breakdown just below the cave entrance. The stream meandered through the passage and was easily followed upstream for 91 m until a 9-m waterfall was encountered in a 12 m high room; the passage and stream continued above the waterfall. The substrate of the cave stream consisted of a mixture of sand, cobble, and bedrock littered with various-sized flat, limestone rocks. The twilight zone extended ca. 20 m into the cave.

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**TABLE 1.** Number of eggs per clutch, developmental stage, clutch surface area, and distance from cave entrance for nine Southern Two-lined Salamander (*Eurycea cirrigera*) clutches observed during three visits (26 Jan, 09 Feb, and 26 Feb 2005) at a privately-owned cave on Short Mountain, Cannon County, Tennessee. Numbers to the left of parentheses are the number of eggs observed in the clutch. Numbers within parentheses are the stages of development according to Harrison (1969); HT = eggs in clutch hatched. Clutches 1, 2, 4, 5 were observed more than once before hatching.

Clutch	Distance from Entrance (m)	Clutch Surface Area (mm <sup>2</sup> )	26 Jan	09 Feb	26 Feb
1	46	1408	61 (11) <sup>1</sup>	61 (31) <sup>1</sup>	60 (42)
2	53	1440	56 (26) <sup>1</sup>	53 (40) <sup>1</sup>	HT
3	56	1400	58 (29)	-	-
4	59	1443	32 (37)	31 (43)	HT
5	64	986	61 (10)	46 (29)	HT
6	38	1426	54 (38)	HT	HT
7	91	1080	-	65 (19)	-
8	38	2183	-	-	71 (21) <sup>1</sup>
9	61	1512	-	-	38 (38)

<sup>1</sup>Adult female found in attendance of clutch.

**Cave Surveys.**—On 26 January 2005, we searched the cave stream for aquatic salamanders as part of a project to determine the status and distribution of the Tennessee Cave Salamander (*Gyrinophilus pallescens*). We examined the stream bottom and carefully overturned rocks within pool, run, and riffle habitats. We returned rocks to their original positions to minimize habitat disturbance. During this initial survey, we found clutches of Southern Two-lined Salamanders, some with attending females, on the undersurfaces of submerged rocks that were embedded in the stream bottom. We marked rocks with attached eggs to aid in relocation. We conducted subsequent searches to locate additional clutches and to check the status of previously discovered clutches on 09 February and 26 February 2005. To determine the extent to which larvae and adult utilized the cave, we also searched the stream on 15 September, 15 October, 29 October, 2 December, and 20 December 2005.

We also searched 40 epigeal streams within the Inner Nashville Basin of adjacent Rutherford County, from 16 February to 14 April 2005, to determine timing of oviposition of epigeal populations of Southern Two-lined Salamanders within middle Tennessee. We searched streams ca. 100 m upstream and downstream from county road crossings. We carefully lifted rocks within both pool and riffle habitat to locate eggs or larvae, and rocks and other cover objects adjacent to streams to locate adults. Lifted rocks and other cover objects were returned to their original positions to minimize habitat disturbance. Additionally, during May 2006, we searched for salamanders in an epigeal stream in Coffee County, Tennessee.

Upon discovery, we photographed clutches with a Sony Cybershot<sup>®</sup> DSC-F707 digital camera. A metric ruler was positioned adjacent to each clutch prior to photography. We analyzed the digital image with Photoshop<sup>®</sup> 6.0 (Adobe Systems, Inc., San Jose, California, USA) to determine the total number of eggs per clutch, clutch surface area, stage of development, size of ova (mean diameter), and size of embryos (mean total length). Embryonic stage of development within each clutch was determined according to Harrison (1969). Little variation in stage of development was observed within clutches and the stage reported is the mode of each clutch. We reported all measurements as the mean ± one standard deviation unless indicated otherwise. We captured, measured, determined mass, and released females attending clutches. Water temperature and other cave fauna were recorded during each visit to the cave.

We statistically analyzed the differences between means for number of eggs/clutch with and without females in attendance using a Student's *t*-test. We used standard non-linear regression (logarithmic, exponential, and second order polynomial) curve fitting to describe correlations between embryo length and Harrison stage of development. We conducted all statistical analyses in SAS 9.1.3 (SAS Institute 2004) and accepted statistical significance at  $\alpha < 0.05$ .

## RESULTS

We observed and photographed nine clutches during the first three cave visits (Table 1). Clutches consisted of eggs that were attached singly in a monolayer on the undersurfaces of rocks submerged in the cave stream (Fig. 1A). We found six clutches on 26 January 2005, one clutch on 09 February 2005, and two clutches on 26 February 2005. All clutches occurred well beyond the

**TABLE 2.** Number of eggs per clutch, developmental stage, and clutch surface area of four Southern Two-lined Salamander (*Eurycea cirrigera*) clutches observed in two epigeal streams in the Inner Nashville Basin of Rutherford Co., Tennessee. Females were found in attendance of all clutches observed (A = Tributary to the Middle Fork Stones River at Broiles Road, B = Big Springs Creek at Big Springs).

Clutch	Date	Locality	Clutch Surface Area (mm <sup>2</sup> )	Number of Eggs	Stage of Development
1	18 Feb 2005	A	991	79	21
2	18 Feb 2005	A	1440	53	23
3	18 Feb 2005	A	345	29	13
4	23 Feb 2005	B	483	51	9

twilight zone of the cave, within the deep cave zone (mean distance from cave entrance =  $56.4 \pm 16.2$  m). Three females attended individual clutches (snout-vent length [SVL] =  $43.7 \pm 2.5$  mm, total length [TL] =  $94.7 \pm 8.7$  mm, wet mass  $\pm 1$  SD =  $1.7 \pm 0.5$  g). Stream water temperature varied little during the January and February searches ( $12.8 \pm 0.3^\circ\text{C}$ ).

Number of eggs/clutch (based on egg count of first observation of clutches located more than once) averaged  $55.1 \pm 12.5$  eggs (range 32-71; Table 1). Number of eggs/clutch did not differ significantly between clutches with and without females in attendance ( $t = 1.341$ ,  $df = 7$ ,  $P = 0.2235$ ). Clutch surface area averaged  $1430.9 \pm 334.5$  mm<sup>2</sup>, but the surface area of clutches discovered more than once increased over time. Embryo length also increased over time and as stage of development increased (Fig. 2). Embryo length was  $2.7 \pm 0.2$  mm (range 2.0-3.0,  $n = 122$ ) for all clutches before Harrison stage 13. After Harrison stage 39, embryo length for all clutches was  $12.0 \pm 1.4$  mm (range 9.0-16.0,  $n = 121$ ). Embryo length of each clutch was correlated with stage of development (Fig. 2).

We observed four clutches more than once, and two clutches were not relocated during this study (Table 1). Hatching occurred by 26 February 2005 in four clutches (Table 1, Fig. 1). Egg loss occurred in all clutches discovered more than once. Based on stage and rate of development at  $13^\circ\text{C}$ , we estimated that oviposition occurred from 06 January – 21 January and hatching from 17 February – 01 March. We observed hatchlings in the cave stream during the 26 February 2005 search. Clutch #8, with a female in attendance, was found only during the 26 February 2005 search and the associated embryos were Harrison stage 21 (Table 1). Although we observed this clutch only once, the stage and rate of embryonic development in other clutches suggest oviposition occurred during mid-February and that hatching followed during mid- to late March.

In addition to finding adult females brooding clutches, other females, both gravid and spent, were observed in the cave stream during the January and February searches. Neither males nor juveniles occurred in the cave during January or February. Also, neither adults nor larvae were found during the September or October searches of the cave. However, we found three gravid females (two in the twilight zone, one in the deep cave zone) on 2 December 2005, and two males and four gravid females in the cave stream on 20 December 2005. A single clutch was found on 28 January 2006.

We found four clutches in two epigeal streams between 18 February and 23 February 2005 in the Inner Nashville Basin of Rutherford County, and one clutch with late stage embryos on 3 May 2006 in the Eastern Highland Rim of

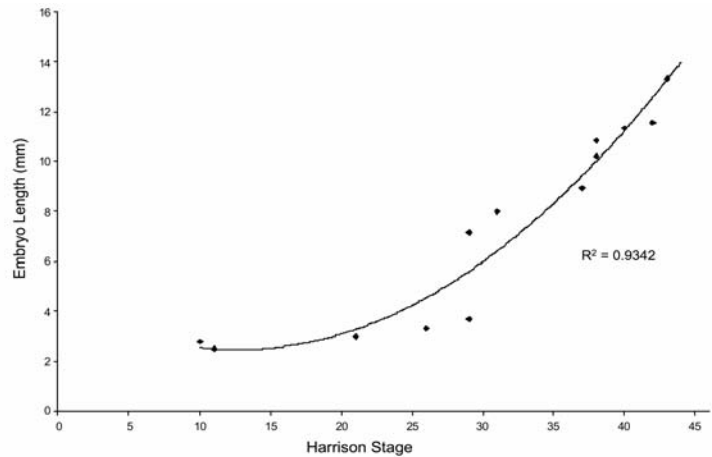


FIGURE 2. Relationship between mean embryo length (mm) and Harrison (1969) stage for nine Southern Two-lined Salamander (*Eurycea cirrigera*) clutches discovered in a privately-owned cave on Short Mountain, Cannon County, Tennessee.

Coffee County (Table 2). All epigeal clutches were attached to the undersurface of flat, submerged rocks with females in attendance.

## DISCUSSION

This study documents the use of a cave stream for reproduction by Southern Two-lined Salamanders, a member of the *E. bislineata* species complex. Although there are reports of adult and larval Southern Two-lined Salamanders from caves (Carey 1973; Carey 1982; Pauley 1993; Himes et al. 2004; Osbourn 2005), to our knowledge, this is the first report of clutches found in a cave. The absence of females outside the breeding period suggests that females migrate (up to 91 m) into the cave to oviposit during late-autumn or early winter and exit sometime after their eggs hatch in late winter or early spring. Larvae presumably drift downstream passively and exit the cave during heavy spring rains. Previous reports document downstream displacement of larvae (Stoneburner 1978; Bruce 1986; Petranka 1998).

Physical parameters, such as # of eggs/clutch, clutch surface area, ova diameter, and hatchling size of clutches from this subterranean breeding population are similar to those of populations reproducing in epigeal environments throughout the range of the species (Duellman and Wood 1954; Barbour 1971; Mount 1975; Baumann and Huels 1982; Dundee and Rossman 1989; Brophy 1995; Pauley and Watson 2005). The duration of the embryonic period depends on the water temperature and is highly variable; reports in the literature range from 4-10 weeks (Smith 1961; Petranka 1998; Pauley and Watson 2005). Although oviposition occurred relatively early at the Short Mountain cave, the relatively rapid development of the eggs and

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embryos to hatching (5-6 weeks) implies that water temperature in the subterranean environment was relatively high in comparison with water temperatures in epigeal habitats during this time period.

Southern Two-lined Salamanders from our main study site oviposit between early-January and mid-February. Based on stages of development of clutches observed in Rutherford County, Tennessee, epigeal populations oviposit between early-February and late February. However, during May 2006 one of us (BTM) observed a late stage clutch in an epigeal stream in Coffee County, Tennessee, suggesting oviposition may occur as late as mid-April in middle Tennessee. Subterranean oviposition in middle Tennessee is earlier than that reported from other regions: April to early May in Indiana (Sever 1988; Minton 2001); April and May in Lafayette Co., Mississippi (Marshall 1996); April in eastern Tennessee (King 1939); late January to mid-April on the Virginia Coastal Plain (Wood 1953); and late March to early April in southwest West Virginia (Brophy 1995). Early oviposition by the cave-breeding population may be associated with the more stable aquatic cave environment compared to epigeal streams during late winter/early spring in middle Tennessee. Eggs and larvae that develop underground may have higher survivorship compared to those that develop at the surface because of reduced predation and reduced exposure to harsh and variable environmental conditions. These hypotheses warrant further research.

Although all clutches observed more than once suffered egg mortality, those with brooding females lost fewer eggs (average of two eggs) than those without brooding females (average of eight eggs). However, egg mortality may be greater than observed as several clutches were not discovered during early development. We found two clutches without brooding females in mid-development (3 and 7) that suffered complete mortality. Few reports of predation on eggs and larvae of Southern Two-lined Salamanders exist in the literature. Known predators include Mosquitofish (*Gambusia sp.*) (Wood 1953), Brook Charr (*Salvelinus fontinalis*) (Resetarits 1991), crayfish (*Cambarus sp.*) (Resetarits 1991), and Spring Salamander (*Gyrinophilus porphyriticus*) larvae (Gustafson 1994). Although no fish occur in the cave stream, crayfish (*Cambarus sp.*), larval *G. porphyriticus*, and both adult and larval Red Salamanders (*Pseudotriton ruber*) reside in the cave stream and may prey on eggs and larvae of Southern Two-lined Salamanders. Stygobitic planarians (*Sphalloplana sp.*) also occur on the undersurfaces of submerged rocks in the vicinity of clutches. Although data regarding the diet of subterranean planarians are limited, epigeal forms are known predators of salamander eggs and larvae (Petranka 1998).

Based on the few records of larvae or adults observed in caves before this study, Southern Two-lined Salamanders have been considered an occasional troglone or accidental inhabitant of caves (Barr 1963; Osbourn 2005).

Although most Southern Two-lined Salamander populations range-wide only occasionally or accidentally enter subterranean habitats, female Southern Two-lined Salamanders from Short Mountain intentionally enter the deep cave zone to oviposit and brood their eggs. Following the classification of Barr (1963), we consider this unique population to be troglone. Historically, the range-wide tendencies of a species drive the proper cave-associated classification, rather than the tendencies of a local population. We agree with Poly and Boucher's (1996) assessment that unique populations of epigeal species capable of hypogean survival 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." Some epigeal species may be preadapted for subterranean existence (Dearolf 1956; Greenwood 1967; Poulson 1963; Poly and Boucher 1996), and research on such species may potentially increase our understanding on the origins of troglone organisms (Poly and Boucher 1996).

*Acknowledgements.*—We thank the private landowners for allowing access to the cave on their property. For assistance in the field, we thank J. M. Todd, B. Glorioso, T. Lee, J. H. Miller, J. A. Miller, and R. Timmons. Funding for this project was provided (in part) by the Tennessee Wildlife Resources Agency and the Department of Biology at Middle Tennessee State University. Field work was conducted under authorization of the Tennessee Wildlife Resources Agency (Permit Nos. 1415 and 1450).

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