

## OBSERVATIONS ON EGG-LAYING BEHAVIOR AND INTERACTIONS AMONG ATTENDING FEMALE RED SALAMANDERS (*PSEUDOTRITON RUBER*) WITH COMMENTS ON THE USE OF CAVES BY THIS SPECIES

BRIAN T. MILLER<sup>1,3</sup>, MATTHEW L. NIEMILLER<sup>2</sup>, AND R. GRAHAM REYNOLDS<sup>2</sup>

<sup>1</sup>Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, USA

<sup>2</sup>Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA

<sup>3</sup>Corresponding author e-mail: [bmiller@mtsu.edu](mailto:bmiller@mtsu.edu)

**Abstract.**—Spelerpine salamanders are generalized plethodontids that typically exhibit biphasic life histories (aquatic eggs and larvae metamorphosing into semi-aquatic or terrestrial adults). However, most species of *Eurycea* and all species of *Gyrinophilus* are obligate or facultative cavernicoles with cave-associated morphologies and behaviors. The trend toward subterranean inhabitation is not recognized in any species of remaining spelerpine genera (*Stereochilus marginatus*, *Pseudotriton ruber*, and *P. montanus*). Nevertheless, *P. ruber* is found often, and is known to nest, in caves, at least within the cave-rich Cumberland Plateau physiographic province of the eastern United States. We studied the salamander community in a privately-owned cave on Short Mountain, Cannon County, Tennessee, USA to assess use and potential importance of subterranean habitat to *P. ruber*. During 10 surveys conducted from 26 January 2005 to 30 September 2006, we found 270 salamanders representing seven species: *Plethodon glutinosus* (n = 1), *Desmognathus conanti* (n = 1), *G. porphyriticus* (n = 1), *E. lucifuga* (n = 35), *E. longicauda* (n = 38), *E. cirrigera* (n = 29), and *P. ruber* (n = 165). *Pseudotriton ruber* was the most commonly encountered salamander (61% of observations), and the only species encountered during each survey. Furthermore, we found nests of *P. ruber* in lotic and lentic environments, observed a female oviposit, and witnessed attending females defend their eggs from a conspecific female that momentarily abandoned her nest to attempt conspecific oophagy. Based on past reports and our observations, we argue that *P. ruber* is an unrecognized facultative cavernicole.

**Key Words.**—cave-dwelling; Cumberland Plateau; nest defense; oophagy; *Pseudotriton ruber*; Red Salamander; Spelerpinae; subterranean reproduction

### INTRODUCTION

The Plethodontidae is a large, monophyletic assemblage of lungless salamanders found largely in the Americas. Traditional taxonomy, based largely on morphology, splits the Plethodontidae into two subfamilies, the Desmognathinae and Plethodontinae, with the latter subfamily further subdivided into three tribes: Hemidactyliini, Plethodontini, and Bolitoglossini (Wake 1966). However, recent molecular analyses do not support traditional taxonomy and the systematics of the Plethodontidae is in a state of turmoil (Mueller et al. 2004; Chippindale et al. 2004). Chippindale et al. (2004) suggest resurrection of the subfamily Spelerpinae, a monophyletic clade comprising four genera and about 34 species: *Eurycea* (27 species), *Pseudotriton* (2 species), *Stereochilus* (1 species), and *Gyrinophilus* (4 species). The life history of all spelerpine species includes aquatic eggs and a prominent larval period (Petranka 1998). Most species of *Eurycea* (at least 15) and three of the four species of *Gyrinophilus* are associated with subterranean environments. Furthermore, some populations of the fourth species of *Gyrinophilus* (*G. porphyriticus*) are also associated with caves (Miller and Niemiller 2008). Accordingly, many

species of these two genera have developed cave-associated morphologies and behaviors, such as paedomorphosis, decreased pigmentation, reduced eye size, and increased number of lateralis receptors (Petranka 1998; Chippindale 2000; Chippindale et al. 2000; Hillis et al. 2001). Most of these species are obligate (e.g., *E. tridentifera*, *E. troglodytes*, *E. latitans*, *E. rathbuni*, *E. robusta*, *E. wallacei*, *G. palleucus*, *G. gulolineatus*) or facultative (e.g., *E. neotenes*) aquatic, cavernicolous paedomorphs (Chippindale 2000; Chippindale et al. 2000; Hillis et al. 2001). However, a few species associated with subterranean habitats do exhibit a biphasic life history and are either obligate (*E. spelaea*, *G. subterraneus*) or facultative (*E. longicauda*, *E. lucifuga*) cavernicoles (Hutchison 1958; Brandon 1971; Besharse and Holsinger 1977; Fenolio et al. 2006).

In contrast to *Gyrinophilus* and *Eurycea*, paedomorphosis has not evolved within *Stereochilus* or *Pseudotriton* and none of the species within these two genera are obligate or facultative cavernicoles. However, the Red Salamander (*P. ruber*), a robust spelerpine that inhabits first- and second-order streams (Petranka 1998) occasionally occurs in caves in Alabama (Knight 1969), Georgia (Buhlmann 2001), Mississippi (Brode 1958), Tennessee (Barr 1949), and West Virginia

(Green and Brant 1966; Carey 1973; and Osbourn 2005). Also, other reports of egg masses located deep within springs (Bishop 1925, 1941; Fowler 1962) and in cave streams (Miller and Niemiller 2005; Niemiller et al. 2006) suggest that reproduction is at least occasionally and perhaps frequently associated with subterranean habitats. Here, we describe relative abundance, nesting location, oviposition behavior, and nest defense from oophagous conspecifics in a population of cave-dwelling Red Salamanders. Moreover, we suggest that this species, although lacking obvious morphological characters associated with subterranean life, is nonetheless adapted to live a part of its life in caves and should, therefore, be classified as a troglophile or facultative cave-dwelling species.

**MATERIALS AND METHODS**

**Study area.**—As part of a larger project examining amphibian cave communities, we conducted surveys from January 2005 through September 2006 in a privately-owned cave on the southwest slope of Short Mountain, near the head of Mountain Creek in Cannon County, Tennessee, USA. Short Mountain is an outlier of the Cumberland Plateau and is located on the western margin of the Eastern Highland Rim. The cave is developed in St. Louis Limestone at an elevation of 384 m. The cave passage averages 1 m high and 2-3 m wide, and a shallow (< 0.25 m) out-flowing stream emerges from breakdown just below the cave entrance. The stream meanders through the passage and is easily accessible upstream for 91 m until a 9 m waterfall is encountered in a 12 m high room. The passage and stream continue on above the waterfall. The waterfall

cascades into a small pool surrounded by a jumble of breakdown boulders. Although the walls of the room are essentially vertical layered limestone, the lower north wall is flowstone and supports several small, shallow rimstone pools. The substrate of the cave stream is a mixture of sand, cobble, and bedrock littered with various-sized flat, limestone rocks. The twilight zone extends ca. 20 m into the cave.

We searched the cave for amphibians on 10 occasions (26 January, 09 February, 26 February, 09 September, 15 October, 29 October, 02 December, and 20 December in 2005, and 28 January and 30 September in 2006) by slowly walking along or wading through the cave stream and thoroughly scanning the streambed with the beams of our headlights. We carefully lifted rocks and small cobble and sifted through detritus and other surface debris washed into the cave under which amphibians might seek refuge. We returned lifted rocks and other cover objects to their original positions to minimize habitat disturbance. We searched within the twilight zone, the walls of the cave and crevices within the walls. However, we did not search cave walls and crevices within the aphotic zone unless water bodies within the cave were within 2 m of a cave wall. We made a concerted effort to capture each salamander encountered with small bait nets. We recorded life-history stage (larva, juvenile, and adult), habitat, and approximate location within the cave of each salamander encountered.

**RESULTS**

We found 270 salamanders during the surveys (Table 1). The salamander community of the cave

**TABLE 1.** Survey dates and number of individuals<sup>1</sup> of each salamander species observed within a privately-owned cave located on Short Mountain, Cannon County, Tennessee, USA in 2005 and 2006. The numbers are metamorphosed individuals / larvae.

Species	Survey Dates										Total
	2005								2006		
	26 Jan	15 Feb	26 Feb	9 Sept	15 Oct	29 Oct	2 Dec	20 Dec	28 Jan	30 Sept	
<i>Desmognathus conanti</i>	-	-	-	-	-	-	-	0/1	-	-	0/1
<i>Plethodon glutinosus</i>	-	-	-	-	1/0	-	-	-	-	-	1/0
<i>Gyrinophilus porphyriticus</i>	0/1	-	-	-	-	-	-	-	-	-	0/1
<i>Eurycea cirrigera</i>	4/0	3/0	5/7	-	-	-	3/0	6/0	1/0	-	22/7
<i>Eurycea lucifuga</i>	1/0	0/2	2/0	3/0	13/3	2/0	-	-	0/1	7/1	28/7
<i>Eurycea longicauda</i>	4/0	-	2/0	7/0	10/1	3/0	1/0	-	-	10/0	37/1
<i>Pseudotriton ruber</i>	2/7	0/2	1/19	4/6	15/9	8/7	4/18	3/30	1/9	16/4	54/111
Total	11/8	3/4	10/26	14/6	39/13	13/7	8/18	9/31	2/10	33/5	270

comprised seven species. Of these, five spelepine species accounted for 99% of all observations (Table 1). *Pseudotriton ruber* was the most commonly encountered salamander and the only species found during every search of the cave (Table 1). We found adult *P. ruber* during nine of the surveys and they typically occurred beyond the twilight zone.

**Nesting observations.**—During 2005, we found two freshly laid *P. ruber* egg masses on 15 September, an egg mass with early stage larvae on 15 October, and three more masses with late stage larvae on 2 December. Four of the six egg masses discovered during 2005 occurred in flowing water, attached either to the undersurface of rocks or to gravel beneath rocks in the shallow stream. By contrast, the other two egg masses occurred in small, shallow rimstone pools associated

with flowstone (Fig. 1). Regardless of the location, one adult attended each nest and, as evidenced by hatchlings within nest sites, eggs hatched during late November or early December. For example, 15 of the 18 larvae found on 2 December and 25 of the 30 larvae found on 20 December were hatchlings yolk-laden and in their nests (Fig. 2). During 2006, we found five freshly laid egg masses on 30 September. Each nest was in a small, shallow rimstone pool and was attended by a single adult.

**Oviposition observations.**—While counting *P. ruber* eggs in one nest on 30 September 2006, we witnessed oviposition behavior by the attending female. During our observations, which lasted approximately 10 min, the female attached two eggs to the margin of a rimstone pool. Oviposition started when the attending female



**FIGURE 1.** A female *Pseudotriton ruber* guarding eggs deposited along the margin of a small rimstone pool located in the dark zone of a cave on Short Mountain in Cannon County, Tennessee, USA. Based on the early developmental stage of the embryos, the eggs were recently laid and the clutch may not have been complete. Photograph by Matthew L. Niemiller on 15 October 2005.



**FIGURE 2.** *Pseudotriton ruber* hatchlings in a rimstone pool located in the dark zone of a cave on Short Mountain in Cannon County, Tennessee, USA. The hatchlings were laden with yolk, but were no longer attended by a female. Photograph by Brad Glorioso on 2 December 2005.



**FIGURE 3.** A female *Pseudotriton ruber* positioned on her back in a small rimstone pool in preparation for oviposition. The rimstone pool is in the dark zone of a cave on Short Mountain in Cannon County, Tennessee, USA. Photograph by R. Graham Reynolds on 30 September 2006.



**FIGURE 4.** A female *Pseudotriton ruber* in the act of ovipositing an egg to the upper margin of a small rimstone pool located in the dark zone of a cave on Short Mountain in Cannon County, Tennessee, USA. Photograph by R. Graham Reynolds on 30 September 2006.

attempted to turn upside down. To accomplish this, she began to twist her body rapidly, which rolled her onto her back; however, she promptly righted herself. She repeated this activity thrice, and remained on her back during the fourth attempt. While so positioned, she arched her back and thrust her cloaca upward; her tail was used as a brace to position her body off of the substrate and she utilized her limbs to grip the walls of the pool and to stabilize her body position (Fig. 3). She had noticeably contracted caudal muscles and a tightened abdominal wall. She remained in this position for about eight min, at which time she extruded an egg from her cloaca and attached it to the upper edge of the rimstone pool (Fig. 4). Following oviposition, the female righted herself rapidly with a lashing of her tail and inadvertently dislodged several previously attached eggs, which settled to the bottom of the shallow pool.

**Conspecific Aggressive Behavior.**—During the 30 September 2006 survey, we observed conspecific interactions of attending *P. ruber* females nesting in close proximity to each other. Specifically, we observed repeated attempts at oophagy by an attending female as she invaded three adjacent conspecific nests. These repeated attempts occurred over a period of 20 min. Shortly after watching the female oviposit, attending female #2 abandoned her clutch and tapped the ground with her snout as she walked along the nest-bearing rimstone. She walked directly into an adjacent small rimstone pool that contained the nest of attending female #3. The intruding female attempted to eat an egg, but the resident female immediately rebuffed her efforts. The two salamanders thrashed about in the small pool and attempted to bite each other. The intruding female exited the pool and once again tapped the ground with her snout as she walked along the rimstone. Within a minute she proceeded to the bottom of the rimstone, approximately 0.5 m from the nest she had previously attacked, and entered into the talus. We lost sight of the female for about 90 seconds, but she then exited the talus and walked back up the rimstone. She continued to tap the substrate with her snout, and headed directly into another small pool occupied by attending female (#4). She attempted to consume an egg, but was once again rebuffed physically by the attending female (#4). The intruding female (#2) exited the pool and the attending female (#4) followed. The attending female (#4) only came part way out of the nest pool and then returned to her eggs. The intruding female (#2) tapped the ground with her snout, relocated, and re-entered her nest.

## DISCUSSION

As with most secretive spelerpine salamanders, relatively little information exists about the reproductive habits or behaviors of *P. ruber* (Petranka 1998;

Hunsinger 2005). Although rarely discovered, reports of *P. ruber* nests generally occur in lotic habitats, either springs or cave streams where water flows over the eggs (Bishop 1925, 1941; Fowler 1962; Miller and Niemiller 2005; Niemiller et al. 2006; this study). However, our observations indicate that *P. ruber* also utilize lentic habitats for oviposition.

Although neither Bishop (1925, 1941) nor Fowler (1962) reported adults attending nests, Petranka (1998) suggested that females brood their eggs until hatching. He based this on the observation that females disappeared from surface sites during autumn and remained rare until hatchlings emerged in late autumn or early winter. In contrast to earlier reports, there are several recent observations of nests with attending adult *P. ruber* (Miller and Niemiller 2005; Niemiller et al. 2006). However, multiple individuals occurred with nests in these latter reports, obfuscating the role of attending adults. Single adults attended eggs in each of the 10 nests we found in the cave during 2005 and 2006. These observations, coupled with those of oviposition and nest defense, indicate that each adult in attendance is a female actively guarding her eggs. The lack of adult attendance in earlier reports is puzzling. Possibly, the individuals who discovered the nests overlooked attending females, or perhaps these were abandoned nests. We also are uncertain what significance to attribute to the presence of two adults in attendance of eggs (Miller and Niemiller 2005) or to aggregations of nests and adults (Niemiller et al. 2006). One possible explanation is that specific nest site attributes limit suitable sites and force *P. ruber* to cluster and, at least occasionally, oviposit under the same rock or within neighboring rimstone pools.

Although the functions of adult attendance are poorly understood or documented for most salamander species, a considerable body of evidence indicates that protection from predators, including conspecific females, is important (Crump 1995; Nussbaum 2003). Much of this evidence comes from observations of attending adults from diverse lineages that actively defend their nests from intruders: Cryptobranchidae; *Andrias japonicus* (Kawamichi and Ueda 1998); Ambystomatidae; *Ambystoma opacum* (Croshaw and Scott 2005); Plethodontidae; *Aneides lugubris* (Ritter 1903); *Desmognathus ochrophaeus* (Forester 1983); and *Plethodon cinereus* (Highton and Savage 1961; Bachman 1984). Additional evidence stems from the observation that loss of eggs occurs rapidly in unattended clutches, e.g., *Aneides aeneus* (Gordon 1952). Our observations provide additional evidence that attending female salamanders in general, and *P. ruber* in particular, actively guard their eggs from intruding conspecific females.

One suggested cost of female attendance is the reduction in foraging rates and commensurate decrease

in food intake (Organ 1961; Montague and Poiniski 1978; Krzysik 1980; Hom 1987; Juterbock 1987). However, relatively little information exists on the foraging behavior of attending females, and the information that exists is contradictory. For example, Brode (1961) reported that attending female *Desmognathus fuscus* occasionally leave their nests during the night, presumably to feed, and Tilley (1972) found that little weight loss occurred in female *D. ochrophaeus* while attending eggs. Similarly, Kaplan and Crump (1978) did not find an energetic cost to reduced foraging in attending female *A. opacum*. Our observations indicate that attending female *P. ruber* may temporarily abandon their eggs to forage, at least shortly after oviposition. Although we do not know how important such foraging bouts are to attending females, the location of nests close together may encourage conspecific oophagy.

The feeding mechanism used by *P. ruber* in complete darkness is unknown, as is the diet of cave-dwelling individuals and attending females. *Pseudotriton ruber* protract their tongue to seize prey on land, but metamorphosed individuals use jaw prehension to capture prey in water (Deban and Marks 2002). Metamorphosed salamanders often rely on visual cues to orient their snout prior to tongue projection (Deban et al. 2007), but such orientation is not possible in the aphotic zone. Because *P. ruber* often oviposits in total cave darkness (Miller and Niemiller 2005; Niemiller et al. 2006; this study), foraging females must use chemical or tactile cues, rather than visual cues, to locate prey. Regardless, conspecific eggs may provide oophagous females with nutrients in an environment otherwise difficult for metamorphosed salamanders to locate and capture prey. We do not know what, if any, significance the relatively close spacing of *P. ruber* nests has with regard to foraging and parental care. However, this phenomenon appears common for *P. ruber* (Niemiller et al. 2006) and other plethodontid species (e.g., *Desmognathus ocoee*: Pope 1924; Martof and Rose 1963; Forester 1977).

The ability of nesting females to find their nest after displacement or foraging bouts exists in several species of desmognathine salamanders (e.g., *D. fuscus*, Brode 1961; Dennis 1962; *D. ocoee*, Forester 1979), but not for spelerypine species. Desmognathine salamanders reportedly use chemical cues to locate their nests (Forester et al. 1983; Forester 1986) and to recognize their eggs (Forester 1986). In plethodontid salamanders, nose-tapping delivers odorants from the moist substrate through the nasolabial grooves to the vomeronasal organs (Brown 1968; Dawley and Bass 1988). Our observations of nose-tapping provide further evidence that attending *P. ruber* females use chemical cues to relocate their nest when returning from foraging bouts,

locate nests of conspecifics, and distinguish their eggs from those of conspecifics.

The placement of eggs on the undersurface of rocks or other objects occurs in several lineages of lotic-nesting salamanders, including *Ambystoma barbouri* (Petranka 1984), *Gyrinophilus porphyriticus* (Bishop 1941), *Necturus maculosus* (Bishop 1941; Trauth et al. 2004), and *P. ruber* (Miller and Niemiller 2005; Niemiller et al. 2006). Presumably, females flip onto their backs to oviposit on the undersurface of objects, but descriptions of this behavior exists for relatively few species (e.g., *A. barbouri*, Petranka 1984; *Eurycea bislineata*, Noble and Richards 1932; *G. porphyriticus*, Bishop 1941; *N. maculosus*, Bishop 1941). The oviposition behavior of *P. ruber* is similar to that of other spelerypine salamanders including *Stereochilus marginatus* and *E. bislineata*. In each of these species, the female braces herself with her front and hind limbs, arches her tail and back, and protrudes her cloacal lips and then places them against the substrate upon which the eggs are to be laid. Noble and Richards (1932) report that *S. marginatus* turn over on their backs before egg-laying, even when ovipositing on vegetation that could be approached from any direction. Similarly, *P. ruber* flip onto their backs to oviposit in the rimstone pools even though ovipositing in this location did not necessitate such behavior. Ovipositing while on the back appears to be the ancestral condition for spelerypine salamanders and it appears that all of these species retain it. This behavior is used by most individuals, even in those situations where eggs are no longer deposited on the undersurface of objects in flowing water.

*Pseudotriton ruber* occur in small headwater streams, seepages, and spring-fed bogs (Bishop 1941; Pflingsten 1989; Petranka 1998; Means 2000; Hunsinger 2005). Although these relatively cool aquatic habitats represent locations where subterranean streams resurge to the surface, the affiliation of *P. ruber* beyond the resurgence location and into the subterranean streams has been heretofore underestimated or overlooked. Consequently, most authors omit *P. ruber* from summaries of cave-associated salamanders (Weber 2004; Durand 2005). Furthermore, recent conservation assessments (Hunsinger 2005) and habitat guidelines (Bailey et al. 2006) ignore caves as important habitat for this species. This is particularly surprising as *P. ruber* inhabits caves throughout much of the southeast (e.g., West Virginia: Green and Brant 1966; Carey 1973; Osbourn 2005; Tennessee: Barr 1949; Miller and Niemiller 2005; Georgia: Buhlmann 2001; Niemiller et al. 2006; Alabama: Knight 1969; Mississippi: Brode 1958; Brode and Gunter 1958). Moreover, *P. ruber* commonly nest in caves within the southeast (Miller and Niemiller 2005; Niemiller et al. 2006; this study). The distinction between spring habitat and cave-stream habitat may seem trivial, but has important management



ramifications. Management practices that protect springs inhabited by *P. ruber* will often protect cave habitat. Conversely, springs commonly suffer from environmental damage that seldom impinges the deeper cave stream. For example, the spring associated with the cave reported here flows from the cave mouth into a cobble stream that continues 10 m before entering a pasture. Cattle use the spring as a water source and regularly penetrate the cave entrance. Although cattle certainly impact the spring and surrounding habitat, the interior cave stream is unaffected and supports a rich and diverse assemblage of amphibians. In addition to consequences associated with habitat management, recognizing *P. ruber* as a troglophile contributes to our understanding of the evolution of cave-dwelling organisms in general and *P. ruber* in particular, and of the importance of caves to amphibian communities.

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**BRIAN MILLER** is a Professor of Biology at Middle Tennessee State University where he teaches comparative vertebrate anatomy, vertebrate zoology, herpetology and freshman biology courses. He received his B.S. and M.A. from the University of Missouri and his Ph.D. from Washington State University. His research focuses on the natural history, morphology, and conservation of amphibians and reptiles, especially that of salamanders. Here he is holding a *Gyrinophilus subterraneus* from General Davis Cave in West Virginia, USA. Photographed by Dante Fenolio.



**MATTHEW L. NIEMILLER** is currently a Ph.D. student in the Department of Ecology and Evolutionary Biology at the University of Tennessee, USA. He received his B.S. and M.S. from Middle Tennessee State University working under Brian T. Miller. His current research focuses on the ecology, phylogeography, and conservation genetics of cave organisms with an emphasis on cave fishes and salamanders. Photographed by Brian Miller.



**R. GRAHAM REYNOLDS** is a Ph.D. student in the Department of Ecology and Evolutionary Biology at the University of Tennessee. He received his B.A. from Duke University, where he studied female mate choice in poison-dart frogs. His research interests include conservation genetics, biogeography, and phylogeography of tropical island reptiles, as well as testing predictions of theoretical population genetics using empirical data. His current work is with reptiles in the Bahamas and Turks and Caicos Islands. Photographed by Stesha Pasachnik.