Notes on the Reproduction of the Streamside Salamander, *Ambystoma barbouri*, from Rutherford County, Tennessee

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Abstract - Populations of *Ambystoma barbouri* (Streamside Salamander) have recently been identified in the Central Basin of middle Tennessee. Little is known regarding the distribution, natural history, and health of populations in Tennessee, resulting in the salamander being "deemed in need of management" by state agencies. Here we provide information regarding reproduction for the only known extant populations in Rutherford County, TN. In February 2005, fifty-one egg masses from three first-order streams were digitally photographed and analyzed to determine size of egg mass, stage of development, ovum diameter, and embryo length. Number of eggs per mass, size of ova, and size of embryos were comparable to that reported in the literature. Egg masses varied noticeably in stage of embryonic development; we estimate that oviposition in 2005 occurred between early December and early February. *Eurycea cirrigera* (Southern Two-lined Salamanders) oviposited in the same first-order streams and during the same time period as Streamside Salamander. Continued deforestation and land development associated with urban sprawl from the city of Murfreesboro threaten existing populations of Streamside Salamander. The information in this study can be used to provide a basis for developing conservation plans for the Streamside Salamander in middle Tennessee and for scheduling construction activities such that they least affect breeding activities.

Introduction

Ambystoma barbouri Kraus and Petranka (Streamside Salamander) is a stream-breeding member of the Family Ambystomatidae (mole salamanders) with a contiguous distribution in southeastern Indiana and southwestern Ohio into central and northern Kentucky; isolated populations are known from Livingston and Russell counties, KY, Wayne County, WV, and Davidson, Jackson, and Rutherford counties in middle Tennessee (Kraus and Petranka 1989, Niemiller et al. 2006, Petranka 1998, Regester and Miller 2000, Scott et al. 1997). Tennessee populations have only recently been assigned to the Streamside Salamander (Scott et al. 1997) from reevaluation of specimens collected during 1967 in Davidson County, 1973 in Jackson County, and 1996 in Rutherford County that were originally identified as *A. texanum* (Matthes) (Small-mouthed Salamander). The streams and surrounding countryside used by Streamside Salamander in Davidson County (Ashton 1966) have been greatly altered during the past 40 years with the growth of metropolitan Nashville, and several attempts by B.T. Miller and

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his students to locate breeding sites in this area during the past decade have been unsuccessful. The status of the Jackson County population is currently unknown, but breeding populations are extant in Rutherford County (Niemiller et al. 2006, Regester and Miller 2000).

Most of the information on Streamside Salamander natural history is derived from populations in Kentucky. Outside of the breeding period, adults inhabit upland deciduous forests in the vicinity of ephemeral first- and second-order streams with beds formed of exposed limestone slabs or bedrock (Petranka 1998). Adults migrate to breeding streams from late October through March (Petranka 1984), and females oviposit from mid-January to early April (Barbour 1971, Keen 1975, Petranka 1984). Females typically deposit eggs singly in a monolayer on the undersurface of submerged, flat rocks (Ashton 1966; Kraus and Petranka 1989; Niemiller et al., in press; Petranka 1982; Regester and Miller 2000). Autumn breeding migrations have not been reported for the Tennessee populations, but breeding activities, as determined by the presence of egg masses, begin during early December and extend into March (Niemiller et al. 2006).

The distribution, natural history, and health of Streamside Salamander populations within Tennessee remain largely unknown, with information on these topics limited to that contained in the brief reports of Ashton (1966), Scott et al. (1997), Regester and Miller (2000), and Niemiller et al. (2006). Because of the limited distribution of the species and lack of information on natural history, Streamside Salamander is "deemed in need of management" by the Tennessee Wildlife Resources Agency (TWRA; Withers et al. 2004). This state listing is analogous to the "special concern" category of other states, and is used by the TWRA when the executive director believes that a species (or subspecies) should be investigated so that a database can be created on distribution, demography, habitat needs, limiting factors, and other pertinent natural history information (Withers et al. 2004). The database is then used to develop management measures to ensure the continued survival of the populations. The purpose of our study was to provide information on reproduction of the only known populations of the Streamside Salamander in Tennessee.

Materials and Methods

On 16 February and 23 February 2005, we searched for egg masses of Streamside Salamander ca. 100 m upstream and downstream from road crossings of the three streams east of Christiana in Rutherford County: a first-order tributary to Long Creek (35º43'03"N, 86º22'29"W; datum NAD27; 16 Feb 2005), a first-order tributary to Middle Fork Stones River (35º40'52"N, 86º20'59"W; 16 Feb 2005), and a first-order tributary to Hurricane Creek (35º43'59"N, 86º17'43"W; 23 Feb 2005). These localities are found within the Inner Nashville Basin ecological subregion of the Interior Plateau in the Stones River watershed (Griffith et al. 1997). This region is characterized by gently rolling terrain with shallow soils and outcrops of Ordovician-age limestone. These streams are of low gradient, flow over large expanses of exposed bedrock, and are ephemeral, becoming reduced to isolated pools or flowing underground during late summer and fall.

Rocks within pool, run, and riffle habitat were carefully lifted to locate egg masses. Lifted rocks and other cover objects were returned to their original positions to minimize habitat disturbance. Egg masses from the three localities were photographed with a Sony Cybershot® DSC-F707 digital camera from a distance of 10–20 cm. Photographs were taken under natural light conditions with a flash, and a ruler was positioned adjacent to each mass to reference size. A digital image (1280 x 960 pixels) of each egg mass was analyzed in Photoshop 6.0 (Adobe Systems, Inc., San Jose, CA) to determine total number of eggs per mass (EPM), stage of development, ovum size (mean diameter), and embryo length (mean total length). Stage of development was determined according to Harrison (1969). Most eggs forming a mass were at the same stage, although a few eggs varied as much as two stages from the modal stage. We used the modal stage of each mass for all calculations. Because of accumulation of silt and the growth of algae on eggs, not all ova or embryos could be measured within an individual mass (Fig. 1). Furthermore, internal ova of females collected from middle Tennessee populations in 2002 and accessioned into the Middle Tennessee State Herpetology Collection were counted to determine potential clutch size.

Results

Fifty-one egg masses were photographed. EPM varied markedly at all three localities, ranging overall from 1–211 (Table 1). Although the mean EPM did not differ significantly between localities ($F_{2,48} = 3.02$, $P = 0.058$), small sample size makes it difficult to interpret these results. Masses with few eggs were at an advanced stage of development, and many were near

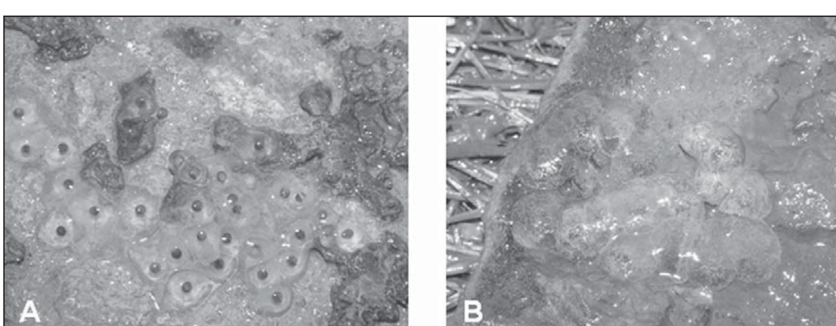

Figure 1. Comparative images of *Ambystoma barbouri* (Streamside Salamander) egg masses observed on the undersurfaces of flat submerged rocks. Ova and embryos are easily observed and counted in recently laid masses or masses laid in sediment-free sections of streams (A), but visibility of developing ova and embryos may be obscured by the accumulation of silt and algal growth in stream sections subject to siltation (B).

hatching when discovered. Consequently, mean EPM may be skewed towards a smaller size. Among all three localities, stage of development of egg masses ranged from Harrison stage 10 to stage 42, with the majority of masses either at stages 10–14 (19.6%; $n = 9$) or stages 37–42 (68.6%; $n = 35$). Mean stage of development could not be determined for two masses because of accumulation of silt and algal growth. Mean diameter of stage 10–14 ova ranged from 2.3 mm to 4.2 mm and was significantly different between sites ($t = 7.93$, df = 502, $P < 0.0001$; Table 1). All ova from Hurricane Creek tributary were at stage 37 or greater. Mean total length of stage 37–42 embryos differed significantly between localities ($F_{2,415} = 31.85$, $P < 0.0001$). Females examined from museum specimens collected in 2002 contained 98–278 mature ova ($n = 3$).

Discussion

As in other states, Tennessee populations of Streamside Salamander breed in ephemeral first-order streams, depositing eggs singly in monolayers on the undersurfaces of flat, limestone rocks. Nearly all masses were found within stream runs or pools; one egg mass with two eggs was found attached to submerged grasses along the shoreline of a pool at the tributary to Hurricane Creek. Streamside Salamanders breed syntopically with *Eurycea cirrigera* (Green) (Southern Two-lined Salamander) in Rutherford County. Three egg masses, two with attending females, of Southern Two-lined Salamander were discovered on the undersurface of submerged rocks in riffles (two egg masses) and runs (one egg mass) within two meters of Streamside Salamander egg masses in the tributary to the Middle Fork of the Stones River. Larval Southern Two-lined Salamander were also present at this locality. Both larvae and adult Southern Two-lined Salamander were observed at another Streamside Salamander breeding locality; however, egg masses of Southern Two-lined Salamander were not observed. The effects, if any, of competition for oviposition sites, egg and larval development, and egg

Table 1. Reproductive data for 51 egg masses of *Ambystoma barbouri* (Streamside Salamander) from three first-order streams in Rutherford County, TN. Locality = tributary of stream; # = number of egg masses observed at locality; EPM = mean number of eggs per egg mass ± 1SD; *n* = number of eggs measured; Diameter = mean diameter of ova in mm ± 1SD; TL = mean total length of embryos ± 1SD. Stages according to Harrison (1969).

		Ova (stages 10–14)					Embryos (stages 37–42)		
Locality	#	EPM	Min–Max	n[1]	Diameter	Range	n[1]	TL	Min–Max
Long Creek	14	38.1 ± 43.8	2–170	67	2.7 ± 0.2	2.3–3.3	59	14.0 ± 1.7	11.8–18.5
Middle Fork Stones River	12	79.3 ± 58.6	16–191	437	3.1 ± 0.3	2.3–4.2	81	12.8 ± 0.9	10.8–14.3
Hurricane Creek	25	35.7 ± 54.4	1–211	NA	NA	NA	278	14.2 ± 1.6	10.9–18.6
Total	51	46.6 ± 54.8	1–211	504	3.0 ± 0.3	2.3–4.2	418	13.9 ± 1.6	10.8–18.6

[1]Not all eggs or embryos in an individual egg mass could be measured because of algal or silt accumulation.

and larval survivability between Streamside Salamander and Southern Two-lined Salamander are unknown, but warrant further study.

EPM in the present study is comparable to that reported in the literature. Petranka (1984) reported from 8 to 1142 EPM, considerably greater than the 1 to 211 range observed in this study. Regester and Miller (2000) also reported low EPM from nearby Puckett's Creek in Rutherford County (range 5–65, *n* = 10). The low EPM may suggest lower fecundity of Tennessee Streamside Salamander populations, but it might be attributable to timing of oviposition. Forty-five percent of the egg masses observed during this study contained 20 or fewer eggs. Of these 23 egg masses, 78.3% were beyond stage 37 (mean stage of development of eggs within a mass). The jelly coating of the egg capsule deteriorates soon after hatching; consequently, egg masses with few eggs may represent the last remaining eggs of larger clutches. Egg masses with low numbers of eggs also may be reflective of females that were disturbed during oviposition, or those egg masses that were partially ingested by predators. Few reports of the natural predators of eggs or adults of Streamside Salamander exist, although *Lepomis cyanellus* Rafinesque (Green Sunfish), crayfish, *Nerodia sipedon* Linnaeus (Northern Watersnake), and the planarian *Phagocata gracilis* (Halderman) have been reported to feed on larvae (Holomuzki 1989, Kats 1986, Petranka 1998, Petranka et al. 1987). Kats and Sih (1992) found that pools that contained predatory fish had significantly lower densities of egg masses than nearby pools without fish. Although *Lepomis* were not observed at the three stream localities, *Cottus carolinae* (Gill) (Banded Sculpin) and *Cambarus* sp. (crayfish) were noted. Additionally, *N. sipedon pleuralis* (Cope) (Midland Watersnakes) are locally common and may be active on warm, rainy nights in late winter/early spring (M.L. Niemiller, pers. observ.).

Petranka (1984) found an average of 262 (min-max = 184–397) mature ova per adult female (*n* = 14) examined. These values exceeded those observed in Rutherford County, TN (Regester and Miller 2000, present study). However, our mean diameter of early stage ova lies within the min-max values (2.4–3.8) reported by Petranka (1998). Consequently, it is unlikely that differences in observed EPM between Kentucky and Tennessee populations are the result of a tradeoff between ovum size and clutch size. Rather, female Streamside Salamanders in Rutherford County, TN, likely distribute their clutches among multiple, smaller egg masses under separate rocks. Other species of *Ambystoma* (e.g., *A. talpoideum* (Holbrook) [Mole Salamander] and Small-mouthed Salamander) have been reported to distribute eggs in smaller masses or scatter their eggs singly (Semlitsch and Walls 1990, Trauth et al. 1990) rather than deposit their entire clutch in a single mass.

Both diameter of ova and total length of late-term embryos differed significantly between localities. Rather than being correlated to tradeoffs between ovum size and clutch size, the observed difference may be an artifact of small sample size. Although diameter of ova differed between two of the localities surveyed (Long Creek and Middle Fork Stones River), means and min-max values of both localities fall within the values reported by Petranka (1998).

We conclude that female Streamside Salamander at our field sites oviposited between early December 2004 and early February 2005. This estimate is based on the observation of egg masses in differing stages of embryonic development and assumes a 29–82 day incubation period (Petranka 1998). Furthermore, hatchlings were observed on 23 February 2005 in a roadside ditch hydrologically connected to the Hurricane Creek tributary. Timing of oviposition during this study is consistent with the late December through mid-February period reported by Regester and Miller (2000), and the late December through early April period reported by Ashton (1966) for middle Tennessee populations. By comparison, breeding occurs in central Kentucky from late December to mid-April (Petranka 1998).

Streamside Salamander populations in middle Tennessee are threatened by deforestation and development of land around breeding streams (Niemiller et al. 2006). Road and other construction associated with the expansion of the city of Murfreesboro are occurring near or adjacent to known Streamside Salamander breeding sites (Niemiller et al. 2006, Regester and Miller 2000). Such habitat disturbance is also the main threat facing many populations of Streamside Salamander outside of Tennessee (Petranka 1998, Watson and Pauley 2005). This urban sprawl will continue to threaten Streamside Salamander populations in Rutherford County; the human population of the county was predicted to increase nearly 75% from 2000 to 2025 (Arnwine et al. 2003). Roads heavily dissect the area inhabited by Streamside Salamander in Rutherford County (Niemiller et al. 2006) and, during the breeding season, adult salamanders migrating to breeding streams are killed unintentionally by motorists during evening and nighttime rainstorms (B.T. Miller and M.L. Niemiller, pers. observ.). Several forested riparian zones that support terrestrial activities of Streamside Salamander are being converted to residential lawns (B.T. Miller, pers. observ.). The continued loss of forested riparian habitat will ultimately pose an insurmountable obstacle for the survival of Rutherford County populations of Streamside Salamander. Loss of forested riparian habitat increases insolation, water temperature, and exposure to ultraviolet light (Corn et al. 2003), and also increases sedimentation and silt load, which can negatively impact development of amphibian eggs (Corn and Bury 1989, Corn et al. 2003). Nevertheless, the reproductive information presented in this study should be used by both state and local agencies as they develop conservation plans for these threatened populations. As development escalates in southern Rutherford County, construction activities should be curtailed from December through April to least affect breeding and developing Streamside Salamander embryos. Limiting construction to non-breeding periods will also reduce the amount of heavy traffic during periods when transformed Streamside Salamander are surface active. Moreover, forested land adjacent to first- and second-order stream breeding sites should be maintained and preserved in an effort to protect breeding habitat and surrounding forests inhabited by adult Streamside Salamander.

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